DIVERSITY OF PHOSPHATE SOLUBILIZING BACTERIA IN JHUM FALLOW SOIL OF SERCHHIP DISTRICT, MIZORAM

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CERTIFICATE

This is to certify that the thesis work entitled**, "Diversity of Phosphate Solubilizing Bacteria in Jhum Fallow Soil of Serchhip District, Mizoram"**, submitted by Remruattluanga Hnamte (**MZU/Ph.D./744 of 22.05.2015)** in partial fulfillment of the requirement for the degree of Doctor of Philosophy in Botany is a record of bonafide work carried out by her under my supervision and guidance.

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DECLARATION BY THE CANDIDATE

I, **Remruattluanga Hnamte** hereby declare that the subject matter of this thesis entitled "**Diversity of Phosphate Solubilizing Bacteria in Jhum Fallow Soil of Serchhip District, Mizoram**" 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.

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TABLE OF CONTENTS

Preface

Jhum cultivation also known as slash and burn agriculture is a common agricultural practice in tropical hilly areas of Southeast Asia, the Pacific, Latin America, the Caribbean, and Africa for millennia. In India, jhum cultivation is mainly practice in the tribal areas of the states of Bihar, Orissa, Madhya Pradesh, Maharashtra, Gujarat, Rajasthan, Tamil Nadu, Kerala, Karnataka, Andhra Pradesh and North Eastern India (NEI). In North Eastern India, jhum cultivation is not only the source of livelihood but also has high cultural importance among the people. The extent of such cultivation is highest in Nagaland, accounting for 38.18% of its geographical area, followed by Mizoram and Manipur

In jhum cultivation fire is deliberately used to change soil physico-chemical properties, and both the release of nutrients by fire and the value of ash have been known to cause alteration in soil biochemical properties. In course of time, different adverse effects emerge because of Jhum cultivation which has detrimental effect on the soil nutrients status and biological properties due to deliberate slashing and burning of vegetation involved in the process.

Phosphorus (P) which is an essential nutrient for the growth and development of plants undergo large volatilization and convective losses during jhum cultivation. The amount available P in soil for plant utilization is very low and the new sources to replace the lost available P mainly comes from non-plant available P in soil and from parent materials which needs to be solubilized through the action of enzymes known as *phosphatase.* These enzymes are secreted by a group of microorganisms known as Phosphate Solubilizing Microorganism (PSM), among which Phosphate Solubilizing Bacteria (PSB) are predominant. These PSB(s) can be used for the rapid solubilization of insoluble P, thereby supplementing the low available P level in soil. Hence these PSB can be developed into location specific fertilizers to maintain the sustainability of jhum cultivation. The study was undertaken to assess the diversity of PSB in different length jhum fallow so as to developed into location specific biofertilizer which will serve as a mean to ensure the sustainability of jhum cultivation in future.

The thesis is broadly categorized into nine (9) chapters. Chapter 1 and 2 deals with the general introduction and review of literature respectively. Chapter 3 deals with the study of the soil physico- chemical properties of jhum and jhum fallow soils. Chapter 4 deals with the study of soil biochemical properties including soil enzymes and soil microbial biomass carbon and nitrogen of the study sites. Chapter 5 covers metagenomic analysis of bacterial diversity in the study sites. Chapter 6 includes the isolation and molecular characterization of phosphate solubilizing bacteria using 16Srrna gene sequencing. Chapter 7 deals with the biochemical characterization and assessment of plant growth promoting characters of the isolated PSB. Chapter 8 deals with the in vitro evaluation of selected psb to alleviate rice growth performance under different treatments. lastly the findings of the thesis are summarised and concluded in Chapter 9.

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

β-GSD	β-Glucosidase
µM	micromolar
µl	microliter
AN	Available Nitrogen
ANOVA	Analysis of variance
AK	Available potassium
AP	Available phosphorus
APase	Acid phosphatase
ARS	Arylsulfatase
BD	Bulk density
BLAST	Basic Alignment Search Tool
bp	base pair
C	Celsius
CFU	Colony Forming Unit
cm	centimeter
CTAB	Cetyl Trimethyl Ammonium Bromide
DAS	Days After Sowing
DES	Deep Eutectic Solvents
DHA	Dehydrogenase
dNTPs	Deoxyribonucleotide triphosphate
EC	Electrical conductivity

EDTA	Ethylene diamine tetraacetic acid
Fig	Figure
g	gram
GA	Gibberellic acid
ha	hectare
hrs	hours
IAA	Indole acetic acid
in	inch
K	Potassium
kg	kilogram
L	Litre
M	Molar
mg	milligram
Mha	million hectare
ml	mililiter
mm	milimeter
mM	Milimolar
MEGA	Molecular Evolutionary Genetics
MT	Metric Ton
MUB	Modified universal buffer
ng	nano gram
N	Nitrogen

N_2	Dinitrogen
N_{nin}	ninhydrin-reactive N
NEI	North Eastern India
NGS	Next generation sequencing
NH_3	Ammonia
p-NPP	p-nitrophenyl phosphate
pmol	picomole
ppm	parts per million
OD	Optical Density
OUT	Observed Operational Taxonomic unit
P	Phosphorous
PCR	Polymerase Chain Reaction
PGP	Plant Growth Promoting
PGPR	Plant Growth Promoting Rhizobacter
PSB	Phosphate Solubilzing Bacteria
PSM	Phosphate Solubilzing Microbe
QIIME	Quantitative Insights into Microbial Ecology
rDNA	ribosomal Deoxyribo Nucleic Acid
RNA	Ribo Nucleic Acid
rRNA	ribosomal Ribo nucleic acid
rpm	revolutions per minute
Secs	seconds
SEW-M	salt/ethanol wash solution

SOC	Soil organic carbon
SOM	Soil organic matter
SMC	Soil moisture content
sp.	Species
spp.	Species (plural)
sq cm	square centimeter
TN	Total nitrogen
U	unit
URS	urease
UV-vis	Ultra violet visible
v/v	volume by volume
w/v	weight by volume

Chapter 1

Introduction

Shifting cultivation also known as Jhum cultivation is a widely practiced primitive agricultural land use believed to have originated around 7000 BC during the Neolithic period (Borthakur, 1992). Jhum cultivation also known as slash and burn agriculture has been a common agricultural practice in tropical hilly areas of Southeast Asia, the Pacific, Latin America, the Caribbean, and Africa for millennia. In Southeast Asia, jhum cultivation is mainly practice in Sri Lanka, Northern Burma, Thailand, Sumatra, Borneo, Indonesia, Philippines, Korea, Vietnam and India. (Ramakrishnan, 1992; Stromgaard, 1992; Craswell *et al.*, 1997; Cairns and Garrity, 1999; Kato *et al.,* 1999; Lawrence and Schlesinger, 2001; Eastmond and Faust, 2006 Thomaz, 2009).

The total estimated area under the shifting cultivation in India is about 0.9 million ha which includes both current jhum (53%) and abandon jhum (47%). In India, jhum cultivation is mainly practice in the tribal areas of the states of Bihar, Orissa, Madhya Pradesh, Maharashtra, Gujarat, Rajasthan, Tamil Nadu, Kerala, Karnataka, Andhra Pradesh and North Eastern India (NEI). The NEI contributes 83% of the total shifting cultivation in India. According to Waste Land Atlas of India (2010), the area under jhum cultivation in North Eastern India is about 0.76 Mha. In Northeast India, especially in the hilly regions comprising of the states of Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura, jhum cultivation is not only the source of livelihood but also has high cultural importance among the people. The extent of such cultivation is highest in Nagaland, accounting for 38.18% of its geographical area, followed by Mizoram and Manipur **(**Das *et al.,* 2017).

The state of Mizoram is one amongst the eight sister states of NEI. A landlocked state, it extends between 21º 58' - 24º 35' N and 92o 15' - 93 º 29' E, covering an area of 2,108,700 hectares. According to the Census of India 2011, total population of Mizoram was 1,091,014. The term 'Mizoram' refers to the land of the "Mizo(s)", the ethnic tribe who settle in the area. The state economy is based upon agriculture and mainly dependent on the traditionally cultivated crops. About 80% people are engaged in agricultural practices.

Out of the total geographical area (2,108,700 hectares), total crop area accounts for 188 hectares out of which 186 hectares are sown area. Fallow land other than current fallow accounts 106 hectares while current fallow or current jhum accounts for only 45 hectares. (Statistical hand book of Mizoram, 2018). The principal crop sown in these jhum fields is paddy or rice (*Oryza sativa* L.) which sometimes is mixed with other crops like maize, cucumber, beans, arum, ginger mustard, sesame and cotton. Due to increase population and land pressure, the fallow cycle under jhum cultivation in Mizoram has been decreased from 20-25 years to 2-3 years. Therefore, it has put excessive burden on the land thus, soil fertility has been reduced. In Mizoram, the economic and cultural life of the people has always been centered on shifting cultivation. The total consumption of rice in Mizoram is 1, 80,000 MT whereas, it produces only 44,950 MT rice (25%). These rice produce by the state is mainly from the jhum cultivation for the past few decades (Sing *et al.,* 2017).

In jhum cultivation, a piece of forest land was cleared, left to dry and the dried biomass is burned *in situ* followed by cropping phase. Once the fallow lands regain its fertility, the farmers come back to the same piece of land for cultivation. The shifting of jhum fields from one site to another result in a dynamic landscape (Zimmermann and Eggenberg, 1994), consisting of a mosaic of jhum fields, different aged fallows and mature forest which can be under constant change (Fox, 2000; Metzger, 2003; Brown, 2006).

The process of Jhum cultivation consists of three basic phases such as conversion, cultivation and fallow. Conversion involves the clearing of native vegetation and the burning of the dried biomass. This slash and burn process uncovered the soil for planting, eliminates competing plant cover and improves soil fertility by altering pH and increase availability of nutrients due to ash deposition (Kleinman *et al.,* 1995). The cleared lands are cultivated for one or two years, depending upon the region. The field is then abandoned or left fallow to restore soil fertility through natural regeneration, and cultivation is shifted to other forested area (Tawnenga, 1997; Grogan *et al.,* 2012; Yadav, 2013). During the fallow period, the abandoned field is colonized by native herbaceous, shrub or tree species or by adventive species that find the ecological conditions favourable (Norman *et al.,* 1995). Jha (1997) noted that shifting cultivation is an agricultural practice which is characterized by a rotation of fields rather than of crops, by a short period of cropping alternating with long fallow periods (up to 10 and more years but often as short as 6–8 years). The duration of the fallow period varies from 5 to more than 10 years or more but is always longer than the cultivated period (Eden and Andrade, 1987; Kleinman *et al.,* 1995; Mertz *et al.,* 2009). This intervening duration between two successive slashing is termed as jhum cycle which is influenced by many factors such as population pressure, terrain, angle slope, soil and average annual rainfall. Jhum cultivation leads to the formation of mosaic landscape of cleared cultivated land mix with secondary forests in different stages of regeneration, along with a mature forest matrix that helps to sustain them (Conklin, 1961; Harris, 1971; Hiraoka and Yamamoto, 1980; Egger, 1981; Altieri *et al.,* 1987; McGrath, 1987). It is hypothesise that the fallowing of jhum fields up to 10 years or more would allow the recuperation of the soil fertility to such extend that the site can be cultivated again for another jhum cycle without compromising its sustainability (Toky and Ramakrishnan 1981; Tawenga *et al.,* 1997; Manjunatha and Singh, 2020)

The basic principles of sustainable jhum cultivation are that plant-available nutrients lost during the cropping phase should be regained by new supply over the full rotational cycle, and that all other components of soil quality (e.g. texture and organic matter content that together determine water holding capacity) should be

restored. In other words, nutrient removal within the harvested crop plus nutrient losses due to run-off, leaching and fire volatilization should not surpass plant-available nutrient inputs from *in situ* soil biogeochemical transformations from non-available forms such as phosphorus-containing compounds, soil carbon and nitrogen etc. (Lawrence and Schlesinger, 2001).

In jhum cultivation fire is deliberately used to change soil physico-chemical properties, and both the release of nutrients by fire and the value of ash have been known to cause alteration in soil biochemical properties. Removal of natural vegetation for cropping interrupts natural mineral cycling between soils and plants, leaving the soil more vulnerable to wind erosion and water erosion. It loosens the soils and caused an increased rate of oxidation of organic matter and weakens soil structure (Matthews, 2003).

In course of time, different adverse effects emerge because of jhum cultivation. With the increasing population, more stress is being exerted on the land due to shorter jhum cycles. Short fallow periods are no longer capable to restore the soil productive capacity. Therefore; as a result, there is decline in crops yield. The main reason why the jhum cultivation persisted lies in its compatibility with the physical environment. Total aboveground nutrient loss during slash-and-burn clearing of forest land is among the highest of any disturbance known. Aboveground nitrogen (N) can undergo large, nearly parallel losses with carbon (C) during biomass burning (Raison *et al.,* 1985), with release rates ranging from 30% to 90% of total aboveground stock (Buschbacher *et al.,* 1988; Kauffman *et al.,* 1995a). Phosphorus (P) in aboveground biomass can also undergo large volatilization and convective losses during slash burning (Kauffman *et al.,* 1995; Giardina *et al.,* 2000). The new sources of P to replace losses include non-plant available P in soil and in parent material, low levels of atmospherically deposited P and fertilizer (Romanyà *et al.,* 1994).

Phosphorus is the second most important macro nutrient required by the plants, after nitrogen. It is a vital nutrient for plant growth and productivity. It

accounts for between 0.2 and 0.8% of the dry weight of plants and it is contained within nucleic acids, enzymes, co-enzymes, nucleotides, and phospholipids. P is essential in every aspect of plant growth and development, from the molecular level to many physiological and biochemical plant activities including photosynthesis (Sharma *et al.,* 2013), development of roots, strengthening the stalks and stems, formation of flowers and seeds, crop maturity and quality of crop, energy production, storage and transfer reactions, root growth, cell division and enlargement, N fixation in legumes, resistance to plant diseases, transformation of sugar to starch, and transporting of the genetic traits (Khan *et al.,* 2009; Satyaprakash *et al.,* 2017; Kumar *et al.,* 2018).

Phosphate compounds present in soil can be classified into three groups, viz. soluble orthophosphate, insoluble inorganic phosphate, and insoluble organic phosphate. Orthophosphate is the only form that can be taken up by the plants. It reacts with numerous inorganic and organic constituents of soil and therefore becomes least mobile and unavailable for uptake by plants (Behera *et al.,* 2014). Inorganic P occurs in soil, mostly in insoluble mineral complexes, some of them appearing after frequent application of chemical fertilizers. These insoluble, precipitated forms cannot be absorbed by plants. Organic matter is also an important reservoir of immobilized P that accounts for 20–80% of P in soils. Only 0.1% of the total P exists in a soluble form available for plant uptake because of its fixation into an unavailable form due to P fixation (Sharma *et al.,* 2013).

In jhum cultivation, the burning of the plants affects the microbial population and also the enzyme activities of the soil. Living microbial populations are the most dynamic among soil components and they control the fertility and productivity of soil ecosystems. Top soil is the most active zone of decomposition, nutrient cycling and mycorrhizal formation and becomes the reservoir of bacterial and fungal spores and other propagules of microbes. The physical and chemical properties of soils can directly influence the structure, spatial distribution and activity of microbial population and enzymes in soils, which are potential early indicator of soil health and quality (Schnurer *et al.,* 1985; Dick, 1994).

According to DeBano and Conrad (1978) fire affects soil in several ways. During clearing of jhum fields, upper soil is exposed to very high temperature and even small rise in temperature of the soils significantly alters the soil physic-chemical properties (Mukherjee, 1954). The jhum cultivators set fire to clear land and this volatilizes nutrients and alters above and below ground composition of soil micro-flora. Consequently, the soil microbial population is altered, which changes the biochemical nature of soil and reduces productivity. Unfortunately, this biological component has largely been ignored as an important aspect of ecosystem functioning. Re-vegetation of fallow lands followed by shifting cultivation is usually a slow process that does not restore soil microorganisms to their pre disturbance state (Greipsson and El-Mayas, 1999).

The differentiation between the different pools of enzyme activities in soils is important, because enzyme activities can be used as indices of nutrient cycling and the health of agricultural ecosystems (Klose and Tabatabai, 1999). Soil enzymes are essential for catalyzing reactions necessary for organic matter decomposition (Ajwa *et al.,* 1999) and their activities are strongly influenced by organic matter content of the soil (Speir, 1997). The enzyme activities have often been used as indices of microbial activity and soil fertility (Dick and Tabatabai, 1992). Human activities that minimize addition of the organic matter to the soil may reduce enzyme activities and could alter the availability of nutrients for plant uptake (Dick *et al.,* 1998).

Soil enzyme activity is the total sum activity of accumulated enzymes and enzymatic activity of proliferating microorganisms (Kiss *et al.,* 1975). They are usually associated with viable proliferating cells, but enzymes can be excreted from a living cell or released into the soil solution from dead cells. Study of soil enzymes gives information about the release of nutrients in soil by means of organic matter degradation and microbial activity as well as indicators of ecological change. Soil enzymes analysis helps to establish correlation with soil fertilization, microbial activity, biochemical cycling of various elements in soil, degree of pollution (heavy metals) and to assess the succession stage of an ecosystem. So, measurements of

enzyme activity in degraded soils have useful in examining impacts of environmental change or management on soil enzyme activities.

A large number of microbial organisms including bacteria, fungi, actinomycetes, and algae exhibit P solubilization and mineralization ability, these microbes are known as Phosphate solubilizing microorganisms (PSMs). PSMs are beneficial microorganisms capable of hydrolyzing organic and inorganic phosphorus compounds from insoluble compounds. Phosphate Solubilizing Microbes plays an important role in plant nutrition through increase in phosphate uptake by plants and used as biofertilizers of agricultural crops (Karpagam and Nagalakshmi, 2014).

Application of PSMs as biofetilizer in soil present to be a productive way to convert the insoluble P compounds to soluble plant available P form, ensuing in better plant growth, crop yield, and quality. *Bacillus, Pseudomonas*, *Rhizobium, Aspergillus, Penicillium*, and arbuscular mycorrhizal fungi are the most efficient P solubilizers for increasing bioavailability of P in soil (Kalayu, 2019). PSMs incite immediate plant growth by providing easily absorbable soluble P and production of plant growth hormones such as IAA and GA. Besides, PSM also acts as a biocontrol against plant pathogens via production of antibiotics, hydrogen cyanate (HCN) and antifungal metabolites (Ahemad and Kibret, 2014). PSMs represent potential substitutes for inorganic phosphate fertilizers to meet the P demands of plants, improving yield in sustainable agriculture. Their application is an ecologically and economically sound approach (Alori *et al.*, 2017).

Since bacteria are predominant amongst the phosphate solubilizing microbes and proved more effective in phosphorus solubilization than fungi and other microbes, they perform sustainably to enhance the productivity of crops in soil as biofertilizer agents (Prabhu *et al.*, 2018). Considering the immense potential of Phosphate Solubilizing Bacteria (PSB), in future, performing a detailed study to understand the ecology of phosphate-solubilizing microorganisms, especially under jhum land is a must. Therefore, the present study is the first attempt to study

biodiversity of phosphate solubilizing bacteria inhabiting jhum and it fallow land of Mizoram, in particular Hmuntha village, Serchhip District.

Therefore, it is crucial to explore the diversity of PSB in jhum and its fallow soil to further investigate the presence of potent bacteria which can be developed into biofertilizer which will serve as a mean to ensure the sustainability of jhum cultivation in future. Hence the study was undertaken to achieve the following aspects-

1. To isolate, screening and assessment of 16SrRNA gene profiling of phosphate solubilizing bacteria from the study sites.
2. To perform biochemical characterisation and to study the efficacy of phosphate solubilizing bacteria as potential biofertilizer.
3. To study biochemical and physico-chemical properties of soil from different jhum lands.

Chapter 2

Review of Literature

2.1. Jhum cultivation

Jhum cultivation has been a subject of research interest for several decades in different parts of the world where it is being practiced. Major research contribution on jhum cultivation was made by researchers like Conklin (1957), Spencer (1966), Jordan (1989), Ramakrishnan (1993), and Fox *et al.* (2000) who made extensive studies in parts of Africa and South East Asia. Jhum system of cultivation systems and their impacts on soil physico-chemical properties as well as soil microbial population have been widely studied by many researchers such as Juo and Manu (1996); Palm *et al.* (1996); Nandwa and Bekunda (1998); Giardina *et al.* (2000); Lawrence and Foster (2002); Bruun *et al.* (2009) and Mertz *et al.* (2009).

Greater part on the study of jhum cultivation in India is carried out in the Northeast region as it is a biodiversity hotspot and jhum cultivation is the predominant form of agricultural practice in the region. Significant pioneering studies on shifting cultivation in northeast India were carried out by Borthakur and his co-workers in Umaim and Meghalaya (Borthakur *et al.,* 1978), Ramakrishnan and his co-workers in parts of Meghalaya and Arunachal Pradesh (Ramakrishnan, 1993). Tawnenga and his co-workers in Mizoram (Tawnenga *et al.,* 1996 and1997) and Choudhury (1998) in parts of Meghalaya (West Garo Hills) and Nagaland (Makokchung) also contributed to the studies of jhum cultivation in northeast India. Apart from the abovementioned researchers' such as Choudhury and Singh (1998); Mokhopadhyay (2000); Darlong *et al*. (2001); Tiwari (2001); Cairns (2004); Upadhyay and Jain (2004) have contributed to the study of jhum cultivation and its impact on the agriculture, soil, forest cover and socio-economic condition of Northeast India

In Mizoram, the studies on ecological impact of shifting cultivation have been carried out by Tawnenga (1990) and Tawnenga *et al.* (1997).

Gogan *et al.* (2012) write a review on the management options and research priorities for jhum cultivation in Mizoram state. Sapriliana *et al*. (2016) studied the impact of jhum cultivation on litter accumulation, soil enzymes and soil properties of jhum soils in Mizoram. Lungmuana *et al.* (2019) carried out the study on impact of secondary forest fallow period and transformation of jhum to plantations on soil microbial biomass carbon and enzyme activity dynamics. Sahoo *et al*. (2019) also contribute to the study of jhum cultivation by highlighting the effect of jhum cultivation on soil organic carbon pool in Mizoram. Apart from these researchers, various workers such as

The sustainability of jhum cultivation is a current topic of discussion in scientific as well as institutional communities; however, there is no present agreement. According to Watters (1971) jhum cultivation is an ideal subsistence agricultural practice in the humid tropics. Researchers such as Kleinman *et al.* (1995), Johnson *et al.* (2001) and Pedroso-Junior (2009) noted that with low population densities and long fallow periods enough to restore soil fertility jhum cultivation can provide a sustainable agricultural practice for the tribal people. This agricultural system is ecologically stable and meets a variety of human needs with great efficiency, particularly with regard to labour and other agricultural inputs. However, other researchers such as Altieri *et al.* (1987), Mertz (2002), Van Vliet *et al.* (2012), and Bruun *et al.* (2009) called attention that in many areas where shifting cultivation has been practiced successfully for centuries, a growing concern has developed for the sustainability of Jhum cultivation and the food security of subsistence farmers due to the rapid change in climatic, economic and political condition that has occurred in recent decades. Sanchez (1977) communicated that increase population leads to more pressure on the land use resulting in the reduction of the fallow period, hence recovery of the nutrient lost is not sufficient for sustainable agriculture. Eckholm (1976) also agreed that declining yields in jhum agriculture are often caused by declining soil fertility and poor plant nutrition as result of the fallow short fallow cycle. Unfortunately, no alternative agricultural practices have yet been proven biologically and economically feasible for replacement of jhum system in many areas of the tropics.

2.2. Soil physico- chemical properties

As summarised by Conklin (1957) and Watters (1960) the phases of jhum cultivation can be broadly divided into three *viz.* Conversion, cultivation and fallowing. These different phases of jhum cultivation have different impact on the soil physicochemical properties as well as biological properties. McGrath (1987) and Ramakrishnan (1992) defined the conversion phase as the clearing of selected areas of its native vegetation to space for the crops plants and the burning of the dried vegetation to increase the productivity of the crop, for this phase the majority of the impacts on the physical properties of the soil were negative.

This practice physically exposes the soil for planting, eliminates competing plant cover, and improves soil fertility by leaving it less acidic and with a greater availability of nutrients. However, Cunningham (1963) mentioned that until the first crop is fully established on the cleared land, rapid organic decomposition and the battering effect of raindrops on the soil can cause degradation of soil properties. Kato *et al.* (1999) and Hattori *et al.* (2005) also reported that the clearing and burning of vegetation has a detrimental effect on the soil temperature which affects texture, structure and biota of the soil.

The soil chemical properties are also altered after the conversion phase of jhum cultivation. However, in contrast to the impact on physical properties, the effects on soil chemical properties are mainly positive. According to Pedroso-Junior *et al.* (2009) and Bruun *et al.* (2009) these positive effects were expected due to the release of nutrients in the dried vegetation through burning. Thus, a considerable portion of the macronutrients stored in the burned biomass (vegetation) becomes available for the cultivation phase. This ensures that the productivity of the cultivation phase does not depend on external resources. Lessa *et al.* (1996) and Béliveau *et al.* (2014) noted an in increase in pH due to alkalinisation from addition ash from the burnt biomass. This improves the cation exchange capacity, nutrient availability and the microbial community of the soil. Also they reported a significant decline in the total soil nitrogen and carbon due to vitalization of these nutrients due

to fire. Other researchers Obale-Ebanga *et al.* (2003) and Yemefack *et al.* (2006) are also in agreement with these findings.

The exposure of the soil surface during the clearing and use of fire during the conversion phase also has a profound impact on the soil microflora. Yemefack *et al.* (2005) and Kendawang *et al.* (2005) report a negative effect on the soil microbial community due to increase in temperature and removal of moisture during the conversion phase of jhum cultivation.

During the cultivation phase, alteration in the soil physico-chemical and biological properties is reported by many researchers. As cited by Hölscher *et al.* (1997) the growth of the cultivar i.e. *Oryza sativa* L. has a positive effect on the soil physical characters such as soil texture, moisture content and temperature. There is recuperation of the soil structure and texture with increase CEC and soil organic matters. Soil temperature and moisture content are also positively impact due to the plant coverage. Bhadauria and Ramakrishnan (1989) reported the same findings.

Roder *et al.* (1995) and Lawrence *et al.* (2005) reported decrease in nutrient lost and progressive increase in nutrient availability due to coverage provided by the crops. According to Sillitoe and Shiel (1999) there is up keep of the pH in the soil changed by the ash, favouring the availability of nutrients. Throughout the cultivation period, the pH returns to its original, more acidic condition which favours the availability of nutrients. Christanty *et al.* (1986) cited the fallow period as a period in which the land is prepared for the next shifting cultivation cycle by controlled natural reforestation and forest enrichment which is important for re-establishing soil fertility.

The removal of vegetation and exposure of the soil after harvest of cultivars has a negative on the soil physico-chemical properties. There is compaction of the soil structure and texture, decrease inthe absorption capacity and moisture content; and increase in temperature leeching and erosion during the beginning of the fallow phase as reported by Mapa and Kumaragamage (1996); Rerkasem *et al.* (2009) and others. Roder *et al.* (1997) and Sakurai *et al.* (2005) report a loss of soil macro nutrients and temporary decease in the amount of soil organic matter due to the

increase surface runoff and leeching. Leeching also leads to the alteration of soil pH as a result of cations being washed away as mentioned by Weisbach *et al.* (2002)

These adverse changes are compromised as the length of the fallow periods increases. After 5 to 10 years or more of fallowing, the soil physico-chemical began to recover along with microbial community. With the successional stages of fallow period, there is an increase in the CEC and restoration of soil pH towards the climax ecosystem. There is an overall increase in the soil fertility and organic matter accumulation as well as increase availability of macro nutrients. These changes in the soil dynamics are reported by various workers such as Neergaard *et al.* (2008) and Mendoza-Vega and Messing (2005).

Similar patterns are observed for the soil microbial community. The adverse alteration of the soil physico-chemical properties during the initial stage of the fallow phase has a negative impact on the soil microbial community in terms of both quantity and quality. Garcia-Oliva *et al.* (1999) observed that the diversity as well as density of soil microbes is reduced due to alteration in nutrient availability and alteration in abiotic factors. The succession of initial bush fallows (2 - 5 years' fallow) to mature forest fallow increase the organic matter content of the soil and creates abiotic condition favourable for the increase microbial biodata in terms of diversity as well as biomass accumulation. Castellanos *et al.* (2001) and Okore *et al.* (2007) reported the same pattern as observed in the past.

2.3. Soil microbial population

It is important to understand the impact of slash and burn on soil microorganism as they are crucial to the stability, regulation and functioning of all ecosystems (Reichle, 1997). Ewel *et al*. (1981) and Uhl (1987) reported that the slashing and felling of native vegetation during jhum cultivation can disrupt the populations of soil micro- and macro-fauna. After clearing, slash is usually allowed to dry before burning. The investigation of microbial community changes following slash and burn is particularly important for understanding the extent and duration of community alterations. In jhum fields burning is an important event and it affects microbes to a large extent. According to DeBano (1991) heating of soil directly

affects microorganisms by either killing them directly or altering their reproductive capabilities resulting in the decrease in microbial population and diversity immediately after burning. Dunn *et al*. (1985) stated that microbial groups differ significantly in their sensitivity to temperature. Immediate decrease in amounts and diversity of microbes after the burning followed by a gradual recovery, which usually occurs within days was reported by Sharma (1981); Deka and Mishra (1983) and recovery within months was reported Ahlgren and Ahlgren (1965); Tiwari and Rai (1977); Theodorou and Bowen (1982) and van Reenen *et al.* (1992).

Dkhar and Mishra (1987) observed that the soil microbial population was lower in jhum cultivation as compared to permanent agricultural lands. Greipsson and El-Mayas (1999) through their experiment reported that fallowing did not return microbial communities to their pre disturbance state, although a shift in the communities did occur with both short (3- month) and longer (15-month) term fallowing which implies the change in soil microbial community dynamics with the change in length of the fallowing period.

The isolation and identification of bacterial communities from jhum and jhum fallow soil to assess the impact of jhum cultivation on the soil bacterial diversity was carried by various workers. Adeduntan (2010) revealed the presence that *Bacillus cereus, Proteus vulgaris, Clostridium sporogenes, Aeromanas hydrophylla and Vibrio anguillarum,* in both the burn and un-burnt plots of jhum in Ondo State, Nigeria. Three different genera of bacteria: *Coccus, Bacillus* and *Streptococcus,* were identified from jhum cultivation in Bangladesh by Miah *et al*. (2010). Pandey *et al.* (2011) reports the recovery of *Bacillus* and *Pseudomonas* spp. from the 'fired plots' under shifting cultivation in northeast India. Zothansanga *et al*. (2016) also reported the isolation of *Bacillus thuringiensis* from the jhum and jhum fallow soil of Mizoram.

2.4. Soil enzyme dynamics

Soil is a living system where all biochemical activities proceed through enzymatic process. Kiss *et al.* (1975) mentioned that soil enzymes play an important role in soil mineralization processes and have been related to other soil biological

properties. Alef and Nannipieri (1995) reported the potential use of enzyme activity as an index of soil productivity or microbial activity. The enzyme activities have often been used as indices of microbial activity and soil fertility (Kennedy and Papendick, 1995). Among the different types of soil enzymes, oxidoreductase (dehydrogenase) and hydrolases (phosphatase and urease) are thoroughly studied due to their specific importance in organic matter transportation processes, phosphorus cycle and agricultural practices. Several workers have studied dehydrogenase, urease and phosphatase activities in different systems under different climate, land use and soil conditions (Baruah and Mishra, 1984; Rao and Ghai, 1985; Tiwari *et al*., 1989). Many workers such as Doran *et al.* (1996), Das and Verma (2010) and Alkorta *et al.* (2013) and have highlighted the potential use of soil enzymes as a biological indicator for soil health.

Dick *et al.* (1998) state that anthropogenic activities such as tillage and agricultural land uses including jhum cultivation may reduce enzyme activities and could alter the availability of nutrients for plant uptake. Pang and Kolenko (1986); and Fox and Comerford (1992) from their experiments reported the adverse effect of tillage, agricultural practices, removal of natural vegetation and application of fertilizers especially p- fertilizers have decreased the enzymatic activity in rhizosphere soils.

Jha *et al.* (1992) reported that in north-eastern India the activities of some soil enzymes, including dehydrogenase, urease, and phosphatase, were higher in the less degraded than in the more degraded forest soils based on the differences in the fungal and bacterial population numbers between them. Reza *et al.* (2014) also observed a decline in the soil enzymes activities of jhum cultivation but also stated that as the vegetation regrows after abandonment of cultivation on jhum land, enzyme activity gradually increased with age of the secondary successional communities on the jhum fallow. The decline reduction in the phosphatase, dehydrogenase and urease enzyme activities under jhum agriculture and horticultural practices in the hilly region of Northeast India was also noted by Ralte *et al.* (2005).

2.5. Soil microbial biomass

Soil microbial biomass is an important component in soil quality assessment because of its important roles in nutrient dynamics, decomposition of natural and organic amendments and physical stabilization of aggregates (Franzluebbers *and* Dick, 1993 and Franzluebbers *et al.,* 1999). Powlson *et al.* (1987) and Srivastava and Singh (1991) had proposed the use of soil microbial biomass as tool for understanding and predicting the long term effects of changes in soil microbial biomass responds much more rapidly than the total organic matter to any change in organic inputs.

Soil microbial biomass is the primary catalyst of biogeochemical processes and also acts as an energy and nutrient reservoir (Bailey *et al.,* 2002; Hargreaves *et al.,* 2003; Hoffman *et al*., 2003 and Moussa *et al.,* 2007). Hence in relatively low fertility soils like those of jhum cultivation systems, the study of soil microbial biomass and the proportion of nutrients (N, C and P) immobilized in the biomass is of prime importance. However, few studies are available on the effect of slash and burning on the dynamics of soil microbial biomass in the tropical forest (Ayanaba *et al*., 1976; Srivastava and Singh, 1991; Saranath and Singh, 1995).

Extensively study was carried out by Arunachalam and his co-workers (1994; 2002; 2003) on the dynamic of soil microbial biomass in jhum and jhum fallow soil. Based on their research they reported that the process of jhum cultivation as a detrimental effect on the soil microbial biomass, which gradually recuperate during the fallow period. According to Borggaard *et al.* (2003) increased land use pressure has resulted in shortened fallow periods which in turn led to reduction in microbial biomass, nutrient depletion and low productivity. Yadava and Devi (2004) reported that due to the addition of large amount of organic matter in the form of ash, the higher values of microbial biomass C, N and P in the recent slash and burnt site than that of the forest site. Binarani and Yadava (2010) also reported that slash and burn cultivation has a detrimental effect on the soil microbial biomass which is regenerated with increased in fallow period. Joshi *et al.* (2019) also carried out the study on the impact of jhum cultivation on the soil microbial biomass and its

implication. The impact of various land use system on soil microbial biomass was also carried out by other workers such as Moore *et al.* (2000); Hargreaves (2003) and Maharjan *et al*. (2017).

2.6. Soil phosphorous nutrient

Sharma *et al.* (2013) cited Phosphorus (P) as one of the major macronutrients essentially required by plants. Major growth-limiting macronutrients required for proper plant growth, particularly in tropical areas, due to its low availability in the soil which accounts for between 0.2 and 0.8% of the dry weight of plants. After uptake by plants, P plays a critical role in photosynthesis, energy transfer, signal transduction, macromolecular biosynthesis, and respiration. It also stimulates root development and facilitates flower formation and quality and quantity of fruits and seed formation as reported by Ahmad *et al.* (2009). Also, Molla et al. (1984) mentioned that, sufficient P concentration may increase the resistance ability of plants to diseases and adverse conditions.

As noted by Alam *et al.* (2002) and Mehrvarz *et al.* (2008), P is the second most important macronutrient required by the plants; next to nitrogen the availability of soluble forms of P for plants in the soils is limited because of its fixation as insoluble phosphates of iron, aluminium, and calcium in the soil. Most soils possess considerable amounts of P, but a large proportion is bound to soil constituents. According to Barber (1984) and Vassileva *et al. (*1988) Phosphorus contents of soil vary from 0.02-0.5 %. However, a greater part of soil phosphorus, approximately 95-99 % is present in the form of insoluble phosphates and hence cannot be utilized by the plants. Behera *et al.* (2014) classified Phosphate compounds present in soil into three groups, *viz.* soluble orthophosphate, insoluble inorganic phosphate, and insoluble organic phosphate. Orthophosphate is the only form that can be taken up by the plants. It reacts with numerousinorganic and organic constituents of soil and therefore becomes least mobileand unavailable for uptake by plants.

Popenoe (1959) found that the available phosphorus level of a soil increases upon clearing and burning as a result of the phosphorus content of the ash. Slight increase of available phosphorus is shown immediately after the burn, but a markedly

increased after a month is reported by Toky and Ramakrishnan (1981). Joachim and Kandiah (1948) cited that supply of available phosphorus is augmented by addition of ash after burning. Nye and Greenland (1964) estimated phosphorus as much as 27 kg ha^{-1} is provided by the ash of a 10-year forest fallow.

Sanchez (1977) considered that the decline in available phosphorus conceivably could be one of the most important reasons for abandoning the field to forest regrowth. Many workers such as Nye and Greenland (1964) along with Zinke *et al.* (1978) stated that a decline in soil fertility during cropping is naturally expected due to the net uptake of nutrients by growing crop may be responsible partly for lowering the nutrient levels despite a continuous supply through mineralization. The decrease in available phosphorus with cropping can also be attributed to the removal in the harvested crop or phosphorous fixation due to a drop in pH particularly when iron and aluminium oxides. Leaching losses are negligible.

According to Nye and Greenland (1960) total available phosphorus showed marked and steady increase during the fallow period. This suggests that a longer fallow period favours greater improvement in humus and some of these nutrients inorder to sustain slash and burn agriculture, an observation also noted by others wormers such as Toky and Ramakrishnan (1981).

2.7. Mechanism of P solubilization

According to Richardson (1994) there are two components of P in soil such as organic and inorganic phosphates. A large proportionof soil P is present in insoluble forms and therefore not available for plant nutrition. Insoluble forms of inorganic P such as tricalcium phosphate [$Ca_3(PO_4)_2$], aluminium phosphate (Al_3PO_4), iron phosphate (Fe_3PO_4) etc. may be converted to soluble P by phosphate solubilizing organisms in habiting different soil ecosystems as reported by Gupta *et al.* (2007) and Song *et al.* (2008). Organic forms of P may constitute 30–50% of the total phosphorus in most soils, although it may range from as low as 5% to as high as 95% as reported Paul and Clark (1988). Dalal (1977) and Anderson (1980) stated that organic P in soil is largely in the form of inositol phosphate (soil phytate).

As observed by Rodríguez and Fraga (1999), Sharma *et al.* (2013) and other researchers, the principal mechanism in inorganic P solubilization is the production of mineral dissolving compounds such as organic acids, siderophores, protons, hydroxyl ions and CO_2. According to Kpomblekou and Tabatabai (1994) and Stevenson (2005), inorganic P solubilization is mainly carried out by the action of organic and inorganic acids secreted by phosphate solubilizing microbes.

Sharma *et al.* (2013) and Altori *et al.* (2017) suggested the release of H^+ in exchange for cation uptake or with the help of H^+ translocation ATPase as an alternate mechanism to organic acid production for solubilization of mineral phosphates.

Mineralization of soil organic P plays an important role in phosphorus cycling of a farming system. Hilda and Fraga (2000) indicated that the principal mechanism for mineralization of soil organic P is the production of *acid phosphatases*. Most of these organic compounds are high molecular-weight materials that are generally resistant to chemical hydrolysis and must therefore be bio-converted to either soluble ionic phosphate (Pi, HPO_4^{2-}, $H_2PO_4^-$) or low molecular-weight organic phosphate to be assimilated by the cell as cited by Peix *et al.* (2001). Release of organic anions, and production of siderophores and acid phosphatase by plant roots and microbes or alkaline phosphatase enzymes hydrolyze the soil organic P or split P from organic residues as reported by Tarafdar and Claasen (1988) and Yadaf and Tarafdar (2001). As reported by Cosgrove (1967) and Tarafdar *et al.* (1988) almost half of the microorganisms in soil and plant roots possess P mineralization potential under the action of phosphatases. Alkaline and acid phosphatases use organic phosphate as a substrate to convert it into inorganic form. The largest portion of extracellular soil phosphatases is derived from the microbial population as noted by Dodor and Tabatabai (2003). Kucey *et al.* (1989) reported that mixed cultures of PSMs (*Bacillus, Streptomyces, Pseudomonas* etc.) are most effective in mineralizing organic phosphate.

2.8. Phosphate Solubilizing Bacteria

Almost all soils harboured phosphate-solubilizing microorganisms but the population abundance varied with soil climate (Sperber, 1958; Katznelson and Bose, 1959; Banik and Dey, 1982; Kucey, 1983; Thomas *et al*, 1985). Khan *et al.* (2007) reports naturally occurring rhizospheric PSMs dates back to 1903. Studies of Kucey *et al*. (1989) revealed the occurrence and abundance of phosphate solubilizers in soil Among the whole microbial population in soil, P solubilizing bacteria comprise 1–50% and P solubilizing fungi 0.1 to 0.5% of the total respective population (Chen *et al*., 2006; Khan *et al.*, 2009; Walpola and Yoon, 2012). According to Sharma *et al.* (2013) in soil P solubilizing bacteria constitute 1-50% and fungi 0.1-0.5% of the total respective population

Swaby and Sperber (1958) reported the solubilization of mineral phosphate by rhizosphere microorganisms isolated from neutral and alkaline soils. The principal genera were *Pseudomonas, Arthrobacter, Xanthomonas, Achromobacter, Flavobacterium, Streptomyces, Aspergillus* and *Penicillium*. Sundara Rao and Sinha (1963) isolated phosphate solubilizing microorganisms viz., *Bacillus megaterium, Escherichia freundii, Escherichia intermedia* and *Bacillus circulans* from rhizosphere of wheat and found that soil inoculation with pure cultures of these organisms increased the acid extractable P_2O_5. Subbarao (1988) and Kucey *et al.* (1989) mentioned *Bacillus megaterium, B. circulans, B. subtilis, B. polymyxa, B. sircalmous, Pseudomonas striata*, and *Enterobacter* as the most important phosphate solubilizing microbial strains. Tilak *et al*. (2005) based on their research report Bacillus and Pseudomonas amongst bacteria and Aspergillus and Penicillium amongst fungi as the most efficient phosphate-solubilizing microorganisms belong to genera. Bacterial genera like *Azotobacter, Bacillus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Microbacterium, Pseudomonas, Rhizobium* and *Serratia* are reported as the most significant phosphate solubilizing bacteria (Bhattacharyya and Jha, 2012). Whitelaw (2000) referred to strains from bacterial genera *Pseudomonas, Bacillus, Rhizobium* and *Enterobacter* along with *Penicillium* and *Aspergillus* fungi as the most powerful P solubilizers. Igual *et al.* (2001) and Alam *et al.* (2002) cited that bacteria are more effective in phosphorus solubilization

than fungi and among the soil bacterial communities, ectorhizospheric strains from *Pseudomonas* and *Bacilli*, and endosymbiotic rhizobia have been described as effective phosphate solubilizers. Genera able to solubilize phosphate include, Rhizobium (Chabot *et al*. 1996), Bacillus (Sahin *et al*., 2004), Burkholderia (Tao *et al*., 2008), Enterobacter (Sharma *et al*., 2005), Pseudomonas (Malboobi *et al*., 2009) and Streptomyces (Chang and Yang, 2009)

The phosphate solubilizing ability of microorganisms has been demonstrated through their growth on culture media supplemented with tricalcium phosphate, dicalcium phosphate, hydroxyapatite, phosphoric rock and other minerals containing insoluble P as sole phosphate source as stated by Igual *et al.* (2001). The phosphate-solubilizing activity can be assessed by the formation solubilization halos (light zones) around the microbial colonies (Mikánova and Nováková, 2002), when they grow on plates of distinct culture media. Various growth mediums are being used for isolation and characterization of PSM. The method given by Pikovskaya (1948) was the most reliable approach for preliminary screening and isolation of potential PSM. NBRIP medium (Nautiyal, 1999) and NBRIP-BPB medium (Mehta and Nautiyal, 2001) are also used by researchers for purpose of PSM identification and isolation. The basic method used for the screening of PSM was determination of solubilization index (SI) and solubilized P (Banik *et al.*, 1989; Alam *et al.*, 2002; Suliasih *et al.*, 2005; Fankem *et al.*, 2006; El-Yazeid *et al.*, 2007; Epsionosa - Victoria *et al.*, 2009; Chakkaravarthy *et al.*, 2010; Nenwani *et al.*, 2010; Prasanna *et al.*, 2011)

The application of molecular biological methods to investigate the occurrence and distribution of bacteria in the environment has the advantage of providing direct information on community structure. The culture-based methods recover merely a fraction of the natural population. Torsvik and Ovreas (2002) alluded that more than 99% of soil microorganisms have not been cultured successfully. Therefore, culture-independent methods are required to study the function and ecology of microbes involved in P cycling in soils Molecular approaches for such culture-independent methods have been developed in the recent past. Vandamme *et al.* (1996) indicated that polyphasic taxonomical studies, which include phenotypic, genetic and phylogenetic information, have been widely used in microbial diversity studies.

Eisen (1995) reported the used of molecular techniques such as gene sequencing for genetic and phylogenetic characterization of microbes. Woese (1987) stated that the concept of phylogenetic relationships among bacteria has revolutionizeddue to the application of molecular techniques to microbial systematic andidentification of ribosomal RNA as a premier molecule for evaluating evolutionaryrelationships.

Based on polyphasic taxonomical studies new PSB species have been identified such as *P. rhizospharae* (Peix *et al.,* 2003), *P. lutea* (Peix *et al.,* 2004) and *Microbacterium ulmi* (Rivas *et al.,* 2004). Kumar *et al.* (2010) isolated and identified six PSB from paddy fields of Eastern Uttar Pradesh Using 16SrRNA gene sequencing. Valverde *et al.* (2003) also cited polyphasic characterization based on molecular tools, of PSB from rhizospheric soil of the north-eastern region of Portugal. Chen *et al.* (2006) reported the isolation, screening and characterization of 36 strains of phosphate solubilizing bacteria from Central Taiwan carried out using 16SrRNA sequencing. Khiangte and Lalfakzuala (2016) reported the isolation and characterisation of 17 PSBs based on 16s rRNA gene profiling from the paddy field of Mizoram, India which is the first report from the region. Based on 16S rRNA Gene Sequencing Chawngthu *et al.* (2020) also characterised 44 PSBs from the rhizosphere soil of wetland paddy field of Mizoram, India

2.9.1. PSB as potential Bio fertilizer

Goldstein (1986) and Sundara *et al.* (2002) has estimated that in some soil up to 75% of applied phosphate fertilizer may become unavailable to the plantbecause of mineral phase precipitation. This greatly reduced the efficiency of applied phosphate fertilizers in agricultural field. Hence a need to develop alternate means to supply the limited but essential P for plant utilization becomes necessary.

In the past few years, exploring the potential of beneficial microbes and the use of PGPR for sustainable agriculture has drawn the attention of many researchers from various parts of the world. According to Davies (1995) PGPR are group of bacteria that actively colonize plant roots region and increase plant growth and yield. Among PGPR, phosphate solubilizing bacteria (PSB) are considered as favourable biofertilizers since they can supplyplants with phosphorus (P) from sources

otherwise poorly available. Pichu (1989) and Glick (1995) reported various species of bacteria like *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus* and *Serratia*as PGPR. Various authors such as Antoun *et al.* (1998), Chabot *et al.* (1998), Pal (1998), Sarawgi *et al.* (1999), Tomar *et al.* (1996) described the beneficial effects of the inoculation with PSB tomany crop plants. Gulati *et al.* (2007) noted that the use of PSB as inoculants to increase P uptake in several plants. The application of PSB by inoculating in soil appears to be an efficient way to convert the insoluble P compounds to plant-available P form as reported by Kalayu (2019), which result in better plant growth, crop yield, and quality. *Pseudomonas sp., Bacillus circulans, Acidithiobacillus ferroxidans, B. mucilaginosus, Burkholderia sp.* And *Paenibacillus* sp. are some efficient microbes as reported by Liu *et al.* (2012), Verma *et al.* (2015) and Saiyad *et al.* (2015).

Chapter 3

Soil Physico-Chemical Properties

3.1. Introduction

Soil is crucial to life on earth. Soil is a dynamic natural body, non-renewable resource, composed of mineral and organic solids, gases, liquids, and living organism which serve as medium for plant growth and monitoring its fertility is an important objective in the sustainable development of agro-ecosystems The quality of soil depends in part on its natural composition, and also on the changes caused by human use and management (Larson and Pierce, 1993). Soil is the primary resource for land use and contributes to principal functions within a landscape: nutrient cycling; water-holding capacity; habitats and biodiversity; storing, filtering, buffering, and transforming compounds; and provision of physical stability and support (Blum, 1993; Young and Crawford, 2004; Koch *et al.*, 2013). It is a dynamic system and an important component of the ecosystem. It contributes an ecological niche where constant biological activity regulates the chemical nature of its parent material and the type of plant growth it supports.

Soil quality has been defined as "the capacity of soil to function within the land-use and ecosystem boundaries, to sustain biological productivity, maintain environmental quality and promote plant, animal and human health (Doran and Parkin, 1994 and 1996). Soil quality is a key factor for the growth of crop plants and the deciding factor for the availability of plant nutrients. Soil quality depends on a large number of physical, chemical, biological, microbiological and biochemical properties. The undesirable changes in any of these properties lead to the degradation of soil quality. Maintaining soil quality is a key determinant for ecosystem services and benefits management and climate change mitigation (Lal, 1997 and 2010; Foley, 2005; Bringezu *et al.*, 2014).

Since soil is the base resource for about all land utilizes and the most noteworthy constituent of sustainable agriculture (Nambiar *et al.*, 2001; Bouma, 2002), appraisal of soil quality and its course of change with time are the perfect and essential indicator of sustainable agrarian land management (Doran, 2002; Karlen *et al.*, 1997). Estimating the changes in soil physical, chemical and biological properties could be utilized as markers to monitor agricultural land management (Hartemink, 1998; Wang and Gong, 1998; Arshad and Martin, 2002).

3.2. Study site and soil sampling

Soil sampling was carried out from jhum fields of Hmuntha village (23.4939° N and 92.9419°E) which is situated in Serchhip District, Mizoram, India. Soil samples were collected from current jhum field (Jh), 2 years jhum fallow land (2F), 5 years jhum fallow land (5F),10 years jhum fallow land (10F) and undisturbed forest (UD) which was serving as reference (control). Soil sample was collected during in the month of February, May, August and November representing the slash- unburnt, post burnt, cultivation period and post-harvest period of the jhum field respectively.

Soils were collected randomly from five points (approximately 20 meters distant) from each study sites from the surface (0-20 cm) layer. The collected soil samples were mixed thoroughly to obtain one composite sample for each site. Collected soil samples were kept in a sterile polybags and stored at 4^{o}C till further analysis. During collection of samples necessary measure were taken to avoid contamination.

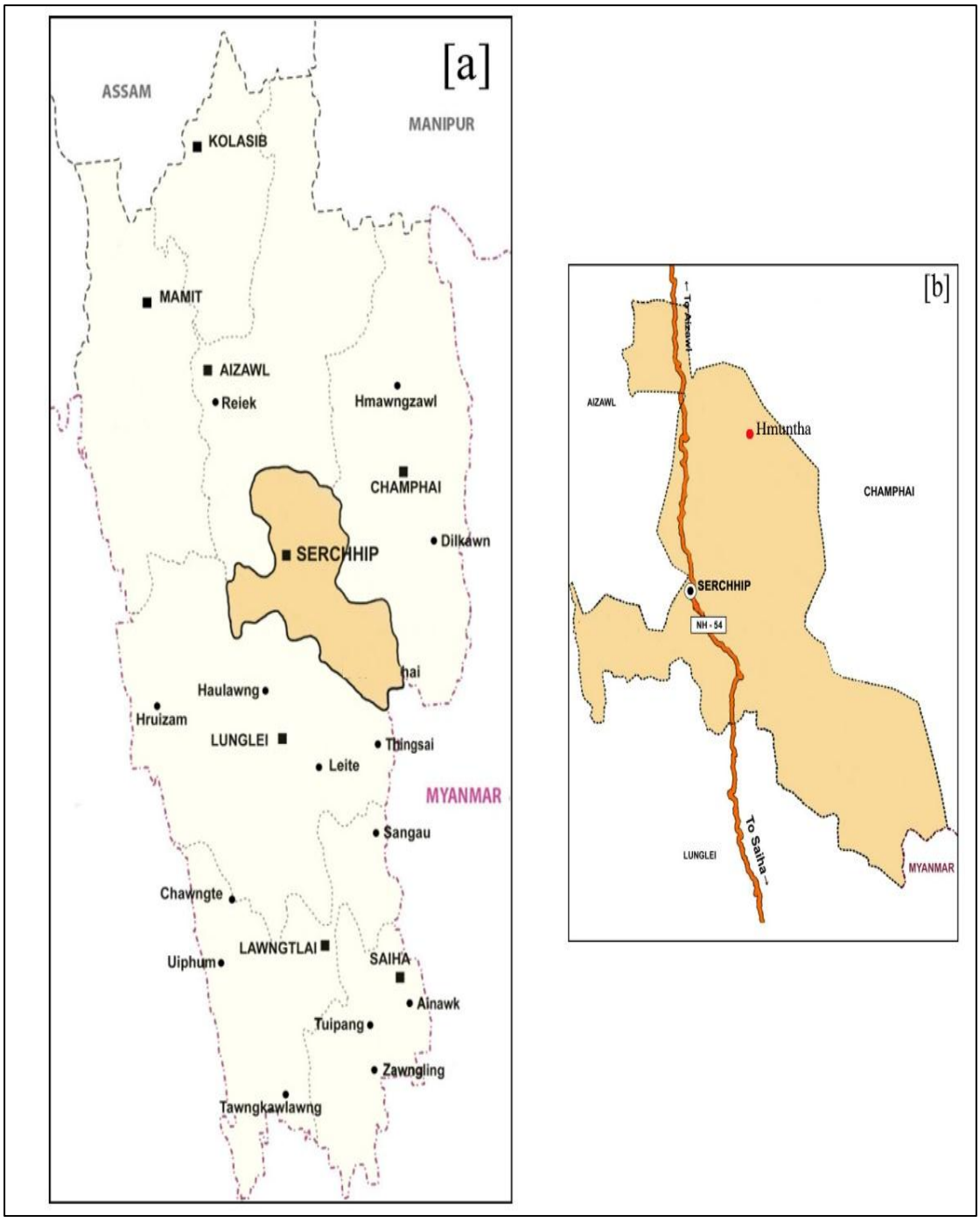

Fig. 3.1. a) Map of Mizoram highlighting Serchhip district

b) Map of Serchhip district showing the location of Hmuntha village

Fig 3.2. Phases of jhum cultivation of the study site

a. Dried slash native vegetation.
b. Burning of dried vegetation.
c. Burnt jhum field covered in ash and debris.
d. Rice cultivation in jhum field
e. Jhum fallow

3.3. Methodology

3.3.1. Soil Moisture Content

Estimation of soil moisture content (SMC) was carried out following the method given by Misra (1968). 20gm of soil samples were taken in moisture boxes of known weight. The moisture boxes with soil samples were then oven dried to a constant mass at 105˚C for 24 hours. The soil moisture content was determined by the following formula-

$$\text{Soil Moisture content (\%)} = \frac{W^1 - W^2}{W^1} \times 10$$

Where, W_1 = initial weight

W_2 = final weight

3.3.2 Soil Bulk Density

Soil bulk density was measured using the method described by Anderson and Ingram (1993). After clearing the soil surface, metal tube of known weight and volume was inserted into the soil surface. The soil was unearthed from around the tube and the soil underneath the tube bottom was cut. The excess soil was removed from the tube ends. The soil samples were dried at 105°C until constant weight. The bulk density determined using the following formulae.

$$\text{Soil Bulk Density (BD)} = \frac{Weight\ of\ oven\text{-}dried\ soil\ (g)}{Volume\ of\ soil\ core\ (cm^2)}$$

Where,

Volume of soil core $=3.14r^2h$

r =inside radius of cylinder (cm)

h =height of cylinder

3.3.3 Soil Water Holding Capacity

Soil water holding capacity (WHC) was measured using the keen box method described by Piper (1944). The bottom of a keen- box with a perforated hole (approximately 0.75mm in diameter) was covered with Whatman No. 1 filter paper

and the weight was recorded. 10 g of oven-dried soil (100-105°C) was taken and placed on the bottom of keen box and weighed. The keen-box along with soil samples were partially submerged in water (up to one fourth) overnight. The keen-box along with the soil was taken out and allowed to stand to drain off the excess water before mass was determined. Water holding capacity of a soil sample is determined with the help of the following formula:

$$\text{Soil Water Holding Capacity (\%)} = \frac{B-(C+D)}{(C-A)} \times 100$$

Where,

A = Weight of empty Keen's box
B = Weight of saturated soil + Keen's box
C = Weight of oven dried soil + Keen's box
D = Weight of wet filter paper

3.3.4 Soil pH

For measurement of soil pH method given by Schofeild and Taylor (1955) was followed. 1:5 soil-water suspensions were prepared by taking about 20 g soil and appropriate volume of distilled water in a beaker and shake vigorously for 5 minutes. The mixture was allowed to stand for 1 hour and the pH reading was then taken. The electrode was washed with a jet of distilled water and dried with the help of tissue paper in between measurement of each sample.

3.3.5. Soil Temperature

For measuring the soil temperature of the study sites, a standard digital thermometer is used.

3.3.6. Soil Organic Carbon

Organic carbon has been determined using titration method given by Walkey and Black (1934). 1.0 g of dried soil sample was taken in a 500 ml dry conical flask to which 10 ml of 1(N) $K_2Cr_2O_7$ solution and 20 ml of concentrated H_2SO_4 acid were added. After swirling for 2 minutes, the mixture was allowed to stand for 30 minutes. The contents were diluted to 200 ml with distilled water and then 10 ml of

orthophosphoric acid and 1 ml of diphenylamine indicator were added. The color of the solution turns blue-violet. The solution was titrated with 0.5 (N) ferrous ammonium sulphate till the color changes to brilliant green. A blank titration was also run simultaneously. The amount of organic carbon in percentage and the total organic matter were calculated by using the following formulae:

$$\text{Soil Organic Carbon (\%)} = \frac{10\ (B - T\ )}{B} \times 0.003\ \times \frac{100}{S}$$

Where,

B= Volume of ferrous ammonium sulphate required for blank titration in ml.

T= Volume of ferrous ammonium sulphate needed for soil sample in ml.

S= Weight of soil in gram.

3.3.7 Available Soil Nitrogen

Available nitrogen of the soil sample was determined by method given by Subbiah and Asija, (1956). 20 g of soil sample was taken in a distillation flask and 20 ml water and 100 ml of 0.32% $KMnO_4$ solution were added. 20 ml of 0.02N H_2SO_4 was taken in a 250 ml conical flask and two to three drops of methyl red indicator was added. Flask was placed below the receiver tube with the tip of the receiver tube well dipped into the H_2SO_4 solution. 100 ml 0.32% $KMnO_4$ and 100 ml of 2.5% NaOH were added to the distillation flask containing soil and flask was immediately connected with the distillation apparatus. Distillation flask was heated. H_2SO_4 containing excess ammonia is titrated with 0.02N NaOH. The amount of available nitrogen is calculated using the given formula and express as $kgha^{-1}$

Soil Available Nitrogen kg ha^{-1}= (X-Y) x 0.00028/20 x 2240000

or = (X-Y) x 28 x 1.12 = (X-Y) x 31.36

X = Volume of N/50 H2SO4 taken in ml

Y = Volume of N\50 NaOH used in ml

3.2.8 Available Soil Phosphorus

Available phosphorus is determined by ammonium molybdate method given by Olsen *et al.,* 1954. Soil sample of 2.5 g was taken in 100 ml conical flask and activated charcoal and 50 ml of Olsen's reagent (0.5M $NaHCO_3$; pH 8.5) was added. The contents were shaken for 30 min on a mechanical shaker and then were filtered through Whatman No.1 filter paper. 5 ml of the clear filtrate was taken in to a 25 ml volumetric flask. To this solution, 5 ml of ammonium molybdate solution (containing 400 ml of 10N HCl per litre) was gradually added. The contents were shaken slowly to drive out the CO2 released from the solution. Distilled water was added, washing down the sides, to bring the volume to about 22 ml. 1 ml of freshly diluted $SnCl_2$ solution was added and the contents were shaken a little and the volume was made up to 25 ml with distilled water. The developed blue color intensity was read at 660 nm against a blank. Calculation for available phosphorous was done using the given formula and expressed in terms of kg/ha.

$$\text{Soil Available Phosphorus } \left(\frac{\text{Kg}}{\text{ha}}\right) = R \times \frac{V}{v} \times \frac{1}{S} \times \frac{(2.24 \times 10^6)}{10^6}$$

Where,

V = total volume of extractant (ml)
v = volume of aliquot taken for analysis (ml)
S = weight of soil (g)
R = weight of phosphorus in the aliquot in µg (from standard graph)

3.2.9 Exchangeable Soil Potassium

Exchangeable potassium content of soil sample was determined by the flame photometer method described by Ghosh *et al.* (1983).) using flame photometer after extracting with 1N ammonium acetate solution. 5 g of soil sample was shaken with 25 ml of 1N ammonium acetate solution for 5 minutes and filtered through Whatman No. 1. The first few ml of the filtrate was rejected. The potassium concentration in the extract was determined by flame photometer using K filter.

$$\text{Soil Exchangeable Potassium } \left(\frac{\text{Kg}}{\text{ha}}\right) = R \times \frac{V}{W} \times 224 \times \frac{10^6}{10^6}$$

Where,

R = ppm of K in the extract (obtained from standard graph)

V = Volume of the soil extract in ml

W = Weight of dry sample taken for extraction in gram

Statistical analysis

All the experiments were performed in triplicate and the mean values with ±SE were calculated. One-Way ANOVA and Correlation coefficient (r) were calculated for each parameter by using SPSS16. Statistical significance at $p \leq 0.05$ was considered.

3.4. Results

3.4.1. Soil pH

The soil pH ranges from 4.7 in Jh field during February to 5.5 in UD site during May were observed. Although there was no significant seasonal varaiation in pH for the study sites except for Jh site which showed a marked increase from 4.7 in February to 5.0 in May.

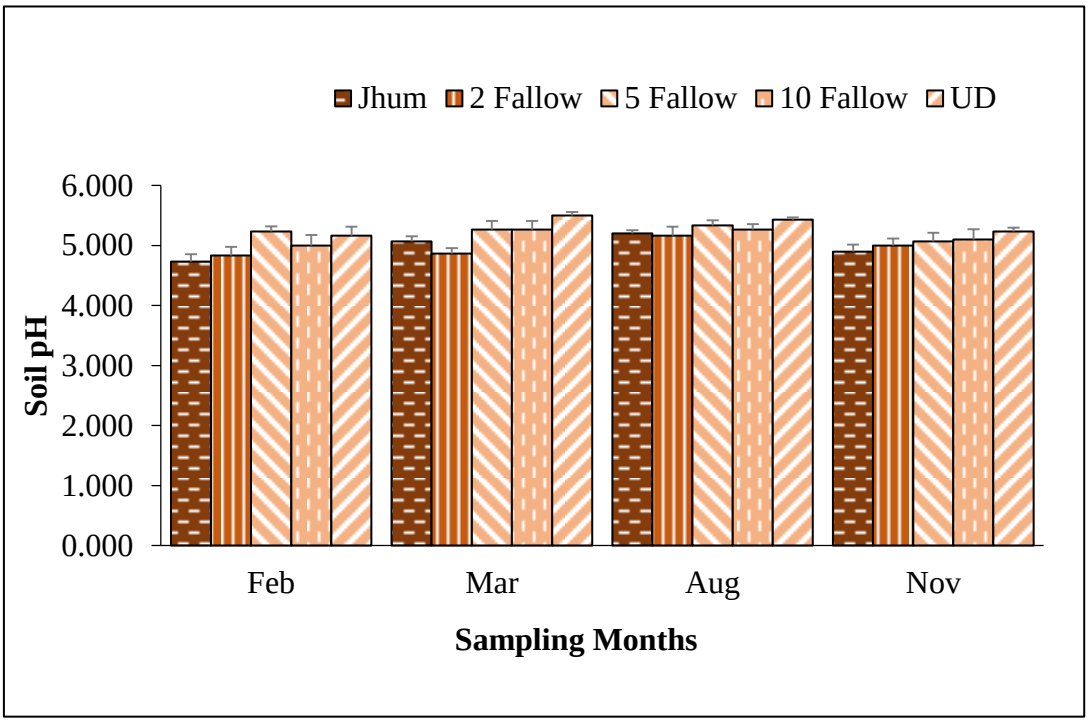

Fig. 3.3. Soil pH of different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD).

Table 3.1. One-way analysis of variance (ANOVA) of pH among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD). Marked effects are significant at $p \leq 0.05$.

S.N.	Source of variation	F-value	p-value
1.	Jh x 2F x 5F x 10F x UD	1.132E3	0.000*
2.	Jh x 2F	0.529	0.520
3.	Jh x 5F	41.350	0.008*
4.	Jh x 10F	30.546	0.012*
5.	Jh x UD	131.760	0.001*
6.	2F x 5F	81.491	0.003*
7.	2F x 10F	74.506	0.003*
8.	2F x UD	293.390	0.000*
9.	5F x 10F	3.183	0.172
10.	5F x UD	30.128	0.012*
11.	10F x UD	81.767	0.003*

3.4.2 Soil Moisture Content

The soil moisture content (SMC) showed seasonal variation as well as variation between different study sites. The collected data indicate that moisture percentage of soil at 10F and UD sites were always higher than the other sites. Highest SMC percent was recorded at UD site during in the month of August (32.63%) while lowest was recorded at the 2 years' fallow (2F) site (12.5 %) during in the month of February. SMC (%) was considerably lower at 2F site during the whole study period. All study sites showed a gradual increase in SMC (%) from February to August with a steep drop during November. Maximum SMC (%) for all sites was recorded during August.

One-way ANOVA was performed to compare the variation between the mean SMC of different study sites present that significant variation in SMC (%) was observed between Current jhum and 2 years fallow (Jhx2F), Current jhum and Undisturbed Forest (JhXUD), 2FX5F, 2Fx10F, 2FXUD and 5FXUD. While no significant variation was observed between JhX5F, JhX10F, 5FX10F and 10FXUD.

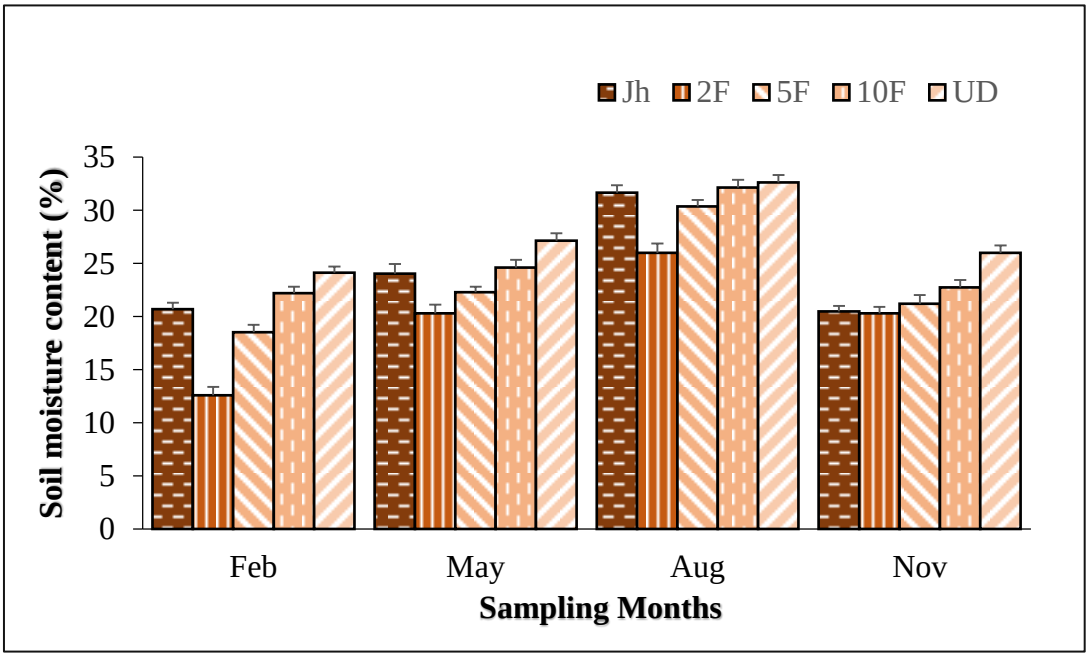

Fig 3.4. Soil moisture content (%) of different soil samples such as current jhum (Jh),2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD).

Table 3.2 One-way analysis of variance (ANOVA) of soil moisture content (%) among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD). Marked effects are significant at $p \leq 0.05$.

S.N.	Source of variation	F-value	p-value
1.	Jh x 2F x 5F x 10F x UD	58.656	0.000*
2.	Jh x 2F	134.363	0.000*
3.	Jh x 5F	6.683	0.061
4.	Jh x 10F	1.412	0.300
5.	Jh x UD	8.147	0.046*
6.	2F x 5F	43.645	0.003*
7.	2F x 10F	78.128	0.001*
8.	2F x UD	95.11	0.001*
9.	5F x 10F	7.501	0.052
10.	5F x UD	16.259	0.016*
11.	10F x UD	1.892	0.241

3.4.3 Bulk Density

Highest Bulk density of 1.37 gcm^{-1} was recorded at the Jh site during the month of May (i.e. after burning of current jhum field). The Jh site showed the highest bulk density for all the sampling periods which gradually decline towards the end of the sampling. The result for Bulk density showed a progressive decline as the fallow site recovers and succession take place towards climax community. Significant variation at $p \leq 0.05$ for one-way ANOVA was observed between all the sampling sites for Bulk density.

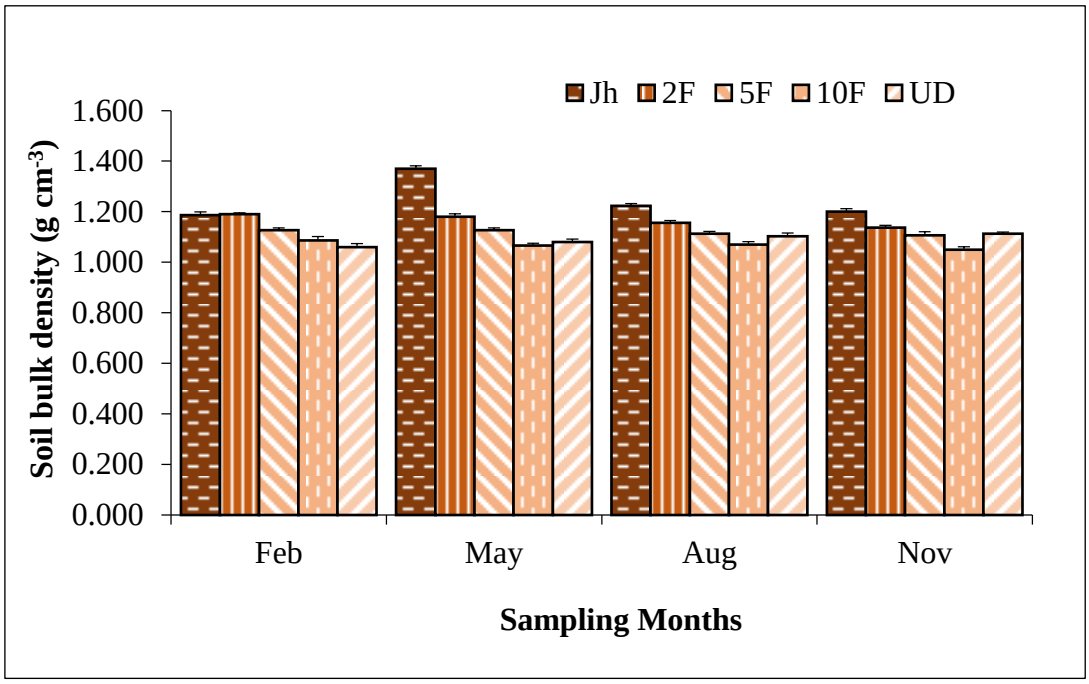

Fig 3.5. Soil bulk density (gcm^{-1}) of different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD).

Table 3.3. One-way analysis of variance (ANOVA) of soi bulk density among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD). Marked effects are significant at $p \leq 0.05$.

S.N.	Source of variation	F-value	p-value
1.	Jh x 2F x 5F x 10F x UD	61. 32E3	0.000*
2.	Jh x 2F	306.934	0.000*
3.	Jh x 5F	1.29E+03	0.000*
4.	Jh x 10F	3.24E+03	0.000*
5.	Jh x UD	2.15E+03	0.000*
6.	2F x 5F	141.028	0.000*
7.	2F x 10F	720.300	0.000*
8.	2F x UD	395.267	0.000*
9.	5F x 10F	465.327	0.000*
10.	5F x UD	121	0.000*
11.	10F x UD	101.769	0.001*

3.4.4 Water holding capacity

The water holding capacity (%) showed variation among the study sites ranging from 60.32% in 2F site to 73% in UD site. However, there was negligible variation in the WHC (%) of the samples with respect to the sampling period i.e. the WHC (%) remains relatively constant with the change in season for each sampling site. However, WHC (%) of the sampling sites showed a constant increase with increase in the fallowing period with the WHC (%) of 10F and UD being almost equivalent. The Jh site showed a decrease in WHC (%) during May which gradually increases as the cropping phase of jhum cultivation progress. One-way ANOVA ($p \leq 0.05$) performed for the obtained result confirmed the observation. Significant variation was observed between all the sampling sites except for Tf x UD.

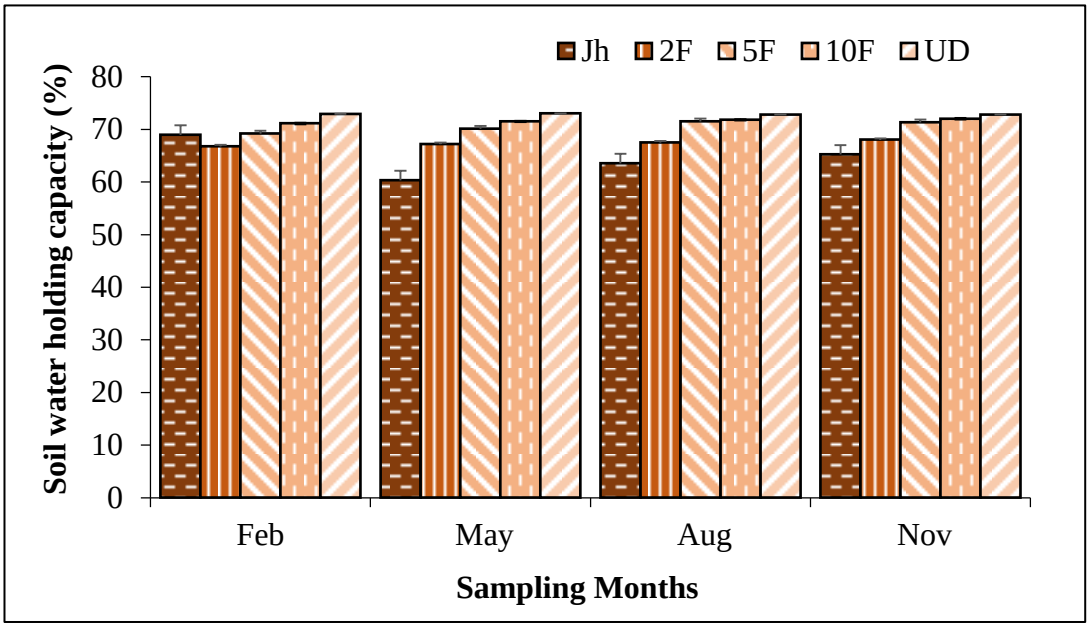

Fig 3.6. Soil water holding capacity (%) of different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD).

T**able 3.4**. One-way analysis of variance (ANOVA) of soil water holding capacity (%) of among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD). Marked effects are significant at $p \leq 0.05$.

S.N.	Source of variation	F-value	p-value
1.	Jh x 2F x 5F x 10F x UD	1.132E3	0.000*
2.	Jh x 2F	107.449	0.000*
3.	Jh x 5F	340.434	0.000*
4.	Jh x 10F	533.44	0.000*
5.	Jh x UD	722.839	0.000*
6.	2F x 5F	266.041	0.000*
7.	2F x 10F	2.39E+03	0.000*
8.	2F x UD	6.06E+03	0.000*
9.	5F x 10F	1.222	0.002*
10.	5F x UD	37.09	0.004*
11.	10F x UD	107.449	0.331

3.4.5. Soil Temperature (^{o}C)

Variation in soil temperature was observed between different sampling sites as well as sampling months. The soil temperature ranges from 12.07^{o}C at UD site during November to 26.11^{o}C at 2F site during the month of August. Highest soil temperature was shown by the 2F site followed by the Jh site for all sampling seasons.

Test of significance using one-way ANOVA showed a significant variation in mean soil temperature between all the study sites except between Jhx2F; Jhx5F; 2FX5Fand 10FxUD.

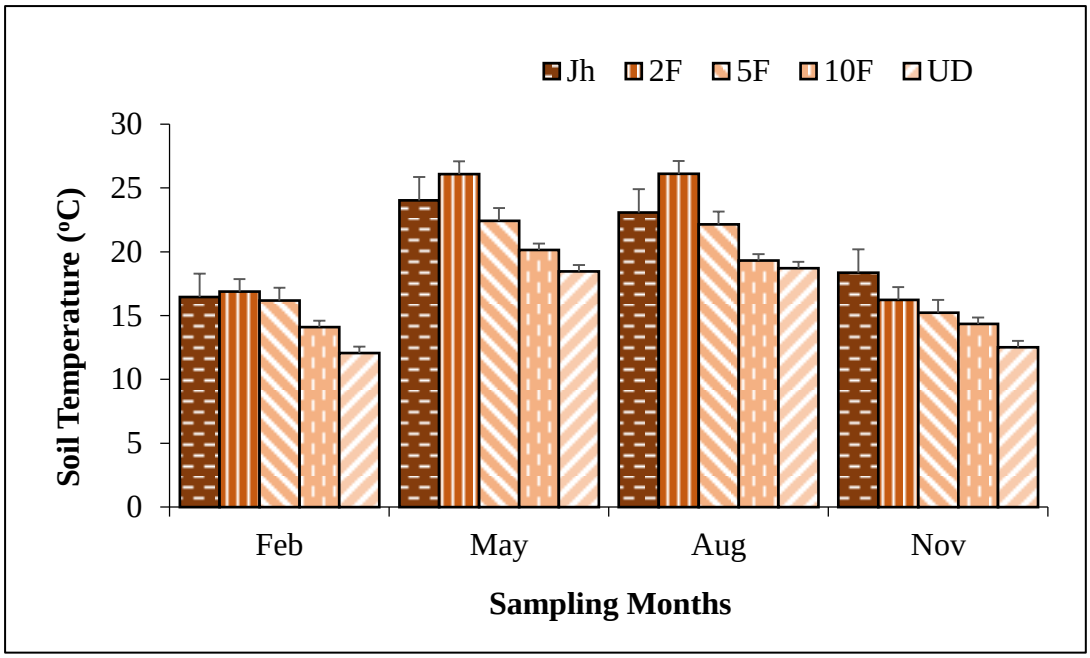

Fig 3. 7. Soil temperature (^{o}C) of different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD).

Table 3.5. One-way analysis of variance (ANOVA) of soil temperature (^{o}C) of among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD). Marked effects are significant at $p \leq 0.05$.

S.N.	Source of variation	F-value	p-value
1.	Jh x 2F x 5F x 10F x UD	50.358	0.000*
2.	Jh x 2F	1.943	0.258
3.	Jh x 5F	5.508	0.101
4.	Jhx10F	29.017	0.013*
5.	Jh x UD	52.385	0.005*
6.	2F x 5F	9.634	0.503*
7.	2F x 10F	32.955	0.010*
8.	2F x UD	54.519	0.005*
9.	5F x 10F	9.415	0.050*
10.	5F x UD	27.476	0.014*
11.	10F x UD	4.422	0.126

3.4.6 Soil organic carbon content

Highest SOC of 4.793% was shown by the UD site during November while Jh site showed the lowest SOC reading of 2.567% during February. The SOC of Jh, 2F and 5F site were found to be comparatively lower than 10F and UD sites. UD site showed a much higher SOC reading as compared to all the other sites for all sampling periods. In Jh site, there was a spike in the amount of soil SOC during May which gradually falls as phases of jhum cultivation continues

Significant variation was observed between all the sampling sites while performing one-way ANOVA taking significant level of $p \leq 0.05$. This implies that the amount of SOC content of the sampling sites differ from one another at a significant level which can affect the soil health

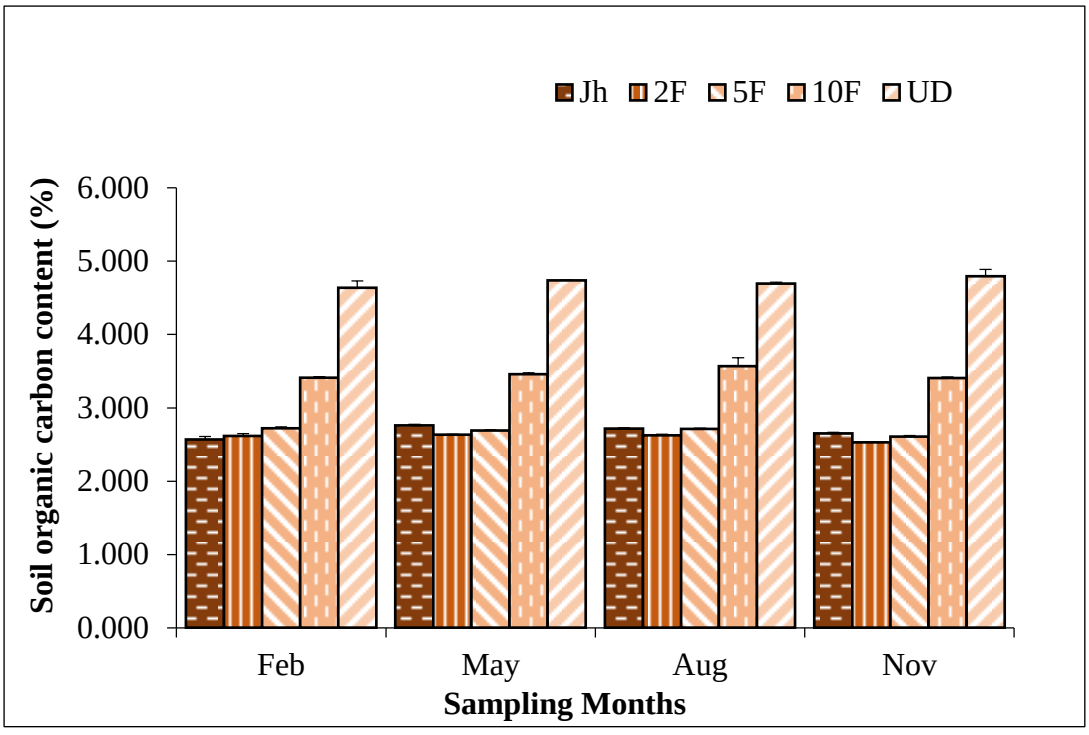

Fig 3. 8. Soil organic carbon content (%) of different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD).

Table 3.6. One-way analysis of variance (ANOVA) of soil organic carbon content (%) among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD). Marked effects are significant at $p \leq 0.05$.

S.N.	Source of variation	F-value	p-value
1.	Jh x 2F x 5F x 10F x UD	2.63E+03	0.000*
2.	Jh x 2F	19.018	0.012*
3.	Jh x 5F	0.575	0.490*
4.	Jh x 10F	311.444	0.000*
5.	Jh x UD	4.03E+03	0.000*
6.	2F x 5F	17.144	0.014*
7.	2F x 10F	484.385	0.000*
8.	2F x UD	4.60E+03	0.000*
9.	5F x 10F	167.114	0.000*
10.	5F x UD	2.35E+03	0.000*
11.	10F x UD	2.10E+03	0.000*

3.4.7 Soil Available Nitrogen

Soil available nitrogen showed variation with respect to the length of the fallowing period of the sampling sites. UD site and 10F showed the highest available Nin soil without any significant variation at $p \leq 0.05$ between them. Lowest available N was shown by 2F site for all sampling months. Jh site showed an increase level of available N, from 158 $mgha^{-1}$ in Feb to 188 $mgha^{-1}$ which was subsequently followed by decrease in the amount of available N.

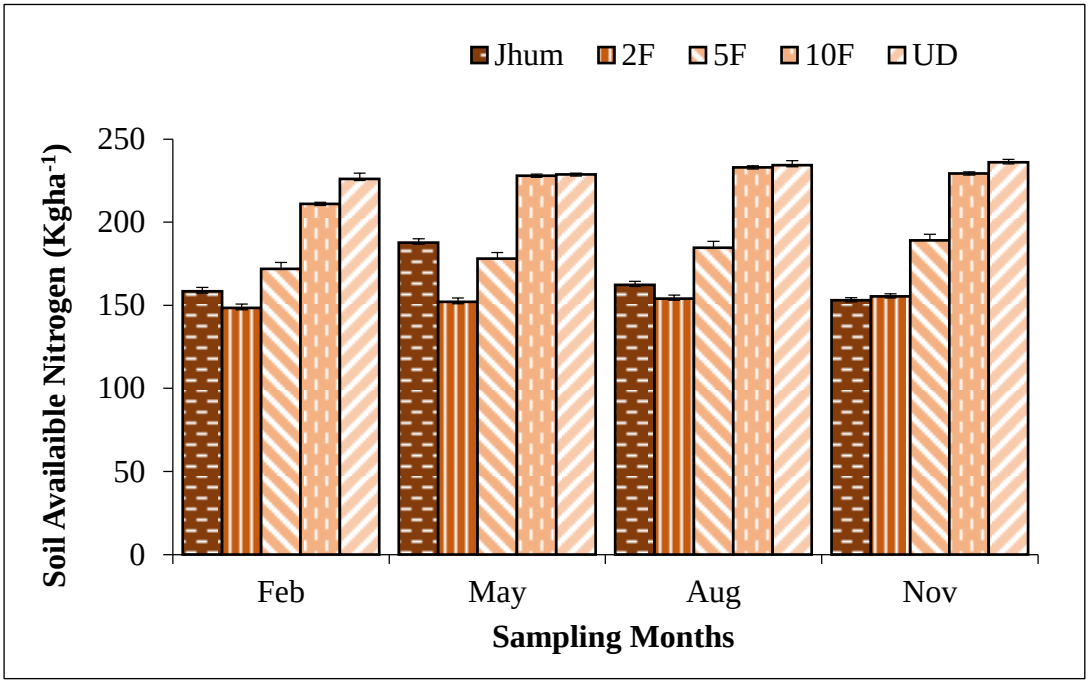

Fig 3.9. Soil available N ($Kgha^{-1}$) of different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD).

Table 3.7. One-way analysis of variance (ANOVA) of soil available N ($Kgha^{-1}$) among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD). Marked effects are significant at $p \leq 0.05$.

S.N.	Source of variation	F-value	p-value
1.	Jh x 2F x 5F x 10F x UD	1.132E3	0.000*
2.	Jh x 2F	16.079	0.016*
3.	Jh x 5F	16.764	0.015*
4.	Jh x 10F	182.661	0.000*
5.	Jh x UD	318.016	0.000*
6.	2F x 5F	96.113	0.001*
7.	2F x 10F	365.514	0.000*
8.	2F x UD	740.118	0.000*
9.	5F x 10F	120.204	0.000*
10.	5F x UD	239.469	0.000*
11.	10F x UD	1.441	0.296

3.4.8 Soil Available Phosphorous

Soil available phosphorous showed a wide range of variation between different sampling sites. Highest level of available P (15.7Kgha^{-}1) was recorded from UD site during August while lowest level of available P (8.1 Kgha^{-}1) was recorded from 2F site during November. For all the sampling periods, 2F site showed a drastically low available P level as compared to the other sampling sites. One-way ANOVA performed for the obtained data revealed a significant level of variation at $p \leq 0.05$, between all the sampling sites except between 10F and UD sites.

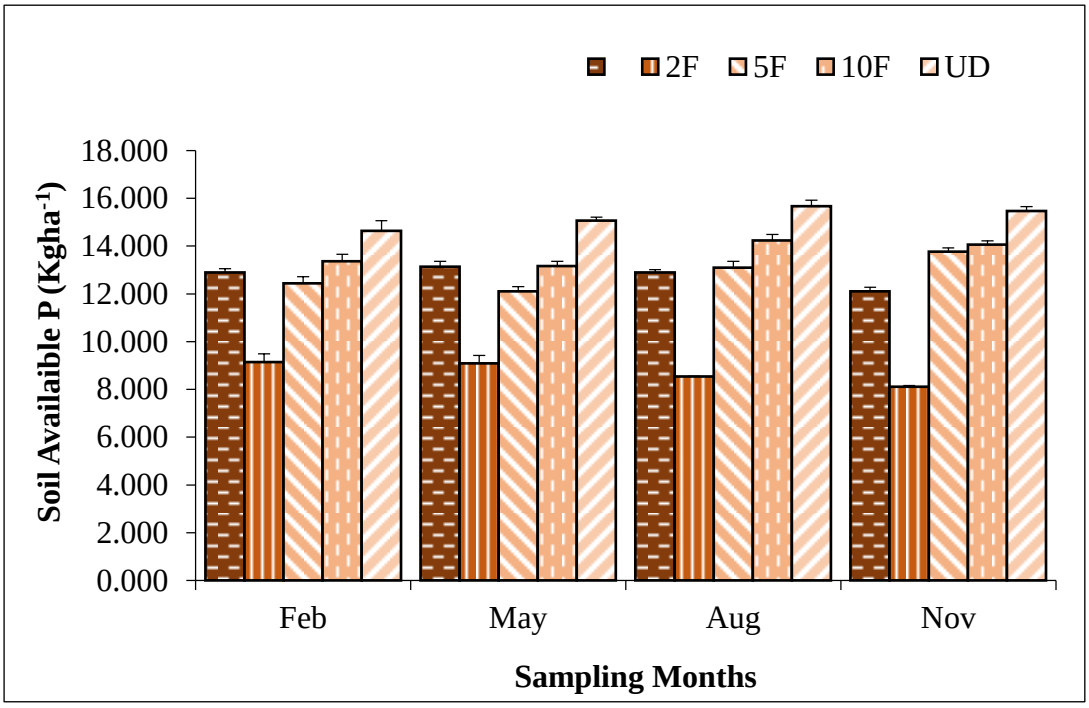

Fig 3.9.1. Soil available P (Kgha^{-1}) of different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD). Marked effects are significant at $p \leq 0.05$.

Table 3. 8. One-way analysis of variance (ANOVA) of soil available P ($Kgha^{-1}$) among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD). Marked effects are significant at $p \leq 0.05$.

S.N.	Source of variation	F-value	p-value
1.	Jh x 2F x 5F x 10F x UD	1.132E3	0.000*
2.	Jh x 2F	16.079	0.016*
3.	Jh x 5F	16.764	0.015*
4.	Jh x 10F	182.661	0.000*
5.	Jh x UD	318.016	0.000*
6.	2F x 5F	96.113	0.001*
7.	2F x 10F	365.514	0.000*
8.	2F x UD	740.118	0.000*
9.	5F x 10F	120.204	0.000*
10.	5F x UD	239.469	0.000*
11.	10F x UD	1.441	0.296

3.4.9. Soil Available Potassium

Soil available K ranges from 144 $kgha^{-1}$ in 2F site during February to 234 $kgha^{-1}$ at UD site during August, indicating a wide range of variation both with sites and sampling season. The Jh site showed an increase in the amount of available K from 158 $kgha^{-1}$ in February to 216 $kgha^{-1}$ in May. Statistical analysis showed that there was a signifcant variation in the amount of available K between the different sites at the level of $p \leq 0.05$.

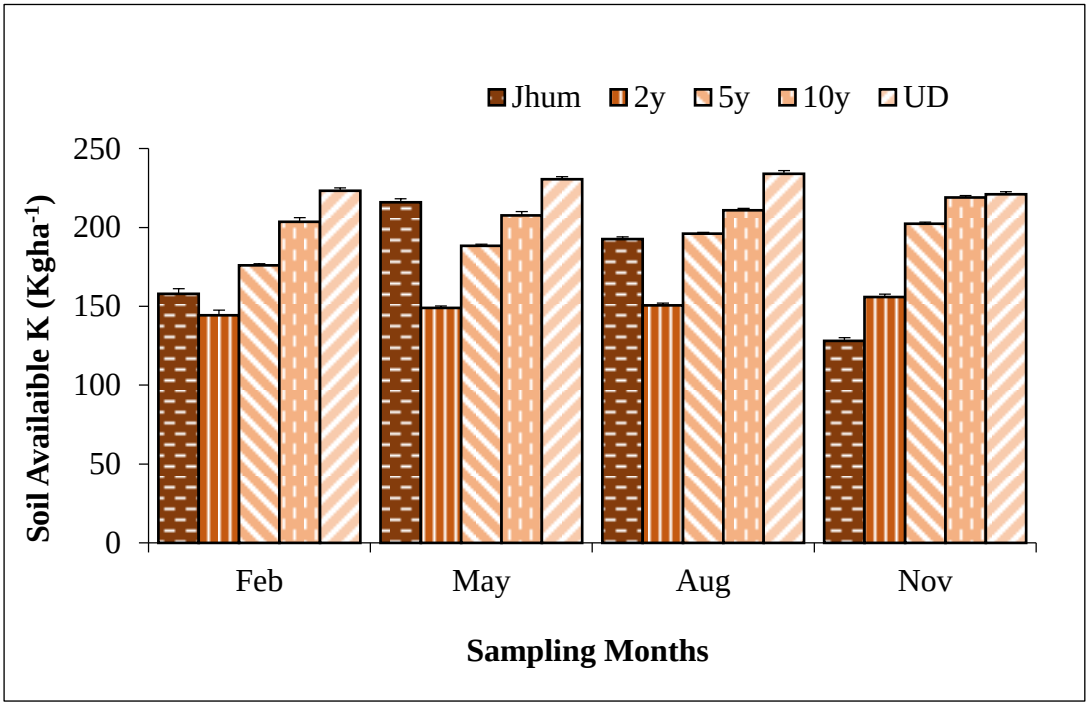

Fig.3.9.2. Soil Available K ($Kgha^{-1}$) of among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD).

Table 3.9. One-way analysis of variance (ANOVA) of soil available K ($Kgha^{-1}$) among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD). Marked effects are significant at $p \leq 0.05$.

S.N.	Source of variation	F-value	p-value
1.	Jh x 2F x 5F x 10F x UD	156.33	0.000*
2.	Jh x 2F	123.045	0.000*
3.	Jh x 5F	25.279	0.007*
4.	Jh x 10F	309.613	0.000*
5.	Jh x UD	299.502	0.000*
6.	2F x 5F	166.511	0.000*
7.	2F x 10F	1.35E+03	0.000*
8.	2F x UD	740.808	0.000*
9.	5F x 10F	40.66	0.003*
10.	5F x UD	91.377	0.001*
11.	10F x UD	36.962	0.004*

3.5. Discussion

In the present study variation in the soil phsyico-chemical properties was observed both in time and space. The status of the soil physico-chemical properteis changes with cultivation of the jhum field length of the fallow period with the UD site serving as a reference for the rate of nutrient recovery. Changes in the soil physico- chemical properties was also observed with the change in the sampling season/month. Also, in the Jh site dynamic changes in the soil physico-chemical properties was obeserved during the different phases of cultivation.

A comparative account of different soil parameters across the different study sites viz. Jh, 2F, 5F, 10F and UD, reveal that from 2 years fallow land (2F) towards the 10 years fallow land (10F), there was a steady reestablishment of the initial conditions of the forest ecosystem prior to crop management. This ecosystem recuperation is caused by the natural ecological processes related to ecological succession (Filho *et al.,* 2013).

The soil physical properties such as Bulk density, SMC WHC and Soil temperature showed a progressive improvement as the length of the fallow phase increases. Bulk density is defined as the mass (weight) per unit volume of a dry soil including pore space. There was a decrease in the soil bulk density from younger fallow (2F) site to natural forest (UD) site due to less compaction of the soil particles which could be attributed to more deposition of more organic matters and penetration by fine plant roots. (Mendoza- vega and Messing, 2005).

WHC is defined the mass of water held per unit mass of soil. It represents the amount of water in a soil when the drainage is restricted and the total pore space is filled up with water. Under natural field conditions, only poorly drained soils are at their maximum water holding capacity for long time. It is generally expressed as percentage. Soil WHC is inversely correlated to the soil bulk density; hence there is an increase in the WHC as the length of the fallow period increases reaching a record maximum of 73% of the soil mass at the UD site (Archer and Smith, 1972)

SMC represents the total amount of water content in the soil on its dry weight basis. Soil water content affects the moisture and amount of nutrients available to plants and soil aeration status. The SMC of the study sites also showed significant variation between the different study sites as well as seasonal variation. Maximum SMC was recorded during August during the monsoon season for all study sites. Comparison of acquired data's showed that between the different study sites UD showed the highest SMC while 2F showed the least. According to Weisbach *et al.* (2002) and Kendawang *et al.* (2004), low SMC of young fallow is due to the lack of foliage cover and high SMC of older fallow and forest are due to plant cover which impedes the soil exposure and moisture lost by increasing humidity. The exposure of the young fallow soil and plant coverage of the older fallow soil and forest soil also resulted in a significant variation in the Soil temperature. Exposed soil tends to have higher temperature while foliage covered soil showed a relatively lower temperature according to Hattori *et al.* (2005).

Analysis of the soil nutrient status and chemical properties of the study sites also showed a wide range of variation some of which are quite significant in determining the soil health and nutrient recovery in the fallow sites. The pH of the soil samples were all found to be acid in nature ranging between 4.7 in the pre-bunt Jh site to 5.2 in the UD site. The steady increase in the soil pH from current jhum field to undisturbed forest is found to be caused by the deposition of organic matters and the release of cations from decaying organic matters (Tanaka *et al.,* 2010).

The soil nutrients dynamic also showed an increase in the amount of available nutrients from 2F site to the UD site. The availability of N, P and K for plant utilization increases as the amount of organic matter deposited to the soil increases. This also increase the amount of soil organic carbon thereby leading to the recovery of nutrients available for plant utilization soil covered by plants reduced the nutrient lost as well as adding organic matters which increase nutrient availability (Mishra and Ramakrishnan, 1983; Ketterings *et al.,* 1999).

Since 2F site is the youngest fallow it lacks plant cover and the soil is directly exposure to the surrounding environments. Hence, the soil physico- chemical properties showed a poor characteristic such as high bulk density, low SMC, low pH and low available nutrients due to leeching, erosion and low organic matter input. (Bhadauria and Ramakrishnan, 1983). 5F site represent the intermediate period of a fallow length, its soil physico-chemical properties showed a slight improvement from the 2F site. These improvements are brought about by the process of ecological succession as the shrub vegetation is now being replaced by small trees and bushes, which provide shades, increase deposit of organic matters and reduced erosion. However, the 5 years fallowing period is still considered as a short fallow period as the nutrient loss during the cultivation period are not restored in a balanced condition. Hence, cultivation of a 5 years fallow land hinders the renewal of the soil fertility thereby compromising its sustainability (Bruun *et al.,* 2006). Although a significant variation is observed between the 10 years fallow site and the undisturbed forest with respect to the soil physico-chemical properties, 10 years fallow lands are considered to have restored the soil fertility prior to cultivation. Therefore, it is considered as being suitable for agricultural purpose without compromising its productivity (Toky and Ramakrishnan, 1983; Are *et al.,* 2009).

For the current jhum, alteration of the soil physico- chemical properties was observed from the conversion phase i.e. the clearing of the native vegetation and burning of the dried biomass throughout the cultivation phases till the jhum is abandoned for fallowing. The clearing of the native vegetation and burning of the dried biomass lead to the compaction of the soil particles thereby increasing erosion and leeching of soil nutrients. This leads to the decrease in SMC and WHC while increasing the bulk density and soil temperature thereby compromising the soil physical condition as a whole (Kato *et al.,* 1999; McDonald *et al.,* 2002; Obale-Ebanga *et al.,* 2003). These soil physical properties slowly recuperate with the onset of the cultivation phase. The growth of cultivars slowly restores the soil structure to the condition prior to the conversion phase, therefore increase WHC and SMC is overserved with decrease in soil temperature and bulk density. This process of restoration of soil physical properties continues till the time of harvest. Then the

abandonment of jhum site leaves the soil exposed again and the soil physical conditions deteriorate again which is gradually restored during the fallow periods.

The soil chemical properties such as available N, P and K; SoC and pH also exhibit the similar pattern as the soil physical properties. The clearing of the native vegetation led to the acidification of the soil due increase decomposition and loss of soil cations due to run-off (Ellingson *et al.,* 2000; Neergaard *et al.,* 2008). Destruction of organic matter in burning has several consequences on micro environmental conditions of the soil, particularly soil surface. While porosity, aeration, field and water holding capacity, infiltration and surface moisture are lowered, erosion losses of soil and nutrients through runoff are intensified (Ahn 1974; Jha *et al.,* 1997) and solar radiation reaching the soil is increased. The burning of dried biomass release ash to the soil leading alkalization and increase in pH which improves the nutrient availability and other soil properties. The cultivation phase and fallowing of the jhum field, there is upkeep and gradual change of the soil pH to initial condition before the start of the jhumming process (Sillitoe and Shiel, 1999; Tanaka *et al.,* 2004).

According to Toky and Ramakrishnan (1983) the percentage carbon in the surface soil was significantly higher ($P = < 0.05$) before burning the slash in a jhum cycle and there is reduction in carbon content up to a period of 30 days to 90 days depending upon the length of the fallow site which is being cultivated. This is due to the volatization of the soil carbon during the burning process and the loss of organic carbon due to leeching and runoff. After this period, the soil organic carbon began to slowly increase due to deposition and decomposition of organic matters during the cultivation period. As such, the current study reported a slight increase in the SoC during the May which is approximately 90 days from the date of burning the jhum field.

Nitrogen is an essential nutrient for plant growth, development and reproduction. Despite nitrogen being one of the most abundant elements on earth, nitrogen deficiency is probably the most common nutritional problem affecting

plants. 95% to 99 % of the potentially available nitrogen in the soil is in organic forms, either in plant and animal residues, in the relatively stable soil organic matter, or in living soil organisms, mainly microbes such as bacteria. This nitrogen is not directly available to plants, but some can be converted to available forms by microorganisms. Available nitrogen is defined as nitrogen in a chemical form that can be readily absorbed by plant roots. Plant available forms of nitrogen (N) are inorganic and include nitrate (NO_3), and ammonium, (NH_4) (Saunders, 1957; Bartholomew, 1965). Although burning of dried biomass is expected to increase total N due intensified nitrification due to rise in pH and surface temperature (Jaiyebo and Moore, 1963) or removal of chemical inhibitors (Smith *et al.*,1968; Rice, 1974), decline in total N is often reported (Joachim and Kandiah, 1948; White *et al.*,1973; Kumada *et al.*,1985). This reduction in total nitrogen is attributed to the conversion of organic nitrogen to volatile forms during pyrolysis (Debell and Ralston, 1980). However, the data collected that from the current study showed an increase in available N, corresponding to short-term changes in N availability. This may be attributed to the increase the N mineralization rates under the influence of slash and burning as reported by Matson *et al.* (1987).

Soil available P is the fraction of total P in soil that is readily available for absorption by plant roots. Orthophosphate (HPO_4^{-2} and H_2PO^4ions) are the dominant form of inorganic phosphate that can be absorbed directly by plant and microbial cells. Polyphosphates (including pyrophosphate) are another form of inorganic P of biological origin that may be present in soils, and generally in low concentrations relative to orthophosphate which are available for plant absorption (Condron *et al.*, 2005). The increase in soil temperature during burning between 170 and 300◦C results in the increase in plant-available inorganic P, as determined by pH dependent extracts which is matched by declines in organic P as P is thermally mineralized from soil organic matter (Kang and Sajjapongse, 1980; Giovannini *et al*., 1990; Giardina *et al*., 2000). Also supply of available phosphorus is augmented by addition of ash afterburning (Joachim and Kandiah, 1948). The results of the current study are in agreement with these findings as there is an increase in available P from the pre-burnt to post- burnt phase of the jhum cycle.

Soil K may be divided into four fractions: soil solution K, exchangeable K, non-exchangeable K and K in soil minerals. These fractions constitute a dynamic system with reversible K transfer between the fractions (Sparks, 1987). Exchangeable K is readily available K; which plants can easily absorb. The proportion of the total potassium in soils held in soluble and exchangeable forms is usually relatively small. The majority of this resides in K-bearing minerals. It is found in equilibrium with the soil solution and is easily released when plants absorb potassium from the soil solution. The increase in available K from pre-bunt to post- burnt phase was reported in thus study as addition of ash dramatically increased exchangeable cation concentrations which is in agreement with Toky and Ramakrishnan (1981), Oya and Tokashiki (1984).

Soil organic carbon (SOC) refers to the carbon component of organic compounds in soil. SOC is the basic indicator of soil quality and soil health (Doran and Parkin, 1994). The quality can be defined as fitness of specific kinds of soil to function within its capacity and within natural and managed ecosystem boundaries. It is very much essential to sustain productivity, air and water purification, support human health and habitation to various life forms (Arshad and Martin, 2002). SOC is one of the most important indicators of soil fertility, productivity, quality and decline in SOC adversely affects the land productivity (Vander Werf *et al.,* 2009). Organic carbon which decreases after slashing and burning, showed a slight increase after 90 days but decreased further during first cropping phase indicating a net loss after a year of cropping. The available N, P and K which showed increment after the burnt phase also decreased as the cultivation phase of the jhum continues. This may be attributed to the net uptake of nutrients by growing crop which is responsible for lowering the nutrient levels despite a continuous supply through mineralization, resulting in the decline in soil fertility during cropping (Nye and Greenland, 1964; Zinke *et al.,* 1978; Toky and Ramakrishnan, 1981).

Chapter 4

Soil Enzyme Activity and Microbial Biomass

4.1. Introduction

The structure, spatial distribution and activity of microbial population and enzymes in soils are directly influence, by the physical and chemical properties of soils; hence they may serve as potential early indicator of soil health and quality (Schnurer *et al.,* 1985; Dick *et al,* 1994). Microorganisms are the important sources of available enzymes in soil. The factors that affect the activity of an enzyme include substrate concentrations, pH, ionic strength and nature of salts present, and temperature. It plays an important role in degradation of organic matter, releasing nutrient in an available form to plants. The enzyme activities have often been used as indices of microbial activity and soil fertility (Kennedy and Papendick, 1995; Burns *et al.,* 2013)

Soil enzymes are the key players in biochemical process of organic matter recycling in the soil system and their activities are closely related to soil organic matter (SOM), soil physical properties, and microbial activity (Rao, 2017). Soil enzymes are essential for catalysing reactions necessary for organic matter decomposition (Ajwa *et al.,* 1999) and their activities are strongly influenced by organic matter content of the soil (Speir, 1997). Soil enzymes play an important role in maintenance of fertility in the soil. These enzymes exist in soil due to secretions or excretion products of activities of microorganisms in soil or may be coming from plant roots. Approximately 250 types of enzymes are found in soils (Kurehekar, 2005). Soil enzymes are the mediators and catalysts of important soil functions that include: decomposition of organic inputs; transformation of native soil organic matter; release of inorganic nutrients for plant growth; N_2 fixation; nitrification; gentrification; and detoxification of xenobiotics (Dick, 1997). In addition, soil enzymes have a crucial role in C (β-glycosidase and β-galactosidase), N (urease), P

(phosphates) and S (sulphatase) cycle (Karaca *et al.,* 2011). The types and quantity of enzymes depend on the soil quality and environmental conditions; hence, enzyme activities may be used as good indicators for soil fertility under different land use system. The soil enzymes such as hydrolases (β-glucosidases, phosphatases, ureases) and oxidoreductases (dehydrogenases) play an important role as indicator of soil health, they catalyse many organic matter transformation process (Khan, 1970) and all biochemical and physiochemical transformation dependent on, or related to the presence of enzyme.

Soil microbial biomass (SMB) has been reported to be significantly more sensitive indicator of changing soil conditions than the total soil organic matter content (Powlson and Jenkinson, 1976). As an early indicator of change in soil organic matter and soil quality status, assessment of SMB is a more useful parameter than total soil organic matter and (Bremer *et al.,* 1994; Gregorich *et al.,* 1994; Chilima *et al.,* 2002; Li *et al.,* 2004). Soil microbial biomass is also related to climate (Dyer *et al.,* 1990), soil moisture (Taylor *et al.,* 1999), soil texture (Bauhas *et al.,* 1998, Hassink 1994, Wardle 1992), plant productivity (Zak *et al.,* 1994) and organic matter quality (Taylor *et al.,* 1989, Zak *et al.,* 1990). Soil with high organic matter and easy available soil organic matter compound tend to have higher microbial biomass content and activities due to high presence of desired energy sources for microorganisms. Microbial biomass can be used for evaluating soil perturbation and restoration (Ross *et al.,* 1982; Smith and Paul, 1990) as well as for assessing soil quality of different types of vegetation (Goffman *et al.,* 2001; Zeng *et al.,* 2009).

4.2. Methodology

4.2.1. Dehydrogenase Activity (DHA)

To determine dehydrogenase activity of the soils, method given by Casida *et al.* (1964) was followed. 1g of fresh soil was placed in a test tube (15 x 2 cm) and 0.1g of $CaCO_3$ was added. 1 ml of 1% 2,3,5 Triphenyl Tetrazolium Chloride (TTC) solution was added and swirled for a few seconds. The tubes were then plugged and

incubated at 30°C for 24 hrs. The resulting slurry was filtered through Whatman No.1 filter paper. Triphenyl formazan (TPF) was extracted with successive aliquots of concentrated methanol in a 50 ml volumetric flask. The developed pink colour was read out with the help of spectrophotometer (Dynamica Halo DB-20) at 485 nm using methanol as blank (without soil). The enzyme activity was expressed in terms of µgTPF ml^{-1} 24 hrs^{-1}.

4.2.2. Acid Phosphatase (APase) Activity

Determination of soil acid phosphatase activity was done using the protocol descrbied by Tabatabai and Bremner (1969**).** 0.1g of air-dried soil sample was weight and 0.25 ml toluene was added and allowed to stand for 10 minutes. Then 4 ml of modified universal buffer (MUB pH-6.5) and 1 ml of 0.115M p-Nitrophenyl Phosphate (p-NP) solution was added. The mixture was swirled for a few seconds and plugged with cotton stopper and incubated for 1 hr at 37° C. Then, 1 ml of 0.5M $CaCl_2$ and 4 ml of 0.5M NaOH solutions were added simultaneously into the mixture before transferring into Whatman No.1 filter paper. The yellow colored filtrate of p-nitrophenol phosphate (phosphoric acid) was read out with the help of spectrophotometer (Dynamica Halo DB-20) at 410 nm. For blank, 1 ml p-NP was added after $CaCl_2$ and NaOH were added into the mixture without soil just before filtration. The enzyme activity was expressed in terms of µg p-NP ml^{-1} hr^{-1}.

4.2.3. Urease Activity (URS)

Method given by McGarity and Myers (1967) was followed for determination of soil urease activity**.** 1g of fresh soil was placed in a 100 ml volumetric flask and 1 ml of toluene was added. The mixture was then allowed to stand for 15 minutes for complete penetration of toluene into the soil. 10 ml of pH-7 Modified Universal Buffer (MuB) and 5 ml of 10 % urea solution (freshly prepared) was added. After a thorough mixing, the flask was incubated for 3 hrs at 37° C. Blank was prepared by replacing 5 ml of 10 % urea solution with 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. The solution was then filtered through Whatman No.5 filter paper. The filtrate was taken into a 25 ml volumetric flask and 5 ml of distilled water, 2 ml of phenolate solution and of 1.5 ml of sodium

hypochlorite solution were added simultaneously. The final volume of the flask was made up to 25 ml with distilled water and the blue color was read by using spectrophotometer at 630 nm. The enzyme activity was expressed in terms of mg NH_4^+-N ml^{-1} $3hrs^{-1}$.

4.2.4. Arylsulfatase (ARS) activity

Soil arylsulfatase was determined using method given by Tabatabai and Bremner, (1970). 1g (2 mm sieved) soil was taken in 50 ml conical flasks. 0.25 ml toluene was added to the soil sample and allowed to stand for 10-15 minutes. 4 ml of acetate buffer (pH 5.8) and 1 ml of 0.025M substrate, p-nitrophenyl sulfate solution was added. The flask was swirled for few seconds to mix the contents and incubated at 37°C for one hour. After incubation, 1 ml of 0.5M $CaCl_2$ and 4 ml of 0.5M NaOH were added and swirled for few seconds. The suspensions were filtered through Whatman No.2 filter paper. The yellow color intensity of the filtrates was measure spectrophotometrically at a wavelength of 410 nm.

4.2.5. β-Glucosidase (β-GSD) activity

For determination of soil β-Glucosidase (β-GSD) activity method described Tabatabai and Bremner (1970) was followed using p-nitrophenyl - β –glucoside as substrate. 1g of soil was taken in a 50-ml Erlenmeyer flask and treated with 0.25 ml of toluene and allow to stand for 10-15 minutes. 4 ml of pH 6 Modified Universal Buffer (MUB) and 1 ml of the 0.05M p-nitrophenyl - β –glucoside solution was added. The flask was swirled or a few seconds, plugged and incubated at 37°C. After 1hour, of incubation 1 ml of 0.5M $CaCI_2$ and 4 ml of 0.1M THAM was added. The flask was swirled for a few seconds to mix the contents, and the soil suspension was filtered through WhatmanNo.1. Absorbance of the colored filtrate was measured by using spectrophotometer at 410 nm wavelength.

4.2.6. Estimation of soil microbial biomass Carbon and Nitrogen

Method given by Pothoff (2008) was followed for determination of soil microbial biomass carbon and nitrogen. 10 g of fresh soil sample was collected in a petridish and fumigated by adding 5ml chloroform. The sample was incubated in a

dessicator at 25°C for 24 hours in the dark. Another batch of soil (10g) was collected similarly without fumigation. Both the fumigated and non-fumigated samples were placed in 100ml flasks, and extracted with 40ml of 0.5M K_2SO_4 (extractant to soil ratio of 4:1) for 30 min by oscillating shaking at 200 rpm. It was then filtered through a folded filter paper. 0.6ml each of K_2SO_4 soil extracts and blank were collected in a test tube and 1.4ml citric acid buffer was added. Ninhydrin reagent (1ml) was slowly added, mixed thoroughly, and closed with loose aluminium lids. The test tubes were heated vigorously in a boiling water bath for 25 min to dissolve any precipitate formed during the addition of the reagents. Finally, 4ml of ethanol-to-water mixture (1:1) was added. The solution was mixed thoroughly and the absorbance was read at 570nm in a spectrophotometer.

4.3. Results

4.3.1. Dehydrogenase Activity

Dehydrogenase activity (DHA) showed variation among different seasons; it steadily increased from a minimum level during winter to maximum level during autumn for all study sites. Highest activity was recorded in the UD site (0.9 mg TPF g dry soil^{-1} 24hr^{-1}) and lowest (0.22 mg TPF g dry soil^{-1} 24hr^{-1}) in Jh site. On the fallows sites DHA gradually increased from the 2F, 5F and then 10F sites. It varied among the sites, with a sharp difference between the fallow sites and undisturbed site. Significant ($p<0.05$) variation was observed among the different sampling sites except between JhX2F and 2FX5F.

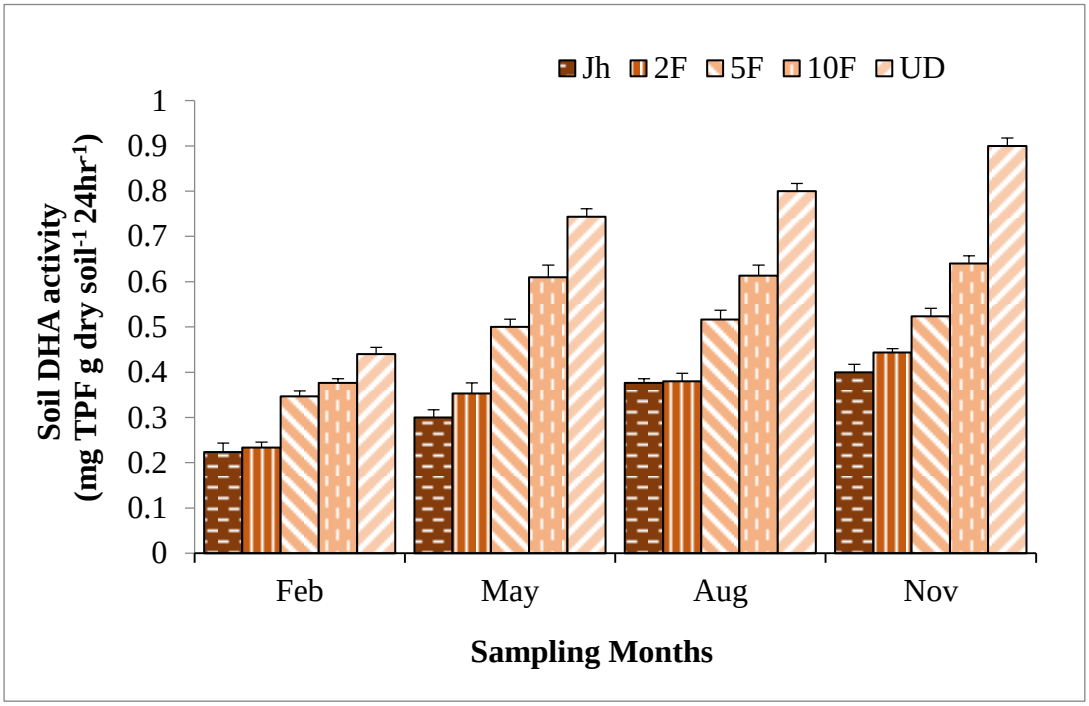

Fig 4.1. Soil dehydrogenase enzyme activity (mg TPF g dry soil^{-1} 24hr$^{-1)}$) of different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD).

Table 4.1. One-way analysis of variance (ANOVA) of DHA among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD). Marked effects are significant at $p \leq 0.05$.

S. N	Source of variation	F-value	P-value
1.	Jh x 2F x 5F x 10F x UD	1.33E+03	**0.000***
2.	Jh x 2F	0.792	0.424
3.	Jh x 5F	28.735	0.006*
4.	Jh x 10F	101.954	0.001*
5.	Jh x UD	1.10E+03	0.000*
6.	2F x 5F	5.904	0.072
7.	2F x 10F	31.996	0.005*
8.	2F x UD	141.083	0.000*
9.	5F x 10F	15.649	0.017*
10.	5F x UD	142.222	0.000*
11.	10F x UD	38.529	0.003*

4.3.2. Acid Phosphatase Activity

Acid phosphatase (Apase) activity the undisturbed forest site showed a significantly higher phosphatase activity as compared to the other sites. There was gradually increase of phosphatase enzyme as the fallow length increase. Enzyme activity ranges from 421.00 µg p-NP g^{-1} dry soil h^{-1} at the 2F site during February to 1495.66 µg p-NP g^{-1} dry soil h^{-1} at the UD site during August. Seasonal variation was observed with little or no significance among the study sites except for UD, which showed a prominent variation in the enzyme activity with respect to change in season.

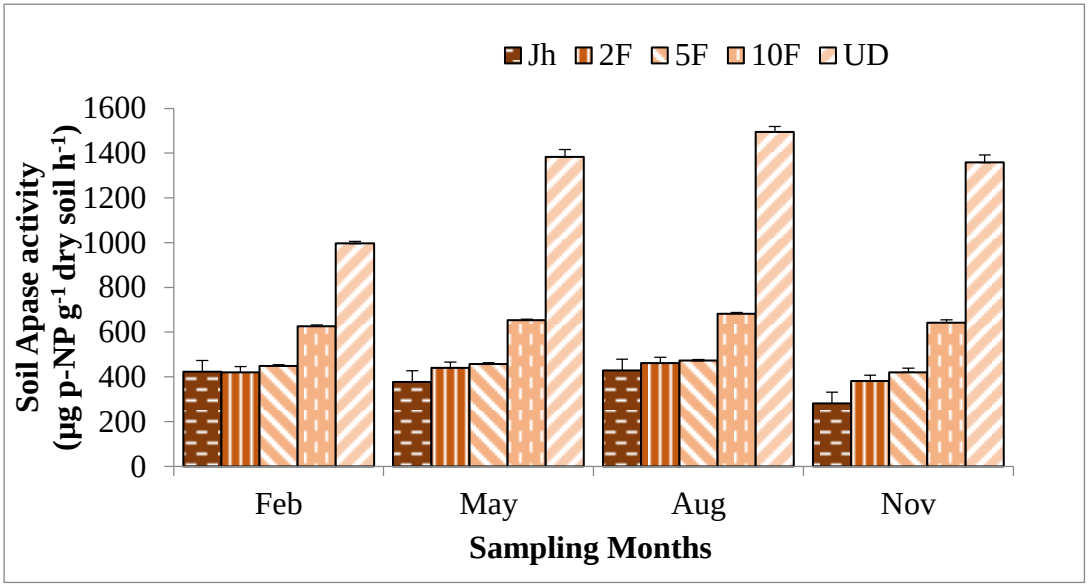

Fig 4.2. Soil acid phosphatase activity (g p-NPP g^{-1} dry soil h^{-1}) of different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD).

Table 4.2. One-way analysis of variance (ANOVA) of Apase among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD). Marked effects are significant at $p \leq 0.05$.

S.N	Source of variation	F-value	P-value
1.	Jh x 2F x 5F x 10F x UD	2.456E+03	0.000*
2.	Jh x 2F	96.479	0.001*
3.	Jh x 5F	255.693	0.000*
4.	Jh x 10F	3.38E+03	0.000*
5.	Jh x UD	2.59E+04	0.000*
6.	2F x 5F	28.274	0.006*
7.	2F x 10F	2.29E+03	0.000*
8.	2F x UD	2.33E+04	0.000*
9.	5F x 10F	2.19E+03	0.000*
10.	5F x UD	2.47E+04	0.000*
11.	10F x UD	1.37E+04	0.000*

4.3.3. Urease Activity

Highest urease enzyme activity was recorded at UD site (34µg NH_3 g dry $soil^{-1}$ $3hr^{-1)}$ where lowest (10 mg NH_4 –N dry $soil^{-1}$ $3hr^{-1)}$ was recorded at the 2F site. The 2 years fallow site showed a low enzyme activity which gradually increases as the fallow length increases from 5 years to 10 years. The undisturbed forest (UD) has the highest overall urease activity while the jhum (Jh) site has the lowest overall urease enzyme during the sampling periods. Significant variation in the urease enzyme activity was observed between the all the study sites.

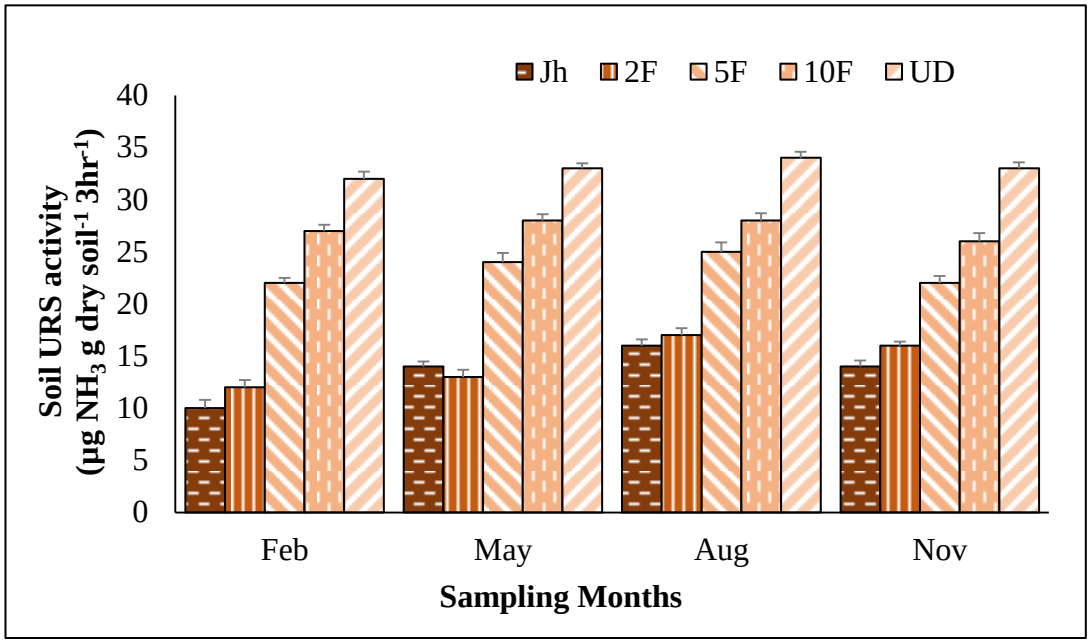

Fig 4.3. Soil urease activity (mg TPF g dry soil^{-1} 24hr$^{-1)\ of}$ different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD)

Table 4.3. One-way analysis of variance (ANOVA) of urease enzyme activity among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD). Marked effects are significant at $p \leq 0.05$.

S.N	Source of variation	F-value	P-value
1.	Jh x 2F x 5F x 10F x UD	115.5625	0.000*
2.	Jh x 2F	10	0.034*
3.	Jh x 5F	393.3902	0.000*
4.	Jh x 10F	932.2581	0.000*
5.	Jh x UD	1889.161	0.000*
6.	2F x 5F	457.96	0.000*
7.	2F x 10F	1500	0.000*
8.	2F x UD	3285.6	0.000*
9.	5F x 10F	115.5625	0.000*
10.	5F x UD	115.5625	0.000*
11.	10F x UD	864	0.000*

4.3.4. Arylsufatase Activity

The ARS activity of the study site ranges from 71 µg PNP dry soil^{-1} hr^{-1} in 2F soil during May to 320 µg PNP dry soil^{-1} hr^{-1} in UD soil during May and August. For the Jh soil, the ARS activity drastically increases from 111 µg PNP dry soil^{-1} hr^{-1} in Feb to 249 µg p-PNP dry soil^{-1} hr^{-1} in May. The ARS enzyme activity showed a significant variation between the different study sites.

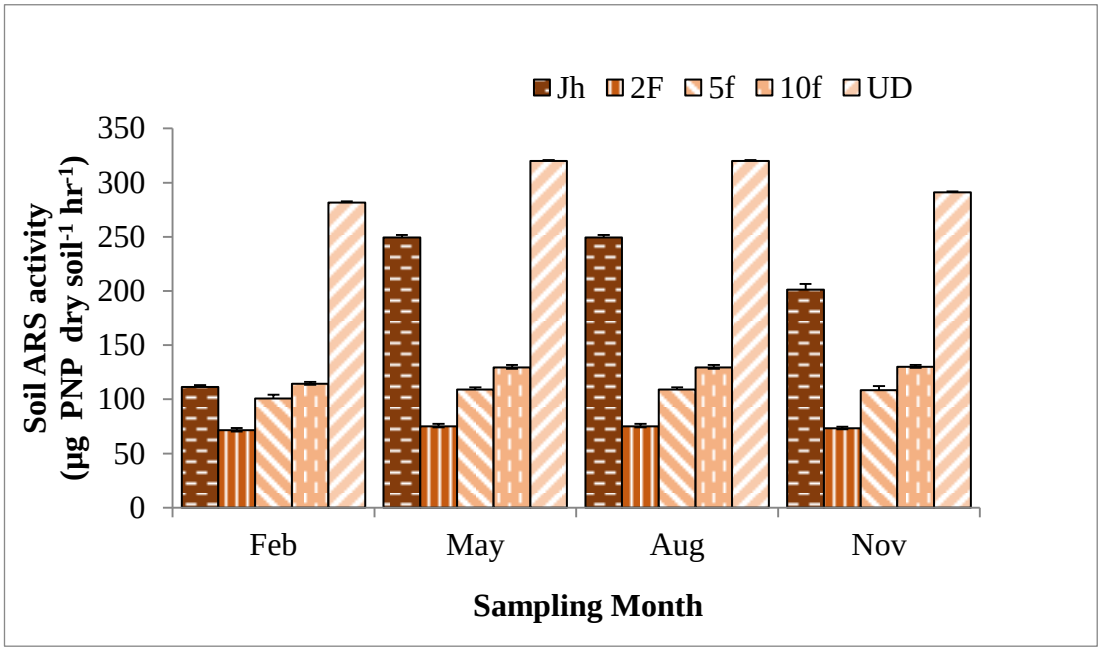

Fig 4.4. **S**oil arylsulfatase activity (µg PNP dry soil^{-1} hr^{-1}) of different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD).

Table 4.4. One-way analysis of variance (ANOVA) of ARS among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD). Marked effects are significant at $p \leq 0.05$.

Sl.no	Source of variation	F-value	P-value
1.	Jh x 2F x 5F x 10F x UD	2E+03	**0.000***
2.	Jh x 2F	4E+03	0.000*
3.	Jh x 5F	2E+03	0.000*
4.	Jh x 10F	2E+03	0.000*
5.	Jh x UD	2E+03	0.000*
6.	2F x 5F	4E+02	0.000*
7.	2F x 10F	4E+02	0.000*
8.	2F x UD	1E+04	0.000*
9.	5F x 10F	1E+02	0.000*
10.	5F x UD	9E+03	0.000*
11.	10F x UD	7E+03	0.000*

4.3.5. β - glucosidase Activity

Beta- glucosidase showed a significant variation between the different study sites. Season variation was also observed among different study sites. Highest β - glucosidase activity of 211.45 µg PNP dry soil^{-1} hr^{-1} was recorded at UD site during May while lowest reading of 58.29 µg PNP dry soil^{-1} hr^{-1} was recorded at the 2F site during February. The enzymes activity increases significantly with the increase in fallow length from 2 years fallow to 10 years' fallow. The variation of β - glucosidase activity was found to between significant between all the study site except for 10F site which does not showed significant variation in β - glucosidase activity when compared to the other study sites.

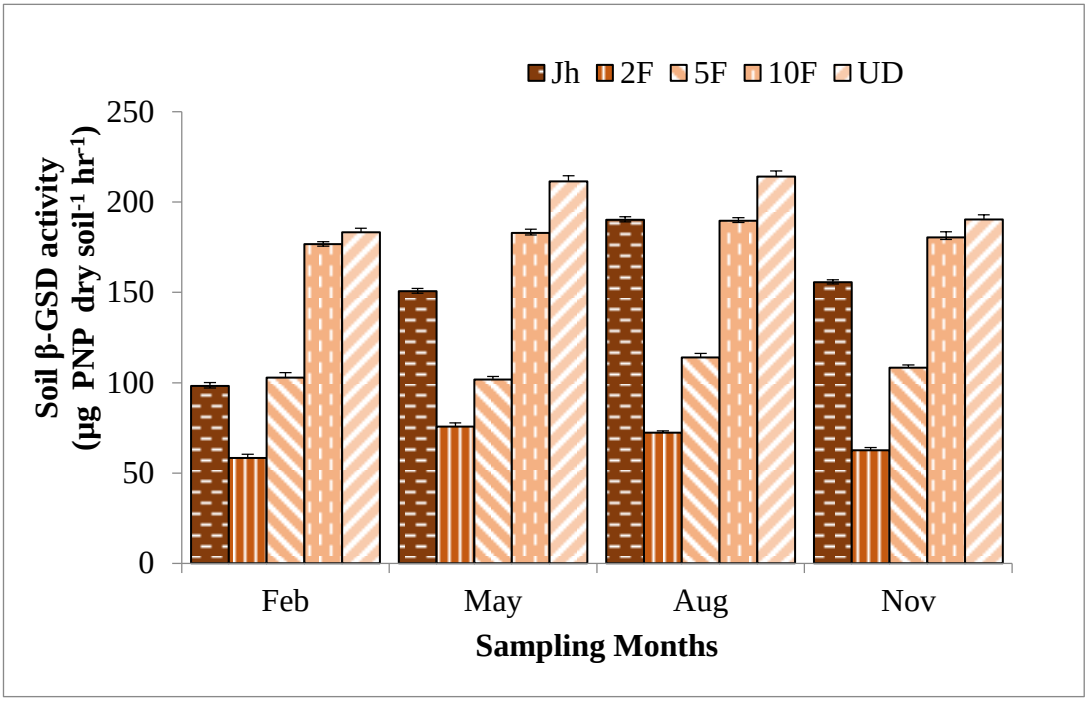

Fig 4.5. Soil β-Glucosidase activity (µg PNP dry soil^{-1} hr^{-1}) of different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD). Marked effects are significant at $p \leq 0.05$.

Table 4.5. One-way analysis of variance (ANOVA) of β-Glucosidase activity among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD). Marked effects are significant at $p \leq 0.05$.

S.N	Source of variation	F-value	P-value
1.	Jh x 2F x 5F x 10F x UD	2.11E+03	0.000*
2.	Jh x 2F	2.43E+03	0.000*
3.	Jh x 5F	638.262	0.000*
4.	Jh x 10F	0	0.987
5.	Jh x UD	941.879	0.000
6.	2F x 5F	563.177	0.000*
7.	2F x 10F	6.354	0.065
8.	2F x UD	6.40E+03	0.000*
9.	5F x 10F	1.723	0.260
10.	5F x UD	3.11E+03	0.000*
11.	10F x UD	2.423	0.195

4.3.6. Soil Microbial Biomass

The soil microbial biomass exhibits both variation in both time and space. Among the study site highest Microbial Biomass Carbon (C_{mic}) content of 164 $mgkg^{-1}$ of soil was observed in the undisturbed site during August while lowest content of 34 $mgkg^{-1}$ of soil was recorded at the 2 years fallow site during February. Soil Microbial Nitrogen (N_{mic}) showed the similar pattern of variation as to that of C_{mic}. The C_{mic} and N_{mic} are positively correlated, hence the highest N_{mic} was observed at UD site during August and lowest was observed in 2F site during February.

For both C_{mic} and $N_{mic,}$ there is significant variation between the different sites, an increase in both N_{mic} and C_{mic} was noted with increase length of the fallow period. The undisturbed site showed significantly higher microbial biomass as compared to the other study sites. For the current jhum, a drastic increase in microbial biomass is observed in May, which declines with the progress of the cropping phase.

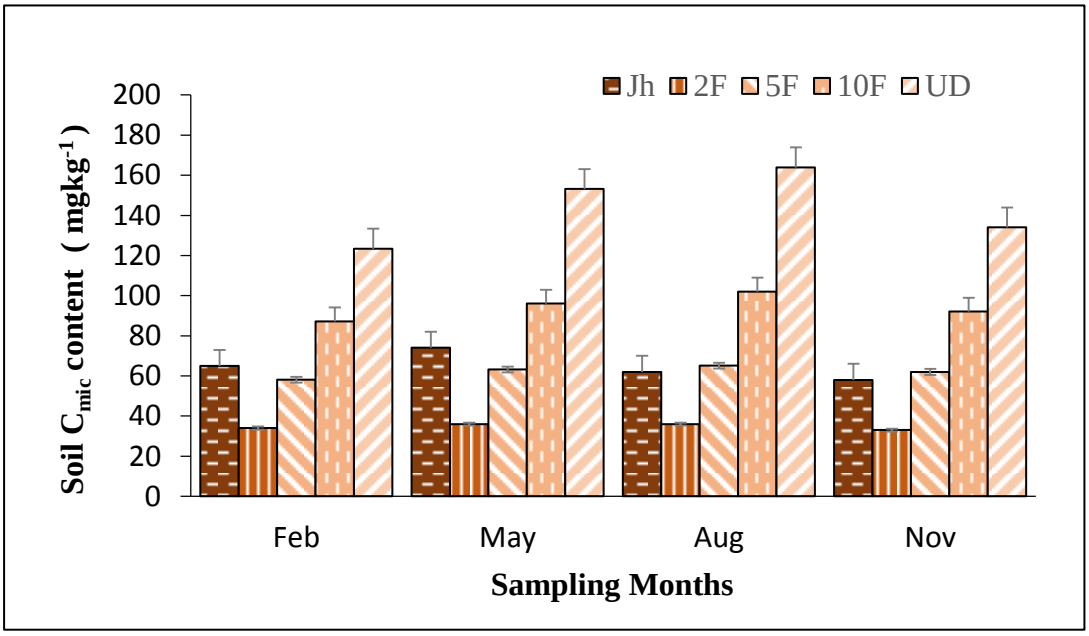

Fig 4.6. Microbial biomass carbon (C_{mic}) content of different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD). sampling period.

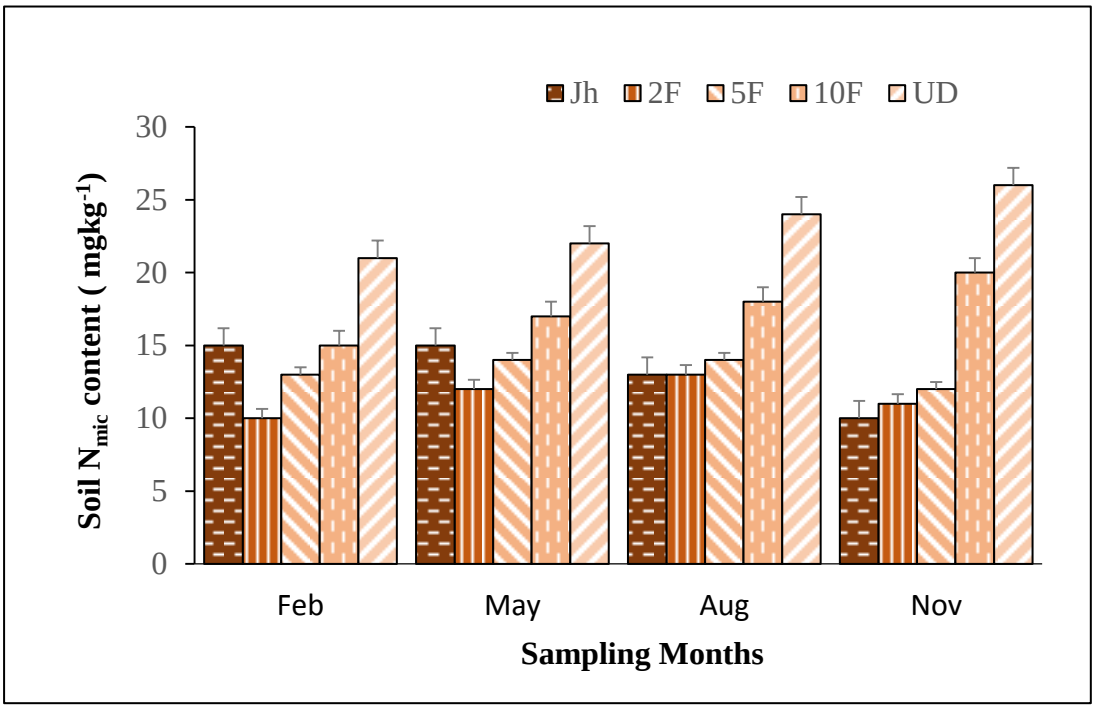

Fig 4.7. Microbial biomass nitrogen (N_{mic}) content of different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD).

Table 4.6. One-way analysis of variance (ANOVA) of C_{mic} among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD). Marked effects are significant at $p \leq 0.05$.

C_{mic}			
S.N	**Source of variation**	**F-value**	**P-value**
1.	Jh x 2F x 5F x 10F x UD	356	0.000*
2.	Jh x 2F	1.85E+03	0.000*
3.	Jh x 5F	486	0.000*
4.	Jh x 10F	2.34E+03	0.000*
5.	Jh x UD	2.12E+04	0.000*
6.	2F x 5F	4.54E+03	0.000*
7.	2F x 10F	1.24E+04	0.000*
8.	2F x UD	3.83E+04	0.000*
9.	5F x 10F	470.206	0.000*
10.	5F x UD	1.58E+04	0.000*
11.	10F x UD	1.62E+04	0.000*

Table 4.7. One-way analysis of variance (ANOVA) of N_{mic} among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD). Marked effects are significant at $p \leq 0.05$.

N_{mic}			
S.N	**Source of variation**	**F-value**	**P-value**
1.	Jh x 2F x 5F x 10F x UD	3.13E+04	0.000*
2.	Jh x 2F	14.912	0.018*
3.	Jh x 5F	1.920	0.238
4.	Jh x 10F	4.267	0.108
5.	Jh x UD	138.720	0.000*
6.	2F x 5F	21.146	0.010*
7.	2F x 10F	21.689	0.010*
8.	2F x UD	161.634	0.000*
9.	5F x 10F	0.923	0.391
10.	5F x UD	84.375	0.001*
11.	10F x UD	39.000	0.003*

Table 4.8. Correlation coefficient (r) between soil enzymes and microbial biomass (C_{mic} and N_{mic}) among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD). Marked (*) are significant at $p = 0.05$

	Jh		**2F**		**5F**		**10F**		**UD**	
	C_{mic}	**N_{mic}**	**C_{mic}**	**N_{mic}**	**C_{mic}**	**N_{mic}**	**C_{mic}**	**N_{mic}**	**C_{mic}**	**N_{mic}**
Apase	0.971* 0.077	0.993* 0.037	0.999* 0.013	0.999* 0.013	0.541* 0.318	0.999* 0.015	0.993* 0.037	0.993* 0.037	0.961* 0.089	0.923* 0.126
URS	0.817* 0.196	0.737* 0.236	1.000* 0.000	1.000* 0.000	1.000* 0.000	0.500* 0.333	0.500* 0.333	0.500* 0.333	0.500* 0.333	0.596* 0.297
DHA	0.971* 0.077	0.993* 0.037	1.000* 0.000	1.000* 0.000	0.998* 0.021	0.557* 0.312	0.866* 0.166	0.866* 0.166	0.866* 0.167	0.918* 0.130
Beta	0.998* 0.022	0.998* 0.018	0.998* 0.018	0.998* 0.018	1.000* 0.000	0.500* 0.333	0.582* 0.302	0.582* 0.302	0.311 0.399	0.418* 0.363
ARS	0.971* 0.971	0.993* 0.993	1.000* 0.000	1.000* 0.000	1.000* 0.000	0.500* 0.333	0.500* 0.333	0.500* 0.333	0.500* 0.333	0.596* 0.297

Discussion

Lowest soil dehydrogenase enzyme activity was observed in the current jhum (Jh) soil for all the sampling periods, while a significant increase in soil DHA enzyme activity was observed with the increase in fallow length from 2F to 10F and highest DHA activity was observed in the undisturbed forest (UD) soil. Since DHA enzyme is considered to exist as an integral part of intact cells but does not accumulate extra-cellular in the soil (Das and Varma, 2011), it can be assumed that the DHA activity of the study sites were greatly influenced by the microbial activity of the given site. Dehydrogenase is an enzyme that oxidizes soil organic matter by transferring protons and electrons from substrates to acceptors and plays a significant role in the biological oxidation of soil organic matter (Zhang *et al.,* 2010). hence, it maybe established that the site with the highest soil DHA activity will have the highest microbial activity and highest nutrient turnover rate while the sites with lower soil DHA activity will have lower nutrient turnover rate and lesser microbial activity.

Undisturbed forest (UD) site showed the highest soil Apase enzyme activity while 2 years fallow (2F) site have the lowest the Apase enzyme activity. A gradual increase in the amount of soil Apase enzyme activity was observed with the increase in fallow length from 2F to 10F, with a drastic increase in the undisturbed forest (UD) soil. Since the amount of available P in soil is consider as an indicator of soil health (Cardoso et al., 2013), the gradual increase in the Apase activity with increase fallow length maybe considered a sign of regeneration of soil health. Phosphatase is an oxidoreductase, an important enzyme in soil which plays a key role in P-cycle of the environment as they catalyze the hydrolysis of ester–phosphate bonds, leading to the release of phosphate (P), which can be taken up by plants or microorganisms (Cosgrove, 1967; Halstead and McKercher, 1975; Quiquampoix and Mousain, 2005) Hence with increase in soil Apase enzyme activity, increase in soil available P is expected. Therefore, longer jhum fallows are expectect to be more fertile in interms of available P as compared to shorter jhum fallow sites.

Urease activity showed a gradual increase with the increase fallow period and is highest in the UD site. Since URS is a hydrolase enzyme responsible for hydrolytic conversion of the substrate, urea into carbon dioxide and ammonia, the amount of plant available N is expected to show a gradual increase with the length of the fallow period. 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; Stott and Hagedon, 1980). The low vegetation covers and absence of large trees in the 2F site may account for the low urease enzyme activity in the site. The change in urease activity which showed an increase from Feb to Aug a minor decrease in Nov for all study sites reflected change in the vegetation cover and the quantity of organic matter incorporated into the soil.

Highest soil ARS enzyme activity was observed in Jh site showed a drastic increase after the burning of the dried vegetation; this may be due to the addition of sulfur compound by the burning process which increases the available substrate for the ARS producing microbes. The drop in the ARS activity in 2F, 5F and 10F may attribute to leaching of sulfur compound thereby limiting the available substrate. Although ester of sulfates is considered to be the most labile form of soil organic S, they are unavailable to plants and must be hydrolyzed to inorganic SO_4^{2-} before plant uptake. Arylsulphatases are typically widespread in nature (Dodgson *et al.*, 1982) as well as in soils and are responsible for the hydrolysis of sulphate esters in the soil and are secreted by bacteria into the external environment as a response to sulphur limitation. The fluctuation in the soil ARS activity between the different sites indicates the sulphur availability in the different sites.

Lowest soil β –glucosidase (β -GSD) enzyme activity was observed in the 2 years fallow (2F) site while highest β –GSD activity was recorded in undisturbed forest (UD) site. A regular increase in the soil β –GSD activity was observed with the increase in fallow length with no significant variation between the 10F and UD site. Since β-Glucosidase enzyme activity give a reflection of past biological

activity, the capacity of soil to stabilize the soil organic matter, and is very sensitive to changes in pH, and soil management practices (Chen *et al.,* 2018), the low level of β -GSD activity in 2F site may result in limited degradation of plant debris and lower soil organic matter, while in UD site rapid degradation of plant debris and higher soil organic matter. Anthropogenic disturbance to the soils such as agricultural practice is highly reflected on the level β-Glucosidase enzyme activity.

Overall, the sudden drop in soil enzyme activities in 2-years old jhum fallow is due to accelerates the loss of organic matter and nutrients from the soil as a result of removal of vegetal cover, Garcia *et al*. (1997). The relative increase in the enzyme activities with the increase in fallow length can be attributed to the increase in substrate availability for the microbes (Saha *et al.,* 2011; Gispert *et al.,* 2013). O'Toole *et al. (*1985), Pancholy and Rice (1973) and Tiwari (1988) conveyed that sites such as forest and older fallows with higher substrate availability for microbes have higher enzyme activity. Palma and Conti (1990) reported that variation in in enzyme activity is related to the type of vegetation and organic matter.

Soil microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}), and enzyme activities have been used as indicators of changes in soil organic matter status that will occur in response to alterations in land use, cropping system, tillage practice and soil pollution (Sparling *et al.,* 1992). For the current jhum (Jh), a drastic increase in microbial biomass was observed in May, which declines with the progress of the cropping phase. This was attributed to the burning, by which large amounts of nutrients from the microbes were released. Increase in soil C_{mic} and N_{mic} was observed with the increase in the fallow length from 2F to 10F with a drastic increase in undisturbed forest (UD) soil. The higher microbial biomass in the older fallows and UD sites may be attributed to dense growth of woody plants and to greater availability of moisture and nutrients in soil on account of greater accumulation of litter and fine roots during community regeneration (Arunachalam et al. 1997b, 1999). The increase in soil C_{mic} and N_{mic} with the increase length of fallow period from 2F to UD was expected as soil C_{mic} and N_{mic} had positive

relationships with soil nutrients, organic matter, and soil enzyme activities, which changed with different stages of revegetation (Shao-Shan *et al.,* 2010).

High positive correlation between the microbial biomass (C_{mic} and N_{mic}) with soil enzymes activities was observed both of which were directly correlated to the length of the fallow period. The higher enzyme activities of the soil after re-vegetation confirmed our first hypothesis, for which one possible reason should be owing to the accumulated microbial biomass. Because the soil enzymes are mainly excreted by microbes to mineralize soil carbon, nitrogen, and phosphorus from SOC, the activities of enzymes could therefore be partially explained by the distribution of soil microbial biomass.

Chapter 5

Metagenomic Analysis of Bacterial Diversity

5.1. Introduction

Soil harbours the most complex and dynamic microbial communities in the entire biosphere. It is a complex ecosystem which is composed of a variety of microhabitats with different physicochemical and environmental conditions. These ecological factors influence microbial activities and play very important roles in determining the dynamics of micro-organisms in natural environments (Curtis *et al.,* 2002; Fenchel, 2006). The soil microbial communities are relatively diverse (Robe *et al.,* 2003), with prokaryotic diversity being the highest (Roesch *et al.,* 2007). As reported by Knietch *et al.* (2003), 1 gram of soil may contain up to 10 billion microorganisms and belonging thousands of different species.

Soil microbiological characters are more sensitive to changes in management and environmental conditions as compared to chemical and physical properties. Changes in the composition of soil microflora are a crucial indicator of the functional integrity of soil (Insam, 2001). Activity and growth of microorganisms is restricted by soil environmental factors such as temperature, moisture, pore size, distribution and nutrient availability and therefore, indirectly by cultivation practice. Microbial populations can also provide an early indication of changes in soil long before it can be measured by changes in organic matter (Powlson *et al.,* 1987). Metagenomics is a study of collective set of genetic materials extracted directly from environmental samples, and does not rely on cultivation or prior knowledge of the microbial communities (Riesenfeld *et al.,* 2004). Thus, it is a powerful tool to unravel environmental genetic diversity without potential biases resulting from culturing or isolation. Metagenomics is also known by other names, such as environmental genomics or community genomics, or microbial ecogenomics (Rastogi and Sani, 2011). The two major aim of metagenomics analysis are to determine the diversity of

the microbes and the metabolic processes are being carried out in the community (Allen and Banfield, 2005). The surveyed for microbial diversity was mainly based on 16SrRNA gene profiling, the prevalent marker gene for identification of prokaryotic species (Weisburg *et al.*, 1991). Metagenomic investigations have been conducted in several environments, ranging from the oceans to soil, the phyllosphere and acid mine drainage, and have provided access to phylogenetic and functional diversity of uncultured microorganisms (Handelsman, 2004).

Next-generation sequencing (NGS) also known as high-throughput sequencing, is the term used to describe a number of different metagenomics sequencing technologies. It is a powerful tool which can be employed for the study of structural composition of the soil microbiome in details. There are a number of different NGS platforms using different sequencing technologies such as sequencing which works by simultaneously identifying DNA bases, as each base emits a unique fluorescent signal, and adding them to a nucleic acid chain, Roche 454 sequencing based on pyrosequencing, a technique which detects pyrophosphate release, again using fluorescence, after nucleotides are incorporated by polymerase to a new strand of DNA and Ion Torrent: Proton / PGM sequencing which measures the direct release of H+ (protons) from the incorporation of individual bases by DNA polymerase and therefore differs from the previous two methods as it does not measure light. For this study the Illumina (Solexa) Miseq was used due to its high genome coverage and low error rate (0.4%) (Quail *et al.*,2012).

5.2. Methodology

5.2.1 Bacterial DNA extraction from Jhum and undisturbed soil samples

The extraction of metagenomic DNA from the Jhum and undisturbed soil samples was performed using the FastDNA® SPIN soil Kit (Qbiogene, Inc., CA) The DNA extraction was performed according to the manufacturer's recommendations for each kit with slight modification suggested by De Mandal *et al.* (2015)

200 mg of soil were put to Lysing Matrix A tube and 1.0 ml Cell Lysis Solution (CLS) was added for homogenization. The mixture was homogenized in the FastPrep® Instrument for 40 seconds at a speed setting of 6.0. The homogenized mixture was the centrifuge at 14,000 x g for 5-10 minutes to pellet the debris. The supernatant (600 – 700µl) were transfer to a 2.0 ml microcentrifuge tube and an equal volume of Binding Matrix was added. The content of the tubes was thoroughly mix by inverting the tubes and incubate with gentle agitation for 5 minutes at room temperature on a rotator. The samples were then centrifuge at 14,000 x g for 10 seconds to pellet the Binding Matrix. Supernatant were discarded and 500µl prepared SEWS-M was added and the pellets were gently suspended. Then suspended Binding Matrix was transferred to a SPIN™ Filter and centrifuge at 14,000 x g for 1 minute. The contents of Catch Tube were discarded and replaced. The content of the SPIN™ Filter were then centrifuge a second time at 14,000 x g for 1 minute. DNA was eluted by gently suspending Binding Matrix above the SPIN filter in 100µl of DES. The suspended DNA was then incubated for 5 minutes at 55°C in a water bath and Centrifuge at 14,000 x g for 1 minute to bring eluted DNA into the clean catch tube. The eluted DNA used for downstream applications and store at -20°C.

5.2.3. 16SrRNA gene amplification ()

The V3 hypervariable region of the 16SrRNA gene was amplified using F 341/R518 primer combination (5′-CCTACGGGAGGCAGCAG-3′; 5′-ATTACCGCGGCTGCTGG-3′) as given by De Mandal *et al.* (2015). The amplification was carried out using 10 pmol/µl of each forward and reverse primer mix with PCR Master Mix containing 2 µL of each primer, 0.5 µL of 40 mM dNTP (NEB, USA), 5 µL of 5X Phusion HF reaction buffer (NEB, USA), 0.2 µL of 2U/µL F-540 Special Phusion HS DNA Polymerase (NEB, USA), 5 ng input DNA and water in a total volume to 25 µL. The cycling conditions were 98ºC for 30 seconds of initial denaturing, 30 cycles of 98ºC for 10 secs and 56ºC for 30 sec, 72ºC for 10 sec, and Final extension 72ºC for 10 minutes.

5.2.4. 16SrRNA gene amplicon sequencing and library preparation

Gene amplicon sequencing and library preparation was carried out following the method of De Mandal *et al.* (2015). The amplicons of 470 bp were gel purified and proceed with library preparation using NEB Next Ultra DNA Library Prep Kit. The libraries were sequenced in HiSeq 2500 for a 2x250 read length. The metagenome sequencing was performed at AgriGenome Labs Pvt Ltd, Cochin.

Cutadapt was used to remove the barcode and primer sequences from the sequenced amplicons after demultiplexing. The sequenced amplicons were the analysed using DADA2 plugin of QIIME2 (version 2019.4) to denoise the forward and reverse reads with quality filtering (Q > 25) and merged, which was followed by removal of chimeric sequences. (Herlemann, *et al.*, 2011; Martin 2011; Bolyen 2019)

5.2.4. Taxonomic and diversity analyses

Following the method given by Wang *et al.,* (2007) and Segata *et al*. (2011), the amplicon sequencing variants (ASVs) were assigned to the pre-trained Greengenes (13_8 version) reference database, which was trimmed in the V3-V4 primer regions using the naïve Bayesian taxonomic classifier. ASVs that matched mitochondria, chloroplasts, and unassigned variants of a known taxon were excluded from downstream analysis. Major classified phyla, families, and genera present in >50% of the jhum and undisturbed samples were mainly evaluated in this study. The alpha-diversity of each sample was examined for species richness, evenness, phylogenetic diversity, Shannon's index, and Simpson's index based on rarefied ASV tables using 78,006 sequences per sample.

Principal coordinates analysis (PCoA) was computed based on both the unweighted and weighted UniFrac distance matrices to compare the dissimilarity of prokaryotic microbiota among different marbling score groups. The linear discriminant analysis effect size (LEfSe) method was used to identify differentially abundant taxa between sample groups with a linear discriminant analysis score of 2 as the cutoff. The number of shared and exclusively identified taxa between jhum, jhum fallows and undisturbed groups at the level of the collapsed phylum, family, and genus was determined and visualized.

5.3. Results

5.3.1 Read count and sequence count of illumina sequencing

Illumina paired end sequencing carried out to assess the bacterial community composition in the soils samples gives a Raw read count ranging from 361709 to 654599. Sequence count and feature counts of the samples is given in table1 which summarised the data used for downstream processes such as assessment of bacterial diversity and community composition of the soil samples.

Table 5.1: Raw read statistics (number of read counts, read elgnth GC% sequencing count and feature counts) by preprocessing raw sequencing data from different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD).

Samples	Read count	Read length (bp)	GC%	Sequence count	Feature count
Jh_R1	489893	250	57	489893	91426
Jh_R2	489893		57		
2F_R1	398379		56	398379	74514
2F_R2	398379		56		
5F_R1	361709		57	361709	54883
5F_R2	361709		57		
10F_R1	654599		56	654599	113307
10F_R2	654599		56		
UD_R1	374525		56	374525	65358
UD_R2	374525		56		

5.3.2 Bacterial species diversity

At the kingdom level, representatives of Archaea are found in all samples except 5F. The highest percentage of Archaea was observed in UD (1.98%). Actinobacter are dominant Phylum in JF representing 37.198% of the total bacterial

phyla while Proteobacteria are the dominant class in the others sites accounting for 33.777%, 30.078%, 33.978% and 31.917% of the bacterial population in 2F, 5F, 10F and UD respectively.

The 10F site showed the highest number of unique bacterial species based on the NGS data generated. 730 bacterial species belonging to 541 genera were identified from the UD site. The 2F showed the second highest number of bacteria species with 582 species from 446 different genera. UD and 5F sites the showed the least number of bacterial species at 545 belonging to 406 genera and 408 genera respectively

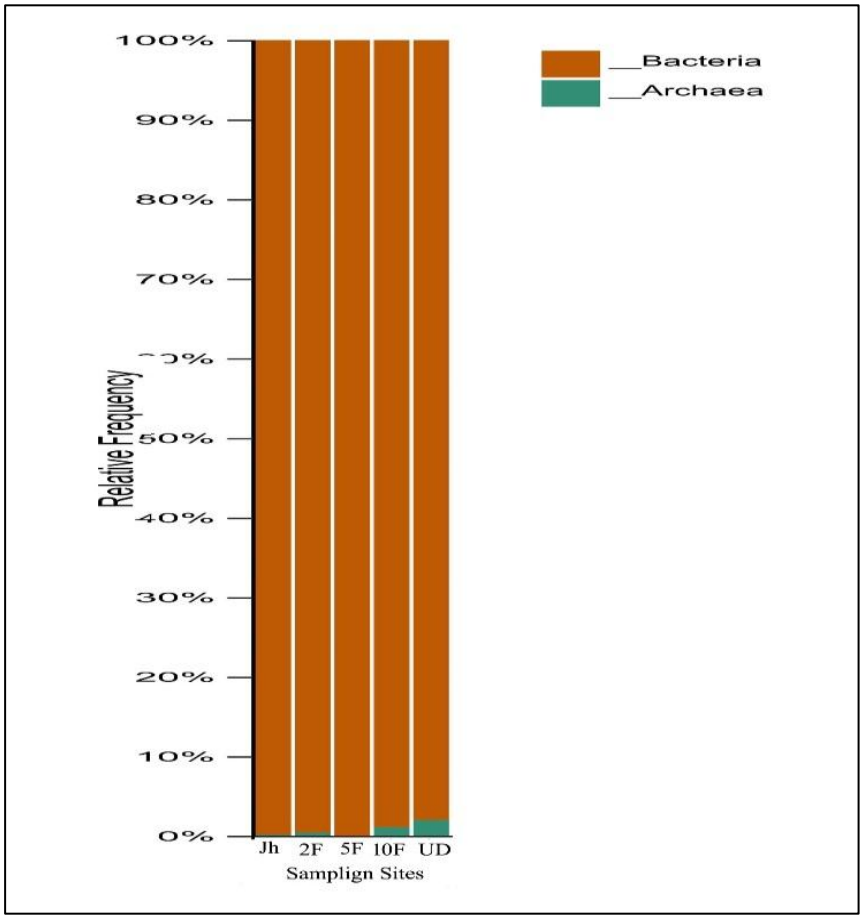

Fig 5.1. Histogram of the soil bacterial community composition at kingdom level among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD).

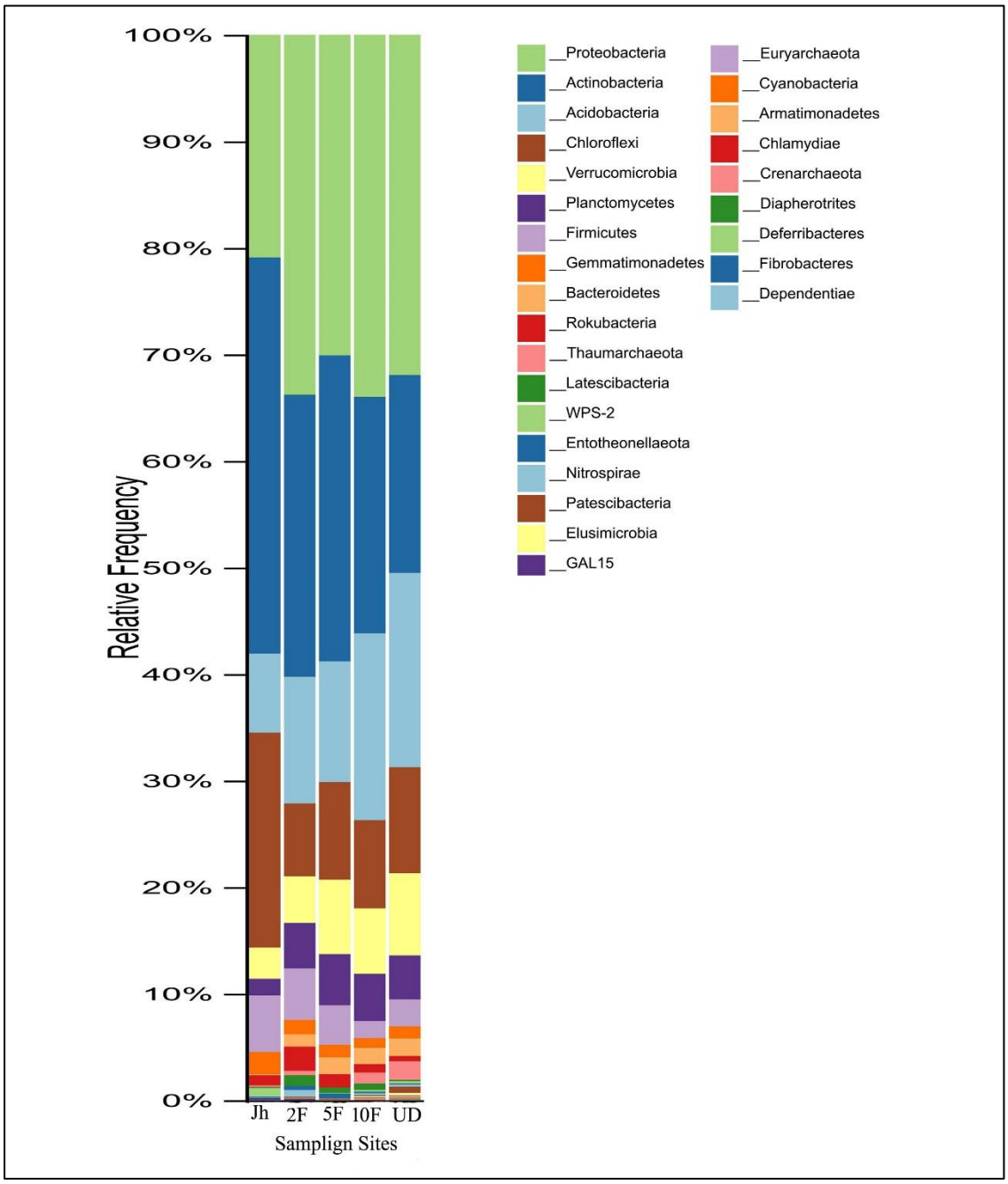

Fig 5.2. Histogram of the soil bacterial community composition at phylum level among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD).

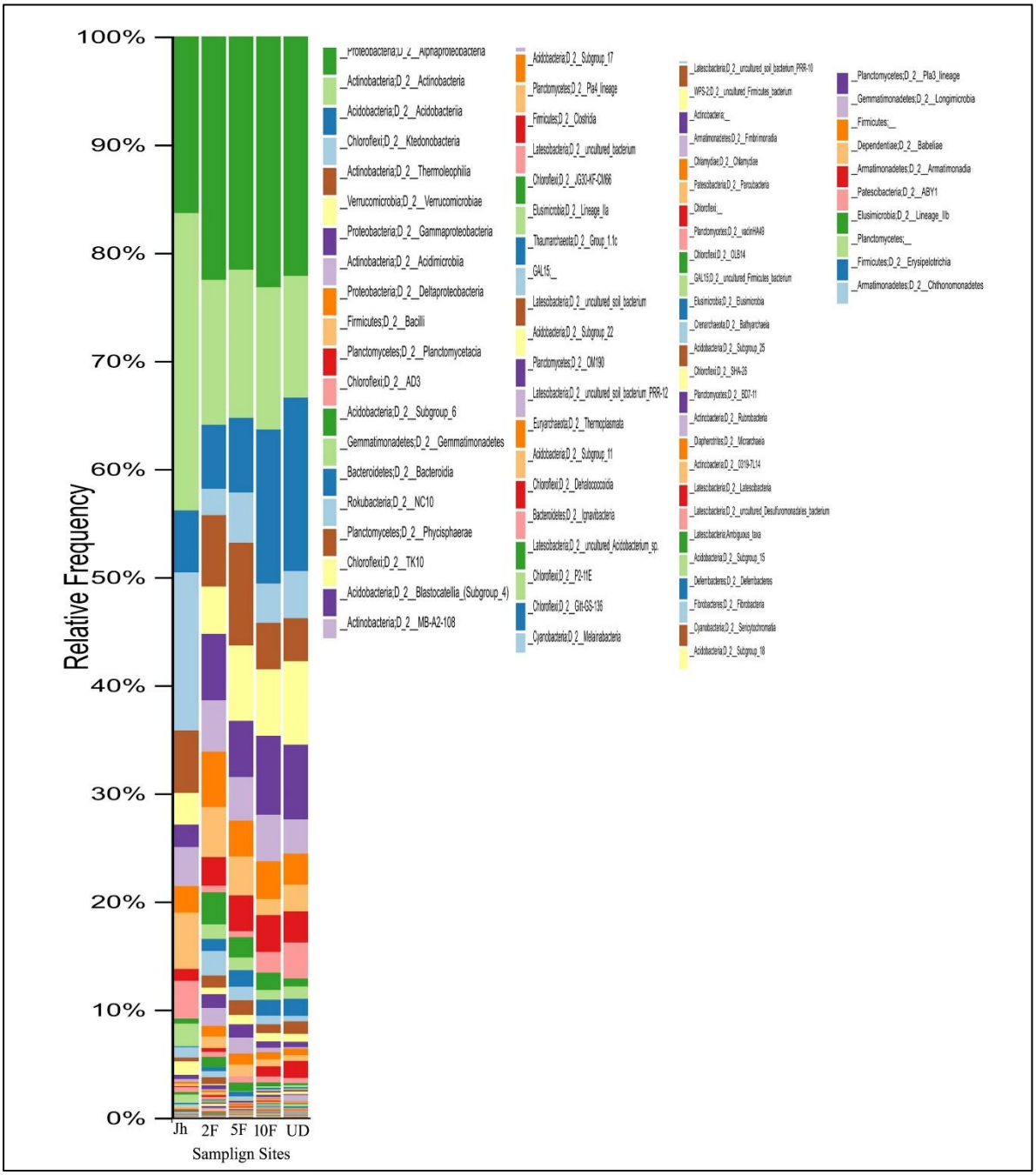

Fig 5.3. Histogram of the soil bacterial community composition at the order level among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD).

Table 5.2. Number of different bacterial community in different study sites (from Class to Genus) based on NGS data analysis among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD).

	Jh	**2F**	**5F**	**10F**	**UD**
Class	53	59	59	72	65
Order	112	169	156	187	152
Family	195	274	252	327	260
Genus	332	446	408	541	406
Species	452	582	545	730	545

5.3.3 Diversity Index

Observed Operational Taxonomic Unit (OTU) was found to be highest in 10F and lowest in Jh sample. Marginal variation was observed between the 2F, 5F and UD samples. The rarefaction curves for OTUs vs sequences in each sample showed that with increase in the sequencing depth the observed OTUs also increase.

Comparison of alpha diversity metrics showed variation in bacterial diversity between the different soil samples. 10F showed the highest Shanon diversity index. (10.169) while lowest was observed in Jh site (9.048). Phylogenetic diversity analysis based on Faith phylogenetic diversity index also follows the same distribution as that of Shanon diversity index.

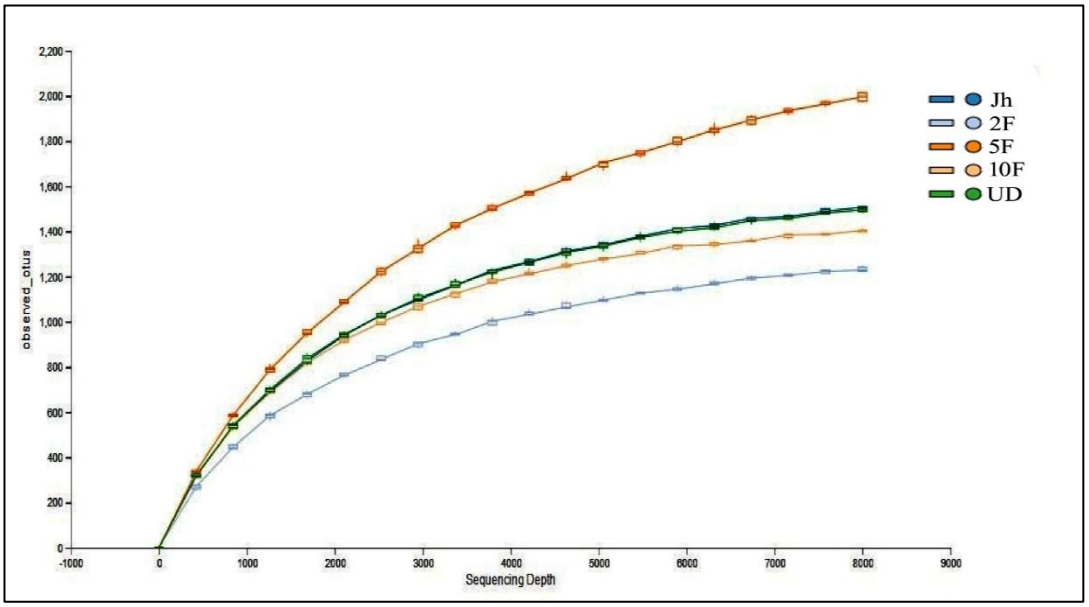

Fig 5.4. Comparison of alpha diversity metrics between jhum and fallow soil microbiome based on Observed OTU among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD).

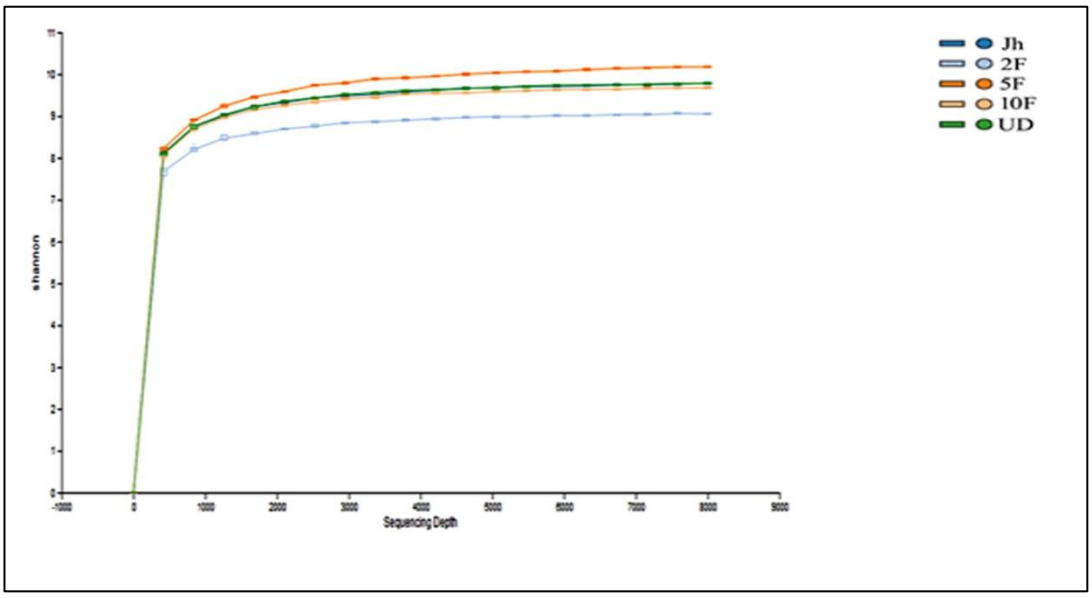

Fig 5.5. Comparison of alpha diversity between jhum and fallow soil microbiome based on Shanon diversity indices among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD).

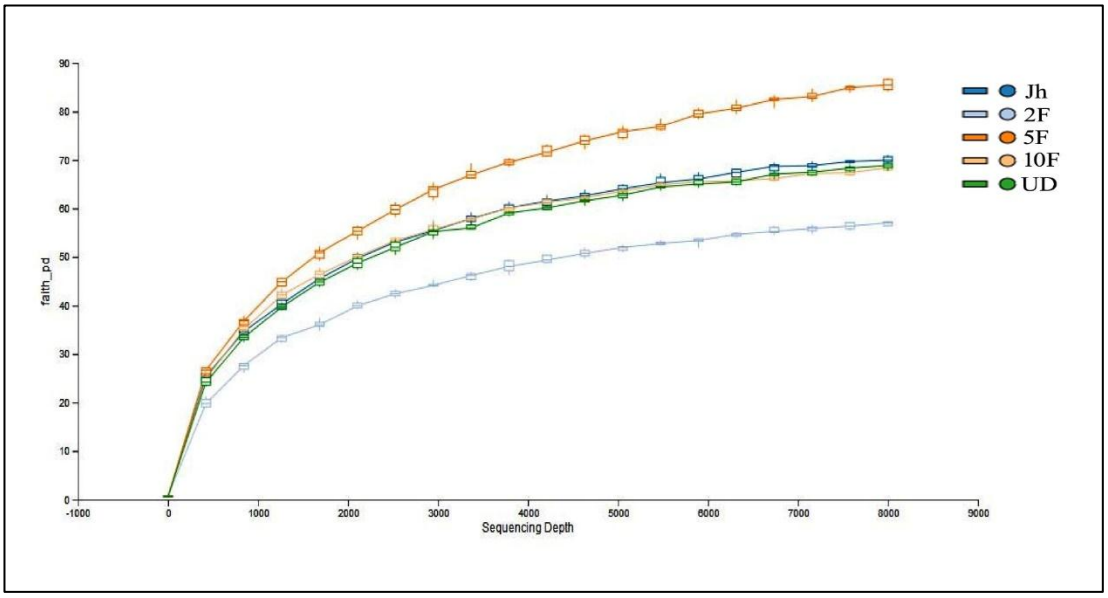

Fig 5.6. Comparison of phylogenetic diversity between jhum and fallow soil microbiome based on Faith's phylogenetic diversity among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (10F), 10 years jhum fallow (5F) and undisturbed forest (UD).

Bacteria beta- diversity between the study sites was analysed using Principal Coordinates Analysis (PCoA). The PCoA plot of Jaccard similarity index showed that there is no significant similarity between the bacterial diversity of all the sites. The PCoA plot of Bray Crutis index also showed dissimilarity in the bacterial diversity of the study sites.

Weighted-UniFrac takes into account the relative abundance of species/taxa shared between samples, whereas unweighted-UniFrac only considers presence/absence of species or taxa. According to the calculated Weighted-UniFrac and unweighted-UniFrac distance generated using PCoA, 2F and 5F showed similarity in terms of abundance was well as diversity of bacterial species while the other sites showed no similarity in terms of abundance or diversity.

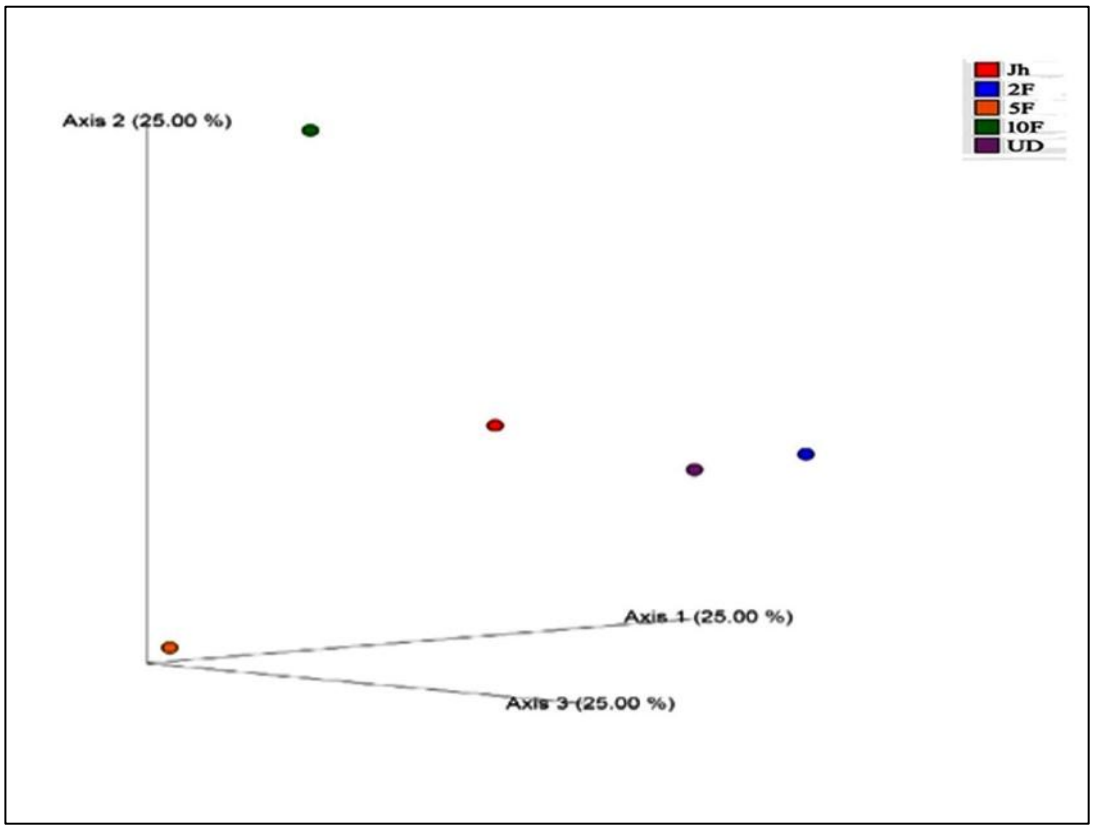

Fig 5.7. PCoA analysis of jhum and undisturbed soil microbiome samples. PCoA plots of Jaccard distance among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD).

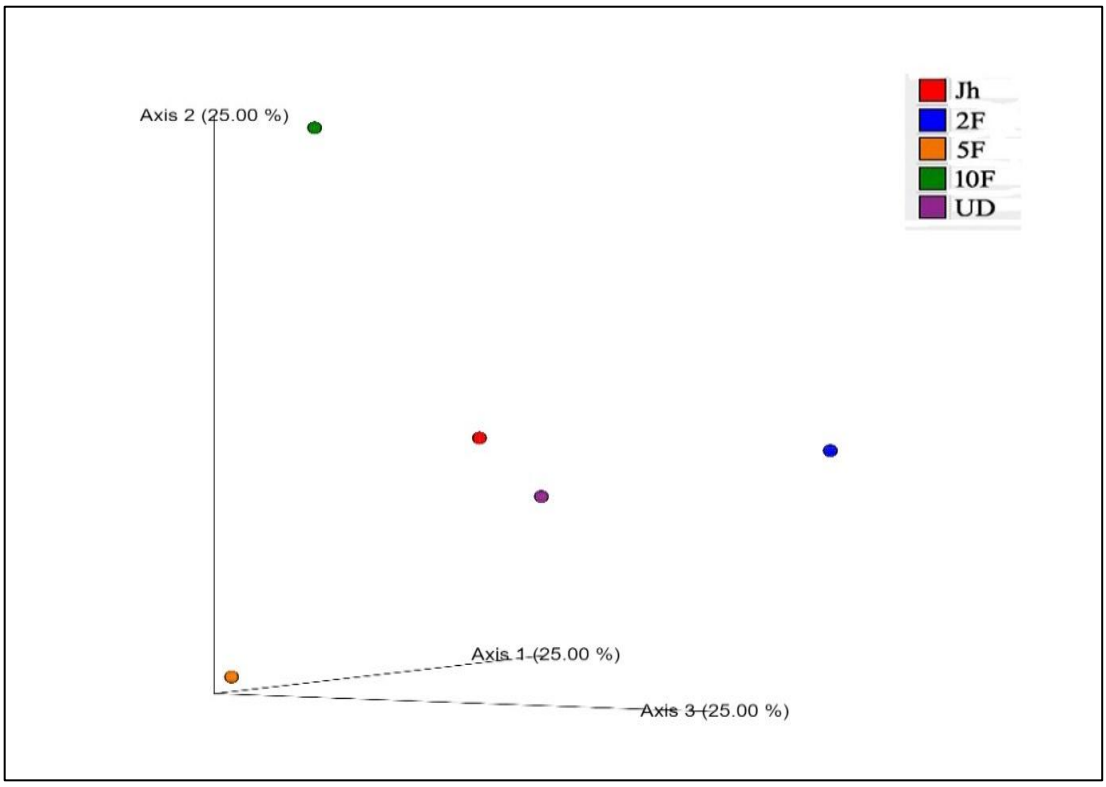

Fig 5.8. PCoA analysis of jhum and undisturbed soil microbiome samples based on Bray- curtiss distance among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD).

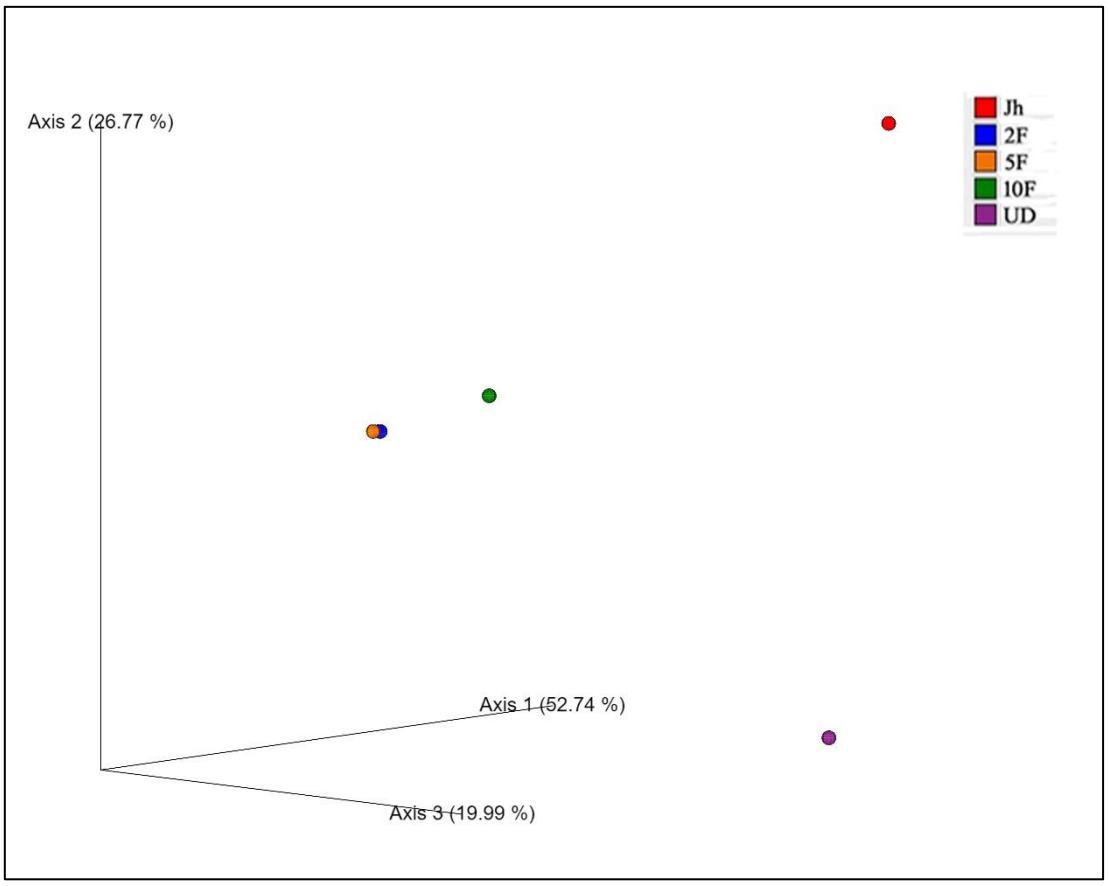

Fig 5.9. PCoA analysis of jhum and undisturbed soil microbiome samples based on weighted_unifrac distance among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD).

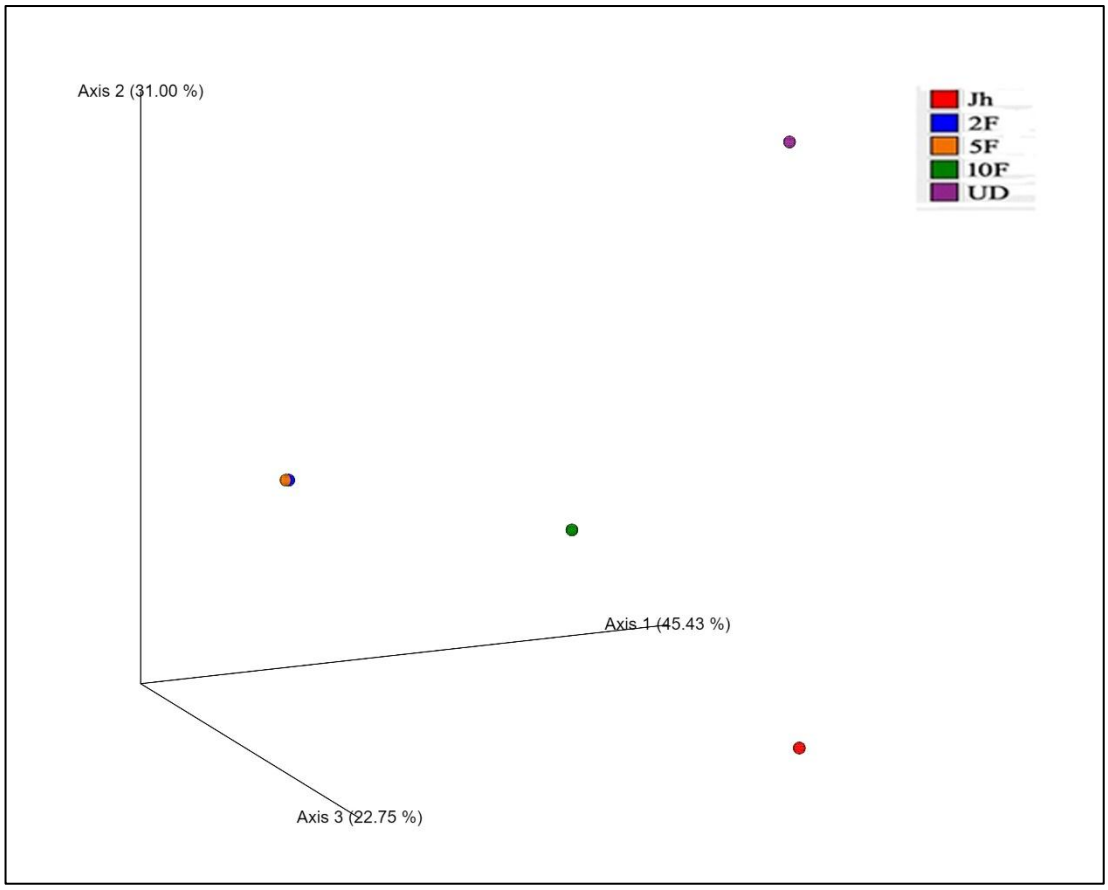

Fig 5.9.1 PCoA analysis of jhum and undisturbed soil microbiome samples based on Unweighted_unifrac_distance among different soil samples such as current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and undisturbed forest (UD).

5.4. Discussion

Studies of microbial diversity are important in order to understand the microbial ecology in soil and other ecosystems) as they play an important role in both natural and agroecosystems (Atlas, 1984; Reid, 1994). An estimate of microbial diversity is a prerequisite for understanding the functional activities of microorganisms in ecosystems (Garland and Mills, 1994; Zak *et.al.*, 1994).

Analysis of metagenomic data revealed a total of 27 bacterial phyla across the different study sites where Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Verrucomicrobia were the dominant phyla. Proteobacteria was found to be the dominant bacteria phylum in all study sites except Jh where Actinobacter was

found to be most dominant, indicating the response of bacterial community to jhum system of cultivation.

Community composition of bacteria within above observed phyla was significantly different for the different samples. In jhum (Jh) sample Actinobacteria was the dominant order contributing 27.497% while Alphaproteobacteria was found to be dominant in the other samples contributing approximately 20% of the observed bacteria community. Rhizobiales, Frankiales, Ktedonobacterales and Solibacterales were found to be the major class of bacteria in the samples. While Rhizobiales were the major class in all fallow sites (2F, 5F and UD) along with UD, Ktedonobacterales were found to be the dominant class in Jh sample.

Microbial community composition is related to soil biological and chemical properties. The physical and chemical parameters of the soil influence the microbial community and its activity. Bacterial species are highly sensitive to the change in the soil physico-chemical properties and Soil bacterial community composition was strongly linked to land use system (Furtak *et al.,* 2019, Hemans *et al.,* 2020). Hence variation in the bacterial community composition was observed with the change in soil physicochemical properties and the length of the fallow period. At the family level Xanthobacteracaea, Acidothermaceae, Ktedonobacteracea, Solibacteraceae and Micromonosporaceae were found to be most dominant in all the samples. However, their level of abundance varied between the different soil samples. Xanthobacteraceae which was the dominant bacterial family in jhum soil showed a gradual increase in its percentage contribution as the length of the fallow increases with marginal decease in the undisturbed (UD) sample. Micromonosporaceae showed a high degree of variation between the Jh sample and samples collected from the other. While it accounts for 11.419% of the total observed bacterial family in Jh sample, its contribution falls to 1.115% in 10F and further to 0.710% in the UD sample, showing that these bacterial families are highly sensitive to the change in the soil physico-chemical properties.

At the genus and species level, many taxonomically unclassified sequences were found to be present which indicates the presence of novel bacterial diversity. Presence of these large numbers of unidentified OTU and variation in the observed

bacterial community suggests that each soil sample has unique bacteria diversity (Kumar *et al*., 2019)

Bacterial alpha-diversity indexes (OTU, Shanon diversity, and Faith phylogenetic diversity) was found to be highest in the 10F site while the lowest alpha diversity indices were observed in 2F site. 10F showed the highest Shanon diversity index hence will comprised of highest bacterial community richness with high evenness while the 2F site having the least Shanon diversity index will show the lowest bacterial community richness. Based on the calculated Shanon diversity index, it was observed that Jh, 5F and UD sites comprised of bacterial community which were more or less similar in terms of richness and evenness.

Phylogenetic diversity based on Faith phylogenetic diversity index revealed that the phylogenetic diversity was highest in 10F while it was lowest in 2F site. Similar to Shanon- diversity index, Jh, 5F and UD showed similar bacterial phylogenetic diversity. As the phylogenetic diversity represents the evolutionary relations between species, higher number of distanced and unique bacterial sepcies was observed in 10F site as compared to the other study sites (Lean and Maclaurin, 2016).

The Beta- diversity of bacterial community based on species abundance and richness was analysed using Jaccard index and Bray- Curtiss index of the different study sites. PCoA of Jaccard and Bray-Curtiss index of similarity showed the bacterial species composition markedly varied across the different study sites. Unifrac beta- diversity calculated using weighted and unweighted unifrac distance showed that the phylogenetic beta- diversity of the different site also varied significantly between the different sites except for 2F and 5F sites which showed an almost similarity in terms of phylogenetic diversity of the bacterial community. The similarity in the bacterial phylogenetic diversity between 2F and 5F may arise due to the similarity of soil physic-chemical properties.

Bacterial diversity was associated with increased environmental heterogeneity. Reflecting the pattern of soil heterogeneity and the difference in soil physico-chemical properties among the study sites, the alpha and beta diversity varied markedly among the different sites (Curb *et al.* 2018). Diversity can vary with a number of factors such as disturbances and stress, in addition to ecological

interactions (predator-prey interactions) (Atlas, 1984). Soil microbial communities remain some of the most difficult communities to characterize, because of their immense phenotypic and genotypic diversity.

Chapter 6

Isolation, Screening and 16SrRNA Gene Profiling of Phosphate Solubilizing Bacteria

6.1. Introduction

The traditional identification of bacteria on the basis of phenotypic characteristics is generally not as accurate as identification based on genotypic methods. Comparison of the bacterial 16SrRNA gene sequence has emerged as a preferred genetic technique for bacterial identification. 16SrRNA gene sequence analysis can better identify poorly described, rarely isolated or phenotypically aberrant strains (Clarridge III, 2004). The 16SrRNA gene sequence is about 1550-bp long and is composed of both variable and conserved regions. The gene is large enough, with sufficient interspecific polymorphisms of 16SrRNA gene, to provide distinguishing and statistically valid measurements. The identification of new bacterial isolates widely relies on the 16SrRNA gene sequence homology analysis by comparison with existing sequences in the reference databases.

The characterization and differentiation of bacteria using 16SrRNA gene sequencing is mostly subjected to comparisons against known sequences availaible in public databases such as Greengenes, SILVA, RDP, GenBank and EZ-biocloud. At present, the sequences of type strains of ~99% of prokaryotic species with validly published names are accessible in these public databases (Chun and Rainey, 2014). The identification of bacterial, reclassification of genera and species, classification of uncultivable bacteria establishment of phylogenetic relationships and the discovery and classification of novel bacterial species have been facilitated by the utilization of 16s rRNA gene profiling (Woo *et al.,* 2008). Because of the experimental simplicity and the availability of public databases of 16S rRNA gene sequences, the use of this gene as a single marker for species circumscription has been well received.

However, being a highly conserved molecule, the 16SrRNA gene does not always provide sufficient resolution at species and strain level (Konstantinidis *et al.,* 2006).

Soil bacteria are essential parts of soil ecosystems and are involved in mineralizing organic matter, biogeochemical cycling of carbon and nitrogen and many other soil processes. PSBs notably *Bacillus, Pseudomonas,* etc. secrete phosphatase enzymes and organic acid which helps in the solubilization of insoluble phosphate compounds thereby playing an important role in P- cycle (Bardgett, *et al.,* 2008, Burton *et al.,* 2010). The analysis of the diversity of PSB using molecular technique such as i16s rRNA gene profiling is important to understand pattern of distribution and the role of these bacteria in the soil ecosystem and can be helpful in the selection of potential PSB strains to be developed into location specific biofertilizers (Cavigelli and Robertson, 2000; Torsvik *et al.,* 2002, Sharma *et al.,* 2013).

6.2. Methodology

6.2.1. Screening of phosphate solubilizing bacteria

Phosphate solubilizing bacteria were screened using plate assay given by Pikovskaya (1948). A serial dilution of 8 was prepare by suspending 1 g of soil sample with 10ml of sterile distilled water and thoroughly mixed for 5 minutes on a rotary vortex. 1 ml of the above solution was again transferred to 9 ml of sterile distilled water to form 10-2 dilution. Similarly, serial dilution was performed till 8 dilutions. 0.2 ml of each dilution was taken using pipettes and spread on Pikovskaya's agar (PVK) medium and inoculated petriplates containing medium were incubated in inverted position in incubator for up to 24 hours at 30° C.

Pure cultures of phosphate solubilizing bacterial was maintained by sub-culturing positive isolates on fresh petriplates containing medium for plate assay. Axenic cultures were isolated by incubating the plates in inverted position in incubator for up to 24 h at 37°C.

6.2.2. Determination of solubilization index (SI)

The phosphate solubilizing potential of the bacterial isolate was determined by calculating their solubilisation index following the method given by Elias *et al.* (2016). Bacterial isolates showing phosphate solubilizing activity were screened for their TCP solubilizing efficacy on PKV plates. Isolates were spot inoculated on the centre of PVK agar plate aseptically. All the plates were incubated at 28°C ± 2°C for 7 days. A clear zone around a growing colony which indicates phosphate solubilization was measured as phosphate solubilization index (SI). SI was calculated as the ratio of the total diameter (colony + halo zone) to the colony diameter

$$Solubilization\ Index\ (SI) = Colony\ diameter + Halo\ zone\ diameter$$

6.2.3. Bacterial Genomic DNA extraction

Extraction of bacterial genomic material was done using the phenol: chloroform extraction method described by Kumar *et al.* (2010) with slight modification. Purified PSB isolates were grown in 10ml of nutrient broth at 3^0C for 24 hours. Bacterial cells were harvested by centrifuging 2 ml of the bacterial culture at 8000 rpm for 5 minute. Pellet was washed with TE buffer pH 8.0 (Tris- EDTA buffer) and centrifuged at 8000 rpm for 3 min. the supernatant was discarded and the cell pellets were lysed using 250 µl TE buffer containing 2 mg/ml lysozyme. The suspension was incubated at 37°C. After 30 mins of incubation, 20 µl proteinase K (20 mg/ml) and 50 µl of 10 % SDS was added and incubated at 37 °C for another 1 hr. 100 µl of 5 M NaCl was added and mixed thoroughly. Pre-warmed 80 µl of cetyl trimethyl ammonium bromide (CTAB, 10 %) was added to the mixture and then incubated at 65°Cfor 10 minutes.

The samples were then allowed to cool down to room temperature and equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) was added and vortexed gently. The mixture was subjected to centrifugation at 12,000 rpm for 10 minutes at 4 °C and the upper aqueous phase was aspirated out. Again, equal volume of chloroform/isoamyl alcohol (24:1) was added to the aqueous phase and mixed

properly. The samples were centrifuged at 12,000 rpm for 10 minutes at 4°C. The clear aqueous phase was collected in a clean 2ml centrifuge tube and precipitated using double volume of chilled ethanol and one tenth volume of 3 M sodium acetate (pH 5.2).

Samples were left overnight at -20°C to allow DNA precipitation. Precipitated DNA pellet was collected by centrifugation at 12,000 rpm for 10 minutes at 4°C. Pellet was washed with 70 % ethanol and air dried followed by suspension in 50 µl of TE buffer (pH 8.0). The quality of the extracted genomic DNA was checked by gel electrophoresis of 2 µl extracted DNA on 0.8% of agrose gel stained with ethidium bromide 0.5 ug/ml.

6.2.4. PCR amplification

Amplification of the extracted bacterial genomic DNA was carried out using the method given by Osbornet *et al.* (2005). The supernatant containing genomic DNA was directly used as template in PCR reaction. PCR amplification of 16SrRNA gene was carried out with specific PCR amplification of 16SrRNA gene using 27f (5'-GAGTTTGATCCTGGCTCAG-3') and 1525r (5'-AGAAAGGAGGTGTACCAGCC-3') primers in 25 µL final volume of reaction mixture containing 2 µl of template DNA (100 ng), 2.5 µL of 10X Buffer, 2.5 mM dNTPs, 2.5 µl of 25 mM of $MgCl_2$, 10 pmol of each primer and 2 U/µl Taq DNA polymerase. The thermocycling conditions consisted of initial denaturation at 95 °C for 5 minutes, followed by 30 cycles of denaturation at 94 °C for 1 minute, annealing at 57 °C for 40 second, extension at 72 °C for 1 minute 30 second and a final extension of 10 minutes at 72 °C. Expected PCR product of around l .5 kb was checked by electrophoresis of 5 µl of PCR product on 1% agarose gel in 1X TBE buffer and stained with ethidium bromide 0.5 µg/ml.

6.2.5. Sequencing and Phylogenetic analysis

The amplified PCR products were out sourced to Scigenom Lab Pvt. Ltd., Cochin, Kerala, India for sequencing. The obtained sequenced strands were then edited using the BioEdit software (Hall, 2013). The nucleotide sequences were

compared with those in the NCBI databases using the Basic Local Alignment Search Tool (BLAST, http:// www.ncbi.nlm.nih.gov/BLAST). The sequences obtained for the bacterial isolates were aligned with each other by using Clustal W multiple alignment programme of Molecular Evolutionary Genetics Analysis (MEGA) 7 software. From the aligned sequences, a phylogenetic tree was constructed. The evolutionary models were selected based on lowest BIC (Bayesian Information Criterion) value using MEGA 7. A neighbour joining phylogenetic tree was constructed based on model Kimura-2 parameter (Saitou and Nei, 1987). The data sets were subjected to 1000 bootstraps replicates.

6.3. Results

6.3.1. Screening and isolation of phosphate solubilizing bacteria

A total of 44 phosphate solubilizing bacteria were screened and isolated from the different study sites based on their ability to solubilize TCP on PVK medium. These isolates were designated as MZUR1, MZUR2, MZUR3, MZUR4, MZUR5, MZUR6, MZUR7, MZUR8, MZUR9, MZUR10, MZUR11, MZUR12, MZUR13, MZUR14, MZUR15, MZUR16, MZUR17, MZUR18, MZUR19, MZUR20, MZUR21, MZUR22, MZUR24, MZUR25, MZUR26, MZUR27, MZUR28, MZUR29, MZUR30, MZUR31, MZUR32, MZUR33, MZUR34, MZUR35, MZUR36, MZUR39, MZUR40, MZUR41, MZUR42, MZUR43 and MZUR44.

The entire isolates exhibit the formation of halo-zone around their colonies and which was used to determined their solubilization index. Highest solubilization index was shown by the MZUR6 (3.45) and lowest was shown by MZUR4 (1.98) while lowest SI was observed for MZUR4 (1.95).

Table 6.1. Solubilization index of potential PSB on PVK agar medium

S.N	Strain	SI
1.	MZUR1	2.22±0.081
2.	MZUR2	2.33±0.22
3.	MZUR3	2.28±0.01
4.	MZUR4	1.98±0.75
5.	MZUR5	2.8±0.59
6.	MZUR6	3.45±0.49
7.	MZUR7	2.03±0.59
8.	MZUR8	2.22±0.06
9.	MZUR9	2.45±0.57
10.	MZUR10	2.28±0.49
11.	MZUR11	2.96±0.08
12.	MZUR12	2.21±0.22
13.	MZUR13	2.7±0.25
14.	MZUR14	2.17±0.75
15.	MZUR15	2.23±0.97
16.	MZUR16	2.67±0.49
17.	MZUR17	3.27±0.04
18.	MZUR18	2.01±0.43
19.	MZUR19	2.83±0.33
20.	MZUR20	2.33±0.63
21.	MZUR21	2.77±0.83
22.	MZUR22	2.02±0.49

S. N	Strain	SI
23.	MZUR24	2.36±0.61
24.	MZUR25	2.24±0.49
25.	MZUR26	2.76±0.08
26.	MZUR27	2.54±0. 49
27.	MZUR28	2.91±049
28.	MZUR29	2.78±0.18
29.	MZUR30	2.62±0.15
30.	MZUR31	2.45±0.08
31.	MZUR32	3.01±0.09
32.	MZUR33	2.89±0.38
33.	MZUR34	2.84±0.08
34.	MZUR35	3.12±0.17
35.	MZUR36	2.22±0.24
36.	MZUR37	2.41±0.49
37.	MZUR38	2.78±0.13
38.	MZUR39	2.35±0.05
39.	MZUR40	2.22±0.21
40.	MZUR41	2.57±0.83
41.	MZUR42	2.66±0.83
42.	MZUR43	2.72±0.22
43.	MZUR44	2.47±0.07
44.	MZUR45	2.81±0.23

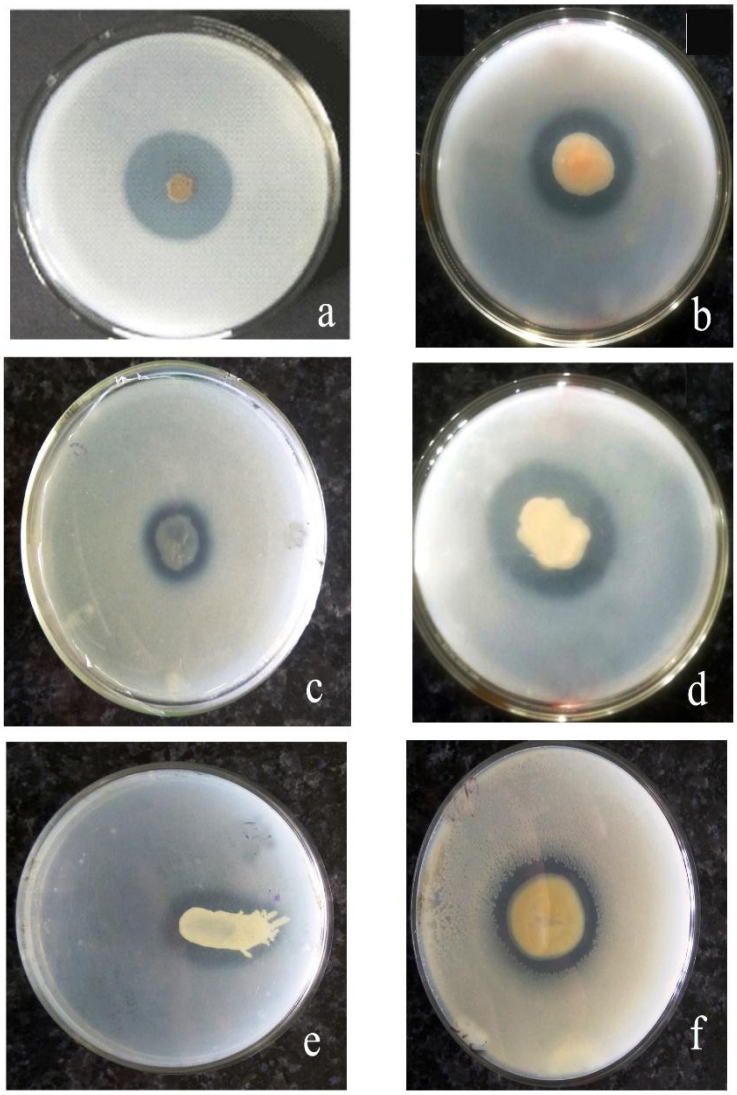

Fig 6.1. Potential PSB isolates on PVK agar medium showing Solubilization zone.

a. MZUR6 (highest SI)
b. MZUR11
c. MZUR32 (lowest SI)
d. MZUR18
e. MZUR35
f. MZUR18

6.3.2. PCR amplification of isolated bacterial genomic DNA

The expected amplicon size of the bacterial genomic 16srRNA PCR product was 1500-bp (1.5 kb). Visualization of the agarose gel after electrophoresis of the PCR product showed a single band against the 1.5 kb position of the DNA ladder, conforming that the genomic DNA of the bacterial isolates were successful amplified in the 16SrRNA region.

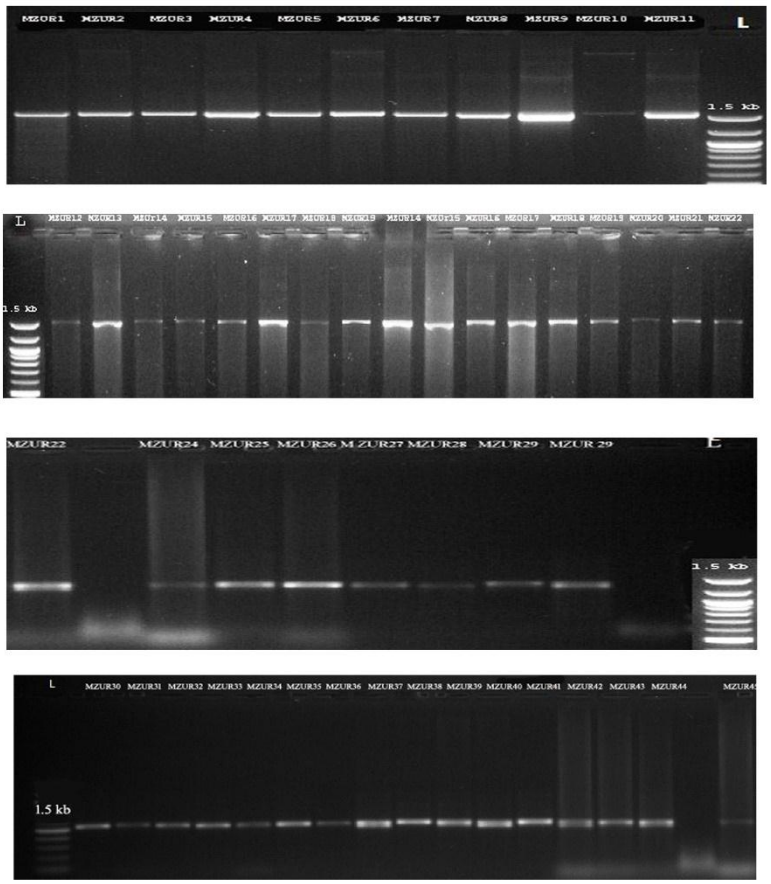

Fig 6.2. Amplification 16SrRNA gene of potential PSB strain. L; low range DNA Ladder (100bp – 1.5 kb)

6.3.3. Identification of bacterial isolates based on sequence homology

The nucleotide sequences obtained were compared with existing 16SrRNA sequences in the using NCBI databank using BLASTn for sequence homology. Based on the sequence similarity the bacteria isolates were grouped into 4 genera viz. *Bacillus*, *Burkholderia*, *Pseuodomonas* and *Alcaligenes*. The nucleotide sequences were further compared with heir corresponding type strains retrieved from EzTaxon-database (http://www.ezbiocloud.net/eztaxon). The sequences were assigned accession numbers after being deposited in NCBI Genbank database.

Table 6.2. List of the potential PSB strains with their strain and similarity isolates name, accession number at NCBI and study site from which they were isolated.

S. N	Strain	Similarity Isolates name	NCBI accession number	Site of isolation
1.	MZUR1	*Bacillus thuringensis*	MT086198	Jh
2.	MZUR2	*Bacillus cereus*	MT086199	Jh
3.	MZUR3	*Bacillus cereus*	MT086200	Jh
4.	MZUR4	*Bacillus cereus*	MT086201	Jh
5.	MZUR5	*Bacillus subtilis*	MT086202	Jh
6.	MZUR6	*Burkholderia glumae*	MT102411	Jh
7.	MZUR7	*Bacillus sp.*	MT102430	Jh
8.	MZUR8	*Bacillus sp.*	MT086203	Jh
9.	MZUR9	*Pseudomonas bifermetnans*	MT086204	Jh
10.	MZUR10	*Bacillus cereus*	MT086205	2F
11.	MZUR11	*Burkholderia glumae*	MT102412	2F
12.	MZUR12	*Alcaliegens faecalis*	MT086206	2F
13.	MZUR13	*Bacillus sp.*	MT086207	2F
14.	MZUR14	*Bacillus sp.*	MT086208	2F
15.	MZUR15	*Bacillus cereus*	MT086209	2F
16.	MZUR16	*Bacillus paramycoides*	MT086210	2F

S. N	Strain	Similarity Isolates name	NCBI accession number	Site of isolation
17.	MZUR17	*Burkholderia vietnameses*	MT086211	2F
18.	MZUR18	*Burkholderia gladioli*	MT096514	2F
19.	MZUR19	*Pseudomonas putida*	MT102121	2F
20.	MZUR20	*Pseudomonas sp.*	MT102439	5F
21.	MZUR21	*Bacillus thuringiensis*	MT102133	5F
22.	MZUR22	*Bacillus cereus*	MT102134	5F
23.	MZUR24	*Bacillus nitratireducens*	MT102135	5F
24.	MZUR25	*Bacillus sp.*	MT102136	5F
25.	MZUR26	*Pseudomonas sp*	MT102440	5F
26.	MZUR27	*Bacillus parmycoides*	MT102137	10F
27.	MZUR28	*Alcaligenes faecalis*	MT102138	10F
28.	MZUR29	*Burkholderia gladioli*	MT102441	10F
29.	MZUR30	*Bacillus paramycoides*	MT087561	10F
30.	MZUR31	*Bacillus cereus*	MT087562	10F
31.	MZUR32	*Pseudomonas sp.*	MT102122	10F
32.	MZUR33	*Burkholderiasp.*	MT102123	10F
33.	MZUR34	*Alcaligenes sp.*	MT087563	10F
34.	MZUR35	*Burkholderia cepacia*	MT102124	10F
35.	MZUR36	*Bacillus cereus*	MT087564	10F
36.	MZUR37	*Burkholderia gladioli*	MT102125	UD
37.	MZUR38	*Pseudomonas oryzihabitans*	MT102126	UD
38.	MZUR39	*Bacillus cereus*	MT102442	UD
39.	MZUR40	*Bacillus cereus*	MT102443	UD
40.	MZUR41	*Alcaligenes sp.*	MT102433	UD
41.	MZUR42	*Alcaligenes sp.*	MT102432	UD
42.	MZUR43	*Alcaligenes sp.*	MT102431	UD
43.	MZUR44	*Bacillus cereus*	MT087567	UD
44.	MZUR45	*Burkholderia glumae*	MT102413	UD

6.3.4. Phylogenetic analysis

The evolutionary history was inferred by using Neighbour joining method based on Kimura 2- parameter model (K2+G) (Saitou and Nei, 1987).The estimated transition/transversion bias (R) ratio is 1.15. The model was selected based on the lowest Bayesian information criterion (8324.105) value. The phylogenetic tree was divided two different clades (Clades I and Clades II). In Clade I, gram positive Bacillus was grouped together with type strains from EzTaxon databases with bootstrap value of 100%. In Clade II, the gram negative bacteria are clustered under two sub-clades, *in* Clade IIA *Pseudomonas* was clustered separately while *Alcaligenes* and *Burkholderia* were clustered together under one sub-clade (Clade IIB) with bootstrap value of 100%.

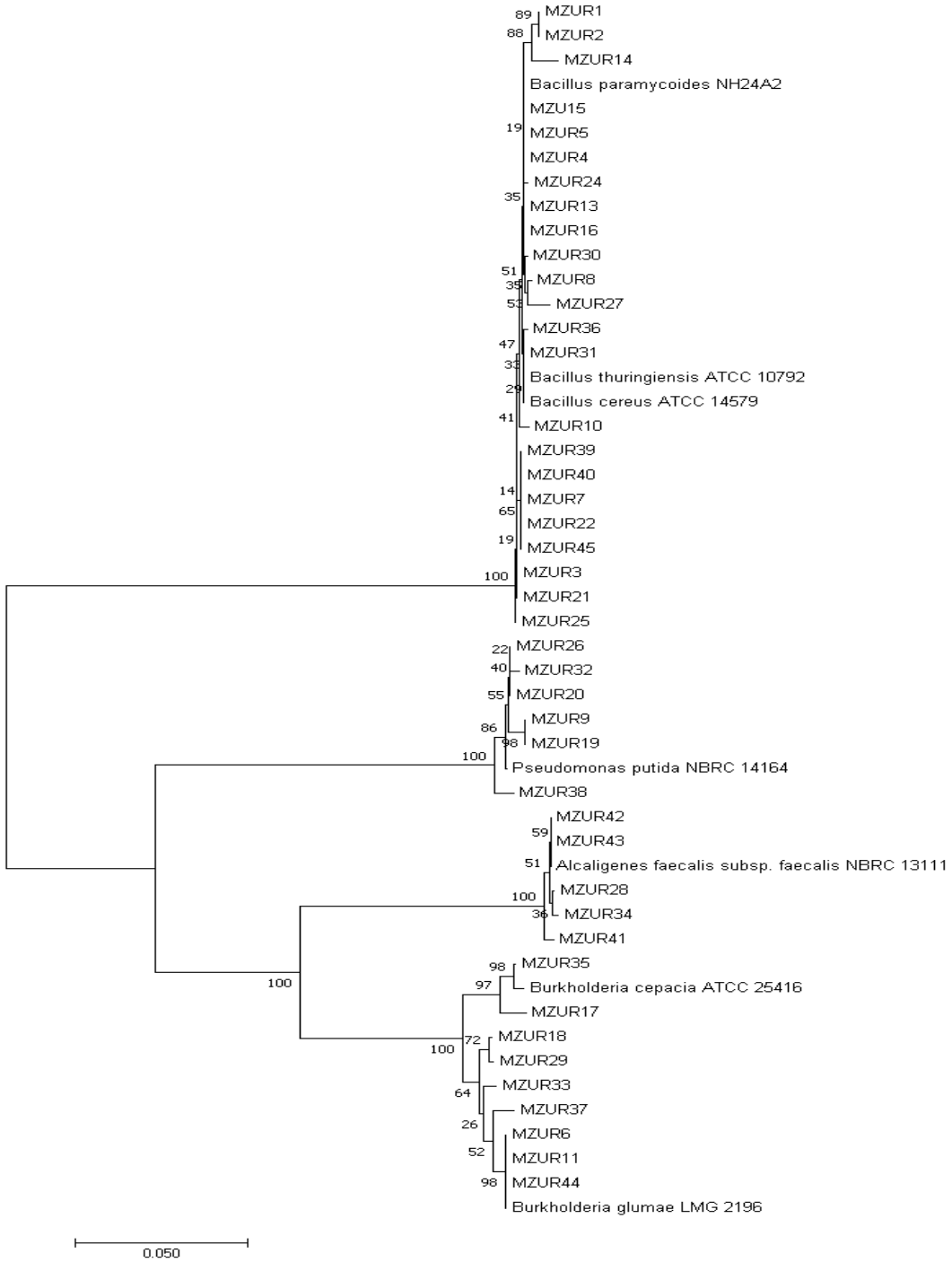

Fig 6.2. Constructed Phylogenetic tree based on16SrRNA sequence using Kimura-2 model with neighbour-joining model under 1000 bootstrap replicates.

6.4. Discussion

Phosphorus (P) is the second limiting nutrient required for plant growth and development involved in important metabolic pathways like nutrient uptake, biological oxidation and energy metabolism (Nesme *et al.,* 2018). The total P in soil accounts roughly for 0.04–0.1% (w/w), only a very tiny proportion of P (soluble $H_2PO_4^-$ or HPO_4^{2-}) can directly be assimilated by plants (Chen *et al.,* 2008). The large portion of P in soils exists in inorganic insoluble form [e.g., $Ca_3 (PO_4)_2$] and organic insoluble/soluble form (e.g. phytate and nucleic acid) (Liu *et al.,* 2015; Neal *et al.,* 2017).

Phosphate solubilizing bacteria (PSBs) convert unavailable P (both P_i and P_o) into available P to satisfy the requirements of plants through dissolution and absorption. P solubilizing activities were detected by the formation of clear halo (a sign of solubilization) around their colonies when grown in PVK agar medium (Park *et al.,* 2010). All 44 isolates showed formation of halo zone around their colonies when grown in PVK agar medium which showed their P solubilization actvitiy. Highest solubilization index was shown by *Burkholderia glumae* MZUR6 (3.45±0.49) followed by *Burkholderia viaetnamenses* MZUR17 (3.27), *Burkholderia cepacia* MZUR35 (3.12) and *Pseudomonas sp.* MZUR32 (3.01). High SI of *Burkholderia* and *Pseudomonas* were also observed by Tripti *et al.* (2012) and Walpola and Yoon (2013). The ability to form solubilization zone depends on the production of organic acids and phosphatase enzyme which indicates that the isolates are all potential P solubilizers (Sharma *et al.,* 2013; Park *et al.,* 2010).

From this study, we found that 7 out of 9 PSB isolated from the Jh soil belongs to *Bacillus* genus, the others being *Pseudomonas* and *Burkholderia*. From the 2F site 10 PSBs were isolated, where 5 of the isolates belong to *Bacillus*, 3 isolates belong to *Burkholderia* and 1 each isolate belongs to *Pseudomonas* and *Alcaligenes*. Least number of PSB was isolated from the 5F site which was made up of 3 *Bacillus* and 3 *Pseudomonas*. Most of the PSB isolated from 10F site were found to be gram negative, with 3 isolates belonging to *Burkholderia*, 2 isolates belonging to *Alcaligenes* and 1 isolate belonging to *Pseudomonas*. 3 isolates from

10F were gram positive bacteria belonging to *Bacillus*. The PSB isolated from UD site showed a consortium of bacteria composed of 3 *Alcaligenes*, 2 *Burkholderia*, 1 *Pseudomonas* and 3gram-positive *Bacillus*. *Bacillus* accounts for 52.23% of the total isolates while *Burkholderia, Pseduodmonas* and *Alclaligenes* accounts for 22.73%, 12.66% and 11.36% respectively.

The diversity and richness of established PSB varies from soil to soil depending upon the physicochemical properties. P solubilizing ability of PSB also varies from organism to organism and even in different strains of the same species (Kumar, 2016). Although the overall taxonomic composition of the P-solubilizing community is affected by soil type, a core community of PSB (taxa present in all samples) was identified. Numerically, these are dominated by *Bacillus* and *Burkholderia.*

Soil nutrient availability, plant characteristics, soil properties and the interaction of roots with microorganisms governs the diversity and distribution of PSB. Soil rich in available nutrients such as 10F and UD site showed high number of PSB. The large amount of available nutrients supports high bacterial counts which are highly correlated to total number of PSB. This is because, the quantity of organic matter was high, so number of macro- and micro faunal population exists to accomplish their decomposition (Reza *et al.,* 2014). In rejuvenating sites such as 2F, the growth of new vegetation was much more dependent on efficient and positive root-microbe interactions. Occurrence of high PSB indicated that under stressed situation the root system of plant supports the proliferation of beneficial bacteria for their growth (Mander *et al.*, 2012). The transition from shrub vegetation to small trees in 5F site along with poor nutrient available maybe accountable for the low number of PSB isolated from the site (Deka *et al,* 2013). In Jh site, burning process plays a critical role in determining the composition of total bacterial community and PSB (Miah *et al.* 2014). The high temperature and addition of large number led to the homogenous distribution of PSB which is mainly dominated by *Bacillus*

A phylogenetic tree is an estimate of the relationships among taxa (or sequences) and their hypothetical common ancestors (Nei and Kumar

2000; Felsenstein, 2004; Hall, 2011). Kumar *et al*. (2014) stated that phylogenetic tree of bacteria must be divided into two clades, i.e., gram positive clade and gram negative clade. Accordingly, the phylogenetic tree constructed based on neighbour joining method using Kimura-2 model clustered the bacterial isolates into two major clades. Clade I is constituted of the gram positive *Baccili* while Clade II consist of *Pseudomonas, Burkholderia* and *Alcaligenes* which were gram negative. The constructed phylogenetic tree showed that all the isolates were clustered together with their corresponding reference strains viz. *Burkholderia glumae* LMG 2196 *Burkholderia cepacia* ATCC 25416, *Bacillus paramycoides* NH 24A2, *Pseudomonas putida* NBRC 14164, *Bacillus cereus* ATCC 14579, *Bacillus thuringiensis* ATCC 10792 and *Alcaligenes faecalis* sub sp. *Faecalis* NBRC 13111 obtained from EzBioCloud.net.

One noticeable pattern observed from this study is that with the increase in the length of the fallow period, the number of gram negative PSB isolated also increases. Also the diversity of the PSB isolates also showed a positive relation with the length of the fallow period. This is evident from the observation made that the Jh site is mainly dominated by *Bacillus* while the diversity of PSB isolates from 10F and UD site were more even. Also, the genus *Alcaligenes* were found to be more regularly distributed in older fallows soil.

Chapter 7

Biochemical Characterisation of Phosphate Solubilizing Bacterial Isolate

7.1. Introduction

The identification and characterisation of bacteria can be carried out using both the conventional and molecular methods. The convention methods often called the classical methods of bacteria identification and classification relies on the observation of bacterial samples under specific conditions. This conventional method includes both morphological and biochemical characterisation and identification of bacteria. Classical identification of individual bacterial species in environmental samples typically involves isolation, laboratory culture and then taxonomic characterization. The classification of bacteria into families, genera and species is based on a wide range of phenotypic characteristics (Holt *et al.,* 1994). These include culture conditions, colony morphology, biochemical characteristics and detailed morphology. Although many high-tech methods have been developed for the identification of bacteria such as polymerase chain reaction (PCR), biochemical tests are still widely applied as they are inexpensive, reliable, and easily applied in all laboratories.

Biochemical tests are used for the identification of bacterial species based on the differences in the biochemical activities of different bacteria. All bacteria have unique biochemical characteristics which are governed by their cellular metabolism which is responsible for the secretion of intra and extra cellular enzymes. Intracellular enzymes include carbohydrate fermentation, nitrate reduction, catalase reactions, urease test, oxidase test, litmus milk reaction, H_2S production and IMViC test (Cappucino and Sherman, 2014). Extracellular enzymes include starch hydrolysis, lipid hydrolysis, casein hydrolysis and gelatine hydrolysis. As a result of

activities of these enzymes, metabolic products are formed and excreted by the cell into the environment. Assay of these end products can be used for the detection and classification of bacteria.

Bacteria that colonize the rhizosphere and plant roots, and enhance plant growth by any mechanism are referred to as PGPR. PGPR can exhibit a variety of characteristics responsible for influencing plant growth. Plant growth-promoting rhizobacteria (PGPR) have ability to colonize plant roots and increase plant growth by wide range of mechanisms. Solubilization of phosphate and production of IAA are the most common mechanisms of plant growth promoting rhizobacteria to promote productivity and growth in plants (Datta *et al*., 2011).

PSB are considered to be PGPR as they as they play a major role in the phosphorous cycle. They mediate the mineralization of organic phosphorous in soil which is necessary for maintaining the level of soil available phosphorous (Greaves and Webley, 1965; Tarafdar and Junk, 1987; Garcia *et al*., 1992; Gupta *et al*., 2015). The production of enzyme phosphatase and organic acids by the bacteria is vital for the mineralization and solubilization of phosphate in soil. The enzyme phosphatase is the most commonly secreted enzymes that hydrolyze phosphoric acid into P ion and a free OH group molecule; hence a special attention is given to its study.

Bacteria synthesize auxins in order to perturb host physiological processes for their own benefit (Shih-Yung, 2010). Indole acetic acid (IAA) is one of the most physiologically active auxins. IAA is a common product of L- tryptophan metabolism produced by several microorganisms including Plant Growth-Promoting Rhizobacteria (PGPR) (Lynch, 1985). Important plant growth stimulating hormone is an Indole acetic acid, which increases root hair density, enhanced seed germination and improvement in the growth of the rice plant (Vessey, 2003; Ashrafuzzaman *et al.,* 2009)

7.2. Methodology

Biochemical characterizations of PSB isolates was performed by using the methods given by Bergey's Manual of Determinative Bacteriology (1995). All tests

were carried out in triplicates and a controlled blank (without bacteria) was carried out alongside for all tests performed.

7.2.1. Gram staining

Thin smear of bacterial culture was made on clean glass slide, air dried and heat fixed. The smear was covered with crystal violet for 30 seconds and the slide was washed with distilled water. The smear was covered with Grams iodine solution for 60 seconds and the slide was washed with 95% ethyl alcohol and then distilled water. Again the smear was covered with safranin for 30 seconds and washed with distilled water and blot dried and observed under microscope. Bacterial smears retaining the crystal violet colour were scored as gram positive while those smears retaining the red/pink safranin were scored as gram negative.

7.2.2. Carbohydrate fermentation

For carbohydrate fermentation test, Phenol red broth containing different carbohydrate *viz.* sucrose or glucose was used as a fermentation media. After autoclaving at 121^{o}C for 15 minutes, the media was cooled down to room temperature and inoculated with the test bacterial strains. The inoculated media were then incubated at 35^{o}C for 24 hours. Fermentation of sucrose or glucose was detected by the change in colour of media to yellow from pink.

7.2.3. Nitrate reduction

Nutrient broth supplemented with 0.1% potassium nitrate (KNO_3) was inoculated with the bacterial strains and incubated at 35°C for 48 hours. After incubation a few drops of Sulfanilic acid (Nitrate reagent A) and reagent B (alpha-naphthylamine) was added to the culture. The development of red colour was observed. If no change of coloration occurs, a pinch of zinc powder was added to the cultures. The cultures which showed red coloration were scored as having nitrate reducing potential while those with no colour change are scored as negative nitrate reducing bacteria.

7.2.4. Casein hydrolysis

Bacteria to be tested for casein hydrolysis were aseptically streaked inoculated in Skim milk agar medium. After incubated at 35°C for 24 hours, the development of clear halo zone around the bacterial colony was observed in culture having proteolysis activity.

7.2.5. Catalase test

To test the catalase production, 24 hours old bacterial cultures were placed on a clean, sterile glass slides and a few drops of 3% H_2O_2 was added to it. The rapid development of bubbles indicates the production of catalase enzymes.

7.2.6. Gelatin hydrolysis

Tubes containing sterile nutrient gelatin medium were stabbed inoculated with the bacterial isolates and incubated at 35°C. After every 24 hours for 14 days, the tubes were taken out and put in 4°C for 15 minutes to check for gelatin liquefaction. The tubes were tilted to observe if gelatin has been hydrolysed. Total or partial liquefaction of the medium recorded as positive gelatin hydrolysis.

7.2.7. Starch hydrolysis

Starch hydrolysis of bacteria was tested by making a single streak inoculation of the bacteria to be tested was made into the centre of a starch agar plates. After incubation at 35°C for 48 hours, the plates were flooded with Iodine solution for 30 seconds and the excess iodine solution was removed. Positive starch hydrolysis was indicated by the presence of a clear zone around the bacterial colony.

7.3. Plant growth promoting activities

7.3.1. Acid phosphatase production

Bacterial acid phosphatase activity was measured using Tabatabai and Bremner (1969). PSB were inoculated in NBRIP medium and incubated at 30°C for 24 hrs. To 3 ml of the culture, 1 ml of acetate buffer (pH 5.6) and 1ml 0.115 M p-nitrophenyl phosphate were added. After incubation the mixture at 37^0C for 1 hr, 20 ml 0.5 N NaOH was added to stopped the reaction. The mixture was then transferred to a 50 ml volumetric flask and the volume was made to with distilled water. The absorbance was then read in a spectrophotometer at 410 nm.

7.3.2. IAA production

IAA production was determined following the method described by Gutierrez *et al.* (2009). 100 ml liquid nutrient medium supplemented with 1 ml of 0.2 % L-tryptophan were inoculated with the bacterial strains and then incubated for 72 hrs with continuous shaking at 30°C. After 72 hours of incubation, the cultures were centrifuged for 10 minutes at 12,000 rpm. 1 ml of the clear supernatant was then taken in a clean test tube and mixed with 4 ml of freshly prepared Salkowski's reagent. The mixture was incubated in the dark at 37°C for 30 minutes. Development of pink colour indicates the IAA production and optical density was measured at 530 nm.

7.4. Results

7.4.1. Biochemical test

52 % of the PSB isolates were found to be gram positive which were hypothetically identified as *Bacillus.* The remaining 48% was contributed by gram negative bacteria hypothetically belonging to *Burkholderia, Alcaligenes* and *Pseudomonas.*

Out of the 44 isolates, 93% of the isolates showed catalase enzymes production. 57% of the isolates were capable of performing starch hydrolysis, gelatin hydrolysis test was positive for 80% of the isolates, while 60% of the isolate were capable of casein hydrolysis. 95% of the isolates were capable of fermenting glucose and sucrose while 2% of the isolates do not perform either scudoes or glucose fermentation. 97% of the isolates were found to be capable of reducing nitrate to nitrite.

Table 7.1. Qualitative biochemical screening of isolated potential phosphate solubilizing bacteria.

S. N	Strain	Gram stain	Catalase test	Starch hydrolysis	Gelatin hydrolysis	Casein hydrolysis	Sucrose	Glucose	Nitrate reduction
1	MZUR1	+	+	+	-	+	+	+	+
2	MZUR2	+	+	+	+	+	+	+	+
3	MZUR3	+	+	+	+	-	+	+	+
4	MZUR4	+	+	+	+	+	+	+	+
5	MZUR5	+	+	+	+	+	+	+	+
6	MZUR6	-	+	-	-	-	+	+	+
7	MZUR7	+	+	+	+	+	+	+	+
8	MZUR8	+	+	+	+	+	+	+	+
9	MZUR9	-	+	-	+	+	+	+	+
10	MZUR10	+	+	+	+	+	+	+	+
11	MZUR11	-	+	-	+	+	+	+	+
12	MZUR12	-	+	-	+	+	+	+	-
13	MZUR13	+	+	+	-	+	+	+	+
14	MZUR14	+	+	+	-	+	+	+	+
15	MZUR15	+	+	+	-	+	+	+	+
16	MZUR16	+	+	+	-	-	+	+	+
17	MZUR17	-	+	-	+	-	+	+	+
18	MZUR18	-	+	+	+	+	+	+	+
19	MZUR19	-	+	-	+	-	+	+	+
20	MZUR20	-	+	-	-	+	+	+	+
21	MZUR21	+	+	+	+	+	+	+	+
22	MZUR22	+	+	+	+	-	-	+	+
23	MZUR24	+	+	+	+	+	+	+	+

S. N	Strain	Gram stain	Catalase test	Starch hydrolysis	Gelatin hydrolysis	Casein hydrolysis	Sucrose	Glucose	Nitrate reduction
24	MZUR25	+	+	+	+	+	+	+	+
25	MZUR26	-	-	-	-	+	+	+	+
26	MZUR27	+	+	+	+	-	+	+	+
27	MZUR28	-	+	-	+	+	+	+	+
28	MZUR29	-	+	-	+	+	+	+	+
29	MZUR30	+	+	+	+	-	-	+	+
30	MZUR31	+	+	+	+	+	+	+	+
31	MZUR32	-	+	-	+	+	+	+	+
32	MZUR33	-	+	-	+	-	+	+	+
33	MZUR34	-	+	-	+	-	+	+	+
34	MZUR35	-	-	-	-	+	+	+	-
35	MZUR36	+	+	+	+	-	+	+	+
36	MZUR37	-	+	-	+	-	+	+	+
37	MZUR38	-	+	-	+	+	+	+	+
38	MZUR39	+	+	+	+	+	+	+	+
39	MZUR40	+	+	+	+	+	+	+	+
40	MZUR41	-	+	-	+	+	+	+	+
41	MZUR42	-	+	-	+	-	+	+	+
42	MZUR43	-	-	-	+	+	+	+	+
44	MZUR44	-	+	+	+	-	+	+	+
45	MZUR45	+	+	+	+	+	+	+	+

7.4.2. Acid phosphatase and IAA production.

All bacterial isolates showed phosphatase production after 24 hours of culturing on NBRIB medium. Quantitatively isolated bacteria showed phosphate solubilization range between 0.271 to 37.604 µg/ml. MZUR22 showed highest P-solubilization values, whereas MZUR35 the minimum.

All 44 isolates could produce IAA in the presence of externally provided L-tryptophan in the broth medium. The highest IAA production was shown by MZUR37 and least IAA was shown by MZUR35.

Table 7.2. Quantification of acid phosphatase activity and IAA production of isolated PSB

S. N	Strain	Apase (p- NPP $\mu g^{-1} hr\ ml^{-1} hr^{-1}$)	IAA (IAA $\mu g\ ml^{-1}$)
1.	**MZUR1**	5.641±0.231	18.402±2.261
2.	**MZUR2**	5.604±0.37	18.291±1.002
3.	**MZUR3**	6.456±0.161	16.146±0.029
4.	**MZUR4**	9.197±0.898	15.946±0.128
5.	**MZUR5**	6.011±0.74	16.091±0.263
6.	**MZUR6**	7.271±0.643	15.946±0.649
7.	**MZUR7**	10.9±0.740	15.602±0.192
8.	**MZUR8**	5.715±0.206	15.446±0.785
9.	**MZUR9**	34.419±0.723	13.935±0.203
10	**MZUR10**	10.53±0.641	12.613±0517
11	**MZUR11**	6.382±0.225	12.024±3.334
12	**MZUR12**	13.382±0.37	11.724±0.414
13	**MZUR13**	32.123±1.397	11.524±0.222
14	**MZUR14**	11.234±0.89	11.091±0.495
15	**MZUR15**	22.234±3.121	10.957±1.113
16	**MZUR16**	5.53±0.64	11.035±0.353
17	**MZUR17**	12.493±0.185	10.679±0.029
18	**MZUR18**	6.197±0.557	9.968±0.656
19	**MZUR19**	21.715±0.481	8.046±0.656
20	**MZUR20**	21.641±0.78	8.113±0.495
21	**MZUR21**	7.049±0.98	7.879±0.124
22	**MZUR22**	37.604±0.64	6.302±0.426
23	**MZUR24**	8.011±0.76	6.291±0.422
24	**MZUR25**	10.021±0.64	4.264±0.45
25	**MZUR26**	9.382±0.234	4.613±0.235
26	**MZUR27**	6.086±0.64	4.124±0.319
27	**MZUR28**	16.197±0.643	4.079±0.095
28	**MZUR29**	6.493±0.334	4.068±0.084
29	**MZUR30**	5.382±0.303	4.046±0.078
30	**MZUR31**	12.123±0.234	2.946±1.473
31	**MZUR32**	0.937±0.243	1.757±0.228

S. N	Strain	Apase (p- NPP µg^{-1}hr ml^{-1}hr^{-1})	IAA (IAA µg ml^{-1})
32	**MZUR33**	9.641±0.525	1.624±0.206
33	**MZUR34**	3.567±0.225	0.879±0.472
34	**MZUR35**	0.271±0.316	0.502±0.135
35	**MZUR36**	7.9±0.392	7.787±0.135
36	**MZUR37**	9.419±0.64	23.957±0.135
37	**MZUR38**	5.641±0.28	21.207±0.135
38	**MZUR39**	8.234±0.196	19.392±0.145
39	**MZUR40**	7.974±0.357	16.737±0.135
40	**MZUR41**	20.826±0.98	19.866±0.165
41	**MZUR42**	8.271±098	18.091±0.164
42	**MZUR43**	6.493±0.365	16.861±0.135
43	**MZUR44**	2.493±0.376	20.645±0.35
44	**MZUR45**	1.764±0.224	1.275±0.786

7.5. Discussion

The ability of bacteria to form organic compounds by metabolizing certain carbohydrates and related compounds is a widely used method for the identification of microorganisms. Carbohydrate fermentation is the process microorganisms use to produce energy. In this study all bacterial isolates except MZUR22 and MZUR30 were capable of fermenting both sucrose and glucose and hence can utilise both carbohydrates as source of energy.

Bacterial species may be differentiated on the basis of their ability to reduce nitrate to nitrite or nitrogenous gases. The nitrate reduction test is based on the detection of nitrite in the medium after incubation with an organism. MZUR35 is the sole isolate which is incapable of nitrate reduction while rest of the isolates can reduce nitrate to nitrite.

Casein is a macromolecule composed of amino acids linked together by peptide bonds, CO-NH. Some microorganism has the ability to degrade the casein protein by producing proteolytic exoenzyme, called proteinase (caseinase). Following inoculation and incubation of the agar plate enhanced with casein or milk,

bacteria secreting proteases will exhibit a zone of proteolysis, which is demonstrated by a clear area surrounding the bacterial colony (Brown, 2009). Out of the 44 isolates, 30 isolates were found to exhibit clear area around their colonies when grown in Skim milk agar. Hence, it can be concluded that these bacterial isolates produce caseinase enzymes for casein hydrolysis.

Catalase is an enzyme, which is produced by microorganisms that live in oxygenated environments to neutralize toxic forms of reactive oxygen metabolites. Catalase is capable of converting hydrogen peroxide to water and oxygen. The presence of the enzyme in a bacterial isolate is evidenced when a small inoculum introduced into hydrogen peroxide (H_2O_2) causes rapid elaboration of oxygen bubbles (Willey *et al.*, 2008). Among all the bacterial isolates except MZUR26, MZUR35 and MZUR43 produce rapid elaboration of bubbles when introduce to H_2O_2, hence it can be concluded that all isolates except these strains have a protective mechanism against reactive oxygen metabolites.

Gelatin hydrolysis test is used to determine the ability of an organism to produce extracellular proteolytic enzymes (gelatinases) that liquefy gelatin, a component of vertebrate connective tissue. Therefore, all isolates found to produces gelatinase, and liquefies the growth medium by hydrolysing gelatin present in the medium will be capable of liquefying animal gelatins (Smith and Goodner, 1958).

The ability to degrade starch is used as a criterion for the determination of amylase production by a microbe. Performing a starch hydrolysis test or starch test is used to determine the presence or absence of an amylase enzyme. Bacterial isolates grown on starch agar medium which produce clear halo zone around their colony when flooded with iodine solution are considered to have amylolytic activity. The clear zone indicates hydrolysis of starch into monosaccharides which cannot bind the iodine molecule and appear as the clear zone around bacterial growth (Tille, 2014).

In both cultivated and natural soil environments, soil microbes play a key role in improving soil health by promoting plant growth. These microbes improve

plant growth through traits such as phosphate solubilization, auxin synthesis, and siderophore production (Sharma *et al.,* 2014). From the present study all 44 bacterial isolates were found to be able to produce phosphatase enzyme and IAA during 72 hours of in vitro culture. Quantitatively, isolate MZUR22 showed the highest phosphatase production (37.604±0.64 p- NPP $\mu g^{-1}hr\ ml^{-1}hr^{-1}$) while MZUR37 MZUR35 (0.271 p- NPP $\mu g^{-1}hr\ ml^{-1}hr^{-1}$) showed the lowest phosphatase production showed the highest IAA production (23.957±0.135 μgml^{-1}) during the culture period. The highest IAA production was shown by MZUR37 (23.957±0.135 IAA $\mu g\ ml^{-1}$) and least IAA was shown by MZUR35 0.502±0.135 IAA $\mu g\ ml^{-1}$). during the culture period.

The production of phosphatase enzyme and IAA by PGPB were reported by many workers such as Gope (2006), Glick (2012) and Behera *et al.* (2017). The plant growth promoters present in the soils of jhum and jhum fallow which are capable of phosphate solubilization and IAA production, can be used as soil amendments for eco-restoration and for increasing crop productivity (Chaiharn and Lumyong, 2010).

Chapter 8

In vitro Evaluation of Selected Phosphate Solubilizing Bacteria to Alleviate Rice Growth Performance

8.1 Introduction

Agriculture is one of the most crucial economic sectors for human and rice (*Oryza sativa* L.) cultivation is one of the most important agricultural practices. It is estimated that nearly 50% of the world population consumed rice as the main staple food. In India, rice cultivation occupies about 23.3% of gross cropped area contributing 43% of the total food grain production and 46% of the total cereal production of the country. Owing to high nutritional value and main staple food of half of the world population, rice production needs to be improved to meet the rapid expanding world population. About 60% of the total workers in Mizoram are engaged in agriculture of which rice cultivation is the main sector. Rice is grown under varying eco-systems on a variety of soils under varying climatic and hydrological conditions. Rain fed upland rice cultivation also called traditional jhum/shifting rice cultivation is regular way of rice cultivation in Mizoram. Although phosphorous is an essential macronutrient for plant growth and development, 95-99% of it, present in the soil is insoluble and cannot be utilized by plants (Vassileva *et al.,* 2000). This unavailability is due to P-fixation, either it is adsorbed on the soil minerals or get precipitated by free $Al^{3+}$and Fe^{3+}in the soil solution (Havlin *et al.,* 1999). To increase the availability phosphorus to plants, a large amount of phosphorous is quickly applied to soil, a large portion of which is quickly transferred into insoluble form (Omar *et al*., 1998) and very little percentage of applied phosphorus is available to plants making continuous application fertilizer necessary (Abd-Alla *et al*., 1994).

Approximately 52.3 billion tons of P-based fertilizers are applied annually to maintain available P levels in soil–plant systems (FAO, 2017). Whereas, only, about 0.2%, i.e., <10 μM of this huge amount, is used by plants (Alori *et al*., 2017; Islam *et al*., 2019) and the rest is precipitated by metal cations in soil such as Fe, Al, Mg, Ca, etc. The extensive use of P may leads to inevitable depletion of world reserves of rock phosphate (Leghari *et al*., 2016). This depletion will be concomitant with the increase in the cost of these commercial fertilizer products. Recently, scientists from all over the world have focused their attention on sustainable agriculture by exploiting beneficial microbes in order to increase the contribution of biofertilizers to food and fibre production (Khan *et al*., 2007; Granada *et al*., 2018; Yadav and Sarkar, 2019).

Biofertilizers are commonly defined as "Preparation containing live microbes which helps in enhancing the soil fertility either by fixing atmospheric nitrogen, solubilizing phosphorus or decomposing organic wastes or by augmenting plant growth by producing growth hormones with their biological activities" (Chaparro *et al*., 2014; Okur, 2018). Many strains, some of which have interesting biotechnological potential have been isolated from the extensive research programs on plant-beneficial microorganisms (Sivasakthivelan and Saranraj, 2013; Umesha *et al*., 2018; Nafis *et al*., 2019).

Thus, solubilization and mineralization of phosphorous by phosphate-solubilizing microorganisms (PSMs) is one of the most important microbial physiological traits in soil biogeochemical cycles (Jeffries *et al*., 2003) as well as in plant growth promotion by PGPMs (Richardson, 2001). Most of the PSMs reported so far include fungi, bacteria and soil yeasts, which were found to solubilize unavailable inorganic phosphates both in culture media and soil through the production of organic acids. The use of phosphate solubilizing bacteria (PSBs) as inoculants simultaneously increases phosphorous uptake by the plant and crop yield.

The plant growth promoters present in these soils, capable of phosphate solubilization and IAA can be used as soil amendments for eco-restoration and for increasing crop productivity (Chaiharn and Lumyong, 2010). The present study

aimed to identify PGPRs native to jhum agricultural fields and to explore their PGP potential for the development of a possible bioinoculant consortium for the eco-restoration of jhum fallows.

8.2 Experimental design

In order to assess the efficacy of PSB isolate on the growth of rice, an in vitro pot culture experiment was conducted using selected PSB isolates as inoculants. Two isolates such as *Bacillus cereus* MZUR22 and *Burkholderia gladioli* MZUR37 were selected based on their most efficient phosphatase activity and IAA production respectively.

8.2.1. Preparation of soil seedbed

Soil samples were collected from pre-burnt jhum field. The soil samples were divided into two sets; first set was autoclaved at 15psi for 15 minutes while the second set was left autoclaved. 100g of soil of each set were kept in 6 sterile plastic containers and triplicate of each soil samples were maintained. 10g of ash collected from the jhum field was added to three triplicates of each set.

The sterilised soil was used in order to limit the interference of native microbial community of the soil with action of the bacterial inoculants. Unsterilized soil was used in the experiment so as to demonstrate the effect of the interaction between the native microbial community and bacterial inoculants on the growth of rice plants. Both sterilized and unsterilized soil were amended with ash obtain from the burnt jhum field so as to observed the effect of ash addition on the growth of rice along with the addition of bacterial inoculants. The unsterilized soil amended with ash without bacterial inoculants will mimic the jhum field.

8.2.2. Preparation of bacterial inoculum

The selected bacterial strains were cultured in nutrient broth (NB) medium (Difco manual, 1953) at 30°C for 24 hrs under constant agitation for biomass propagation. The cultured bacterial strains were then used as inoculants *in vitro* condition.

8.2.3. Seed surface sterilization (Amin *et al*. 2004)

Rice seeds were first washed with sterilised distilled water and dried. The dried seeds were t agitated in 70 % ethanol for 5 minutes and then washed in sodium hypochlorite solution comprising 3 % ChloroxTM (2.6 % NaOCl). The seeds were again rinsed with sterile distilled water followed by washing with 2% sodium thiosulphate solution to neutralize chloramines residue. Finally, the seed were washed again with sterile distilled water and dried on a sterile paper towel.

8.2.4. Inoculation and in vitro culture of rice seed

Surface sterilised rice seeds were soaked in nutrient broth media which already contain selected PSB strains cultured overnight. The soaked seeds were then transferred to the plastic containers. The rice plants were grown for 21 days in in vitro condition. Shoot length and dried biomass were measured at 14 and 21 days after sowing.

8.3. Methodology

Quantification of dry biomass, shoot and root length were carried out after 14 and 21 DAS. The dry biomass content of rice plant will be estimated by oven-drying the plant samples freed of debris in an oven at 60 °C till constant weight was obtained. The dried plants biomasses were weighted in an electronic balance and the mean were expressed in grams (g) for each replicates. The shoot and root length were measured for each replicates using a standard ruler and the mean were expressed in cms.

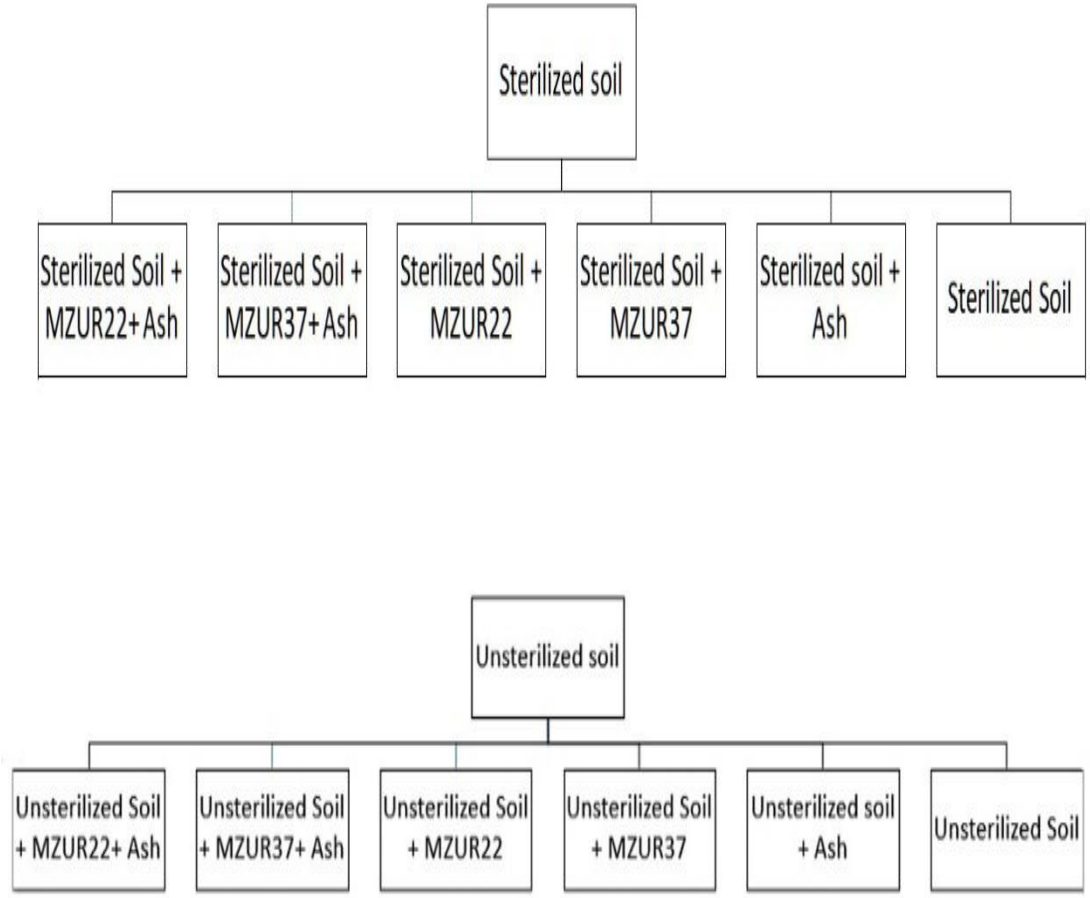

Fig 8.1. Experimental design for *in- vitro* test of selected Phosphate Solubilizing Bacteria efficacy as potential bio fertilizer

8.4. Results

The observed results from the *in vitro* growth of rice under different treatments showed that the rice seeds treated with PSB inoculum showed better growth performance in terms of root and shoot length as well as biomass accumulation during the observed period. Soil amended with ash also showed a positive effect on the growth of rice which are inoculated with PSB.

Rice seeds treated with bacteria inoculum MZUR22 *Bacillus cereus* grown on unsterilized soil amended with ash showed the highest shoot and root length along with highest biomass accumulation after 21 DAS, followed by rice seeds treated with MZUR37 *Burkholderia gladioli* grown on unsterilized soil amended with ash. Lowest growth performance of rice was observed in the control treatment for sterilised soil. The detail results of the experiment were shown in the following fig 8.3- 8.5

One-way ANOVA performed considering significant level of $p \leq 0.05$ showed that significant variation exists among the different rice inoculated with PSBs which were given different combination treatment with Ash. The rice inoculated with MZUR22 in combination with ash treatment showed the most significant variation for growth performance and biomass accumulation from the other treatments for both sterilised and unsterilized soils. Inoculated rice seed with MZUR37 in combination with ash also showed significant variation in growth performance and biomass accumulations as well. Rice seed inoculated with PSBs which were not amended with ash also showed variation from the control rice plants, however their effect was found to less prominent as to those amended with ash.

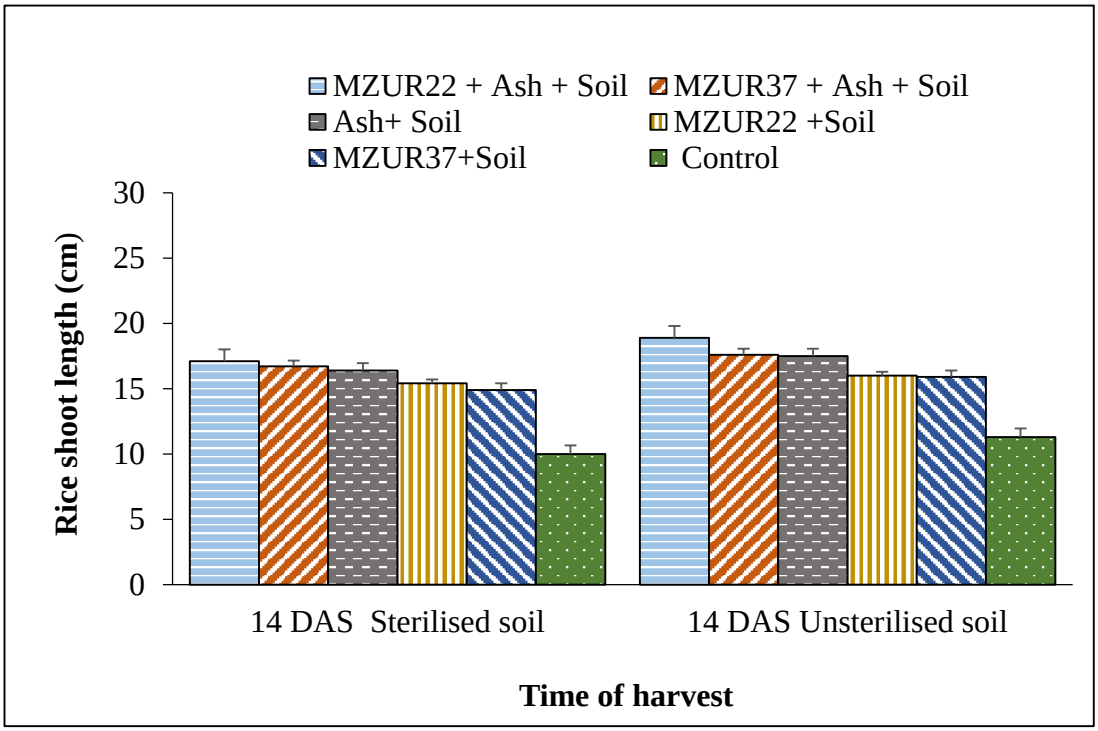

Fig 8.2. Shoot growth performance of rice treated with PSBs under different treatments on sterilized and unsterilized soil at 14 DAS

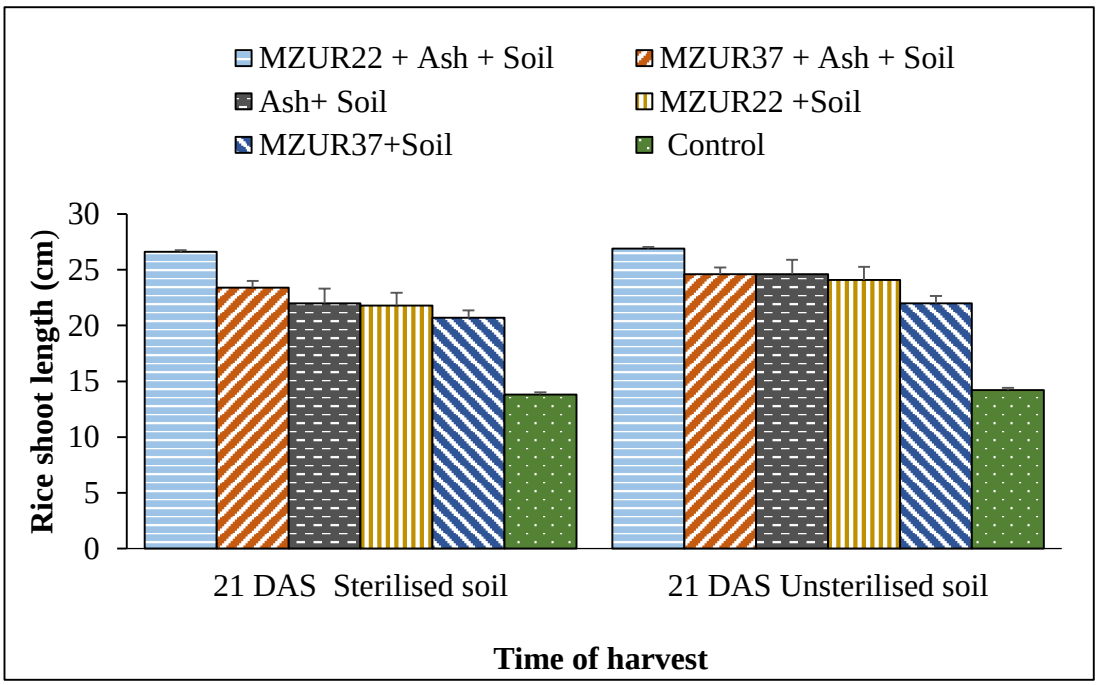

Fig 8.3. Shoot growth performance of rice treated with PSBs under different treatments on sterilized and unsterilized soil at 21 DAS.

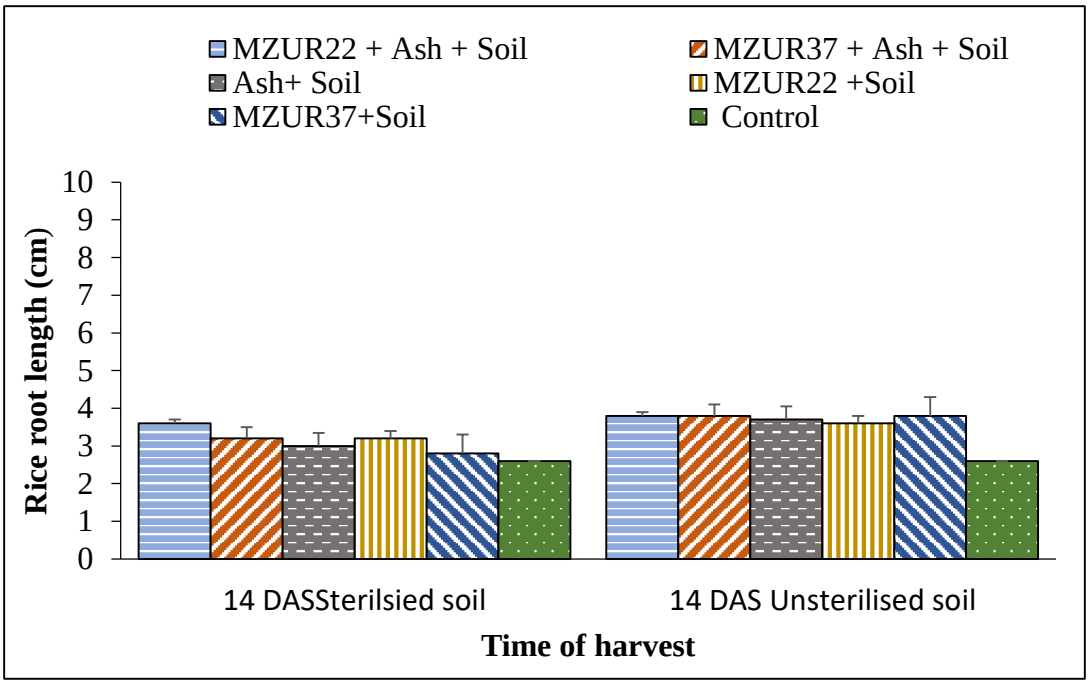

Fig 8.4. Root growth performance of rice treated PSBs under different treatments on sterilized and unsterilized soil at 14 DAS.

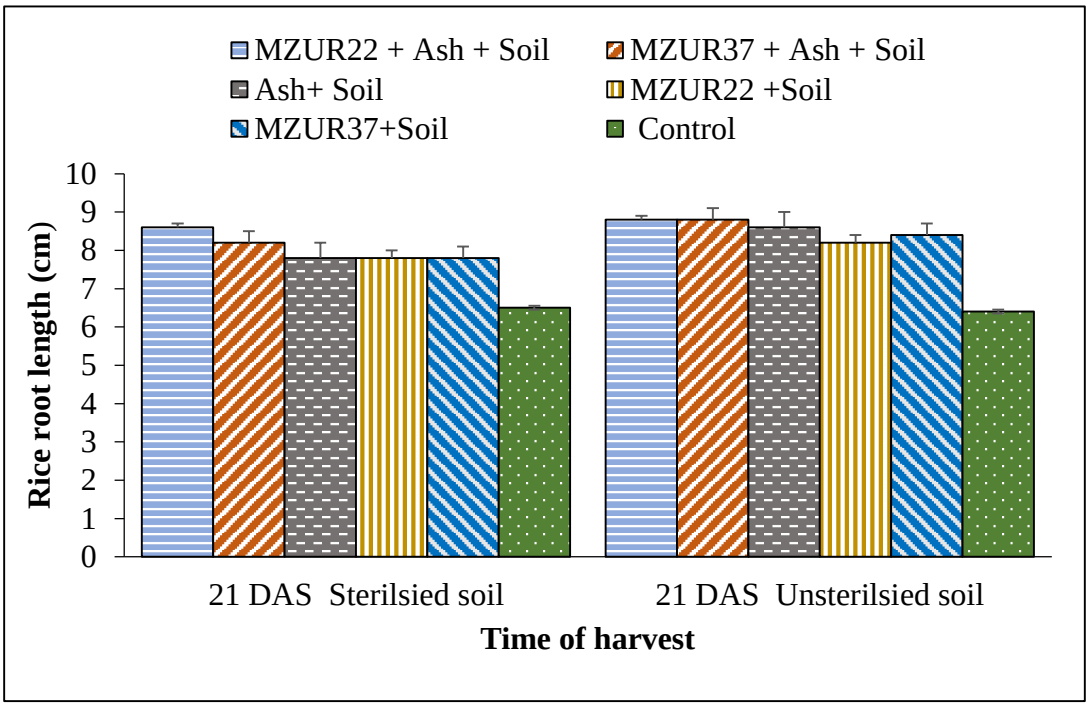

Fig 8.5. Root growth performance of rice treated PSBs under different treatments on sterilized and unsterilized soil at 21 DAS

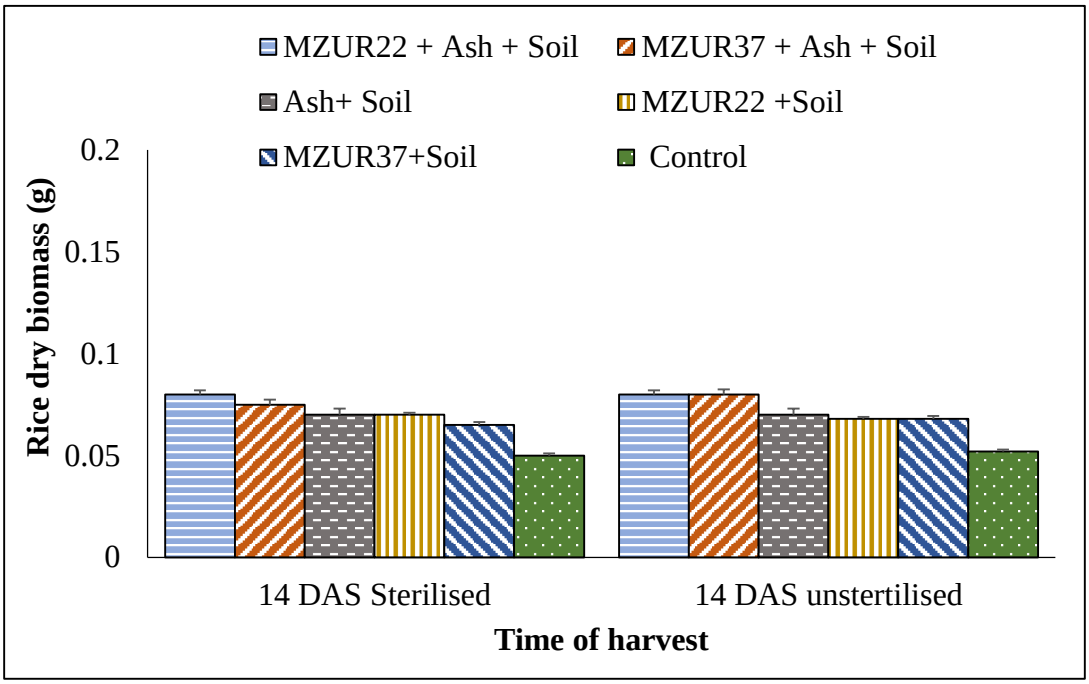

Fig 8.6. Dry biomass (g) of rice treated with PSBs under different treatments on unsterilized a soil at 14 DAS.

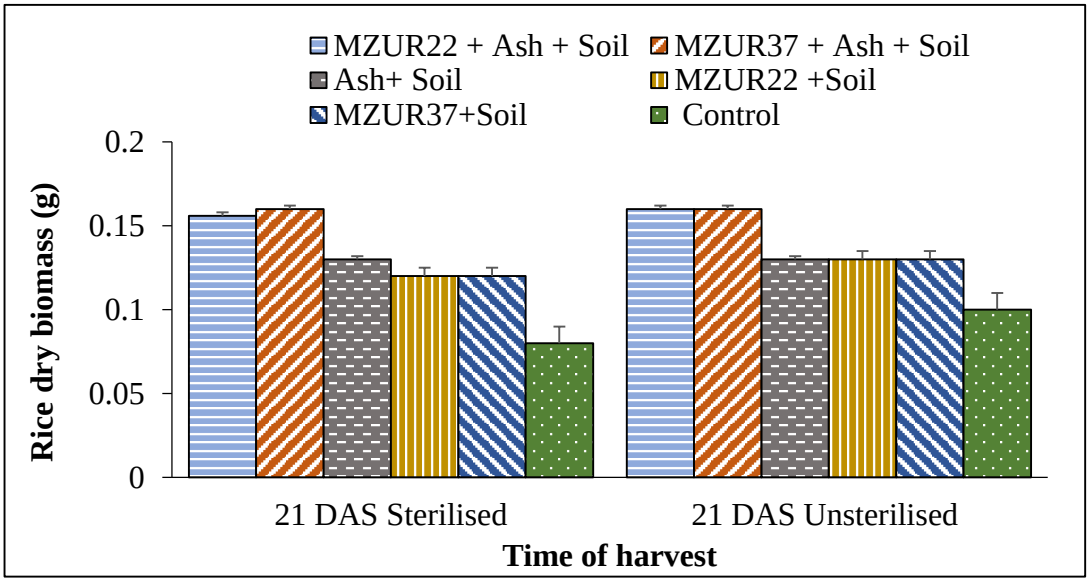

Fig 8.8. Dry biomass (g) of rice treated with PSBs under different treatments on sterilized a soil at 21 DAS.

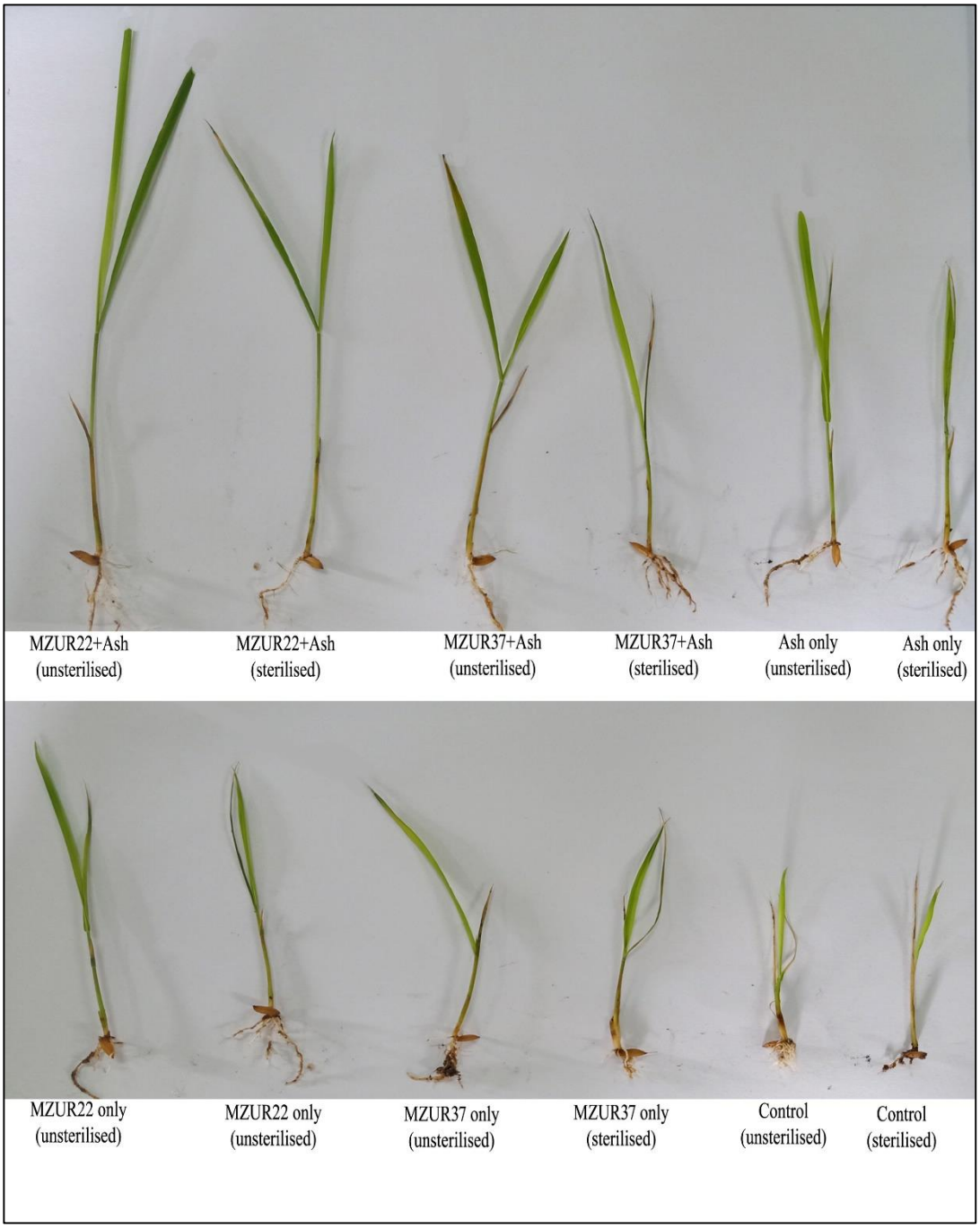

Fig.8.7. Rice growth performance under different treatments with PSB isolates and ash at 21 DAS.

Table 8.1. One way analysis of variance (ANOVA) of rice shoot growth between rice seeds inoculated with isolated potential PSB strains under different treatment grown on sterilized soil at 21 DAS. Marked effects are significant at $p \leq 0.05$.

S. N	Source of variation	F-value	p-value
1.	MZUR22 +Ash x MZUR37 + Ash	61.264	0.001*
2.	MZUR22 + Ash x Ash	12.982	0.023*
3.	MZUR22 + Ash x MZUR22	24.962	0.008*
4.	MZUR22 + Ash x MZUR37	31.291	0.005*
5.	MZUR22 + Ash x CONTROL	46.775	0.002*
6.	MZUR37 + Ash x Ash	215.063	0.000*
7.	MZUR31 + Ash x MZUR22	1.877	0.243
8.	MZUR37 + Ash x MZUR37	4.612	0.098
9.	MZUR37 x CONTROL	11.382	0.028*
10.	MZUR22 x Ash	128.847	0.000*
11.	MZUR22 x MZUR37	0.729	0.441
12.	MZUR22 x CONTROL	4.328	0.106
13.	MZUR37x CONTROL	103.380	0.001*

Table 8.2. One way analysis of variance (ANOVA) of rice root growth between rice seeds inoculated with isolated potential PSB strains under different treatment grown on sterilized soil at 21 DAS. Marked effects are significant at $p \leq 0.05$.

S.N	Source of variation	F-value	p-value
1.	MZUR22 +Ash x MZUR37 + Ash	42.025	0.003*
2.	MZUR22 + Ash x Ash	6.000	0.070
3.	MZUR22 + Ash x MZUR22	9.600	0.036*
4.	MZUR22 + Ash x MZUR37	12.100	0.025*
5.	MZUR22 + Ash x CONTROL	12.100	0.025*
6.	MZUR37 + Ash x Ash	12.100	0.025*
7.	MZUR31 + Ash x MZUR22	2.400	0.196
8.	MZUR37 + Ash x MZUR37	2.500	0.189
9.	MZUR37 x CONTROL	2.500	0.189
10.	MZUR22 x Ash	2.500	0.189
11.	MZUR22 x MZUR37	0.053	0.830
12.	MZUR22 x CONTROL	0.053	0.830
13.	MZUR37x CONTROL	25.350	0.007*

Table 8.3. One way analysis of variance (ANOVA) of rice shoot growth between rice seeds inoculated with isolated potential PSB strains under different treatment grown on unsterilized at 21 DAS. Marked effects are significant at $p \leq 0.05$.

S.N	Source of variation	F-value	p-value
1.	MZUR22 +Ash x MZUR37 + Ash	23.562	0.008*
2.	MZUR22 + Ash x Ash	2.765	0.172
3.	MZUR22 + Ash x MZUR22	2.765	0.172
4.	MZUR22 + Ash x MZUR37	5.524	0.078
5.	MZUR22 + Ash x CONTROL	10.217	0.033*
6.	MZUR37 + Ash x Ash	75.002	0.001*
7.	MZUR31 + Ash x MZUR22	0.000	1.000
8.	MZUR37 + Ash x MZUR37	0.116	0.751
9.	MZUR37 x CONTROL	1.788	0.252
10.	MZUR22 x Ash	37.875	0.004*
11.	MZUR22 x MZUR37	0.116	0.751
12.	MZUR22 x CONTROL	1.788	0.252
13.	MZUR37x CONTROL	37.875	0.004*

Table 8.4. One way analysis of variance (ANOVA) of rice root growth between rice seeds inoculated with isolated potential PSB strains under different treatment grown on unsterilized at 21 DAS. Marked effects are significant at $p \leq 0.05$.

S.N	Source of variation	F-value	p-value
1.	MZUR22 +Ash x MZUR37 + Ash	1.500	0.288
2.	MZUR22 + Ash x Ash	6.000	0.070
3.	MZUR22 + Ash x MZUR22	21.600	0.010*
4.	MZUR22 + Ash x MZUR37	9.600	0.036*
5.	MZUR22 + Ash x CONTROL	345.600	0.000*
6.	MZUR37 + Ash x Ash	1.500	0.288
7.	MZUR31 + Ash x MZUR22	15.000	0.018*
8.	MZUR37 + Ash x MZUR37	5.400	0.081
9.	MZUR37 x CONTROL	317.400	0.000*
10.	MZUR22 x Ash	9.600	0.036
11.	MZUR22 x MZUR37	2.400	0.196
12.	MZUR22 x CONTROL	290.400	0.000*
13.	MZUR37x CONTROL	1.500	0.288

8.4. Discussion

From the experimental result and statistical analysis, it was observed that the rice seed inoculated with PSB isolates showed better root and shoot growth as well as biomass accumulation in the early stage of rice growth. MZUR22 + Ash treatment showed the best growth performance in terms of shoot and root length along with biomass accumulation followed by MZUR37 + Ash treatment. This was in agreement with numbers of researchers such as Anand et al., (2016), Banerjee *et al*., (2017) etc. who demonstrated the used of native microbes from jhum cultivation as bio-inoculant improves rice growth. The addition of ash to the soil greatly influence the growth and biomass accumulation of the rice plant since supply of nutrients especially available phosphorus is augmented by addition of ash (Joachim and Kandiah 1948). Hence the combination of PSBs inoculum with soil amended with ash showed the highest growth performance and biomass accumulation (Ramakrishnan and Toky 1981; Tawnenga *et al.,* 1997, Sapriliana *et al.,* 2016)

The rice seeds inoculated with bacterial isolates exhibiting high phosphatase (MZUR22) showed higher shoot and root growth as well as biomass accumulation as compared to rice seed inoculated with bacteria having the highest IAA production (MZUR37) under all treatment conditions. Since low available P in soil is one of the main limiting factor for plant growth, the production of phosphatase which increase the amount of available P in soil have greater influence on the growth of rice plant. This was demonstrated by Kalayu (2019) who mentioned that using *Bacillus* and *Pseudomonas* as bio-inoculant to be an efficient way to convert the insoluble P compounds to plant-available P form, resulting in better plant growth, crop yield, and quality ; Sharma *et al*. (2007) who reported the enhancement in seed germination and seedling growth enhancement with the combined inoculation of *Pseudomonas fluorescens* and *Bacillus megateriumand* Stephen *et al.* (2015) who state that the use of *Gluconacetobacter* sp. strain MTCC 8368 and *Burkholderia*sp. strain MTCC 8369 as inoculants increased the growth, yield, phosphorus content and nutrient uptake of rice (*Oryza sativa*) to name a few.

The use of beneficial microbes such as PSBs as bio-fertilizers is a growing trend in the field of agriculture and is necessary for its sustainability. Bacteria isolated from jhum and jhum fallows with combined PGP properties (P-solubilization and IAA production) were obtained which were able to enhance plant growth. The use of microbes as bio-fertilizers in jhum fields is not widely practice; therefore, the use of native PGPB for improving crop productivity and aiding the eco-restoration and sustainability of the degraded soil system is required.

Chapter 9

Summary and conclusion

Jhum cultivation is one of the most ancient systems of farming which is a common agricultural practice among the tribal people of South America, Africa, and Southeast Asia. In India it is commonly practiced in the hilly areas of south, central, and North-Eastern India. About 0.76 Mha of land is under jhum cultivation in North-Eastern India. Among the seven North-Eastern states of India, the area under jhum cultivation is highest in Nagaland, accounting for 38.18% of its geographical area, followed by Mizoram and Manipur.

In jhum cultivation, a piece of forest land was cleared, left to dry and the dried biomass is burned *in situ* followed by the cropping phase. Once the fallow lands regain their fertility, the farmers come back to the same piece of land for cultivation. In jhum cultivation fire is deliberately used to change soil physico-chemical properties, and both the release of nutrients by fire and the value of ash have been known to cause alteration in soil biochemical properties. In course of time, different adverse effects emerge because of jhum cultivation which has a detrimental effect on the soil nutrients status and biological properties due to deliberate slashing and burning of vegetation involved in the process.

Phosphorus (P) which is an essential nutrient for the growth and development of plants undergoes large volatilization and convective losses during jhum cultivation. The amount available P in the soil for plant utilization is very low and the new sources to replace the lost available P mainly comes from non-plant available P in soil and from parent materials which needs to be solubilized through the action of enzymes known as *phosphatase.* These enzymes are secreted by a group of microorganisms known as Phosphate Solubilizing Microorganism (PSM), among which Phosphate Solubilizing Bacteria (PSB) are predominant. These PSB(s) can be used for the rapid solubilization of insoluble P, thereby supplementing the low available P level in the soil. Hence these

PSB can be developed into location-specific fertilizers to maintain the sustainability of jhum cultivation.

In this study soil samples were collected from the current jhum field, different length jhum fallows viz. current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and an undisturbed forest (UD) of Hmuntha village (23.4939° N and 92.9419°E), Serchhip district, Mizoram, India. Soil samples were collected during February, May, August and November. The soil physico-chemical properties such as soil temperature, bulk density (BD), soil moisture content (SMC), water holding capacity (WHC), soil pH, soil organic carbon (SoC), available nitrogen (AN), available phosphorous (AP) and available potassium (AK) were analyzed. Soil enzymes assay for dehydrogenase, urease, phosphatase, arylsulfatase, and β- glucosidase were also carried out. Analysis of soil microbial biomass carbon (Cmic) and microbial biomass nitrogen (Nmic) was also done. Estimation of soil bacterial diversity based on metagenomic sequencing and isolation and molecular identification of PSB was carried out using 16s rRNA gene sequencing

Metagenomics diversity of soil bacteria was assessed by amplification of V3-V4 hypervariable region of the 16SrRNA gene. The isolation of PSB was carried out based on their ability to solubilize TCP on PVK medium and 16SrRNA gene sequencing was also done for the potential isolates to assert their identity. Biochemical characterization including assessment of PGP traits such as IAA and phosphatase enzyme production was done to screen for potential bio-fertilizers. Based on their PGP trait, the effect of selected bacterial isolates on rice growth performance as carried out in *in-vitro* experiments under different treatments such as the addition of ash and sterilization of soil.

Analysis of soil physico- chemical properties showed that UD site was the most productive and fertile site while 2F was observed to be the poorest and least fertile. Statistical analysis showed that there is significant variation among soil physico-chemical properties of different sampling sites. Maximum soil temperature and BD were found to highest in 2F soil, while UD recorded the lowest for both parameters. Soil BD ranges from 1.05g cm^3 in 10F during November to 1.37 g cm^3 in Jh during May.

Soil BD and WHC are inversely correlated hence lowest WHC% (60.32 %) were observed in Jh during May and the highest WHC% (73%) was observed in UD during May. The SMC was highest in August for all sampling sites where UD showed the highest SMC of 32.63% while the Jh site showed the lowest SMC (12.58%) during February.

The soil nutrients *viz.* SoC, AN, AP, and AK were found to be highest in UD site and lowest in 2F site. The soil pH ranges from 4.7-5.5, highest soil temperature of 26^{o}C was recorded in 2F during May and lowest of 12.07^{o}C during November in Jh site Highest SoC (4.793 %) was observed at UD site during November while Jh site showed the lowest SOC (2.567%) during February. Soil available nitrogen showed variation with respect to the length of the fallowing period of the sampling sites. The highest AN 236 kgha-1 was observed in UD site during November and the lowest AN (148 kg ha^{-1}) was observed in 2F during February. Soil AP showed a wide range of variation between different sampling sites. The highest level of available P (15.7 kg ha^{-1}) was recorded from UD site during August while the lowest level of available P (8.1 kg ha^{-1}) was recorded from 2F site during November. Soil available K ranges from 144 kg ha^{-1} in 2F site during February to 234 kg ha^{-1} at UD site during August, indicating a wide range of variation both with sites and sampling season.

Soil biochemical properties such as enzymes dynamic and soil microbial biomass carbon and nitrogen showed a significant variation between the samples site. For soil enzyme dynamic, the highest DHA activity was recorded in the UD site (0.9 mg TPF g dry soil^{-1} 24hr^{-1}) and lowest (0.22 mg TPF g dry soil^{-1} 24hr^{-1}) in Jh site. Acid phosphatase (Apase) phosphatase enzyme activity ranges from 42.100 µg p-NP g-1 dry soil hr^{-1} at 2F site in February to 149.566 µg p-NP g^{-1} dry soil hr^{-1} at the UD site during August. The highest urease (URS) enzyme activity was recorded at UD site (34 µg NH_3 g dry soil^{-1} 3hr^{-1}) where the lowest (10 µg NH_3 g dry soil^{-1} 3hr^{-1}) was recorded at the 2F site. The ARS activity of the study site ranges from 71µg p-NP dry soil^{-1} hr^{-1} in 2F soil during May to 320 µg p-NP dry soil^{-1} hr^{-1} in UD soil during May and August and the highest Beta- glucosidase activity of 211.45 µg p-NP dry soil^{-1} hr^{-1} was recorded at UD site during May while lowest reading of 58.29 µg p-NP dry soil^{-1} hr^{-1} was recorded at the 2F site during February.

Metagenomics analysis of soil bacterial population using NGS revealed that at kingdom level, archaea were found in all samples except 5F. The highest percentage of Archaea was observed in UD (1.98%). Actinobacteria are the dominant Phylum in Jh representing 37.198% of the total bacterial phyla while Proteobacteria are the dominant class in the other sites accounting for 33.777%, 30.078%, 33.978%, and 31.917% of the bacterial population in 2F, 5F, 10F, and UD respectively. The 10F site showed the highest number of unique bacterial species based on the NGS data generated. 730 bacterial species belonging to 541 genera were identified from the UD site. The 2F showed the second-highest numbers of bacteria species with 582 species from 446 different genera while from the Jh site 452 species genera belong to 332 genera were recorded. UD and 5F sites showed the least number of bacterial species at 545 belonging to 406 genera and 408 genera respectively.

Alpha diversity analysis revealed that there was significant variation in the bacterial composition between the sampling site. 10F showed the highest Shanon diversity index (10.169) while the lowest was observed in Jh site (9.048). Phylogenetic diversity analysis based on Faith phylogenetic diversity index also follows the same distribution as that of Shanon diversity index with diversity index ranging from 57.8 for Jh to 85.9 for 10F. PCoA of Beta-diversity analysis based on Bray-Curtis index and Jaccard distance showed dissimilarity between the bacterial populations of the different sites. While PCoA of weighted and unweighted infrac distance showed that there is dissimilarity in the phylogenetic diversity of bacterial community shared between the study sites except for 2F and 5F which showed an almost similar phylogenetic diversity of bacteria.

Base on their ability to solubilized tri-calcium phosphate and formation of halo zone on PVK medium, 44 bacteria were identified as having phosphate solubilizing potential and were isolated and maintained in pure-culture. They were then designated as MZUR1, MZUR2, MZUR3, MZUR4, MZUR5, MZUR6, MZUR7, MZUR8, MZUR9, MZUR10, MZUR11, MZUR12, MZUR13, MZUR14, MZUR15, MZUR16, MZUR17, MZUR18, MZUR19, MZUR20, MZUR21, MZUR22, MZUR24, MZUR25, MZUR26, MZUR27, MZUR28, MZUR29, MZUR30, MZUR31, MZUR32, MZUR33, MZUR34, MZUR35, MZUR36, MZUR39, MZUR40, MZUR41, MZUR42, MZUR43

and MZUR44. Their SI was calculated and the highest solubilization index was shown by the MZUR6 (3.45) and the lowest was shown by MZUR4 (1.98) while the lowest SI was observed for MZUR4 (1.95).

Genomic DNA and molecular characterization using 16srRNA gene profiling were performed on all of the isolated PSBs. The 16SrRNA gene amplification was performed by using the primer 27f and 1525r. The PCR products were sent for sequencing commercially at AgriGenome Labs Pvt. Ltd. Kochi, Kerala, India. After sequencing of the PCR purified product, the sequence data were compared for similarity level with the reference strains of PSB from genomic database banks. The 16SrRNA nucleotide sequences were compared by BLASTn analysis tool to look for 16SrRNA gene homology and based on the results the bacterial isolates placed under the genera *Burkholderia, Pseudomonas, Alcaligenes*, and *Bacillus*. *Bacillus* accounts for 52.23% of the total isolates while *Burkholderia, Pseduodmonas*, and *Alclaligenes* account for 22.73%, 12.66%, and 11.36% respectively. The 16s rRNA nucleotide sequences nucleotide were deposited in NCBI Genbank database bearing the accession MT086198- MT086211, MT087561- MT087567, MT096514, MT102121- MT102138, MT102411- MT102443.

Based on the 16SrRNA sequence result, 7 out of 9 PSB isolated from the Jh soil belongs to *Bacillus* genus, the others being *Pseudomonas* and *Burkholderia*. From the 2F site 10 PSBs were isolated, where 5 of the isolates belong to *Bacillus*, 3 to *Burkholderia* and 1 each to *Pseudomonas* and *Alcaligenes*. The least number of PSB were isolated from the 5F site which was made up of 3 *Bacillus* and 3 *Pseudomonas*. Most of the PSB isolated from 10F site were found to be gram-negative, with 3 isolates belonging to *Burkholderia*, 2 to *Alcaligenes*, and 1 to *Pseudomonas*. 3 isolates from 10F were gram-positive bacteria belonging to Bacillus. The PSB isolated from UD site showed a consortium of bacteria composed of 3 *Alcaligenes*, 2 *Burkholderia*, 1 *Pseudomonas*and 3-gram positive *Bacillus*.

The evolutionary history was inferred by using the Neighbor-joining method based on Kimura 2- parameter model (K2+G). The estimated transition/transversion bias (R) ratio is 1.15. The model was selected based on the lowest Bayesian information

criterion (8324.105) value. The phylogenetic tree was divided into two different clades (Clades I and Clades II). In Clade I, gram-positive Bacillus was grouped with type strains from EzTaxon databases with a bootstrap value of 100%. In Clade II, the gram-negative bacteria are clustered under two sub-clades, *in* Clade IIA *Pseudomonas* was clustered separately while *Alcaligenes* and *Burkholderia* were clustered together under one sub-clade (Clade IIB) with a bootstrap value of 100%.

The biochemical characterization of the 44 bacterial isolates showed that Out of the 44 isolates, 93% of the isolates showed catalase enzyme production. 57% of the isolates were capable of performing starch hydrolysis, gelatin hydrolysis test was positive for 80% of the isolates, while 60% of the isolate were capable of casein hydrolysis. 95% of the isolates were capable of fermenting glucose and sucrose while 2% of the isolates do not perform either scudoes or glucose fermentation. 97% of the isolates were found to be capable of reducing nitrate to nitrite.

All bacterial isolates showed phosphatase and IAA production after 24 hours of culturing. Quantitatively, isolated bacteria showed a phosphate solubilization range between 0.271 to 37.604 p- NPP μg^{-1}hr ml^{-1}hr-1. MZUR22 showed the highest P-solubilization values, whereas MZUR35 the minimum. The highest IAA production was shown by MZUR37 (23.957±0.135 IAA µg ml^{-1}) and the least IAA was shown by MZUR35 0.502±0.135 IAA µg ml^{-1}).

The *in vitro* growth of rice under different treatments showed that the rice seeds treated with PSB inoculum showed better growth performance in terms of root and shoot length as well as biomass accumulation during the observed period. Soil amended with ash also showed a positive effect on the growth of rice inoculated with PSB. Rice seeds treated with bacteria inoculum MZUR22 -*Bacillus cereus* grown on unsterilized soil amended with ash showed the highest shoot and root length along with highest biomass accumulation after 21 DAS, followed by rice seeds treated with MZUR37- *Burkholderia gladioli* grown on unfertilized soil amended with ash. The lowest growth performance of rice was observed in the control treatment for sterilized soil. One-way ANOVA performed considering a significant level of $p \leq 0.05$ showed that there was significant variation in the shoot and root growth as well as rate of biomass

accumulation between rice inoculated with PSBs and rice which were not inoculated with PSB. It was also observed that the addition of ash played an important role in the early growth of rice plants.

From this study, it can be concluded that the deliberate and intentional use of fire for land preparation during jhum cultivation has a long-term effect on the soil physico-chemical, biochemical and biological properties. A fallowing period of at least 10 years is recommended for the rejuvenation of the soil property before it is being cleared for another jhum cycle. However, due to the increase in population and increase in land pressure the fallowing period has been reduced to around 4-5 years, hence compromising the sustainability of the jhum cultivation. The use of native microbes as bio-fertilizer has a huge potential. The isolated PSB which have PGP trait may be developed as bio-fertilizers to help increase the rice production from jhum field, allow a sustainable 2nd year cropping and help in the fast recuperation of soil nutrient especially P during the fallowing period.

PARTICULARS OF THE CANDIDATE

NAME OF THE CANDIDATE	: Remruattluanga Hnamte
DEGREE	: Ph.D.
DEPARTMENT	: Botany
TITLE OF THESIS	:**Diversity of Phosphate Solubilizing Bacteria in Jhum Fallow Soil of Serchhip District, Mizoram**
DATE OF ADMISSION	: 19.08.2014
APPROVAL OF RESEARCH APPROVAL	
1. DRC	: 11.08.2014
2. BOS	: 14.08. 2014
3. SCHOOL BOARD	: 22.05.2015
MZU REGISTRATION No.	: 3075 of 2007-08
PH.D REGISTRATION No. & DATE	: MZU/Ph.D./744 of 22.05.2015

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References

A.C. Kennedy., & R.I. Papendick. (1995). Microbial characteristics of soil quality, *Journal of Soil and Water Conservation*, 50 *(*3*)* 243-248.

Abd-Alla, M.H. (1994). Phosphatases and the utilization of organic P by Rhizobium leguminosarum biovar viceae. *Lett Appl Microbiol* 18:294-6.

Adeduntan, S.A., (2010). Effects of slash and burning on soil microbial diversity and abundance in the tropical rainforest ecosystem, Ondo State, Nigeria. *African J. Plant Sci.* 4, 322–329.

Ahemad, M., & Kibret, M. (2014). Mechanisms and applications of plant growth promoting rhizobacteria. Current perspective. *Journal of King Saud University – Science* 26, 1–20.

Ahlgren, I. F. & Ahlgren, C. E. (1965). Effects of prescribed burning on soil microorganisms in a Minnesota jack pine forest. *Ecology* 46: 304-310.

Ahmad, M., Hannan, A., Yasin, M., Ranjha, A.M., & Niaz. A., (2009). Phosphorus application to cotton enhances growth, yield, and quality characteristics on a sandy loam soil. *Pak. J. Agric. Sci.* 46 (3): 169-173.

Ahmed, A., & Hasnain. S. (2010). Auxin-producing *Bacillus* sp.: auxin quantification and effect on the growth of *Solanum tuberosum*. Pure and Applied Chemistry. (2010);82(1):313–319

Ahn, P. A. (1974). Properties and management of soils in the tropics (New York: John Wiley)

Ajwa, H. A., Dell, C. J. &Rice, C. W. (1999). Changes in enzyme activities and microbial biomass of tallgrass prairie soil as related to burning and nitrogen fertilization. *Soil Biol. Biochem*. 31: 769–777.

Alam S., Khalil, S., Ayub, N. & Rashid, M. (2002). In vitro solubilization of inorganic phosphate by phosphate solubilizing microorganism (PSM) from maize rhizosphere. Intl. J. *Agric Biol*. 4:454-458.

Alef, K. & P. Nannipieri (Eds.) (1995). Methods in applied soil microbiology and biochemistry. Academic Press, London.

Allen, E.E. and Banfield, J.F. (2005). Community genomics in microbial ecology and evolution. *Nat Rev Microbiol*.3 (6):489-498.

Alori, E. T., Glick, B. R., & Babalola, O. O. (2017). Microbial Phosphorus Solubilization and Its Potential for Use in Sustainable Agriculture. *Frontiers in microbiology*, *8*, 971.

Altieri, M. A., Anderson, M. K., & Merrick, L. C. (1987). Peasant agriculture and the conservation of crop and wild plant resources. *Conservation Biology*, v. 1, n. 1, p. 49-58.

Amin, M.A., Uddin, M.A. & Hossain, M.A. (2004). Regeneration study of some Indica rice 7 cultivars followed by Agrobacterium-mediated Transformation of highly generable cultivar 8 BR-8. J. Biol. Sci. 4: 207–211.

Anand, K., Kumari, B. & Mallick, M.A., (2016). Phosphate solubilizing microbes: an effective and alternative approach as bio-fertilizers. Int. J. Pharm. Sci. 8 (2), 37–40.

Anderson, G. (1980). Assessing organic phosphorus in soils. In: Khasawneh FE, Sample EC, Kamprath EJ (eds) The role of phosphorus in agriculture. *Amer. Soc. Agronomy*, Madison, pp 411–432

Anderson, J. M., & Ingram, J. S. I. (1993). *Tropical Soil Biology and Fertility A handbook of Methods* CA B International Wallingford, U K.

Anonymous (2018). Mizoram statistical handbook. Directorate of information & public relations, Government of Mizoram

Anonymous. (2010). Wasteland Atlas of India Ministry of Rural Development, Department of Land Resources, Government of India, New Delhi and Land Use Division, Land Resources Group, RS & GIS Application Area NRSC, ISRO, Hyderabad.

Antoun, H., Beauchamp, C.J., Goussard, N., Chabot, R., & Lalande, R. (1998). Potential of Rhizobium and Bradyrhizobium species as plant growth promoting rhizobacteria on non-legumes: Effect on radishes (Raphanus sativus L.). *Plant and Soil* 204, 57–67.

Are, K. S., Oluwatosin, G. A., Adeyolanu, O. D., & Oke, A. O. (2009). Slash and burn effect on soil quality of an Alfisol: soil physical properties. *Soil and Tillage Research*, v. 103, n. 1, p. 4-10.

Armando, S.N., Darwin, L., Andersen, W., & Moir. J. (1996). Fine root mineralization, soil organic matter and exchangeable cation dynamics in slash and burn agriculture in the semi-arid northeast Brazil. *Agriculture, Ecosystems & Environment*, 59(3): 191−202.

Arshad, M.A., & Martin, S. (2002). Identifying critical limits for soil quality indicators in agroecosystems. Agriculture, Ecosystems and Environment 88: 153-160

Arunachalam, A., Boral, L. & Maithani, K. (1994). Effects of ground fire on nutrients in soil land litter in a sub-tropical forest of Meghalaya. J. Hill Res. 7: 13-16.

Arunachalam, A. (2002). Dynamics of soil nutrients and microbial biomass during first year cropping in an 8-year jhum cycle. *Nut. Cycl. Agroecosys*., 64: 283-291.

Arunachalam, A. (2003). Role of microbial biomass in soil nutrient dynamics along a jhum cycle gradient. J. Trop. Fo. Sci. 15 (2): 279-288.

Ashrafuzzaman, Hossen, Md., Ismail, F.A., Hoque, M., Islam, Md., Shahidullah, M.Z., Meon, S., & Sariah. (2010). Efficiency of plant growth-promoting rhizobacteria PGPR for the enhancement of rice growth. *African J. Biotechnol.8(7):1247-1252*

Atlas, R.M. (1984). Diversity of microbial communities. *Advances in microbial ecology,* Vol. 7. Plenum press New York. pp. 1-47

Ayanaba, A., Tuckwell, S.B. & Jenkinson, D.S. (1976). The effect of clearing and cropping on the organic matter reserves and biomass of tropical forest soils. *Soil Biol. Biochem.* 8:519- 525.

Bailey, V.L., Peacock A.D., Smith J.L. & Bolton Jr. H. (2002). Relationships between soil microbial biomass determined by chloroform fumigation-extraction, substrate-induced respiration, and phospholipid fatty acid analysis. *Soil Biology & Biochemistry.* 34: 1385-1389.

Banerjee, A., Donald, A., & Joshi, S.R. (2017). Native microorganisms as potent bioinoculants for plant growth promotion in shifting agriculture (Jhum) systems. *J. Soil Sci. Plant Nutr*. 17, 127-140

Banik, S. & Dey, B.K. (1982) Available phosphate content of an alluvial soil as influenced by inoculation of some isolated phosphate-solubilizing micro-organisms. *Plant Soil* 69, 353–364.

Banik, S. Bhattacharyya, S. K. & Pandey, S. N. (1989). Rock phosphate as substitute for single super phosphate in tossa jute (Corchorus olitohus) cultivation. Indian Journal of *Agricultural Sciences.* 59(10): 676-679.

Barber, S.A. (1984). Soil Nutrient Bioavailiability; A Mechanistic Approach. John Wiley and Sons, New York, pp. 398.

Barber, S.A., & Mackay, A. (1986). Root growth and phosphorus and potassium uptake by two corn genotypes in the field. *Fertilizer Research* 10, 217–230.

Bardgett, R. D., Freeman, C.& Ostle, N. J. (2008). Microbial contributions to climate change through carbon cycle feedbacks. The *ISME journal* 2, 805–814.

Bartholomew, W. V., & Clark, F. E. (1965). Soil Nitrogen. *American Society of Agronomy*, Madison, Wisconsin.

Baruah, M. & Mishra, R.R. (1984). Dehydrogenase and urease activities in rice-field soils. Soil *Biol. & Biochem*. 16: 423-424.

Bauhas, J., Pare, D., & Cote, L. (1998). Effects of tree species stand age and soil type on soil microbial biomass and its activity in a southern boreal forest. *Soil Biology and Biochemistry* 30: 1077-1089

Behera, B.C., Singdevsachan, S.K., Mishra, R.R., Dutta, S.K., & Thatoi, H.N. (2014) Biocatalysis and Agricultural Biotechnology Diversity, mechanism and biotechnology of phosphate solubilising microorganism in mangrove—A review. *Biocatal. Agric. Biotechnol*, 3, 97–110

Béliveau, A., Davidson, R., Lucotte, M., Do Canto Lopes, L. O., PaqueT, S., & Vasseur, C. (2014). Early effects of slash-and-burn cultivation on soil physicochemical properties of small-scale farms in the Tapajós region, Brazilian Amazon. *The Journal of Agricultural Science*, 153(02), 205–221.

Bhadauria, T., & Ramakrishnan, P. S. (1989). Earthworm population dynamics and contribution to nutrient cycling during cropping and fallow phases of shifting agriculture (Jhum) in North-East India. *The Journal of Applied Ecology*, v. 26, n. 2, p. 505-520.

Bhattacharyya, P.N. & Jha, D.K. (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture.*World J Microbio Biotechnol* 28, 1327–1350.

Binarani, R. K. & Yadava, P.S. (2010). Effect of Shifting Cultivation on Soil Microbial Biomass C, N and P under the Shifting Cultivations Systems of Kangchup Hills, Manipur, North- East India. *J Exp Sci* Vol. 1, Issue 10, Pages 14-17.

Blake, G. R. (1965). Bulk Density. Agronomy Monograph.Blum, W. E. H. (1993). Soil protection concept of the Council of Europe and integrated soil research. *In Eijsackers, H. J. P. and Hamers, T. (eds.) Integrated Soil and Sediment Research: A Basis for Proper Protection. Kluwer Academic Publisher, Dordrecht. pp. 37–47*

Borggaard, O.K., Gafur A. & Petersen L. (2003). Sustainability appraisal of shifting cultivation in the Chittagong Hill Tracts of Bangladesh. *Ambio.* 32, 118-123.

Borthakur, D. N. (1992). Agriculture of north eastern region. *Guwahati: BeeCee* Prakashan.

Borthakur, D.N., Singh, A., Awasthi, R.P. & Rai, R.N. (1978). Shifting cultivation in the north-eastern region. In: Resources Development and Environment in the Himalayan Region. Dep. Sci. Tech., Govt. of India, pp. 330--342.

Bouma, J. (2002). Land quality indicators of sustainable land management across scales.*Agric. Ecosyst. Environ. 88, 129–136*

Bowman, M.G., & Tabatabai, M, A. (1978). Phosphodiesterase activity of soils. *Soil Science Society of America Journal* 42: 284-290.

Bremer, E., Janzen, H. H.& Johnston, A. M. (1994). Sensitivity of total, light fraction and mineralizable organic matter to management practices in a Lethbridge soil. Canandian *Journal of Soil Sciences*. 74: 131-138.

Bringezu, S., Schütz, H., Pengue, W., O'Brien, M., Garcia, F., Sims, R., Howarth, R.W., Kauppi, L., Swilling, M., & Herrick, J. (2014). Assessing global land use: balancing consumption with sustainable supply. *UNEP*.

Brown, D.R. (2006). Personal preferences and intensification of land use: their impact on southern Camaroonian slash-and-burn agroforestry systems. *Agrofor. Syst*. 68, 53–67.

Bruun, T. B., Mertz, O., & Elberling, B. (2006). Linking yields of upland rice in shifting cultivation to fallow length and soil properties. *Agriculture, Ecosystems & Environment*, v. 113, n. 1-4, p. 139-149, (2006).

Bruun, T.B., de Neergaard, A., Lawrence, D. and Ziegler, A.D. (2009). Environmental consequences of the demise in swidden cultivation in Southeast Asia: carbon storage and soil quality. *Hum Eco*, 37:375-388

Burket, J., & Dick, R. (1998). Microbial and soil parameters in relation to N mineralization in soils of diverse genesis under differing management systems. *Biol Fertil Soils* 27, 430–438.

Burns, G.R., DeForest, L.J., Marxsen, J., Sinsabaugh, L.R., Stromberger, E.M., Matthew D., Wallenstein, D.M., Michael, N., Weintraub, N.M., & Zoppini.A. (2013). Soil enzymes in a changing environment: Current knowledge and future directions. *Soil Biology and Biochemistry, 58, 216–234*

Burton, J., Chen, C., Xu, Z., & Ghadiri, H. (2010). Soil microbial biomass, activity and community composition in adjacent native and plantation forests of subtropical Australia. *Journal of Soils and Sediments* 10, 1267–1277.

Buschbacher, R., Uhl, C., & Serrao, E.A.S. (1988). Abandoned pastures in eastern Amazonia. II. Nutrients stocks in the soil and vegetation. *Journal of Ecology* 76, 682–699.

Cairns M., & Garrity D.P. (1999). Improving shifting cultivation in Southeast Asia by building on indigenous fallow man-agement strategies. *Agrofor Syst* 47:37–48

Cairns, M. (2004). Fuel and Fertility: Alder's Role in Land Use Intensification. Panel Discussion Presentation on Shifting Cultivation {Jhum): Policy Imperatives for North East India. July 7 (Reproduced by LEAD-India and ICIMOD).

Cappuccino, J. G., & N Sherman, N. (1992). Microbiology Laboratory Manual

Cardoso, E.J.B.N Rafael., Vasconcellos, L.F., Bini, D., *et al*. (2013). Soil health: looking for uitable indicators. What should be considered to assess the effects of use and management on soil health?. Sci. Agric. v.70, n.4, p.274-289

Casida, L.E., Klein, D.A., & Santoro, T. (1964). Soil dehydrogenase activity. *Soil Science* 98: 371-376..

Castellanos, J., Jaramillo, V. J., Sanford Jr., R. L., & Kauffman, J. B. (2001). Slash-and-burn effects on fine root biomass and productivity in a tropical dry forest ecosystem in México. Forest Ecology and Management, v. 148, n. 1-3, p. 41-50.

Chabot, R., Beauchamp, C. J., Kloepper, J. W., & Antoun, H. (1998). Effect of phosphorus on root colonization and growth promotion of maize by bioluminescent mutants of phosphate-solubilizing Rhizobium leguminosarum biovar phaseoli. *Soil Biology and Biochemistry*, 30(12), 1615–1618.

Chaiharn, M. & Lumyong, S. (2008). Phosphate solubilization potential and stress tolerance of rhizobacteria from rice soil in Northern Thailand. World *Journal of Microbiology and Biotechnology*. 25. 305-314.

Chakkaravarthy, V. M., Arunachalam, R., Vincent, S., Paulkumar, K. & Annadurai, G. (2010). Biodegradation of tricalcium phosphate by phosphate solubilizing bacteria. *Journal of Biological Sciences*. 10 (6): 531 -535.

Chaparro, J. M., Badri, D. V., &Vivanco, J. M. (2014). Rhizosphere microbiome assemblage is affected by plant development. *ISME J.* 8, 790–803.

Charana Walpola, B., & Yoon, M.-H. (2013). Phosphate solubilizing bacteria: Assessment of their effect on growth promotion and phosphorous uptake of mung bean (Vigna radiata [L.] R. Wilczek). *Chilean Journal of Agricultural Research*, 73(3), 275–281.

Chatterjee, A., Vance, G. F., Pendall, E., & Stahl, P. D. (2008). Timber harvesting alters soil carbon mineralization and microbial community structure in coniferous forests. *Soil Biology and Biochemistry* 40, 1901–1907.

Chawngthu, L., Hnamte, R., & Lalfakzuala, R. (2020). Isolation and Characterization of Rhizospheric Phosphate Solubilizing Bacteria from Wetland Paddy Field of Mizoram, India. *Geomicrobiology Journal, 1–10.*

Chen, S., Arrouays, D., Angers, D. A., Martin, M. P., & Walter, C. (2018). Soil carbon stocks under different land uses and the applicability of the soil carbon saturation concept. *Soil and Tillage Research.*

Chen, Y.P., Rekha, P.D., Arun, A.B., Shen, F.T., Lai, W.A., & Young, C.C. (2006). Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Applied Soil Ecology* 34: 33-41.

Chilima, J., Huang, C. Y., & Wu, C. F. (2002). Microbial biomass carbon trends in black and red soils under single straw application: Effect of straw placement, mineral N addition and tillage. *Pedosphere* 121: 5-72.

Choudhury, D. (1998). Conserving Genetic Heterogeneity in North East India: Indigenous Knowledge Systems and Opportunities for Sustainable Development. *North East India: The Human Interface.* Gyan Publishing House, New Delhi, pp. 147- 162.

Christanty, L., Mailly, D., & Kimmins, J. P. (1996). Without bamboo, the land dies: Biomass, litterfall, and soil organic matter dynamics of a Javanese bamboo talun-kebun system. *Forest Ecology and Management*, 87(1-3), 75–88.

Chun, J., & Rainey, FA. (2014.) Integrating genomics into the taxonomy and systematics of the Bacteria and Archaea. *Int J Syst Evol Micr* 64:316–324

Clarridge J. E., 3rd. (2004). Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clinical microbiology reviews*, *17*4, 840–862.

Condron, L.M., B.L. Turner, & B.J. Cade-Menun. (2005). Chemistry and dynamics of soil organic phosphorus. *Agron. Monogr. 46.* p. 87–121

Conklin, H. C. (1957). *Hanunoo agriculture, FAD Forestry Development Paper No. 12, FAD, Rome*, 109 pp.

Conklin, H. C. (1961). The study of shifting cultivation. *Current Anthropology*, v. 2, n. 1, p. 27-61.

Cosgrove, D. J. (1967). Metabolism of organic phosphates in soil. *Soil Biochem.* 216-228, pp 216–228

Craswell, E.T., Sajjapongse, A., Howlett, D.J.B., & Dowlin, g A.J. (1997). Agroforestry in the management of sloping lands in Asia and the Pacific. *Agrofor Syst* 38:121–13

Cunningham, R. K. (1963). The effect of clearing a tropical forest soil, *J. Soil Sci*., 14,334- 335.

Curd, E. E., Martiny, J. B. H., Li, H., & Smith, T. B. (2018). *Bacterial diversity is positively correlated with soil heterogeneity. Ecosphere, 9(1),*

Curtis, T.P., Sloan, W.T.& Scannell, J.W. (2002). Estimating prokaryotic diversity and its limits. *Proc. Natl Acad Sci U S A.* 99(16):10494-10499

Dalal, R.C. (1977). Soil organic phosphorus. *Adv Agron* 29:83–117

Darlong, V.T. (2001). Community Based Fire Management: Tools and Techniques of the Mizos in India. In: Shifting Cultivation: Towards Sustainability and Resource Conservation in Asia. *International Institute of Rural Reconstruction*, Philippine (IIRR), pp. 86-92.

Das, S. K., and Varma, A. (2010). Role of Enzymes in Maintaining Soil Health. *Soil Biology, 25–42.*

Datta, M.P., Sengupta, R., Pandit, C., Banerjee, M.K., & Samiran. (2011). Plant growth promoting rhizobacteria enhance growth and yield of chilli Capsicum annuum L. under field conditions. *Australian Journal of Crop* Science. 5.

Davies, P.J. (1995). Plant Hormones: Physiology, Biochemistry, and Molecular Biology. Kluwer Academic Publishers, Netherlands

De Mandal, S., Zothansanga, Lalremsanga, H. T., & Kumar, N. S. (2015). Bacterial diversity of Murlen National Park located in Indo-Burman Biodiversity hotspot region: A metagenomic approach. *Genomics Data, 5, 25–26.*

Debano, L. F., & Conrad, C. E. (1978). The Effect of Fire on Nutrients in a Chaparral Ecosystem. *Ecology*, 59(3), 489–497.

DeBano, L.F. (1991). The effect of fire on soil properties. In: Harvey, Alan E.; Neuenschwander, Leon F., compilers. Proceedings-management and productivity of western-montane forest soils; 1990 April 10-12;

Debell, D. S., & Ralston, C. W. (1980). Release of nitrogen by burning light forest fuels; Soil Sci. Soc. *Am. Proc. 34 936-938.*

Deka, H. K. & Mishra, R. R. (1983). The effect of slash burning on soil microflora. Plant and Soil 73: 167-175.

Dick, R.P., (1994). Soil enzyme activities as indicators of soil quality. In: J.W. Doran, D.C. Coleman, D.F. Bezdicek and B.A. Stewart *(*Editors*)*, Defining Soil Quality for a Sustainable Environment. ASA and SSSA Spec. Publ., Madison, WI, in press.

Dick, R. P., Sandor, J. A., & Eash, N. S. (1995). Soil enzyme activities after 1500 years of terrace agriculture in the Colca Valley, Peru. *Agriculture, Ecosystems and Environment, 502, 123–131.*

Dick, R.P. (1997). Soil Enzyme Activities as Integrative Indicators of Soil Health. - In: Pankhurst, C. E., Doube, B. M., Gupta, V.V.S.R. eds. *Biological Indicators of Soil Health*, CABI Publishing. USA.

Dick, R.P., Rasmussen, P.E., & Kerle, E.A., (1988). Influence of long-term residue management on soil enzyme activities in relation to soil chemical properties of a wheat-fallow system. *Biol. Fertil. Soils*, 6:159-164.

Dick R.P., Rasmussen P.E. & Kerle E.A. (1998). Influence of long-term resides management on soil enzyme activity in relation to soil chemical properties of a wheat-fallow system. *Biology and Fertility of Soils* 6:159-164.

Dick, W. A. & Tabatabai, M.A. (1992). Significance and potential uses of soil enzymes. In: Meeting FBJr (ed) Soil microbial ecology. Marcel Dekker, New York, Basel, Hong Kong, pp 95–127.

Dkhar, M.S. & Mishra, R.R. (1987). Microbial population, fungal biomass and CO_2 evolution in maize (*Zea mays* L.) field soils. *Plant Soil* 99, 277–283.

Dodgson K. S., White G., & Fitzgerald J. W. (1982) Sulfatase Enzymes of Microbial Origin, Vol. 1. CRC Press, Florida.

Dodor, D.E., & Tabatabai, M.A., (2003). Amidohydrolases in soils as affected by cropping systems. *Appl. Soil Ecol*. 24, 73–90.

Doran, J. W. (2002). Soil health and global sustainability: translating science into practice. *Agriculture, Ecosystems & Environment*, 88(2), 119–127.

Doran, J.W., & Parkin, T.B. (1994). Defining and assessing soil quality. Pages 3-21, In: Doran, J.W.; Coleman, D.C; Bezdieck, D.F; and Stewart, B.A. (Editors), Defining Soil Quality for a Sustainable Environment, *SSSA Special Publication* No. 35. Soil Science Society of America, Madison, WI, USA.

Doran, J.W., & Parkin, T.B. (1996). Quantitative indicators of soil quality: a minimum data set. Pages 25-37, In: Doran, J.W. and Jones, A.J. (Editors), Methods for Assessing Soil Quality. *SSSA Special Publicatio*n No. 49, Soil Science Society of America, Madison, WI, USA

Dunn, P. H., Barro, S. C. & Poth, M. (1985). Soil moisture affects survival of microorganisms in heated chaparral soil. *Soil Biology and Biochemistry* 17: 143-148.

Dyer, M.L., Meentemeyer, V., & Berg, B. (1990). Apparent controls of mass loss of leaf littermass regional scale. *Scandinavian Journal of Forest Research* 5:312-323

Eastmond, A., & Faust, B. (2006). Farmers, fires, and forests: a green alternative to shifting cultivation for conservation of the Maya forest. *Landsc Urban Plan* 74:267–284

Eckholm, E. P. (1976). Losing ground: environmental stress and world food prospects; New York, N.Y. Norton and Company

Eden, M. J., & Andrade, A., (1987). Ecological aspects of swidden cultivation among the Andoke and Witoto Indians of the Colombian Amazon. *Human Ecology,* v. 15, n. 3, p. 339-359.

Egger, K. (1981). Eco farming in the tropics - characteristics and potentialities. *Plant Research and Development,* v. 13, n. 1, p. 96-106.

Eisen, J.A. (1995). The RecA protein as a model molecule for molecular systematic studies of bacteria: comparison of trees of RecAs and 16S rRNAs from the same species. *Journal of Molecular Evolution* 41: 1105-1123.

Eivazi, F., & Tabatabai, M.A. (1977). Phosphatases in soils. Soil Biology and Biochemistry 9:167-172.

Elias, F., Woyessa, D., & Muleta, D. (2016). Phosphate Solubilization Potential of Rhizosphere Fungi Isolated from Plants in Jimma Zone, Southwest Ethiopia. *International Journal of Microbiology, (2016), 1–11.*

Ellingson, L. J., Kauffman, J. B., Cummings, D. L., Sanford Jr., R. L., & Jaramillo, V. J. (2000). Soil N dynamics associated with deforestation, biomass burning, and pasture conversion in a Mexican tropical dry forest. *Forest Ecology and Management,* v. 137, n. 1-3, p. 41-51.

El-Yazeid, A. A., Abou-Aly. H. E., Mady, M. A. and Moussa, S. A. M. (2007). Enhancing growth, productivity and quality of squash plants using phsosphate dissolving microorganisms (Biophos-phor) combined with boron foliar spray. Research *Journal of Agriculture and Biological Sciences.* 3(4):274-286.

Espinosa-Victoria, D., Lopez-Reyes, L. &Cruz Benitez, A.D. (2009) Use of 16S rRNA gene for characterization of phosphate solubilizing bacteria associated with com. Rev. *Fitotec Mex* 32(1): 31-37

Ewel, J., C. Berish, B. Brown, N. Price, & J. Raich. (1981). Slash and burn impacts on a Costa Rican wet forest site, Ecology, 62, 816 – 829.

Fankem, H., Nwaga, D., Deubel, A., Dieng, L., Merbach, W.& Etoa, F.X. (2006). Occurance and functioning of phosphate solubilizing microorganisms from oil palm tree (Elaeis guineensis) rhizosphere in Cameroon. *African Journal of Biotechnology*. 5(24): 2450-2460.

FAO. (2018). The State of Food Security and Nutrition in the World. Building climate resilience for food security and nutrition. Global Food Insecurity Report. Rome.

FAO. (2018). *The State of Agricultural Commodity Markets (2018). Agricultural trade, climate change and food security. Rome*.

Felsenstein, J. (2004). Inferring phylogenies. Sunderland MA: Sinauer Associates

Fenchel, T.& Finlay B.J. (2006). The diversity of microbes: resurgence of the phenotype. *Phil. Trans. R. Soc*. B.361:1965–1973

Findlay, S. E. G., Sinsabaugh, R. L., Sobczak, W. V., & Hoostal, M. (2003). Metabolic and structural response of hyporheic microbial communities to variations in supply of dissolved organic matter. *Limnology and Oceanography*, 48(4), 1608–1617.

Foley, J.A. (2005). Global consequences of land use. *Science* 309, 570–574

Fox, J. (2000). How Blaming 'slash and burn' Farmers is Deforesting Mainland Southeast Asia. *Asia-Pacific Issues,* East-West Center, Honolulu, Hawaii

Fox, J., Truong D.M., Rambo A. T., Tuyen N. P., Cuc L.T., & Leisz S. (2000). Shifting Cultivation: A New Old Paradigm for Managing Tropical Forests, *BioScience*, Volume 50, Issue 6, June, Pages 521–528

Fox, T.R, & Comerford, N.B. (1992). Rhizosphere phosphatase activity and phospohatase hydrolyzable orgamc phosphorus m two forested spodosols. *Soil Biol Biochem,* 24: 579-583

Frankenberger, W.T., & Dick. W.A., (1983). Relationships between enzyme activities and microbial growth and activity indices in soil. *Soil Sci. Soc. Am. J*. 47,945-951.

Franzluebbers, A, J., Hons FM. & Zuberer. D.A. (1995). Tillage induced seasonal changes in soil physical properties affecting soil C02 evolution under intensive cropping. *Soil Till.* Res. 34:41-60.

Furtak, K., Grządziel, J., Gałązka, A., & Niedźwiecki, J. (2019). Analysis of Soil Properties, Bacterial Community Composition, and Metabolic Diversity in Fluvisols of a Floodplain Area. *Sustainability* 11, 3929.

Garcia C., Hernandez T., & Roldan A (1997). Changes in microbial activity after abandonment of cultivation in a semiarid Mediterranean environment. *Journal of Environmental Quality* 26: 285-291.

García, C., Hernández, T., Costa, C., Ceccanti, B., Masciandaro, G., & Ciardi, C. (1993). A study of biochemical parameters of composted and fresh municipal wastes. *Bioresource Technology*, 441, 17–23.

Garcia-oliva, F., Sanford Jr., R. L., & Kelly, E. (1999). Effects of slash-and-burn management on soil aggregate organic C and N in a tropical deciduous forest. *Geoderma*, v. 88, n. 1-2, p. 1-12.

Garland, J.L., & Mills, A.L. (1994). A community-level physiological approach for studying microbial communities. In: Beyond the Biomass (Ritz K. Dighton J. Giller K.E., Eds.), pp. 77–83. John Wiley and Sons, Chichester.

Ghosh, A.B., Bajaj, J.C., Hasan, R., & Singh, D. (1983). Soils and water testing methods. IARI, New Delhi pp.66.

Giardina, C. P. Sanford JR., R. L., & Døckersmith, I. C. (2000). Changes in soil phosphorus and nitrogen during slash-and-burn clearing of a dry tropical forest. *Soil Science Society of America Journal, v. 64, n. 1, p. 399-405.*

Giovannini, G., Lucchesi, S., & Giachetti M. (1990) Effects of heating on some chemical parameters related to soil fertility and plant growth. *Soil Science* 149, 344350.

Gispert, M., Emran, M., Pardini, G., Doni, S., & Ceccanti, B. *(*2013). *The impact of land management and abandonment on soil enzymatic activity, glomalin content and aggregate stability. Geoderma,* 202-203, 51–61.

Glick, B.R. (1995). The enhancement of plant growth by free living bacteria, *Canadian Journal of Microbiology* 41: 109-114.

Goldstein, A. H. (1986). Bacterial solubilization of mineral phosphates: Historical perspective and future prospects. *American Journal of Alternative Agriculture,* 1(02)

Goldstein, A. H. (2003). Future trends in research on microbial phosphate solubilization: one hundred years of insolubility. *Developments in Plant and Soil Sciences*, 91–96. doi:10.1007/978-1-4020-5765-6_11

Granada, C. E., Passaglia, L. M. P., de Souza, E. M., and Sperotto, R. A. (2018). Is phosphate solubilization the forgotten child of plant growth-promoting rhizobacteria? *Front. Microbiol.* 9:2054.

Grant, C., Bittman, S., Montreal, M., Plenchette, C., & Morel, C. (2005). Soil and fertilizer phosphorus: Effects on plant P supply and mycorrhizal development. *Canadian Journal of Plant Science, 85(1), 3–14.*

Greaves, M. P., & Wilson, M. J. (1970). The degradation of nucleic acids and montmorillonite-nucleic-acid complexes by soil microorganisms. *Soil Biology and Biochemistry, 24, 257–268.*

Gregorich, E. G., Carter, M. R., Angers, D. A., MonrealL, C. M., & Ellert, B. H. (1994). Towards a minimum data set to assess soil organic-matter qualityin agricultural soils. *Canadian Journal of Soil Science*. 74:367-385.

Greipsson, S., & El-Mayas, H. (1999). *Large-scale reclamation of barren lands in Iceland by aerial seeding. Land Degradation & Development, 10(3), 185–193.*

Groffman, M. P., Driscoll, T.C., Fahey, J.T., Hardy, P.J., Fitzhugh D.R., & Tierney, L.G., (2001) Colder soils in a warmer world: A snow manipulation study in a northern hardwood forest ecosystem. *Biogeochemistry* 56, 135–150.

Groffman, M. P., Hardy, J., Fashu-Kanu, S., Driscoll, C., Cleavitt, N., Fahey, T., & Fisk, M. (2011). Snow depth, soil freezing and nitrogen cycling in a northern hardwood forest landscape. *Biogeochemistry,* 102, 223–238

Grogan, P., Lalnunmawia, F., & Tripathi, S.K. (2012). Shifting cultivation in steeply sloped regions: a review of management options and research priorities for Mizoram state, Northeast India. *Agrofor. Syst*. 84 (2), 163–177.

Grzadziel, J., & Gałazka, A. (2018). Microplot long-term experiment reveals strong soil type influence on bacteria composition and its functional diversity. *Appl. Soil Ecol. 124*, 117–123

Gulati, A., Rahi, P., & Vyas, P. (2007). Characterization of phosphate solubilizing fluorescent *Pseudomonas* from the rhizosphere of sea buckthorn growing in the cold desert of Himalayas. Current Microbiology 56: 73-79.

Gupta, G., Parihar, S.G., Ahirwar, K.N., K.S., & Vinod Singh, V. (2015). Plant Growth Promoting Rhizobacteria PGPR: Current and Future Prospects for Development of Sustainable Agriculture. *Journal of Microbial Biochemical Technology, 0702.*

Gupta, N., Sabat, J., & Parida, R. (2007). Solubilization of tricalcium phosphate and rock phosphate by microbes isolated from chromite, iron and manganese mines. *Acta Botanica Croatica* 66: 197-204.

Gyaneshwar, P., Kumar, G.N., Parekh, L.J., & Poole, P.S. (2002). Role of soil microorganisms in improving P nutrition of plants. *Plant and Soil*, vol. 245, no. 1, pp. 83–93.

Hall, B.G. (2011). Phylogenetic trees made easy: a how-to manual. 4th ed. Sunderland MA: Sinauer Associates.

Hall, B.G. (2013). Building Phylogenetic Trees from Molecular Data with MEGA, *Molecular Biology and Evolution*, Volume 30, Issue 5, May (2013), Pages 1229–1235

Halstead R.L., & McKercher R.B. (1975). Biochemsitry and cycling of phosphorus. In: Paul EA, McLaren AD eds *Soil biochemistry, vol* 4. Marcel Dekker, New York, pp 31–63.

Handelsman, J., Rondon, M.R., Brady, S.F., Clardy, J., & Goodman, R.M. (1998) Molecular biological access to the chemistry of unknown soil microbes: A new frontier for natural products. *Chem. Biol. 5*, 245–249.

Hargreave,s P.R., Brookes, P.C,. Ross, G.J.S. & Poultion P.R. (2003). Evaluating soil microbial biomass carbon as an indicator of ong-term environmental changes. *Soil Biology & Biochemistry*. 35:401-407.

Harris, D. R. (1971). The ecology of swidden cultivation in the upper Orinoco rain forest, Venezuela. The Geographical Review, v. 61, n. 4, p. 475-495.

Hartemink, A. E. (1998). Soil chemical and physical properties as indicators of sustainable landmanagement under sugar cane in Papua New Guinea.*Geoderma85,283–306.*

Hassink, J. (1994). Effects of soil texture on the size of the microbial biomass and on the amount of C mineralized per unit of microbial biomass in Dutch grasslands soils. *Soil Biology and Biochemistry 26:* 1573-1581.

Hattori, D., Sabang, J., Tanaka, S., Kendawang, J. J., Ninomiya, I., & Sakurai, K. (2005). Soil characteristics under three vegetation types associated with shifting cultivation in a mixed dipterocarp forest in Sarawak, Malaysia. *Soil Science & Plant Nutrition*, v. 51, n. 2, p. 231-241.

Hattori, T., Inanaga, S., Araki, H, An, P., Morita, S., Luxová, M., & Lux, A. (2005) Application of silicon enhanced drought tolerance in Sorghum bicolour. *Physiol Plant* 123:459–466.

Havlin, J., Beaton, J., Tisdale, S.L., & Nelson, W. (1999). Soil fertility, and fertilizers. An introduction to nutrient management. Prentice Hall, Upper Saddle River, NJ

Hermans, S. M., Buckley, H. L., Case, B. S., Curran-Cournane, F., Taylor, M., & Lear, G. (2016). *Bacteria as emerging indicators of soil condition. Applied and Environmental Microbiology, AEM.02826–16.*

Hilda, R., & Fraga, R. (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advance* 17(4-5): 319-339.

Hiraoka, M., & Yamamoto, S. (1980). Agricultural development in the upper Amazon of Ecuador. *Geographical Review*, v. 70, n. 4, p. 423-445.

Hoffman, J., Bezchlcbova, J., Dusck, L., Dolczal, L., Holoubek, I., Andel P. & Ansorgova Maly S. (2003). Novel approach to monitoring of soil biological quality. *Environmental International*. 28:771-778.

Holscher, D., Moller, R.F., Denich, M., & Folster, H. (1997). Nutrient input-output budget in shifting-agriculture from Eastern Amazonia. *Nutr cycl agroecosys* 47: 49-57.

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Stanley, J. T., & Williams, S. T. (1994). Bergey's Manual of Determinative Bacteriology, 9th ed. Williams Wilkins

Igual, J. M., A. Valverde, E. Cervantes & E. Velázquez. (2001). Phosphate-solubilizing bacteria asinoculants for agriculture: use of updated molecular techniques in their study. *Agronomie*.21:561-568

Insam, H. (2001). Developments in soil microbiology since the mid-1960s. *Geoderma*, 100(3-4), 389–402

Iqbal, M., Wagi, S., & Ahmed, A. (2017). Phyllospheric bacterial treatments improve growth in *Helianthus annuus* L. RADS *Journal of Biological Research and Applied Sciences*. 91:2521–8573.

Islam, M. K., Sano, A., Majumder, M. S. I., Hossain, M. A., & Sakagami, J. I. (2019). Isolation and molecular characterization of phosphate solubilizing filamentous fungi from subtropical soils in Okinawa. *Appl. Ecol. Env. Res*. 17, 9145–9157.

J.R. Archer & P.D. Smith. J. (1972). The relation between bulk density, available water capacity and air capacity of soils: *Soil Sci.*23 (4), 475–480

Jacoby, R., Peukert, M., Succurro, A., Koprivova, A., & Kopriva, S. (2017). *The Role of Soil Microorganisms in Plant Mineral Nutrition—Current Knowledge and Future Directions.* Frontiers in Plant Science, 8.

Jaiyebo, E. O., & Moore, A. W. (1963). Soil Nitrogen Accretion under Different Covers in a Tropical Rain-Forest Environment. *Nature*, 197(4864), 317–318.

Janda, J. M., & Abbott, S. L. (2007). 16S rRNA Gene Sequencing for Bacterial Identification in the Diagnostic Laboratory: Pluses, Perils, and Pitfalls. *Journal of Clinical Microbiology*, 459, 2761–2764.

Jeffries, Peter & Gianinazzi, Silvio & Perotto, Silvia & Turnau, Katarzyna & Barea, Jose. (2003). The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biology and Fertility of Soils. 37. 1-16.

Jha, D.K.; Sharma, G.D. & Mishra, R.R. (1992). Soil microbial population numbers and enzyme activities in relation to altitude and forest degradation. *Soil Biology and Biochemistry*, v.24, p.761-767.

Jha, L. K. (1997) Shifting Cultivation. *APH Publishing Corporation, New Delhi.* pp.1-38.

Jha, L.K., & Lalnunmawia, F. (2003) Agroforestry with bamboo and ginger to rehabilitate degraded areas in North East India. *J Bamboo Rattan* 2:103–109

Jha, M. N., Pande, P., & Pathak, T. C. (1979). Studies on the changes in the physio-chemical properties of Tripura soils as a result of Jhuming. *Indian For.* 105, 436-443.

Joachim, A. W. R., & Kandiah, S. (1948). The efect of shifting (Chena) cultivation and subsequent regeneration of vegetation on soil composition and structure; *Trop. Agric. 104 3-11*

Johnson, C.M., Vieira I.C.G., Zarin, D.J., Frizano, J., & Johnson, A.H. (2001). Carbon and nutrient storage in primary and secondary forests in eastern Amazonia. *For. Ecol. Manag.,* 147(2-3): 245-252

Jordan, C.F. (1989). An Amazonian Rain Forest. The Structure and Function of a Nutrient Stressed Ecosystem and the Impact of Slash-and-Burn Agriculture. *Man and Biosphere Series*. The Parthenon Publishing Group, Lancs, UK.

Joshi, S, R., Bareh, D., & Banerjee, A. (2019). Soil Microbiota and Sustainable Jhum. *Agroecosystem Microbial Diversity in Ecosystem Sustainability and Biotechnological Applications*

Juo, A.S.R., & Manu A., (1996). Chemical dynamics in slash-and-burn agriculture. *Agricul. Ecosyst. Envion*. 58(1): 49-60

Kalayu, G., (2019). Phosphate Solubilizing Microorganisms: Promising Approach as Biofertilizers. *International Journal of Agronomy*, Article ID 4917256, 7

Kang, B. T., & Sajjapongse, A. (1980). Effect of heating on properties of some soils from Southern Nigeria and growth of rice. *Plant and Soil, 55(1), 85–95.*

Karaca A., Cetin S.C., Turgay O.C., & Kizilkaya R. (2011). Soil enzymes as indication of soil quality. In: Shukla G., Varma A. eds.: *Soil Enzymology. Springer-Verlag, Berlin, Heidelberg*, 119–148

Karlen, D.L., Mausbach, M.J., Doran, J.W., R.G. Cline, Harris, R.F., & Schuman, G.E. (1997). Soil quality: A concept, definition, and framework for evaluation. *Soil Sci. Soc. Am. J. 61:4-10*

Karpagam, T., & Nagalakshmi P.K. (2014). Isolation and characterization of Phosphate Solubilizing Microbes from Agricultural soil. *Int.J. Curr.Microbiol. App.Sci* 3(3): 601-614

Kato M.S.A., Kato O.R., Denich M., & Vlek P.L.G. (1999). Fire-freealternatives to slash-and-burn for shifting cultivation in theeastern Amazon region: the role of fertilizers. *Field Crops Research* 62:225–237

Kauffman, J., Cummings, D., Ward, D., & Babbitt, R. (1995a). Fire the Brazilian Amazon: I. Biomass, nutrient pools, and losses in slashed primary forests. *Oecologia* 104:397–408.

Kauffman, J., Sanford Jr, R., Cummings, D., Salcedo, I., & Sampaio, E. (1995). 1993. Biomass and nutrient dynamics associated with slash fires in neotropical dry forests. *Ecology* 74:140–151.

Kendawang, J. J., Tanaka, S., Shibata, K., Yoshida, N., Sabang, J., Ninomiya, I., and Sakurai, K. (2005). Effects of shifting cultivation on soil ecosystems in Sarawak, Malaysia. III. Results of burning practice and changes in soil organic matter at Niah and Bakam experimental sites. *Soil Sci. Plant Nutr*. 51, 515–523.

Kennedy, A. C., & K. L. Smith. (1995). Soil microbial diversity and the sustainability of agricultural soils. Plant Soil. 170: 75-86.

Kennedy, A.C.& R.I. Papendick (1995). Microbial characteristic of soil quality. *Soil Water Conserv.*, 50 (3): 243-248.

Kennedy, A.C., & Papendick, R.I. (1995). Microbial characteristics of soil quality. J. *Soil Water Conserv*. 50: 243-248.

Ketterings, Q. M., Wibowo, T. T., Noordwijk, M., & Penot, E. (1999). Farmers' perspectives on slash-and-burn as a land clearing method for small-scale rubber producers in Sepunggur, Jambi Province, Sumatra, Indonesia. *Forest Ecology and Management*, v. 120, n. 1-3, p. 157-169, (1999).

Khan, M. S & Zaidi, A. (2007). Synergistic effects of the inoculation with plant growth-promoting rhizobacteria and an arbuscular mycorrhizal fungus on the performance of wheat. *Turk J Agric* 31:355–362

Khan, M.S., Zaidi, A. & Wani, P.A. (2007). Role of phosphate-solubilizing microorganisms in sustainable agriculture—a review. *Agron Sustain Develop* 27: 29–43.

Khan, S.W. (1970). Enzymatic activity in a gray wooded soil as influenced by cropping systems and fertilizers. *Soil Biol. Biochem.* 2: 137-139.

Khan, V., Jilani, M. S., Akhtar1, S. M. Naqvi S., &. Rasheed, M. (2009) Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. *Journal of Agricultural and Biological Science*, vol. 1, pp. 48–58.

Khiangte, L. & Lalfakzuala, R. (2017). *In vitro* production of growth regulator (IAA) and phosphatase by phosphate solubilizing bacteria. *Science & Technology Journal. Vol.* 5 (1): 33-35

Kiss, S., Dragan-Bularda, M. & Radulescu, D. (1975). Biological significance of enzymes accumulated in soil. *Adv Agron* 27:25-87

Kiss, S.M., Dragan-Bularda, M. & Radulescu, D. (1975). Biological significance of enzymes accumulated in soil. *Advance in Agronomy*. 27: 25-87.

Kleinman, P. J., Pimentel, D., & Bryant, R. B. (1995). The ecological sustainability of slash-and-burn agriculture. *Agriculture, Ecosystems & Environment*, v. 52, n. 2-3, p. 235-249.

Klose, S., & Tabatabai, M. A. (1999). Urease activity of microbial biomass in soils. *Soil Biology and Biochemistry, 31(2), 205–211*

Knietsch, A., Waschkowitz, T., Bowien, S., Henne, A., & Daniel R. (2003). Metagenomes of complex microbial consortia derived from different soils as sources for novel genes conferring formation of carbonyls from short-chain polyols on Escherichia coli. *J Mol Microbiol Biotechnol*. 5(1):46-56.

Koch, A., McBratney, A.B., Adams, M., Field, D.J., Hill, R., Lal, R., Abbott, L., Angers, D., Baldock, J., Barbier, E., Bird, M., Bouma, J., Chenu, C., Crawford, J., Flora, C.B., Goulding, K., Grunwald, S., Jastrow, J., Lehmann, J., Lorenz, K., Minansy, B., Morgan, C., O'Donnell, A., Parton, W., Rice, C.W., Wall, D.H., Whitehead, D., Young, I.& Zimmermann, M., (2013). Soil Security: Solving the Global Soil Crisis. *Global Policy, 4(4), 434–441*

Konstantinidis, K.T., Ramette, A., & Tiedje, J.M. (2006). Toward a more robust assessment of intraspecies diversity, using fewer genetic markers. *Appl Environ Microbiol* 72: 7286–7293

Kozdrój, J. (2013). Metagenome—A new source of information about soil microorganisms. *Post˛epy Mikrobiologii. 52*, 185–200. (In Polish)

Kpomblekon, A.K., & Tabatabai, M.A. (1994). Effect of organic acid on release of phosphorus from phosphate rock. *Soil Science* 158: 442- 453.

Krishnaraj, U., & Dahale, S. (2014). Mineral Phosphate Solubilization: Concepts and Prospects in Sustainable Agriculture. *Proc Indian Natn Sci Acad* 80 No. 2 Spl. Sec. pp. 389-40

Kucey, R. M. N. (1983). Phosphate-solubilizing bacteria and fungi in various cultivated and virgin alberta soils. *Canadian Journal of Soil Science,* 63(4), 671–678.

Kucey, R. M. N., Janzen, H. H., & Leggett, M. E. (1989). Microbially Mediated Increases in Plant-Available Phosphorus. *Advances in Agronomy* v 42, 199–228.

Kumada, K., Ohta, S., Arai, S., Kitamura, M., & Imai, K. (1985). Changes in soil organic matter and nitrogen fertility during the slash and burn and cropping phases in experimental shifting cultivation; *Soil Sci. Plant Nutr. 31 611-623*

Kumar A., Maurya B. R., Raghuwanshi R. (2014). Isolation and characterization of PGPR and their effect on growth, yield and nutrient content in wheat *Triticum aestivum* L.. *Biocatal. Agric. Biotechnol.* 3 121–128.

Kumar, A., Bhargava, P., & Rai, L.C. (2010). Isolation and molecular characterization of phosphate solubilizing *Enterobacter* and *Exiguobacterium* species from paddy fields of Eastern Uttar Pradesh, India. *African Journal of Microbiology Research* 4(9): 820-829.

Kumar, A., Kumar, A., & Patel H. (2018). Role of microbes in phosphorus availability and acquisition by plants. *International Journal of Current Microbiology and Applied Sciences*, vol. 7, no. 5, pp. 1344–1347, (2018).

Kurehekar J.V. (2005). Effect of some agrochemicals on physico chemical and microbial parameters of soil and sustainable farming. *Nature Environment and pollution Technology*. 4: 381-384.

Lal, R. (1997). Residue management, conservation tillageand soil restoration for mitigating greenhouse effect by CO2 enrichment. *Soil Tillage Res. 43: 81–107.*

Lal, R. (2010). Enhancing Eco-efficiency in Agro-ecosystems through Soil Carbon Sequestration. *Crop Science, 50(Supplement_1), S–120*

Larson, W. E., & F. J. Pierce. (1993). The dynamics of soil quality as a measure of sustainability. Proceedings of a Symposium on Soil Quality, ASA annual meeting, November 1-6, 1992, Minneapolis, MN

Lawrence, D., & Foster D., (2002). Changes in forest biomass, litter dynamics and soils following shifting cultivation in Southern Mexico: an overview. *Interciencia*, v. 27, n. 8, p. 400-408,

Lawrence, D., Suma, V., & Mogea, J. P. (2005). Change in species composition with repeated shifting cultivation: limited role of soil nutrients. *Ecological Applications*, v. 15, n. 6, p. 1952-1967.

Lawrence. D., & Schlesinger, W.H. (2001) Changes in soil phosphorus during 200 years of shifting cultivation in Indonesia. *Ecology* 82:2769–2780

Lean, C., & Maclaurin, J. (2016*).* The Value of Phylogenetic Diversity. *Topics in Biodiversity and Conservation, 19–37.*

Leghari, S. J., Buriro, M., Jogi, Q., Kandhro, M. N., & Leghari, A. J. (2016). Depletion of phosphorus reserves, a big threat to agriculture: challenges and opportunities. *Sci. Int.* 28, 2697–2702.

Lemaire, G., Franzluebbers, A., Carvalho, P.C., & Dedieu, B. (2014). Integrated crop-livestock system: Strategies to achieve synergy between agricultural production and environmental quality. *Agric. Ecosyst. Environ. 190*, 4–8.

Lessa, A., Anderson, D., & Moir, J. (1996) Fine root mineralization, soil organic matter arid exchangeable cation dynamics in slash and burn agriculture in the semi-arid northeast of Brazil. Agric. Ecosyst. Environ. 59, 191–202.

Li, P., Zhang, T., Wang, X., & Yu, D. (2013). Development of biological soil quality indicator system for subtropical China. *Soil Tillage Res.126*, 112–118

Li, Q., Lee A.H., & Wollum, I. (2004). Microbial biomass and bacterial functional diversity in forest soils: effects of organic matter removal, compaction, and vegetation control. *Soil Biology and Biochemistry* 36: 571-579.

Liu, D., Lian, B., & Dong, H., (2012). Isolation of Paenibacillus sp. and assessment of its potential for enhancing mineral weathering. *Geophys. J. R. Astron. Soc*. 29, 413421

Liu, J., Cade-Menun, B. J., Yang, J., Hu, Y., Liu, C. W., Tremblay, J., LaForge k., Schellenberg, M., Hamel., C., & Bainard, L. D. (2018). Long-Term Land Use Affects Phosphorus Speciation and the Composition of Phosphorus Cycling Genes in Agricultural Soils. *Frontiers in Microbiology, 9.*

Liu, J., Hu, Y., Yang, J., Abdi, D., &Cade-Menun, B. J. (2015). Investigation of soil legacy phosphorus transformation in long-term agricultural fields using sequential fractionation, P K-edge XANES and solution P NMR spectroscopy. *Environ. Sci. Technol.* 49, 168–176.

Lungmuana, Singh, S. B., Choudhury, B. U., Vanthawmliana, Saha, S., & Hnamte, V. *(2019).* Transforming jhum to plantations: Effect on soil microbiological and biochemical properties in the foot hills of North Eastern Himalayas, India. *CATENA*, 177, 84–91.

Lynch, J.M. (1985). Origin, nature and biological activity of aliphatic substances and growth hormones found in soil. In: Vaughan, D., Malcom, R. E. Eds. *Soil Organic Matter and Biological Activity.* Martinus Nijhoff /Dr. W. Junk Publishers. Dordrecht, Boston, Lancaster. pp. 151-174

Maharjan, M., Sanaullah, M., Razavi, B. S., & Kuzyakov, Y. (2017). Effect of land use and management practices on microbial biomass and enzyme activities in subtropical top-and sub-soils. *Applied Soil Ecology,* 113, 22–28.

Malboobi, M. A., Behbahani, M., Madani, H., Owlia, P., Deljou, A., Yakhchali, B., Moradi, M. &Hassanabadi, H. (2009). Performance evaluation of potent phosphate solubilizing bacteria in potato rhizosphere. *World Journal of Microbiology and Biotechnology*, 25(8), 1479–1484.

Malcolm, R.E. (1983). Assessment of phosphatase activity in soils. *Soil Biol Biochem* 15: 403 – 408

Mander, C., Wakelin, S., Young, S., Condron, L., & O'Callaghan, M. (2012). Incidence and diversity of phosphate-solubilising bacteria are linked to phosphorus status in grassland soils. *Soil Biology and Biochemistry,* 44(1), 93–101.

Mapa, R.B., & Kumaragamage, D. (1996). Variability of soil properties in a tropical Alfisol used for shifting cultivation. *Soil Technol*. 9, 187–197.

Martínez, J. R. R. (1968). Organic phosphorus mineralization and phosphatase activity in soils. *Folia Microbiologica, 132, 161–174.*

Mathews, J. (2003) *The Encyclopaedic Dictionary of Environmental Change. Oxford University Press Inc.*, New York. pp.12,363,578.

Matson P. A. Vitousek P. M. Ewe1 J. J., Mazzarino M. J., & Robertson G. P. (1987). Nitrogen transformations following tropical forest fefling and burning on a volcanic soil. *Ecology 68, 491-502.*

McDonald, M. A., Healey, J. R., & Stevens, P. A. (2002). The effects of secondary forest clearance and subsequent land-use on erosion losses and soil properties in the Blue Mountains of Jamaica. *Agriculture, Ecosystems & Environment,* v. 92, n. 1, p. 1-19.

McGarity, J.W. and Myers, M.G. 1967. A survey of urease activity in soil of northern New South Wales. *Plant Soil.* 27:217-238.

McGill, W.B., & Cole, C.V. (1981). Comparative aspects of cycling of organic C, N, S, and P through soil organic matter. *Geoderma* 26:267-286

Mcgrath, D. G. (1987). The role of biomass in shifting cultivation. *Human Ecology*, v. 15, n. 2, p. 221-242, 1987.

Mehrvarz, S., Chaichi, M.R., & Alikhani, H.A. (2008). Effects of phosphate solubilizing microorganisms and phosphorus chemical fertilizer on yield and yield components of barely (*Hordeum vulgare L.*). *American-Eurasian Journal of Agricultural and Environmental Sciences* 3: 822-828.

Mehta, S., & Nautiyal, C.S. (2001). An efficient method for qualitative screening of phosphate- solubilizing bacteria. *Current Microbiology* 43: 51-56.

Mendoza-Vega, J., & Messing, I. (2005). The influence of land use and fallow period on the properties of two calcareous soils in the humid tropics of southern Mexico. *Catena, v. 60, n. 3, p. 279-292.*

Mertz, O. (2002). The relationship between length of fallow and crop yields in shifting cultivation: a rethinking. *Agrofor. Syst*, 55(2):149-159

Mertz, O., Padoch, C., Fox, J., Cramb, R. A., Leisz, S. J., Lam, N. I., & Vien, T. D. (2009). Swidden change in Southeast Asia: understanding causes and consequences. *Human Ecology*, v. 37, n. 3, p. 259-264.

Metzger, J.P., (2003). Effects of slash-and-burn fallow periods on landscape structure. *Environmental Conservation* 30. pp. 325–333

Miah, S., Dey, S., & Sirajul Haque, S. M. (2010). Shifting cultivation effects on soil fungi and bacterial population in Chittagong Hill Tracts, Bangladesh. *Journal of Forestry Research*, 21(3), 311–318.

Mikánova, O., & Nováková, J (2002). Evaluation of the P-solubilizing activity of soil microorganisms and its sensitivity to soluble phosphate. *Rostlinná Výroba* 48:397-400.

Mishra, B. K., & Ramakrishnan, P. S. (1983). Slash and burn agriculture at higher elevations in North-Eastern India. I. Sediment, water and nutrient losses. *Agriculture, Ecosystems & Environment, 9(1), 69–82*

Misra, R. (1968). Ecology Work Book. Oxford and IBH Publishing Company, Calcutta

Mokhopadhyay, S. (2000). Shifting Cultivation: From Knowledge to Unsustainability; Can Agroforestry Remediate it. *Agroforestry and Forest Products. Proceedings of International Workshop,* Aizawl, November 28-30, pp. 15-24.

Molla, M.A.Z., Chowdhury, A.A., Islam, A., & Hoque, S. (1984). Microbial mineralization of organic phosphate in soil. Plant Soil 78, 393–399.

Moore, J., Klose, S. & Tabatabai, M. (2000). Soil microbial biomass carbon and nitrogen as affected by cropping systems. *Biol Fertil Soils* 31, 200–210.

Moussa, A,.S. Rensburg, L.V., Kellner, K. & Bationo A. (20070. Soil microbial biomass in semi-arid communal sandy rangelands in the western Bophirima district, South Africa. *Applied Ecology and Environmental Research.* 5(1): 43-56.

Mukherjee, H.N. (1954). Improvement of soil fertility by the application of heat. Proc. Bihar Acad. *Agric. Sci.* 21-30

Myers, M. G., and McGarity, J. W. (1968). The urease activity in profiles of five great soil groups from northern New South Wales. *Plant and Soil,* 281, 25–37.

Nafis, A., Raklami, A., Bechtaoui, N., Khalloufi, F. E., Alaoui, A. E., Glick, B. R., et al. (2019). Actinobacteria from extreme niches in morocco and their plant growth-promoting potentials. *Diversity* 11:139.

Nambiar, K. K., Gupta, A., Fu, Q. and Li, S. (2001). Biophysical, chemical and socio-economic indicators for assessing agricultural sustainability in the Chinese coastal zone. *Agriculture, Ecosystems & Environment, 87(2), 209–214*

Nandwa, S. M., & Bekunda, M. A. (1998). Research on nutrient flows and balances in East and Southern Africa: state-of-the-art. *Agric Ecosyst Environ*, v. 71, n. 1-3, p. 5-18, (1998).

Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G. and Renella, G. (2003). Microbial diversity and soil functions. *Eur. J. Soil Sci.54*, 655–670

Nautiyal, C.S. (1999). An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiology Letters 170: 265-270.

Neal, A. L., Rossmann, M., Brearley, C., Akkari, E., Guyomar, C., Clark, I. M., … Hirsch, P. R. (2017). Land-use influences phosphatase gene microdiversity in soils. *Environmental Microbiology*, 19(7), 2740–2753.

Neal, A.L., Blackwell, M., Akkari, E., Guyomar, C., Clark, I., & Hirsch, P.R. (2017). Phylogenetic distribution, biogeography and the effects of land management upon bacterial non-specific Acid phosphatase Gene diversity and abundance. *Plant Soil.* 427, 175–189

Neergaard, A., Magid, J., & Mertz, O. (2008). Soil erosion from shifting cultivation and other smallholder land use in Sarawak, Malaysia. *Agriculture, Ecosystems & Environment*, v. 125, n. 1-4, p. 182-190.

Nei, M., & Kumar S. (2000) Molecular Evolution and Phylogenetics. Oxford University Press, New York.

Nenwani, V., Doshi, P., Saha, T. and Rajkumar, S. (2010). Isolation and characterization of a fungal isolate for phosphate solubilization and plant growth promoting activity. *Journal of Yeast and Fungal Research.* 1(1): 9-14.

Nesme, T., Metson, G. S., & Bennett, E. M. (2018). *Global phosphorus flows through agricultural trade. Global Environmental Change, 50, 133–141.*

Norman, M. J. T., Pearson, C. J., & Searle, P. G. E. (1995) Tropical food crops in their Environment. *Cambridge University Press, Great Britain* 2:5-11

Nye, P. H., & Greenland, D. J. 1964. Changes in the soil after clearing tropical forest. *Plant and Soil, 21(1), 101–112.*

Obale-Ebanga, F., Sevink, J., de Groot, W., Nolte, C., (2003). Myths of slash and burn on physical degradation of savannah soils: impacts on Vertisols in north Cameroon. Soil Use Manage. 19, 83–86.

Ocham, H, Lerat, E., & Daubm, V. (2005). Examining Bacterial Species under the Specter of Gene Transfer and Exchange. *Proc Natl Acad. Sci*, USA, Vol 102 Pp 6595-6599

Okore, I. K., Tijani-eniola, H., Agboola, A. A., Aiyelari, E. A. (2007). Impact of land clearing methods and cropping systems on labile soil C and N pools in the humid zone Forest of Nigeria. Agriculture, Ecosystems & Environment, v. 120, n. 2-4, p. 250-258.

Okur, N. (2018). A review: bio-fertilizers- power of beneficial microorganisms in soils. *Biomed. J. Sci. Tech. Res.* 4: BJSTR. MS.ID.001076.

Olsen, S.R., Cole, C.V., Watanabe, F.S., & Dean, L.A. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate, US Government Printing Office, USDA Washington, D.C. Circ. pp. 939

Omar, S.A. (1998). The role of rock phosphate solubilizing fungi and vesicular-arbuscular mycorrhizae VAM in the growth of wheat plant fertilized with rock phosphate. *World J Microbiol Biotechnol* 14;211-8.

Osborne, C. A., Galic, M., Sangwan, P., & Janssen, P. H. (2005). PCR-generated artefact from 16S rRNA gene-specific primers. *FEMS Microbiology Letters, 2482, 183–187.*

O'Toole P., Morgan M.A., & McGarry S.J. (1985). A comparative study of urease activities in pasture and tillage soils. Communications in Soil Science and Plant Analysis 16: 759-773.

Oya, K., & Tokashiki. J. (1984). Soil fertility in a shifting ciltivation on Iromote Island of Okinawa I. Nutrients stored in the forest and their input to the soil; *Jpn. J. Trop. Agric. 28 218-222*

Pace, N.R. (1997). A molecular view of microbial diversity and the biosphere. *Science*. 276(5313):734-740.

Pal, S.S. (1998). Interactions of an acid tolerant strain of phosphate solubilizing bacteria with a few acid tolerant crops. *Plant and Soil* 198, 169–177.

Palm, C. A., Swift, M. J., & Woomer, P. L. (1996). Soil biological dynamics in slash-and-burn agriculture. *Agric Ecosyst Environ*, v. 58, n. 1, p. 61-74.

Palma R.M., & Conti M.E. (1990). Urease activity in Argentine: Field studies and influence of sample treatment. Soil Biology and Biochemistry 22: 105-108.

Pancholy S.K., & Rice E.L. (1973). Soil enzymes in relation to old-field succession: amylase, cellulase, invertase, dehydrogenase and urease. Soil Science Society of America Jouma/37: 47-50.

Pandey, A., Chaudhry, S., Sharma, A., Choudhary, V. S., Malviya, M. K., Chamoli, S., … Palni, L. M. S. (2010). Recovery of Bacillus and Pseudomonas spp. from the "Fired Plots" Under Shifting Cultivation in Northeast India. *Current Microbiology*, 62(1), 273–280

Pang P.C.K. & Kolenko H. (1986). Phosphomonoesterase activity in forest soils. *Soil Biology and Biochemistry,* 18:35-40.

Park, J., Bolen, N., Megharaj, M., & Naidu, R. (2010). Isolation of phosphate solubilizing bacteria and characterization of their effects on lead immobilization. *Journal of Hazardous Materials* 1852-3: 829-836

Park, J.H., Bolan, N., Megharaj, M., & Naidu, R. (2011) Isolation of phosphate solubilizing bacteria and their potential for lead immobilization in soil. *J Hazard Mater* 186:829–836.

Paul, E.A., & Clark, F.E. (1988). Soil microbiology and biochemistry. Academic, San Diego

Pedroso, Jr, N.N., Murrieta, R.S.S., & Adams, C. (2009). Slash-and-burn agriculture: a system intransformation. *Current Trends in Human Ecology*. p.12-34

Peix, A., Mateos, P. F., Rodríguez-Barrueco, C., Martínez-Molina, E., & Velázquez, E. (2001). Growth promotion of common bean (*Phaseolus vulgaris* L.) by a strain of Burkholderia cepacia under growth chamber conditions. *Soil Biol. Biochem*. 33, 1927–1935.

Peix, A., Rivas, R., Mateos, P.F., Martínez-Molina, E., Rodríguez- Barrueco, C., & Velázquez, E. (2003). *Pseudomonas rhizosphaerae* sp. nov., a novel species that actively solubilizes phosphate in vitro. *International Journal of Systematic and Evolutionary Microbiology* 53: 2067-2072.

Peix, A., Rivas, R., Santa-Regina, I., Mateos, F.P., Martı´nez-Molina, E., Claudino Rodrı´guez-Barrueco1 & Vela´zquez, E. 2 (2004) Pseudomonas lutea sp. nov., a novel phosphate-solubilizing bacterium isolated from the rhizosphere of grasses. *International Journal of Systematic and Evolutionary Microbiology*, 54, 847–850

Peix, A., Velazquez, E., & Martinez-Molina, E. (2007). Molecular methods for biodiversity analysis of phosphate solubilizing microorganisms (PSM), *Development of Plant and Soil Sciences 1*02: 97-100.

Pichu, R. (1989). Free-living bacterial inoculate for enhancing crop productivity. *Trends in Biotechnology* 7: 39-44.

Pierce, F. J., & Gilliland, D. C. (1997). *Chapter 9 Soil quality control. Developments in Soil Science, 203–219.*

Pikovskaya, R.I. (1948). Mobilization of phosphates in soil in connection with vital activity of some microbial species. *Microbiologia* 17: 362-370

Piper, C.S. (1944). *Soil and Plant Analysis*. New York

Popenoe, H. (1959). The influence of the shifting cultivation cycle on soil properties in Central America. Proc. 9th Pacific Sci. Gong, 7, 148-160.

Potthoff, M. Jorgensen, R., Dormann, G., Wachendorf, C., Richter, K. & Formowitz, B. (2008). Ecological soil microbiology. University of Kassel.

Powlson, D. S. and Jcnkinson, D. S. (1976). The cflirts of biocidal trcatmcnts on metabolism in soil. II. Gamma radiation. autoclaving. air-drying and fumigation. *Soil Biology and Biochemistry* II, 179 – 188

Powlson, D. S., Brookes P. C. and Christensen, B. T. (1987). Measurement of soil microbial biomass provides an early indication of changes in total soil organic matter due to straw incorporation. *Soil Biology and Biochemistry* 19, 159-164.

Prabhu N., Borkar S., Garg. (2018). Phosphate solubilization mechanisms in alkaliphilic bacterium *Bacillus marisflavi* FA7. *Current Science,* VOL.114, NO. 4.

Prasanna, A., Deepa, V. Murthy, P.B., Deecaraman, M., Sridhar, R. &Dhandapani, P. (2011). Insoluble phosphate solubilization by bacterial strains isolated from rice rhizosphere soils from Southern India. *International Journal of Soil Sciences.* 6(2): 134-141.

Quail, M.A., Smith, M., Coupland, P. Otto, D. T., Harris, S. R., Connor, R, T., Bertoni, A., Swerdlow, P, H.& Gul, Y. (2012). A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers. *BMC Genomics* 13, 341.

Quiquampoix, H., & Mousain. D. (2005). Enzymatic hydrolysis of organic phosphorus. In: *Turner BL, Frossard E, Baldwin DS eds Organic phosphorous in the environment.* CABI, Wallingford, pp 89–112

Raison, R.J., Khanna, P.K., & Woods, P.V. (1985). Transfer of elements to the atmosphere during low intensity prescribed fires in three Australian sub-alpine eucalypt forest. *Can. J. For. Res*., 15: 657-664

Ralte, V., Pandey, H. N., Barik, S. K., Tripathi, R. S., & Prabhu, S. D. (2005). Changes in microbial biomass and activity in relation to shifting cultivation and horticultural practices in subtropical evergreen forest ecosystem of north-east India. *Acta Oecologica*, 28(2), 163–172.

Ramakrishnan, P. S., & Toky, O. P. (1981). Soil nutrient status of hill agro-ecosystems and recovery pattern after slash and burn agriculture (Jhum) in north-eastern India. *Plant and Soil, 60(1), 41–64.*

Ramakrishnan, P.S. (1992). Shifting agriculture and sustainable development: an interdisciplinary study from north-eastern India Parthenon, Paris

Ramakrishnan, P.S. (1993). Shifting agriculture and sustainable development: an interdisciplinary study from north-eastern India. *Unesco and Oxford University Press*; ρ 424

Rao D.L.N. (2017) Microbial and Biochemical Origins of Soil Organic Matter: Insights from History and Recent Ecological and Bio-molecular Advances. *Souvenir 82nd Annual Convention and National Seminar of Indian Society of Soil Science, Kolkata, Dec11*-14, 2017, pp. 77-89.

Rao, D.N.L.& Ghai, S.K. (1985). Urease and dehydrogenase activity of alkali and reclaimed soils. *Aus. J. Soil Res.* 23: 661-665.

Rastogi, G., & Sani, R. K. (2011). Molecular Techniques to Assess Microbial Community Structure, Function, and Dynamics in the Environment.*Microbes and Microbial Technology, 29–57.*

Rastogi, G., & Sani, R. K. (2011). *Molecular Techniques to Assess Microbial Community Structure, Function, and Dynamics in the Environment. Microbes and Microbial Technology, 29–57.*

Reichle, D.E. (1997): The role of Soil invertebrates in nutrient cycling, Soil organisms as Components of ecosystems. Ed. V. Lohm, and T. Person, Swedish Natural Science Research Council, Stockholm.

Reid, M.F., & Fewson, C.A. (1994). Molecular characterization of microbial alcohol dehydrogenases. *Crit Rev Microbiol*. 20(1):13-56.

Reid, W.V. (1994). Setting objectives for conservation evaluation. *Systematics Association Special* Vol. No. 50. Oxford: Clarendren Press.

Rerkasem, K., & Rerkasem, B. (1995). Montane mainland south-east Asia: *Agroecosystems in transition. Global Environmental Change 5:313-322.*

Rerkasem, K., Lawrence, D., Padoch, C., Schmidt-Vogt, D., Ziegler, A. D., & Bruun, T. B. (2009). Consequences of Swidden Transitions for Crop and Fallow Biodiversity in Southeast Asia. *Hum Ecol* 37, 347–360.

Reza S. K., Baruah U., Nath D.J., Sarkar D. & Gogoi D. (2014). Microbial biomass and enzyme activity in relation to shifting cultivation and horticultural practices in humid subtropical North-Eastern India. *Range Manag. Agrofor*; 35: 78-84

Ribeiro Filho, Alexandre Antunes, Adams, Cristina, Murrieta, & Rui Sergio Sereni. (2013). The impacts of shifting cultivation on tropical forest soil: a review. *Boletim do Museu Paraense Emílio Goeldi. Ciências Humanas, 8(3), 693-727.*

Rice, E. L. (1974). Allelopathy (New York: Academic Press)

Richardson, A. (2001). Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Functional Plant Biology. 28. 897-906. 10.1071/PP01093.

Richardson, A.E. (1994). Soil microorganisms and phosphorus availablility. *'Soil biota: management in sustainable farming systems*. (Eds CE Pankhurst, BM Doube, VVSR Gupta, PR Grace) pp. 50–62

Riesenfeld, C, S, Schloss, P, D., & Handelsman, J. (2004) Metagenomics: genomic analysis of microbial communities. *Annu Rev Genet.;*38:525-52.

Riesenfeld, C.S., Schloss, P.D., & Handelsman, J. (2004). Metagenomics: genomic analysis of microbial communities. *Annu Rev Genet*.38:525-552.

Rivas, R., Trujillo, M.E., Sánchez, M., Mateos, P.F., Martínez-Molina, E. 7 Velásquez, E. (2004) *Microbacterium ulmi* sp. Nov. a xilanolytic, phosphate-solubilizing bacterium isolated from sawdust of *Ulmus nigra*. *International Journal of Systematic and Evolutionary Microbiology* 54: 513-517.

Robe, P., Nalin, R., Capellano, C., & Vogel, T.M. (2003). Extraction of DNA from soil. Eur. J. Soil Biol. 39:183–190.

Roder, W., Phengchanh, S.& Keoboulapha, B. (1995). Relationships between soil, fallow period, weeds and rice yield in slash-and-burn systems of Laos. *Plant and Soil*, v. 176, n. 1, p. 27-36.

Roder, W., Phengchanh, S., & Maniphone, S. (1997). Dynamics of soil and vegetation during crop and fallow period in slash-and-burn fields of northern Laos. *Geoderma*, v. 76, n. 1-2, p. 131-144.

Rodriguez, H., & Fraga, R. (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advances* 17: 319-339.

Rodriguez, H., Gonzalez, T., & Selman, G. (2000). Expression of a mineral phosphate solubilizing gene from *Erwinia herbicola* in two rhizobacterial strains. *Journal of Biotechnology* 84: 155-161.

Roesch, L.F., Fulthorpe, R.R., Riva A, Casella, G., Hadwin, A.K.M., Kent, D.A., Daroub, S.H., Camargo, F.A.O., Farmerie, W.G., & Triplett.E.W. (2007). Pyrosequencing enumerates and contrasts soil microbial diversity. *ISME J*.1 (4):283-290.

Rogers H. T. (1942). Dephosphorylation of organic phosphorus compounds by soil catalysts. *Soil Sci.* 54,439-445.

Romanyà, J., Khanna, P. K., & Raison, R. J. (1994). Effects of slash burning on soil phosphorus fractions and sorption and desorption of phosphorus. *Forest Ecology and Management, 65(2-3), 89–103.*

Ross D. J., Speir T. W., Tate K. R., Cairns A., Meyrick K. F. & Pansier E. A. (1982) Restoration of pasture after topsoil removal: effects on soil carbon and nitrogen mineralization, microbial biomass and enzyme activities. Soil Biology & Biochemistry 14, 575-581.

Ross, D. J., Speir, T. W., Kettles, H. A., & Mackay, A. D. (1995). Soil microbial biomass, C and N mineralization and enzyme activities in a hill pasture: Influence of season and slow-release P and S fertilizer. *Soil Biology and Biochemistry,* 2711, 1431–1443.

Sabhasri, R, P. J., & Kunstadter, S. 1978. P. Soil fertility aspects of the Lau' forest fallow system of shifting cultivation. *Farmers in the forest: economic development and marginal agriculture in Northern Thailand*. Honolulu: The University Press of Hawaii, 1978. p. 134-159.

Saha, R., Mishra, V. K., *&* Khan, S. K. *(*2011). Soil Erodibility Characteristics Under Modified Land-Use Systems as Against Shifting Cultivation in Hilly Ecosystems of Meghalaya, India. *Journal of Sustainable Forestry*, 30(4), 301–312.

Sahin F., Cakmakci R., Kanta F. (2004). Sugar beet and barley yields in relation to inoculation with N2-fixing and phosphate solubilizing bacteria. *Plant Soil* 265 123–129

Sahoo, U. K., Singh, S. L., Gogoi, A., Kenye, A., & Sahoo, S. S. (2019). Active and passive soil organic carbon pools as affected by different land use types in Mizoram, Northeast India. *PLOS ONE, 14(7).*

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4(4):406-425.

Saiyad, S.A., Jhala, Y.K., & Vyas, R.V., (2015). Comparative efficiency of five potash and phosphate solubilizing bacteria and their key enzymes useful for enhancing and improvement of soil fertility. *Int. J. Sci. Res. Pub*. 5, 16.

Sakurai, K., Okabayashi, Y., Tanaka, S., Watanabe, E., Boonyanuphap, J., & Nouanthasing, L. (2005). Can shifting cultivation on acid soils in the Southeast Asia be sustainable in the future? A case study in Northern Laos. *Soil Science & Plant Nutrition*, v. 51, n. 5, p. 767-770.

Sanchez, P. A. (1977). Properties and Management of Soils in the Tropics. *Soil Sci.*, 124(3), 187

Saplalrinliana, H., Thakuria, D., Changkija, S. & Hazarika, S. (2016). Impact of shifting
cultivation on litter accumulation and properties of jhum soil of North East India. *J. Indian Soc. Soil Sci*. 64, 402–413.

Sarathchandra, S. U., & Perrott, K. W. (1981). Determination of phosphatase and arylsulphatasc activities in soils. *Soil Biology and Biochemistry, 136, 543–545.*

Sarawgi, S. K., Tiwari P. K., & Tripathi, R. S. (1999). Uptake and balance sheet of nitrogen and phosphorus in gram (*Cicer arietinum*) as influenced by phosphorus, biofertilizers and micronutrients under rainfed condition. Indian J. Agron. 44: 768–772

Sarnath, S. & Singh, J.S. (1 9 9 5). Microbial biomass associated with water stable aggregates in forest, savanna and cropland soils o f a seasonally dry tropical region, India. Soil Biol. Biochem. 27:1027-1033.

Satyaprakash, M., Nikitha, T., Reddi, E. U. B., Sadhana, B., & Vani S. S. (2017) "A review on phosphorous and phosphate solubilising bacteria and their role in plant nutrition," *International Journal of Current Microbiology and Applied Scences*, vol. 6, pp. 2133–2144

Saunders, G. W. (1957). Interrelations of dissolved organic matter and phytoplankton. *Rot. Rev. 23: 389-409*

Schmidt G., & Laskowski, M.S.K. (1961). Phosphate ester cleavage survey. In the enzymes P. D. Boyer. H. Lard, and K. Myrback. Ed. 2nd edn. pp. 3 35. Academic Press. New York.

Schnürer, J., Clarholm, M., & Rosswall, T. *(*1985). *Microbial biomass and activity in an agricultural soil with different organic matter contents. Soil Biology and Biochemistry, 17(5), 611–618.*

Schofield, R. K.&Taylor, A. W. 1955. *The Measurement of Soil pH1. Soil Science Society of America Journal, 19(2), 164.*

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., & Huttenhower, C. (2011) Metagenomic biomarker discovery and explanation. *Genome Biol*. 2011 Jun 24;12(6).

Sharma, G. D. (1981). Effect of fire on soil microorganisms in a Meghalaya pine forest. *Folia Microbiologica* 26: 321-327.

Sharma, K., Dak, G., Agrawal, A., Bhatnagar, M. & Sharma, R. (2007). Effect of phosphate solubilizing bacteria on the germination of *Cicer arietinum* seeds and seedling growth. J. *Herb Med. Toxicol.* 1: 61-63.

Sharma, S. B., Sayyed, R. Z., Trivedi, M. H., & Gobi, T. A. (2013). Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *SpringerPlus, 2(1), 587*.

Shih-Yung, H. (2010). IAA production by Streptomyces scabies and its role in plant microbe interaction. Msc thesis, Cornell University

Sillitoe, P., & Shiel, R. S. (1999). Soil fertility under shifting cultivation in the Southern Highlands of Papua New Guinea. *Soil Use and Management*, v. 15, n. 1, p. 49-55.

Sivasakthivelan, P., & Saranraj, P. (2013). *Azospirillum* and its formulations: a review. *Inter. J. Microbiol. Res.* 4, 275–287.

Smith, J. L., & Paul, E. A. (1990). The significance of soil biomass estimations. In *'Soil Biochemistry'*. Vol. 6. Eds J. M. Bollog and G. Stotzky. pp. 357–96. Marcel Dekker: New York

Smith, W. H., Bormann, F. H., & Likens, G. E. (1968). Response of chemo trophic nitrofiers to forest cutting; Soil Sci. 106 471-473

Song, O.R., Lee, S.J., Lee Y.S., Lee, S.C., Kim, K.K., & Choi, Y.L. (2008). Solubilization of insoluble inorganic phosphate by *Burkholderia cepacia* DA 23 isolated from cultivated soil. Brazilian Journal of Microbiology 39: 151-156

Sparks, D. L. (1987). Potassium Dynamics in Soils. *Advances in Soil Science, 1–63.*

Sparling, G. P., Shepherd, T. G. and Kettles, H. A. (1992). Changes in soil organic C, microbial C and aggregate stability under continuous maize and cereal cropping, and after restoration to pasture in soils from the Manawatu region, New Zealand. Soil Tillage Res. 24: 225–241.

Speir, T.W., & Ross, D.J. (1978). Soil phosphatase and sulphatase. In: Burns RG ed Soil enzymes. Academic Press, London, pp 197 – 250.

Speir, T.W., & Ross, D.J. 1978. Soil phosphatase and sulphatase. In: *Burns RG ed Soil Enzymes. Academic Press, London*, pp 197-250.

Spencer, J.E. (1966). Shifting cultivation in SouthEastern Asia. *University of California Press, Berkeley.*

Sperber, J,I, (1958) The incidence of apatite-solubilizing organisms in the rhizosphere and soil. *J Agric Res* 9:778–781.

Srivastava S.C. & Singh, J.S. (1991). Microbial biomass C, N and P in dry tropical forest soils - Effect of alternate landuses and nutrient flux. Soil Biol. Biochem. 23:1 17-124.

Srivastava S.C. & Singh J.S. (1991): Microbial C, N and P in dry tropical forest soils: effects of alternate land uses and nutrient flux. *Soil Biology and Biochemistry*, 23: 117–124.

Stackebrandt, E., & Goebel, B. (1994). Taxonomic note: A place for DNA: DNA reassociation and 16s rRNA sequence analysis in the present spec.

Stephen, J., Shabanamol, S., Rishad K.S., Jisha M.S. (2015). Growth enhancement of rice *Oryza sativa* by phosphate solubilizing *Gluconacetobacter* sp. MTCC 8368 and *Burkholderia* sp. MTCC 8369 under greenhouse conditions. *Biotechnology* 5: 831- 837

Stevenson, F.J. (2005). Cycles of Soil: Carbon, Nitrogen, Phosphorus, Sulfur, Micronutrients. John Wiley and Sons, New York. pp. 231-284.

Stott, D. E., & Hagedorn, C. (1980). Interrelations between selected soil characteristics and arysulfatase and urease activities. *Soil Science and Plant Analysis* 11, 935-955.

Stromgaard, P. (1992). Immmediate and long-term effects of fire and ash fertilization on a Zambian miombo woodland soil. *Agric Ecosyst Environ* 41:19–3

Subbarao, N.S. (1988). Phosphate solubilizing microorganism. In: Biofertilizer in agriculture and forestry. *Regional Biofert. Dev. Centre, Hissar, India*. pp. 133-142.

Subbiah, B. V., & Asija, G. L., (1956). A rapid procedure for the estimation of available nitrogen in soils. *Current Sei. 25, 259.*

Suliasih &Widawati, S. (2005). Isolation and identification of phosphate solubilizing and nitrogen fixing bacteria from soil in Wamena biological garden, Jayawijaya, Papua. *Biodiversitas.* 6(3): 175-177.

Sundara, B., Natarajan, V., & Hari, K. (2002). Influence of phosphorus solubilizing bacteria on the changes in soil available phosphorus and sugarcane and sugar yields. *Field Crops Research* 77: 43-49.

Sundara, Rao, W. V. B., & Sinha, M. K. (1963). Phosphate dissolving microorganisms in the soil and rhizosphere. *Indian J. Agr*. Sci.33, 272–278.

Swaby, R. & Sperber, J. I. (1959). Phosphate Dissolving Microorganisms in the Rhizosphere of Legume, Nutrition Of Legumes; Proc. Univ. Nottingham 5Th Easter Sch. Agril. Sci. (CSIRO Adelaide) *Soils & Fert.* 1958;22, 286. 289–294.

Swensen, B., & Bakken, L.R. (1998). Urease activity and nitrification potential in a mineral subsoil. *Soil Biol. Biochem.* 30: 1333–1341

T. Karpagam and P. K. Nagalakshmi. (2014). Isolation and characterization of Phosphate Solubilizing Microbes from Agricultural soil Int.J. *Curr.Microbiol. App.Sci* 3(3): 601-614

Tabatabai, M. A., & Bremner, J. M. (1970). *Arylsulfatase Activity of Soils1. Soil Science Society of America Journal, 34(2), 225.*

Tabatabai, M.A & Bremner, J.M. (1970). Arylsulfatase activity of soils. *Soil Sci Soc Am Proc* 34:225-229

Tabatabai, M.A. (1994). Soil enzymes. In: Weaver RW, Angle S, Bottomley P, Bezdicek D, Smith S, Tabatabai A, Wollum A eds Methods of soil analysis. Part 2. Microbiological and biochemical properties. *Soil Science Society of America,* Madison, pp 775–833

Tabatabai, M.A., & Bremner, J.M. (1969). Use of p-nitrophenyl phosphate for assay of soil Phosphatase activity. *Soil Biology and Biochemistry* 1: 301-307.

Tanaka, Y., Miyajima, T., & Ogawa, H., (2010). Effects of nutrient enrichment on the release of dissolved organic carbon and nitrogen by the scleractinian coral Montipora digitata. Coral Reefs 29:675–682.

Tarafdar J. C., Rao A. V., & Bala K. (1988) Production of phosphatases by fungi isolated from desert soils. *Folia Microbiologia* 33, 453-457.

Tarafdar, J., & Jungk, A. (1987). Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. *Biology and Fertility of Soils.* 3. 199-204.

Tarafdar, J.C., & Claassen, N. (1988). Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and microorganisms. *Biol Fert Soils* 5, 308–312.

Tawnenga, Shankar., U. & Tripathi., R. S (1996). Evaluating second year cropping on jhum fallows in Mizoram, north-eastern India—Phytomass dynamics and primary productivity; J. *Biosci*. 21 563-575.

Tawnenga, Shankar., U. & Tripathi., R. S. (1997). Evaluating second year cropping on jhum Fallows in Mizoram, north-eastern India: Energy and economic efficiencies; *J. Biosci*. 22 605-613 *(18).*

Tawnenga. (1990). Studies on ecological implications of traditional and innovative approaches to shifting cultivation in Mizoram, Ph D thesis, North-Eastern Hill University, Shillong.

Taylor, B.R., Parkinson, D., & Parsons, W.F.J. 1989. Nitrogen and lignin content as predictors of litter decay rates: a microcosm test. *Ecology* 70: 97-104.

Taylor, L.A., Arthur, M.A., & Yanai, R.D. (1999). Forest floor microbial biomass across a northern hardwood successional sequence. *Soil Biology and Biochemistry* 31: 431-439.

Theodorou, C. & Bowen, G. D. (1982). Effects of a bushfire on the microbiology of a South Australian low open (dry sclerophyll) forest soil. *Australian Forest Research* 12: 317-327.

Thomas, G.V., Shantaram, M.V. & Saraswathy, N. (1985). Occurrence and activity of phosphate-solubilizing fungi from coconut plantation soils. *Plant Soil* 87, 357–364.

Thomaz, E.L. (2009). The influence of traditional steep landagricultural practices on runoff and soil loss. *Agric EcosystEnviron* 130:23–30

Tilak, K.V.B., Ranganayaki, R.N., Pal, K.K., Desaxena, R., Nautiyal, A., Mittal, C., Tripathi, A.K., & Johri, B.N. (2005). Diversity of plant growth and soil health supporting bacteria. *Current Science, 89,* 136-150.

Tiwari B.K. (2001). Domestication of Three Non-Traditional Species by Shifting Cultivators of Northeast India. In: Shifting Cultivation: Towards Sustainability and Resource Conservation in Asia. *International Institute of Rural Reconstruction* (IIRR), Philippine, pp. 98-104

Tiwari S.C. (1988). Studies on microbial communities and their activities in soils of pineapple *(*Ananas comosus L.*)* plantations, Ph. D. Thesis, North-Eastern Hill University, Shillong, India.

Tiwari, M.B., Tiwari, B.K. & Mishra, R.R. (19899. Enzyme activity and carbon dioxide evolution from upland and wet land rice soils under three agricultural practices in hilly regions. *Biol. Fertil. Soils*. 7: 359-364.

Tiwari, V. K. & Rai, B. (1977). Effect of soil burning on microfungi. Plant and Soil 47: 693-697

Toky, O. P.& Ramakfishnan, P. S. (1983). Secondary succession following slash and burn agrieulture in north-eastern India I Biomass, litterfall and productivity; *J. Ecol. 71*

Toky, Q. P., & Ramakrishnan, P. S. (1981). Runoff and infiltration losses related to shifting agriculture (Jhum) in northeastern India. *Environmental Conservation*, 8, 313-321.

Toky, O. P., &Ramakrishnan, P. S. (1981) Cropping and yields in agricultural systems of the north-eastern hill region of India; *Agro-Ecosystems* 7 11-25

Tomar R. K. S., Namdeo K. N., & Ranghu J. S. (1996). Efficacy of phosphate solubilizing bacteria biofertilizers with phosphorus on growth and yield of gram (*Cicer arietinum*). Indian Journal of Agronomy 41: 412–415

Torsvik, V., & Øvreås, L. (2002). Microbial diversity and function in soil: from genes to ecosystems. *Curr Opin Microbiol*;5(3):240-245.

Trasar-Cepeda, M. C., & Gil-Sotres, F. (1988). *Kinetics of acid phosphatase activity in various soils of galicia NW Spain. Soil Biology and Biochemistry, 203, 275–280.*

Tripti, Vipin Kumar & Anshumali. (2012). Phosphate Solubilizing Activity of Some Bacterial Strains Isolated from Chemical Pesticide Exposed Agriculture Soil. *International Journal of Engineering Research and Developmen*t. 39:01-06

Turco, R. F., Kennedy, A. C., & Jawson, M. D. (1994). Microbial indi- cators of soil quality. In Defining Soil Quality for a Sustainable Environment. Eds. J W Doran, D C Coleman, D F Bezdicek and B A Stewart, pp 73-90. *American Society of Agronomy Special Publication* # 35. American Society of Agronomy, Madison

Uhl, C, (1987). Factors controlling succession following slash-and-burn agriculture in Amazonia. *J Ecol* 75:377±407.

Umesha, S., Singh, P. K., & Singh, R. P. (2018). Microbial biotechnology and sustainable agriculture in *Biotechnology for Sustainable Agriculture*, eds R. L. Singh and S. Monda Sawston: Woodhead Publishing, 185–205.

Upadhyay, S. & Jain, S. (2004). Community Forestry and Policy in Northeast India: An Historical Legal Analysis. *Community Forestry International*, Santa Barbara, California, USA

Valverde, A., Ingual, J.M., Cervantes, E. (2003). Polyphasic characterization of phosphate-solubilizing bacteria isolated from rhizospheric soil of the north-east region of Portugal. In: *Proceeding of First International Meeting of Microbial Phosphate Solubilization* pp. 273-276.

Van der Werf, G.R., Morton, D., Defries, R., Olivier, G.J.J., Kasibhatla, P.S., Jackson, R. B., Collatz, G.J., & Randerson, J.T. (2009). CO2 emissions from forests. *Nature Geoscience, 2.*

van Reenen, C. A., Visser, G. J. & Loos, M. A. (1992). Soil microorganisms and activities in relation to season, soil factors and fire. In: Van Wilgen, B. W., Richardson, D. M., Kruger, F. J. & Van Hensbergen, H. J. (eds). Fire in South African Mountain Fynbos. *Ecosystem, Community and Species Response at Swartboskloof. Springer-Verlag, Berlin*. pp. 258-272.

Van Vliet, N., Mertz, O., Heinimann, A., Langanke, T., Pascual, U., Schmook, B., Adams, C., Schmidt-Vogt, D., Messerli, P., & Leisz, S. (2012). Trends, drivers and impacts of changes in swidden cultivation in tropical forest-agriculture frontiers: a global assessment. *GLOBAL ENVIRON CHANG*, 22: 418-429.

Vandamme, P, Pot B, Gillis M, de Vos P, Kersters K., & Swings J. (1996). Polyphasic taxonomy, a consensus approach to bacterial systematics. *Microbiol Rev*. 60(2):407-438.

Vassileva, M., Azcon, R., Barea, J.M.& Vasslev, N. (2000). Rock phosphate solubilization by free and encapsulated cells of Yarowia lipolytica. *Proc Biochem*;35;693-7.

Vassileva, M., Vassilev, N., & Azcon, R. (1998). Rock phosphate solubilization by *Aspergillus niger* on olive cake-based medium and its further application in a soil-plant system. *World* Journal of *Microbiology* and *Biotechnology* 14: 281-284

Vaughan, D., & R.E., Malcolm. (1985). Influence of humic substances on growth and physiological processes, p. 37-75. In D. Vaughan and R.E. Malcolm ed. *Soil organic matter and biological activity.* Dordrecht, Boston.

Vaz-Moreira I., Figueira V., Lopes A. R., De Brandt E., Vandamme P., Nunes O. C., & Manaia C. M. (2011); *Candidimonas nitroreducens* gen. nov., sp. nov. and *Candidimonas humi* sp. nov., isolated from sewage sludge compost. *Int J Syst Evol Microbiol* 61:2238–2246

Vela´zquez, E., Trujillo, M. E., Peix, A., Palomo, J. L., Garcı´aBenavides, P., Mateos, P. F., Ventosa, A., &Martı´nez-Molina, E. (2001). Stable low molecular weight RNA analyzed by staircase electrophoresis, a molecular signature for both prokaryotic and eukaryotic microorganisms. *Syst. Appl. Microbiol*. 24, 490–49

Verma, P., Yadav, A.N., Khannam, K.S., Panjiar, N., Kumar, S., Saxena, A.K., & Suman A. (2015). Assessment of genetic diversity and plant growth promoting attributes of psychrotolerant bacteria allied with wheat (*Triticum aestivum* L.) from the northern hills zone of India. *Ann. Microbiol*

Vessey, J.K. (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil* 255, 571–586.

Walkley, A., & Black, I.R. 1934. An examination of Degtijareff method for determining soil organic matter and proposed modification of the chromic acid titration. Soil Science 37:29-38.

Wang, Q., Garrity, G.M., Tiedje, J.M., & Cole, J.R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73, 5261–5267.

Wang, X. J., & Gong, Z. T. (1998). Assessment and analysis of soil quality changes after eleven years of reclamation in subtropical China. *Geoderma. 81: 339–355.*

Ward, D.M., Weller, R., & Bateson, M.M. (1990). 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community. *Nature.* 345(6270):63-65.

Wardle, D.A. (1992). A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. *Biological Reviews* 67: 321-358.

Watters R F. (1960). Some forms of shifting cultivation in the south west Pacific; J. *Trop. Geogr.* 14 35-50

Watters R. F. (1971). Shifting cultivation in Latin America, *FAO Forestry Development Paper No. 17, FAO, Rome*, 305 pp

Weisbach, C., Tiessen, H., & Jimenez-Osornio, J. J. (2002). Soil fertility during shifting cultivation in the tropical Karst soils of Yucatan. *Agronomie,* v. 22, n. 3, p. 253-263.

Weisburg, W.G., Barns, S.M., Pelletier, D.A., &Lane, D.J. (1991).16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol*. 173(2):697-703.

White, E. M., Thompson, W. W., & Gartner, F. R. (1973). Heat effect on nutrient release from soils under ponderosa pine; J. *Range Manag. 22 22-24*

Whitelaw, M. A. (2000). Growth promotion of plants inoculated with phosphate solubilizing fungi. *Advances in Agronomy* (Academic Press). 69: 99-151.

Woese, C. R. (1987). Bacterial evolution. *Microbiolology Reviews* 51: 221-271

Yadav, K. K., & Sarka, S. (2019). Biofertilizers, impact on soil fertility and crop productivity under sustainable agriculture. *Environ. Ecol.* 37, 89−93.

Yadav, P.K., (2013). Slash-and-burn agriculture in north-East India. *Exp. Opin. Environ. Biol.* 2, 1–4.

Yadav. R., & Tarafdar, J. (2001). Influence of organic and inorganic phosphorus supply on the maximum secretion of acid phosphatase by plants. *Biol Fertil Soils* 34, 140–143.

Yang S., Liao B., Xiao R., & Li J. (2019). Effects of Amendments on Soil Microbial Diversity, Enzyme Activity & Nutrient Accumulation after Assisted Phytostabilization of an Extremely Acidic Metalliferous Mine Soil. *Applied Sciences*; 9: 1552.

Yemefack M., Rossiter D.G., & Njomgang R. (2005). Multi-scale characterization of soil variability within an agricultural landscape mosaic system in southern Cameroon. *Geoderma* 125(1-2): 117-143

Yemefack, M.; Rossiter, D. G., & Jetten, G. (2006). Empirical modelling of soil dynamics along a chronosequence of shifting cultivation systems in Southern Cameroon. *Geoderma,* v. 133, n. 3-4, p. 380-397.

Young, I. M., & Crawford J.W. (2004). *Interactions and Self-Organization in the Soil-Microbe Complex. Science, 304(5677), 1634–1637.*

Zak, D.R., Grigal, D.F, Gleeson, S., & Tilman, D. (1990). Carbon and nitrogen cycling during old-fields succession: constrains on plant and microbial biomass. *Biogeochemistry* 11:111-129.

Zak, D.R., Tilman, D., Parmenter, R.R., Rice, C.W., Fisher, F.M., Vose, J., Milchunas, D., &Martin, C.W. (1994). Plant production and soil microorganisms in late- successional ecosystems: a continentalscale study. *Ecology* 75: 2333-2347.

Zak, J., Willig, M., Moorhead, D., & Wildman, H. (1994). Functional diversity of microbial communities: A quantitative approach. *Soil Biology and Biochemistry, 26(9), 1101–1108.*

Zeng, D. H., Hu, Y. L., Chang, S. X., & Fan, Z. P. 2009. Land cover change effects on soil chemical and biological properties after planting Mongolian pine Pinus sylvestris var. mongolica in sandy lands in Keerqin, northeastern China. *Plant Soil* 317, 121–133.

Zhang, N., He, X., Gao, Y., Li, Y., Wang, H., Ma, D., Zhang, R., & Yang, S. (2010). Pedogenic carbonate and soil dehydrogenase activity in response to soil organic matter in *Artemisia ordosica* community. *Pedosphere*, *20*, 229–235.

Zimmermann, K., & Eggenberg, S. (1994). Comparison of vegetation and geomorphology: problems and approach. *Rev. Valdôtaine Hist. Nat.* 48 (suppl.), 357–365.

Zinke, P.J., Sabhasri, S., & Kundstadter, P. (1978). Soil fertility aspects of the 'Lus' forest fallow system of shifting cultivation. In: P. Kundstadter, E.C. Chapman and S. Sabhasri (Editors), Farmers in the Forest. East West Centre, Honolulu, HI.

Zothansanga, R., Senthil Kumar, N., & Gurusubramanian, G. (2016). Diversity and Toxicity of Bacillus thuringiensis from Shifting Cultivation (Jhum) Habitat. *Biocontrol Science*, 21(2), 99–111.

Appendix – I

Media composition for PSB

1. Pikovskaya's medium (Pikovskaya, 1948)

Glucose	-	10.00 g
$MgSO_4.7H_2O$	-	10.00 ml
$CaCl_2$	-	10.00 g
Tricalcium phosphate ($Ca_3 (PO_4)_2$)	-	5.00 g
Distilled water	-	1000 ml
Agar	-	18.00 g
pH	-	7.00

2. Nutrient medium (Difco Manual, 1953)

Peptone	–	5.00 g
Beef extract	–	3.00 g
Sodium chloride	–	8.00 g
Distilled water	–	1000 ml
pH	–	7.30

3. NBRIP medium (Nautiyal, 1999)

Glucose	–	10.00 g
$Ca_3(PO_4)_2$	–	5.00 g
$MgCl_2.6H_2O$	–	5.00 g
$MgSO_4.7H_2O$	–	0.25 g
KCl	–	0.20 g
$(NH_4)_2SO_4$	–	0.10 g
Distilled water	–	1000 ml
pH	–	7.00

Appendix-II

Reagents prepared for soil physico-chemical and biochemical properties

1% 2,3,5 triphenyl tetrazolium chloride (TTC)

TTC	–	1.0 g
Distilled water	–	100 ml

0.115 M p-nitrophenyl phosphate (p-NPP)

p- NPP	–	4.26 g
Distilled water	–	100 ml

0.5 M p-nitrophenyl-β-glucoside (PNG)

PNG	-	0.654g
MUB (pH 6)	-	50 ml

0.25M p-nitrophenyl sulfate

Potassium p-nitrophenyl sulfate	-	0.614g
Acetate buffer	-	50 ml

Modified universal Buffer (MUB pH 6.5)

Tris (hydroxyaminomethane)	–	2.42g
Maleic acid	–	2.3 g
Citric acid	–	2.8 g
Boric acid	–	1.26 g
MilliQ water	–	800 ml

Then adjusted to pH 6.5 with 10 M sodium hydroxide (NaOH)

10 M NaOH

NaOH	–	40 g
Distilled water	–	100 ml

0.5 N NaOH

NaOH	–	2 g
Distilled water	–	100 ml

0.5 M $CaCl_2$

$CaCl_2$	–	7.351 g
Distilled water	–	100 ml

10 % urea solution

Urea	–	10.0 g
Distilled water	–	100 ml

Phenolate solution preparation:

Solution A: Dissolve 62.5 g of phenol in 20 ml of methanol. Then, add 18.5 ml of acetone to it and the mixture is then made up to 100 ml with ethyl alcohol.

Solution B: Dissolve 27 g of NaOH in 100 ml of distilled water.

For phenolate solution, mix together 20 ml of solution A and B and make up the whole volume to 100 ml with distilled water. This has to be prepared fresh before use.

1N potassium dichromate solution

Potassium dichromate	–	29.418 g
Distilled water	–	100 ml

0.5 N ferrous ammonium sulphate solution

Ferrous ammonium sulphate	–	19.606 g
Distilled water	–	100 m

0.32% $KMnO_4$ solution

Potassium permanganate ($KMnO_4$)	–	3.2 g
Distilled water	-	1000

1% Boric acid

Boric acid	–	20 g
Distilled water	–	1000 ml

2.5 % NaOH

Sodium Hydroxide (NaOH)	–	25 g
Distilled water	–	1000 ml

0.05 M Sodium bicarbonate ($NaHCO_3$)

$NaHCO_3$	–	21 g
Distilled water	–	100 ml
pH	–	8.5

0.02 N Sulphuric acid (H_2SO_4)

Conc.H_2SO_4	–	1 ml
Distilled water	–	1.8 L

Dickman's and Bray's reagent

Ammonium molybdate	–	15 g
Distilled water	–	600 ml
10 N HCl	–	400 ml

40 % stannous chloride ($SnCl_2.2H_2O$)

$SnCl_2.2H_2O$	–	10 g
Conc. HCl	–	25 ml

Diluted Stannous chloride solution (Make a fresh solution)

40 % $SnCl_2.2H_2O$	–	0.5 ml
Distilled water	–	66 ml

1 M Ammonium acetate (NH_4OAc)

NH_4OAc	–	77.09 g
Distilled water	–	1000 ml
pH	–	7.0

Neutral Ammonium acetate solution

99.5% Glacial acetic acid (CH_3COOH)	–	57 ml
Distilled water	–	700 ml
Concentrated (NH_4OH)	–	69 ml (Diluted upto 900 ml with dist.water)
pH (adjusted by 3 N NH_4OH or 3 N CH_3COOH)	–	7.0

Appendix-III

Media and Reagents Used For Biochemical Screening of Potential Phosphate Solubilizing Bacteria

Phenol red carbohydrate broth medium

Peptone	-	5.00 g
Beef extract	-	3.00 g
Sodium chloride	-	8.00 g
Carbohydrate source	-	10 g
Distilled water	-	1000 ml
pH	-	7.3±0.1
Phenol red indicator	-	few drops till medium turn pink

Starch agar medium

Beef extract	-	3g
Peptone	-	5g 2g
Starch, soluble	-	
Agar	-	15 g
Distilled water	-	1000 ml
pH	-	7.2±0.1

Nutrient gelatin medium

Gelatin	-	120g
Peptone	-	5g
Beef extract	-	3g
Distilled water	-	1000 ml
pH	-	7.2±0.1

Skim milk agar medium

Skim milk powder	-	5g mixed with 50 ml distilled water (stir until dissolved)
Agar	-	1 g mixed with 50 ml distilled water. (stir until dissolved). Add the mixture and autoclave

Nitrate broth medium

Peptone	–	5 g
Beef extract	–	3 g
Potassium nitrate (KNO_3)	–	1 g
Distilled water	–	1000 ml
pH	–	7.0±2.0

Appendix-IV

Reagents Used for Isolation and Phylogenetic Affiliation of Phosphate Solubilizing Bacteria

TE Buffer (pH 8.0)

10mM Tris-HCl	-	0.157 g
Distilled water	-	100 ml
1mM EDTA	-	3.722 g
Distilled water	-	10 ml

5M Sodium chloride

Sodium chloride	-	29.22 g
Distilled water	-	100 ml

3M Sodium acetate (pH 5.2)

Sodium acetate	-	24.69 g
Distilled water	-	100 ml
pH	-	5.2

5x Tris- borate- EDTA (TBE) buffer

Tris	-	54 g
0.5M EDTA	-	3.722 g
Boric acid	-	27.50 g
Distilled water	-	1000 ml
pH	-	8.2

1x TBE buffer (500 ml)

5x TBE	-	100 ml
Distilled water	-	400 ml

Composition of the PCR reaction mixture (25 µl tube^{-1})

10 x buffers	-	2.5 µl
$MgCl_2$ (25 mM)	-	1.5 µl
dNTPs (2.5 mM)	-	2.0 µl
Primers	-	0.8 µl
Template DNA (100 ng)	-	1.0 µl
Taq DNA polymerase (2U/µl)	-	0.5 µl
Nuclease free water	-	15.9 µl

0.8% agarose gel (50 ml)

Agarose	-	0.4 g
1x TBE	-	50 ml
Ethedium Bromide (EtBr) (10 mg/ml)	-	2 µl

1.5% agarose gel (50 ml)

Agarose	-	0.75 g
1x TBE	-	50 ml
Ethedium Bromide (EtBr) (10 mg/ml)	-	2 µl

1.5% agarose gel (100 ml)

Agarose	-	1.5 g
1x TBE	-	100 ml
Ethidium Bromide (EtBr) (10 mg/ml)	-	4 µl

70% ethanol (100 ml)

Ethanol (99.9%)	-	70 ml
Distilled water	-	30 ml

10% Cetyl trimethyl ammonium bromide (CTAB)

CTAB	-	10.0 g
Distilled water	-	100 ml

10% Sodium dodecyl sulphate (SDS)

SDS	-	10.0 g
Distilled water	-	100 ml

Lysozyme (2 mg/ml): Dissolve 2 mg of lysozyme in 1 ml of 1x TBE buffer (pH 8.0) and store at -20°C for further use.

Proteinase K (20 mg/ml): Dissolve 20 mg of proteinase K in 1 ml of sterile distilled water and store at -20°C for further use.

Ethidium Bromide (EtBr) (10 mg/ml): Dissolve 10 mg of EtBr in 1 ml of sterile distilled water and store at 4°C for further use.

Appendix-IV

Reagents used for estimation of bacterial acid phosphatase and IAA production

0.2% L-tryptophan

L-tryptophan	–	0.200 g
Distilled water	–	100 ml

Salkowski's reagent

35% perchloric acid	–	50 ml
0.5 M $FeCl_3$	–	1 ml

0.5 M Ferric chloride ($FeCl_3.6H_2O$)

$FeCl_3$	–	6.757 g
Distilled water	–	50 ml

0.115 M p-nitrophenyl phosphate

p- nitrophenyl phosphate		–4.62 g
Distilled water	–	100 ml

0.5 N Sodium hydroxide (NaOH)

NaOh	–	20 g
Distilled water	–	1 L

Bio-data

Name	Remruattluanga Hnamte
Father's name:	Challianchhunga (L)
Mother's name:	B. Lalenpuii
Date of Birth:	24th July, 1988
Nationality:	Indian
Category:	Scheduled tribe
Permanent address	C-55, Bethlehem veng, Aizawl, Mizoram 796001

Academic Profiles:

Examination	Board	Year	Division	Percentage	Achivement
SLET (Life Sciences)	SLET Commision (NE Region)	2013			
M.Sc (Botany)	MZU	2012	First	70.28	Gold medal
B.Sc (Botany)	MZU	2010	First	73	
HSSLC	MBSE	2007	Second	58	
HSLC	MBSE	2005	Dist	75	Letter in Science

Ph.D. Registration No. and Date	MZU/Ph.D./744 of 22.05.2015
Department	Botany
Title of Research	Diversity of Phosphate Solubilizing Bacteria in Jhum Fallow Soils of Serchhip District, Mizoram
Supervisor	Dr. R. Lalfakzuala
Joint supervisor	Dr. H. Lalruatsanga

List of Publications (Journals)

1. R.Lalfakzuala*, Lalrampani, C.Vanlalveni, Lalmuankimi Khiangte and **Remruattluanga Hnamte** (2014); Antibacterial activity of methanolic extracts of selected weeds against two phosphorous solubilizing bacteria; ***Int.J.Curr.Microbiol.App.Sci*** **(2014) 3(4): 1014-1019.**

2. R. Lalfakzuala, C. Vanlalveni, Lalmuankimi Khiangte, Lalrampani, **Remruattluanga Hnamte** and Lianthangpuii (2015); Effects of insecticides on growth of soil fungI Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 17, No. (1): 2015: 199-203

3. Aayushi Biswas, Lalrampani Chawngthu2 C. Vanlalveni, **Remruattluanga Hnamte**, R. Lalfakzuala ∗, and Lalthazuala Rokhum (2018); Biosynthesis of Silver Nanoparticles Using *Selaginella bryopteris* Plant Extracts and Studies of Their Antimicrobial and Photocatalytic Activities; Journal of Bionanoscience Vol. 12, 227–232, 2018

4. **Remruattluanga Hnamte** and R. Lalfakzuala (2018); Phosphate Solubilizing Bacteria in Traditional Jhum Field of Mizoram; Science and Technology Journal Vol. 6 Issue: I January 2018 ISSN: 2321-3388

5. Lalrampani Chawngthu; **Remruattluanga Hnamte**; R. Lalfakzuala (2019) Isolation and Characterization of Rhizospheric Phosphate Solubilizing Bacteria from Wetland Paddy Field of Mizoram, India; Geomicrobiology Journal0149-0451 (Print) 1521-0529 (Online)

Conference Proceeding

6. **Remruattluanga Hnamte** and Lalfakzuala, R. (2016). Diversity of Phosphate Solubilizing Bacteria in Traditional Jhum Field of Mizoram. *Proceeding of the Mizoram Science Congress*. Page 1-4, ISBN: 978-93-85926-49-5.
7. Lalfakzuala, R., **Remruattluanga Hnamte** and Lalremruata. (2016). Effect of Soil Amended with Decomposed Weeds on Microbial population and Growth Performance of Maize. *Proceeding of the Mizoram Science Congress*. Page 17-20. ISBN: 978-93-85926-49-5.
8. **Remruattluanga Hnamte**, Lalruatsanga, H. and Lalfakzuala, R**.** (2017). Effect of Insecticide on Soil Fungal Diversity. *Proceeding of the Biodiversity, Conservation and Utilization of Natural Resources with reference to Northeast India*. Page 81-84. ISBN: 978-818653578-0.
9. **Remruattluanga Hnamte** and lalfakzuala, R. (2018). Soil physico properties and soil enzyme ctivity in different jhum fallow land. Proceedings of the National Seminar on Shifting Cultivation and its environemental impact in North- eadt India. ISBN 978-81-935083-5-0

Seminar and Workshop Attended

1. Short term training course on "PCR Based Detection and Characterization of Micorbial Pathogens" Instituitonal Biotech Hub, College of Veterinary Sciences and Animal Husbandry, 24th Feb 2014- 5th March 2014.
2. State Level Seminar on Fostering Scientific Temper organised by Mizo Academy of Sciences in collaboration with Directorate of Science and Technology, Government of Mizoram catalysed and supported by the National Council for Science & Technology Communications, Department of Science and Technology, New Delhi on 3rd October 2014 at Central YMA Hall, Tuikhuahtlang, Aizawl, Mizoram.

3. UGC- Sponsored Short Termed Course on Resrerch Methodology for Research Scholars; 20th-26th June 2016; Human Resource Development Center, Mizoram University.
4. Mizoram Science Congress held at Mizoram University during 13th-14th October 2016 organized by: MISTIC, MSS, MAS, STAM, MMS, GSM & BIOCONE.
5. Seminar on Make in India: Science and Technology Driven Innovations organised by Mizo Academy of Sciences in collaboration with the Mizoram Science, Technology & innovation Council (MISTIC), Government of Mizoram catalysed by National Council for Science & Technology Communications, Department of Science and Technology, New Delhi on 4th November 2016 at Pi Zaii Hall, Synod Conference Centre, Aizawl, Mizoram.
6. National Seminar on Biodiversity and Utilization of National Resources with Reference to Northeast Inida; 30th-31st March 2017, Department of Botany, Mizoram Univeristy.
7. "Statistical and Computing Methods for Life-Science Data Analysis" organised by Department of Botany, Mizoram University and Indian Statistical Institute, Biological Anthropology Unit, Kolkata on 5th-10th March, 2018 at Department of Botany, Mizoram University.
8. National Seminar on Shifting Cultivation and its Environmental Impact in North-East India; 15th-16th March 2018, Dept. Of Geography, Pachhunga Univeristy College.
9. One-day Awareness Proagramme cum Workshop on Invasive Alien Plants In Himalaya; Status, Ecological Impact and Mangement (Mizoram and Tripura) 26th April 2019; Botanical Survey of Inida in collaboration with Department of Botany, Mizoram Univeristy.
10. National workshop ion IPR and Plant Protection with special refernce to NE inida; 18th December 2019, Departmetn of Botany, Mizoram Univeristy in colloboration with Dept of Horticulture, Govt. Of Mizoram.

Abstract

Jhum cultivation is an ancient agricultural land use system practiced widely among the hill tribes of North-East India. In jhum cultivation, a piece of forest land was cleared, left to dry and the dried biomass is burned *in situ* followed by the cropping phase. Once the fallow lands regain their fertility, the farmers come back to the same piece of land for cultivation. In jhum cultivation fire is deliberately used to change soil physico-chemical properties, and both the release of nutrients by fire and the value of ash have been known to cause alteration in soil biochemical properties. In course of time, different adverse effects emerge because of jhum cultivation which has a detrimental effect on the soil nutrients status and biological properties due to deliberate slashing and burning of vegetation involved in the process.

Phosphorus (P) which is an essential nutrient for the growth and development of plants undergoes large volatilization and convective losses during jhum cultivation. The amount available P in soil for plant utilization is very low and the new sources to replace the lost available P mainly comes from non-plant available P in soil and from parent materials which needs to be solubilized through the action of enzymes known as *phosphatase.* These enzymes are secreted by a group of microorganisms known as Phosphate Solubilizing Microorganism (PSM), among which Phosphate Solubilizing Bacteria (PSB) are predominant. These PSB(s) can be used for the rapid solubilization of insoluble P, thereby supplementing the low available P level in the soil. Hence these PSB can be developed into location-specific fertilizers to maintain the sustainability of jhum cultivation.

In this study soil samples were collected from the current jhum field, different length jhum fallows viz. current jhum (Jh), 2 years jhum fallow (2F), 5 years jhum fallow (5F), 10 years jhum fallow (10F) and an undisturbed forest (UD) of Hmuntha village (23.4939° N and 92.9419°E), Serchhip district, Mizoram, India. Soil samples were collected during February, May, August, and November. The soil physico-chemical properties such as soil temperature, bulk density (BD), soil moisture content

(SMC), water holding capacity (WHC), soil pH, soil organic carbon (SoC), available nitrogen (AN), available phosphorous (AP) and available potassium (AK) were analyzed. Soil enzymes assay for dehydrogenase, urease, phosphatase, arylsulfatase and β- glucosidase were also carried out. Analysis of soil microbial biomass carbon (Cmic) and microbial biomass nitrogen (Nmic) were also done. Estimation of soil bacterial diversity based on metagenomic sequencing and isolation and molecular identification of PSB was carried out using 16s rRNA gene sequencing

Metagenomics diversity of soil bacteria was assessed by amplification of the V3-V4 hypervariable region of the 16SrRNA gene. The isolation of PSB was carried out based on their ability to solubilize TCP on PVK medium and 16SrRNA gene sequencing was also done for the potential isolates to assert their identity. Biochemical characterization including assessment of PGP traits such as IAA and phosphatase enzyme production was done to screen for potential bio-fertilizers. Based on their PGP trait, the effect of selected bacterial isolates on rice growth performance as carried out in *in-vitro* experiments under different treatments such as the addition of ash and sterilization of soil.

Analysis of soil physico- chemical properties showed that UD site was the most productive and fertile site while 2F was observed to be the poorest and least fertile. Statistical analysis showed that there is significant variation among soil physico-chemical properties of different sampling sites. Maximum soil temperature and BD were found to highest in 2F soil, while UD recorded the lowest for both parameters. Soil BD ranges from 1.05g cm^3 in 10F during November to 1.37 g cm^3 in Jh during May. Soil BD and WHC are inversely correlated hence lowest WHC% (60.32 %) were observed in Jh during May and the highest WHC% (73%) was observed in UD during May. The SMC was highest in August for all sampling sites where UD showed the highest SMC of 32.63% while the Jh site showed the lowest SMC (12.58%) during February.

The soil nutrients *viz.* SoC, AN, AP, and AK were found to be highest in UD site and lowest in 2F site. The soil pH ranges from 4.7-5.5, highest soil temperature of 26^{o}C was recorded in 2F during May and lowest of 12.07^{o}C during November in Jh site

Highest SoC (4.793 %) was observed at UD site during November while Jh site showed the lowest SOC (2.567%) during February. Soil available nitrogen showed variation with respect to the length of the fallowing period of the sampling sites. The highest AN 236 kgha^{-1} was observed in UD site during November and the lowest AN (148 kg ha^{-1}) was observed in 2F during February. Soil AP showed a wide range of variation between different sampling sites. The highest level of available P (15.7 kg ha-1) was recorded from UD site during August while the lowest level of available P (8.1 kg ha^{-1}) was recorded from 2F site during November. Soil available K ranges from 144 kg ha^{-1} in 2F site during February to 234 kg ha^{-1}at UD site during August, indicating a wide range of variation both with sites and sampling season.

Soil biochemical properties such as enzymes dynamic and soil microbial biomass carbon and nitrogen showed a significant variation between the samples site. For soil enzyme dynamic, the highest DHA activity was recorded in the UD site (0.9 mg TPF g dry soil^{-1} 24hr^{-1}) and lowest (0.22 mg TPF g dry soil^{-1} 24hr^{-1}) in Jh site. Acid phosphatase (Apase) phosphatase enzyme activity ranges from 42.100 µg p-NP g^{-1} dry soil hr^{-1} at 2F site in February to 149.566 µg p-NP g^{-1} dry soil hr^{-1} at the UD site during August. The highest urease (URS) enzyme activity was recorded at the UD site (34 µg NH3 g dry soil^{-1} 3hr^{-1}) where the lowest (10 µg NH_3 g dry soil^{-1} 3hr^{-1}) was recorded at the 2F site. The ARS activity of the study site ranges from 71µg p-NP dry soil-1 hr-1 in 2F soil during May to 320 µg p-NP dry soil^{-1} hr^{-1} in UD soil during May and August and the highest Beta- glucosidase activity of 211.45 µg p-NP dry soil^{-1} hr^{-1} was recorded at UD site during May while lowest reading of 58.29 µg p-NP dry soil^{-1} hr^{-1} was recorded at the 2F site during February.

Metagenomics analysis of soil bacterial population using NGS revealed that at kingdom level, archaea were found in all samples except 5F. The highest percentage of Archaea was observed in UD (1.98%). Actinobacteria are the dominant Phylum in JF representing 37.198% of the total bacterial phyla while Proteobacteria are the dominant class in the other sites accounting for 33.777%, 30.078%, 33.978%, and 31.917% of the bacterial population in 2F, 5F, 10F, and UD respectively. The 10F site showed the highest number of unique bacterial species based on the NGS data generated. 730 bacterial species belonging to 541 genera were identified from the UD site. The 2F

showed the second-highest numbers of bacteria species with 582 species from 446 different genera while from the Jh site 452 species genera belong to 332 genera were recorded. UD and 5F sites showed the least number of bacterial species at 545 belonging to 406 genera and 408 genera respectively.

Alpha diversity analysis revealed that there was significant variation in the bacterial composition between the sampling sites. 10F showed the highest Shanon diversity index (10.169) while the lowest was observed in JH site (9.048). Phylogenetic diversity analysis based on Faith phylogenetic diversity index also follows the same distribution as that of Shanon diversity index with diversity index ranging from 57.8 for Jh to 85.9 for 10F. PCoA of Beta-diversity analysis based on Bray-Curtis index and Jaccard distance showed dissimilarity between the bacterial population of the different sites. While PCoA of weighted and unweighted infrac distance showed that there is dissimilarity in the phylogenetic diversity of bacterial community shared between the study sites except for 2F and 5F which showed an almost similar phylogenetic diversity of bacteria.

Base on their ability to solubilized tri-calcium phosphate and formation of halo zone on PVK medium, 44 bacteria were identified as having phosphate solubilizing potential and were isolated and maintained in pure-culture. They were then designated as MZUR1, MZUR2, MZUR3, MZUR4, MZUR5, MZUR6, MZUR7, MZUR8, MZUR9, MZUR10, MZUR11, MZUR12, MZUR13, MZUR14, MZUR15, MZUR16, MZUR17, MZUR18, MZUR19, MZUR20, MZUR21, MZUR22, MZUR24, MZUR25, MZUR26, MZUR27, MZUR28, MZUR29, MZUR30, MZUR31, MZUR32, MZUR33, MZUR34, MZUR35, MZUR36, MZUR39, MZUR40, MZUR41, MZUR42, MZUR43 and MZUR44. Their SI was calculated and the highest solubilization index was shown by the MZUR6 (3.45) and the lowest was shown by MZUR4 (1.98) while the lowest SI was observed for MZUR4 (1.95).

Isolated bacterial genomic DNA were amplified using 27f (5'-GAGTTTGATCCTGGCTCAG-3') and 1525r (5'-AGAAAGGAGGTGTACCAGCC-3') primers. After sequencing of the PCR purified product, the sequence data were compared for similarity level with the reference strains of PSB from NCBI database

banks. The 16SrRNA nucleotide sequences were compared by BLASTn analysis tool to look for 16SrRNA gene homology and based on the results the bacterial isolates placed under the genera *Burkholderia, Pseudomonas, Alcaligenes,* and *Bacillus*. *Bacillus* accounts for 52.23% of the total isolates while *Burkholderia, Pseudomonas,* and *Alclaligenes* account for 22.73%, 12.66%, and 11.36% respectively. The 16s rRNA nucleotide sequences nucleotide were deposited in NCBI Genbank database bearing the accession MT086198- MT086211, MT087561- MT087567, MT096514, MT102121- MT102138, MT102411- MT102443.

Based on the 16SrRNA sequence result, 7 out of 9 PSB isolated from the Jh soil belongs to *Bacillus* genus, the others being *Pseudomonas* and *Burkholderia*. From the 2F site 10 PSBs were isolated, where 5 of the isolates belongs to *Bacillus*, 3 to *Burkholderia* and 1 each to *Pseudomonas* and *Alcaligenes*. Least number of PSB were isolated from the 5F site which was made up of 3 *Bacillus* and 3 *Pseudomonas*. Most of the PSB isolated from the 10F site were found to be gram-negative, with 3 isolates belonging to *Burkholderia*, 2 to *Alcaligenes*, and 1 to *Pseudomonas*. 3 isolates from 10F were gram-positive bacteria belonging to Bacillus. The PSB isolated from UD site showed a consortium of bacteria composed of 3 *Alcaligenes,* 2 *Burkholderia*, 1 *Pseudomonas,* and 3-gram positive *Bacillus*.

The biochemical characterization of the 44 bacterial isolates showed that Out of the 44 isolates, 93% of the isolates showed catalase enzyme production. 57% of the isolates were capable of performing starch hydrolysis, gelatin hydrolysis test was positive for 80% of the isolates, while 60% of the isolate were capable of casein hydrolysis. 95% of the isolates were capable of fermenting glucose and sucrose while 2% of the isolates do not perform either scudoes or glucose fermentation. 97% of the isolates were found to be capable of reducing nitrate to nitrite.

All bacterial isolates showed phosphatase and IAA production after 24 hours of culturing. Quantitatively, isolate MZUR22 showed the highest phosphatase production (37.604±0.64 p- NPP $\mu g^{-1}hr\ ml^{-1}hr^{-1}$) while MZUR37 MZUR35 (0.271 p- NPP $\mu g^{-1}hr\ ml^{-1}hr^{-1}$) showed the lowest phosphatase production showed the highest IAA production (23.957±0.135 μgml^{-1}) during the culture period. The highest IAA production was

shown by MZUR37 (23.957±0.135 IAA µg ml^{-1}) and the least IAA was shown by MZUR35 0.502±0.135 IAA µg ml^{-1}).

The *in vitro* growth of rice under different treatments showed that the rice seeds inoculated with selected PSBs (MZUR22 and MZUR37) showed better growth performance in terms of root and shoot length as well as biomass accumulation during the observed period. Rice seeds treated with bacteria inoculum MZUR22 -*Bacillus cereus* grown on unsterilized soil amended with ash showed the highest shoot and root length along with highest biomass accumulation after 21 DAS, followed by rice seeds treated with MZUR37- *Burkholderia gladioli* grown on unfertilized soil amended with ash. The lowest growth performance of rice was observed in the control treatment for sterilized soil. One-way ANOVA performed considering a significant level of $p \leq 0.05$ showed that there was significant variation in the shoot and root growth as well as rate of biomass accumulation between rice inoculated with PSBs and rice which were not inoculated with PSB. It was also observed that the addition of ash played an important role in the early growth of rice plants.