

Impact of Slash-Burning and Plant Species on Soil Microbial Community and Processes in *Jhum* Agro-Ecosystem

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Central Agricultural University, Imphal
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by

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COLLEGE OF POST-GRADUATE STUDIES
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Dated:

Umiam, Meghalaya


(Carolyn Zothansiami)

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LIST OF ABBREVIATION

%	Per cent
°C	Degree celcius
ANOVA	Analysis of variance
ASA	Aryl-sulphatase activity
<i>B</i>	<i>Bacillus</i>
BC	Before christ
BFC	Baseline fungal community
bp	Basepair
BSA	Bovine serum albumin
C	Carbon
C	Cytosine
CaCl ₂	Calcium chloride
CaCO ₃	Calcium carbonate
CDB	Cellulose Degrading Bacteria
cm	Centimeter
CMC	Carboxymethyl Cellulose
CO ₂	Carbon dioxide
Conc.	Concentration
CPGS	College of Post Graduate Studies
DAS	Days After Sowing
DGGE	Denaturing Gradient Gel Electrophoresis
DHA	Dehydrogenase activity
DNA	Deoxyribonucleic acid

dNTPs	Deoxynucleoside triphosphate
DOC	Dissolved Organic Carbon
dw	Dry weight
EDTA	Ethylene diamine tetra-acetic acid
<i>et al.</i>	<i>et alia</i>
etc	<i>et cetera</i>
g	Gram
G	Guanine
GOI	Government of India
GSA	β - glucosidase activity
H	Hydrogen
H ₂ SO ₄	Sulphuric acid
H ₃ PO ₄	Phosphoric acid
ha-1	Per hacter
hrs	hour
ICAR	Indian Council of Agricultural Research
ISI	Institute of Scientific Information
K	Potassium
K ₂ Cr ₂ O ₇	Potassium dichromate
K ₂ SO ₄	Potassium sulphate
KCl	Potassium chloride
K _{EC}	
kg	Kilogram
kg ha ⁻¹	Kilogram per hectare

M	Molar
MBC	Microbial biomass carbon
MC	Microbial consortium
MC	Microbial Consortium
MDS	Multidimensional Scalling
meq	milli equivalent
Mg	Magnesium
Mgcl ₂	Magnesium Chloride
MgO	Magnesium oxide
min	Minute
mL	Millilitre
mm	Millimetre
mM	Millimole
MUB	Modified Universal Buffer
N	Nitrogen
N ₂	Di nitorgen
NaOH	Sodium hydroxide
NB	Nutrient broth
NE	North Eastern
NEH	North Eastern Hill
nfb	nitrogen free bromothymol
ng	Nanogram
NH ₄ -N	Ammonium nitrogen
nm	nanometer

OC	Organic Carbon
P	Phosphorus
PCR	Polymerase Chain rReaction
PHA	Phosphomonoesterases activity
pMN	Potentially mineralizable nitrogen
pMN	Potentially mineralizable nitrogen
PNG	<i>p</i> -Nitrophenyl- β -D-glucoside
<i>p</i> -NPP	<i>p</i> -nitrophenyl phosphate
PNS	<i>p</i> -Nitrophenyl sulphate
POM	Particulate organic Matter
ppm	Parts per million
PSB	Phosphate Solubilising Bacteria
rDNA	Ribosomal Deoxyribonucleic acid
rpm	Rotation per minute
SC	Shifting Cultivation
SCS	Shifting Cultivation System
SEA	Southeast Asia
SMBC	Soil Microbial Biomass Carbon
SMBN	Soil Microbial Biomass Nitrogen
SMBP	Soil Microbial Biomass Phosphorus
SOC	Soil Organic Carbon
SOM	Soil Oranic Matter
SPSS	Statistical Package for the Social Sciences
TAE	Tris-acetate-EDTA

Taq-DNA	Thermus aquaticus
TEMED	Tetramethylethylenediamine
THAM	Tris (hydroxymethyl) aminomethane
TPF	triphenyl formazan
TTC	triphenyl -tetrazolium chloride
USDA	United States Department of Agriculture
V	Volt
wt	weight
α	Alpha
β	Beta
μ	Micron
μg	Microgram
$\mu\text{g g}^{-1}$	Microgram per gram
μL	Micro litre
Mm	Micro metre
μM	Micro-molar

ABSTRACT

Terrestrial ecosystem supports the above- and below-ground biota communities that interact to influence ecosystem-level processes and functions. Soil acts as the most important medium for linking the above- and below-ground communities which are very sensitive to any external disturbances. To study the effect of above- and below-ground linkages on soil microbial community composition and soil processes, the short jhum cycle can be considered as important experimental plot in farmers' field. Because the above ground plant biomass generated during fallow phase of the jhum cycle gradually rejuvenate the degraded soil system and upon burning of such biomass suddenly breakdown the linkages between above-ground and below-ground community linkages. In this study, a jhum field (approximate 1.5 ha) during fallow phase of 5 year cycle was considered from Muallungthu village, Aizawl, Mizoram and the one half part of the field was slashed and burned and the other half part of the field was kept unburnt. The bulk quantity (800 kg) of soil (0-10 cm depth) from 4 random spots (1 sqm per spot) was collected immediately next morning of burning night from the burnt field and similar way 800 kg soil from unburnt field were collected and carried to the CPGS Farm Field for setting up of mesocosm (pot) experiments under 4 conditions *viz.* burnt and unburnt soils in presence of rice crop and without rice crop. Under each of 4 conditions, 6 microbial inoculation (MI) treatments were imposed maintaining 6 replicated pots. Each pot contained 4 kg soil. Altogether 144 pots were maintained (4 conditions x 6 MI treatments x 6 replications = 144). The MI treatments were: (T1) PSB+Fungi, (T2) N₂-Fixer+Fungi, (T3) CDB+Fungi, (T4) PSB+N₂-Fixer+CDB+ Fungi, (T5) No bacteria + Fungi and (T6) No inoculation. The rice crop used was a jhum rice landrace Khawlian Buh. The crop was maintained till harvest within a plastic shade house.

The study reveals that the soil bacterial community composition altered significantly due to burning of slashed biomass on soil surface. The introduction of rice crop also altered the bacterial community composition in burnt/unburnt which shows a distinct cluster within the soil type. The Alpha proteobacterial communities of burnt and unburnt without rice crop clustered together and distinctly separated from the clusters of burnt and unburnt soil with rice crop at 45 days of rice growth and at 90 days of rice growth the impact of rice crop on composition of Alpha and beta proteobacterial community was more conspicuous in burnt soil than in unburnt soil. The beta proteobacterial community of burnt and unburnt soil cluster together in absence of rice crop and there cluster was distinctly separated from the cluster due to cultivation. With the progress of time (90 days of rice growth) the effect of rice crop on beta proteobacterial community was more prominent than compared to burnt unburnt factor.

Burning had significant negative effect on the activity of DHA, GSA, PHA, SOC, pMN, POM, and MBC except ASA indicating higher activity in burnt soil. Introduction of rice crop had significant positive influence on the activity of soil enzymes and soil process indicators. There was significant positive interaction on burning and cropping on soil enzymes activities soil process indicators. There was a significant difference in the activity of soil enzymes and soil process indicators among the microbial inoculants treatment soil process indicators. There was significant positive interaction between burnt and microbial inoculants or cropping and microbial inoculants.

The change in bacterial community composition due to burning and cropping factor significantly influence soil process indicator such as ASA, DHA, pMN, POM, and MBC as evident from analysis result of Multi dimensional Scale (MDS) 1 and 2. The change in alpha and beta proteobacterial community composition due to burning and cropping factor significantly influence soil process indicator like PHA, ASA, DHA, pMN, POM, SOC and MBC as evident from their significant correlation with MDS 1 and 2. Burning had significant positive influence on rice growth yield. This study indicated that introduction of crop in burn soil along with microbial inoculation may positively influenced soil processes as well as crop growth.

Key word: Alpha-proteobacteria; Beta-proteobacteria, DGGE fingerprint; Shifting cultivation; Soil bacterial community composition; Soil enzymes, Soil process indicator

Chapter-1

Introduction

Soil is heterogeneous, dynamic and complex system composed of different soil micro-organism, mineral particles and nutrients, water and air and organic matter at different stage of decomposition. It play primary role in sustaining the life of living beings on earth by providing favorable environment to soil micro-organism which in turn play a fundamental task in transformation of organic waste into an useful organic matter. Soil micro-organism determined soil health by degrading the organic compounds such as xenobiotics and naturally occurring polyphenolic compounds. Soil microbes play a leading role in biogeochemical cycles of nutrients which are essential for growth and development and even maintaining the soil structure (Dick, 1994; Tabatabai, 1994; Acosta and Tabatabai, 2000; Nannipieri *et al.*, 2003; Baldrian, 2009; Haifang *et al.*, 2013; Vereset *et al.*, 2013; Zhao *et al.*, 2014). Soil microbes mediated about 80–90% processes that occur in the soil (Coleman & Crossley, 1996; Nannipieri & Badalucco, 2003). For which soil is often term as a living system. Its contribution towards the soil dweller is irreplaceable by none. The soil microbes are divided in to the following groups according to size: macrofauna, mesofauna, microfauna and microflora. Appropriate community structure, abundant diversity and high actions of microorganisms result in functioning of the ecosystem (Bell *et al.*, 2005; Bissett *et al.*, 2007; Zhang *et al.*, 2013 and Zhao *et al.*, 2014). Terrestrial ecosystems consist of above-ground and below-ground components that interact to influence community- and ecosystem-level processes and properties. Soil act as a system of linkages between the above-ground and below-ground components (Bradgett *et al.*, 2008; Suleiman *et al.*, 2013). Soils are known to house the most diverse microbial communities those are responsible for innumerable soil processes. Due to its invisibility to our naked eye and lack of knowledge on how a soil microbe helps in balancing our ecosystem indiscriminate on slaught of soil by various human activities has affected microbial community and their functions. The dynamic of soil microbial biomass, its activity, diversity, abundance and composition is affected by any disturbances that occur in their environment and their unique mechanism presence in themselves helps to response quickly in any environmental disturbances (Zhao *et al.*, 2014). Decomposition of above ground litters in the soil is the main channel for soil microbes to maintain their ecosystem (Arunachalam *et al.*, 1998). Organic matter deposition by leaf-litter, exudates release from roots, organic compounds deposited into the soil environment by

the above ground community results in a unique soil community's i.e below ground community (Bever *et al.*, 1996; Zak *et al.*, 2003). The biological input of the above-ground vegetations modulates the diversity and activity of soil biota communities. As the soil microbes have the capacity to decompose the organic matter, recycling of nutrients and stabilization of terrestrial ecosystems it regulates the soil processes as a feedback mechanism, (Wardle, 2002; Arunachalam *et al.*, 1998).

At rhizospheric zone plant-microbe interactions occur which are responsible for numerous processes like carbon restoration, ecosystem services and nutrient recycling in soil. The soils influence the ability of plants to obtain nitrogen and other nutrients which is influence by the composition and quantity of microbes in the soil (Hoorman, 2011). The potential of soil for enzyme-mediated substrate catalysis is strongly affects composition of soil microbial communities in soil (Tabatabai, 1994) and large group of other enzymes was also secreted by plants in the rhizospheric zone (Baldrian, 2008; Šnajdr *et al.*, 2008). For carbon and nitrogen sources soil microbial communities depend on plant root exudates, thus soil microbial community structure is thought to influence by shift in vegetation. For shaping up the microbial community plant species composition is one of the major contributing factors (Liliensiek *et al.*, 2012). Large group of soil fungi is often to ascribed for production of several polymer-degrading enzymes (Miller *et al.*, 1998; de Boer *et al.*, 2005; Hattenschwiler *et al.*, 2005; Steffen *et al.*, 2007; Baldrian, 2009) and bacterial populations. For proper flow of ecosystem properties and processes soil communities from native origin play primary roles (Wolfe and Kilrinomos, 2005). Native plant community composition and ecosystem function may greatly affected by shifts of soil microbial community composition colonized by invasive plant species (Batten *et al.*, 2006).

The accumulation of microbial biomass gives knowledge regarding ecosystem functioning processes these occurs in soils which were mediated by soil microbes, catalysed and supported by chemicals bound in the biomass or secreted to the environment. The enzyme activities mediated by soil microbial community is then reflecting the current vegetation in an ecosystem (Awasthi *et al.*, 2014).

The different groups of microbes produced different soil enzymes which act as the drivers of major soil biological processes (Veres *et al.*, 2013; Baldrian, 2009; Haifang *et al.*, 2013). The rates of transformation of soil biopolymers into compounds that are accessible for microorganisms and plants are directly affected by the activity of hydrolytic enzymes and ligninolytic oxidases and peroxidases. Upon death the above and below ground communities released extracellular enzymes into the soil that allow them to access energy and nutrients present in complex substrates, catalyzing the

initial step of nutrient mineralization and decomposition of organic matter (Allison and Vitousek, 2005; Awasthi *et al.*, 2014). Soils dominated by various plant species is the functions of variation of soil microbial community (Waldrop *et al.*, 2000). An external disturbance alters the soil enzymes activities. Therefore, it serves as a good indicator of the microbial community function, metabolic requirements and available nutrients. This results in comprehensive understanding the main processes linking nutrient dynamics and microbial populations (Nannipieri *et al.*, 1990; Sinsabaugh and Moorhead, 1994; Hofrichter, 2002; Allison, 2006; Baldrain, 2009; Burns *et al.*, 2013; Kotroczó *et al.*, 2014). Bacteria and fungi play an important role in defense and cellular processes as well as acquisition of carbon (C) and nitrogen (N) (Sinsabaugh, 2010). Once in the soil environment, these enzymes mediate the biogeochemical processes of lignin degradation, carbon mineralization and sequestration, and dissolved organic C export. Stability of ecosystem functioning under variable environment is due to the presence of tolerant species which is the result of high diversity in the community (Yachi and Loreau, 1999).

The destruction of linkages between above-ground vegetations and below-ground biota by anthropogenic activities results in modifications of soil microbial communities, threatening of microbial diversity and result in losses of functions when specific structural patterns or regulation mechanisms was lost (Lavelle, 1997; da Jesus *et al.*, 2009; Alguacil *et al.*, 2014; Köhl *et al.*, 2014).

The predominant farming practice *i.e.* *jhumming* (a common farming practice in the Northeastern Hill States of India) in the slope has been practice by the local people since time immemorial. This *jhum* farming is known for destruction of the above-ground biomass through slash and burn activities followed by cropping in forest lands result in a mass loss of above ground communities, which may lead to alteration of soil microbial communities structure and diversity who are the drivers of major ecosystem processes such as nutrient cycling (van der Heijden *et al.*, 2008; Batten *et al.*, 2006), bioremediation (Gilbert *et al.*, 2012), plant health (Lugtenberg and Kamilova, 2009) and organic matter decomposition and formation (Veres *et al.*, 2013; Baldrain, 2009; Haifang *et al.*, 2013 and Burns *et al.*, 2013). Above all it leads to alteration of functional microbial groups and soil processes. Thus, the disruption of the linkages between above- and below ground biota communities may have altered the natural functioning mechanisms of soil microbial communities leading to detrimental impact on soil processes and overall ecological imbalances in soils of *jhum* agroecosystems. To date, scientific evidence is lacking on how slash and burn practices impact the functional microbial community compositions? Again, the knowledge on how the

combine interactive effects of functional microbial community and plant species can regulate/modify soil processes and functioning is grossly limited.

Keeping the above points in background the following objectives are set up in this research.

1. To examine the response of microbial functional groups in burnt and unburnt soils.
2. To determine the effect of microbial functional groups on soil processes.
3. To assess the relationship of microbial functional groups and soil processes as influence by crop plant (rice).

Chapter-2

Review of Literature

2.1 Brief overview of slash-and-burn cultivation

Slash-and-burn agriculture is a very prehistoric form of agriculture (Li *et al.*, 2014) and is based on the ecological processes of forest ecosystems (Boserup, 1965; Altieri, 1999; Pedroso-Junior *et al.*, 2008). Assume to originated during the Neolithic period around 7000 B.C. (Subhramanyam and Sambamurty, 2000). Mostly practices in the mountainous and hill parts of Latin America, Central Africa and Southeast Asia (SEA) (Van Vliet *et al.*, 2012). This system of cultivation is also known as Swidden in South East Asia, *Milpa* in Latin America, *Tavya* in Africa, *Poddu* in Oddisha, India and *Jhum* in North Eastern (NE) state of India (Thakuria and Sharma, 2014). Globally known as shifting cultivation (SC). This shifting cultivation has been practiced by around 40-50 countries in world (Mertz *et al.*, 2009) where as Institute of Scientific Information (ISI) Web of Science database (8 October 2013), estimated 64 developing countries practice Shifting cultivation system (SCS). It is common agricultural practice in tropical hilly areas of Southeast Asia, the Pacific, Latin America, and millennia for Caribbean and Africa (Ramakrishnan 1992; Stromgaard 1986; Lawrence and Schlesinger 2001). About 300-500 million people in the world depend on shifting cultivation for their livelihood (Brady, 1996). Shifting cultivation occupied about 30% of all arable land but provide food to only 8% of the world population which results in a wide land to man ratio (Kumar, 2008). Generally the ethnic minority groups, tribal people, hill tribes, or other indigenous peoples in South East Asia practices the shifting cultivation (Erni, 2008).

Mertz *et al.* (2009) defined shifting cultivation in Southeast Asia as “a land use system that employs a natural or improved fallow phase, which is longer than the cultivation phase of annual crops, sufficiently long to be dominated by woody vegetation, and cleared by means of fire”. They further described the shifting cultivation scenario of Southeast Asia to be a cycle of short cropping period of 1–3 years succeeds a long fallow period of about 5–20 years where clearing is done by slashing and burning and those parts of the fallow areas are often planted with useful tree crops either for subsistence or cash income. For sustaining the production of food crops fields are rotated rather than rotating the crops thus can say it is a natural resource management strategy (Garrity, 1993; Brookfield, 2001). Shifting cultivation is a multi step process which involves site selection and slash the biomass during a dry

season and sundried for about 2 months and burn it *in-situ* for nutrient management. This burning makes easy land preparation and suppress the weed growth till the onset of monsoon. To coincide with monsoon showers farmers burn the dried slash biomass which is followed by mixed cropping (Ramakrishnan and Toky, 1981). In Southeast Asia and parts of humid West Africa upland rice is the most common crop. Maize and other cereal crops are more common in Latin America and sub-humid Africa, whereas bananas and tuber crops dominate in Central Africa, the Amazon and the Pacific region (Mertz *et al.*, 2008). In shifting cultivation the main aim of the farmer is to sustain the family food requirement and not a commercial crop production.

In shifting cultivation the farmers cultivated the land for a year or two and shift to another area for new cultivation. They abandoned the land to naturally regenerate so as to recover the vegetation and soil fertility. It is a low input system where nutrient input is obtained only from the debris of plant residues and ash after burning of dried slash prior to cropping in each *jhum* cycle (Styger, 2009). The productivity and sustainability of traditional slash-and-burn agriculture relies on the short term disruption, through clearing and burning and subsequent restoration of biological processes and components through fallowing (Palm *et al.*, 1996). The fallow phase in a slash-and-burn cultivation cycle is responsible for soil fertility restoration in which over a period of several years' nutrients are taken up from the subsoil, surface soil and the atmosphere and stored in tissues of growing vegetation. The burning of the forest or fallow biomass results in the release of nutrients that can be utilized by crop plants (Nye and Greenland, 1960; Sanchez, 1976). The successful regeneration of soil productivity via fallows depends on the extent of past soil degradation, the characteristics of the fallow species and succession vegetation community, and the length of the fallow period (Uhl, 1987; Kleinman *et al.*, 1996). This translates into the rate and magnitude of nutrient accumulation in plant biomass and surface soil pools. Biomass production in a fallow is a direct index of ecosystem primary productivity and nutrient cycling.

Slash-and-burn agriculture has a rich traditional ecological knowledge base and remains an important component of forested landscapes in many parts of north-east India states. Indigenous shifting cultivators have a vast store of local knowledge about their particular landscape and how best to use it for survival, and have much to teach the world about the efficient use of their landscape for combined agriculture, forestry and biodiversity conservation (Yadav *et al.*, 2013). However, in any consequences there is always a negative effect which holds true in shifting cultivation as well. Shifting cultivation is known for the major causes of land degradation, deforestation and the burning results in release of greenhouse gases

(Nye and Greenland, 1960 and Comte *et al.*, 2012). Due to its ever increase in human population there is a pressure on land for settlement, infrastructure and for cultivation. Thus, the fallow period have decrease which result in over exploitation of the resources

The primary principles of sustainable shifting cultivation are that losses of plant-available nutrients should be coordinated by new supply over the complete 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 conserved. The main concern of shifting cultivation was that nutrient removal within the harvested crop as well as nutrient losses due to run-off, leaching and fire volatilisation should not exceed plant-available nutrient inputs from nitrogen fixation plus *in situ* soil biogeochemical transformations from non-available forms. (Lawrence and Schlesinger, 2001).

In tropical agricultural systems, especially slash-and-burn situations farmers do not apply fertilizer to upland rice and other crops but have traditionally relied on fallowing their land to restore soil fertility and to reduce problems from insects and weeds (Nye and Greenland, 1960). Farmers from the Yunnan province of China use to plant economic trees (*Cassia siamea*, *Gmelina arborea*, *Cajanus cajan*, *Alnus nepalensis*, *Pinus armandi*, and *Pinus yunnanensis*) during the cropping phase as a tradition and let them grow during the fallow phase of the shifting cultivation (Xu, 1991; Guo and Padoch, 1995).

The deeply rooted culture of shifting cultivation in northeast India has many intricate aspect to be understood scientifically from within. *Jhum* system remains to be a perfect institute as a in situ repository for local germplasm of paddy, cucumber, millet, bajra, maize, pumpkin, chillies, etc. having high resistance to pest and diseases. It has been a long-known practice of grown alder tree (*Alnus nepalensis*) in *jhum* lands of Nagaland to suffice the need for N (Rathore *et al.*, 2010). The mix cropping pattern of this traditional agroecosystem happens to provide biochemicals within itself and maintaining a natural line of defense against invading insects and pest (Ramakrishnan, 1984).

Studies of tropical forests in Thailand and Myanmar indicated that biomass of secondary forest of 40 years after shifting cultivation was almost 95% of uncultivated forests, but species richness was far lower than that of uncultivated forests (Fukushima *et al.*, 2007, 2008).

2.2 Impact of shifting cultivation on soil properties

The nature of shifting cultivation includes clearing of forest, sun dried the biomass for two to three months, burned the dried biomass, mixed cropping for 1-2 years. After the nutrient has depleted the *jhumias* left the land for forest regeneration which is term as a fallow phase. This fallow period is responsible for soil fertility restoration (Styger *et al.*, 2009). Shifting cultivation practice in a steep slopes (44–53%) of NEH region reported the loss of 702.9 kg of organic carbon (OC), 63.5 kg of phosphorus (P) and 5.9 kg of potassium (K) ha⁻¹ (Ram and Singh, 1993). Information on shifting cultivation in relation to chemical and physical soil properties with length of the fallow period, before and after burning during the first year were reported from NE India region (Ramakrishnan and Toky, 1981; Arunachalam, 2002; Sapalrinliana *et al.*, 2016 and Luangmuana *et al.*, 2017).

Burning activities play a major role in nutrient lost and availability. This practices release and incorporates nutrients from burnt biomass, clears rapidly and efficiently, and reduces weeds and pests (Aguilar *et al.*, 2009). The ash from the burn biomass incorporates with the soil and fertilized the soil and makes certain action nutrients Mg, Ca, available P available for plants (Sanchez *et al.*, 1982; Uhl 1987; Maass 1995 and Scheuner *et al.*, 2004). It is generally expected that soil pH will increase after a fire (Gil *et al.*, 2010) and its increment was reported by (Tawnenga *et al.*, 1996 and Arunachalam, 2002) which is due to OH-losses, oxide formation, and alkaline cations released by ashes which can enter the exchange complex and expulse protons to the soil solution (Dikici and Yilmaz, 2006).

Fernandez *et al.* (1997) reported that organic matter was almost completely removed after heating soils up to 400 °C. Post burning soil result in increase in soil organic carbon (SOC) content compare to a forest soil however it decrease during cropping season. Contribution of soil microbial biomass carbon (SMBC) to SOC increased as the duration of cultivation increased (Arunachalam, 2002).

A study conducted in Northeast Indian *jhumming* condition has shows a significant reduction in the carbon content just after burning, with most prominent in 10 and 30 year *jhum* fallow compared to 5 years old fallow. The trend for reduction in carbon continued upto a period of 90 days in 10 and 30 year *jhum* cycle sites and for a period of 30 days only in the site of 5 year cycle. After this period, carbon content in the surface soil showed an increasing trend (Ramakrishnan and Toky, 1981). The

decrement in carbon content had been attributed to the effect of dissimilar intensities of fire from unequal amount of slashed biomass of different *jhum* fallows.

Nitrogen lost by volatilization and inadequacy transfer of mineral N from the aboveground biomass to the soil after burning results in shortfall of available nitrogen for plants which is one of the limiting factor for crop production in shifting cultivation (Mertz *et al.*, 2008; Pandey *et al.*, 2011; Knoepp and Swank, 1993 and Tanaka *et al.*, 2001) reported that mineralization after burning enhanced N supply in the *jhum* field.

Increases in the amount of available phosphorus after burning vegetation are well documented (Ramakrishnan and Toky, 1981; Andriessse, 1987; Roder *et al.*, 1995; Giardina *et al.*, 2000; Lawrence and Schlesinger, 2001 and Bruun *et al.*, 2006). Increase in base cations was also reported by (Nye and Greenland, 1960; Brinkmann and Nascimento, 1973; Stromgaard, 1986; Juo and Manu, 1996; Frizano *et al.*, 2003 and Oliveria, 2008).

Shifting cultivation practice on sloping land result in soil run-off following disappearance of the protective vegetative cover, land degradation and deterioration of surface water quality (Maass *et al.*, 2002; Yadav *et al.*, 2013) and disruption of soil aggregates (García-Oliva *et al.*, 1999b). Severe water limitation in dry tropical ecosystems exacerbates soil degradation after forest conversion to pastures. It exposes soils to desiccation, high temperatures, and rain erosion, besides consuming soil litter, organic matter, and microbiota (García-Oliva *et al.*, 1999a) that are essential for nutrient cycling (Kauffman *et al.*, 1993). Percent water holding capacity increase with burning but decrease after harvesting the crops. Responded increase of bulk density to burning attributed it to the disruption of soil aggregation and loss of organic matter (Ketterings *et al.*, 2000). Due to its static in nature soil texture was not effected by slash and burn activities where as slight changes in particle size distribution in the post fire soil and decrease in total number of pore volume (Are *et al.*, 2009). The disruption of soil aggregates and of the processes maintaining long-term soil nutrient and water availabilities contributes to soil deterioration (García-Oliva *et al.*, 1999b).

The higher amount of soil microbial biomass carbon (SMBC) soil microbial biomass nitrogen (SMBN) and soil microbial biomass phosphorus (SMBP) relatively decline in slash and burn area comparatively with forest area (Arunachalam, 2002; Maass *et al.*, 2002 and Fernandez *et al.*, 1997) which could be due to the abundance of diverse vegetation and vegetation cover which contributes high amount of substrate for the activities of micro organisms in the forest area. Increase in SMBC

increased as the duration of cultivation and vice versa for soil SMBN and SMBP (Arunachalam, 2002). Soil temperatures after burning; stimulate biological activity (Ojima *et al.*, 1994 and Brye, 2006). Colonies of bacteria and fungi were more in number in the forest field and their population decreased after burning and then increased gradually as the duration of cultivation increased (Arunachalam, 2002). Under shifting cultivation in Papumpare district of Arunachal Pradesh, Northeast India, the bacterial (*B. clausii*, *B. licheniformis*, *B. megaterium*, *B. subtilis*, *B. thuringiensis*, *P. aeruginosa* and *P. stutzeri*) and actinomycetes counts were significantly higher in fired plots which signify a higher rate of recovery as compared to the fallow plots (Pandey *et al.*, 2011). Study conducted on forest fire in central Colorado, reveal that there was no significant difference in total microbial biomass between the unburnt and burnt soils. However, the microbial communities in the burnt sites were structurally different from the unburnt sites (Hamman *et al.*, 2007). Several studies conducted some years after forest conversion to pastures have documented a decrease in soil microbial activity and nutrient losses associated with the loss of plant cover, severe erosion, and reduction in soil organic matter and microbial biomass (Maass *et al.* 2002).

2.3. Impact of shifting cultivation on soil microbial community

The microbial structure gets affected with a change in ecosystem functions such as interaction between plants, soil and microorganisms which will influence the ecological processes (Singh *et al.*, 2004). Soil attributes, plant species, land management were important for maintaining the structure and functions of bacterial communities (Nusslein and Tiedje, 1999; Wieland *et al.*, 2001; Steenwerth *et al.*, 2002; Hartman *et al.*, 2008; Lauber *et al.*, 2008; Wakelin *et al.*, 2008; da Jesus *et al.*, 2009 and Blasiak *et al.*, 2014). With respect to soil texture there was a community structure shift among bacteria and fungi (Girvan *et al.*, 2003), soil pH (Lauber *et al.*, 2008) and availability of soil nitrogen (Frey *et al.*, 2004). Faulty agricultural practices and land use result in major loss of biodiversity with negative result consequences for the environment (Doran and Zeiss; 2000; Balvanera *et al.*, 2006 and Navarrete *et al.*, 2010). Structural shift and composition of the microbial community become strong indicators of soil quality and soil biological activity. The microbial community in soil can be affected by fire, land use and change plant species which are the main component in shifting cultivation and this component are discussed below:

2.3.1 Effect of Microbial Community By Land Used

Size, activity and composition of soil microbial communities gets affected by changes in the aboveground vegetation (Carson *et al.*, 2010; Lemos *et al.*,

2011). Microbial communities likely to respond changes made in land use system. However, such responses are poorly characterized. Any changes in land-use can possibly cause a disturbance, which in turn might affect the soil microbial communities (Allison and Martin, 2008). Nevertheless, agricultural practices do not always deplete soil bacterial diversity. Different land uses may have a positive, negative or neutral impact to shifts in microbial diversity and structure. Although changes in soil properties due to continuous cultivation appear to be a slow process, To bridge the gap of a poorly understood on the impact of land-use changes on soil conditions and microbial communities responds studies was carried out by Lauber *et al.* (2008) in Calhoun experimental forest in South Carolina, USA and da Jesus *et al.* (2009) Amazonas State of Brazil at Benjamin Constant municipality. In Calhoun experimental forest in South Carolina, four different land use type (cultivated fields, pasture land, pine forest plantation, and mixed hardwood forest) was studied and the result show that Proteobacteria was the most dominant bacteria phyla in cultivated land, Actinobacteria in pasture and Acidobacteria in Hardwood and pine forest respectively. At Benjamin Constant municipality, Amazonas State, Brazil Six (6) different land use type (crop, pasture, agroforestry, young secondary forest (up to 5-years old), old secondary forest (5- to 30-years old) and primary forest) was studied and reveals that alteration of bacterial community was observed when crop or pasture was grown in shifting cultivation. Bacteroidetes was found to be most dominated in crop and pasture soils where as the primary forest was dominated with betaproteobacteria, firmicutes and burkholderia. In secondary old forest firmicutes and actinobacteria was present with half of alphaproteobacteria sequences were from crops land (da Jesus *et al.*, 2009). This indicated that different land management system / land use alter the soil properties which thus then result in changes of soil microbial community structure. The greatest fungal taxonomic unit was reported in forest sites and arable sites with lowest fungal taxonomic unit (Lauber *et al.*, 2008 and Creamer *et al.*, 2015). In forest the Nitrospira genus, was statistically greater in numbers than in grassland (Suleiman *et al.*, 2013). The importance of C:N ratio for phylogenetic distance shifts in fungal abundance was also reported by (Frey *et al.*, 2004; Lauber *et al.*, 2008).

With a higher abundance of the genus Bradyrhizobium, shifts in bacterial community composition were also reported in slash and burn system. Betaproteobacteria was reported as most abundant bacterial group in slash and burn system (Blasiak *et al.*, 2014).

When a land use was changed from paddy to vegetables in soil bacterial diversity and soil microbial biomass was decreases despite an increase in the

abundance of culturable microorganism (Sun *et al.*, 2011). Changes in soil physical and chemical properties induced with the conversion of land use result in the transformation of soil microbial communities (Yuan *et al.*, 2015).

2.3.2 Effect of fire to microbial community

Most critical threats to forest ecosystems is fire (Xiang *et al.*, 2014). Fires likely have large direct and indirect effects on soil bacterial community composition and diversity. Fire produces a broad spectrum of effects that depend on fuel load and combustion, vegetation type, climate, topography and so on. Heat from fires can kill soil bacteria, reducing microbial biomass (Wang *et al.*, 2012) and directly impacting bacterial community composition and diversity (Hart *et al.*, 2005). It results in aboveground biomass depletion, altering the physical, chemical and biological components of soil ecosystems (Certini, 2005 and Neary *et al.*, 1999). Community composition and diversity of above-ground community after fire have been extensively studied. Despite the central role of bacteria in ecosystem recovery and functioning effects of fire on soil bacterial communities remain largely unexamined (Xiang *et al.*, 2014). There is scanty information about the long term effects of fire on bacterial communities in soils. The sensitivity of soil microbes to fire differed bacteria tend to be more resistant to heat than fungi, and generally increase in relative abundance after a fire (Hamman *et al.*, 2007 and Xiang *et al.*, 2014).

Fire does not only burn out the slashed biomass but also the litter layer, and the uppermost humus layer which constitute to be the major part of the resources and habitats for soil organisms (DeBano *et al.*, 1998).

Xiang *et al.* (2014) in his study on boreal forest in China reported that fire significantly result in the shift of Bacteroidetes and Betaproteobacteria with greater abundance of Alphaproteobacteria and decreased in Acidobacteria, as well as Deltaproteobacteria and Planctomycete are in low abundance and reported that Actinobacteria was not effected by fire and found no significant difference in total microbial biomass between the unburnt and burnt soils.

Hamman *et al.* (2007) reported a structural difference among the microbial communities in the burnt sites from the unburnt sites of forest fire in central Colorado and documented that AMF abundance was high in low severity burn sites relative to the unburned no significant change.

Deka and Mishra, (1983) and Pandey *et al.* (2011) in their studies also reported fungi as the least affected group by fire, actinomycetes population get stimulation by fire and bacteria as the most affected group. Study conducted in Khasi

hills of Meghalaya, India, reported greater microbial population from the burnt plots as compared to unburnt, following two to three showers (Sharma, 1980).

A study conducted in the coast of Jalisco, México has concluded that forest conversion by means of slash-and-burn followed by cultivation results in few immediate changes in the AMF communities, which could be due to low heat conductivity of the soil and rapid combustion of plant residues (Aguilar-Fernández *et al.*, 2009).

2.3.3. Effect of microbial community by plant

Zhang *et al.* (2013) through cluster analysis of DGGE profile showed that soil bacterial community structures under different vegetation and their restoration patterns were different. This unique characteristic association of different plant species with microbial communities was probably due to differences in amount and quality of root exudates and plant species influences on the selection of rhizosphere microbes (Zhang *et al.*, 2013). Ecosystem functioning (plant productivity, soil carbon sequestration, nitrogen mineralization) was strongly affected by the coexistence of multiple plant species with particular functional traits (nitrogen-fixing legumes, C4 grasses) (Balvanera *et al.*, 2006; Tilman, 1984) and may enhance the complexity of soil microorganisms by increasing the heterogeneity of organic substrates during decomposing of litter and living roots (Broughton and Gross, 2000; Stephen *et al.*, 1998). The dependence of ecosystem process on the presence of plant functional types was reported by (Roldan *et al.*, 2005) primary productivity (Crutsinger *et al.*, 2006), nutrient cycling, litter decomposition rates and ecosystem stability and resistance to disturbance. (Madritch and Hunter, 2002 and Madritch *et al.*, 2006). Change in plant community composition results in changes of litter quality that alters nutrient cycling process and resultant soil conditions (Miki *et al.*, 2010). Shifts in vegetation type fungi are more sensitive to it than soil bacteria, particularly mycorrhizal fungi that form symbiotic associations with specific plant types (Heinemeyer and Fitter, 2004) or Basidiomycota which are involved in decomposing lignified plant detritus (Bardgett and Mc Alister, 1999). Shifts in carbon pools may have different effects on these two groups of microorganisms as bacteria and fungi may not mineralize the same types of carbon substrates in soil, (Six *et al.*, 1999; Lauber *et al.*, 2008).

2.4. Impact of Shifting cultivation on Soil microbial community composition

The microbial communities in soil perform a vast range of ecosystem functions. Changes in the composition and function of below-ground communities and can affect composition or species diversity of above-ground communities and vice versa (van der Heijden *et al.*, 2008). Microbial community changes respond to changes in the aboveground vegetation in soil which affect the size, activity, diversity, abundance and composition of soil microbial communities (Nusslein and Tiedji, 1999; da Jesus *et al.*, 2009 and Zhao *et al.*, 2014). These changes had an impact on the ecological services provided by soils, such as soil quality, nutrient cycling and crop productivity of terrestrial agro-ecosystems. Due to their involvement in the geochemical cycles soil organisms have a strong role to play in the cycling of nutrients. There is a significant correlation of functional genes of soil microbial communities and environmental variables. Based on whole metagenomic survey 5 yrs fallow phase have 2 time higher prokaryotic diversity than that of 20 yrs fallow phase in Nagaland (Thakuria and Sharma 2014). This might be supported by diversified aboveground plant species in early succession stages, which is closely related to the findings of Anjaneyulu *et al.*, 2011).

2.5. Soil Enzymes Activities as an indicator of soil processes

Terrestrial ecosystems consist of above- and below-ground components that interact to influence community- and ecosystem-level processes and properties. These above and below ground communities released extracellular enzymes into the soil upon death and lysis or for physiological reasons such as hydrolysis of polymers in oligomers or monomers to be taken up by cells (Allison and Vitousek, 2005; Awasthi *et al.*, 2014). In soil these enzymes are continually synthesised, accumulated, inactivated or decomposed. The activities of these enzymes in soils undergo complex biochemical processes consisting of integrated and ecologically-connected synthetic processes, and in the immobilisation and enzyme stability. As each soil type has different amounts of organic matter content, composition and activity of its microbial communities and intensity of the biological processes thus the enzyme levels in soil systems vary in amount (Xu *et al.*, 2015). As soil is interacted by different plant species the functional capacity of the microbial communities also vary depending upon plant species (Waldrop *et al.*, 2000). Mineral element limitation in the ecosystem leads to high enzyme activity signals in soil (Ndakidemi, 2006). Therefore, enzymatic activities can be used as a direct expression of available

nutrients and microbial community's metabolic requirements and comprehensive understanding of nutrient dynamics microbial populations and the key processes of soil (Sinsabaugh and Moorhead, 1994). Due to the effect of external disturbance on their activity and fast to environmental changes, enzymes can serve as sensitive indicators of microbial function during invasive processes and soil quality (Nannipieri *et al.*, 1990; Dick, 1994; Allison *et al.*, 2006 and Baldrain, 2009). Microbial communities act as a main driver of various soil processes such as nutrient cycling (van der Heijden *et al.*, 2008), bioremediation (Gilbert *et al.*, 2012) and plant health (Lugtenberg and Kamilova, 2009). These microbial processes are catalysed and supported by several enzymes and other chemicals bound in the biomass or secreted in the environment (Awasthi *et al.*, 2014). Enzymes are the main constituent for biological soil processes. Knowledge of soil enzyme activity also indicates the potential of soil to support some of the basic processes necessary for maintaining soil fertility (Hernandez *et al.*, 1997). The various factors of soil processes which are mediated by microbes are describe below. Furthermore, plants may also play important roles in determining soil enzymes activities, as extracellular enzymes are derived mainly from soil microorganisms, plant roots and soil animals.

2.5.1 Arylsulphates (E C 3.1.6.1)

Plant uptake sulphur in the form of inorganic sulphate. However, certain portion of sulphur is bound into organic compounds and become unavailable for the plant uptake. Thus, accessibility to the plants depend on the extracellular hydrolysis of these aromatic sulphate esters or intra cellular oxidation of soluble organic matter absorbed by the micro-organisms to yield energy and carbon skeletons for biosynthesis by which some inorganic sulphate are released as a by-product to the external environment as a response to sulphur limitation (McGill and Colle, 1981 and Dodgson *et al.*, 1982). These mineraliation and immobilization and dependent on arylsulphatases enzymes (Strickland and Fitzgerald, 1984 and Fitzgerald and Strickland, 1987). It is known to play a role in sulphur cycling. Its occurrence in different soil systems is often correlated with microbial biomass and rate of S immobilisation (Klose and Tabatabai, 1999; Vong *et al.*, 2003). The environmental factor that effect the conversion of soluble sulphur to insoluble sulphur is reported by Burns, 1982, heavy metal pollution (Tyler, 1981); changes of soilpH in the soil solution (Acosta-Martinez and Tabatabai, 2000); organic matter content and its type (Tabatabai andBremner, 1970; Ladd, 1978; Sarathchandra and Perrott,1981; Dalal, 1982); the concentration of organic sulphateesters (Dodgson and Rose, 1976). The difference

caused by burning could be a reason for change in ASA activity and similar result was also reported by (Vong *et al.*, 2003).

2.5.2 β -Glucosidase (E C 3.2.1.21)

The β -glucosidase is the most predominant glycosidase activity in soil one among the glycosidases which plays a vital role in the large-scale C cycle, as well as small scale processes of releasing a labile carbon energy source for microorganisms (Acosta *et al.*, 2007). Various organisms i.e. plants, animals, fungi and bacteria produce this enzymes (Esen, 1993). Fungi have been suggested as the predominant source of β -D-glucosidase (Hayano and Katami, 1977; Hayano and Tubaki, 1985) activity in soils. Zygomycetes are identified as sucrose and cellulose degraders (sugar fungi), while some Basidiomycetes are recognized as lignin degraders (Hanson *et al.*, 2008; Xiong *et al.*, 2014). This β -glucosidase breaks down labile cellulose and related carbohydrates with 1–4 glucosidic bonds, degrading plant cell walls and thus contributing to the first phases of plant cell tissues decomposition, which then facilitate the activities of other enzymes such as proteases and phosphatases. Its final product is glucose, an important C energy source of life to microbes in the soil. (Esen, 1993). Its sensitivity to soil management is reported by (Dick *et al.*, 1996; Bergstrom *et al.*, 1998; Bandick and Dick, 1999; Bandick and Dick, 1999; Ndiaye *et al.*, 2000), soil pH (Bergstrom *et al.*, 1998; Bandick and Dick, 1999 and Acosta-Martinez and Tabatabai, 2000) and may give a reflection of past biological activity. A good indicator as an advanced change in organic carbon was reported by (Dick, 1994 and Wick *et al.*, 1998). Thus it served as a good biochemical indicator for measuring ecological changes.

As the plant invasion accelerates nutrient uptake and nutrient cycling in soil higher soil enzymes phosphatase and β -glucosidase activities in association with faster water and nutrient cycles, lower concentrations of some nutrients in the soil and with higher levels of nutrients in stand biomasses and faster growth. Microbes adjust their extracellular release of soil enzymes to maximize the mobilization of substrates rich in their limiting element (Burns *et al.*, 2013). Since β -glucosidase is involved in cellulose catabolism, more abundant and extended litter production in invaded stands may also explain the higher activity of this enzyme in this type of forest (Burns *et al.*, 2013).

2.5.3 DEHYDROGENASE (E C 1.1.1.X)

The dehydrogenase exist in soils as integral parts of intact cells (Taylor *et al.*, 2002) its gives correlative information on microbial populations in soil and soil

biological activity (Wolinska and Stepniewska 2012). At the time of the test it gives immediate metabolic activities of soil microorganism. It is an oxidative degradation process .i.e., dehydrogenation of organic matter by transferring hydrogen and electrons from substrate to acceptors (Makoi and Ndakidemi 2008). Many specific dehydrogenases act as to transfer the nicotinamide adenine dinucleotide or nicotinamide adenine dinucleotide phosphate. Through these co-enzymes the H atoms take part in the reductive processes of biosynthesis. These processes are part of respiration pathways of soil micro-organisms (enzymes of the respiratory metabolism, the citrate cycle and N metabolism) and are closely related to the type of soil and soil air-water conditions (Kandeler *et al.*, 1996). Thus serves as an indicator of the microbiological redox systems and may be considered as a good measure of microbial oxidative activities in soil (Kumar *et al.*, 2013). Several environmental factors, including oxidation reduction potential, soil pH, soil moisture, oxygen availability, organic matter content, temperature, depth of the soil profile, season of the year, soil fertilization, heavy metal contamination or pesticide use can affect significantly DHA in the soil environment (Baruah and Mishra 1984; Dkhar and Mishra 1983; Sinsabaugh *et al.*, 2008; Wolinska and Stepniewska, 2012; Kumar *et al.*, 2013 and Veres *et al.*, 2013). Additionally, dehydrogenase enzyme is often used as a measure of any disruption caused by pesticides, trace elements or management practices to the soil as well as a direct measure of soil microbial activity (Skujins, 1978; Trevors, 1984; McCarthy *et al.*, 1994 and Garcia and Hernandez, 1997). It can also indicate the type and significance of pollution in soils. For example, dehydrogenase enzyme is high in soils polluted with pulp and paper mill effluents but low in soils polluted with fly ash (Pitchel and Hayes, 1990). Similarly, higher activities of dehydrogenases have been reported at low doses of pesticides, and, lower activities of the enzyme at higher doses of pesticides (Baruah and Mishra, 1986). Dehydrogenase is an enzyme that exists only in viable microbial cells. It is often used as an indicator of microbial activity (Skujins, 1976; Nannipieri *et al.*, 1990; Dick, 1994.). Dehydrogenase activity was originally used as a test for measuring respiration of soil micro-organisms in situ (Casida *et al.*, 1964).

2.5.4 Acid Phosphatases (E C 3.1.3.2)

Phosphatase enzymes play an important role in phosphorus cycling (Speir and Ross, 1978; Zhang *et al.*, 2013). It is capable of catalysing hydrolysis of esters and anhydrides of phosphoric acid (Schmidt and Lawoski 1961). An excellent indicator of the organic phosphorus mineralization potential and biological activity of soils (Dick and Tabatabai, 1993). Apart from being good indicators of soil fertility, phosphatase enzymes exercise key roles in the soil system (Dick and Tabatai, 1992;

Eivazi and Tabatabai, 1977). Environmental conditions affected this activity but also reflects and feeds back on community composition (Sinsabaugh *et al.*, 2002). This enzyme is produced by many organisms in the soil. Bacteria and fungi that break down insoluble nutrient sources in the soil produce extracellular enzymes. These are proteins that are produced inside the cell and exported out into the soil solution. Its purpose is to remove the phosphate molecule from organic compounds such as phospholipids and nucleic acids. Once the phosphate is cleaved it becomes soluble and can be taken up by the cell. Land plants have evolved many morphological and enzymatic adaptations to tolerate low phosphate availability. Scientists in their studies reported that phosphatase activity was correlated with organic matter in various studies (Guan 1989; Jordan and Kremer, 1994; Aon and Colaneri, 2001), soil pH (Eivazi and Tabatabai, 1977; Tabatabai, 1994; Acosta- Martínez and Tabatabai, 2000; Makoi *et al.*, 2008), soil and vegetation conditions (Herbien and Neal, 1990), responds to changes in management, and seasonal changes in soil temperature and moisture. Many organisms, including soil fungi, release phosphatases into their environment. These phosphatases are introduced into the soil by active exudation, leakage or cell lyses. P deficiency often enhances extracellular phosphatase activity from plant roots, fungi and other microorganisms (Li *et al.*, 1997). There is no doubt that the presence of plants has an effect on enzyme activities, including phosphatase (Kiss *et al.*, 1975 and Juma and Tabatabai 1977). The effect of plants can be direct when the roots secrete acid phosphatase and indirect when related to changes in soil organic matter content and microbial populations (rhizosphere effect). Enzyme activity was higher in rhizosphere soils than in before planting and non – rhizosphere soils. Increase phosphatase activity with an increase in P deficiency caused by increased root density and decreased soluble inorganic P levels was reported by Sarapatka 2002. The positive correlation between phosphatases and C and N is in good agreement with the results of (Šarapatka and Kršková 1997) the correlation between acid phosphatase and pH is similar to the observations of (Herbien and Neal, 1990). Fire decreases the acid phosphatase activity and serve as an indicator of overall microbial activity, (Eivasi and Bayan, 1996; Boerner *et al.*, 2000).

Some enzyme functions are associated with the microbes themselves, such as dehydrogenase activity of which the main localization is the plasma membrane of bacteria or in mitochondrial membranes of fungi. Other enzymes are synthesized and secreted extracellularly by bacteria or fungi (e.g. phosphatases, urease, cellulases, pectinases). Microbially-secreted enzymes may take part of the soil matrix as extracellular enzymes, also called abiotic (Dick, 1994; Sinsabaugh, 1994).

Activities of β -glucosidase and phosphatase have been shown to increase in a tall-grass prairie following fire (Ajwa, 1999), and to decrease or be unchanged in oak-hickory forests (Boerner *et al.*, 2000; Boerner and Brinkman, 2003; Eivazi and Bayan, 1996). Greater soil phosphatase activity has been reported in forests that were burned and thinned compared to unburned or burned only treatments, although these differences were only apparent a growing season later (Boerner *et al.*, 2005). In contrast, when the activities of various soil enzymes were examined in eight North American forest ecosystems being restored by the use of prescribed fire and mechanical thinning, fire lowered phenol oxidase activity but did not affect phosphatase or chitinase activity (Boerner *et al.*, 2008). However, when the effects on forests in the eastern United States were pooled together, phosphatase activity was lower in areas that were both burned and thinned (Boerner *et al.*, 2008). Changes in soil enzyme activity in the years following a fire have also been shown in grasslands, with wildfires decreasing overall activity by 10-20% in the first year, 25-50% the following year, and enzyme activities returning to pre-fire levels after three years (Gutknecht *et al.*, 2010). Overall, the effects of fire on soil microbial enzyme activity are variable and may depend on both ecosystem type and past site history, as well as time since fire event (Fritze *et al.*, 1994). Microbial enzyme activity has been linked to rates of decomposition (Sinsabaugh *et al.*, 1991, 1994), suggesting that fire-induced changes in enzyme activity could affect organic matter decomposition rates. β -glucosidase may be regulated primarily by substrate availability, whereas phosphatase and NAGase are regulated by the soil microclimate and chemical factors (Sinsabaugh *et al.*, 1992). Therefore, any changes in β -glucosidase activity could indicate substrate alteration by fire, while changes in phosphatase may indicate physicochemical alteration. Long-term N fertilization significantly increased activities of β -glucosidase and acid phosphatase but decreased urease activity (Ajwa *et al.*, 1999). By examining organically cultivated soils of different ages, Monokrousos *et al.* (2006) found that acid phosphatase activity increased from newest to oldest organic systems whereas the activities of amidohydrolase and alkaline phosphatase were highest in the middle-aged ones.

2.6. Soil Biochemical Activities In Jhum Agro Ecosystem

Burning plays an important role in *jhum* agro-ecosystem. During burning nutrients are released from parent material and biomass and become available for crop uptake, escape via volatilization, leaching or surface runoff, or remain bound in recalcitrant ash complexes (Ramakrishnan, 1992). Researchers have the view that within a shifting cultivation schedule, burning of the forest or fallow biomass results in

the release of nutrients that can be utilized by crops planted after the burning process is over (Sanchez, 1976). Incorporation of cations from nutrient-rich ash into the cation exchange pool result in short-term increment of soil fertility (Nye and Greenland, 1960). Several soil biochemical activities (Soil Organic Carbon, Dissolve Organic Carbon, Particulate Organic Matter, Microbial Biomass Carbon, Potentially Mineralizable Nitrogen) were taken into consideration and describe below

The past studies have widely describe the decrease in SOC content after burning (Nye and Greenland, 1960; Fernandez *et al.*, 1997; Sapalrinliana *et al.*, 2016; Lungmuana *et al.*, 2017). Topsoil temperature ($242^{\circ}\text{C}\pm 10^{\circ}\text{C}$) during burning alters the SOC content, burning of forest floor litters leads to limited incorporation of litter materials in burnt plots (Tinker *et al.*, 1996). Findings indicated that the content of SOC increased with the increase in the length of fallow phase and the differences in SOC contents among fallow phases were significant. The single most important indicator of soil productivity was soil organic matter (Haynes, 2005). As its role for maintaining soil fertility and soil structure, soil organic matter reflects the productivity of shifting agricultural systems.

Significant reduction ($P = < 0.05$) of carbon content just after burning in Northeast Indian *jhumming* condition and the phenomenon was more prominent in 10 and 30 year *jhum* fallow compared to 5 years old fallow. Upto a period of 90 days in reduction of carbon continued in 10 and 30 year *jhum* cycle sites and 30 days for 5 year cycle. Carbon content in the surface soil showed an increasing trend after this period (Ramakrishnan and Toky, 1981). A study conducted in Beforona, the central eastern Madagascar has shown that slash-and-burn of a 5-year-old fallow resulted in a decrease of the topsoil carbon concentration by approximately 20% which indicates that fire affected the organic matter in the first few centimetres of topsoil (Brand and Pfund, 1998).

Soil microbial biomass reflects to soil organic pool which is responsible for organic matter decomposition affecting soil nutrient content and primary productivity in most biogeochemical processes in terrestrial ecosystems (Franzluebbers *et al.*, 1999; Gregorich *et al.*, 2000; Haney *et al.*, 2001; Sharma *et al.*, 2004). Microbes are the primary decomposers of plant material due to the diversity of the enzymes produced and their unique ability to produce enzymes to break down both simple molecules such as cellulose and more complex plant derived compounds such as lignin. While the microbial (bacterial and fungal) community are commonly associated with transformations of SOC in soils (Tardy *et al.*, 2015) it has also been shown that the interaction between microbes and soil fauna (including mites, earthworms,

collembolans, enchytraeids and nematodes) aid this process and typically simulate decomposition thus affecting carbon cycling. Soil microbial biomass and fungi plays a key role for carbon sequestration in soil (Clemmensen *et al.*, 2013). Inclusion of soil microbial biomass, respiration and fungal richness have been found to be key indicators of carbon cycling and potential storage of soil organic carbon. (Creamer *et al.*, 2015).

Burning results in reduction of MBC and MBN (Prieto-Fernández *et al.*, 1998; Ajwa *et al.*, 1999 and Liu *et al.*, 2010). Past findings indicated that many of the soil decomposer community would get reduced or totally die because of the burning effect (DeBano *et al.*, 1998; Malmström, 2012). The remaining species that may have survived would also be suppressed because of the sudden change in the environment like change in pH, temperature and the low soil moisture content which are outcome of the burning activities. Reduction in microbial activity may also be attributed to the loss of SOC and N after burning operations (Choromanska and DeLuca, 2002).

Dissolved organic carbon (DOC) is the primary form of C that is transported from forest floor to mineral soils (Yano *et al.*, 2000). It is produced by soil enzyme-catalyzed depolymerization of organic matter and is comprised of low molecular weight chemicals that are often water soluble and thus more accessible to microbial assimilation as energy, carbon and nutrient sources. Microbial utilization of these soluble compounds leads to microbial immobilization as well as soil C and N mineralization. Heterotrophic microbes in soils play important roles in N dynamics, and that their metabolism is often restrained by the availability of C in the soil (Starr and William, 1993). Due to differences in chemical compositions of soil organic matter and plant litters dissolved organic matter, soil enzyme activity, and soil C decomposition relationship seem to be inconsistent across various ecosystems. (DeForest *et al.*, 2004 and Waldrop and Zak, 2006). Increase in the concentration of dissolved organic carbon in forest soil was recorded after the application of synthetic N fertilizer where as the oxidative enzymes activities decline (Sinsabaugh *et al.*, 2005).

Relationships among dissolved organic matter, soil enzyme activity, and soil C decomposition has been studied on forest and grassland ecosystems (Carreiro *et al.*, 2000; Saiya-Cork *et al.*, 2002; Waldrop *et al.*, 2000; Sinsabaugh *et al.*, 2005; Zeglin *et al.*, 2007).

Particulate organic matter (POM) is mainly derived from plant material, and only to a limited extent derived from soil macro-fauna. A study conducted in slopy upland in Thailand shows a greatest proportion of in the tilled wetland rice soil followed

by the relatively undisturbed forest soils and with the lowest content in the recently afforested soils. The highest in wetland rice field cultivation could be due to actual breaking and turning of the soil matrix. Topsoil profiles of C and particulate organic matter were affected by land use but unaffected by slope position in landscape, indicating minor effects of erosion Aumtong *et al.*, 2009.

The aboveground biomass and dissolved organic carbon increase with increasing N input across various ecosystems (Liu and Greaver, 2010; Liu *et al.*, 2014). Soil microbial biomass and respiration reduces under N enrichment condition (Treseder, 2008; Liu and Greaver, 2010; Liu *et al.*, 2010). Low levels of N inputs stimulate soil microbial biomass C, N, and soil microbial respiration (Treseder and Holden, 2013; Liu *et al.*, 2010).

Potentially Mineralizable Nitrogen (pMN) can be defined as the fraction of organic nitrogen converted to plant available (or mineral) forms under specific conditions of temperature, moisture, aeration, and time. Determination of pMN levels can provide an estimate of available N in the soil. pMN originates mainly from microbial biomass and plant and animal tissues—the main source of the organic nitrogen pool. It represents the fraction of nitrogen easily decomposable by soil microorganisms and is considered an indirect measure of nitrogen availability during the crop growing season (if measured during that period). While anaerobic N mineralization potential may be a good indicator of the potential for soil to deliver N, it does not necessarily reflect microbial biomass N levels. It was suggested that the ratio of N mineralized to total organic nitrogen N could serve as a sensitive indicator of differences in soil organic matter (SOM). As a readily available fraction of total N, pMN is an important potential source of N for crop growth and yield, especially in synthetic N-fertilizer-free agricultural operations (eg. organic farming). pMN can be a source of available N for microorganisms and indirectly enhances microbial growth and activities, including C and N cycling. In well drained soils, PMN is made available to plants and microorganisms, mostly in the form of nitrate, through aerobic mineralization. In poorly drained soils (such as rice fields), pMN is made available, in the form of ammonium, through anaerobic mineralization.

At temperatures above 300°C, soil organic N is lost during the thermal oxidation of organic matter in the form of oxidized N gases and N₂ (Raison, 1979). The content of NO₃-N has a declining trend after burning (Ramakrishnan and Toky, 1981). Loss of nitrogen at temperature as high as 100°C was reported by DeBano *et al.* (1979), which in the case of conventional *jhumming* usually exceed this threshold temperature.

2.7 Denaturing gradient gel electrophoresis (DGGE) fingerprinting

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

Although there are many advantages to using PCR-DGGE for microbial community analysis, method limitations must be recognized for interpretation of result. The greatest challenge in performing DGGE and attaining reproducible results is minimizing variation between gel gradients. It is very difficult to exactly reproduce gel gradients; this must be kept in mind when performing between-gel comparisons. Another limitation of DGGE is that the number of bands observed in a profile cannot be interpreted to be the exact numbers of populations in a community. In some cases investigators have found that a single laboratory isolate can produce multiple bands by DGGE (Satokari *et al.*, 2001), and conversely a single band may represent multiples populations (Yang and Crowley, 2000). Combining DGGE profiling with other techniques, such as sequencing of bands or hybridization with probes can reduce the ambiguity of band identification (Stephen *et al.*, 1998). Nevertheless, the method can estimate of richness that enables researches to determined subsequent analyses that

can be conducted and a means of choosing samples representing unique or representative communities.

2.8. Research gaps:

Literature review revealed that scientific evidence is lacking on how slash and burn practices impact the functional microbial community compositions. Scanty of research data on how interactive effects of functional microbial community and plant species can regulate/modify the soil processes and thereby soil functioning as well as to what extent such combined effects are altered due to slash and burn practices in *jhum* agroecosystem are not known. The knowledge on the relationship among microbial functional groups and plant species under the influence of slash and burn practices is grossly limited.

Chapter-3

Materials and Methods

3.1 Description of the sampling site

Mizoram is one among the North Eastern states where we can find that shifting cultivation is practiced extensively. Due to the increase in population the area for agricultural land has decreased and thus led to a pressure on land which results in reducing the *jhum* cycle into 3-5 years from 10-20 years in many areas. Keeping this in view five (5) years *jhum* cycles from Muallungthu village Aizawl district Mizoram was selected as a study area which lies between 23°36.279' N latitude and 92°42.909' E Longitude at an altitude of 841-857 m. As shifting cultivation is part of the Mizo culture till date it is impossible for the people to completely change their type of cultivation.

3.2 Climate and soil

Aizawl district of Mizoram experiences warm humid tropical climate. Muallungthu falls on the southern part of Aizawl district which experiences hot-humid weather during the summer months with an average temperature of 25°C during May to August. Winter months usually remain cold with moderate summer months. Maximum and minimum temperature in Muallungthu ranges from 20.8°C to 31.6°C and 7.1°C to 20.2°C, respectively. Out of total annual rainfall (2115 mm), about 81 % occurs during May to August and relative humidity ranges between 60.7 to 80.9% during most of the periods.

Soils of Aizawl district fall under the red soil group and the dominant soil order is Inceptisols followed by Entisols and Ultisols. Soils are mostly light to medium texture (sandy loam and clay loam) with depth ranging from deep to very deep.

3.3 Soil sampling and processing for microcosm experiment

From the identified 5 years *jhum* cycle before slashing the biomass soil samples at a depth of 0 to 15 cm of about 5 quintals were collected. Then, the next day after burning the biomass before sowing of seeds soil samples at a depth of 0 to 15 cm of about 5 quintals were again collected. The soil samples which were collected before slashing the biomass will represent as unburnt soils as well the soil samples which were collected after burning will represent as burnt soils. The collected bulk soils both burnt and unburnt soils were carried to research farm at College of Post Graduate Studies, Central Agricultural University, Umiam, Meghalaya for microcosm

experiments. The collected soils from burnt and unburnt situations were allowed to pass through 2 mm sieve separately and removed all visible fine roots and other organic debris and keep it ready for the microcosm experiment.

3.4 Location of the Microcosm experiment

To study the Impact of slash-burning and plant species on soil microbial community and processes in *Jhum* agro-ecosystem microcosm experiment was carried out set under poly house at the Experimental Farm of the College of Post Graduate Studies, Umiam, Meghalaya. The study area is located at 91°54.643" E, 25°40.929" N latitude and 950 m above mean sea level. The maximum and minimum temperature in the study site ranges from 20.9°C to 28.3°C and 4.4°C to 19.1°C, respectively. Out of total annual rainfall (2190 mm), about 76% occurs during May to September. The relative humidity ranges between 50.91 to 83.87% during most of the periods.

3.5 Microcosm experiment

The sieved soil mentioned in 3.3 was used for microcosm experiment. A series of pots were arranged in 4 groups as depicted in figure 1. In two groups i.e burnt and unburnt soil *jhum* rice was grown where in the other 2 groups no crop was grown. The pot filled with 4.0 kg sieved soil and 2 kg sieved soil. The bulk density of the pot soil was adjusted based on weight by volume basis to mimic the bulk density in field situations. Only after the pot soil mimic bulk density of the field situation functional microbial groups were inoculated. Only after functional microbial inoculation rice seed was sown in the pot. The pot which was filled only by 4.0 kg sieved soil was used for growing rice crop and no crop was grown in the pot which was filled by only 2 kg sieved soil. Soil moisture in the pot was maintained at field capacity throughout the experimental period. Just before inoculating the functional microbial groups a soil sample of approximately 100gm was collected from each groups and store the soil sample at 4°C for further analysis.

3.5.1 Treatment details

Each group of pot experiment was treated with different bacterial functional groups and a synthetic fungal community. Factorial Complete Randomized Design was used as an experimental design in these studies. Three functional bacterial groups are: (1) N₂- fixers, (2) Phosphate Solubilising Bacteria (PSB), and (3) Cellulose Degrading Bacteria (CDB). All together six (6) treatment combinations was imposed viz. T1: 5 strains PSB + 5 strains synthetic fungal community, T2: 5 strains

N₂-fixers+ 5 strains synthetic fungal community, T3: 5 strains CDB + 5 strains synthetic fungal community, T4: 5 strains each of PSB + N₂-fixers + CDB +5 strains synthetic fungal community, T5: No bacteria + 5 strains synthetic fungal community and T6: No inoculation.

Upland rice variety khawlian buh was used as a test crop. In each pot rice seeds (3 seeds per pot) was sown where rice is to be grown and no seeds were sown where only 2 kg of soil were kept in the pot.

3.6 Background of the native functional microbial groups (bacteria)

A repository of 300 bacterial cultures was maintained in the Microbial Ecology Laboratory, College of Post Graduates Studies, CAU, Umiam, Meghalaya. These bacteria was isolated from the roots and rhizosphere of wild and cultivated rice species of North East India under a research programme funded by the department of Biotechnology, GOI, New Delhi. These isolates were preliminarily screened for their ability to produce ammonia in nitrogen free bromothymol (nfb) semi solid agar, solubilization zone in CMC amended minimal agar, tri calcium amended Pikovskaya's agar. Based on their behavior in three media isolates were tentaviley categorized into 3 groups: cellulose decomposing bacteria (C-group), nitrogen fixing bacteria (N-group) and phosphorus solubilising bacteria (P-group) and on the basis of their screening result 5 efficient isolates were considered and farther used for investigating their role on soil processes. The pure culture isolates hereafter referred as C1,C2,C3,C4,C5 for C group; N1,N2,N3,N4,N5 for N group and P1,P2,P3,P4,P5 for P group.

3.7 Isolation of Fungi

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

3.8 Production of microbial consortium

Well decomposed compost collected from CPGS farm was used as a carrier material. The compost was air dried, grinded and sieved through 1mm sieve to obtain an uniform and homogenous powdered forms for easy handling and utilization. These carriers was then packed in an auto-clavable plastic bags, air-tighten with a rubber band and performed tyndallisation process of sterilization in order to avoid contamination from unwanted microbes. The bags are than labeled and stored in a clean chamber at room temperature until mixing with the microbial formulation.

Five efficient functional bacteria from each 3 groups were grown as a pure culture in 5 ml of nutrient broth (NB) and were incubated ($30^{\circ}\text{C}\pm 0.5$ at 160 rpm) for 22 hrs till attainment of maximum viable cells. One milliliter of mother culture from each bacterial groups was transferred aseptically in a series of 150 ml NB and incubated in an environmental shaker cum incubator at $30^{\circ}\text{C}\pm 0.5$ at 160 rpm for 22 hrs.

After incubation 150 ml broth of each functional microbial groups (150 ml X 5=750 ml in 1000g of compost for each group i.e. C, N and P) was mixed uniformly using aseptically sterilized gloves, trays and beakers. The carrier and inoculant mix were checked properly in order to obtain uniformly mixed. The mixing should not be too dry or too wet. The microbial consortium (MC) was then packed in a sterilized plastic bags and labeled properly.

Five efficient synthetic fungal community was grown as a pure culture in 100 ml of Rose Bengal Agar Media and were incubated ($30^{\circ}\text{C}\pm 0.5$) for 5 days till attainment of maximum viable cells. The full grown spores was grind by using autoclaved mortal and pestal. After grinding the synthetic fungal community aseptically it was mixed with 1000 gm sterile soil uniformly using sterilized glove, trays and beaker.

3.9 Application of microbial consortium

As per the microcosm experiment lay out in fig1 10 gm of different microbial consortium 5 strains N_2 - fixers, 5 strains PSB, 5 strains CDB and 10 gm of 5 strains synthetic fungal community were used as soil inoculants before sowing of seeds. The consortium and the pot soils were mixed uniformly.

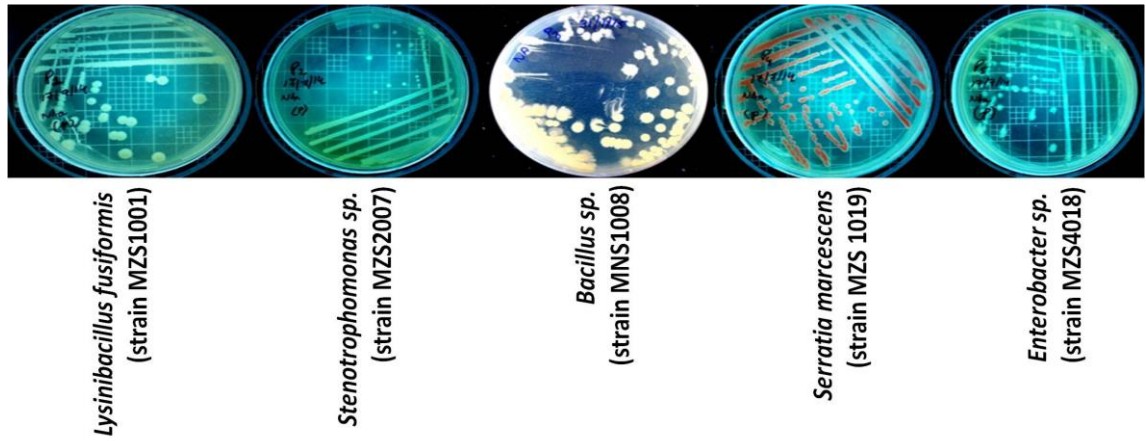


Plate No. 3.1: Synthetic PSB community used as an inoculants

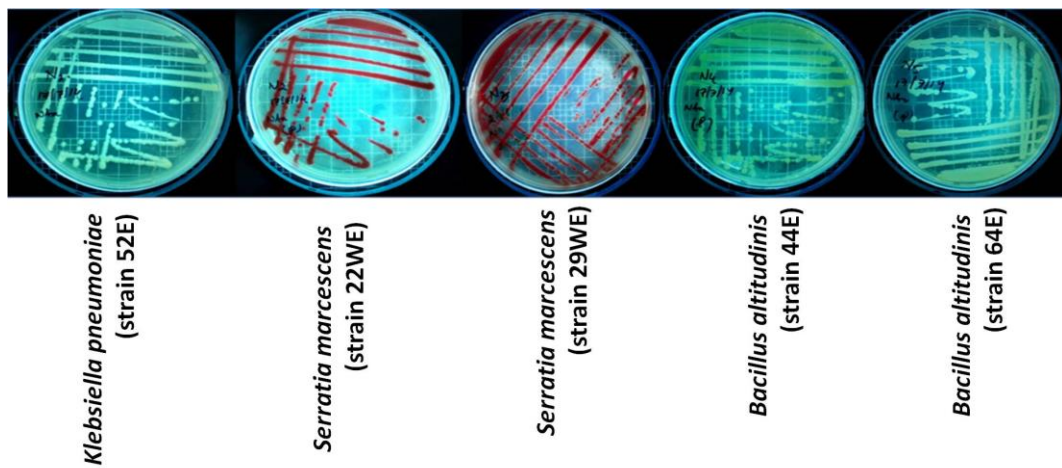


Plate no. 3.2. Synthetic di-nitrogen fixer community used as an inoculants

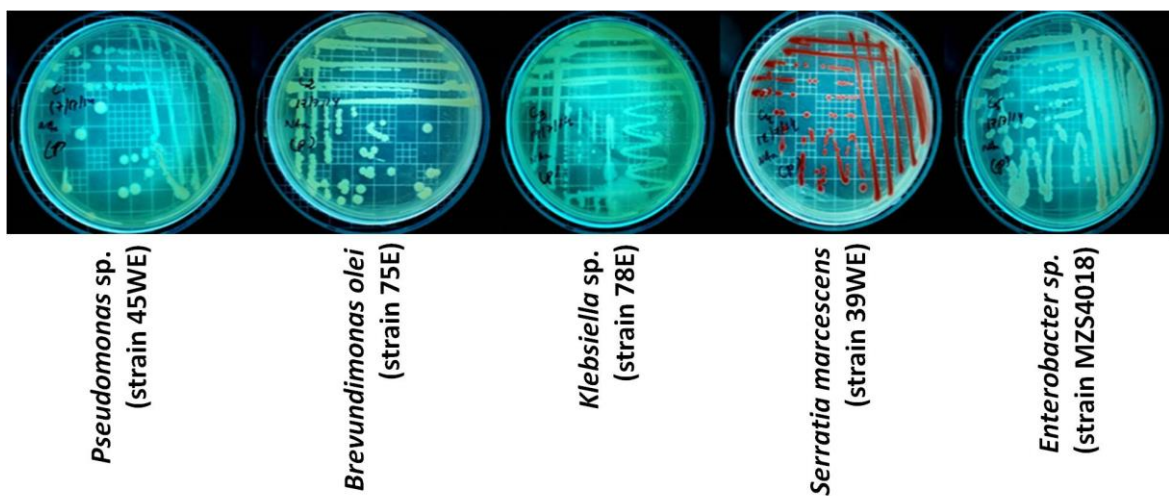


Plate no 3.3. Cellulase Degrading bacterial community used as an inoculants

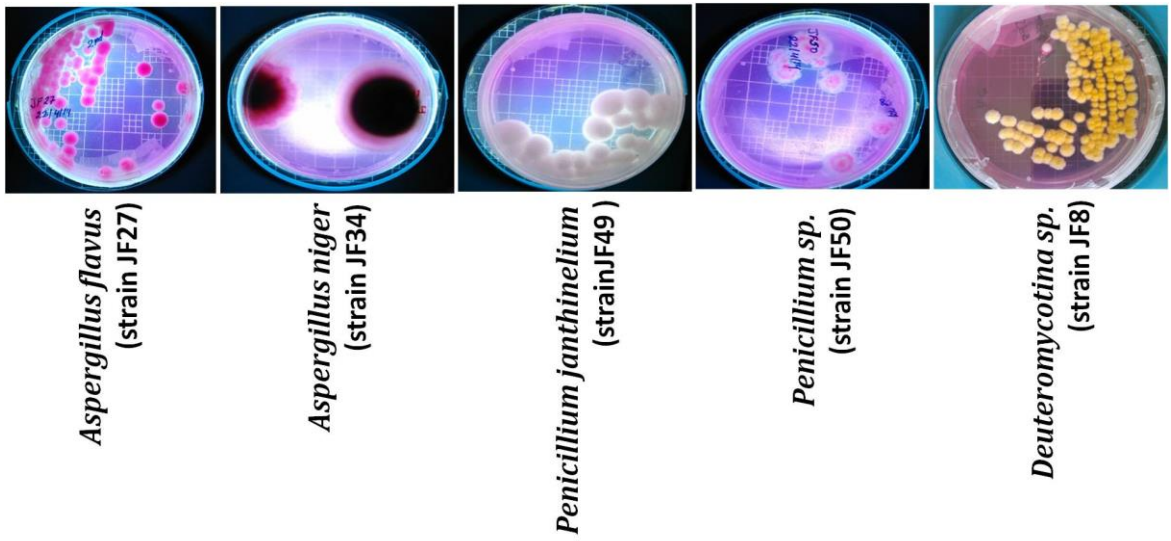


Plate no. 3.4. Synthetic fungal community used as an inoculant

BURNT

UNBURNT

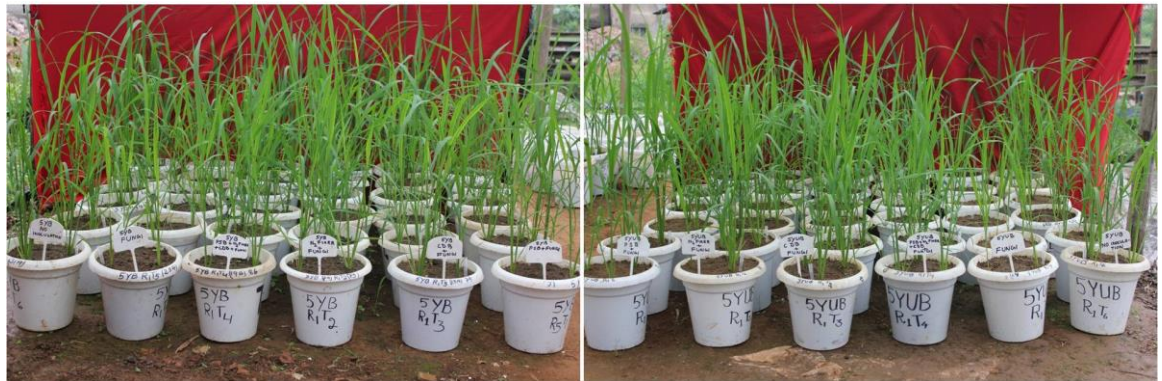


Plate no. 3.5 Plant growth at 45 days

3.10 Sampling

Soil cores (0 to 10 cm depth) were collected from each pot using a core sampler of 2.5 cm diameter at 0, 10, 45, 90 and 120 days after sowing (DAS) of *jhum* rice and packed in sterile polythene bags. Each soil was stored at 4°C for analysis of their biological and biochemical properties.

3.11 Soil chemical and bio-chemical properties

3.11.1 Soil physico-chemical properties

3.11.1.1. Bulk density (BD)

Bulk density of the soil was determined by Keen (Rackzowski) box method as described by Baruah and Barthakur (1997).

3.11.1.2. pH

Soil samples were analyzed for pH (1:2.5 soil/water suspension) using a standard pH meter (Mettler Toledo, Switzerland).

3.11.1.3. Electrical conductivity (EC)

Soil samples were analyzed for electrical conductivity (1:5 soil/water suspension) using a standard conductivity meter (Mettler Toledo, Switzerland).

3.11.1.4. Soil Available Nitrogen (Avl N)

Fresh soil (0.5 g) sieved through 1 mm sieve was used for determination of available nitrogen by alkaline permanganate oxidation method as described by Subbiah and Asija (1956). Soil sample was taken in a distillation tube and to this 40 mL of 0.32% KMnO_4 and 40 mL of 2.5% NaOH were added and distilled for 6 min in an automated distillation chamber (Classic DX, Pelican Equipment, Chennai). Ammonia generated during distillation was collected in 2% boric acid containing few drops of mixed indicator (Composition of mixed indicator: 0.1 g bromocresol green with 0.07 g of Methyl red and dissolved this mixture in 100 mL ethanol) in a conical flask and finally amount of boric acid used for absorption of ammonia was determined by titrating with standard 0.02 N H_2SO_4 . Available nitrogen was expressed in kg ha^{-1} .

3.11.1.5. Soil Available Phosphorus (Avl P)

Available phosphorus in soil was determined by following the stannous chloride blue colour method (Bray and Kurtz, 1945; Page *et al.*, 1982). Finely grinded air dried soil (0.5 g) was extracted with 50 mL of 0.03N NH_4F in 0.025N HCl for 5 min in a reciprocating shaker. After shaking, the soil suspension was filtered through Whatmann No. 42. A 5 mL volume of filtrate was taken for developing blue colour by adding 5 mL of Dickman Bray's reagent and 1 mL stannous chloride. Finally, intensity of blue colour was measured at 660 nm using microtiter plate reader (Multiskan, Thermo Scientific, USA) and concentration of P was obtained from the standard curve. Available phosphorus was expressed as kg ha^{-1} .

3.11.1.6. Soil Available Potassium (Avl K)

Soil available potassium was determined by following the ammonium acetate method described by Hanway and Heidel, (1952). An aliquot (25 mL) of neutral normal ammonium acetate was added to 5 g finely grinded air dried soil sample and shook for 5 min in a shaker. After shaking, the soil suspension was filtered through Whatman No.1 and the concentration was measured using a flame photometer.

3.11.1.7. Soil Organic Carbon (SOC)

Finely grinded (< 0.1 mm) air dried soil was used to determine SOC by following the potassium dichromate wet oxidation method described by Walkley (1947). Soil sample (0.5 g) was mixed with 10 mL of 1N $\text{K}_2\text{Cr}_2\text{O}_7$ and 20 ml of conc. H_2SO_4 in a 500 mL conical flask and kept for 30 minutes. After oxidation, 200 mL distilled water and 5 mL H_3PO_4 was added to the flask. The residual $\text{K}_2\text{Cr}_2\text{O}_7$ was titrated with freshly prepared 0.5 N ferrous ammonium sulphate in presence of diphenylamine indicator. SOC content in soil was expressed in $\text{g } 100\text{g}^{-1}$ soil (%).

3.11.1.8. Microbial Biomass Carbon (MBC)

Freshly collected soil samples were used for MBC determination by the procedure chloroform-fumigation-extraction method (Brookes and Joergensen, 2006). First the root debris and all the visible organic residues were removed from the sample. Each soil sample was divided into two sub samples (each sub sample weight was 25 g). Each sub sample was taken in 50 mL beaker. One sub-sample was fumigated for 24 h with chloroform vapour in a desiccator and the other sub-sample kept in a desiccator as check without fumigation for 24 h. After incubation for 24 h, the residual chloroform in the fumigated sample was removed by releasing the pressure maintaining valve. To each sub-sample 100 mL 0.5 M K_2SO_4 (1:4 ratio) was added

and the samples were shaken for 30 min at 200 rpm in an orbital shaker (Rotek LES, Pelican Equipment, Chennai). Then, soil suspensions were filtered through a Whatman No. 42 filter paper and 10 mL of the supernatants were used for determination of C by wet oxidation method similar to that described for determination of SOC in the section 3.4.4. The difference in C content between fumigated and non-fumigated sub-samples was determined and then, MBC was calculated using a conversion factor, $K_{EC} = 0.38$ (Vance *et al.*, 1987; Wu *et al.*, 1990; Dilly and Munch, 1998). MBC content was expressed in $\mu\text{g g}^{-1}$ (dw) soil.

3.11.1.9. Potentially mineralizable nitrogen (pMN)

Static anaerobic incubation procedure was used to determine pMN in freshly collected soil samples. Each soil sample was divided into 2 sub-samples. From one sub-sample, 16 g portion of soil was taken in test tube and 40 mL distilled water was added. Trapped air was removed by lightly tapping the tubes and then, the mouth is closed using screw caps. Soils were incubated at 40°C for 7 days. The other sub sample is added with same amount of distilled water but is kept as such without incubation. The inorganic $\text{NH}_4\text{-N}$ produced during 7 days incubation under anaerobic situation was extracted using 40 mL of 4M KCl solution by shaking the sample for 1 h and then filtered through Whatman No. 42 filter paper. 10 mL of the aliquot taken in digestion tube and 0.5 g of freshly ignited MgO is added into the tube. Mineralized N content was determined in the samples by following the regular Kjeldahl method. The mineralisable N during 7 days incubation was calculated by the difference between incubated and non-incubated samples (Page *et al.*, 1982; Franzluebbbers *et al.*, 2000; Tirol-Padre *et al.*, 2007). pMN is expressed in $\mu\text{g g}^{-1}$ (dw) soil.

3.11.1.10. Dissolve Organic Carbon (DOC)

Extraction of DOC in the field moist was done by using 1M KCl solution at a ratio of 5:1 (v/w). In 10 g of field moist soil 50 ml of 1M KCl solution was added and shake it for 30 minutes at 160 rpm. Soil suspension was filtered through 0.45 μm membrane filter (Zsolnay, 1996). 10 ml of the filtered solution was for determination of carbon by wet oxidation methods. DOC content is expressed in $\mu\text{g g}^{-1}$ (dw) soil.

3.11.1.11. Particulate Organic Matter (POM)

Physically uncomplexed organic matter is composed of particles of organic matter that are not bound to soil mineral particles and can be isolated from soil by density or size fractionation. Uncomplexed organic matter isolated by sieve is usually referred to as 'particulate organic matter' (Cambardella and Elliott 1992) but has also referred as 'sand size organic matter' or 'macro-organic matter' (Gregorich

and Ellert 1995). It is isolated by dispersing the soil and collecting the sand fraction on a sieve. The soils are first pass through a 2mm sieve. The POM recovered on a 53 μ m sieve can be defined as ranging in size from 53 to 2000 μ m in diameter and such represent a quantifiable component of thw whole soil organic matter.The field moist soil was allowed to pass through 2mm sieve and air dry it. 25 gm of air dried soil was placed in a bottle having a lid and mixed with 100 ml of sodium hexametaphosphate solution and shake it for 16 hrs. The suspension was poured to a 53 μ m sieve and rinse with a little amount of water. By this a silt+clay sized fraction which includes minerals and fine organic matter was washed and the aggregates were gently crush with a rubber policeman. The POM (sand+large particles of organic matter) was retained in the sieve. The particles which retained in sieve was transferred to a moisture cane box and oven dried it at 60^oc for overnight. By using a mortar and pestle the dried samples was grinded uniformly and was sieved it using a 250 μ m

3.11.1.12 Arylsulphatase Activity (ASA)

Arylsulphatase was measured following the principle described by Tabatabai and Bremner, (1970) which was based on determination of *p*-nitrophenol released after incubation of soil with *p*-nitrophenyl sulphate (PNS). Moist sieved (1 mm) soil was added with 0.25 mL toluene, 4 mL 0.5 M acetate buffer (pH 5.8) solution, 25 mM 1 mL *p*-nitrophenyl sulphate and incubated at 37^oC for 1 h. After incubation, 1 mL of 0.5 M CaCl₂ and 4 mL 0.5 M NaOH were added to the soil suspension and filtered using Whatman No. 42 filter paper. Intensity of yellow colour was measured in the filtrate at 400 nm using microtiter plate reader (Multiskan, Thermo Scientific, USA). The concentration of *p*-nitrophenol in the filtrate was determined against a standard curve prepared by using *p*-nitrophenol standard solution. Arylsulphatase enzyme activity was expressed as μ g (PNP) g⁻¹ (dw) soil h⁻¹.

3.11.1.13. β -glucosidase Activity (GSA)

β -glucosidase was determined following the assay outlined by Tabatabai (1982) and Eivazi and Tabatabai (1988). Moist sieved (1 mm) soil was added with 0.25 mL toluene, 4 mL MUB (Modified Universal Buffer, pH 6.0) solution, 1 mL *p*-nitrophenyl- β -D-glucoside (PNG) and incubated at 37^oC for 1 h. After incubation, 1 mL of 0.5 M CaCl₂ and 4 mL 0.1M THAM buffer, pH 12.0 were added to the soil suspension and filtered using Whatman No. 2v folded paper. Intensity of yellow colour was measured in the filtrate at 400 nm using microtiter plate reader (Multiskan, Thermo Scientific, USA). The concentration of *p*-nitrophenol in the filtrate was

determined against a standard curve prepared by using *p*-nitrophenol standard solution. β -glucosidase enzyme activity was expressed as μg (PNP) g^{-1} (dw) soil h^{-1} .

3.11.1.14. Dehydrogenase Activity (DHA)

Dehydrogenase was determined in air dried soil samples as per the method described by Casida *et al.* (1964). Soil sample (10 g) was mixed with 0.1g CaCO_3 and then, the mixture was divided into three parts (each part weighed 3 g) and transferred to three screw cap test tubes. To each test tube 0.5 mL of 1% 2,3,5-triphenyl-tetrazolium chloride (TTC) and 1.25 mL of distilled water were added and mixed thoroughly by gentle tapering and incubated it at 37°C for 24 h. After 24 h incubation, the soil suspension was filtered through glass funnel fitted with absorbent cotton. Methanol was added to extract the soil suspension until the cotton plug's colour became white and the final volume was made up to 50 mL. Intensity of reddish colour was measured by using microtiter plate reader at a wavelength of 485 nm (Multiskan, Thermo Scientific, USA). The concentration of triphenyl formazan (TPF) in the supernatant was determined against a standard graph prepared using known concentrations of TPF. DHA was expressed as μg (TPF) g^{-1} (dw) soil h^{-1} .

3.11.1.15. Phosphomonoesterase Activity (PHA)

Fresh sieved (1 mm) soil samples were used for phosphomonoesterase determination following the protocol described by Tabatabai and Bremner (1969). Soil sample (1 g) was taken in an Erlenmeyer flask and to this 4 mL of MUB (Modified Universal Buffer, pH 6.5), 0.25 mL of toluene and 1 mL of *p*-NPP (*p*-nitrophenyl phosphate) were added and incubate at 37°C for 1 h. After incubation, 1 mL of 0.5 M CaCl_2 and 4 mL 0.5M NaOH were added to the soil suspension and filtered using funnel fitted with cotton plug. Intensity of yellow colour was measured in the filtrate at 400 nm using microtiter plate reader (Multiskan, Thermo Scientific, USA). The concentration of *p*-nitrophenol in the filtrate was determined against a standard curve prepared by using *p*-nitrophenol standard solution. phosphomonoesterase enzyme activity was expressed as μg (PNP) g^{-1} (dw) soil h^{-1} . sieve openings. Then the carbon content was analysed by wet oxidation methods.

3.12 Soil Biological Analysis

3.12.1 Extraction of soil DNA

Soil DNA was extracted based on the principle of *in-situ* lysis DNA extraction method. An amount (0.25 g) of fresh soil was used for extraction of soil DNA using the Power Soil™ DNA isolation kit (MoBio Laboratories Inc., Carlsbad, USA)

according to the manufacturer's protocol, with the following modification to improve DNA yield. The Power Bead tube with soil sample was incubated at 60°C for 15 min in a Thermomixer compact (Eppendorf Make, Germany) at 60°C for 15 min and at 300 rpm. After incubation, rest steps were followed as per manufacturer's instruction. DNA was eluted in 50 µl elution buffer and stored at -20°C.

3.12.2 Agarose gel electrophoresis

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

3.12.3 Spectrophotometry

The absorption spectrum of DNA extracts (230-280nm) was determined using a Nano-drop® 2000 spectrophotometer (Thermo Scientific, USA) according to the manufacturer's instructions. DNA was quantified based on the absorption at 260 nm and expressed as µg g⁻¹ (dw) soil. A decrease in absorption ratio at 260/230 and 260/280 nm was used as indicator of humic acid, polysaccharide and protein impurities (Krsek and Wellington, 1999; Stach *et al.*, 2001; Thakuria *et al.*, 2008).

3.13. Polymerase Chain Reaction (PCR)

3.13.1. PCR amplification of bacterial 16S rRNA genes from soil DNA

Extracted soil DNA was amplified in a Gradient Master Cyclor 5331 (Eppendorf Make, Germany) with primer pair (27f and 1492r) specific to bacterial domain (Lane *et al.*, 1991). This primer pair is specific to 16S rRNA genes of the bacterial domain and yields amplified product size of approximate 1465 bp. The amplification reactions were carried out in a 25µl volume containing 25 ng soil DNA as template, 100 nM of each oligonucleotide primers [27f (5'-AGAGTTTGTATCCTGGCTCAG-3') and 1492r (5'-GGTTACCTTGTTACGA CTT-3')], 12.5 µl of Go-Taq DNA polymerase (Promega, USA) along with bovine serum albumin (BSA concentration 0.1 µg µl⁻¹; New England Biolabs, UK) and the required quantity of PCR grade water (HiMedia, India). The PCR reaction condition included an initial 3

min denaturation at 94°C, and was followed by 30 thermal cycles of 1 min at 94°C, 1min at 62°C and 2 min at 72°C. Amplification was completed with the final extension step at 72°C for 7 min. All PCR products were examined by electrophoresed in an agarose gel (1% w v⁻¹) using a 100 bp DNA ladder (New England Biolabs, UK).

3.13.2. PCR amplification for α -proteobacteria

The extracted DNA was amplified in thermo scientific thermocycler using a primer pair of 203f α and 1494 r (203f α : 5'-CCGCATACGCCCTACGGGGGAAAGATTTAT and 1494r: 5'-CTACGGTCTAGCCTTGTTACGAC). The PCR reaction consist of 1X buffer with 1.5 mM MgCl₂ 100 μ M dNTPs 100mM of each 203f and 1494r primers, 5.0 μ l BSA (concentration 0.1 μ g μ l⁻¹), 1U of Taq-DNA polymerase and 1-2 μ l DNA template. PCR reaction conditions consisted of an initial denaturation step (94°C for 3min) followed by 30 cycles of 45 second of denaturation at 94°C, 45 second at 62°C for primer annealing, and 1 min at 72°C for primer extension, followed by final step at 72°C for 7 min and hold at 10°C. Products were first analyzed by electrophoresis in an agarose gel (1% w v⁻¹) using a 100bp DNA ladder (New England Biolabs, UK). The amplified template was re-amplified using a primer pairs of 984fgc and 1378r (984fgc: 5'-CGCCCGGGCGCGCCCGGGCGGGGC GGGGGCACGGGGGGAACGCGAAGAACCTTAC and 1378r 5'-GCGGTGTGTACAA GGCCCGGGAACG). Amplification was carried out in the same manner as discussed in section 3.13.3.

Table: 3.1 Different Primers used for studying soil bacteria community

Sl. no.	Specificity	Primer pair	Annealing temperature	Reference
1.	α - proteobacteria	203fa:(5'-CCGCATACGCCCTACGG GGGAAAGATTTAT) 1494r: (5'-CTACGGTCTAGCCTTGT TACGAC) 984fgc:5'- (CGCCCGGGGCGCGCCCCGGGC GGGGCGGGGGCACGGGGGGAAC GCGAAGAACCTTAC) 1378r (5'-GCGGTGTGTACAAGG CCCGGGAACG)	62°C 62.2 °C	Heuer and Smalla. 1999.
2.	β - proteobacteria	F948 β gc: (5'-CGCCCGGGGGGCGC CCCGGGCGGGGCGGGGGCACG GGGGCCGCACACGCGGTGGATG A) 1494r: (5'- CTACGGTCTAGCCTTGTACGAC).	63.1 °C	Gomes <i>et al.</i> (2001).
3.	Soil Bacterial DNA	27f (5'-AGAGTTTGATCCTGGCT CAG-3') 1492r (5'-GGTTACCTTGTACGA CTT-3')	62 °C	Weisburg <i>et al.</i> , 1991 and Heuer, 2007.

3.13.3. PCR amplification for β -proteobacteria

The extracted DNA was amplified in Thermo scientific thermocycler using a primer pair of F948 β gc and 1494 r (F948 β gc: 5'-CGCCCGGGGGCGCCCCGGGCGGGGCGGGGGCACGGGGGCCGCACACGCGG TGGATGA and 1494r: 5'-CTACGGTCTAGCCTTGTACGAC). The PCR reaction consist of 1X buffer with 1.5 mM MgCl₂ 100 μ M dNTPs 100mM of each F948 β gc and 1494r primers, 5.0 μ l BSA (concentration 0.1 μ g μ l⁻¹), 1U of Taq-DNA polymerase and 1-2 μ l DNA template. PCR reaction conditions consisted of an initial denaturation step (94°C for 3min) followed by 30 cycles of 45 second of denaturation at 94°C, 45 second at 63.1°C for primer annealing, and 1 min at 72°C for primer extension, followed by final step at 72°C for 7 min and hold at 10°C. The PCR reaction mixture and electrophoresis in an agarose gel analysed was similar as with the amplification of bacterial 16S rDNA products mention in 3.13.1.

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

Denaturing gradient gel electrophoresis (DGGE) was performed using Ingeny PhorU2 system (Ingeny International BV, The Netherlands). The PCR products (amplified with DGGE primer: mention in table 3.1) were loaded on to 8% (w v⁻¹) polyacrylamide gel with a denaturing gradient of 40% and 60% (100% denaturant tends for 7M urea plus 40% formamide in 1 X TAE buffer). Approximate 500 ng of PCR product was loaded into each well and electrophoresis was carried out for 18h at constant 90V in presence of 1X TAE buffer (242 g Trizma, 57.1 ml glacial acetic acid and 100 ml 0.5M EDTA and volume made up to 1 l; pH adjusted to 8.0) at a constant temperature of 60°C. After electrophoresis the gel was stained with Gel-Red fluorescent nucleic acid dye (working concentration 0.6X; Biotium, USA) for 30 min. The stained gel was visualized and image was captured using gel documentation system (BioRad, CA, USA).

From the image of each DGGE fingerprint a reference DGGE profile was created that contained all maximum possible bands of DGGE profiles. Then, DGGE profiles were scored visually for presence or absence of bands comparing to the reference profile. Thus, a binary presence-absence matrix was developed for each DGGE fingerprint (Kropf *et al.*, 2004). These binary matrices were exported to Excel worksheet 4.0 (Microsoft Office Excel, 2003) and arranged in a single sheet according to band numbers. This combined binary matrix was finally exported to PRIMER v6.1.9

software (Primer-E Ltd, Plymouth, UK) and dataset was standardised by total method (Magurran, 1988). This standardised dataset finally used for all multivariate analyses.

3.14. STATISTICAL ANALYSIS

3.14.1 Univariate statistics

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

3.14.2 Multivariate statistics

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

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

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

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

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

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

Chapter-4

Result

There were 3 objectives in this investigation and the objective-wise results are presented in this chapter.

4.1 Objective 1

4.1.1 Physico-chemical properties of soil

Physico-chemical properties (BD, pH, EC, SOC, Avl N, Avl P and Avl K) of soils under burnt and unburnt conditions is presented in Table 1. Values of BD, pH, EC, Avl P and Avl K in burnt soil were significantly higher relative to their values in unburnt soil under 5 year jhum cycle. On the other hand the contents of SOC and Avl N under burnt soil were significantly lower relative to their values in unburnt soil.

Table 4.1. Influence of burning on physico-chemical attributes of *jhum* soils

5 year Jhum cycle	BD (Mg m ⁻³)	pH 1:2.5 (soil: water)	EC (μS s ⁻¹)	SOC (%)	Avl N (kg ha ⁻¹)	Avl P (kg ha ⁻¹)	Avl K (kg ha ⁻¹)
Unburnt soil	1.52±0.07a	4.70±0.12a	24.8±2.7a	1.44±0.04b	273±23b	6.18±1.6a	220±10a
Burnt soil	1.65±0.08a	5.08±0.16b	33.7±3.4b	1.18±0.06a	212±18a	9.56±1.7b	258±14b

The response of soil bacterial community to application of culture of microbial species mixture (PSB community, N₂-fixer community, CDB community and fungal community) in presence or absence of rice crop under both burnt and unburnt soils of 5 year *jhum* cycle was studied in mesocosm experiment. The changes in soil bacterial community (using domain specific primer set 27 f and 1492 r) was studied at 10 days of rice seedling growth and the phylum level (alpha-proteobacteria and beta-proteobacteria) community composition at 45 and 90 days of rice plant growth. The PCR-DGGE fingerprint technique was used to decipher the bacterial community composition.

4.1.2 Effect of burning, cropping and microbial inoculation on soil bacterial community

4.1.2.1. Soil bacterial community at 10 days of rice growth

The PCR-DGGE fingerprint of the bacterial community composition in burnt and unburnt soils in presence or absence of rice crops at 10 days of rice plant growth is presented in (Fig.4.1). The number and the relative intensity of bands are clearly visible in the PCR-DGGE fingerprint. The MDS plot developed on binary matrix of PCR-DGGE fingerprint showed distinct clustering between burnt and unburnt soils (Fig 4.2). The bacterial community of burnt soil or unburnt soil clustered separately according to the absence and presence of rice crop at 80% similarity level and the separation among clusters were positively differ from each other as confirmed by Hierarchical cluster analysis (incorporating SIMPROF test at 95% confidence limit) (Fig 4.3). Interestingly, the clusters representing bacterial communities of burnt soil distinctly separated out from the clusters of unburnt soil irrespective of rice crop present or absent (Fig. 4.2). Within burnt or unburnt soil conditions, the two distinct clusters were represented by the bacterial communities in presence or absence of rice crop. Though there were variations in bacterial community compositions among the treatments, these treatment induced variations couldn't mask the effect of burning and cropping. The stronger factor of variability in shaping the soil bacterial community composition was found to be in the order burning > cropping > microbial inoculation treatment (Fig.4.1, Fig.4.2 and Fig.4.3).

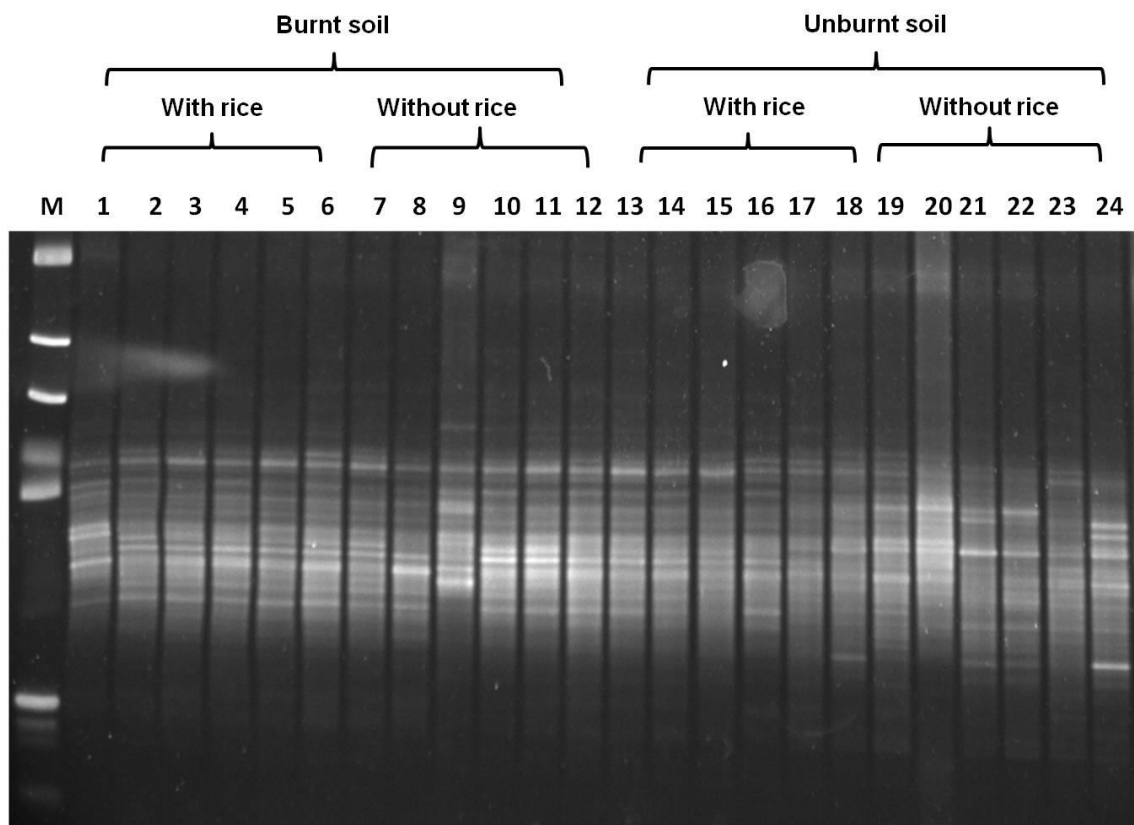


Fig.4.1: Soil bacterial community at 10 days growth of rice plant

The DGGE fingerprint depicting soil bacterial community at 10 days in *jhum* rice rhizosphere and bulk soils under burnt and unburnt situations of 5 years *jhum* cycle as influenced by application of microbial community composed of N₂-fixers (NF), phosphate solubilizing bacteria (PSB), cellulose degrading bacteria (CDB), NF+PSB+CDB, fungi, and control (no inoculants).

M – 100 bp marker, lane 1 -PSB+Fungi, lane 2 -N₂-fixers+Fungi, lane 3 - CDB+Fungi, lane 4 -NF+PSB+CDB +Fungi, lane 5 -Fungi, lane 6 -Control(no inoculation) lane 7 -PSB+Fungi, lane 8 -N₂-fixers+Fungi, lane 9 -CDB+Fungi, lane 10 - NF+PSB+CDB +Fungi, lane 11 -Fungi, lane 12 -Control (no inoculation) lane – 13 PSB+Fungi, lane 14 -N₂-fixers+Fungi, lane 15 -CDB+Fungi, lane 16 - NF+PSB+CDB +fungi, lane 17- Fungi, lane 18 -Control (no inoculation), lane 19 -PSB+Fungi, lane 20 -N₂-fixers+Fungi, lane 21 - CDB+Fungi, lane -22 NF+PSB+CDB +Fungi, lane 23 - Fungi, lane 24 - Control(no inoculation)

Lane 1-6 Burnt with rice, Lane 7-12 Burnt without rice, Lane 13-18 Unburnt with rice, Lane 19-24 Unburnt without rice

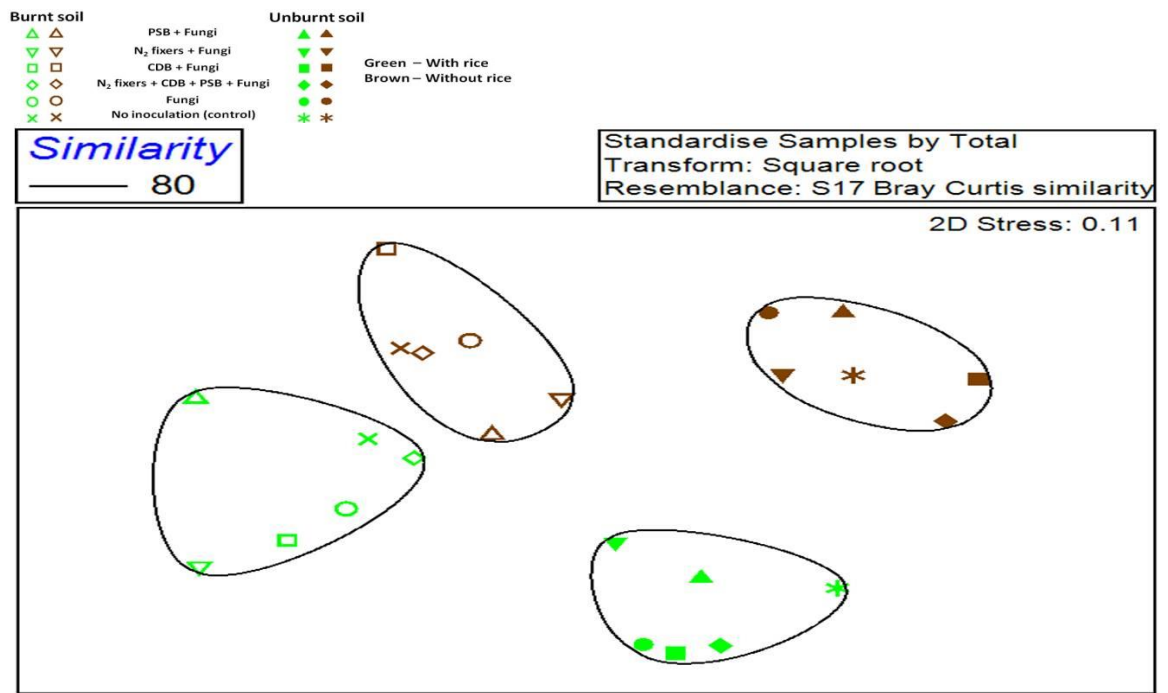


Fig.4.2: MDS Clustering of bacterial Community at 10 days of rice growth in response to burning, cropping and microbial inoculants in soils of 5 years *Jhum* Cycle.

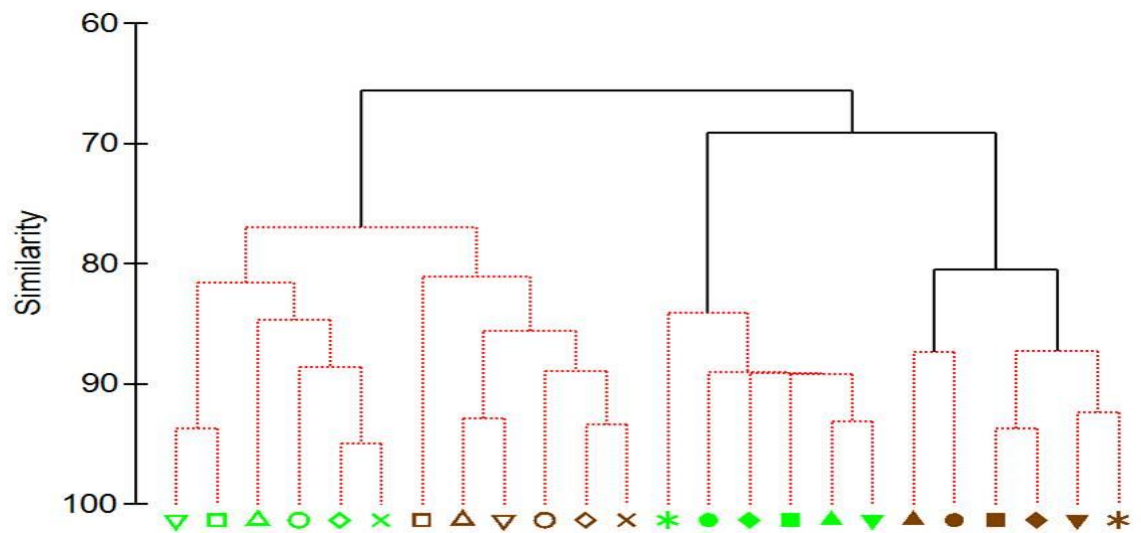


Fig.4.3: Hierarchical Clustering of bacterial Community at 10 days of rice growth in response to burning, cropping and microbial inoculants in soils of 5 years *Jhum* Cycle

4.1.2.2 Soil bacterial (alpha-proteobacteria) community at 45 days of rice growth

The changes in alpha-proteobacteria (using domain specific primer set 203fa:5' and 1494r: 5' nested with 984fgc:5' and 1378r 5'.) was studied at 45 days of rice plant growth in burnt and unburnt soils with presence or absence of rice crop. The PCR-DGGE fingerprint revealing the alpha-proteobacteria community composition at 45 days of rice plant growth is depicted in (Fig. 4.4.). The number and the relative intensity of bands are clearly visible in the PCR-DGGE fingerprint. The MDS plot developed on binary matrix of PCR-DGGE fingerprint showed distinct clustering between burnt and unburnt soils (Fig. 4.5.). The bacterial community of burnt soil or unburnt soil clustered separately according to the presence and absence of rice crop at 72% similarity level and the separation among clusters were significantly different from each other as confirmed by Hierarchical cluster analysis (incorporating SIMPROF test at 95% confidence limit) (Fig.4.6.). Interestingly, the clusters representing bacterial communities of burnt soil distinctly separated out from the clusters of unburnt soil according to the present or absent of rice crop (Fig. 4.5.). Within burnt or unburnt soil conditions, the two distinct clusters were represented by the bacterial communities in presence or absence of rice crop. Though there were variations in bacterial community compositions among the treatments, these treatment induced variations couldn't mask the effect of burning and cropping. The stronger factor of variability in shaping the soil alpha-proteobacterial community composition was found to be in the order burning > cropping > microbial inoculation treatment (Fig.4.4, Fig. 4.5. and Fig. 4.6).

4.1.2.3. Soil bacterial (alpha-proteobacteria) community at 90 days of rice growth

The changes in alpha-proteobacterial (using domain specific primer set 203fa:5' and 1494r: 5' nested with 984fgc:5' and 1378r 5'.) was studied at 45 days of rice plant growth in burnt and unburnt soils with presence or absence of rice crop. The PCR-DGGE fingerprint revealing the alpha-proteobacteria community composition at 90 days of rice plant growth is depicted in (Fig.4.7). The number and the relative intensity of bands are clearly visible in the PCR-DGGE fingerprint. The MDS plot developed on binary matrix of PCR-DGGE fingerprint showed distinct clustering between burnt and unburnt soils (Fig. 4.8.). The bacterial community of burnt soil or unburnt soil clustered separately according to the presence or absence of rice crop at 58% similarity level and the separation among clusters were significantly different from each other as confirmed by Hierarchical cluster analysis (incorporating SIMPROF test at 95% confidence limit).

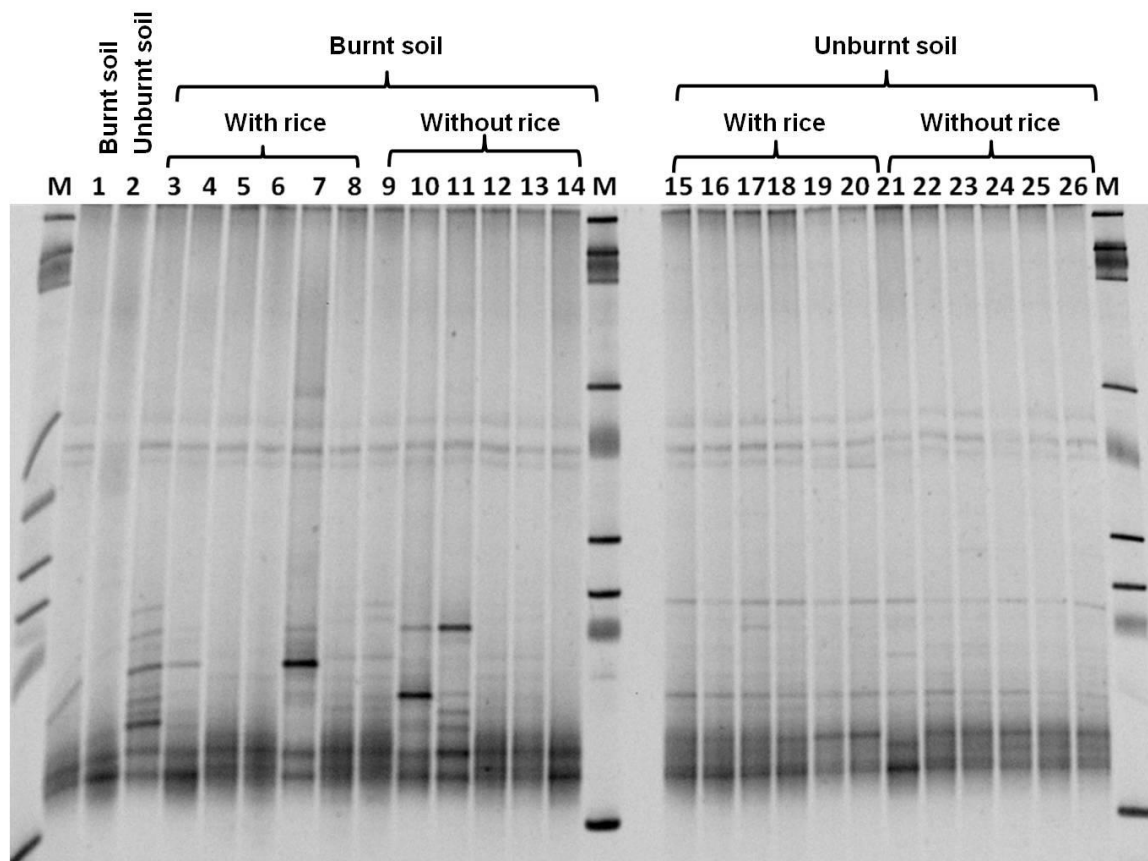


Fig.4.4: PCR DGGE of Alpha-proteobacterial group at 45 days growth of rice plant

The DGGE fingerprint depicting alpha-proetobacterial community at 45 days in *jhum* rice rhizosphere and bulk soils under burnt and unburnt situations of 5 years *jhum* cycle as influenced by application of microbial community composed of N₂-fixers (NF), phosphate solubilizing bacteria (PSB), cellulose degrading bacteria (CDB), NF+PSB+CDB, fungi, and control (no inoculants).

M – 100 bp marker, lane 1 –Burnt at 0 days, lane 2 -unburnt at 0 days, lane 3 - PSB+Fungi, lane 4 -N₂-fixers+Fungi, lane 5 -CDB+Fungi, lane 6 -NF+PSB+CDB +Fungi, lane 7 - Fungi, lane 8 -Control(no inoculation) lane 9 -PSB+Fungi, lane 10 -N₂-fixers+Fungi, lane 11 - CDB+Fungi, lane 12 -NF+PSB+CDB +Fungi, lane 13 -Fungi, lane 14 -Control (no inoculation) lane 15 -PSB+Fungi, lane 16 -N₂-fixers+Fungi, lane 17 -CDB+Fungi, lane 18 -NF+PSB+CDB +fungi, lane 19 -Fungi, lane 20 -Control (no inoculation), lane 21 -PSB+Fungi, lane 22 -N₂-fixers+Fungi, lane 23 -CDB+Fungi, lane 24 -NF+PSB+CDB +Fungi, lane 25- Fungi, lane 26 - Control(no inoculation)

Lane 3-8 Burnt with rice, Lane 9-14 Burnt without rice, Lane 15-20 Unburnt with rice, Lane 21-26 Unburnt without rice

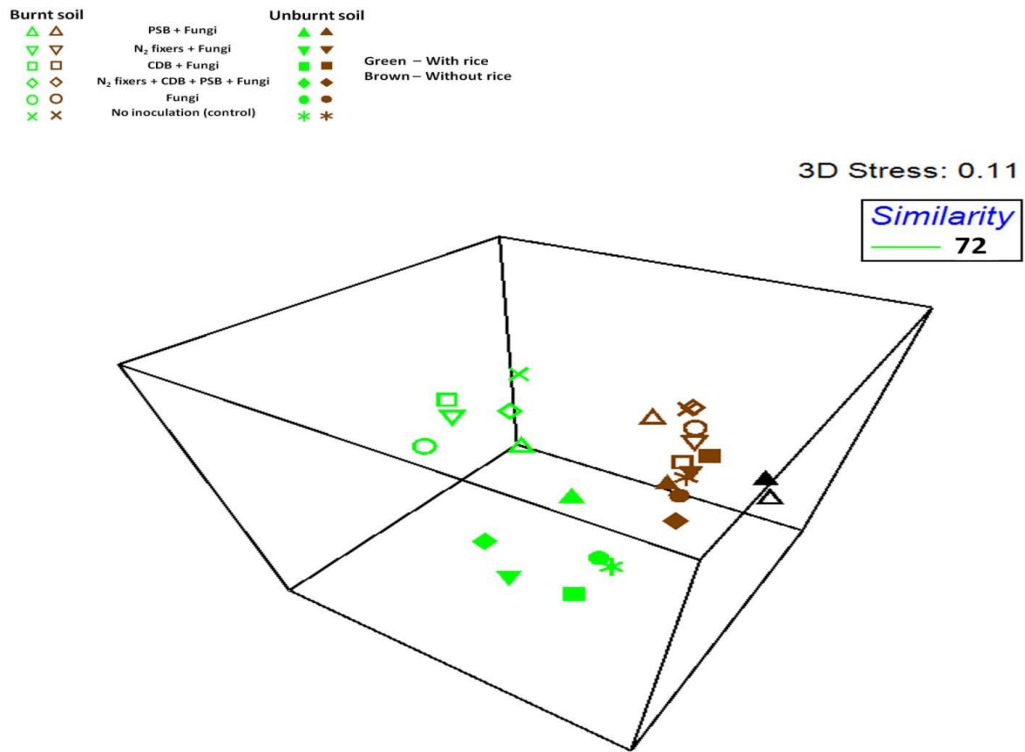


Fig.4.5: MDS Clustering of alpha-proteobacterial Community at 45 days of rice growth in response to burning, cropping and microbial inoculants in soils of 5 years *Jhum* Cycle.

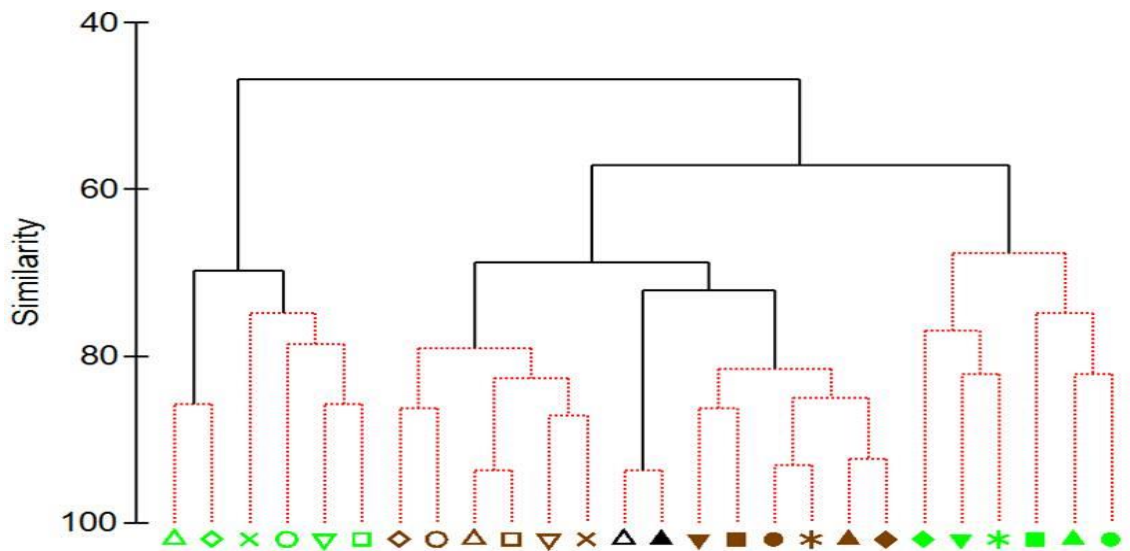


Fig.4.6: Hierarchical Clustering alpha-proteobacterial Community at 45 days of rice growth in response to burning, cropping and microbial inoculants in soils of 5 years *Jhum* Cycle.

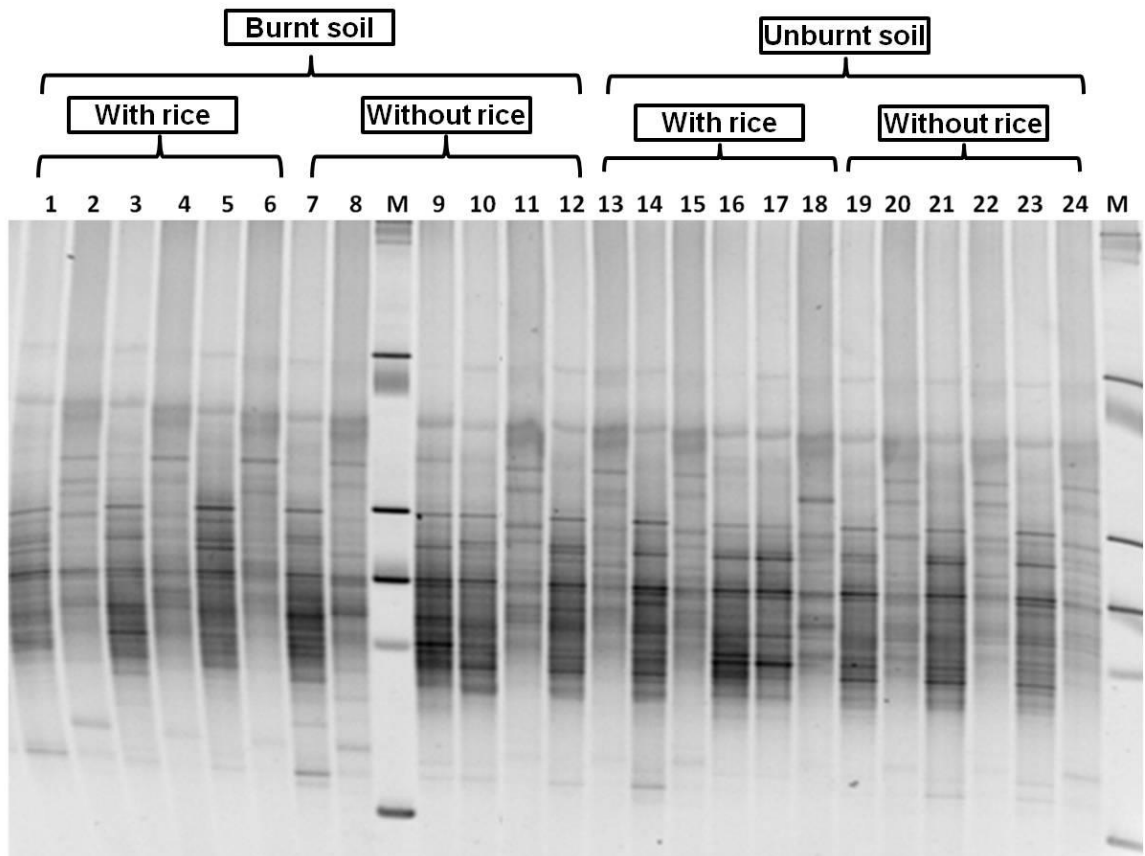


Fig.4.7: The PCR-DGGE OF Alpha-proteobacterial at 90 days of rice plant growth

The DGGE fingerprint depicting alpha-proetobacterial community at 90 days in *jhum* rice rhizosphere and bulk soils under burnt and unburnt situations of 5 years *jhum* cycle as influenced by application of microbial community composed of N₂-fixers (NF), phosphate solubilizing bacteria (PSB), cellulose degrading bacteria (CDB), NF+PSB+CDB, fungi, and control (no inoculants).

M – 100 bp marker, lane 1 -PSB+Fungi, lane 2 -N₂-fixers+Fungi, lane 3 - CDB+Fungi, lane 4 -NF+PSB+CDB +Fungi, lane 5 -Fungi, lane 6 -Control(no inoculation) lane 7 -PSB+Fungi, lane 8 -N₂-fixers+Fungi, lane 9 -CDB+Fungi, lane 10 - NF+PSB+CDB +Fungi, lane 11 -Fungi, lane 12 -Control (no inoculation) lane – 13 PSB+Fungi, lane 14 -N₂-fixers+Fungi, lane 15 -CDB+Fungi, lane 16 - NF+PSB+CDB +fungi, lane 17- Fungi, lane 18 -Control (no inoculation), lane 19 -PSB+Fungi, lane 20 -N₂-fixers+Fungi, lane 21 - CDB+Fungi, lane -22 NF+PSB+CDB +Fungi, lane 23 - Fungi, lane 24 - Control(no inoculation)

Lane 1-6 Burnt with rice, Lane 7-12 Burnt without rice, Lane 13-18 Unburnt with rice, Lane 19-24 Unburnt without rice.

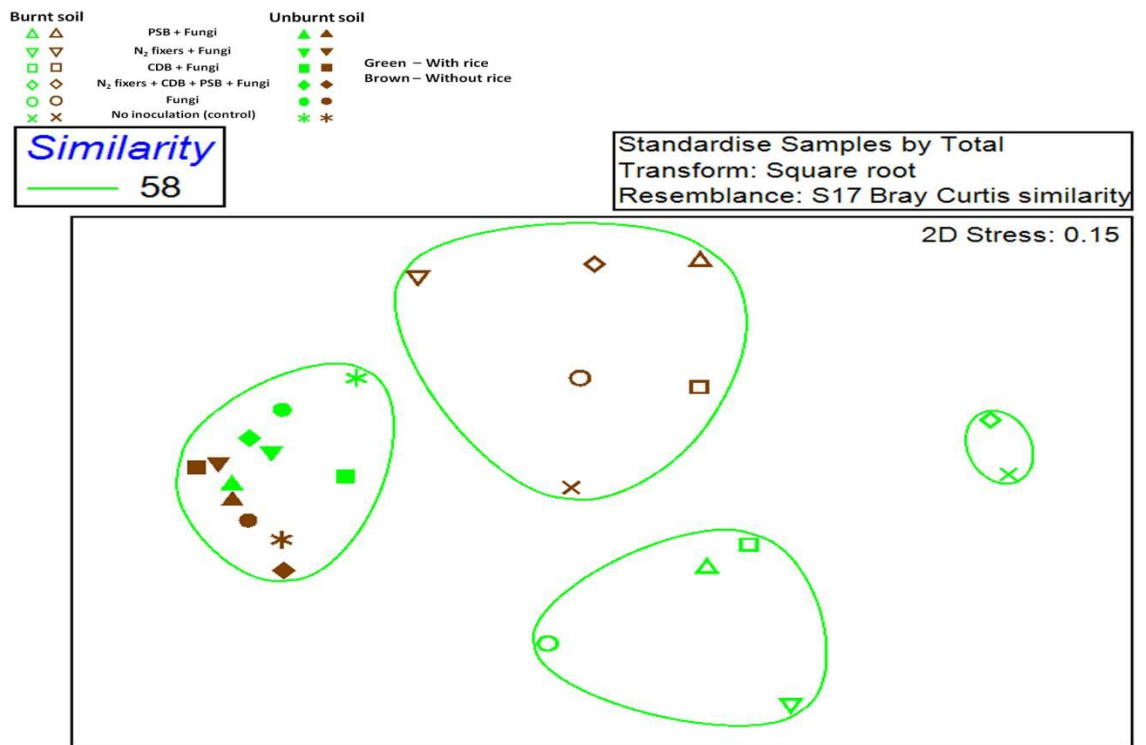


Fig.4.8: MDS Clustering of alpha-proteobacterial Community at 90 days of rice growth in response to burning, cropping and microbial inoculants in soils of 5 years *Jhum* Cycle.

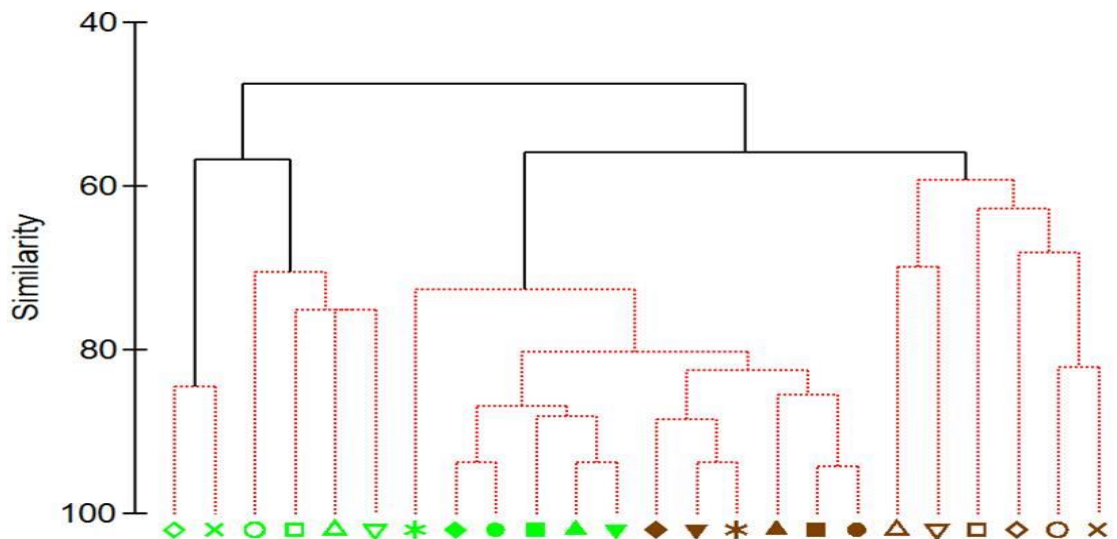


Fig.4.9: Hierarchical Clustering alpha-proteobacterial Community at 90 days of rice growth in response to burning, cropping and microbial inoculants in soils of 5 years *Jhum* Cycle

(Fig. 4.9.). Interestingly, the clusters representing bacterial communities of burnt soil distinctly separated out from the clusters of unburnt soil according to the present or absent of rice crop (Fig. 4.8.). Within burnt or unburnt soil conditions, the two distinct clusters were represented by the bacterial communities in presence or absence of rice crop. Though there were variations in bacterial community compositions among the treatments, these treatment induced variations couldn't mask the effect of burning and cropping. The stronger factor of variability in shaping the soil alpha-proteobacterial community composition at 90 days was found to be in the order burning > cropping > microbial inoculation treatment (Fig.4.7, Fig.4.8 and Fig.4.9).

4.1.2.4. Soil bacterial (beta-proteobacterial) community at 45 days of rice growth

The changes in beta-proteobacteria (using domain specific primer set F948 β gc: 5' and 1494r: 5'.) was studied at 45 days of rice plant growth in burnt and unburnt soils with presence or absence of rice crop. The PCR-DGGE fingerprint showed the beta-proteobacteria community composition at 45 days of rice plant growth is depicted in (Fig.4.10). The number and the relative intensity of bands are clearly visible in the PCR-DGGE fingerprint. The MDS plot developed on binary matrix of PCR-DGGE fingerprint showed distinct clustering between burnt and unburnt soils (Fig. 4.11). The bacterial community of burnt soil or unburnt soil clustered separately according to the presence or absence of rice crop at 75% similarity level and the separation among clusters were significantly different from each other as confirmed by Hierarchical cluster analysis (incorporating SIMPROF test at 95% confidence limit) (Fig. 4.12.). Interestingly, the clusters representing bacterial communities of burnt soil distinctly separated out from the clusters of unburnt soil according to the present or absent of rice crop (Fig. 4.11.). Within burnt or unburnt soil conditions, the two distinct clusters were represented by the bacterial communities in presence or absence of rice crop. Though there were variations in bacterial community compositions among the treatments, these treatment induced variations couldn't mask the effect of burning and cropping. The stronger factor of variability in shaping the beta-proteobacterial community composition was found to be in the order cropping > burning > microbial inoculation treatment (Fig.4.10, Fig.4.11. and Fig.4.12.).

4.1.2.5. Soil bacterial (beta-proteobacterial) community at 90 days of rice growth

The changes in beta-proteobacterial (using domain specific primer set F948 β gc: 5' and 1494r: 5'.) was studied at 90 days of rice plant growth in burnt and unburnt soils with presence or absence of rice crop. The PCR-DGGE fingerprint revealing the beta-proteobacteria community composition at 90 days of rice plant growth is depicted in (Fig.4.13). The number and the relative intensity of bands are clearly visible in the PCR-DGGE fingerprint. The MDS plot developed on binary matrix of PCR-DGGE fingerprint showed distinct clustering between burnt and unburnt soils (Fig. 4.14.). The bacterial community of burnt soil or unburnt soil clustered separately according to the presence or absence of rice crop at 80% similarity level and the separation among clusters were significantly different from each other as confirmed by Hierarchical cluster analysis (incorporating SIMPROF test at 95% confidence limit) (Fig.4.15). Interestingly, the clusters representing bacterial communities of burnt soil distinctly separated out from the clusters of unburnt soil according to the present or absent of rice crop (Fig. 4.14.). Within burnt or unburnt soil conditions, the two distinct clusters were represented by the bacterial communities in presence or absence of rice crop. Though there were variations in bacterial community compositions among the treatments, these treatment induced variations couldn't mask the effect of burning and cropping. The stronger factor of variability in shaping the beta-proteobacterial community composition was found to be in the order cropping > burning > microbial inoculation treatment (Fig.4.13, Fig.4.14 and Fig.4.15).

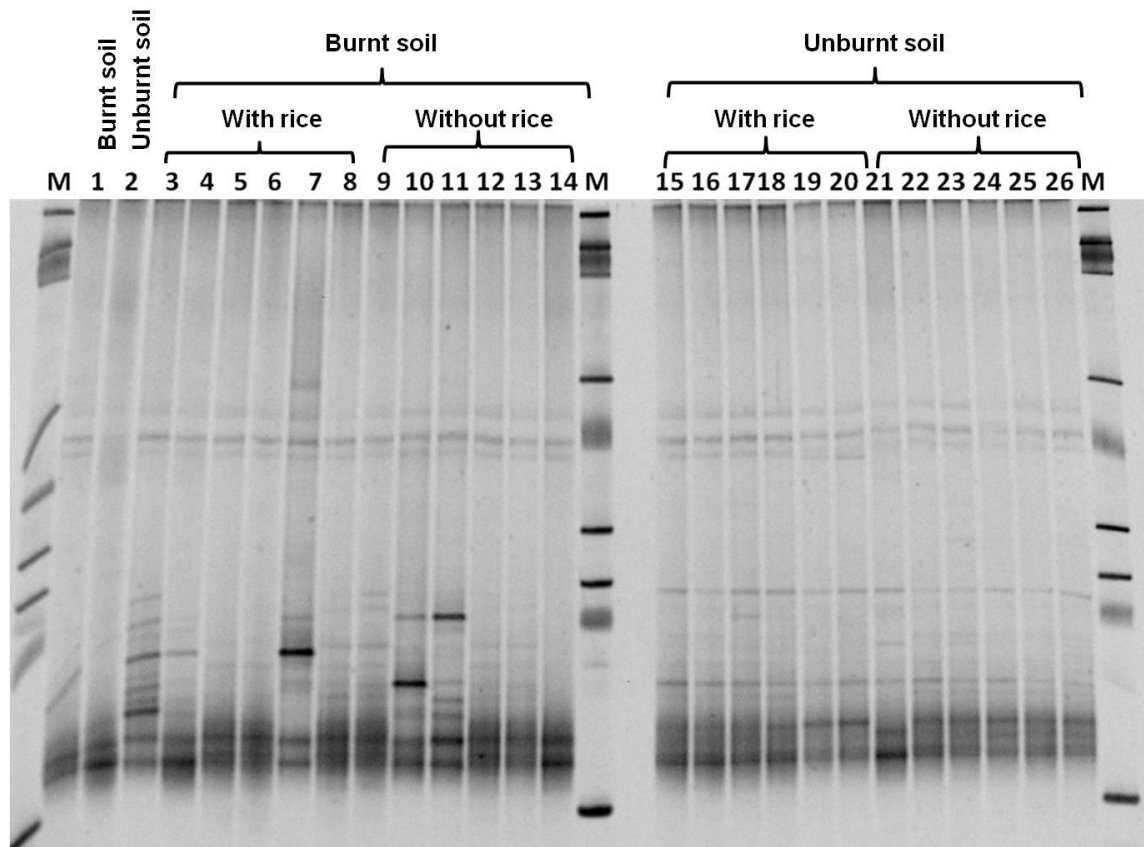


Fig.4.10: The PCR-DGGE of Beta-proteobacterial at 45 days of rice plant growth

The DGGE fingerprint depicting Beta-proetobacterial community at 45 days in *jhum* rice rhizosphere and bulk soils under burnt and unburnt situations of 5 years *jhum* cycle as influenced by application of microbial community composed of N₂-fixers (NF), phosphate solubilizing bacteria (PSB), cellulose degrading bacteria (CDB), NF+PSB+CDB, fungi, and control (no inoculants).

M – 100 bp marker, lane 1 –Burnt at 0 days, lane 2 -unburnt at 0 days, lane 3 - PSB+Fungi, lane 4 -N₂-fixers+Fungi, lane 5 -CDB+Fungi, lane 6 -NF+PSB+CDB +Fungi, lane 7 -Fungi, lane 8 -Control(no inoculation) lane 9 -PSB+Fungi, lane 10 -N₂-fixers+Fungi, lane 11 -CDB+Fungi, lane 12 -NF+PSB+CDB +Fungi, lane 13 -Fungi, lane 14 -Control (no inoculation) lane 15 -PSB+Fungi, lane 16 -N₂-fixers+Fungi, lane 17 -CDB+Fungi, lane 18 -NF+PSB+CDB +fungi, lane 19 -Fungi, lane 20 -Control (no inoculation), lane 21-PSB+Fungi, lane 22 -N₂-fixers+Fungi, lane 23 -CDB+Fungi, lane 24 -NF+PSB+CDB +Fungi, lane 25 -Fungi, lane 26 -Control(no inoculation)

Lane 3-8 Burnt with rice, Lane 9-14 Burnt without rice, Lane 15-20 Unburnt with rice, Lane 21-26 Unburnt without rice.

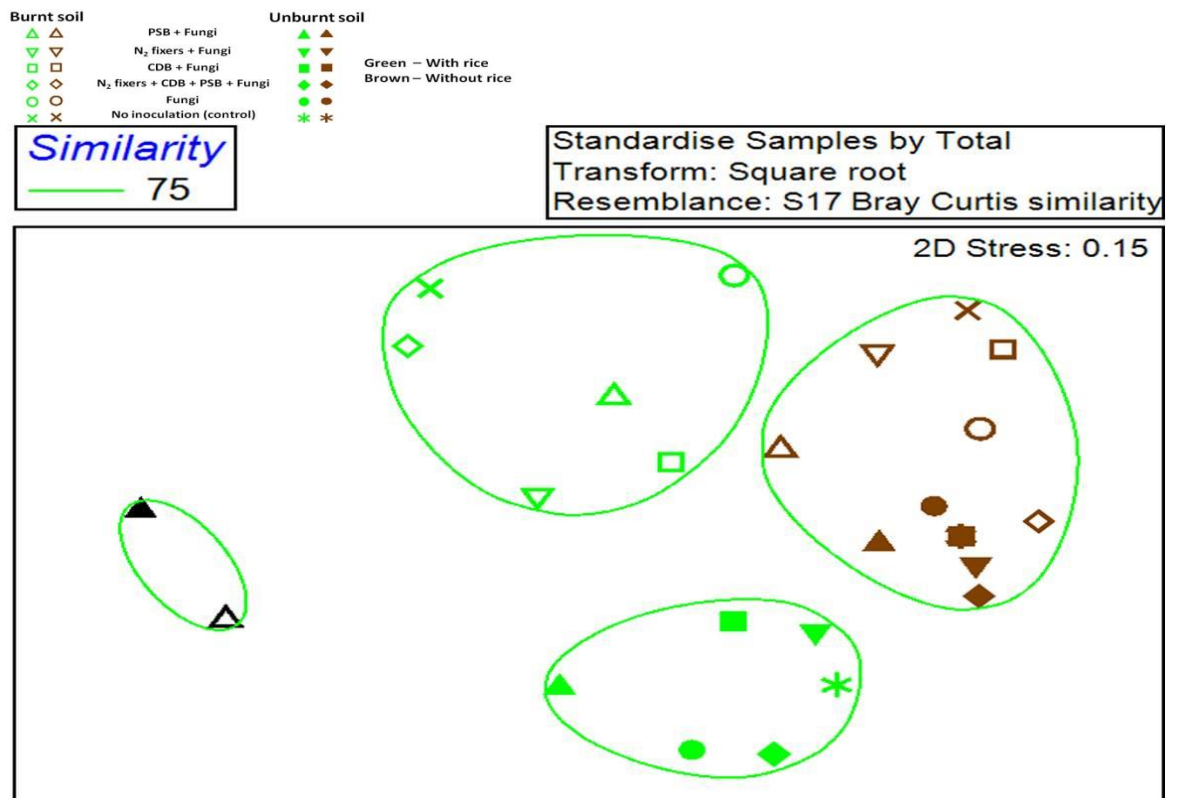


Fig.4.11: MDS Clustering of beta-proteobacterial Community at 45 days of rice growth in response to burning, cropping and microbial inoculants in soils of 5 years *Jhum* Cycle.

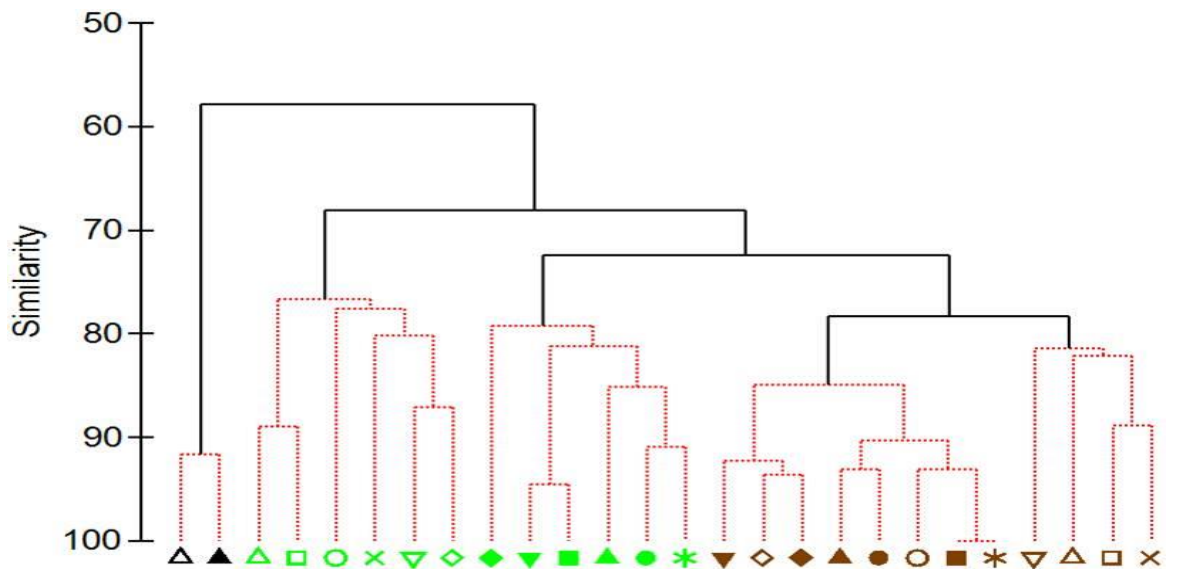


Fig.4. 12: Hierarchical Clustering beta-proteobacterial Community at 45 days of rice growth in response to burning, cropping and microbial inoculants in soils of 5 years *Jhum* Cycle

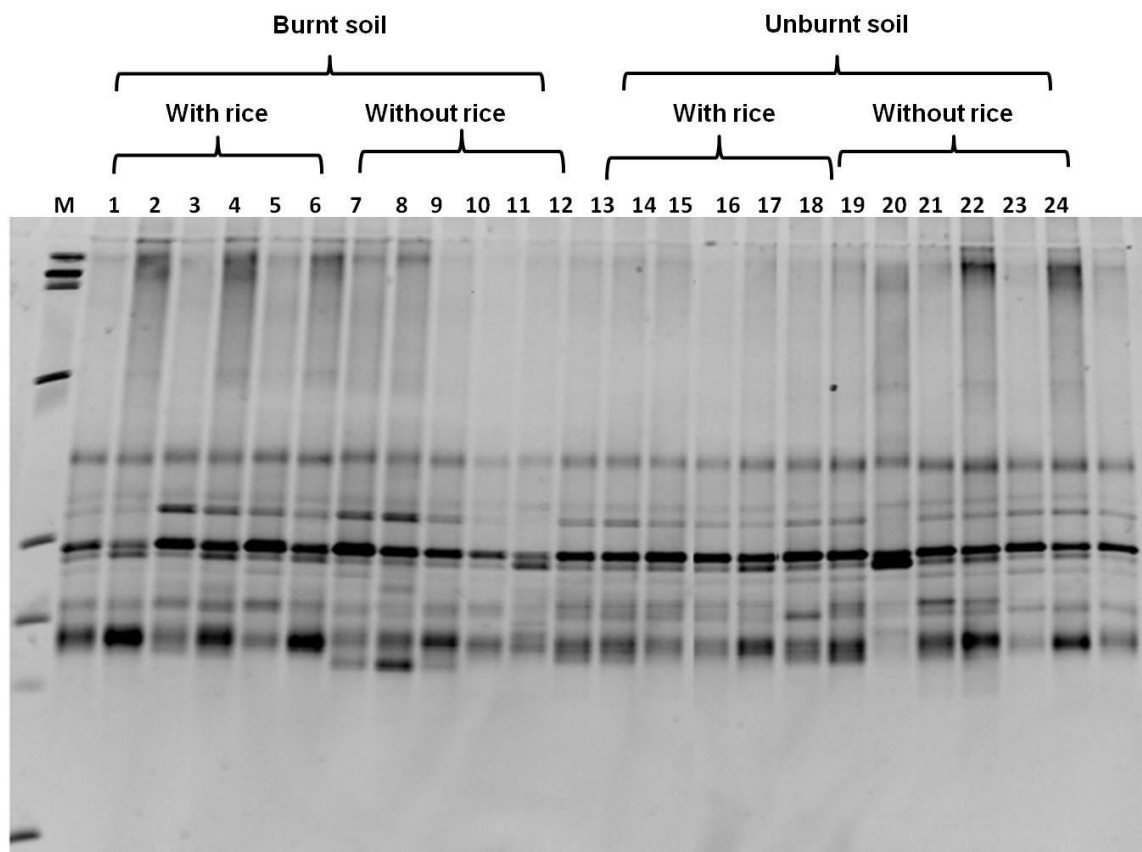


Fig.4.13: The PCR-DGGE of Beta-proteobacterial at 90 days of rice plant growth

The DGGE fingerprint depicting Beta-proetobacterial community at 90 days in *jhum* rice rhizosphere and bulk soils under burnt and unburnt situations of 5 years *jhum* cycle as influenced by application of microbial community composed of N₂-fixers (NF), phosphate solubilizing bacteria (PSB), cellulose degrading bacteria (CDB), NF+PSB+CDB, fungi, and control (no inoculants).

M – 100 bp marker, lane 1 -PSB+Fungi, lane 2 -N₂-fixers+Fungi, lane 3 -CDB+Fungi, lane 4 -NF+PSB+CDB +Fungi, lane 5 -Fungi, lane 6 -Control(no inoculation) lane 7 -PSB+Fungi, lane 8 -N₂-fixers+Fungi, lane 9 -CDB+Fungi, lane 10 -NF+PSB+CDB +Fungi, lane 11 -Fungi, lane 12 -Control (no inoculation) lane – 13 PSB+Fungi, lane 14 -N₂-fixers+Fungi, lane 15 -CDB+Fungi, lane 16 - NF+PSB+CDB +fungi, lane 17- Fungi, lane 18 -Control (no inoculation), lane 19 - PSB+Fungi, lane 20 -N₂-fixers+Fungi, lane 21 - CDB+Fungi, lane -22 NF+PSB+CDB +Fungi, lane 23 - Fungi, lane 24 - Control(no inoculation)

Lane 1-6 Burnt with rice, Lane 7-12 Burnt without rice, Lane 13-18 Unburnt with rice, Lane 19-24 Unburnt without rice

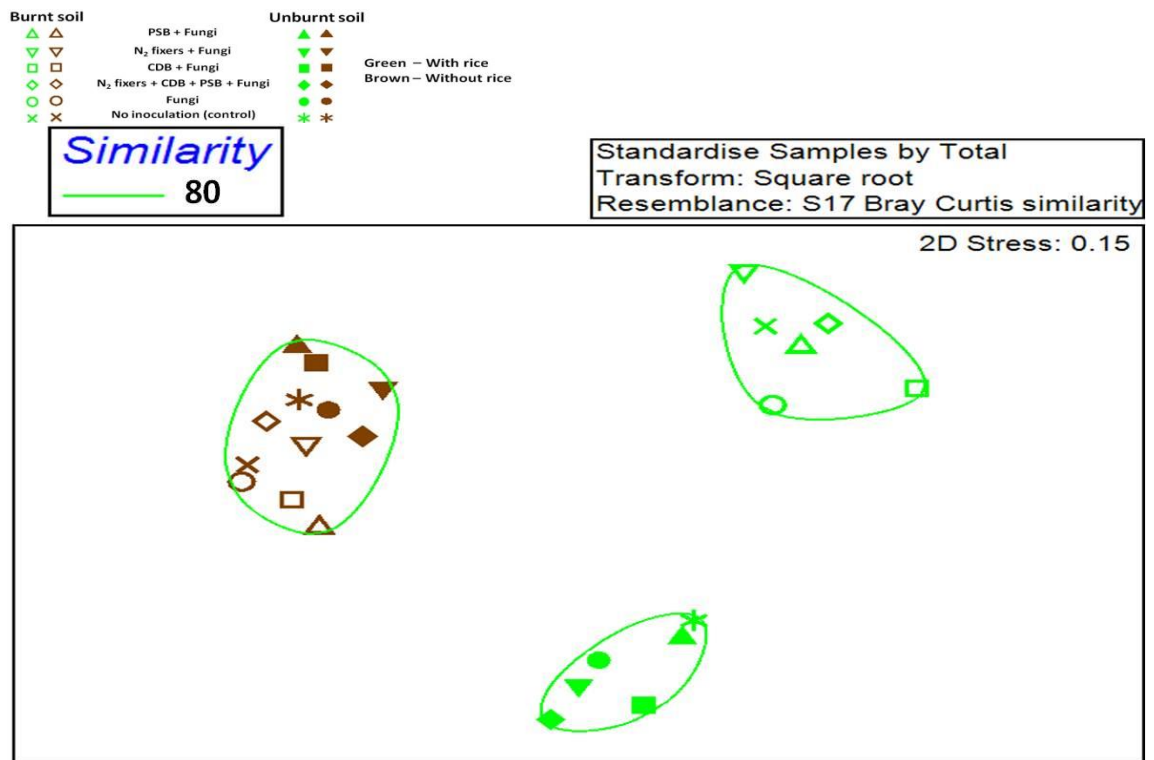


Fig.4.14: MDS Clustering of beta-proetobacterial Community at 90 days of rice growth in response to burning, cropping and microbial inoculants in soils of 5 years *Jhum* Cycle.

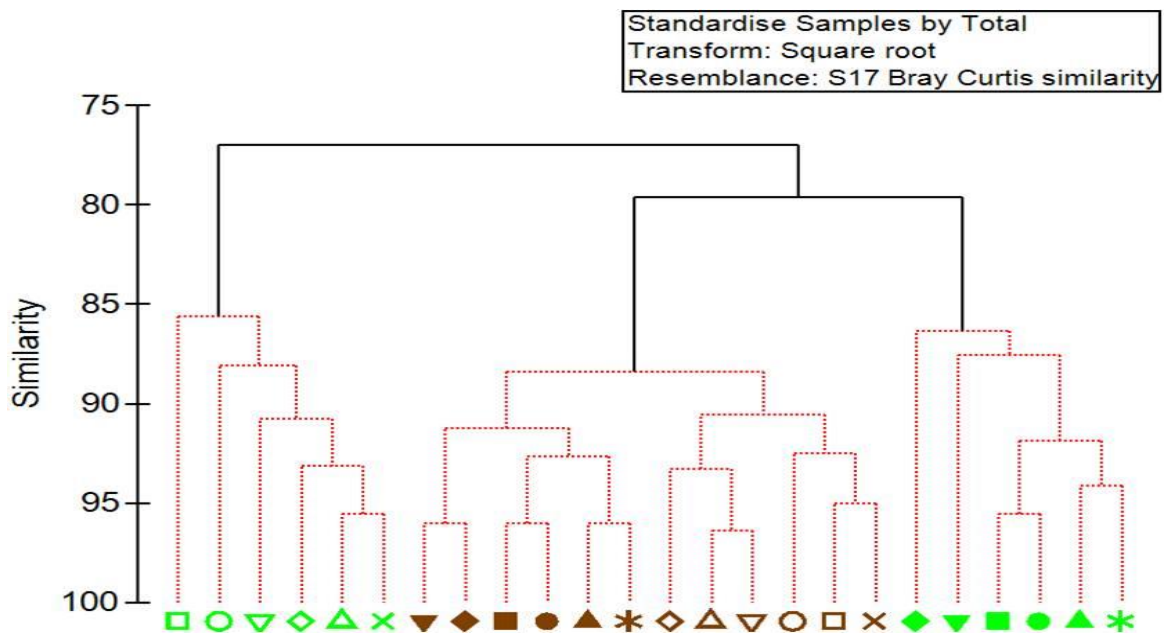


Fig.4.15: Hierarchical Clustering beta-proetobacterial Community at 90 days of rice growth in response to burning, cropping and microbial inoculants in soils of 5 years *Jhum* Cycle.

4.2 Objective - 2

The response of soil bacterial community to application of microbial community (PSB community, N₂-fixer community, CDB community and fungal community) in presence or absence of rice crop under both burnt and unburnt soils of 5 year *jhum* cycle was studied in mesocosm experiment. The change in soil enzymes and biochemical properties was studied at 10, 45, 90 and 120 days of rice plant growth.

4.2.1 Effect of burning, cropping and microbial inoculation on soil enzymes activity as an indicator of soil processes

4.2.1.1 Soil enzymes activities at 10 days of rice plant growth

The soil enzymes such as DHA, GSA, ASA and PHA were strongly influence by the burning, cropping and microbial inoculation (Table 4.2 and annexure 4.1, 4.2, 4.3, 4.4.). The activities of DHA, GSA, and PHA were decrease in burnt soil where as a slight increase in the activity of ASA was found in burnt soil.

Cropping had a great influence to soil enzymes which shows the enzymes activity increase in presence of rice crop than the bulk soils.

Each of the microbial inoculation responds was differ to each enzymes activity. The inoculation of synthetic N₂ fixer had a greater impact to soil DHA, CDB had greater impact to GSA, PSB inoculants affect the PHA and fungi inoculation greater impact at ASA at 10 days of rice plant growth.

The interaction between *jhumming** cropping; *jhumming* * microbial inoculation; cropping * microbial inoculation and *jhumming* * cropping * microbial inoculation were significance at a level $P \leq 0.01$.

4.2.1.2 Soil enzymes activities at 45 days of rice plant growth

The soil enzymes such as DHA, GSA, ASA and PHA were strongly influence by the burning, cropping and microbial inoculation (Table 4.3 and annexure 4.5., 4.6., 4.7., 4.8.). The activities of DHA, GSA, and PHA were decrease in burnt soil where as a slight increase in the activity of ASA was found in burnt soil.

Cropping had a great influence to soil enzymes which shows the enzymes activity increase in presence of rice crop than the bulk soils.

Table 4.2: Interaction effect of burning, cropping and microbial communities on soil enzyme activities in 5 years *Jhum* cycle at 10 days growth of rice plant

Treatments	DHA	GSA	PHA	ASA
	$\mu\text{g TPF g}^{-1}$ (dry) soil h^{-1}	$\mu\text{g PNP g}^{-1}$ (dry) soil h^{-1}		
Jhumming (J)				
Burnt	0.51b	365.8b	560.4b	74.06b
Unburnt	1.39a	383.4a	612.1a	104.4a
Cropping (C)				
With rice	0.90b	340.9b	571.9b	90.71a
Without rice	0.99a	408.2a	600.6a	87.80b
Microbial Inoculation (MI)				
PSB + Fungi	1.1b	428.1b	646.0b	94.40b
N ₂ fixer + Fungi	1.2a	384.6c	626.7c	85.15c
CDB + Fungi	1.0c	452.4a	705.4a	86.07c
PSB + N ₂ fixer + CDB + Fungi	0.8d	319.3e	529.7e	83.69c
Fungi	0.7e	342.7d	547.0d	101.3a
No inoculation (control)	0.6f	320.3d	462.7f	84.88c
Interactions				
J * C	**	**	**	**
J * MI	**	**	**	**
C * MI	**	**	**	**
J * C * MI	**	**	**	**

DHA – Dehydrogenase activity; GSA – beta-glucosidase activity; PHA – acid phosphomonoesterase activity; ASA – aryl sulphatase activity. *,** are levels of significance at the probability $P \leq 0.05$ and 0.01 , respectively.
 Within a parameter the values followed by different letters are significant different at ≥ 0.05 within a factor (J, C, MI)

Table 4.3: Interaction effect of burning, cropping and microbial communities on soil enzyme activities in 5 years *Jhum* cycle at 45 days growth stage of rice plant

Treatments	DHA	GSA	PHA	ASA
	$\mu\text{g TPF g}^{-1}$ (dry) soil h^{-1}	$\mu\text{g PNP g}^{-1}$ (dry) soil h^{-1}		
Jhumming (J)				
Burnt	08.30b	321.6a	524.8a	110.8b
Unburnt	16.10a	306.1b	502.2b	118.3a
Cropping (C)				
With rice	14.48a	296.7b	520.6a	131.2a
Without rice	09.92b	330.9a	506.5b	98.09b
Microbial Inoculation (MI)				
PSB + Fungi	12.5c	298.3d	559.a	116.4bc
N ₂ fixer + Fungi	14.1b	260.1e	511.3d	109.7c
CDB + Fungi	15.7a	433.3a	543.4a	130.1a
PSB + N ₂ fixer + CDB + Fungi	10.8d	337.5b	436.3f	116.7bc
Fungi	10.0d	308.6c	527.2c	121.0b
No inoculation (control)	10.0d	245.2f	503.3e	93.6d
Interactions				
J * C	**	**	**	**
J * MI	**	**	**	**
C * MI	**	**	**	**
J * C * MI	**	**	**	**

DHA – Dehydrogenase activity; GSA – beta-glucosidase activity; PHA – acid phosphomonoesterase activity; ASA – aryl sulphatase activity. *,** are levels of significance at the probability $P \leq 0.05$ and 0.01 , respectively.
 Within a parameter the values followed by different letters are significant different at ≥ 0.05 within a factor (J, C, MI)

Table 4.4: Interaction effect of burning, cropping and microbial communities on soil enzyme activities in 5 years *Jhum* cycle at 90 days growth stage of rice plant

Treatments	DHA	GSA	PHA	ASA
	$\mu\text{g TPF g}^{-1}$ (dry) soil h^{-1}	$\mu\text{g NP g}^{-1}$ (dry) soil h^{-1}		
Jhumming (J)				
Burnt	10.64b	379.6b	336.0b	47.73b
Unburnt	18.86a	396.5a	497.1a	102.3a
Cropping (C)				
With rice	17.97a	405.1a	402.8b	88.70a
Without rice	11.53b	371.1b	430.3a	61.37b
Microbial Inoculation (MI)				
PSB + Fungi	16.1c	426.4c	420.1c	66.2d
N ₂ fixer + Fungi	16.8bc	345.6d	363.6e	82.0a
CDB + Fungi	71.5b	304.3f	461.3a	76.7b
PSB + N ₂ fixer + CDB + Fungi	18.9a	321.0e	424.0b	72.3c
Fungi	10.7d	443.0b	405.1d	77.1b
No inoculation (control)	8.4e	488.0a	426.0b	75.8b
Interactions				
J * C	**	**	**	**
J * MI	**	**	**	**
C * MI	**	**	**	**
J * C * MI	**	**	**	**

DHA – Dehydrogenase activity; GSA – beta-glucosidase activity; PHA – acid phosphomonoesterase activity; ASA – aryl sulphatase activity. **,*** are levels of significance at the probability $P \leq 0.05$ and 0.01 , respectively.
 Within a parameter the values followed by different letters are significant different at ≥ 0.05 within a factor (J, C, MI)

Table 4.5: Interaction effect of burning, cropping and microbial communities on soil enzyme activities in 5 years *Jhum* cycle at 120 days growth stage of rice plant

Treatments	DHA	GSA	PHA	ASA
	$\mu\text{g TPF g}^{-1}$ (dry) soil h^{-1}	$\mu\text{g NP g}^{-1}$ (dry) soil h^{-1}		
Jhumming (J)				
Burnt	07.475b	308.9b	351.397	68.12b
Unburnt	20.356a	425.2a	323.021	81.54a
Cropping (C)				
With rice	16.151a	355.6b	514.652	86.02a
Without rice	11.680b	378.6c	159.766	63.64b
Microbial Inoculation (MI)				
PSB + Fungi	15.8a	327.0f	336.1c	60.4e
N ₂ fixer + Fungi	15.3a	337.1e	296.8e	76.5bc
CDB + Fungi	13.1b	371.7c	350.2b	83.6a
PSB + N ₂ fixer + CDB + Fungi	13.7b	400.0b	367.6a	75.5bc
Fungi	13.3b	403.3a	349.6b	78.6b
No inoculation (control)	12.2c	363.3d	322.7d	74.4d
Interactions				
J * C	**	**	**	**
J * MI	**	**	**	**
C * MI	**	**	**	**
J * C * MI	**	**	**	**
DHA – Dehydrogenase activity; GSA – beta-glucosidase activity; PHA – acid phosphomonoesterase activity; ASA – aryl sulphatase activity. ***, ** are levels of significance at the probability $P \leq 0.05$ and 0.01 , respectively.				
Within a parameter the values followed by different letters are significant different at ≤ 0.05 within a factor (J, C, MI)				

Each of the microbial inoculation responds to soil enzymes activity differently. The inoculation of CDB + Fungi had impact to soil DHA, GSA and ASA enzyme activities where as inoculations of PSB + fungi effect the PHA activity at 45 days of rice plant growth.

The interaction between jhumming* cropping; jhumming * microbial inoculation; cropping * microbial inoculation and jhumming * cropping * microbial inoculation were significance at a level $P \leq 0.01$.

4.2.1.3 Soil enzymes activities at 90 days of rice plant growth

The soil enzymes such as DHA, GSA, ASA and PHA were strongly influence by the burning activity (Table 4.4. and annexure 4.9., 4.10., 4.11., 4.12.). The activities of DHA, GSA, and PHA were decrease in burnt soil where there was a slight increased in the activity of ASA in burnt soil.

Interestingly the activities of all the enzymes were found higher in soils where rice crop was grown as compared to bulk soils.

Each of the microbial inoculation responds to soil enzymes activity differently. The soil inoculation of PSB + N₂ fixer + CDB with fungi had a greater impact to soil DHA, inoculation of CDB and Fungi effect the GSA, inoculations of PSB + fungi influence the PHA activity and N₂ fixer + fungi had a greater impact to ASA at 90 days of rice plant growth.

The interaction between jhumming* cropping; jhumming * microbial inoculation; cropping * microbial inoculation and jhumming * cropping * microbial inoculation were significance at a level $P \leq 0.01$.

4.2.1.4. Soil enzymes activities at 120 days of rice plant growth

The soil enzymes such as DHA, GSA, ASA and PHA were strongly influence by the burning activity (Table 4.5. and appendix 4.13., 4.14., 4.15., 4.16.). The activities of DHA, GSA, and PHA were decrease in burnt soil where the activity of ASA was slightly increased in burnt soil.

Interestingly the activities of all the enzymes were found higher in soils where rice crop had grown increased and cropping and GSA were taken as an early indicator of soil processes.

The microbial inoculation responds was different to each enzymes activity. The inoculation of synthetic N₂ fixer had a greater impact to soil on DHA and CDB had a greater impact to GSA. PSB inoculants affect the PHA and sole fungi inoculation had a greater impact to ASA at 120 days of rice plant growth.

Each of the microbial inoculation responds to soil enzymes activity differently. The inoculation of synthetic N₂ fixer had a greater impact to soil DHA is impacted by inoculations of PSB + fungi and application of N₂ fixer + fungi and sole fungi had greater impact on GSA. PSB with N₂ fixer and CDB with fungi inoculants effect the PHA. CDB with Fungi inoculation had greater impact to ASA at 120 days of rice plant growth.

The interaction between jhumming* cropping; jhumming * microbial inoculation; cropping * microbial inoculation and jhumming * cropping * microbial inoculation were significance at a level $P \leq 0.01$.

4.2.2.1. Soil biochemical properties at 10 days growth stage of rice plant in response to burning, cropping and synthetic microbial inoculants

The soil biochemical properties such as SOC, pMN, POM, MBC and DOC were studied and the result shows that this biochemical properties of soil were strongly affected by burning, cropping and synthetic microbial inoculation (Table 4.6. and annexure 4.17., 4.18., 4.19., 4.20. and 4.21.).

The higher amount of SOC, pMN, POM and MBC were observed in unburnt soils where a slight increase was observed in case of DOC.

In cropping conditions all the tested soil biochemical properties were found higher in a condition where rice crop was grown than the bulk soils.

The SOC was found to be highest in a condition where soils was inoculated with sole fungi and pMN was found highest where soil was inoculated with CDB + fungi. POM and MBC were found highest where soil was inoculated with PSB + fungi and the inoculation of N₂ fixer + fungi had a great impact on DOC content in soil.

The interaction between jhumming* cropping; jhumming * microbial inoculation; cropping * microbial inoculation and jhumming * cropping * microbial inoculation were significance at a level $P \leq 0.01$.

Table 4.6.: Interaction effect of burning, cropping and microbial communities on soil biochemical activities in 5 years *Jhum* cycle at 10 days growth stage of rice plant

Treatment	SOC %	pMN $\mu\text{g g}^{-1}(\text{dw})\text{soil}$	POM %	MBC $\mu\text{g /g}$	DOC $\mu\text{g g soil}$
Jhumming (J)					
Burnt	3.06b	16.87a	4.44a	166.6b	145.7a
Unburnt	3.17a	11.88b	5.47b	186.9a	116.7b
Cropping					
Burnt	3.10b	18.92a	6.42b	201.3a	134.3a
Unburnt	3.14a	09.84b	6.48a	152.1b	128.1b
Microbial Inoculation (MI)					
PSB + Fungi	3.01c	13.18d	7.24a	212.9a	136.8c
N ₂ fixer + Fungi	3.02c	16.67b	6.36c	204.1b	170.2a
CDB + Fungi	3.18b	18.00a	6.94b	178.0c	143.2b
PSB + N ₂ fixer + CDB + Fungi	3.16b	15.10c	6.17c	162.9d	117.2d
Fungi	3.29a	10.49e	5.99e	160.2e	110.9e
No inoculation (control)	3.03c	12.83d	6.02e	142.4f	108.7f
INTERACTION					
J * C	**	**	**	**	**
J * MI	**	**	**	**	**
C * MI	**	**	**	**	**
J * C * MI					

SOC – Soil Organic Carbon; pMN; Potentially Mineralizable Nitrogen; POM – Particulate Organic Matter; SMBC – Soil Microbial Biomass Carbon DOC – Dissolve Organic Carbon **, ** are levels of significance at the probability $P \leq 0.05$ and 0.01 , respectively. Within a parameter, the value followed by different letters are significantly differed at $P \leq 0.05$ within a factor (J, C, MI)

Table 4.7.: Interaction effect of burning, cropping and microbial communities on soil biochemical activities in 5 years *Jhum* cycle at 45 days growth stage of rice plant

Treatment	SOC %	pMN $\mu\text{g g}^{-1}(\text{dw})\text{soil}$	POM %	MBC $\mu\text{g /g}$	DOC $\mu\text{g g soil}$
Jhumming (J)					
Burnt	3.09b	17.0a	8.14b	89.44b	181.5a
Unburnt	3.13a	14.3b	9.14a	130.2a	135.8b
Cropping					
Burnt	3.14a	21.1a	8.22b	133.2a	194.7a
Unburnt	3.07b	10.1b	9.06a	86.37b	122.6b
Microbial Inoculation (MI)					
PSB + Fungi	2.93e	08.8e	8.51b	129.6b	230.9a
N ₂ fixer + Fungi	3.03d	22.2a	8.44bc	134.7a	148.1c
CDB + Fungi	3.18b	19.2b	8.38bc	117.4c	145.9d
PSB + N ₂ fixer + CDB + Fungi	3.10c	16.4c	9.67a	108.2d	126.0e
Fungi	3.37a	11.9d	8.27c	93.99e	154.5b
No inoculation (control)	3.03d	15.2c	8.57b	74.86f	146.9cd
INTERACTION					
J * C	**	**	**	**	**
J * MI	**	**	**	**	**
C * MI	**	**	**	**	**
J * C * MI					
SOC – Soil Organic Carbon; pMN; Potentially Mineralizable Nitrogen; POM – Particulate Organic Matter; SMBC – Soil Microbial Biomass Carbon DOC – Dissolve Organic Carbon **,* are levels of significance at the probability $P \leq 0.05$ and 0.01 , respectively. Within a parameter, the value followed by different letters are significantly differed at $P \leq 0.05$ within a factor (J, C, MI)					

Table 4.8: Interaction effect of burning, cropping and microbial communities on soil biochemical activities in 5 years *Jhum* cycle at 90 days growth stage of rice plant

Treatment	SOC %	pMN $\mu\text{g g}^{-1}(\text{dw})\text{soil}$	POM %	MBC $\mu\text{g /g}$	DOC $\mu\text{g g soil}$
Jhumming (J)					
Burnt	3.04b	09.52a	7.87a	31.69b	136.6b
Unburnt	3.14a	07.63b	6.15b	42.18a	139.3a
Cropping					
Burnt	3.15a	10.30a	6.78b	61.79a	143.9a
Unburnt	3.02b	06.86b	7.24a	12.08b	132.0b
Microbial Inoculation (MI)					
PSB + Fungi	3.04c	08.91b	6.72d	41.22b	157.1b
N ₂ fixer + Fungi	3.06c	07.58c	6.15e	42.06ab	125.0d
CDB + Fungi	3.11b	05.75d	8.00a	42.74a	122.5e
PSB + N ₂ fixer + CDB + Fungi	3.20a	06.16c	7.73b	38.99c	119.3f
Fungi	3.21a	11.33a	6.24e	35.57d	145.3c
No inoculation (control)	2.90d	11.75a	7.21c	21.04e	158.7a
INTERACTION					
J * C	**	**	**	**	**
J * MI	**	**	**	**	**
C * MI	**	**	**	**	**
J * C * MI					

SOC – Soil Organic Carbon; pMN; Potentially Mineralizable Nitrogen; POM – Particulate Organic Matter; SMBC – Soil Microbial Biomass Carbon DOC – Dissolve Organic Carbon **,* are levels of significance at the probability $P \leq 0.05$ and 0.01 , respectively. Within a parameter, the value followed by different letters are significantly differed at $P \leq 0.05$ within a factor (J, C, MI)

Table 4.9.: Interaction effect of burning, cropping and microbial communities on soil biochemical activities in 5 years *Jhum* cycle at 120 days growth of rice plant

Treatment	SOC %	pMN $\mu\text{g g}^{-1}(\text{dw})\text{soil}$	POM %	MBC $\mu\text{g /g}$	DOC $\mu\text{g g soil}$
Jhumming (J)					
Burnt	2.50a	15.98a	7.43a	54.56a	204.7b
Unburnt	2.36b	14.55b	5.56b	53.96b	208.6a
Cropping					
Burnt	2.41b	18.25a	8.50a	51.195b	204.0b
Unburnt	2.46a	12.27b	6.33b	57.339a	209.3a
Microbial Inoculation (MI)					
PSB + Fungi	2.43b	10.56d	07.23c	66.61a	231.4a
N ₂ fixer + Fungi	2.37c	13.94c	07.74b	54.87c	220.6c
CDB + Fungi	2.41c	18.95a	07.70b	57.27b	154.1f
PSB + N ₂ fixer + CDB + Fungi	2.49a	16.47b	08.07a	66.33a	224.2b
Fungi	2.48a	13.22c	07.05c	48.26d	203.0e
No inoculation (control)	2.42b	18.43a	06.70d	32.24e	206.9d
INTERACTION					
J * C	**	**	**	**	**
J * MI	**	**	**	**	**
C * MI	**	**	**	**	**
J * C * MI					

SOC – Soil Organic Carbon; pMN; Potentially Mineralizable Nitrogen; POM – Particulate Organic Matter; SMBC – Soil Microbial Biomass Carbon DOC – Dissolve Organic Carbon **,** are levels of significance at the probability $P \leq 0.05$ and 0.01 , respectively. Within a parameter, the value followed by different letters are significantly differed at $P \leq 0.05$ within a factor (J, C, MI)

4.2.2.2. Biochemical properties of soil at 45 days growth stage of rice plant in response to burning, cropping and microbial inoculants

The soil biochemical properties such as SOC, pMN, POM, MBC and DOC were strongly affected by burning, cropping and microbial inoculation (Table 4.7. and annexure 4.22., 4.23., 4.24., 4.25. and 4.26.).

The higher amount of SOC, pMN, POM and MBC were observed in unburnt soils where a slight increase was observed in case of DOC.

In cropping conditions all the tested soil biochemical properties were found higher in a condition where rice crop was grown than the bulk soils.

The SOC was found to be highest in a condition where soils was inoculated with sole synthetic fungi. pMN was highest in soil was inoculated with N₂ fixer + fungi. POM was highest in soil inoculated with CDB, N₂ fixer and PSB + fungi. The MBC were found highest where soil was inoculated with N₂ fixer + fungi and the inoculation of PSB + fungi had a great impact on DOC content in soil.

The interaction between jhumming* cropping; jhumming * microbial inoculation; cropping * microbial inoculation and jhumming * cropping * microbial inoculation were significance at a level $P \leq 0.01$.

4.2.2.3 Biochemical properties of soil at 90 days growth stage of rice plant in response to burning, cropping and microbial inoculants

The soil biochemical properties such as SOC, pMN, POM, MBC and DOC were strongly affected by burning, cropping and microbial inoculation (Table 4.8. and annexure 4.27., 4.28., 4.29., 4.30. and 4.31.).

At 90 days of rice growth there was changes in MBC and DOC content in soil. The higher amount of SOC, pMN, POM and DOC were observed in unburnt soils interestingly a slight increase was observed in case of MBC at burnt soil.

The cropping does had an effect on biochemical properties. In comparison with bulk soil higher amount of soil biochemical properties was recorded in soil where rice crop was grown.

The SOC and pMN was found to be highest in a condition where soils was inoculated with sole fungi and pMN was highest in soil inoculated with N₂ fixer + fungi, POM and MBC were highest in soil inoculated with CDB + fungi and at 90 days the microbial inoculation doesn't had any effect on DOC content in soil.

The interaction between jhumming* cropping; jhumming * microbial inoculation; cropping * microbial inoculation and jhumming * cropping * microbial inoculation were significance at a level $P \leq 0.01$.

4.2.2.4 Biochemical properties of soil at 120 days growth stage of rice plant in response to burning, cropping and microbial inoculants

The soil biochemical properties such as SOC, pMN, POM MBC and DOC were strongly affected by burning, cropping and synthetic microbial inoculation (Table 4.9. and annexure 4.32., 4.33., 4.34., 4.35. and 4.36.).

Higher amount of SOC, pMN, POM and MBC were observed in burnt soils where the DOC content shows higher value in an unburnt soil.

The cropping does had an effect on biochemical properties. In comparison with bulk soil higher amount of soil biochemical properties was recorded in soil where rice crop was grown however higher amount of DOC content was found in soil where rice crop was not grown.

The SOC was found to be highest in a condition where soils was inoculated with sole synthetic fungi, pMN in soil was inoculated with CDB + fungi, POM and MBC in soil inoculated with PSB + N₂ fixer + CDB + fungi and highest DOC content was recorded with soil inoculated with PSB + fungi.

The interaction between jhumming* cropping; jhumming * microbial inoculation; cropping * microbial inoculation and jhumming * cropping * microbial inoculation were significance at a level $P \leq 0.01$.

OBJECTIVE- III

4.3.1 Relationship of soil properties with bacterial community

The relationship of soil properties with bacterial community profile was determined by super imposing soil data matrix on Multidimensional (MDS) plot. The weightage of individual soil properties in separating the bacterial communities was determined by Pearson co-relation co-efficient along with the MDS axis. The super impose soil data matrix on MDS plot shows that soil properties had played role in separating bacterial (Fig.4.16.). The bacterial community based MDS plot at 10 days of rice growth indicated that DHA, ASA, pMN, POM played significant role in separating bacterial communities in MDS axis 1 according to the presence and absence of rice. On the other hand MBC played significant role in separating burn and unburn soil along the MDS axis 2 (Table 4.10.). The other soil properties though they have show co-relation with MDS axis there effect was not significant.

4.3.2. Relationship of soil properties with Alpha proteobacteria at 45 days of rice growth stage

The relationship of soil properties with bacterial community profile was determined by super imposing soil data matrix on Multidimensional (MDS) plot. The weightage of individual soil properties in separating the bacterial communities was determined by Pearson co-relation co-efficient along with the MDS axis. The super impose soil data matrix on MDS plot shows that soil properties had played role in separating bacterial (Fig.4.17.). Alpha-Proteobacterial group based MDS plot at 45 days of rice growth indicated that pMN, POM and DOC played significant role in separating bacterial communities in MDS axis 1 according to the presence and absence of rice. On the other hand DHA played significant role in separating burn and unburn soil along the MDS axis 2 (Table 4.11.). The other soil properties though they have show co-relation with MDS axis there effect was not significant.

4.3.3. Relationship of soil properties with Alpha proteobacteria at 90 days of rice growth stage

The relationship of soil properties with bacterial community profile was determined by super imposing soil data matrix on Multidimensional (MDS) plot. The weightage of individual soil properties in separating the bacterial communities was determined by Pearson co-relation co-efficient along with the MDS axis. The super impose soil data matrix on MDS plot shows that soil properties had played role in

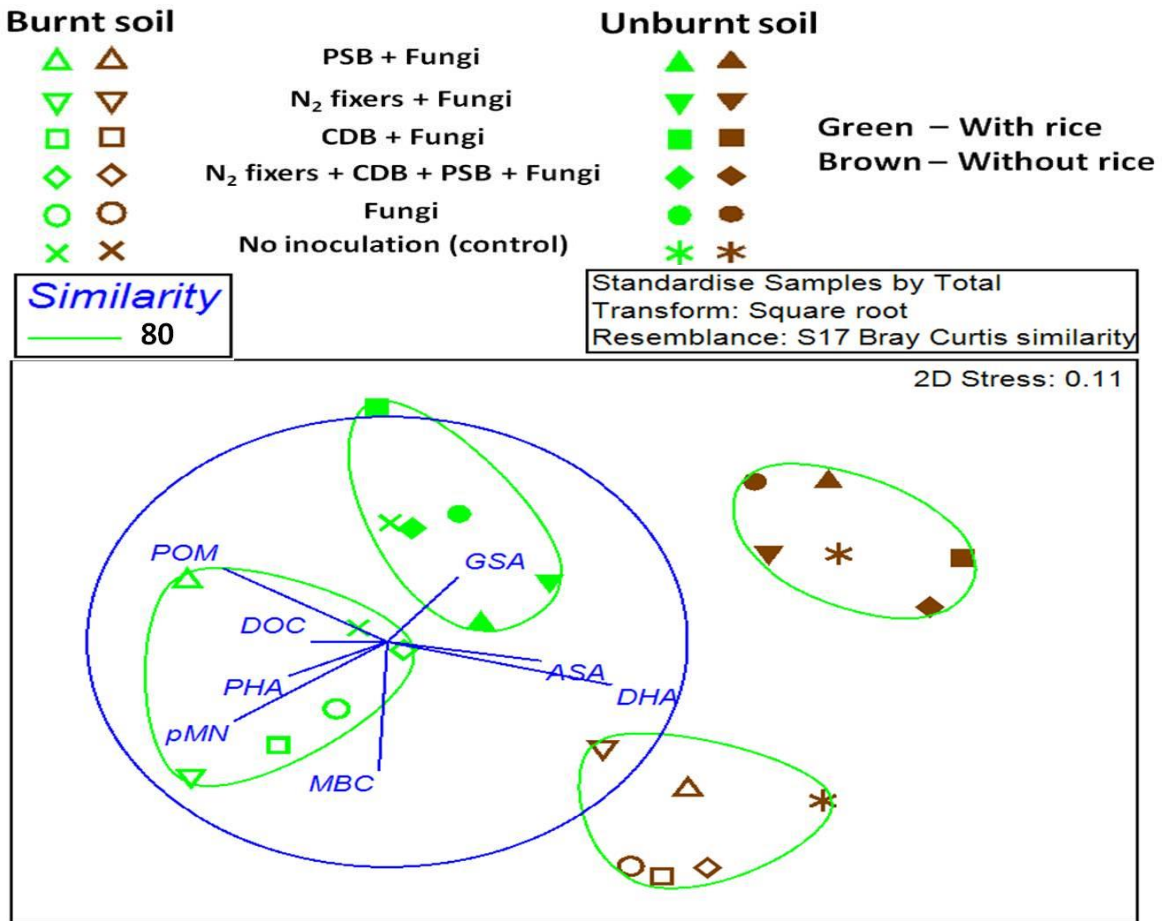


Fig.4.16: Principal Component Analysis plot showing relationship of bacterial community and soil properties at 10 days of rice growth

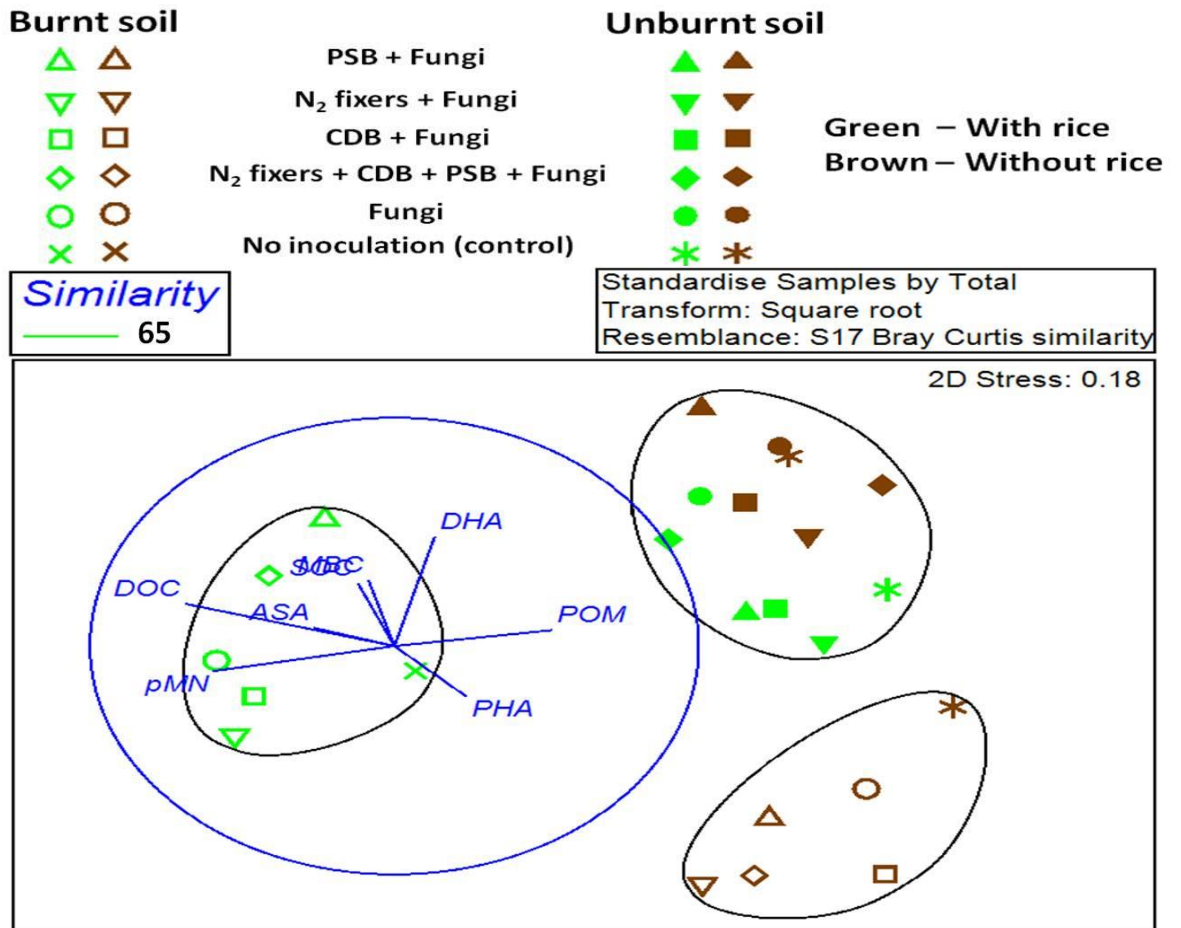


Fig.4.17: Principal Component Analysis based plot showing relationship of Alpha Proteobacterial community and soil properties at 45 days of rice growth

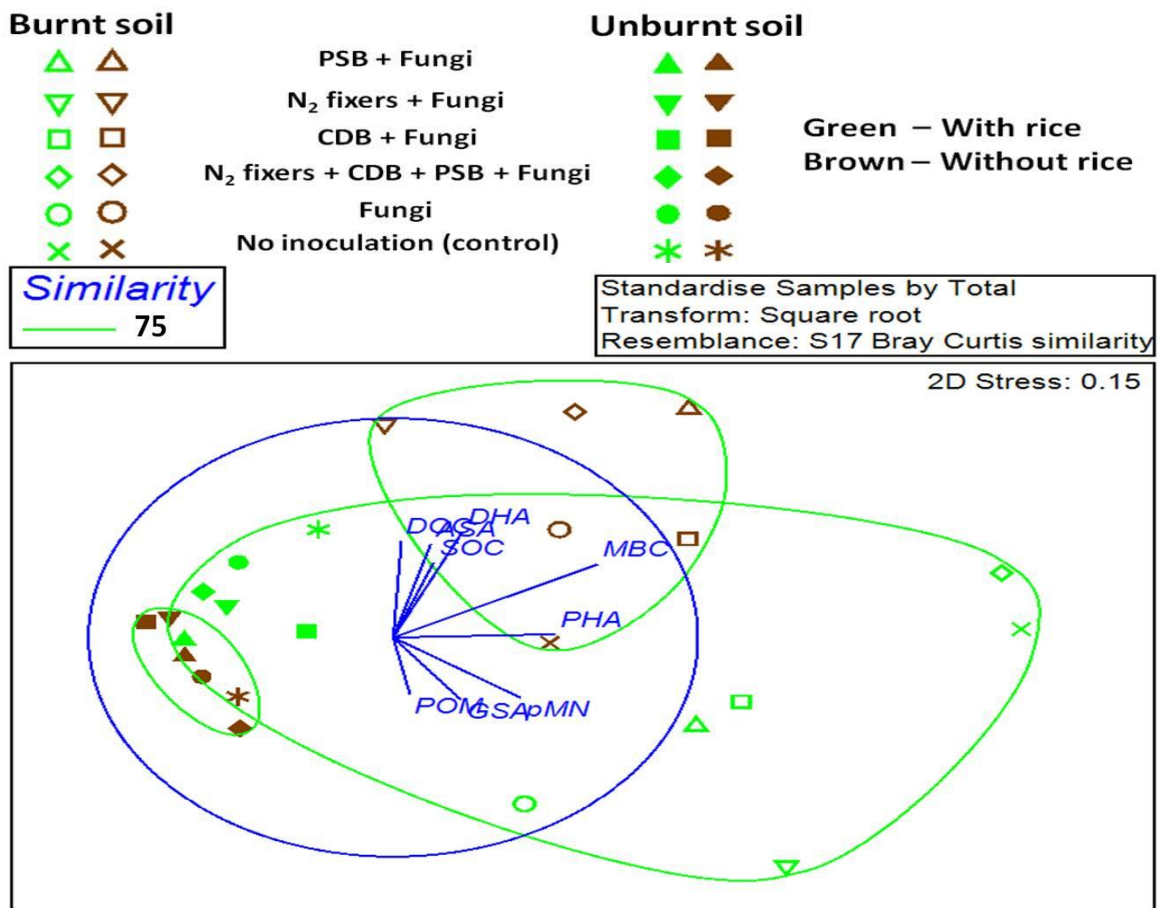


Fig.4.18: Principal Component Analysis based plot showing relationship of Alpha Proteobacterial community and soil properties at 90 days of rice growth

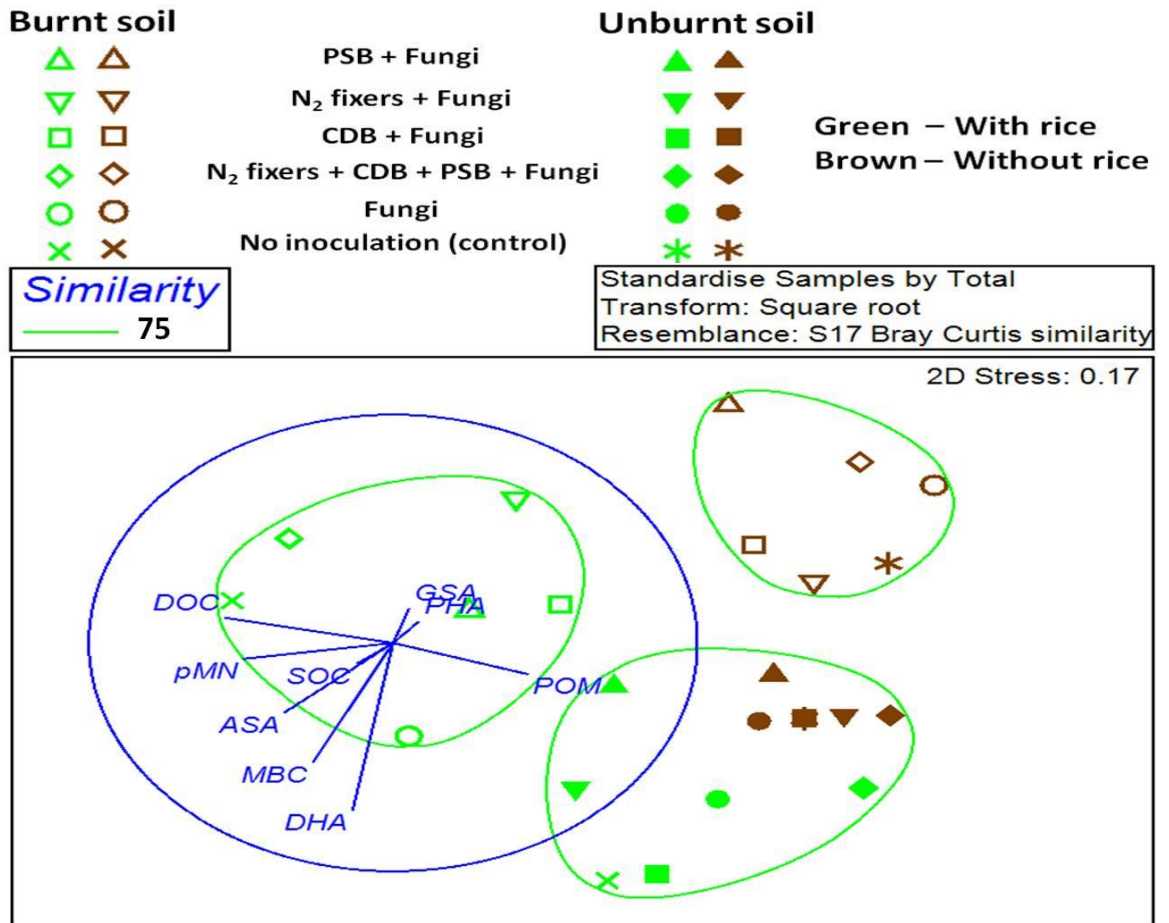


Fig.4.19: Principal Component Analysis based plot showing relationship of Beta Proteobacterial community and soil properties at 45 days of rice growth

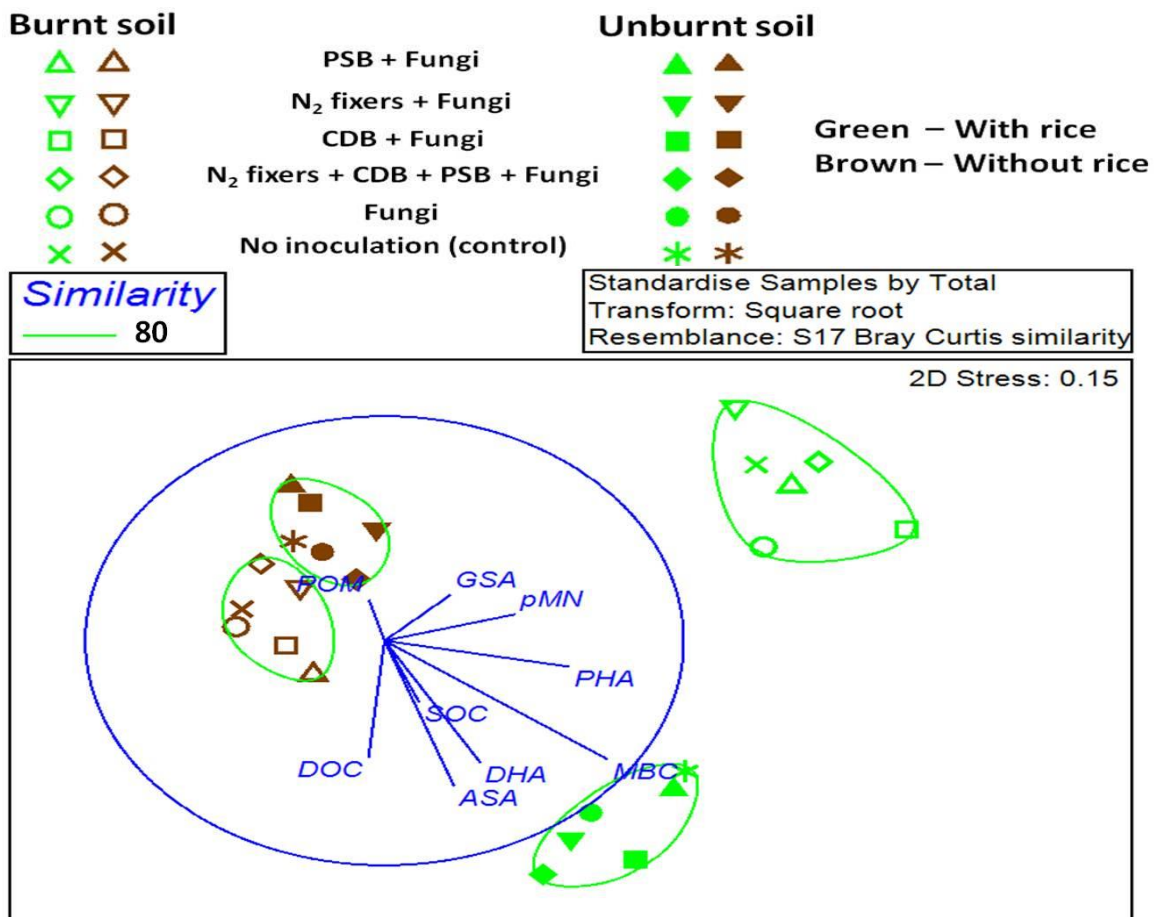


Fig. 4.20: Principal Component Analysis based plot showing relationship of Beta Proteobacterial community and soil properties at 90 days of rice growth

Table 4.10: Pearson's correlation coefficient (r) for the relationship of bacterial community in MDS axis with soil properties at 10 days of rice plant growth

	MDS 1	MDS 2
GSA	0.24	0.29
ASA	0.51**	-0.08
PHA	-0.33	-0.15
DHA	0.75**	-0.19
pMN	0.51**	-0.35
MBC	-0.03	-0.57**
DOC	-0.25	0.00
SOC	-0.07	0.10
POM	-0.55**	0.33

* and ** indicate level of significance at $P \leq 0.005$ and 0.01 respectively.

Table 4.11: Pearson's correlation coefficient (r) for the relationship alpha proteobacterial community along the MDS axis with soil properties at 45 days of rice plant growth

	MDS 1	MDS 2
GSA	-0.06	0.05
ASA	-0.26	0.08
PHA	0.24	-0.22
DHA	0.13	0.48*
pMN	-0.60**	-0.11
MBC	-0.09	0.29
DOC	-0.69**	0.19
SOC	-0.12	0.27
POM	0.52**	0.07

* and ** indicate level of significance at $P \leq 0.005$ and 0.01 respectively.

Table 4.12: Pearson's correlation coefficient (r) for the relationship alpha proteobacterial community along the MDS axis with soil properties at 90 days of rice plant growth

	MDS 1	MDS 2
DOC	0.03	0.44*
GSA	0.22	-0.28
ASA	0.12	0.43*
PHA	0.53**	0.01
pMN	0.42*	-0.27
MBC	0.67**	0.33
SOC	0.13	0.34
DHA	0.23	0.48
POM	0.05	-0.26

* and ** indicate level of significance at $P \leq 0.005$ and 0.01 respectively.

Table 4.13: Pearson's correlation coefficient (r) for the relationship beta proteobacterial community along the MDS axis with soil properties at 45 days of rice plant growth

	MDS 1	MDS 2
GSA	0.05	0.15
ASA	-0.36	-0.31
PHA	0.09	0.09
pMN	-0.50*	-0.07
SOC	-0.12*	-0.09
DOC	-0.55**	0.11
DHA	-0.13	-0.73**
MBC	-0.26	-0.52**
POM	0.44	-0.14

* and ** indicate level of significance at $P \leq 0.005$ and 0.01 respectively.

Table 4.14: Pearson's correlation coefficient (r) for the relationship beta proteobacterial community along the MDS axis with soil properties at 90 days of rice plant growth

	MDS 1	MDS 2
DOC	-0.05	-0.52**
GSA	0.22	0.20
ASA	0.23	-0.65**
PHA	0.62**	-0.12
pMN	0.44**	0.12
MBC	0.75**	-0.53**
SOC	0.12	-0.27
DHA	0.32	-0.54**
POM	-0.05	0.18

* and ** indicate level of significance at $P \leq 0.005$ and 0.01 respectively.

separating bacterial (Fig.4.18). Alpha-Proteobacterial group based MDS plot at 45 days of rice growth indicated that PHA, pMN, MBC played significant role in separating bacterial communities in MDS axis 1 according to the presence and absence of rice. On the other hand DOC and ASA played significant role in separating burn and unburn soil along the MDS axis 2 (Table 4.12.). The other soil properties though they have show co-relation with MDS axis there affect was not significant.

4.3.4. Relationship of soil properties with Beta proteobacteria at 45 days of rice growth stage

The relationship of soil properties with bacterial community profile was determined by super imposing soil data matrix on Multidimensional (MDS) plot. The weightage of individual soil properties in separating the bacterial communities was determined by Pearson co-relation co-efficient along with the MDS axis. The super impose soil data matrix on MDS plot shows that soil properties had played role in separating bacterial (Fig.4.19.). Beta-Proteobacterial group based MDS plot at 45 days of rice growth indicated that SOC, pMN, DOC played significant role in separating

bacterial communities in MDS axis 1 according to the presence and absence of rice. On the other hand DHA and SOC played significant role in separating burn and unburn soil along the MDS axis 2 (Table 4.13). The other soil properties though they have show co-relation with MDS axis there effect was not significant.

4.3.5. Relationship of soil properties with Beta- proteobacteria at 90 days of rice growth stage

The relationship of soil properties with bacterial community profile was determined by super imposing soil data matrix on Multidimensional (MDS) plot. The weightage of individual soil properties in separating the bacterial communities was determined by Pearson co-relation co-efficient along with the MDS axis. The super impose soil data matrix on MDS plot shows that soil properties had played role in separating bacterial (Fig.4.20.). Alpha-Proteobacterial group based MDS plot at 90 days of rice growth indicated that PHA, pMN, MBC played significant role in separating bacterial communities in MDS axis 1 according to the presence and absence of rice. On the other hand DOC, ASA, MBC and DHA played significant role in separating burn and unburn soil along the MDS axis 2 (Table 4.14.).The other soil properties though they have show co-relation with MDS axis there effect was not significant.

Chapter-5

Discussion

5.1 Soil Physio-chemical Properties

The burning event caused variations in the physico-chemical attributes of *jhum* soils. Burning activities have reduced the organic matter content from the secondary forests which were found to reduce the bulk density of soil (Alegre and Cassel 1996; Granged *et al.*, 2011; Biswas *et al.*, 2012). Rise in pH after burning was more prominent in acidic soils than in alkaline soils and was reported to be contributed by loss of OH, oxide formation and release of alkaline cations like Ca, Mg, and K (Certini, 2005). Results from this study also indicated that EC of the soil has increased immediately after burning. Similar increment in EC was previously observed by Terefe *et al.* (2008) when the soil was exposed to beyond 150°C. It was reported that the soil temperature rose up to 242°C± 10°C during burning of slashed biomass in *Jhum* fallows of NE India (Thakuria, 2015).

Decrease in SOC content after burning has been vividly described in the past (Nye and Greenland, 1960; Fernandez *et al.*, 1997). Alteration in SOC content can be accounted for higher topsoil temperature (242°C± 10°C) during burning that causes burning of FFLs leading to limited incorporation of litter materials in burnt plots (Tinker *et al.*, 1996). At temperatures above 300°C, soil organic N is lost during the thermal oxidation of organic matter in the form of oxidized N gases and N₂ (Raison, 1979). The content of NO₃-N has a declining trend after burning (Ramakrishnan and Toky, 1981). In this study, soil Avl N content decreased after burning. Past findings indicated that burning activity decreased the content of soil Avl N. Depletion of N from the topsoil attributed to leaching loss caused by heavy monsoon rainfall and absorption by fast sprouting weed species (Wallbrink *et al.*, 2005), which is a common phenomenon in *jhum* fields across Northeast India. Loss of nitrogen at temperature as high as 100°C was reported by DeBano *et al.* (1979), which in the case of conventional *jhumming* usually exceed this threshold temperature. Increase in the content of Avl P after burning is a common phenomenon in *jhum* scenarios across Northeast India. Recently, Adeyolanu *et al.* (2014) has also cited an increase of Avl P while studying a slash and burn situation in tropical rainforest of Nigeria. The increase in Avl P can be attributed to the incorporation of P from the slashed biomass in the form of ash as indicated by Raison *et al.*, 1985). Ramakrishnan and Toky (1981) reported increase in P and K concentrations after burning as well as an increasing

trend with increase in fallow length and summarized the phenomenon to be controlled by quantum of slashed and burned biomass of the above-ground plant species. Neff *et al.* (2005) have however reported about a mild shift in the K content after burning.

5.2. Responses of soil microbes to burnt and unburnt soils

In slash and burn agriculture burning activities play a major role in altering soil microbial community. Good indicator of soil biological activity i.e enzymes and soil health altered as soil microbial community structure change. Soil microbes are sensitive to fire. Structural differences of microbial communities from burnt to unburnt was well documented (Hamman *et al.*, 2007; Xiang *et al.*, 2014). The aboveground vegetation affects the size, abundance, diversity, composition and activity of microbial community in soils (Nusslein and Tiedji, 1999 and da Jesus *et al.*, 2009; Zhao *et al.*, 2014). The microbial structure gets affected with a change in ecosystem functions such as interaction between plants, soil and microorganisms which lead to manipulation in ecological processes (Singh *et al.*, 2004). Land management, soil attributes and plant species were important for maintaining the structure and functions of bacterial communities (Nusslein and Tiedje, 1999; Wieland *et al.*, 2001; Steenwerth *et al.*, 2002; Hartman *et al.*, 2008; Lauber *et al.*, 2008; da Jesus *et al.*, 2009 and Blasiak *et al.*, 2014). The microbial community in soil can be affected by fire, land use and change in plant species which are the main component in shifting cultivation (Carson *et al.*, 2010).

Soil microbial community composition and diversity was directly or indirectly affected by fire. Fire produces heat which kills the soil micro-organism and reduces the microbial biomass (Wang *et al.*, 2012). Thus, fire become the threats to forest ecosystems (Bond and Keeley, 2005; Barcenas *et al.*, 2011; Xiang *et al.*, 2014). Shift of Bacteroidetes and Betaproteobacteria with greater abundance. Alphaproteobacteria and Acidobacteria with reduced at boreal forest of China (Xiang *et al.*, 2014). With a higher abundance of the genus Bradyrhizobium, shifts in bacterial community composition was also reported in slash and burn system. Betaproteobacteria was reported as most abundant bacterial group in slash and burn system (Blasiak *et al.*, 2014).

From our present study using PCR-DGGE fingerprint, the soil bacterial community was found to be distinctly separated out between burnt and unburnt soils. In burnt and unburnt soils the bacterial communities cluster separately according to the presence or absence of rice crop. The stronger factor of variability in shaping the soil bacterial community at 10 days of rice plant growth and alpha-proteobacterial

community composition at 45 and 90 days of rice plant growth was found to be in the order burning > cropping > microbial inoculation treatment. The beta-proteobacterial community at 45 and 90 days of rice plant growth clustered separately according to the presence or absence of rice crop in burnt soil or unburnt soil. The stronger factor of variability in shaping the beta-proteobacterial community composition was found to be in the order cropping > burning > microbial inoculation treatment. Hamman *et al.* (2007) reported the difference in structural soil microbial communities in burnt soils from unburnt soils of forest fire in central Colorado which was in agreement with our findings. Our studies indicate that cropping/ presence of plants in soils shows a distinct separation in soil microbial community which could be due to the variation in secretion of root exudates by plants in a different quantity and quality leads to selection of rhizospheric microbes which in turn results in different microbial communities in soil (Nguyen, 2003 and Zhang *et al.*, 2013). The presence of various plant species with special functional traits (C4 grasses, nitrogen-fixing legumes) affects the eco-system functioning (Balvanera *et al.*, 2006; Fornara and Tilman, 2008) and improves the intricacy of soil microorganisms by rising the heterogeneity of organic substrates during decomposition of living roots and litter (Broughton and Gross, 2000). The restoration patterns of soil bacterial community differ under different vegetation (Zhang *et al.*, 2013). Deka and Mishra, 1983 and Pandey *et al.*, 2011) in their studies reported fire stimulates the actinomycetes population, fungi as the least affected group by fire and bacteria as the most affected group. Study conducted in Khasi hills of Meghalaya, India, reported that after two to three showers microbial population increased in the burnt plots as compared to unburnt (Sharma *et al.*, 2004). With specific plant types the Mycorrhizal fungi and basidiomycota form symbiotic associations and the lignified plant detritus gets decomposed respectively and fungi are more sensitive to change in types of vegetation than soil bacteria (Bardgett and Mc Alister, 1999).

Microbial inoculation affects the soil microbial community, however, its effect was less prominent in our studies. Plant–soil-microorganism interaction results in a complex reaction. The inoculations of PGP (*Azospirillum brasilense*) with maize results in more number of roots in the soil (Dobbelaere *et al.*, 2003), higher enzymes activity and microbial densities in the rhizospheric zone is due to the inoculation of soils with the beneficial micro-organism (Mawdsley & Burns, 1994 and Sowinski *et al.* 2007). When rhizobia (*Sinorhizobium meliloti*) was used as inoculants in field the diversity of bacterial community in the root zone was quantitatively and qualitatively affected. The proteobacteria groups largely get affected by this inoculations which

results in reduction in numbers of γ -proteobacteria whereas the α -proteobacteria were increased in numbers (Schwieger and Tebbe, 2000). Plant nutrient uptake, growth, development and yield of plants are effected by the inoculation of Rhizobium culture by different mechanism. As they are capable to fixing nitrogen, that accelerate the nitrogen-fixing free-living bacteria in soils which in turns results in increase the production of the essential nutrients such as phosphorus and iron for plants. Alterations of soil microbial community due to inoculation of various beneficial micro-organism may occur through secretion of antibiotic compound and their influence on rhizopheric bacterial communities. Spatial and temporal effects on rhizobacterial or fungal population noticed as the root zone was colonized with antibiotic-producing fluorescent pseudomonads, 2,4-diacetylphoroglucinol or phenazine compound produced (De Leij *et al.*, 1995; Girlanda *et al.*, 2001; Naseby & Lynch, 2001; Bakker *et al.*, 2002 and Sowinski *et al.* 2007). The trifolitoxin-sensitive bacteria (Alphaproteobacteria) gets reduce in the root zone of bean due to secretions of trifolitoxin by *Rhizobium etlidue* (Robleto *et al.*, 1998; Trabelsi and Mhamdi, 2013).

The importance of plant functions, nutrient cycling, primary productivity, litter decomposition and ecosystem stability and resistance to disturbance rates to ecosystem process was reported by (Madritch and Hunter, 2002 and Madritch *et al.*, 2006). Change in litter quality was a result of alteration in plant community composition that disturbed soil conditions and nutrient cycling processes.

5.3. Effect of burning, cropping and microbial inoculation on soil enzymes activity as an indicator of soil processes

Soil enzymes get reduced as their hydrological enzyme gets disturbed by burning activities (Boerner *et al.*, 2005). Change in their environment and oxidation of the available compounds by fire also directly affect the microbial activities in soil. Fire change the soil energy pathway which reflects in taxonomic shift in soil microbial communities (Bisset and Parkinson 1980).The soil enzymes such as DHA, GSA, ASA and PHA were strongly influenced by the burning, cropping and microbial inoculation throughout the rice crop growing season in our investigation. With the consequence of seasonal moisture changes, soil temperature and land management, soil and vegetation conditions the phosphatase activity in soil gets effected (Herbien and Neal, 1990; Speir and Cowling, 1991). It also reflects and feedback on community composition (Sinsabaugh *et al.*, 2002). As PHA is directly affected by various factors the effect of PHA due by fire which results in decrease of PHA activity in soil(Ajwa *et al.*, 1999; Boerner *et al.*, 2000). This past findings was concurrent with our present

finding that PHA enzymes activity decrease in burnt soil as comparison to the unburnt soils. The higher amount of PHA in soil with rice plant in comparisons to bulk soil was observed when there was P deficient in a soil plant roots secrete which enhance the solubilisation and remobilization of phosphate in soil and in bulk soil there was no such things which can release the phosphatase enzymes (Nakas *et al.*, 1987; Chrost, 1991; Muchhal *et al.*, 1996; Li *et al.*, 1997; Daram *et al.*, 1999; Kai *et al.*, 2002; Versaw and Harrison, 2002). PHA enzymes activity was high in soils inoculated with functional PSB + synthetic fungi. Soil microclimate, SOC and the availability of P in the soil governed phosphatase enzyme and involved in P-cycling (Hamman *et al.*, 2008).

GSA a good indicator of advanced changes in soil organic carbon (Dick, 1994; Wick *et al.*, 1998). Thus, become a good biochemical indicator for measuring ecological changes. In present studies show a decreasing trend of GSA in burnt soil throughout the rice growing session and a similar results of decrease in GSA activity after burning was reported by (Ajwa *et al.*, 1999; Sapalrinliana *et al.*, 2016 and Lungmuana *et al.*, 2017). Under the influence of rice plant GSA activity was found higher throughout the rice growing session. This could be that most dominant glucosidase i.e β -glucosidase was released to the soil largely by plants, animals, fungi and bacteria (Esen, 1993). The most predominant source of β -D-glucosidase activity in soils was reported to be Fungi (Hayano and Katami, 1977; Hayano and Tubaki, 1985). This enzyme activity play a fundamental role in release of labile carbon to microorganism as well as C cycling in large scale (Acosta *et al.*, 2007). GSA was found highest in soil inoculated with synthetic CDB + Fungi. CDB being involved in C-cycling this can be the result where highest GSA activity was found in soil inoculated with functional CDB + synthetic fungi.

The labile cellulose was broken down by β - glucosidase, which involves in plant cell tissues decomposition at the first phases. This cell wall decompositions activities trigger the other enzymes such as proteases and phosphatases (Turner *et al.*, 2000; Sardans *et al.*, 2008). Glucose as its final product it becomes an important C energy source to microbes in soil. (Esen, 1993). Its sensitivity to land management and soil pH was reported by (Dick *et al.*, 1996; Bergstrom *et al.*, 1998; Acosta-Martinez and Tabatabai, 2000; Ndiaye *et al.*, 2000) and also reflects the past biological activity.

The decrease of DHA in burnt soils was observed throughout the rice growing session in comparison with unburnt soils. Our present study was in harmony with the past findings of (Ajwa *et al.*, 1999; Wolińska and Stępniewska, 2012; Sapalrinliana *et al.*, 2016; Lungmuana *et al.*, 2017) who reported the decrease of DHA after burning. Polluted soil with fly ash has lower DHA activity (Pitchel and Hayes,

1990). DHA as an intracellular enzymes it has a close relationship with microbial activity and is often used as an indicator of microbial activity in soil (Skujins, 1976; Nannipieri *et al.*, 1990; Dick, 1994.). Both DHA and GSA activity patterns resembled the associated change in OC as a reflection of change in substrate availability for soil microbial community (Saha *et al.*, 2011; Gispert *et al.*, 2013).

Types and amount of organic matter content (Sarathchandra and Perrott, 1981) and change in soil pH (Acosta-Martínez and Tabatabai, 2000) contributes the reasons for change in ASA activity. Burning the biomass result in increase in soil pH which results to changes in ASA activity after burning and a similar change was also reported by (Vong *et al.*, 2003) Microbial biomass in different soil systems is often correlated to the rate of S immobilisation (Klose and Tabatabai, 1999; Vong *et al.*, 2003). With the introduction of rice crop ASA activity increases in comparison to bulk soil. This is due to the fact that where a crop is grown there was stress of available sulphur content in soil which results in increased secretion of sulphatase to cope up with the ecological demand thus results in higher ASA content in soils where rice crop was grown (Saplalrinliana *et al.*, 2016; Lungmuana *et al.*, 2017).

The possible causes of negative impact of burning on soil enzyme activities are: (1) depletion of hydrolytic enzyme pools due to breakdown of above- and below-ground community (2) sudden reduction in soil biota population and (3) nutrient enrichment in soils after burning reduce the dependency of crop plants on enzyme activities.

5.4. Burning, cropping, and microbial inoculation effect to soil biochemical properties

Soil biochemical properties get affected by burning activities. Our studies show that there was a decline in soil biochemical properties after burning. SOC, POM, MBC were gradually decreased during the cropping season. In first year of paddy cultivation by 17 to 31.3% the SOC decrease after burning in NE India (Ramakrishnan and Toky, 1981). The soil biochemical properties decreases throughout the cropping season by 6.1% after burning in Mizoram (Lungmuana *et al.*, 2017). Burning of slash biomass results in alteration of SOC content which was reduced by 10 to 22.3% in *jhum* agriculture as burning activities accelerated soil erosion process greatly (Hatten *et al.*, 2005; Choudhury *et al.*, 2015). The decrease in SOC, MBC content after burning reflects the immediate degradation of soil by burning and as POM is sensitive to land management its amount decrease in soil after burning

(Aumtong *et al.*, 2009). Our study reveals that soil biochemical activities were higher where rice crop was grown in comparison to bulk soil. Higher microbial biomass in the ecosystem was a result of higher OM input normally due to rhizodeposition (Shao *et al.*, 2015). Root mass, fine roots production from plants increased the accumulation of MBC, SOC become one of the major factors for increasing the soil microbial activity (Ajwa *et al.*, 1999). The MBC and MBN was reduced by 82 and 71% respectively in the 1st year after burning (Xiang *et al.*, 2014).

5.5. Relationship of soil properties with soil bacterial community

Affect of carbon pool by burning was reported by various scientists (Ramakrishnan and Toky, 1981; Aumtong *et al.*, 2009; Lungmuana *et al.*, 2017). Our studies confirm the past findings in which the PCA studies also reveals that the carbon pool had a significant role in separating out the burnt soils and unburnt soils. Mainly MBC and DHA act as the main role in separating the soils from burnt to unburnt soils. DHA as an intracellular enzymes it has a close relationship with microbial activity and is often used as an indicator of microbial activity in soil (Nannipieri *et al.*, 1990; Dick, 1994). Change in OC as a reflects changes in substrate availability for soil microbial community and DHA activity patterns resembled the associated changes in OC and substrate availability (Saha *et al.*, 2011; Gispert *et al.*, 2013). The relationship study of soil properties with bacterial community profile by super imposing soil data matrix on MDS plot shows that soil properties DHA, ASA, pMN, POM had significant role in separating bacterial communities in MDS axis 1 according to the presence and absence of rice at 10 days where MBC played significant role in separating burn and unburn soil. In case of Alpha proteobacteria at 45 and 90 days of rice growth soil properties like pMN, POM, DOC PHA, MBC had significant role in separating bacterial communities according to the presence and absence of rice where as DHA, DOC, ASA played significant role in separating burn and unburn soil and with Beta proteobacteria at 45 and 90 days of rice growth SOC, pMN, DOC PHA, and MBC had significant role in separating bacterial communities according to the presence and absence of rice where as MBC and DHA played significant role in separating burnt and unburnt soil. In slash and burn agriculture due to biomass burning the soil pH shows a gradual increase after burning and also change in organic matter content. This could be the results for ASA activity for separating the burnt and unburnt soils. This finding is in concurrent with the past finding of (Sarathchandra and Perrott, 1981, Acosta-Martínez and Tabatabai, 2000 and Vong *et al.* (2003) who state that ASA activity

results in types, amount of organic matter content and change in soil pH As ASA activity gets affected by change in soil pH.

Soil enzymes activities along with carbon pool played a significant role in separating out the bacterial community in presence and absence of rice plant. This could be due to that change in land management, soil and vegetation conditions the phosphatase activity in soil gets effected (Herbien and Neal, 1990; Speir and Cowling, 1991). With a limitations of available P for plant uptake the plant roots secrete phosphatase which enhance the solubilisation and remobilization of phosphate in soil. (Versaw and Harrison, 2002). DHA as an intracellular enzymes is often used as an indicator of microbial activity in soil (Skujins, 1976, 1984; Nannipieri *et al.*, 1990; Dick, 1994). Both DHA and GSA activity patterns resembled the associated change in OC as a reflection of change in the availability substrate for soil microbial community (Saha *et al.*, 2011; Gispert *et al.*, 2013).

Change in OC is the results of change in substrate availability which results in change in microbial activity. As the carbon pool are sensitive to land management burning activities results change in carbon pool. With the introduction of crops in shifting cultivation root mass, fine roots were produce from plants. The plants roots and fine roots release the exudates which results in selections of microbes in the root zone and with a monsoon shower the soil microbial activity in soils gradually increase. This could be the reason for which the soil carbon pool had a significant role in separating the soil bacterial community in presence and absence of rice crop. This findings was in concurrent with the past findings of sensitivity of carbon pool to land management (Aumtong *et al.*, 2009), increasement of rhizodeposition leads to higher microbial biomass (Shao *et al.*, 2015), increased the accumulation of MBC, SOC from plants root mass, fine roots as major influential factors towards the increased of microbial activity (Ajwa *et al.*, 1999).

Chapter-6

Summary and Conclusion

Soil is the power house for all the terrestrial ecosystems. For biogeochemical cycles it is an essential and through these cycles the soil functions. Both abiotic and biotic factors affect the soil functioning and its processes in soils. The major component of biological soil processes is soil enzymes activity. Soil enzymes play key role in decomposition of organic compounds, formation of organic matter in soil, their mineralization and recycling of nutrients. Due to different composition and activity of its living organisms, organic matter content and intensity of the biological processes, the enzymes level in soil vary. Understanding the relationship of soil enzymes activities, influence of crop plants to soil microbial community and its association to soil nutrient cycling processes has become one of ecology's primary goals in order to effectively maintain and sustain the functioning of an *jhum* agroecosystem. *Jhum* agroecosystem is the unique blending of forest ecology and agroecology in the sense that the fallow phase represents the secondary forest succession and rejuvenation of soils while the cropping phase represents the primitive forms of agricultural practices involving slashing and burning of above-ground forest biomass and breaking the functional linkages with the below-ground counterparts. As such above-ground and below-ground linkages in *jhum* agroecosystem can be influential mutual drivers, with both positive and negative impacts. Thus the impact assessment of *jhum* practices on soil biochemical processes as a reflection of the functioning of soil biota community and how the above-ground biological inputs like accumulated forest floor litters during fallow phase control over the functioning of soil biochemical processes were studied in this research.

The soil bacterial community composition altered significantly due to burning of slashed biomass on soil surface. The introduction of rice crop also altered the bacterial community composition in burnt/unburnt soil as evident from distinct cluster within the soil type. Though microbial inoculants treatment had impact on soil bacterial community composition, but such effect were obscured due to burn and crop factor. The Alpha proteobacterial communities of burnt and unburnt without rice crop clustered together and distinctly separated from the clusters of burnt and unburnt soil with rice crop at 45 days of rice growth. At 90 days of rice growth the impact of rice crop on composition of Alpha and beta proteobacterial community was more permanent in burnt soil than in unburnt soil. In absence of rice crop the beta

proteobacterial community of burnt and unburnt soil cluster together and there cluster was distinctly separated from the cluster of burnt and unburnt soil with rice crop at 45 days of rice growth. With the progress of time (90 days of rice growth) the effect of rice crop on beta proteobacterial community was more prominent than compared to burnt unburnt factor.

Burning had significant negative effect on the activity of enzymes such as DHA, GSA, PHA except ASA indicating higher activity in burnt soil. Introduction of rice crop had significant positive influenced on the activity of soil enzymes. There was significant positive interaction on burning and cropping on soil enzymes activities. There was a significant difference in the activity of soil enzymes among the microbial inoculants treatment. There was significant positive interaction between burnt and microbial inoculants or cropping and microbial inoculants. Burning had significant negative effect on SOC, pMN, POM, and MBC. Introduction of rice crop had significant positive influence on the activity on soil process indicators. There was significant positive interaction on burning and cropping on soil process indicators. There was significant differences in the activity of soil process indicators among the Microbial inoculants treatment. There was significant positive interaction between burnt and microbial inoculants or cropping and microbial inoculants

The change in bacterial community composition due to burning and cropping factor significantly influence soil process indicator like ASA, DHA, pMN, POM, and MBC as evident from their significant correlation with MDS 2. The change in alpha and beta proteobacterial community composition due to burning and cropping factor significantly influence soil process indicator like PHA, ASA, DHA, pMN, POM, SOC and MBC as evident from their significant correlation with MDS 1 and 2.

Burning of slash biomass on surface soil can altered soil bacterial community composition. The influence of rice crop on composition of soil bacterial community was more prominent in burnt soil than unburnt soil. The clustering behavior of soil bacterial community indicated that the greater influencing factors in shaping community composition is in order of burning > cropping > microbial inoculation. However, cropping seems to be greater influencing factor than burning in case of β -proteobacterial community. Burning had significant positive influence on rice growth yield. Therefore, it can be concluded that introduction of crop in burn soil along with microbial inoculation may positively influence soil processes as well as crop growth.

This research showed through pot experiment and laboratory analysis that burning of vegetative biomass does not change alpha and beta proto-bacterial

diversity; however cultivation of rice in the burnt soil cause a significant alteration of bacterial community. Perhaps, the gradual decomposition of the left over unburnt biomass through decomposition brings conspicuous change in soil microbial community upto 45 days of crop growth, as a result the influence of rice plant/ root activity is not seen conspicuously in the bacterial community. This is also reflected in different soil enzymes activities of the two systems i.e., burnt and unburnt. The influence of factor such as biomass burning, cropping and microbial inoculation in shaping microbial community composition is shown as burning cropping microbial inoculation. Thus in a burnt field cropping phase brings a positive change in microbial community and soil process and application of consortia of beneficial bacteria might help in enhcancing crop peoductivity. Such mechanistic studies may be directed in *jhum* system of varying fallow cycle in future research.

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Annexure 4.1. ASA Factorial ANOVA to test the significance of factors at 10 days

Tests of Between-Subjects Effects					
Dependent Variable:	ASA				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	39427.162 ^a	23	1714.224	133.110	.000
Intercept	573653.312	1	573653.312	44544.481	.000
J	16617.678	1	16617.678	1290.371	.000
C	153.265	1	153.265	11.901	.001
MI	2994.840	5	598.968	46.510	.000
J * C	2375.325	5	475.065	36.889	.000
J * MI	4337.312	1	4337.312	336.795	.000
C * MI	6767.468	5	1353.494	105.099	.000
J * C * MI	6181.274	5	1236.255	95.996	.000
Error	618.154	48	12.878		
Total	613698.628	72			
Corrected Total	40045.317	71			

a. R Squared = .985 (Adjusted R Squared = .977)

	C*J		C*MI		J*MI		C*J*MI
C1J1	83.289	C1MI1	99.712	J1MI1	76.366	C1J1MI1	87.017
C1J2	98.150	C1MI2	89.094	J1MI2	112.439	C1J1MI2	112.407
C2J1	64.848	C1MI3	81.932	J1MI3	73.869	C1J1MI3	65.716
C2J2	110.755	C1MI4	88.371	J1MI4	96.433	C1J1MI4	112.472
		C1MI5	98.630	J1MI5	73.220	C1J1MI5	86.402
		C1MI6	73.525	J1MI6	98.936	C1J1MI6	77.461
		C2MI1	94.895	J2MI1	74.717	C1J2MI1	61.336
		C2MI2	72.497	J2MI2	92.675	C1J2MI2	115.405
		C2MI3	85.412	J2MI3	74.854	C1J2MI3	82.699
		C2MI4	117.278	J2MI4	127.837	C1J2MI4	114.561
		C2MI5	83.736	J2MI5	71.383	C1J2MI5	63.740
		C2MI6	86.043	J2MI6	98.395	C1J2MI6	83.311
						C2J1MI1	86.155
						C2J1MI2	103.635
						C2J1MI3	63.279
						C2J1MI4	81.715
						C2J1MI5	82.814
						C2J1MI6	88.010
						C2J2MI1	66.894
						C2J2MI2	167.663
						C2J2MI3	74.645
						C2J2MI4	92.826
						C2J2MI5	68.122
						C2J2MI6	103.964

Annexure 4.2. Factorial ANOVA to test the significance of factors at 10 days of rice plant growth

Tests of Between-Subjects Effects							
Dependent Variable:	DHA						
Source	Type III SS	df	Mean Square	F	Sig.		
Corrected Model	17.862 ^a	23	.777	138.615	.000		
Intercept	64.878	1	64.878	11579.789	.000		
J	13.760	1	13.760	2455.949	.000		
C	.145	1	.145	25.869	.000		
MI	2.435	5	.487	86.919	.000		
J * C	.005	1	.005	.828	.367		
J * MI	.941	5	.188	33.604	.000		
C * MI	.261	5	.052	9.332	.000		
J * C * MI	.315	5	.063	11.245	.000		
Error	.269	48	.006				
Total	83.009	72					
Corrected Total	18.131	71					
a. R Squared = .985 (Adjusted R Squared = .978)							
	C*J		C*MI		J*MI		C*J*MI
C1J1	.459	C1MI1	1.033	J1MI1	.755	C1J1MI1	.654
C1J2	1.350	C1MI2	1.189	J1MI2	.827	C1J1MI2	.845
C2J1	.565	C1MI3	1.085	J1MI3	.615	C1J1MI3	.500
C2J2	1.423	C1MI4	.890	J1MI4	.588	C1J1MI4	.647
		C1MI5	.657	J1MI5	.110	C1J1MI5	.070
		C1MI6	.573	J1MI6	.177	C1J1MI6	.040
		C2MI1	1.217	J2MI1	1.495	C1J2MI1	1.412
		C2MI2	1.173	J2MI2	1.534	C1J2MI2	1.533
		C2MI3	1.027	J2MI3	1.497	C1J2MI3	1.670
		C2MI4	.883	J2MI4	1.185	C1J2MI4	1.134
		C2MI5	.839	J2MI5	1.386	C1J2MI5	1.243
		C2MI6	.826	J2MI6	1.222	C1J2MI6	1.105
						C2J1MI1	.857
						C2J1MI2	.810
						C2J1MI3	.730
						C2J1MI4	.530
						C2J1MI5	.150
						C2J1MI6	.313
						C2J2MI1	1.578
						C2J2MI2	1.535
						C2J2MI3	1.324
						C2J2MI4	1.236
						C2J2MI5	1.528
						C2J2MI6	1.339

Annexure 4.3 . Factorial ANOVA to test the significance of factors at 10 days of rice plant growth

Tests of Between-Subjects Effects					
Dependent Variable:	GSA				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	1038187.437 ^a	23	45138.584	13734.848	.000
Intercept	10102469.798	1	10102469.798	3073997.372	.000
C	5577.317	1	5577.317	1697.076	.001
J	81430.673	1	81430.673	24777.869	.100
MI	192578.185	5	38515.637	11719.606	.000
C * J	403667.309	5	80733.462	24565.721	.000
C * MI	176519.053	5	35303.811	10742.306	.000
J * MI	77201.380	1	77201.380	23490.972	.000
C * J * MI	101213.520	5	20242.704	6159.486	.000
Error	157.749	48	3.286		
Total	11140814.983	72			
Corrected Total	955661.657	71			

a. R Squared = .922 (Adjusted R Squared = .884)

	C*J		C*MI		J*MI		C*J*MI
C1J1	364.896	C1MI1	331.954	J1MI1	318.381	C1J1MI1	212.037
C1J2	317.009	C1MI2	307.631	J1MI2	326.904	C1J1MI2	285.793
C2J1	366.666	C1MI3	383.337	J1MI3	398.698	C1J1MI3	336.277
C2J2	449.759	C1MI4	327.526	J1MI4	312.438	C1J1MI4	408.396
		C1MI5	380.780	J1MI5	445.218	C1J1MI5	558.975
		C1MI6	314.487	J1MI6	393.049	C1J1MI6	387.899
		C2MI1	524.196	J2MI1	537.769	C1J2MI1	451.871
		C2MI2	461.592	J2MI2	442.319	C1J2MI2	329.470
		C2MI3	521.631	J2MI3	506.270	C1J2MI3	430.396
		C2MI4	311.117	J2MI4	326.205	C1J2MI4	246.656
		C2MI5	304.665	J2MI5	240.227	C1J2MI5	202.585
		C2MI6	326.075	J2MI6	247.513	C1J2MI6	241.074
						C2J1MI1	424.724
						C2J1MI2	368.015
						C2J1MI3	461.120
						C2J1MI4	216.479
						C2J1MI5	331.461
						C2J1MI6	398.198
						C2J2MI1	623.668
						C2J2MI2	555.168
						C2J2MI3	582.143
						C2J2MI4	405.755
						C2J2MI5	277.869
						C2J2MI6	253.952

Annexure 4.4. Factorial ANOVA to test the significance of factors at 10 days growth stage of rice Plant

Tests of Between-Subjects Effects					
Dependent Variable:	PHA				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	976372.881 ^a	23	42450.995	20481.787	.000
Intercept	24749250.354	1	24749250.354	11941036.185	.000
J	48270.603	1	48270.603	23289.635	.000
C	14866.365	1	14866.365	7172.734	.000
MI	473112.176	5	94622.435	45653.501	.000
J * C	135732.631	5	27146.526	13097.676	.000
J * MI	43074.885	5	8614.977	4156.560	.000
C * MI	159378.626	1	159378.626	76897.115	.000
J * C * MI	101937.596	5	20387.519	9836.585	.000
Error	99.486	48	2.073		
Total	25725722.721	72			
Corrected Total	976472.367	71			

a. R Squared = 1.000 (Adjusted R Squared = 1.000)

	C*J		C*MI		J*MI		C*J*MI
C1J1	498.982	C1MI1	627.179	J1MI1	612.326	C1J1MI1	528.083
C1J2	644.865	C1MI2	594.284	J1MI2	574.053	C1J1MI2	501.647
C2J1	621.819	C1MI3	743.778	J1MI3	601.581	C1J1MI3	525.253
C2J2	579.506	C1MI4	509.002	J1MI4	548.420	C1J1MI4	504.410
		C1MI5	512.150	J1MI5	563.502	C1J1MI5	482.029
		C1MI6	445.150	J1MI6	462.521	C1J1MI6	452.471
		C2MI1	664.941	J2MI1	679.793	C1J2MI1	726.274
		C2MI2	659.180	J2MI2	679.412	C1J2MI2	686.921
		C2MI3	667.217	J2MI3	809.415	C1J2MI3	962.304
		C2MI4	550.445	J2MI4	511.027	C1J2MI4	513.593
		C2MI5	581.859	J2MI5	530.507	C1J2MI5	542.270
		C2MI6	480.332	J2MI6	462.960	C1J2MI6	437.828
						C2J1MI1	696.569
						C2J1MI2	646.458
						C2J1MI3	677.909
						C2J1MI4	592.430
						C2J1MI5	644.975
						C2J1MI6	472.571
						C2J2MI1	633.312
						C2J2MI2	671.902
						C2J2MI3	656.526
						C2J2MI4	508.460
						C2J2MI5	518.744
						C2J2MI6	488.093

Annexure 4.5: ASA Factorial ANOVA to test the significance of factors at 45 days

Tests of Between-Subjects Effects							
Dependent Variable:	ASA						
Source	Type III SS	df	Mean Square	F	Sig.		
Corrected Model	46459.539 ^a	23	2019.980	26.398	.000		
Intercept	945454.019	1	945454.019	12355.484	.000		
J	1031.631	1	1031.631	13.482	.001		
C	19799.873	1	19799.873	258.751	.000		
MI	9030.957	5	1806.191	23.604	.000		
J * C	2466.684	1	2466.684	32.235	.000		
J * MI	6605.108	5	1321.022	17.264	.000		
C * MI	3153.283	5	630.657	8.242	.000		
J * C * MI	4372.004	5	874.401	11.427	.000		
Error	3673.008	48	76.521				
Total	995586.567	72					
Corrected Total	50132.547	71					
a. R Squared = .927 (Adjusted R Squared = .892)							
	C*J		C*MI		J*MI		C*J*MI
C1J1	121.537	C1MI1	137.528	J1MI1	115.810	C1J1MI1	140.872
C1J2	140.813	C1MI2	131.700	J1MI2	108.469	C1J1MI2	128.970
C2J1	100.077	C1MI3	134.607	J1MI3	120.100	C1J1MI3	110.316
C2J2	95.941	C1MI4	129.704	J1MI4	131.292	C1J1MI4	137.079
		C1MI5	144.688	J1MI5	107.834	C1J1MI5	114.408
		C1MI6	108.824	J1MI6	81.333	C1J1MI6	97.575
		C2MI1	95.322	J2MI1	117.040	C1J2MI1	134.184
		C2MI2	87.751	J2MI2	110.981	C1J2MI2	134.429
		C2MI3	125.650	J2MI3	140.156	C1J2MI3	158.898
		C2MI4	103.772	J2MI4	102.184	C1J2MI4	122.329
		C2MI5	97.132	J2MI5	133.986	C1J2MI5	174.967
		C2MI6	78.425	J2MI6	105.916	C1J2MI6	120.073
						C2J1MI1	90.749
						C2J1MI2	87.969
						C2J1MI3	129.885
						C2J1MI4	125.506
						C2J1MI5	101.261
						C2J1MI6	65.092
						C2J2MI1	99.896
						C2J2MI2	87.534
						C2J2MI3	121.415
						C2J2MI4	82.038
						C2J2MI5	93.004
						C2J2MI6	91.759

Annexure 4.6. DHA factorial ANOVA to test the significance of factors at 45 days

Tests of Between-Subjects Effects							
Dependent Variable:	DHA						
Source	Type III SS	df	Mean Square	F	Sig.		
Corrected Model	2254.460 ^a	23	98.020	65.906	.000		
Intercept	10726.068	1	10726.068	7211.956	.000		
J	1094.338	1	1094.338	735.807	.000		
C	375.041	1	375.041	252.169	.000		
MI	329.313	5	65.863	44.284	.000		
J * C	244.727	5	48.945	32.910	.000		
J * MI	37.703	5	7.541	5.070	.001		
C * MI	11.643	1	11.643	7.829	.007		
J * C * MI	161.696	5	32.339	21.744	.000		
Error	71.389	48	1.487				
Total	13051.917	72					
Corrected Total	2325.849	71					
a. R Squared = .969 (Adjusted R Squared = .955)							
	C*J		C*MI		J*MI		C*J*MI
C1J1	10.187	C1MI1	15.656	J1MI1	8.437	C1J1MI1	12.603
C1J2	18.789	C1MI2	16.929	J1MI2	8.944	C1J1MI2	9.496
C2J1	6.427	C1MI3	18.709	J1MI3	8.496	C1J1MI3	9.238
C2J2	13.420	C1MI4	12.535	J1MI4	8.913	C1J1MI4	12.029
		C1MI5	11.276	J1MI5	7.570	C1J1MI5	7.926
		C1MI6	11.821	J1MI6	7.482	C1J1MI6	9.831
		C2MI1	9.384	J2MI1	16.603	C1J2MI1	18.708
		C2MI2	11.368	J2MI2	19.354	C1J2MI2	24.363
		C2MI3	12.662	J2MI3	22.876	C1J2MI3	28.181
		C2MI4	9.183	J2MI4	12.805	C1J2MI4	13.041
		C2MI5	8.732	J2MI5	12.438	C1J2MI5	14.627
		C2MI6	8.209	J2MI6	12.549	C1J2MI6	13.812
						C2J1MI1	4.270
						C2J1MI2	8.392
						C2J1MI3	7.754
						C2J1MI4	5.796
						C2J1MI5	7.215
						C2J1MI6	5.133
						C2J2MI1	14.498
						C2J2MI2	14.345
						C2J2MI3	17.570
						C2J2MI4	12.570
						C2J2MI5	10.249
						C2J2MI6	11.286

Annexure 4.7. Factorial ANOVA to test the significance of factors at 45 days of rice plant

Tests of Between-Subjects Effects							
Dependent Variable:	GSA						
Source	Type III SS	df	Mean Square	F	Sig.		
Corrected Model	879833.841 ^a	23	38253.645	8354.338	.000		
Intercept	7092739.452	1	7092739.452	1549006.388	.000		
J	4339.782	1	4339.782	947.779	.000		
C	20968.002	1	20968.002	4579.270	.000		
MI	272230.459	5	54446.092	11890.659	.000		
J * C	71399.992	1	71399.992	15593.276	.000		
J * MI	204323.936	5	40864.787	8924.594	.000		
C * MI	133090.370	5	26618.074	5813.208	.000		
J * C * MI	173481.301	5	34696.260	7577.429	.000		
Error	219.787	48	4.579				
Total	7972793.080	72					
Corrected Total	880053.628	71					
a. R Squared = 1.000 (Adjusted R Squared = 1.000)							
	C*J	C*MI	J*MI	C*J*MI			
C1J1	336.053	C1MI1	230.607	J1MI1	359.430	C1J1MI1	294.417
C1J2	257.544	C1MI2	295.732	J1MI2	187.950	C1J1MI2	257.392
C2J1	307.202	C1MI3	402.460	J1MI3	424.643	C1J1MI3	507.041
C2J2	354.656	C1MI4	346.112	J1MI4	309.929	C1J1MI4	394.416
		C1MI5	235.638	J1MI5	395.647	C1J1MI5	303.106
		C1MI6	270.241	J1MI6	252.163	C1J1MI6	259.943
		C2MI1	366.079	J2MI1	237.256	C1J2MI1	166.796
		C2MI2	224.514	J2MI2	332.296	C1J2MI2	334.071
		C2MI3	464.167	J2MI3	441.984	C1J2MI3	297.878
		C2MI4	328.824	J2MI4	365.007	C1J2MI4	297.808
		C2MI5	381.679	J2MI5	221.670	C1J2MI5	168.170
		C2MI6	220.308	J2MI6	238.386	C1J2MI6	280.540
						C2J1MI1	424.444
						C2J1MI2	118.508
						C2J1MI3	342.245
						C2J1MI4	225.442
						C2J1MI5	488.188
						C2J1MI6	244.384
						C2J2MI1	307.715
						C2J2MI2	330.521
						C2J2MI3	586.089
						C2J2MI4	432.207
						C2J2MI5	275.170
						C2J2MI6	196.232

Annexure 4.8. Factorial ANOVA to test the significance of factors at 45 days of rice Plant

Tests of Between-Subjects Effects					
Dependent Variable:	PHA				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	578531.860 ^a	23	25153.559	13809.731	.000
Intercept	18989196.940	1	18989196.940	10425391.292	.000
J	9143.762	1	9143.762	5020.080	.000
C	3540.037	1	3540.037	1943.541	.000
MI	111556.868	5	22311.374	12249.323	.000
J * C	37892.178	5	7578.436	4160.690	.000
J * MI	120203.350	5	24040.670	13198.736	.000
C * MI	263077.036	1	263077.036	144433.756	.000
J * C * MI	33118.628	5	6623.726	3636.538	.000
Error	87.429	48	1.821		
Total	19567816.229	72			
Corrected Total	578619.289	71			

a. R Squared = 1.000 (Adjusted R Squared = 1.000)

	C*J		C*MI		J*MI		C*J*MI
C1J1	471.389	C1MI1	544.369	J1MI1	573.710	C1J1MI1	472.906
C1J2	569.745	C1MI2	559.944	J1MI2	501.355	C1J1MI2	514.234
C2J1	578.260	C1MI3	513.359	J1MI3	538.151	C1J1MI3	462.010
C2J2	434.827	C1MI4	510.900	J1MI4	495.873	C1J1MI4	484.077
		C1MI5	492.116	J1MI5	530.013	C1J1MI5	457.407
		C1MI6	502.715	J1MI6	509.845	C1J1MI6	437.702
		C2MI1	575.223	J2MI1	545.882	C1J2MI1	615.832
		C2MI2	462.588	J2MI2	521.177	C1J2MI2	605.654
		C2MI3	573.431	J2MI3	548.639	C1J2MI3	564.708
		C2MI4	361.678	J2MI4	376.705	C1J2MI4	537.723
		C2MI5	562.403	J2MI5	524.507	C1J2MI5	526.825
		C2MI6	503.935	J2MI6	496.805	C1J2MI6	567.727
						C2J1MI1	674.515
						C2J1MI2	488.475
						C2J1MI3	614.292
						C2J1MI4	507.669
						C2J1MI5	602.618
						C2J1MI6	581.988
						C2J2MI1	475.932
						C2J2MI2	436.701
						C2J2MI3	532.571
						C2J2MI4	215.688
						C2J2MI5	522.188
						C2J2MI6	425.882

Annexure 4.9. ASA Factorial ANOVA to test the significance of factors at 90 days of rice plant

Tests of Between-Subjects Effects							
Dependent Variable:	ASA						
Source	Type III SS	df	Mean Square	F	Sig.		
Corrected Model	78019.658 ^a	23	3392.159	382.665	.000		
Intercept	405353.632	1	405353.632	45727.431	.000		
J	53691.026	1	53691.026	6056.817	.000		
C	13436.847	1	13436.847	1515.794	.000		
MI	1676.564	5	335.313	37.826	.000		
J * C	2172.470	1	2172.470	245.074	.000		
C * MI	2638.540	5	527.708	59.530	.000		
J * MI	1661.840	5	332.368	37.494	.000		
J * C * MI	2742.372	5	548.474	61.873	.000		
Error	425.499	48	8.865				
Total	483798.789	72					
Corrected Total	78445.157	71					
a. R Squared = .995 (Adjusted R Squared = .992)							
	C*J		C*MI		J*MI		C*J*MI
C1J1	55.893	C1MI1	77.281	J1MI1	39.766	C1J1MI1	46.953
C1J2	121.494	C1MI2	93.159	J1MI2	51.407	C1J1MI2	64.791
C2J1	39.557	C1MI3	102.828	J1MI3	43.731	C1J1MI3	62.471
C2J2	83.186	C1MI4	84.782	J1MI4	41.621	C1J1MI4	48.611
		C1MI5	83.714	J1MI5	52.673	C1J1MI5	58.259
		C1MI6	90.398	J1MI6	57.152	C1J1MI6	54.274
		C2MI1	55.268	J2MI1	92.783	C1J2MI1	107.609
		C2MI2	70.802	J2MI2	112.554	C1J2MI2	121.527
		C2MI3	50.595	J2MI3	109.692	C1J2MI3	143.185
		C2MI4	59.924	J2MI4	103.085	C1J2MI4	120.954
		C2MI5	70.415	J2MI5	101.456	C1J2MI5	109.169
		C2MI6	61.226	J2MI6	94.473	C1J2MI6	126.522
						C2J1MI1	32.579
						C2J1MI2	38.024
						C2J1MI3	24.992
						C2J1MI4	34.631
						C2J1MI5	47.087
						C2J1MI6	60.029
						C2J2MI1	77.957
						C2J2MI2	103.581
						C2J2MI3	76.199
						C2J2MI4	85.216
						C2J2MI5	93.742
						C2J2MI6	62.423

Annexure 4.10. Factorial ANOVA to test the significance of factors at 90 days of rice plant

Tests of Between-Subjects Effects					
Dependent Variable:	DHA				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	3468.161 ^a	23	150.790	110.276	.000
Intercept	15671.129	1	15671.129	11460.678	.000
J	1216.301	1	1216.301	889.511	.000
C	745.478	1	745.478	545.186	.000
MI	1054.789	5	210.958	154.279	.000
J * C	261.629	5	52.326	38.267	.000
J * MI	124.850	5	24.970	18.261	.000
C * MI	40.710	1	40.710	29.772	.000
J * C * MI	24.403	5	4.881	3.569	.008
Error	65.634	48	1.367		
Total	19204.924	72			
Corrected Total	3533.795	71			

a. R Squared = .981 (Adjusted R Squared = .973)

	C*J		C*MI		J*MI		C*J*MI
C1J1	13.109	C1MI1	18.905	J1MI1	11.351	C1J1MI1	14.380
C1J2	22.833	C1MI2	18.544	J1MI2	11.911	C1J1MI2	13.031
C2J1	8.177	C1MI3	21.733	J1MI3	14.462	C1J1MI3	18.194
C2J2	14.894	C1MI4	24.381	J1MI4	11.447	C1J1MI4	15.531
		C1MI5	14.043	J1MI5	7.786	C1J1MI5	9.675
		C1MI6	10.219	J1MI6	6.901	C1J1MI6	7.842
		C2MI1	13.382	J2MI1	20.936	C1J2MI1	23.430
		C2MI2	15.068	J2MI2	21.701	C1J2MI2	24.057
		C2MI3	13.297	J2MI3	20.568	C1J2MI3	25.271
		C2MI4	13.512	J2MI4	26.445	C1J2MI4	33.230
		C2MI5	7.293	J2MI5	13.551	C1J2MI5	18.412
		C2MI6	6.660	J2MI6	9.979	C1J2MI6	12.597
						C2J1MI1	8.321
						C2J1MI2	10.792
						C2J1MI3	10.730
						C2J1MI4	7.363
						C2J1MI5	5.897
						C2J1MI6	5.961
						C2J2MI1	18.442
						C2J2MI2	19.344
						C2J2MI3	15.865
						C2J2MI4	19.660
						C2J2MI5	8.690
						C2J2MI6	7.360

Annexure 4.11. Factorial ANOVA to test the significance of factors at 90 days of rice plant

Tests of Between-Subjects Effects					
Dependent Variable:	GSA				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	931088.993 ^a	23	40482.130	15302.017	.000
Intercept	10843347.884	1	10843347.884	4098724.347	.000
J	5135.854	1	5135.854	1941.324	.000
C	20797.441	1	20797.441	7861.316	.000
MI	333728.872	5	66745.774	25229.526	.000
J * MI	85709.132	5	17141.826	6479.514	.000
J * C	182736.826	1	182736.826	69073.490	.000
C * MI	144983.261	5	28996.652	10960.571	.000
J * C * MI	157997.608	5	31599.522	11944.441	.000
Error	126.986	48	2.646		
Total	11774563.864	72			
Corrected Total	931215.979	71			

a. R Squared = 1.000 (Adjusted R Squared = 1.000)

	C*J		C*MI		J*MI		C*J*MI
C1J1	447.003	C1MI1	483.108	J1MI1	441.884	C1J1MI1	490.932
C1J2	363.138	C1MI2	386.677	J1MI2	345.349	C1J1MI2	423.756
C2J1	312.255	C1MI3	352.995	J1MI3	347.798	C1J1MI3	403.820
C2J2	429.904	C1MI4	368.292	J1MI4	265.280	C1J1MI4	367.794
		C1MI5	404.407	J1MI5	437.440	C1J1MI5	473.853
		C1MI6	434.945	J1MI6	440.023	C1J1MI6	521.865
		C2MI1	369.819	J2MI1	411.043	C1J2MI1	475.284
		C2MI2	304.614	J2MI2	345.942	C1J2MI2	349.598
		C2MI3	255.571	J2MI3	260.767	C1J2MI3	302.169
		C2MI4	273.665	J2MI4	376.676	C1J2MI4	368.790
		C2MI5	481.679	J2MI5	448.646	C1J2MI5	334.960
		C2MI6	541.128	J2MI6	536.050	C1J2MI6	348.025
						C2J1MI1	392.836
						C2J1MI2	266.942
						C2J1MI3	291.776
						C2J1MI4	162.767
						C2J1MI5	401.026
						C2J1MI6	358.181
						C2J2MI1	346.802
						C2J2MI2	342.286
						C2J2MI3	219.366
						C2J2MI4	384.563
						C2J2MI5	562.331
						C2J2MI6	724.074

Annexure 4.12. Factorial ANOVA to test the significance of factors at 90 days of rice plant

Tests of Between-Subjects Effects					
Dependent Variable:	PHA				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	893521.208 ^a	23	38848.748	3002.159	.000
Intercept	12495565.282	1	12495565.282	965633.910	.000
J	466749.371	1	466749.371	36069.518	.000
C	13619.452	1	13619.452	1052.486	.000
MI	62480.850	5	12496.170	965.681	.000
J * C	54833.532	5	10966.706	847.487	.000
J * MI	195152.865	5	39030.573	3016.210	.000
C * MI	83170.353	1	83170.353	6427.249	.000
J * C * MI	17514.783	5	3502.957	270.702	.000
Error	621.133	48	12.940		
Total	13389707.623	72			
Corrected Total	894142.341	71			

a. R Squared =.999 (Adjusted R Squared =.999)

	C*J		C*MI		J*MI		C*J*MI
C1J1	288.337	C1MI1	468.929	J1MI1	304.441	C1J1MI1	332.693
C1J2	517.341	C1MI2	428.780	J1MI2	271.641	C1J1MI2	315.700
C2J1	383.819	C1MI3	412.208	J1MI3	426.677	C1J1MI3	315.903
C2J2	476.874	C1MI4	399.651	J1MI4	316.844	C1J1MI4	245.569
		C1MI5	345.249	J1MI5	341.793	C1J1MI5	261.943
		C1MI6	362.219	J1MI6	355.073	C1J1MI6	258.215
		C2MI1	371.933	J2MI1	536.421	C1J2MI1	605.165
		C2MI2	296.434	J2MI2	453.573	C1J2MI2	541.860
		C2MI3	510.419	J2MI3	495.950	C1J2MI3	508.512
		C2MI4	448.377	J2MI4	531.185	C1J2MI4	553.733
		C2MI5	464.939	J2MI5	468.395	C1J2MI5	428.554
		C2MI6	489.975	J2MI6	497.121	C1J2MI6	466.224
						C2J1MI1	276.189
						C2J1MI2	227.582
						C2J1MI3	537.450
						C2J1MI4	388.118
						C2J1MI5	421.643
						C2J1MI6	451.930
						C2J2MI1	467.677
						C2J2MI2	365.286
						C2J2MI3	483.387
						C2J2MI4	508.637
						C2J2MI5	508.235
						C2J2MI6	528.019

Annexure 4.13.ASA. Factorial ANOVA to test the significance of factors at 120 days of rice plant

Tests of Between-Subjects Effects							
Dependent Variable:	ASA						
Source	Type III SS	df	Mean Square	F	Sig.		
Corrected Model	24874.509 ^a	23	1081.500	72.587	.000		
Intercept	403225.345	1	403225.345	27063.227	.000		
J	3244.465	1	3244.465	217.758	.000		
C	9015.308	1	9015.308	605.079	.000		
MI	3646.446	5	729.289	48.948	.000		
J * C	2245.626	5	449.125	30.144	.000		
J * MI	1043.446	5	208.689	14.007	.000		
C * MI	3644.318	1	3644.318	244.595	.000		
J * C * MI	2034.899	5	406.980	27.315	.000		
Error	715.170	48	14.899				
Total	428815.025	72					
Corrected Total	25589.679	71					
a. R Squared = .972 (Adjusted R Squared = .959)							
	C*J	C*MI	J*MI	C*J*MI			
C1J1	72.198	C1MI1	71.415	J1MI1	47.245	C1J1MI1	49.288
C1J2	99.853	C1MI2	91.260	J1MI2	70.690	C1J1MI2	79.529
C2J1	64.047	C1MI3	95.201	J1MI3	80.376	C1J1MI3	75.522
C2J2	63.244	C1MI4	89.787	J1MI4	78.834	C1J1MI4	84.201
		C1MI5	81.837	J1MI5	67.385	C1J1MI5	67.750
		C1MI6	86.653	J1MI6	64.206	C1J1MI6	76.897
		C2MI1	49.356	J2MI1	73.525	C1J2MI1	93.541
		C2MI2	61.656	J2MI2	82.226	C1J2MI2	102.991
		C2MI3	72.096	J2MI3	86.921	C1J2MI3	114.879
		C2MI4	61.241	J2MI4	72.193	C1J2MI4	95.372
		C2MI5	75.350	J2MI5	89.802	C1J2MI5	95.923
		C2MI6	62.175	J2MI6	84.623	C1J2MI6	96.410
						C2J1MI1	45.201
						C2J1MI2	61.851
						C2J1MI3	85.230
						C2J1MI4	73.467
						C2J1MI5	67.020
						C2J1MI6	51.514
						C2J2MI1	53.510
						C2J2MI2	61.461
						C2J2MI3	58.962
						C2J2MI4	49.015
						C2J2MI5	83.680
						C2J2MI6	72.836

Annexure 4.14. DHA factorial ANOVA to test the significance of factors at 120 days of rice plant

Tests of Between-Subjects Effects							
Dependent Variable:	DHA						
Source	Type III SS	df	Mean Square	F	Sig.		
Corrected Model	3917.031 ^a	23	170.306	149.255	.000		
Intercept	13942.756	1	13942.756	12219.318	.000		
J	2986.542	1	2986.542	2617.381	.000		
C	359.823	1	359.823	315.346	.000		
MI	108.772	5	21.754	19.065	.000		
J * C	72.256	5	14.451	12.665	.000		
J * MI	315.244	1	315.244	276.277	.000		
C * MI	38.024	5	7.605	6.665	.000		
J * C * MI	36.372	5	7.274	6.375	.000		
Error	54.770	48	1.141				
Total	17914.558	72					
Corrected Total	3971.801	71					
a. R Squared = .986 (Adjusted R Squared = .980)							
	C*J		C*MI		J*MI		C*J*MI
C1J1	11.803	C1MI1	17.601	J1MI1	7.913	C1J1MI1	10.875
C1J2	20.499	C1MI2	17.118	J1MI2	7.585	C1J1MI2	11.219
C2J1	3.147	C1MI3	16.318	J1MI3	7.333	C1J1MI3	12.466
C2J2	20.213	C1MI4	15.235	J1MI4	7.738	C1J1MI4	12.316
		C1MI5	16.681	J1MI5	7.159	C1J1MI5	12.015
		C1MI6	13.955	J1MI6	7.124	C1J1MI6	11.930
		C2MI1	13.954	J2MI1	23.642	C1J2MI1	24.328
		C2MI2	13.418	J2MI2	22.951	C1J2MI2	23.017
		C2MI3	10.070	J2MI3	19.055	C1J2MI3	20.170
		C2MI4	12.136	J2MI4	19.633	C1J2MI4	18.154
		C2MI5	10.018	J2MI5	19.539	C1J2MI5	21.347
		C2MI6	10.485	J2MI6	17.317	C1J2MI6	15.981
						C2J1MI1	4.951
						C2J1MI2	3.951
						C2J1MI3	2.200
						C2J1MI4	3.159
						C2J1MI5	2.304
						C2J1MI6	2.319
						C2J2MI1	22.957
						C2J2MI2	22.886
						C2J2MI3	17.940
						C2J2MI4	21.112
						C2J2MI5	17.732
						C2J2MI6	18.652

Annexure 4.15. Factorial ANOVA to test the significance of factors at 120 days of rice plant

Tests of Between-Subjects Effects					
Dependent Variable:	ASA				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	46459.539 ^a	23	2019.980	26.398	.000
Intercept	945454.019	1	945454.019	12355.484	.000
J	1031.631	1	1031.631	13.482	.001
C	19799.873	1	19799.873	258.751	.000
MI	9030.957	5	1806.191	23.604	.000
J * C	2466.684	1	2466.684	32.235	.000
J * MI	6605.108	5	1321.022	17.264	.000
C * MI	3153.283	5	630.657	8.242	.000
J * C * MI	4372.004	5	874.401	11.427	.000
Error	3673.008	48	76.521		
Total	995586.567	72			
Corrected Total	50132.547	71			
a. R Squared = .927 (Adjusted R Squared = .892)					

	C*J		C*MI		J*MI		C*J*MI
C1J1	121.537	C1MI1	137.528	J1MI1	115.810	C1J1MI1	140.872
C1J2	140.813	C1MI2	131.700	J1MI2	108.469	C1J1MI2	128.970
C2J1	100.077	C1MI3	134.607	J1MI3	120.100	C1J1MI3	110.316
C2J2	95.941	C1MI4	129.704	J1MI4	131.292	C1J1MI4	137.079
		C1MI5	144.688	J1MI5	107.834	C1J1MI5	114.408
		C1MI6	108.824	J1MI6	81.333	C1J1MI6	97.575
		C2MI1	95.322	J2MI1	117.040	C1J2MI1	134.184
		C2MI2	87.751	J2MI2	110.981	C1J2MI2	134.429
		C2MI3	125.650	J2MI3	140.156	C1J2MI3	158.898
		C2MI4	103.772	J2MI4	102.184	C1J2MI4	122.329
		C2MI5	97.132	J2MI5	133.986	C1J2MI5	174.967
		C2MI6	78.425	J2MI6	105.916	C1J2MI6	120.073
						C2J1MI1	90.749
						C2J1MI2	87.969
						C2J1MI3	129.885
						C2J1MI4	125.506
						C2J1MI5	101.261
						C2J1MI6	65.092
						C2J2MI1	99.896
						C2J2MI2	87.534
						C2J2MI3	121.415
						C2J2MI4	82.038
						C2J2MI5	93.004
						C2J2MI6	91.759

Annexure 4.16. Factorial ANOVA to test the significance of factors at 120 days of rice plant

Tests of Between-Subjects Effects					
Dependent Variable:	PHA				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	2505635.592 ^a	23	108940.678	68952.941	.000
Intercept	8187116.006	1	8187116.006	5181955.303	.000
J	14494.305	1	14494.305	9174.029	.000
C	2267002.404	1	2267002.404	1434877.083	.000
MI	37081.178	5	7416.236	4694.034	.000
J * C	40219.604	5	8043.921	5091.321	.000
J * MI	35906.124	5	7181.225	4545.286	.000
C * MI	72829.979	1	72829.979	46097.025	.000
J * C * MI	38101.999	5	7620.400	4823.258	.000
Error	75.837	48	1.580		
Total	10692827.435	72			
Corrected Total	2505711.428	71			

a. R Squared = 1.000 (Adjusted R Squared = 1.000)

	C*J		C*MI		J*MI		C*J*MI
C1J1	560.645	C1MI1	528.686	J1MI1	368.947	C1J1MI1	613.277
C1J2	468.660	C1MI2	451.993	J1MI2	278.288	C1J1MI2	448.331
C2J1	142.150	C1MI3	565.494	J1MI3	388.688	C1J1MI3	665.633
C2J2	177.382	C1MI4	552.067	J1MI4	395.471	C1J1MI4	617.788
		C1MI5	501.959	J1MI5	331.042	C1J1MI5	515.606
		C1MI6	487.716	J1MI6	345.947	C1J1MI6	503.236
		C2MI1	143.635	J2MI1	303.373	C1J2MI1	444.094
		C2MI2	141.705	J2MI2	315.410	C1J2MI2	455.655
		C2MI3	134.935	J2MI3	311.740	C1J2MI3	465.354
		C2MI4	183.225	J2MI4	339.821	C1J2MI4	486.346
		C2MI5	197.351	J2MI5	368.268	C1J2MI5	488.312
		C2MI6	157.744	J2MI6	299.512	C1J2MI6	472.196
						C2J1MI1	124.618
						C2J1MI2	108.245
						C2J1MI3	111.743
						C2J1MI4	173.154
						C2J1MI5	146.479
						C2J1MI6	188.658
						C2J2MI1	162.652
						C2J2MI2	175.165
						C2J2MI3	158.126
						C2J2MI4	193.295
						C2J2MI5	248.223
						C2J2MI6	126.829

Annexure 4.17. Factorial ANOVA to test the significance of factors at 10 days of rice plant

Tests of Between-Subjects Effects					
Dependent Variable:	DOC				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	129384.178 ^a	23	5625.399	1539.756	.000
Intercept	1239373.320	1	1239373.320	339234.991	.000
J	15077.614	1	15077.614	4126.968	.000
C	708.223	1	708.223	193.851	.000
MI	28924.849	5	5784.970	1583.433	.000
J * C	33788.739	5	6757.748	1849.697	.000
J * MI	21306.964	5	4261.393	1166.407	.000
C * MI	4193.881	1	4193.881	1147.928	.000
J * C * MI	25383.907	5	5076.781	1389.591	.000
Error	175.365	48	3.653		
Total	1368932.863	72			
Corrected Total	129559.543	71			

a. R Squared = .999 (Adjusted R Squared = .998)

	C*J		C*MI		J*MI		C*J*MI
C1J1	156.440	C1MI1	134.450	J1MI1	145.737	C1J1MI1	161.647
C1J2	112.233	C1MI2	182.959	J1MI2	211.661	C1J1MI2	193.356
C2J1	134.903	C1MI3	136.752	J1MI3	157.648	C1J1MI3	180.693
C2J2	121.225	C1MI4	90.162	J1MI4	109.739	C1J1MI4	94.146
		C1MI5	134.276	J1MI5	149.981	C1J1MI5	184.643
		C1MI6	127.422	J1MI6	99.262	C1J1MI6	124.153
		C2MI1	139.152	J2MI1	127.866	C1J2MI1	107.254
		C2MI2	157.598	J2MI2	128.896	C1J2MI2	172.562
		C2MI3	149.763	J2MI3	128.866	C1J2MI3	92.810
		C2MI4	144.259	J2MI4	124.681	C1J2MI4	86.177
		C2MI5	87.645	J2MI5	71.940	C1J2MI5	83.909
		C2MI6	89.967	J2MI6	118.127	C1J2MI6	130.690
						C2J1MI1	129.827
						C2J1MI2	229.966
						C2J1MI3	134.602
						C2J1MI4	125.333
						C2J1MI5	115.319
						C2J1MI6	74.371
						C2J2MI1	148.478
						C2J2MI2	85.229
						C2J2MI3	164.923
						C2J2MI4	163.185
						C2J2MI5	59.971
						C2J2MI6	105.563

Annexure 4.18 factorial ANOVA to test the significance of factors at 10 days growth of rice plant

Tests of Between-Subjects Effects					
Dependent Variable:	SMBC				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	112640.634 ^a	23	4897.419	1379.911	.000
Intercept	2250059.376	1	2250059.376	633983.247	.000
J	7442.610	1	7442.610	2097.051	.000
C	43596.616	1	43596.616	12283.909	.000
MI	44478.205	5	8895.641	2506.462	.000
J * C	202.709	1	202.709	57.116	.000
C * MI	6078.064	5	1215.613	342.515	.000
J * MI	5448.673	5	1089.735	307.047	.000
J * C * MI	5393.756	5	1078.751	303.952	.000
Error	170.356	48	3.549		
Total	2362870.365	72			
Corrected Total	112810.990	71			

a. R Squared = .998 (Adjusted R Squared = .998)

	C*J		C*MI		J*MI		C*J*MI
C1J1	189.541	C1MI1	235.962	J1MI1	190.093	C1J1MI1	198.647
C1J2	213.231	C1MI2	236.002	J1MI2	186.895	C1J1MI2	207.213
C2J1	143.683	C1MI3	201.875	J1MI3	175.843	C1J1MI3	203.320
C2J2	160.661	C1MI4	166.025	J1MI4	161.717	C1J1MI4	175.180
		C1MI5	193.965	J1MI5	157.477	C1J1MI5	195.373
		C1MI6	174.488	J1MI6	127.647	C1J1MI6	157.513
		C2MI1	189.948	J2MI1	235.817	C1J2MI1	273.277
		C2MI2	172.272	J2MI2	221.378	C1J2MI2	264.790
		C2MI3	154.158	J2MI3	180.190	C1J2MI3	200.430
		C2MI4	154.418	J2MI4	158.727	C1J2MI4	156.870
		C2MI5	131.942	J2MI5	168.430	C1J2MI5	192.557
		C2MI6	110.293	J2MI6	157.135	C1J2MI6	191.463
						C2J1MI1	181.540
						C2J1MI2	166.577
						C2J1MI3	148.367
						C2J1MI4	148.253
						C2J1MI5	119.580
						C2J1MI6	97.780
						C2J2MI1	198.357
						C2J2MI2	177.967
						C2J2MI3	159.950
						C2J2MI4	160.583
						C2J2MI5	144.303
						C2J2MI6	122.807

Annexure 4.19. Factorial ANOVA to test the significance of factors at 10 days of rice plant

Tests of Between-Subjects Effects					
Dependent Variable:	pMN				
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4861.047 ^a	23	211.350	101.299	.000
Intercept	14890.519	1	14890.519	7136.971	.000
J	448.706	1	448.706	215.063	.000
C	1483.318	1	1483.318	710.949	.000
MI	454.832	5	90.966	43.600	.000
J * C	491.516	5	98.303	47.116	.000
J * MI	734.281	1	734.281	351.938	.000
C * MI	547.025	5	109.405	52.437	.000
J * C * MI	701.370	5	140.274	67.233	.000
Error	100.147	48	2.086		
Total	19851.713	72			
Corrected Total	4961.194	71			

a. R Squared = .980 (Adjusted R Squared = .970)

	C*J		C*MI		J*MI		C*J*MI
C1J1	24.610	C1MI1	14.115	J1MI1	17.582	C1J1MI1	24.500
C1J2	13.230	C1MI2	26.325	J1MI2	22.193	C1J1MI2	38.500
C2J1	9.145	C1MI3	24.098	J1MI3	23.079	C1J1MI3	34.995
C2J2	10.539	C1MI4	18.873	J1MI4	15.705	C1J1MI4	20.997
		C1MI5	13.233	J1MI5	9.123	C1J1MI5	12.667
		C1MI6	16.875	J1MI6	13.582	C1J1MI6	16.000
		C2MI1	12.247	J2MI1	8.780	C1J2MI1	3.730
		C2MI2	7.026	J2MI2	11.158	C1J2MI2	14.150
		C2MI3	11.913	J2MI3	12.932	C1J2MI3	13.200
		C2MI4	11.332	J2MI4	14.500	C1J2MI4	16.750
		C2MI5	7.747	J2MI5	11.857	C1J2MI5	13.800
		C2MI6	8.788	J2MI6	12.082	C1J2MI6	17.750
						C2J1MI1	10.663
						C2J1MI2	5.887
						C2J1MI3	11.163
						C2J1MI4	10.413
						C2J1MI5	5.580
						C2J1MI6	11.163
						C2J2MI1	13.830
						C2J2MI2	8.165
						C2J2MI3	12.663
						C2J2MI4	12.250
						C2J2MI5	9.913
						C2J2MI6	6.413

Annexure 4.20. POM factorial ANOVA to test the significance of factors at 10 days of rice plant

Tests of Between-Subjects Effects					
Dependent Variable:	POM				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	193.657 ^a	23	8.420	1351.783	.000
Intercept	3001.052	1	3001.052	481809.988	.000
J	70.010	1	70.010	11239.874	.000
C	.071	1	.071	11.440	.001
MI	16.153	5	3.231	518.649	.000
J * C	4.997	5	.999	160.452	.000
J * MI	65.872	1	65.872	10575.558	.000
C * MI	4.044	5	.809	129.862	.000
J * C * MI	32.510	5	6.502	1043.865	.000
Error	.299	48	.006		
Total	3195.008	72			
Corrected Total	193.956	71			

a. R Squared = .998 (Adjusted R Squared = .998)

	C*J		C*MI		J*MI		C*J*MI
C1J1	8.367	C1MI1	7.401	J1MI1	8.693	C1J1MI1	10.471
C1J2	4.482	C1MI2	6.666	J1MI2	7.235	C1J1MI2	8.420
C2J1	6.517	C1MI3	6.974	J1MI3	7.708	C1J1MI3	9.654
C2J2	6.458	C1MI4	6.016	J1MI4	6.974	C1J1MI4	7.856
		C1MI5	5.904	J1MI5	6.781	C1J1MI5	6.754
		C1MI6	5.586	J1MI6	7.261	C1J1MI6	7.049
		C2MI1	7.081	J2MI1	5.789	C1J2MI1	4.331
		C2MI2	6.056	J2MI2	5.487	C1J2MI2	4.911
		C2MI3	6.916	J2MI3	6.182	C1J2MI3	4.295
		C2MI4	6.331	J2MI4	5.372	C1J2MI4	4.176
		C2MI5	6.081	J2MI5	5.204	C1J2MI5	5.055
		C2MI6	6.461	J2MI6	4.786	C1J2MI6	4.124
						C2J1MI1	6.915
						C2J1MI2	6.050
						C2J1MI3	5.763
						C2J1MI4	6.093
						C2J1MI5	6.808
						C2J1MI6	7.474
						C2J2MI1	7.246
						C2J2MI2	6.063
						C2J2MI3	8.070
						C2J2MI4	6.568
						C2J2MI5	5.353
						C2J2MI6	5.448

Annexure 4.21.SOC factorial ANOVA to test the significance of factors at 10 days of rice plant

Tests of Between-Subjects Effects					
Dependent Variable:	SOC				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	9.900 ^a	23	.430	223.242	.000
Intercept	700.837	1	700.837	363470.419	.000
J	.189	1	.189	97.852	.000
C	.027	1	.027	14.022	.000
MI	.771	5	.154	79.989	.000
J * C	.196	1	.196	101.464	.000
J * MI	3.083	5	.617	319.805	.000
C * MI	2.453	5	.491	254.473	.000
J * C * MI	3.181	5	.636	329.979	.000
Error	.093	48	.002		
Total	710.830	72			
Corrected Total	9.993	71			

a. R Squared = .991 (Adjusted R Squared = .986)

	C*J		C*MI		J*MI		C*J*MI
C1J1	3.101	C1MI1	2.991	J1MI1	2.883	C1J1MI1	2.847
C1J2	3.100	C1MI2	2.805	J1MI2	3.014	C1J1MI2	2.742
C2J1	3.036	C1MI3	2.949	J1MI3	3.354	C1J1MI3	3.135
C2J2	3.243	C1MI4	3.394	J1MI4	3.322	C1J1MI4	3.912
		C1MI5	3.574	J1MI5	2.833	C1J1MI5	2.906
		C1MI6	2.889	J1MI6	3.006	C1J1MI6	3.066
		C2MI1	3.039	J2MI1	3.148	C1J2MI1	3.135
		C2MI2	3.237	J2MI2	3.028	C1J2MI2	2.868
		C2MI3	3.431	J2MI3	3.025	C1J2MI3	2.762
		C2MI4	2.935	J2MI4	3.008	C1J2MI4	2.877
		C2MI5	3.010	J2MI5	3.751	C1J2MI5	4.243
		C2MI6	3.184	J2MI6	3.067	C1J2MI6	2.712
						C2J1MI1	2.918
						C2J1MI2	3.286
						C2J1MI3	3.573
						C2J1MI4	2.732
						C2J1MI5	2.761
						C2J1MI6	2.945
						C2J2MI1	3.161
						C2J2MI2	3.187
						C2J2MI3	3.288
						C2J2MI4	3.138
						C2J2MI5	3.259
						C2J2MI6	3.422

Annexure 4.22. Factorial ANOVA to test the significance of factors at 45 days

Tests of Between-Subjects Effects					
Dependent Variable:	DOC				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	399334.080 ^a	23	17362.351	3497.156	.000
Intercept	1813573.369	1	1813573.369	365293.207	.000
J	37530.840	1	37530.840	7559.529	.000
C	93498.119	1	93498.119	18832.559	.000
MI	80507.330	5	16101.466	3243.186	.000
J * C	85710.371	1	85710.371	17263.937	.000
J * MI	40311.251	5	8062.250	1623.913	.000
C * MI	39909.809	5	7981.962	1607.741	.000
J * C * MI	21866.360	5	4373.272	880.872	.000
Error	238.306	48	4.965		
Total	2213145.755	72			
Corrected Total	399572.386	71			

a. R Squared = .999 (Adjusted R Squared = .999)

	C*J		C*MI		J*MI		C*J*MI
C1J1	252.078	C1MI1	303.686	J1MI1	304.478	C1J1MI1	441.089
C1J2	137.411	C1MI2	165.182	J1MI2	160.013	C1J1MI2	217.622
C2J1	111.002	C1MI3	210.287	J1MI3	172.394	C1J1MI3	283.710
C2J2	134.344	C1MI4	141.460	J1MI4	136.279	C1J1MI4	173.185
		C1MI5	172.720	J1MI5	160.677	C1J1MI5	194.028
		C1MI6	175.133	J1MI6	155.400	C1J1MI6	202.836
		C2MI1	157.966	J2MI1	157.175	C1J2MI1	166.283
		C2MI2	131.099	J2MI2	136.268	C1J2MI2	112.743
		C2MI3	81.373	J2MI3	119.267	C1J2MI3	136.864
		C2MI4	110.352	J2MI4	115.533	C1J2MI4	109.735
		C2MI5	136.391	J2MI5	148.434	C1J2MI5	151.412
		C2MI6	118.857	J2MI6	138.590	C1J2MI6	147.430
						C2J1MI1	167.866
						C2J1MI2	102.405
						C2J1MI3	61.077
						C2J1MI4	99.372
						C2J1MI5	127.326
						C2J1MI6	107.964
						C2J2MI1	148.066
						C2J2MI2	159.794
						C2J2MI3	101.669
						C2J2MI4	121.331
						C2J2MI5	145.456
						C2J2MI6	129.750

Annexure 4.23. Factorial ANOVA to test the significance of factors at 45 days growth of rice plant

Tests of Between-Subjects Effects					
Dependent Variable:	SMBC				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	113875.924 ^a	23	4951.127	2725.849	.000
Intercept	868557.600	1	868557.600	478185.423	.000
J	29920.417	1	29920.417	16472.721	.000
C	39622.247	1	39622.247	21814.075	.000
MI	30594.579	5	6118.916	3368.776	.000
J * C	1601.444	1	1601.444	881.677	.000
C * MI	1867.247	5	373.449	205.603	.000
J * MI	3585.029	5	717.006	394.748	.000
J * C * MI	6684.960	5	1336.992	736.083	.000
Error	87.185	48	1.816		
Total	982520.709	72			
Corrected Total	113963.109	71			

a. R Squared = .998 (Adjusted R Squared = .998)

	C*J		C*MI		J*MI		C*J*MI
C1J1	108.190	C1MI1	159.676	J1MI1	108.076	C1J1MI1	121.042
C1J2	158.393	C1MI2	167.158	J1MI2	106.854	C1J1MI2	126.392
C2J1	70.705	C1MI3	146.290	J1MI3	91.925	C1J1MI3	108.205
C2J2	102.044	C1MI4	125.788	J1MI4	90.782	C1J1MI4	113.792
		C1MI5	110.631	J1MI5	80.139	C1J1MI5	103.348
		C1MI6	90.208	J1MI6	58.911	C1J1MI6	76.363
		C2MI1	99.651	J2MI1	151.250	C1J2MI1	198.309
		C2MI2	102.406	J2MI2	162.710	C1J2MI2	207.924
		C2MI3	88.618	J2MI3	142.983	C1J2MI3	184.375
		C2MI4	90.677	J2MI4	125.683	C1J2MI4	137.783
		C2MI5	77.364	J2MI5	107.856	C1J2MI5	117.914
		C2MI6	59.531	J2MI6	90.828	C1J2MI6	104.053
						C2J1MI1	95.111
						C2J1MI2	87.316
						C2J1MI3	75.645
						C2J1MI4	67.771
						C2J1MI5	56.929
						C2J1MI6	41.459
						C2J2MI1	104.192
						C2J2MI2	117.495
						C2J2MI3	101.591
						C2J2MI4	113.583
						C2J2MI5	97.798
						C2J2MI6	77.602

Annexure 4.24. Factorial ANOVA to test the significance of factors at 45 days

Tests of Between-Subjects Effects					
Dependent Variable:	pMN				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	5250.586 ^a	23	228.286	31.858	.000
Intercept	17592.461	1	17592.461	2455.044	.000
J	134.863	1	134.863	18.820	.000
C	2179.041	1	2179.041	304.087	.000
MI	1396.206	5	279.241	38.968	.000
J * C	261.137	1	261.137	36.442	.000
J * MI	311.203	5	62.241	8.686	.000
C * MI	777.471	5	155.494	21.699	.000
J * C * MI	190.664	5	38.133	5.321	.001
Error	343.960	48	7.166		
Total	23187.008	72			
Corrected Total	5594.547	71			

a. R Squared = .939 (Adjusted R Squared = .909)

	C*J		C*MI		J*MI		C*J*MI
C1J1	24.406	C1MI1	12.832	J1MI1	7.955	C1J1MI1	11.664
C1J2	17.860	C1MI2	32.666	J1MI2	26.832	C1J1MI2	42.000
C2J1	9.594	C1MI3	28.832	J1MI3	22.122	C1J1MI3	35.000
C2J2	10.666	C1MI4	18.666	J1MI4	17.500	C1J1MI4	21.000
		C1MI5	15.164	J1MI5	13.704	C1J1MI5	18.664
		C1MI6	18.635	J1MI6	13.886	C1J1MI6	18.106
		C2MI1	4.789	J2MI1	9.666	C1J2MI1	14.000
		C2MI2	11.664	J2MI2	17.498	C1J2MI2	23.332
		C2MI3	9.538	J2MI3	16.248	C1J2MI3	22.664
		C2MI4	14.250	J2MI4	15.416	C1J2MI4	16.332
		C2MI5	8.705	J2MI5	10.165	C1J2MI5	11.664
		C2MI6	11.833	J2MI6	16.582	C1J2MI6	19.164
						C2J1MI1	4.246
						C2J1MI2	11.664
						C2J1MI3	9.244
						C2J1MI4	14.000
						C2J1MI5	8.744
						C2J1MI6	9.666
						C2J2MI1	5.332
						C2J2MI2	11.664
						C2J2MI3	9.832
						C2J2MI4	14.500
						C2J2MI5	8.666
						C2J2MI6	14.000

Annexure 4.30.POM factorial ANOVA to test the significance of factors at 45 days of rice plant

Tests of Between-Subjects Effects					
Dependent Variable:	POM				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	133.954 ^a	23	5.824	126.758	.000
Intercept	5381.127	1	5381.127	117117.238	.000
J	17.843	1	17.843	388.353	.000
C	12.933	1	12.933	281.488	.000
MI	15.795	5	3.159	68.754	.000
J * C	32.275	5	6.455	140.488	.000
J * MI	9.291	1	9.291	202.217	.000
C * MI	29.543	5	5.909	128.595	.000
J * C * MI	16.274	5	3.255	70.837	.000
Error	2.205	48	.046		
Total	5517.287	72			
Corrected Total	136.159	71			

a. R Squared = .984 (Adjusted R Squared = .976)

	C*J		C*MI		J*MI		C*J*MI
C1J1	7.364	C1MI1	7.862	J1MI1	8.679	C1J1MI1	7.626
C1J2	9.078	C1MI2	9.179	J1MI2	8.132	C1J1MI2	7.780
C2J1	8.930	C1MI3	8.428	J1MI3	8.612	C1J1MI3	7.780
C2J2	9.208	C1MI4	8.497	J1MI4	9.119	C1J1MI4	8.115
		C1MI5	7.737	J1MI5	7.545	C1J1MI5	6.997
		C1MI6	7.625	J1MI6	6.797	C1J1MI6	5.887
		C2MI1	9.166	J2MI1	8.349	C1J2MI1	8.098
		C2MI2	7.710	J2MI2	8.757	C1J2MI2	10.578
		C2MI3	8.350	J2MI3	8.166	C1J2MI3	9.076
		C2MI4	10.846	J2MI4	10.223	C1J2MI4	8.878
		C2MI5	8.817	J2MI5	9.009	C1J2MI5	8.477
		C2MI6	9.525	J2MI6	10.353	C1J2MI6	9.362
						C2J1MI1	9.732
						C2J1MI2	8.484
						C2J1MI3	9.443
						C2J1MI4	10.123
						C2J1MI5	8.093
						C2J1MI6	7.707
						C2J2MI1	8.599
						C2J2MI2	6.936
						C2J2MI3	7.257
						C2J2MI4	11.569
						C2J2MI5	9.541
						C2J2MI6	11.344

Annexure 4.26. SOC factorial ANOVA to test the significance of factors at 45 days of rice plant

Tests of Between-Subjects Effects					
Dependent Variable:	SOC				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	11.239 ^a	23	.489	136.330	.000
Intercept	696.828	1	696.828	194407.161	.000
J	.029	1	.029	8.203	.006
C	.098	1	.098	27.447	.000
MI	1.453	5	.291	81.048	.000
J * C	.150	1	.150	41.786	.000
J * MI	2.371	5	.474	132.290	.000
C * MI	5.112	5	1.022	285.247	.000
J * C * MI	2.026	5	.405	113.047	.000
Error	.172	48	.004		
Total	708.240	72			
Corrected Total	11.411	71			

a. R Squared = .985 (Adjusted R Squared = .978)

	C*J		C*MI		J*MI		C*J*MI
C1J1	3.173	C1MI1	2.991	J1MI1	2.883	C1J1MI1	2.847
C1J2	3.123	C1MI2	2.805	J1MI2	3.031	C1J1MI2	2.742
C2J1	3.008	C1MI3	2.949	J1MI3	3.354	C1J1MI3	3.135
C2J2	3.140	C1MI4	3.512	J1MI4	3.271	C1J1MI4	4.010
		C1MI5	3.741	J1MI5	3.000	C1J1MI5	3.239
		C1MI6	2.889	J1MI6	3.006	C1J1MI6	3.066
		C2MI1	2.871	J2MI1	2.980	C1J2MI1	3.135
		C2MI2	3.254	J2MI2	3.028	C1J2MI2	2.868
		C2MI3	3.431	J2MI3	3.025	C1J2MI3	2.762
		C2MI4	2.694	J2MI4	2.935	C1J2MI4	3.014
		C2MI5	3.011	J2MI5	3.752	C1J2MI5	4.243
		C2MI6	3.184	J2MI6	3.067	C1J2MI6	2.712
						C2J1MI1	2.918
						C2J1MI2	3.320
						C2J1MI3	3.573
						C2J1MI4	2.532
						C2J1MI5	2.761
						C2J1MI6	2.945
						C2J2MI1	2.825
						C2J2MI2	3.187
						C2J2MI3	3.288
						C2J2MI4	2.855
						C2J2MI5	3.261
						C2J2MI6	3.422

Annexure 4.27. Factorial ANOVA to test the significance of factors at 90 days

Tests of Between-Subjects Effects					
Dependent Variable:	DOC				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	97177.259 ^a	23	4225.098	1148.631	.000
Intercept	1370835.585	1	1370835.585	372673.938	.000
J	134.947	1	134.947	36.686	.000
C	2535.820	1	2535.820	689.385	.000
MI	19268.386	5	3853.677	1047.657	.000
J * C	8432.671	5	1686.534	458.499	.000
J * MI	37593.994	1	37593.994	10220.264	.000
C * MI	14247.182	5	2849.436	774.645	.000
J * C * MI	14964.259	5	2992.852	813.634	.000
Error	176.562	48	3.678		
Total	1468189.407	72			
Corrected Total	97353.821	71			

a. R Squared = .998 (Adjusted R Squared = .997)

	C*J		C*MI		J*MI		C*J*MI
C1J1	119.699	C1MI1	138.548	J1MI1	164.942	C1J1MI1	117.851
C1J2	168.137	C1MI2	124.528	J1MI2	134.328	C1J1MI2	112.556
C2J1	153.530	C1MI3	129.573	J1MI3	99.022	C1J1MI3	100.335
C2J2	110.567	C1MI4	140.698	J1MI4	121.086	C1J1MI4	93.555
		C1MI5	148.185	J1MI5	145.065	C1J1MI5	122.910
		C1MI6	181.975	J1MI6	155.242	C1J1MI6	170.985
		C2MI1	175.586	J2MI1	149.192	C1J2MI1	159.246
		C2MI2	125.526	J2MI2	115.726	C1J2MI2	136.501
		C2MI3	115.372	J2MI3	145.923	C1J2MI3	158.812
		C2MI4	97.929	J2MI4	117.541	C1J2MI4	187.840
		C2MI5	142.343	J2MI5	145.463	C1J2MI5	173.459
		C2MI6	135.536	J2MI6	162.270	C1J2MI6	192.966
						C2J1MI1	212.034
						C2J1MI2	156.101
						C2J1MI3	97.710
						C2J1MI4	148.617
						C2J1MI5	167.220
						C2J1MI6	139.498
						C2J2MI1	139.138
						C2J2MI2	94.951
						C2J2MI3	133.034
						C2J2MI4	47.242
						C2J2MI5	117.466
						C2J2MI6	131.573

Annexure 4.28. Factorial ANOVA to test the significance of factors at 90 days growth of rice plant

Tests of Between-Subjects Effects					
Dependent Variable:	SMBC				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	55665.691 ^a	23	2420.247	1056.338	.000
Intercept	98240.419	1	98240.419	42877.874	.000
J	1980.535	1	1980.535	864.421	.000
C	44472.044	1	44472.044	19410.205	.000
MI	4040.724	5	808.145	352.722	.000
J * C	2452.548	1	2452.548	1070.436	.000
C * MI	75.352	5	15.070	6.578	.000
J * MI	2326.956	5	465.391	203.124	.000
J * C * MI	317.533	5	63.507	27.718	.000
Error	109.976	48	2.291		
Total	154016.086	72			
Corrected Total	55775.667	71			

a. R Squared = .998 (Adjusted R Squared = .997)

	C*J		C*MI		J*MI		C*J*MI
C1J1	50.710	C1MI1	71.229	J1MI1	37.360	C1J1MI1	64.732
C1J2	72.872	C1MI2	69.835	J1MI2	37.047	C1J1MI2	56.481
C2J1	12.677	C1MI3	72.024	J1MI3	35.722	C1J1MI3	56.651
C2J2	11.494	C1MI4	64.684	J1MI4	33.882	C1J1MI4	53.239
		C1MI5	58.713	J1MI5	29.586	C1J1MI5	47.507
		C1MI6	34.262	J1MI6	16.566	C1J1MI6	25.653
		C2MI1	11.212	J2MI1	45.082	C1J2MI1	77.727
		C2MI2	14.258	J2MI2	47.045	C1J2MI2	83.190
		C2MI3	13.461	J2MI3	49.763	C1J2MI3	87.397
		C2MI4	13.314	J2MI4	44.116	C1J2MI4	76.130
		C2MI5	12.435	J2MI5	41.562	C1J2MI5	69.920
		C2MI6	7.834	J2MI6	25.530	C1J2MI6	42.871
						C2J1MI1	9.988
						C2J1MI2	17.614
						C2J1MI3	14.793
						C2J1MI4	14.524
						C2J1MI5	11.665
						C2J1MI6	7.479
						C2J2MI1	12.436
						C2J2MI2	10.901
						C2J2MI3	12.129
						C2J2MI4	12.103
						C2J2MI5	13.204
						C2J2MI6	8.190

Annexure 4.29. Factorial ANOVA to test the significance of factors at 90 days

Tests of Between-Subjects Effects					
Dependent Variable:	pMN				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	987.500 ^a	23	42.935	38.322	.000
Intercept	5304.500	1	5304.500	4734.595	.000
J	64.222	1	64.222	57.322	.000
C	213.556	1	213.556	190.612	.000
MI	390.833	5	78.167	69.769	.000
J * C	128.778	5	25.756	22.988	.000
J * MI	143.778	5	28.756	25.666	.000
C * MI	24.500	1	24.500	21.868	.000
J * C * MI	21.833	5	4.367	3.898	.005
Error	53.778	48	1.120		
Total	6345.778	72			
Corrected Total	1041.278	71			

a. R Squared = .948 (Adjusted R Squared = .924)

	C*J		C*MI		J*MI		C*J*MI
C1J1	11.833	C1MI1	8.333	J1MI1	8.833	C1J1MI1	10.000
C1J2	8.778	C1MI2	10.500	J1MI2	8.167	C1J1MI2	11.667
C2J1	7.222	C1MI3	6.833	J1MI3	6.167	C1J1MI3	7.667
C2J2	6.500	C1MI4	7.167	J1MI4	5.833	C1J1MI4	7.000
		C1MI5	13.500	J1MI5	15.000	C1J1MI5	17.667
		C1MI6	15.500	J1MI6	13.167	C1J1MI6	17.000
		C2MI1	9.500	J2MI1	9.000	C1J2MI1	6.667
		C2MI2	4.667	J2MI2	7.000	C1J2MI2	9.333
		C2MI3	4.667	J2MI3	5.333	C1J2MI3	6.000
		C2MI4	5.167	J2MI4	6.500	C1J2MI4	7.333
		C2MI5	9.167	J2MI5	7.667	C1J2MI5	9.333
		C2MI6	8.000	J2MI6	10.333	C1J2MI6	14.000
						C2J1MI1	7.667
						C2J1MI2	4.667
						C2J1MI3	4.667
						C2J1MI4	4.667
						C2J1MI5	12.333
						C2J1MI6	9.333
						C2J2MI1	11.333
						C2J2MI2	4.667
						C2J2MI3	4.667
						C2J2MI4	5.667
						C2J2MI5	6.000
						C2J2MI6	6.667

Annexure 4.35. POM factorial ANOVA to test the significance of factors at 90 days of rice plant

Tests of Between-Subjects Effects					
Dependent Variable:	POM				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	241.661 ^a	23	10.507	109.177	.000
Intercept	3541.753	1	3541.753	36801.966	.000
J	52.872	1	52.872	549.385	.000
C	3.781	1	3.781	39.287	.000
MI	35.368	5	7.074	73.501	.000
J * C	30.740	5	6.148	63.884	.000
J * MI	.494	1	.494	5.138	.028
C * MI	66.204	5	13.241	137.583	.000
J * C * MI	52.201	5	10.440	108.484	.000
Error	4.619	48	.096		
Total	3788.032	72			
Corrected Total	246.280	71			

a. R Squared = .981 (Adjusted R Squared = .972)

	C*J		C*MI		J*MI		C*J*MI
C1J1	7.559	C1MI1	6.454	J1MI1	8.435	C1J1MI1	7.757
C1J2	6.010	C1MI2	7.480	J1MI2	7.139	C1J1MI2	9.932
C2J1	8.183	C1MI3	8.047	J1MI3	9.602	C1J1MI3	9.428
C2J2	6.303	C1MI4	7.569	J1MI4	8.186	C1J1MI4	8.412
		C1MI5	5.909	J1MI5	6.802	C1J1MI5	5.179
		C1MI6	5.246	J1MI6	7.060	C1J1MI6	4.643
		C2MI1	7.003	J2MI1	5.023	C1J2MI1	5.152
		C2MI2	4.834	J2MI2	5.176	C1J2MI2	5.028
		C2MI3	7.957	J2MI3	6.402	C1J2MI3	6.667
		C2MI4	7.906	J2MI4	7.289	C1J2MI4	6.726
		C2MI5	6.579	J2MI5	5.686	C1J2MI5	6.639
		C2MI6	9.178	J2MI6	7.364	C1J2MI6	5.850
						C2J1MI1	9.112
						C2J1MI2	4.345
						C2J1MI3	9.775
						C2J1MI4	7.960
						C2J1MI5	8.425
						C2J1MI6	9.477
						C2J2MI1	4.894
						C2J2MI2	5.323
						C2J2MI3	6.138
						C2J2MI4	7.851
						C2J2MI5	4.733
						C2J2MI6	8.879

Annexure 4.31. SOC factorial ANOVA to test the significance of factors at 90 days of rice plant

Tests of Between-Subjects Effects					
Dependent Variable:	SOC				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	4.863 ^a	23	.211	209.862	.000
Intercept	688.140	1	688.140	683019.017	.000
J	.157	1	.157	155.970	.000
C	.305	1	.305	303.118	.000
MI	.813	5	.163	161.469	.000
J * C	.729	5	.146	144.714	.000
J * MI	.152	1	.152	150.578	.000
C * MI	1.077	5	.215	213.717	.000
J * C * MI	1.630	5	.326	323.532	.000
Error	.048	48	.001		
Total	693.051	72			
Corrected Total	4.911	71			

a. R Squared = .990 (Adjusted R Squared = .985)

	C*J		C*MI		J*MI		C*J*MI
C1J1	3.064	C1MI1	3.064	J1MI1	3.011	C1J1MI1	3.033
C1J2	3.249	C1MI2	3.215	J1MI2	2.983	C1J1MI2	3.080
C2J1	3.026	C1MI3	2.950	J1MI3	3.185	C1J1MI3	3.134
C2J2	3.027	C1MI4	3.312	J1MI4	3.201	C1J1MI4	3.265
		C1MI5	3.438	J1MI5	2.967	C1J1MI5	2.834
		C1MI6	2.961	J1MI6	2.922	C1J1MI6	3.038
		C2MI1	3.020	J2MI1	3.072	C1J2MI1	3.094
		C2MI2	2.906	J2MI2	3.137	C1J2MI2	3.350
		C2MI3	3.283	J2MI3	3.048	C1J2MI3	2.766
		C2MI4	3.105	J2MI4	3.216	C1J2MI4	3.359
		C2MI5	2.994	J2MI5	3.465	C1J2MI5	4.043
		C2MI6	2.851	J2MI6	2.890	C1J2MI6	2.884
						C2J1MI1	2.988
						C2J1MI2	2.887
						C2J1MI3	3.236
						C2J1MI4	3.137
						C2J1MI5	3.101
						C2J1MI6	2.805
						C2J2MI1	3.051
						C2J2MI2	2.925
						C2J2MI3	3.330
						C2J2MI4	3.073
						C2J2MI5	2.888
						C2J2MI6	2.896

Annexure 4.32. Factorial ANOVA to test the significance of factors at 120 days

Tests of Between-Subjects Effects					
Dependent Variable:	DOC				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	171944.502 ^a	23	7475.848	2166.091	.000
Intercept	3075903.584	1	3075903.584	891228.269	.000
J	268.382	1	268.382	77.762	.000
C	520.344	1	520.344	150.767	.000
MI	46690.600	5	9338.120	2705.675	.000
J * C	21231.576	5	4246.315	1230.349	.000
J * MI	3870.293	1	3870.293	1121.399	.000
C * MI	54409.132	5	10881.826	3152.957	.000
J * C * MI	44954.176	5	8990.835	2605.051	.000
Error	165.663	48	3.451		
Total	3248013.749	72			
Corrected Total	172110.165	71			

a. R Squared = .999 (Adjusted R Squared = .999)

	C*J		C*MI		J*MI		C*J*MI
C1J1	209.403	C1MI1	228.729	J1MI1	223.020	C1J1MI1	229.609
C1J2	198.601	C1MI2	225.369	J1MI2	207.649	C1J1MI2	227.326
C2J1	200.116	C1MI3	99.370	J1MI3	170.279	C1J1MI3	163.757
C2J2	218.641	C1MI4	243.938	J1MI4	245.316	C1J1MI4	230.093
		C1MI5	234.036	J1MI5	204.524	C1J1MI5	228.876
		C1MI6	192.569	J1MI6	177.770	C1J1MI6	176.757
		C2MI1	234.013	J2MI1	239.722	C1J2MI1	227.849
		C2MI2	215.759	J2MI2	233.479	C1J2MI2	223.412
		C2MI3	208.792	J2MI3	137.883	C1J2MI3	34.983
		C2MI4	204.545	J2MI4	203.167	C1J2MI4	257.783
		C2MI5	172.008	J2MI5	201.520	C1J2MI5	239.197
		C2MI6	221.155	J2MI6	235.954	C1J2MI6	208.381
						C2J1MI1	216.430
						C2J1MI2	187.972
						C2J1MI3	176.801
						C2J1MI4	260.539
						C2J1MI5	180.173
						C2J1MI6	178.782
						C2J2MI1	251.595
						C2J2MI2	243.546
						C2J2MI3	240.783
						C2J2MI4	148.551
						C2J2MI5	163.844
						C2J2MI6	263.527

Annexure 4.33. Factorial ANOVA to test the significance of factors at 120 days growth of rice plant

Tests of Between-Subjects Effects					
Dependent Variable:	SMBC				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	13350.654 ^a	23	580.463	520.031	.000
Intercept	212033.974	1	212033.974	189958.908	.000
J	6.413	1	6.413	5.746	.020
C	679.310	1	679.310	608.586	.000
MI	9942.329	5	1988.466	1781.445	.000
J * C	12.976	1	12.976	11.625	.001
C * MI	1771.046	5	354.209	317.332	.000
J * MI	527.065	5	105.413	94.438	.000
J * C * MI	411.514	5	82.303	73.734	.000
Error	53.578	48	1.116		
Total	225438.206	72			
Corrected Total	13404.232	71			

a. R Squared = .996 (Adjusted R Squared = .994)

	C*J		C*MI		J*MI		C*J*MI
C1J1	51.918	C1MI1	58.958	J1MI1	62.852	C1J1MI1	52.467
C1J2	50.472	C1MI2	50.806	J1MI2	49.653	C1J1MI2	47.585
C2J1	57.213	C1MI3	56.081	J1MI3	66.131	C1J1MI3	67.696
C2J2	57.465	C1MI4	67.264	J1MI4	66.833	C1J1MI4	71.217
		C1MI5	43.979	J1MI5	52.639	C1J1MI5	46.636
		C1MI6	30.084	J1MI6	29.285	C1J1MI6	25.910
		C2MI1	74.276	J2MI1	70.382	C1J2MI1	65.450
		C2MI2	58.947	J2MI2	60.100	C1J2MI2	54.027
		C2MI3	58.461	J2MI3	48.411	C1J2MI3	44.467
		C2MI4	65.398	J2MI4	65.829	C1J2MI4	63.311
		C2MI5	52.546	J2MI5	43.886	C1J2MI5	41.322
		C2MI6	34.404	J2MI6	35.203	C1J2MI6	34.258
						C2J1MI1	73.238
						C2J1MI2	51.722
						C2J1MI3	64.566
						C2J1MI4	62.448
						C2J1MI5	58.641
						C2J1MI6	32.660
						C2J2MI1	75.315
						C2J2MI2	66.173
						C2J2MI3	52.356
						C2J2MI4	68.347
						C2J2MI5	46.450
						C2J2MI6	36.149

Annexure 4.34. Factorial ANOVA to test the significance of factors at 120 days

Tests of Between-Subjects Effects					
Dependent Variable:	pMN				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	4254.792 ^a	23	184.991	39.313	.000
Intercept	16778.779	1	16778.779	3565.678	.000
J	36.832	1	36.832	7.827	.007
C	642.990	1	642.990	136.642	.000
MI	637.000	5	127.400	27.074	.000
J * C	1254.303	5	250.861	53.311	.000
J * MI	51.946	1	51.946	11.039	.002
C * MI	774.261	5	154.852	32.908	.000
J * C * MI	857.459	5	171.492	36.444	.000
Error	225.871	48	4.706		
Total	21259.441	72			
Corrected Total	4480.662	71			

a. R Squared = .950 (Adjusted R Squared = .925)

	C*J		C*MI		J*MI		C*J*MI
C1J1	18.120	C1MI1	14.916	J1MI1	7.916	C1J1MI1	8.832
C1J2	18.388	C1MI2	17.500	J1MI2	15.166	C1J1MI2	21.000
C2J1	13.842	C1MI3	28.166	J1MI3	12.166	C1J1MI3	14.500
C2J2	10.713	C1MI4	17.082	J1MI4	20.027	C1J1MI4	23.832
		C1MI5	12.498	J1MI5	17.998	C1J1MI5	16.332
		C1MI6	19.361	J1MI6	22.611	C1J1MI6	24.222
		C2MI1	6.222	J2MI1	13.222	C1J2MI1	21.000
		C2MI2	10.388	J2MI2	12.722	C1J2MI2	14.000
		C2MI3	9.748	J2MI3	25.748	C1J2MI3	41.832
		C2MI4	15.861	J2MI4	12.916	C1J2MI4	10.332
		C2MI5	13.943	J2MI5	8.443	C1J2MI5	8.664
		C2MI6	17.500	J2MI6	14.250	C1J2MI6	14.500
						C2J1MI1	7.000
						C2J1MI2	9.332
						C2J1MI3	9.832
						C2J1MI4	16.222
						C2J1MI5	19.664
						C2J1MI6	21.000
						C2J2MI1	5.444
						C2J2MI2	11.444
						C2J2MI3	9.664
						C2J2MI4	15.500
						C2J2MI5	8.222
						C2J2MI6	14.000

Annexure 4.32. Factorial ANOVA to test the significance of factors at 120 days growth of rice plant

Tests of Between-Subjects Effects					
Dependent Variable:	POM				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	226.316 ^a	23	9.840	108.156	.000
Intercept	3964.515	1	3964.515	43576.553	.000
J	60.987	1	60.987	670.352	.000
C	84.703	1	84.703	931.030	.000
MI	15.609	5	3.122	34.313	.000
J * C	19.386	5	3.877	42.618	.000
J * MI	2.101	1	2.101	23.088	.000
C * MI	22.558	5	4.512	49.589	.000
J * C * MI	20.972	5	4.194	46.103	.000
Error	4.367	48	.091		
Total	4195.198	72			
Corrected Total	230.683	71			

a. R Squared = .981 (Adjusted R Squared = .972)

	C*J		C*MI		J*MI		C*J*MI
C1J1	7.414	C1MI1	8.432	J1MI1	6.716	C1J1MI1	7.644
C1J2	9.596	C1MI2	10.005	J1MI2	7.328	C1J1MI2	9.510
C2J1	5.586	C1MI3	8.564	J1MI3	7.383	C1J1MI3	9.033
C2J2	7.085	C1MI4	8.914	J1MI4	6.513	C1J1MI4	7.434
		C1MI5	7.880	J1MI5	5.533	C1J1MI5	5.552
		C1MI6	7.237	J1MI6	5.527	C1J1MI6	5.311
		C2MI1	6.042	J2MI1	7.758	C1J2MI1	9.219
		C2MI2	5.482	J2MI2	8.159	C1J2MI2	10.500
		C2MI3	6.849	J2MI3	8.029	C1J2MI3	8.094
		C2MI4	7.240	J2MI4	9.641	C1J2MI4	10.394
		C2MI5	6.237	J2MI5	8.584	C1J2MI5	10.207
		C2MI6	6.164	J2MI6	7.873	C1J2MI6	9.162
						C2J1MI1	5.788
						C2J1MI2	5.147
						C2J1MI3	5.733
						C2J1MI4	5.592
						C2J1MI5	5.515
						C2J1MI6	5.743
						C2J2MI1	6.297
						C2J2MI2	5.818
						C2J2MI3	7.964
						C2J2MI4	8.889
						C2J2MI5	6.960
						C2J2MI6	6.585

Annexure 4.36. SOC factorial ANOVA to test the significance of factors at 120 days of rice plant

Tests of Between-Subjects Effects					
Dependent Variable:	SOC				
Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	1.553 ^a	23	.068	34.407	.000
Intercept	428.226	1	428.226	218233.542	.000
J	.349	1	.349	177.774	.000
C	.058	1	.058	29.725	.000
MI	.118	5	.024	12.072	.000
J * C	.311	5	.062	31.668	.000
J * MI	.024	1	.024	12.420	.001
C * MI	.456	5	.091	46.453	.000
J * C * MI	.236	5	.047	24.097	.000
Error	.094	48	.002		
Total	429.874	72			
Corrected Total	1.647	71			

a. R Squared = .943 (Adjusted R Squared = .915)

	C*J		C*MI		J*MI		C*J*MI
C1J1	2.462	C1MI1	2.357	J1MI1	2.425	C1J1MI1	2.355
C1J2	2.359	C1MI2	2.424	J1MI2	2.394	C1J1MI2	2.424
C2J1	2.555	C1MI3	2.327	J1MI3	2.508	C1J1MI3	2.324
C2J2	2.379	C1MI4	2.396	J1MI4	2.525	C1J1MI4	2.337
		C1MI5	2.423	J1MI5	2.596	C1J1MI5	2.588
		C1MI6	2.535	J1MI6	2.603	C1J1MI6	2.741
		C2MI1	2.519	J2MI1	2.451	C1J2MI1	2.358
		C2MI2	2.333	J2MI2	2.363	C1J2MI2	2.424
		C2MI3	2.502	J2MI3	2.321	C1J2MI3	2.330
		C2MI4	2.590	J2MI4	2.461	C1J2MI4	2.455
		C2MI5	2.552	J2MI5	2.379	C1J2MI5	2.258
		C2MI6	2.307	J2MI6	2.239	C1J2MI6	2.329
						C2J1MI1	2.495
						C2J1MI2	2.364
						C2J1MI3	2.692
						C2J1MI4	2.712
						C2J1MI5	2.604
						C2J1MI6	2.465
						C2J2MI1	2.544
						C2J2MI2	2.301
						C2J2MI3	2.312
						C2J2MI4	2.468
						C2J2MI5	2.500
						C2J2MI6	2.150

VITA

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