SOIL FERTILITY MANAGEMENT UNDER SHIFTING CULTIVATION IN MIZORAM, INDIA

A THESIS SUBMITTED FOR THE PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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DEPARTMENT OF FORESTRY
SCHOOL OF EARTH SCIENCES & NATURAL RESOURCE MANAGEMENT

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SOIL FERTILITY MANAGEMENT UNDER SHIFTING CULTIVATION IN MIZORAM, INDIA

BY

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Department of Forestry

Under the Supervision of

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Submitted

In the partial fulfillment of the requirement of the Degree of Doctor of Philosophy in Forestry of Mizoram University, Aizawl.

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March, 2022

I, Shrayosee Ghosh, hereby declare that the subject matter of this thesis is the original record of work done by me and to do the best of my knowledge, the contents of this thesis did not form basis of the award of any previous degree to me or to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/ Institute.

This is being submitted to the Mizoram University for the degree of Doctor of Philosophy in Forestry.

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CERTIFICATE

This is to certify that the thesis entitled **"*Soil Fertility Management under Shifting cultivation in Mizoram, India*"** submitted to Mizoram University, Aizawl for the award of the degree of Doctor of Philosophy by **Ms. Shrayosee Ghosh (Regd. No.: MZU/Ph. D/1143 of 27.04.2018)**, research scholar in the Department of Forestry, is the record of original research work carried out by her under my supervision. She has been duly registered and the thesis presented is worthy of being considered for the award of the Ph.D. degree. This work has not been submitted elsewhere for any degree in any other Universities.

S.K. TRIPATHI

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ABBREVIATIONS

TC	*Tephrosia candida*
OS	*Oryza sativa*
TC + OS	*Tephrosia candida + Oryza sativa*
FF3	3 years of Forest Fallow
FF5	5 years of Forest Fallow
JF3	3 years of Forest Fallow
JF8	8 years of Forest Fallow
PJS3	Pot 3 years Jhum fallow soil
PJS8	Pot 8 years Jhum fallow soil
MGL	Mixed grass litter
BL	Bamboo litter
MFL	Mixed forest litter
C	Carbon
N	Nitrogen
AP	Available Phosphorus
P	Phosphorus
K	Potassium
Mg	Magnesium
Ca	Calcium
BD	Bulk density
CFU	Colony Forming Unit
H_2O_2	Hydrogen peroxide
CEC	Cation Exchange Capacity
NA	Nutrient Agar
PDA	Potato Dextrose Agar
SCA	Starch Casein Agar

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2. Ghosh, S., & Tripathi, S. K. (2021). Changes in soil microbial population following decomposition of different litters under shifting cultivation in Mizoram. Indian Journal of Ecology, 48(5).
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8. International Conference on Biotechnology and Biological Sciences “BIOSPECTRUM 2019”, University of Engineering and Management, Kolkata, India, 8th-10th August 2019
9. International Conference on Biodiversity, Environment and human Health: Innovations and Emerging Trends, Mizoram University, Mizoram, India, 12th-14th November 2018
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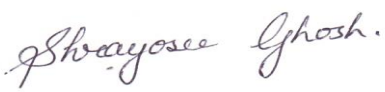
Declaration:

I hereby declare that all the statements made above are true, complete and correct to the best of my knowledge and belief.



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PHOTOPLATES

Field view of 3 years Jhum fallow after burning

Field view of 8 years Jhum fallow after burning

Microcosm carried out in polyhouse for different litter types in soil from different study sites.

Litter bag showing litters during decomposition

ABSTRACT

SOIL FERTILITY MANAGEMENT UNDER SHIFTING CULTIVATION IN MIZORAM, INDIA

A THESIS SUBMITTED FOR THE PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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CHAPTER 1

Introduction

1.1 Shifting cultivation practice: problems and prospects

Shifting cultivation is one of the primitive agricultural practices believed to be as old as the history of any cropping in the world (Tripathi et al., 2017). Archaeological and radio-carbon dating evidences suggest that the origin of this cultivation could be traced back in the Neolithic period which resulted in remarkable change in the mode of food production practices (Conklin, 1961). As per the recent reports this form of cultivation can be traced back to about 8000 BC (Tiwari, 2018). Shifting cultivation is being carried out by about half a billion population in tropical moist forests of the world (Grogan et al., 2012). It is mainly confined to Sub-Saharan Africa, Southeast Asia and South America. In India, this practice continues to be a dominant mode of food production in hilly region of Northeast India. In Northeast India, shifting cultivation or jhum cultivation occupies about 85% area out of total cultivated land (Singh and Singh, 1992).

Mizoram is one among the eight sister states of Northeast India. The state is characterized by highest forest cover on the hilly topography with steep slopes (Grogan et al., 2012). This area is a part of megadiversity hotspots of the world. Shifting agriculture is the chief source of living and economy for the rural people in Mizoram (Maithani, 2005). The term 'Mizoram' denotes 'the land of highlanders'. The forefathers of Mizo people lived a nomadic life and were mainly based on shifting cultivation practices for their living which passed down from generations after generations (Tripathi et al., 2017). The practice of shifting cultivation called "*Lo neih*" in Mizo, which involves several cultural operations i.e., clearing of vegetation of an area (during December and January), allow it to dry (during February-March) and burn *in situ* (during March-April) followed by cropping for 1-2 years (cropping phase) depending on the levels of soil fertility and abandoning the land as fallow for few years (fallow period) to recover soil productivity through natural re-growth (Grogan et al., 2012; Tripathi et al., 2017). In this farming, mixed crops are typically produced such as rice, maize, French bean, yam, chilly, brinjal, cucumber, pumpkin, bitter guard, tapioca, squash, bottle gourd, cow pea, tomato, and

flat beans (Sati and Rinawma, 2014). In past years, the cultivators were capable to abandon the land as fallow for more than 20 years which allowed the land to recover soil fertility and support high crop productivity. However, in recent years, increasing population densities has led to reduce the fallow period (less than 5 years) which substantially reduce the soil and crop productivity. This has posed a serious threat to local farmers for their livelihood generations.

According to the Food and Agricultural Organization, shifting cultivation was described as the extreme hindrance not only to the instant increase in agricultural production, but also to the maintenance of making potential for the future, in the form of forests and soils (FAO, 1957). Since then, shifting cultivation has become an old and economically unproductive form of agriculture which caused soil erosion, watershed siltation and smoke problems in the atmosphere. This observation has triggered the land use management and policy advancement in many affected regions.

In Northeast India, Mizoram has the highest burnt area under shifting cultivation and responsible for emitting highest amount of greenhouse gases in the environment (Chand and Badrinath, 2007). Shifting cultivation has also been criticized as economically unproductive and ecologically damaging practice by Government of Mizoram. The Government has introduced various land use policies to support the farmers to develop alternate of jhuming but all of them have failed because of either one or other reasons (Tripathi et al., 2017). In last one decade, the Government of Mizoram has launched a New Land Use Policy (NLUP) to provide financial support to jhumias. The main objectives of the NLUP were to wean away of jhum practice by developing alternate methods of livelihood options (i.e., dairy, piggery, poultry) for the farmers (Wapongnungsang et al., 2021). This has resulted in lessening of the area under shifting agriculture and converted some area into agroforestry systems. However, it is likely that when Government support will terminate, the area under shifting cultivation will increase again as has happened in the past (Tripathi et al., 2017). Since this form of cultivation has been closely linked with the culture of the region and serving as major source of living option for the majority of rural people, it is difficult to transform this practice by other form of cultivation.

Therefore, there is a great scope for energizing this agricultural practice to be ecologically sound which can meet various human needs with great efficiency through understanding the organic matter dynamics of locally available resources (like forest litters and agricultural residues) by manipulating their decay rates considering the factors responsible for their decomposition (Wapongnungsang et al., 2021). This will provide a potential tool to manipulate organic decay kinetics and nutrient release rates as well as to use forest litters and agricultural residues for optimizing fallow length and synchronizing nutrient availability with that of crop nutrient demand to sustain crop productivity in shifting cultivation sites of Mizoram. This may significantly contribute towards increasing societal value for a place like Mizoram in near future.

1.2 Litter inputs in shifting cultivation

The transfer of matter and energy maintains the ecosystem's integrity, and litterfall in the forest accounts for the majority of the annual gain of energy and matter (Xu and Hirata, 2005). The litters on the forest floor are the major source of minerals and nutrients for plant growth (Christensen, 1975). According to Bisht et al., (2014), leaf litters contribute 75-79% of total litter production. Litter production is primarily influenced by site fertility, although other elements including as air, temperature, soil, water, and nutrient availability all have an impact (Jorgensen et al., 1975). According to Cornwell et al., (2008) chemical composition of litter depends on the length of the fallow land. There is a positive connection between the span of fallow land and the accumulation of litters on forest floor. The longer fallow land produce significantly higher quantity of litters compared to shorter fallow land (Saplalrinliana et al., 2016). In Northeast India, litterfall was reported to be concentrated during the cold dry season, and was substantially influenced by decreased temperature and soil moisture (Pandey et al., 2007). Similarly, in another study it is reported that the two-third of the litterfall occurred during December-March (Pandey et al., 2016).

1.3 Decomposition of leaf litters

The nutrients in the litter are recycled during the decomposition process which allows the release of inorganic nutrients to the soil which promotes plant

productivity and soil fertility (Aponte et al., 2010 and 2012). The availability of nutrients in the soil is thought to be regulated by litter formation and breakdown (Tripathi and Singh, 1992a; Fioretto et al., 2003; Chen et al., 2014; Gavazov et al., 2014).

Litter decomposition is a systematic process in which complex organic compounds are progressively decomposed into simpler molecules, releasing nutrients as a result of the breakdown (Yadav and Malanson, 2007). Litter decomposition involves two processes: a) the mineralization and humification of lignin, cellulose, and other compounds by a series of microbial actions, and b) the discharge of soluble compounds to the soil, where carbon and nitrogen are steadily mineralized (Anderson, 1988). The belowground litters have been reported as an important source for the supply of carbon and nitrogen, as well as critical mechanism for forest restoration (Pandey et al., 2016). Slow decomposition rates result in the accumulation of organic matter and nutrients in the soil, whereas fast breakdown speed up the enhanced availability of soil nutrients and consequently plant uptake (Isaac and Nair, 2005). Litter decomposition is carried out by three groups of factors; abiotic variables, litter quality or substrate quality and soil microorganisms (Swift et al., 1979; McClaugherty and Berg 1987; Tripathi and Singh, 1992a and b; Lalnunzira and Tripathi, 2018). These factors are listed below:

1.3.1 Abiotic variables

Various abiotic variables are responsible for decomposition process among them temperature, humidity, precipitation and soil properties strongly affect degeneration of living matter and nutrient mineralization in soil (Anderson, 1988). Temperature can be considered as the leading aspect for decomposition (Meentemeyer, 1978; Hobbie, 1996). There is exponential rise in microbial action with increase in temperature of soil (Kirschbaum, 1995) up to certain limit which favorably affects decomposition rate, however, after this limit it decreases the rate of decomposition. Litter decomposition is influenced by the physical and chemical qualities of the soil. The most important of them is texture, while other important chemical qualities include nutrients, organic matter content, pH, and cation exchange

capacity (Coleman et al., 1999). In the process of litter breakdown, soil moisture and temperature are inescapable elements (Esperschutz et al., 2011).

1.3.2 Litter chemical properties or substrate quality

Substrate quality (litter chemistry) is one of the main determinants of litter decomposition (Hoorman et al., 2003; Rawat et al., 2010) which critically affect the organic matter pool and nutrient availability in the soil. Decomposition of litters can be predicted from two types of initial litter quality; firstly, percentage of weight of different nutrients i.e., Nitrogen, Carbon, Phosphorus etc. and organic constituents specially lignin and; secondly, nutrient ratio like C:N, C:P and Lignin:N (Coulson and Butterfield, 1978; Berg and Staaf, 1980; Tripathi and Singh 1992a and b). Litter decay has been demonstrated to be regulated by following chemical features of litters, such as the litter nitrogen concentration or the C:N ratio (Coulson and Butterfield, 1978; Berg and Staaf, 1980; Taylor et al., 1989; Tian et al., 1992a), phosphorus concentration and/or C:P ratio (Coulson and Butterfield, 1978; Schlesinger and Hasey, 1981; Staaf and Berg, 1982; Berg et al., 1987; Vitousek et al., 1994) and lignin concentration and/or lignin to nutrients ratio (Berg and Staaf, 1980; Melillo et al., 1982; Berg, 1984; Tian et al., 1992b; Van Vuuren et al., 1993; Aerts and De Caluwe, 1997). Lignin content varies from 15-40% in different litters depending on the amount of cellulose and hemicellulose concentrations (Esperschutz et al., 2013). The rate of decomposition differs between species (Cornalissen, 1996; Wardle et al., 1997), owing to leaf hardiness, lignin, nitrogen, polyphenol concentration, the C:N ratio, and the lignin:N ratio, among other factors (Berg et al., 1993; Cadish and Giller, 1997; Perez-Herguindeguy et al., 2000).

Litters with high N concentration and low C:N ratio were found to decompose more swiftly than those with high N concentration and low C:N ratio (Finzi et al., 1998; Berg and Laskowski, 2006; Kamei et al., 2009). Also, according to Sjoberg et al., (2004) higher N content in litters promotes faster decomposition. Other studies showed that litters of higher altitude species are rich in P content and releases P in soil during decomposition (Upadhyay et al., 1989).

1.3.3 Soil microorganisms

The decomposition of organic matter is introduced by a broad range of microorganisms (mostly bacteria, fungus, and actinomycetes) that live in the soil. The action of numerous microbes, which create a variety of enzymes that aid in the decomposition process, causes organic matter to decompose (Perez et al., 2002). Microorganisms are vital in the organizing and stabilizing of soil organic matter, as well as in the immobilizing of N and the preservation of organic C. Soil contains large number of bacteria, one tenth of actinomycetes and considerable number of fungi which is dominant in undisturbed soil (Hoorman, 2011). Microorganisms encompassing both fungi and bacteria have cellulolytic and lignolytic properties. These organisms produce enzymes in surrounding environment and ultimately results in decomposition. Bacteria are vital in the early phases of decomposition, but actinomycetes and fungi are important in the future phases (Ghosh and Tripathi, 2021). There are a number of unknown microbes in soil that affects the rate of litter decomposition. Several studies revealed that $2/3^{rd}$ of the decomposition was carried out by fungi (Kjoller and Struwe, 1992) and residual $1/3^{rd}$ is generally by the action of bacteria and actinomycetes (Person, 1980; Kurihara and Kikkawa, 1986). During the first phases of breakdown of litter, starch and labile substances are degraded followed by hemicellulose and cellulose in next phase and lignin, pectin and polyphenols in final phases (Taylor et al., 1989; Tripathi and Singh, 1992 a and b).

1.4 Litter decomposition and soil fertility

Although litter decomposition is an important part of nutrient cycling, few researches have looked into how it affects soil fertility in Northeast India. Microbial decomposition of leaf litters can help to improve soil quality and solve problems related with acidic soil (Huang et al., 2007). This process is believed to make nutrients which available to plants, enhancing soil quality and contributing to nutrient and water retention in soil (Rodriguez et al., 2001). The amount of litter added to different soil layers impacts the soil's ability to absorb nutrients and water (Cadish and Giller, 1997; Chaubey et al., 1988). Through the process of nutrient cycling, litter breakdown integrates organic carbon into the soil. Decomposition of litters also enhances soil superiority by moving nutrients from above-ground biomass

to the soil (Vitousek and Sanford, 1986), growing the soil's cation exchange capacity and water retention capacity (Argao et al., 2009). The disintegration process also permits Ca, Mg, and K to be released into the soil (Parker, 1983; Villela and Proctor, 2002; Ngoran et al., 2006).

There is a high demand to conserve our ecosystems to benefit the rural population. The practice of shifting cultivation allows the burning of biomass which releases huge amount of carbon dioxide into the air and causes global warming. Present study presumed that the manipulation of litter decomposition may allow the availability of soil nutrients for crop productivity without burning of biomass. As decomposition depends on litter quality, manipulation of litter species can accelerate decomposition process and improve crop productivity in shifting cultivation sites. Further addition of decomposing microorganisms in soil can enhance the course of litter disintegration. The purpose of this research is to find out the combination of litter species and the decomposing microorganisms to produce bio-compost in future that can optimize the process of litter decomposition, soil nutrients and crop productivity in the jhum field.

1.5 Scope of study

Shifting agriculture has a long history of being linked to the culture of Northeast India, notably Mizoram, and this type of agriculture is the primary source of income for the rural inhabitants. Increased human population has resulted in a substantial drop in soil fertility and crop productivity, raising concerns about soil erosion, food security, and pollution in the region. Therefore, technology interventions are required to energizes this form of cultivation to be ecologically sound and economically feasible that can meet various human need with great efficiency through understanding of the organic matter dynamics of locally available resources (like forest litter and agricultural residues) along with factors responsible for their decay. This will be possible through manipulation of organic decay kinetics and nutrient release rates as well as to use forest litter and agricultural residues for optimizing fallow length and synchronizing nutrient accessibility with that of crop nutrient demands to sustain crop production in shifting cultivation sites of Mizoram.

1.6 Objectives

The present work focuses on decomposition of different litters and their associated microbes in shifting cultivation sites to attain the succeeding chief objectives:

1) To determine litter decomposition rates in forest fallows and shifting cultivation sites and to identify the microbial community on decomposing litters.

2) To assess the role of C/N and lignin/N ratio in decomposition kinetics of different litter types and mixed litter (high vs. low litter quality) decomposition and their nutrient release rates in the field as well as laboratory microcosm.

3) To identify key factors influencing natural decomposition of litters in shifting cultivation and to isolate key microorganisms from decomposing litters to develop effective bio-compost.

CHAPTER 2

Review of literature

2.1 Shifting cultivation and its impact

Shifting cultivation was known to be common in temperate zones of Mediterranean and Northern Europe, as well as in Southwestern and Northeastern pine woodlands of North America, during the nineteenth century (Dove, 1983; Warner, 1991; Brookfield, 1994). It was later practiced in Africa, Asia, and Latin America's tropics (Warner, 1989). Shifting cultivation can be found in a wide range of topographic environments, including tropical moist forests, dry tropical forests, savannas, and grasslands (Thrupp, 1997). Slash-and-burn cultivation, also known as 'jhum' or 'jhuming' in Northeast India, is referred to as shifting cultivation. Rotational bush fallow agriculture, also known as swidden farming or slash and burn agriculture, is an old kind of agriculture that is still practiced in many places of the humid tropics (Raman, 2001). In Mizoram, agriculture is the primary source of income. Approximately 80% of the population partakes in this activity (Sati and Rinawma, 2014). Various indigenous populations in this region have been conducting shifting farming for millennia (Nath et al., 2020). According to the paper, shifting agriculture is inextricably linked to the indigenous people's cultural identity. As a result, its significance extended beyond purely economic considerations (Bhuyan, 2019).

In the past, shifting cultivation was considered sustainable due to a longer fallow period; but, in recent years, this practice has proven unsustainable due to a shorter fallow period (Grogan et al., 2012; Nath et al., 2016; Tripathi et al., 2017). The population-driven reduction in the duration of fallow cycles (3–5 years) has also been noted as making this system obsolete and irrelevant (Tripathi and Barik, 2003; Sati and Rinawma, 2014; Nath et al., 2016; Ovung et al., 2021). The purpose of the fallow time in shifting cultivation is to restore soil fertility (which was reduced throughout the harvesting stage due to crop nutrient transfer and leaching) as well as to manage weeds (Nye and Greenland, 1960; de Rouw, 1995; Smith et al., 1999). Shorter cycle jhum fields, according to Sommer et al., (2004) are a soil-nutrient depleting style of cultivation that enhances nutrient losses through runoff and leaching.

This cultivation has been blamed for soil degradation and greenhouse gas emissions (Nath et al., 2020), depletion of soil organic C (Sarkar et al., 2015), climate alteration, biodiversity damage, reduced timber stock, flooding, siltation, and a shift in forest vegetation from primary to secondary and finally grassland (Nath et al., 2020). Deforestation is primarily caused by clearing forests and burning them for shifting agriculture (Monela and Abdallah, 2007; Zahabu, 2008). In Mizoram's steep parts, the loss of vegetation has increased the evidence of soil erosion (Sfeir – Younis and Draggun, 1993; Shoaib et al., 1998). According to Sati and Rinawa (2014), roughly 16.84 metric tons of soil are lost each year. Shifting cultivation has an impact on some chemical aspects of the soil, including pH, macro and micronutrient dynamics, CEC, and soil organic matter (Filho et al., 2013). Several other studies have found a negative impact on soil fecundity and an increase in destruction (Ewel et al., 1981; Kyuma et al., 1985; Andriesse and Schelhaar, 1987a and b; Brand and Pfund, 1998; Nagy and Proctor, 1999; McDonald et al., 2002). Due to shifting agriculture, soil physical and chemical characteristics ranged from 13.3 to 55.1 percent (Junqueira et al., 2016). From above ground biomass, 97 percent and 94 percent of C, 98 percent and 96 percent of N, 90 percent and 63 percent of P, and 45 percent and 70 percent of K, Mg, and Ca were lost owing to burning in 3.5–7 years fallow land (Sommer et al., 2004). According to a similar nutrient loss study conducted in Northeast India, the annual loss (mg ha^{-1} $year^{-1}$) of top soil, N, and K under shifting farmed areas were 58.9, 7.1, and 4 respectively (Nath et al., 2016). During the cropping phase of shifting agriculture, a reduction of 14–20 percent of SOC in top soil has been documented (Bahr et al., 2014), and it takes 35 years to restore the content comparable to that under primary forest (Palm et al., 1996). Further research into the biochemical and biological features of soil microorganisms in shifting cultivated soil revealed a reduced microbial population when compared to soil that had not been subjected to shifting cultivation (Miah et al., 2014). According to several empirical research, shifting agriculture with shorter fallow is the primary cause of poor soil quality (Ranjan and Upadhyay, 1999; Fox et al., 2000; Raman, 2001; Nath et al., 2016; Mishra et al., 2017; Nath et al., 2020). Despite the fact that a variety of shifting agriculture options have been proposed and tested, little progress has been made in developing viable alternatives. As a result, understanding the

dynamics of commonly accessible organic matter, as well as the elements that cause decay, can be a viable alternative to shifting farming. Decomposition and addition of above-ground organic matter have an impact on many soil variables in diverse ecosystems around the world (Silver and Miya, 2001).

2.2 Litter decomposition in soil fertility management

The primary source of land degradation in Northeast India has been identified as shifting farming (Tripathi et al., 2017). In degraded soils, the process of litter decomposition and nutrient release has been frequently reported as a way to control soil fertility and plant productivity (Wapongnungsang and Tripathi, 2017). Litter is an essential element in ecosystem dynamics because it is a good predictor of nutrient cycling and soil fertility (Ochoa-Hueso et al., 2019). The occurrence of a huge number of litters on the forest base has a major impact on the dynamics of the forest ecosystem (Olson, 1963). The nutrient budget in a forest ecosystem is regulated by nutrient reprocessing of plant litters, and litter decomposition plays a central role in that (Vesterclal et al., 1999; Wedderburn and Carter, 1999).

Nutrient release during litter decay is estimated to contribute for 67–87 percent of yearly forest plant requirement (Warning and Schteslnger, 1985). The decomposition of leaf litters produces a larger amount of dissolved organic C compounds in soil, according to Singh and Gupta (1977). Legumes have nitrogen-rich litters that release nitrogen quickly once they have been incorporated into the soil (Melillo et al., 1982; Hector et al., 2000; White et al., 2017). If N mineralization is not synced with growing crops, fast degradation of legume biomass could result in N loss (Crews and Peoples, 2005; Basche et al., 2014). Plants get very little orthophosphate from litter breakdown (Verhoef and Brussaard, 1990). Organic P is mineralized in most cases, according to Mattingly and Williams (1962). Although K and Mg are necessary minerals for higher plants, they hardly limit microbial activity and were swiftly eliminated from decaying litter (Anderson and Ingram, 1989). Guo and Sims (1999), did a similar investigation in a forest, where litter decomposition returned 140 kg ha^{-1} $year^{-1}$ of N and 8.2 kg ha^{-1} $year^{-1}$ of P to the soil surface. As a result, litter breakdown is a significant method of recycling nutrients, particularly C and N, as well as other elements, in ecosystems (Krishna and Mohan, 2017).

Mixed litters were found to have a favorable effect on decomposition rates and promoted increased nutrient return to the soil in several studies (Guo and Sim, 1999; Hartemink and O'Sullivan, 2001; Sariyildiz and Anderson, 2003; Wang et al., 2007; Blesh and Ying, 2020). Despite the availability of numerous researches on decomposition and nutrient release (Okeke et al., 1992; Montagnini et al., 1993; Lehmann et al., 1995; Palm, 1995; Vanlauwe et al., 1995; Byard et al., 1996; Mugundi, 1997), efforts in Northeast India were restricted (Pandey and Singh, 1982; Upadhyay, 1988; Sharma et al., 1997). There is consistent evidence throughout the study that leaf litter decomposition can improve soil nutrients and, as a result, soil fertility. Litter decomposition control is divided into three tiers, which work in the following order: climate > litter substrate quality > soil microbes (Aerts, 1997).

2.3 Abiotic factors and litter decomposition

Soil characteristics such as pH, bulk density, and texture have been found to be closely linked to litter breakdown and thus affect soil nutrient fillings (Delgado-Baquerizo et al., 2015; Pii et al., 2015; Pena-Pena and Irmler, 2016) because they can influence decomposer reproduction and growth (Mueller, et al., 2012; Fanin and Bertrand, 2016). Serna-Chaveza et al., (2013) for example, found that soil temperature and moisture level affect changes in soil microbial biomass and diversity, which affect litter decomposition. Other research has found that soil pH and bulk density influence not just microbial community dynamics and respiratory characteristics, but also soil enzyme activity, which influences litter degradation (Batty and Younger, 2007; Zhang et al., 2018). Furthermore, soil pH, temperature, and ammonia-N content all had a significant impact on decomposition (Giweta, 2020). Changes in environmental conditions may also have an impact on the nutrient exchange between soil and plants (Pena-Pena and Irmler, 2016; Zhang et al., 2018). In addition, soil N concentration, soil C:N and C:P ratios, organic matter content of soil and fungal: bacterial ratio all slowed litter breakdown (Parson et al., 2014; Veen et al., 2015).

Temperature, rainfall, and other microclimatic conditions could all influence the pace of litter decomposition. Several authors (Pant and Tiwari, 1992; Devi and Yadav, 2007; Tripathi et al., 2009) described that the decomposition rate was

deliberate in the winter and profligate during the rainy season, with the main reasons for the higher decomposition rate in the rainy season being the presence of suitable rainfall, suitable moisture, and a higher microbial population. There have been reports of a very increased litter decomposition rate during the wet season (Pant and Tiwari, 1992; Kumar et al., 2010). Despite the obviousness of this statement, the question of which climatic parameter best predicts decay rate is still contested. For example, climate variables are not necessarily a significant component for litter decomposition, according to Meentemeyer (1978), who found that as long as there is water existing in the soil, actual evo-transpiration is the most important factor for decomposition. Many scholars disagreed with this approach (Gillon et al., 1993; Joffre et al., 2001; Magid et al., 2002) and acknowledged that the relationship between evo-transpiration and litter disintegration did not give a trustworthy source of decay rates.

2.4 Litter substrate quality and decomposition

One of the most important elements determining litter decomposition, according to studies, is the quality of the litter substrate. While litter quantity and quality are important for soil C storage and the nutrient cycle (Aerts and Caluwe, 1997; Hattenschwiler and Gasser, 2005), there is indication that litter substrate quality and decay rate (Mannez-Yrzar et al., 2007) have a significant impact on soil nutrients and forest productivity. Litter substrate quality is one of the primary elements that controls litter decomposition, according to several research from various regions (Swift et al., 1979; McClaugherty et al., 1985; Couteaux et al., 1995; Deng et al., 2016; Zhang et al., 2016). Plant litter's chemical composition is often used to determine its quality (Strickland et al., 2009). The quantity of nutrients (C, N, P, K, etc.) and complex cells (lignin, hemicellulose, and cellulose) existing in litter that regulate decay and nutrient exchange in soil were used to define litter quality (Swift et al., 1979). Since the litter nutrients were directly and correspondingly provided to the soil, variations in substrate quality are well documented to alter and alteration the soil C, N, and P content (Xu et al., 2013). The most important factors of litter breakdown rates are lignin and N (Miller et al., 1936; Minderman, 1968; Fogel and Cromack, 1977; Meentemeyer, 1978; Gartner and Cardon, 2004). The

amount of N in the litter influenced its breakdown. Litter chemical markers include the C:N ratio, P, Ca, lignin:N ratio, and other chemical indicators (Aber et al., 1990; Aerts, 1997). Researchers also discovered that the C:N and lignin:N ratios could predict the breakdown of leaf litters (Melillo et al., 1982). Litters with lower C:N and lignin:N ratios degraded at a faster rate in general (Ostertag and Hobbie, 1999; Wang et al., 2010; Esperschutz et al., 2013). However, a high lignin concentration had a substantial adverse impact on the rate of litter mass loss (Fogel and Cromack, 1977; Berg et al., 1982; Morphy et al., 1998; Osono and Takeda, 2004 and 2005). Lignin level fluctuates between 15 and 40% depending on the species, but owing to differences in syringyl and guaiacyl concentration in litters, lignin content can range from 5 to 50% in some circumstances (Espuschutz et al., 2013). It's also possible that the amount of cellulose and hemicellulose in the litter varies by species (Akpor et al., 2006; Wegener et al., 1983). The rate of decomposition was greatly influenced by such changes in species.

In general, high-quality leaf litters would disintegrate faster than low-quality litters initially. Several studies found differences in decomposition rates among species with varying litter quality (Adamas and Angradi, 1996; Cornelissen, 1996).

2.5 Microorganisms and litter decomposition

Microbial activity has been shown to change the chemical conformation of litter and influence C and N dynamics in soil (Berg and McClaugherty, 2008). Microorganisms' enzymatic activities serve a critical role in litter decomposition and soil formation (Aber and Mellillo, 2001). Algae, actinomycetes, bacteria, and fungi are among the principal soil fauna and microbes linked with litter breakdown (McCarthy, 1987; Schaefer and Schauermann, 1990), and the organization of these microorganisms influences the pace of litter decomposition (McCarthy, 1987; Crawford, 1988). The functional role and metabolic actions of microbial groups are directly related to litter breakdown, nutrient mineralization, and soil fertility (Swift et al., 1979; McCarthy, 1987). Furthermore, the composition of microorganisms has a significant impact on the litter decomposition process, including physical breakdown of litters, organic matter transfer to nutrients, and CO_2 release into the atmosphere (McCarthy, 1987; Dilly et al., 2004; Schinner et al., 2012). Despite the fact that their

roles and mechanisms in the litter disintegration process differ (Giweta, 2020), fungus and bacteria are the primary motors in the process (Swift et al., 1979; McCarthy, 1987).

During the breakdown phase, bacteria using a labile C supply appear to prefer the N-fixation approach (Treseder et al., 2011). Fungi, on the other hand, are important because they may degrade compounds formed from high lignin polymers and humus (Aponte et al., 2010a and b; 2012) and transmit N and C through litter levels via colonizing leaf litters (Vivanco and Austin, 2008; Laganiere et al., 2010). Fungi are also recognized for cellulose and hemicellulose breakdown (Berg and McClaugherty, 2014). Cellulose degrading bacteria were less common, with a prevalence of 4% to 5%. (Tlaskal et al., 2016). Actinomycetes have the ability to breakdown lignocellulose complexes in a variety of ways (Eriksson et al., 1990). Some decomposing microbes have a specific affinity with certain plant species and have evolved to break down their litter (Brown, 1995; Vivanco and Austin, 2008). Fungi decrease and bacteria increase as soil fertility rises, hence the bacterial community structure dictates the soil fertility gradient (Pennanen et al., 1999).

Over the course of litter breakdown, the bacterial community displayed a succession of colonizers. Pseudomonadota, Actinomycetota, and Bacteroidota were the most prevalent species discovered throughout the succession (Urbanova et al., 2015; Purahong et al., 2016; Tlaskal et al., 2016). Phyllosphere bacteria are only existing in the early stages of decomposition, and they are quickly replaced by taxa that produce proteolytic and cellulolytic enzymes (Purahong et al., 2016; Tlaskal et al., 2016). N-fixing bacteria, according to Purahong et al., (2016) and Tlaskal et al., (2016), can help to enhance N availability. The variety of the fungal community in litters increased from the early to intermediate phases (Voriskova and Baldrin, 2012; Purahong et al., 2016). In the second stage, fungi and phyllosphere are replaced by taxa that can utilize cellulose, as detailed in enzymatic characterization, where endo-cellulase and endo-xylanases were the most abundant enzymes (Fioretto et al., 2000; Snajdr et al., 2011). In the late phase, a higher percentage of Basidiomycota is detected. These bacteria can break down lignin and humic acids (Voriskova and Baldrin, 2012; Purahong et al., 2016). Plant growth-promoting characteristics were

discovered in actinomycetes from a shifting cultivated area (Momin and Tripathi, 2019).

Microorganisms create a number of enzymes that aid in the breakdown process (Perez et al., 2002). Previous enzymatic studies on bacterial species revealed cellulase (Rajagopal et al., 2007; Sethi et al., 2013), catalase (Eisenberg et al., 2015; Babiker et al., 2016), and protease (Eisenberg et al., 2015; Babiker et al., 2016) activity (Eisenberg et al., 2015; Pant et al., 2015). Proteolytic enzyme activity was also observed in a number of fungus taxa (Giudice et al., 2012; Debi and Prakash, 2017). As a result, the abundance and activity of decomposers dictate the rate at which leaf litter decomposes (Akpor et al., 2006; Perez et al., 2009; Glebelmann et al., 2011).

2.6 Use of microbial inoculants in bio-compost preparation

Microorganisms are said to decompose organic matter in soil, resulting in the creation of intermediate metabolites (Zucconi et al., 1981). Composting is thus a method of getting a stable product by biological oxidative change comparable to that which occurs naturally in soil (Bertoldi et al., 1982). Composting is divided into numerous stages, each of which is distinguished by the activity of various microbial communities (Gray et al., 1971; Gray and Biddlestone, 1974; Finstein and Morris, 1975; Bertoldi et al., 1979). Compost is made by decaying plants and recycling organic materials in order to improve soil fertility and save farmers money on chemical fertilizers (USEPA 1971). Composting improves soil pH, EC, organic matter, Ca, Mg, K, and P, but the C:N ratio is reduced (Sarwar et al., 2003). Microbial inoculants allow for a faster and more efficient conversion of raw organic waste to compost. It also ensures that healthy bacteria are used to remediate organic waste (Ltibke, 2016). Bacillus sp. and Pseudomonas sp. microbial inoculants have been demonstrated to improve the composting process (Pan et al., 2012). Pretreated straw with microbial inoculants increases lignocellulose degradation and reduces cellulose and hemicellulose content by 8–18% and 20–23%, respectively, improving compost quality (Wu et al., 2019).

CHAPTER 3

Materials and Methods

3.1 Description of site

Mizoram is one of the eight sister states of northeast India. Mizoram is extended between 21º 58'- 24º 35' N and 92º 15'- 93º 29' E and covered an area of 21,087 km^2. It lies in east part making international borders with Bangladesh in west and Myanmar in east Manipur in the northwest and Assam and Tripura in north and northwest bound it. The state is characterized by various climatic conditions due to variations in altitude and vegetation. Mean minimum and maximum annual air temperature varied from 12ºC - 30ºC and total annual rainfall was 2061 mm during the study period. The state of Mizoram has been reported to have about 86.27% of the area under forest cover (ISFR, 2017).

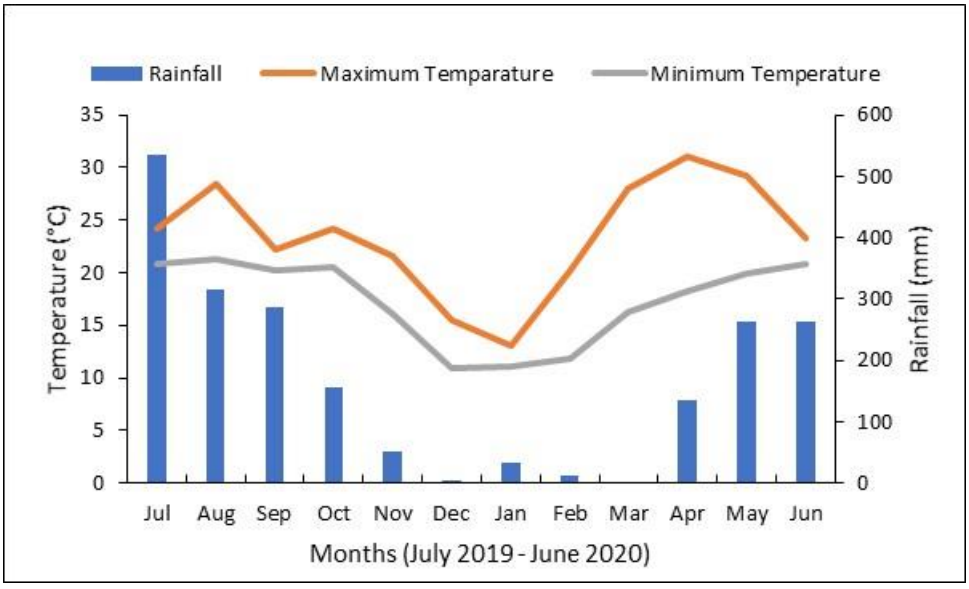

Fig. 3.1 Monthly rainfall, maximum and minimum temperature during July 2019 – June 2020 (Meteorological data of Mizoram 2019 and 2020, Directorate of Economics and Statistic, Government of Mizoram).

The study was conducted in Tanhril, Mizoram on jhum fields and forest fallows. Two jhum fields with fallow periods 3 years (23º 39´ 55´´ N and 92º 33´ 24´´ E) (JF3) and 8 years (23º 43´ 55´´ N and 92º 40´ 4´´ E) (JF8) were selected. The ages of the fallow lands were identified by interrogating with the land owners. Similarly, two

forest fallows have also been selected, e.g., 3 years (23º 44´ 48´´ N, 92º 31´ 48´´E) (FF3) and 5 years (23º 44´ 55.25´´ N and 92º 38´ 36.68´´ E) (FF5) for the study and the ages of the forest fallows were determined by observing the growing of forests vegetation and interviewing the land owner.

Tanhril is roughly 15 km from Aizawl, the capital city of the state. The climate of the area is subtropical, with significant seasonal differences (20-30 ºC in summer and 11-21 ºC in winter). The soil at the research sites was acidic in reaction. The soil was classified as red belonging to the order inceptisols with light to medium texture having a slope of 35º-40º (Hauchhum and Tripathi, 2017).

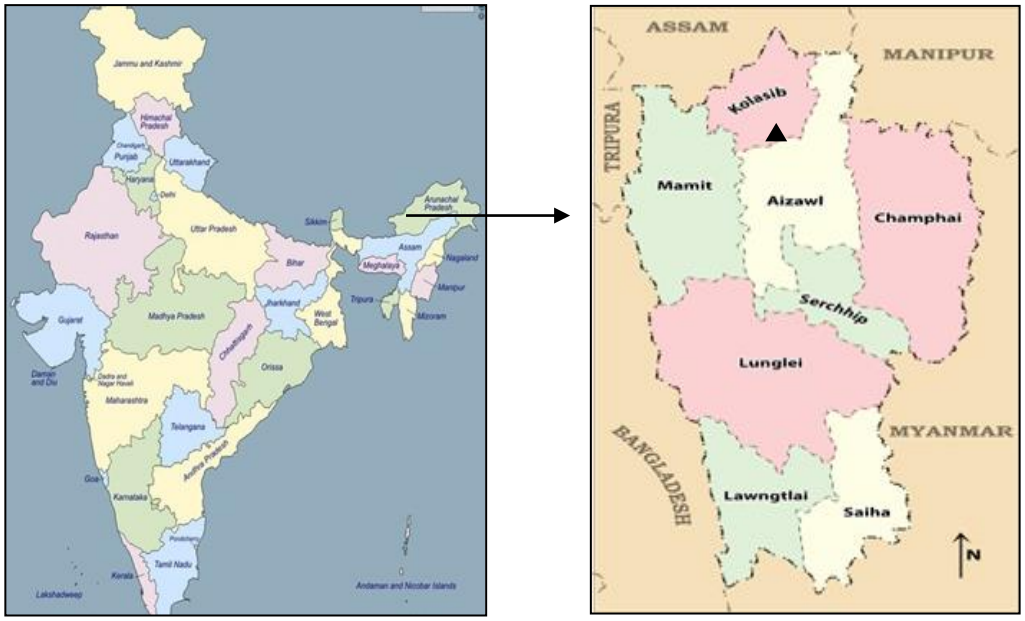

Fig. 3.2 Geographical map of India and the state of Mizoram showing the Aizawl district and the study sites.

3.2 Sampling and experimental design

3.2.1 Field experiment design

Senesced bamboo leaves, mixed forest leaves and mixed grasses were collected from two forest fallows (FF3 and FF5) and leaf litters of *Tephrosia candida* and *Oryza sativa* were collected after slashing of vegetation from JF3 and JF5 fields. All the litters were brought to the laboratory, air-dried and 10g of each litter type were enclosed in nylon net bags measuring 20 cm x 20 cm with 2 mm mesh size.

For forest fallows (FF3 and FF5), a total of 150 litter bags (50 bags for bamboo leaf, 50 bags for mixed forest litters and 50 bags for mixed grasses) were prepared. Out of total litter bags, 100 litter bags (50 for bamboo leaf litter and 50 mixed forest leaf litter) were placed in FF5 and remaining 50 litter bags of mixed grass litter were placed in FF3 at 4 random locations. On the other hand, for jhum fields leaf litter bags of *T. candida, O. sativa* alone and in combination (*T. candida* + *O. sativa* in equal proportions) were prepared. Overall, 50 litter bags of each litter type were positioned at 4 random locations on soil of two jhum fallow (i.e., JF3 and JF8). Litter bags on both forest fallows and jhum fallows were placed in July 2019. Every month four litter bags for each litter type from four sites (2 forest fallows and 2 jhum fallows) were recovered for one year period. A total of 12 retrievals were made for each litter type at every site.

Litter bags were carried to the laboratory and properly rinsed in running water to eliminate any soil particles that had adhered to them. Litters were dried individually for 24 h at 65°C in a hot-air oven to achieve a constant weight. The dried litters' weight was measured. Dry litter samples were crushed and sieved through a 1.5 mm mesh screen before being stored for subsequent analysis.

3.2.2 Microcosm experiment design

Soils were collected from jhum field and brought to the laboratory for setting pot experiment under laboratory condition. Microcosms were made from polybags (15 cm in diameter and 20 cm in height) with sealed bottom and pierced with 0.5 cm holes on sides of polybags for draining out the excess water. A total 300 microcosms (150 with JF3 soil and 150 with JF8 soil) were prepared with 3kg of soil from each jhum field. Similar leaf litter types were used as described in field experiment design on jhum field in section 3.2.1. In each microcosm, 10 g litter samples were directly placed on top soil of microcosm without any litter bags. Hand held mist sprayer was utilized for retaining soil moisture on alternate days with known volume of water. The replicated (4) litters were retrieved at monthly interval for one year. Sampling design was similar to that as explained in section 3.2.1 above.

3.3 Computation of litter decomposition rate

The weight of dried litter samples was recorded at monthly interval and litter decomposition rate was calculated by comparing the difference in the weight of two retrieval dates. The relative decomposition rate (RDR) was calculated by using formula:

RDR ($g\ g^{-1}\ day^{-1}$) = $ln\ (W_t - W_i)/\ (t_1 - t_i)$

Where W_i = mass of litter present at time t_i, W_t= mass of litter at time t_1, and $t_1 - t_i$ = sampling interval (days).

The daily instantaneous decayed constant (*k*) of litter was calculated through the negative exponential decay model of Olson (1963):

$W_t/W_i = exp^{(-kt)}$

Where W_i = initial mass of the litter, and W_t= mass remaining after time t.

As suggested by Olson (1963), the time required for 50% mass loss was calculated as, $t_{50} = 0.693/k$ and for 95% mass loss as $t_{95} = 3/k$.

3.4 Analysis of carbon and nutrients in soil and litter samples

Elements like Carbon, Nitrogen, Phosphorus, Potassium, Magnesium and Calcium were analyzed from collected soil and litter samples. The detail methods for the analysis of various elements are given below:

3.4.1 Soil carbon and nutrient analysis

Every month soil (0-10 cm) beneath the litter bags was recovered. Sampling design and replications were also similar to that of litter bags as described in section 3.2.1. Further, **s**oil samples were pooled in the group of 4 replicates per litter type for different plots. Soil samples were dried for 48 h in a hot-air oven at 80°C until they reached a consistent weight. For nutrient analysis, the samples were crushed, sieved (0.05 mm screen), and placed in zip lock bags. Soil pH, Organic Carbon (OC), Total Nitrogen (TN), Available Phosphorus (AP), Exchangeable Potassium (EP), Magnesium (Mg) and Calcium (Ca) were analyzed (Allen et al., 1989).

3.4.2 Litter carbon and nutrient analysis

The elements were determined using dried and powdered litter samples. The loss on ignition method was used to determine the C content of litters (Mcbrayer and Cromack, 1980). Total nitrogen was identified by micro Kjeldahl method (Jackson, 1973). Lignin content in litter sample was analyzed by acid detergent fiber method (Van Soest and Wine, 1968). Phosphorus, potassium, magnesium and calcium were analyzed by digestion in concentrated tri-acid mixture. Phosphorus was measured calorimetrically through ammonium molybdate blue color method using spectrophotometer and potassium, magnesium and calcium by Atomic Absorption Spectrophotometry (Allen et al., 1989).

3.5 Sampling and analysis of decomposing litters for microbes

Litter samples were collected and microbes were isolated and identified.

3.5.1 Collection of litter samples

Decomposing litters from jhum fields and microcosms were collected at monthly interval and brought to the laboratory. Litter samples were stored in 4ºC for further use (Kasa et al., 2015). The samples were used for microbial analysis within one month of collection.

3.5.2 Sterilization of glass wares

For 12 hours, all glass wares (Petri plate, test tubes, slides, conical flask, inoculum needle, measuring jar, and beaker) were immersed in a cleaning solution. They were then rinsed in soapy water and plain water. These were again washed with distilled water and dried. In an autoclave, the dried glass wares were sterilized at 120 ºC for 15 minutes. The autoclave was used to sterilize all chemical media.

3.5.3 Microbial population count

Microorganisms were isolated from the decaying litters by serial dilution method. Six sterilized test tubes were taken and the first test tube was filled with 10 ml of 0.9% NaCl and remaining test tubes were filled with 9 ml of saline solution. Crushed litter sample (1 g) was dissolved in first test tube and its dilution factor

becomes 10^{-1}. Then 1 ml of the sample was moved from first test tube (10^{-1}) to the second test tube and its dilution factor become 10^{-2}. Similarly, samples were relocated to the remaining test tubes and dilution factor obtained as 10^{-3}, 10^{-4},10^{-5}, and 10^{-6} (Martin, 1950). Then aliquots of 0.1 ml (100 µl) from each dilution was taken and transported to distinct Nutrient Agar (NA) media plate, Potato Dextrose Agar (PDA) plate and Starch Casein Agar (SCA) plate and spread with a L-spreader cautiously which is then incubated at 37±2ºC, 24±2ºC and 28±2ºC for 24 h, 48 h and 72 h for bacteria, fungi and actinomycetes respectively.

Plates were observed after incubation and the number of colonies that had developed was counted under colony counter. The population of microbes was observed and expressed as CFU/g.

Colony Forming Unit (CFU/g) = (Number of colonies X Dilution factor) / (Volume of culture plate)

3.5.4 Isolation of microbial pure culture

Using sterile toothpicks, representatives of bacteria, fungi, and actinomycetes were randomly selected from their growth plates and streaked on their respective media plates to generate pure microbe cultures. The isolates were incubated at their respective temperature and time as mentioned in section 3.5.3. For obtaining pure culture, NA plate was supplemented with Nystatin and Cycloheximide, PDA plates were supplemented with Rose Bengal, Amoxyline and SCA plates were supplemented with Nystatin, Nalidixic acid and Cycloheximide. All these antibiotics were used in amount of 50 µg/ml.

3.5.5 Screening of microbes for enzyme activities

Qualitative enzyme activities of isolated microbes were carried out by following standard method of Kumar et al., (2015). Five enzyme tests were done during the study.

Catalase test: Catalase activity of microbes was identified by using 3% H_2O_2. Pure culture of microbial isolates was transferred by using a sterile loop from their respective growing media to a clean slide and 3% H_2O_2 was added over the culture

on the slide. Immediate and vigorous effervescence was presumptive confirmation of catalase activity in microbes.

Amylase test: The production of amylase enzyme by isolated microbes was estimated by growing the pure microbial isolates on starch agar plates separately. After the microbial growth, the plates were flooded with Gram's Iodine. The clear zone observed on starch agar plates after flooding indicated the confirmation of amylase activity as the microbes were capable of hydrolyzing and iodine does not stain in absence of starch.

Protease test: Pure microbial isolates were inoculated on skim milk agar plates and incubated at different temperature as mentioned in section 3.5.3 for the growth of three different microorganisms. After incubation period, a clear zone around the colony indicated that the isolate was protease producer.

Lipase test: Lipase production by microorganisms was determined by growing the pure microbial isolate on tributyrin agar. A clear region around the growing colony was the possible confirmation of lipase production by microbial isolates.

Cellulase test: Qualitative cellulase activity of isolated microbes was determined by using 1% Congo red indicator. Pure microbial isolates were inoculated on Carboxy Methyl Cellulase (CMC) agar plates and incubated. After the microbial growth on CMC agar plates, it was flooded with 1% Congo red indicator for 1-2 mins and washed with 1 M NaCl solution. A halo zone around the colony was considered positive for cellulase production.

3.5.6 Morphological and microscopical characterization

Aerial mycelia, spore distinctiveness, colony color, and other morphological and microscopic properties of microorganisms with positive enzyme activity were investigated. Gram staining process was used for bacteria and actinomycetes whereas lactophenol and cotton blue was used for fungal staining. Bergey's Manual of Determinative Bacteriology (2000) was used for recognizing bacteria and actinomycetes and Gliman's Manual of Soil Fungi (1957) was used for identification of fungi up to genus level.

3.6 Bio-compost preparation

Biofertilizer containing the isolated and identified decomposing microbes were applied in pot experiment to assess the effect of microbial population on litter decomposition process.

3.7 Statistical analysis

Data was recorded into a computer spreadsheet and thereafter imported into a statistical package. Mass loss of litters was correlated with various abiotic parameters (i.e., rainfall, temperature, humidity) for all sites separately. Regression equations were developed for the variables having significant correlation. Further, to understand the effect of different environmental variables (i.e., rainfall, humidity, air temperature, soil temperature, soil carbon, soil nitrogen and different microbes) on litter decomposition, stepwise multiple correlation (R) analysis was performed. The one-way analysis of variance (ANOVA) was performed between the number of days in decomposition and their nutrients (C, N, C/N, and AP) in soil followed by the Least Significant Difference (LSD, $p<0.05$) test using MS Excel.

CHAPTER 4

Results

4.1 Soil physico-chemical properties of forest and jhum fallows

4.1.1 Forest fallows

Soil pH (4.82), BD (0.95 g cm^3), C (2.97%) and N (0.15%) concentrations were higher in FF5 as compared to FF3 (Table 4.1). Similarly, AP was higher (13.46 µg/g) in FF5 compared to FF3 (12.39 µg/g). In contrast, C/N ratio was higher in FF3 (24.48) compared to FF5 (19.49) (Table 4.1). The mean annual soil temperature was 23.06 ºC in FF3 and 19.6 ºC in FF5 (Fig 4.1).

4.1.2 Jhum fallows

Soil chemical characteristics like pH, C, N and AP were higher in JF8 compared to JF3 (Table 4.1). However, soil C/N ratio was higher in JF3 (20.9) compared to JF8 (17.6) (Table 4.1). Mean annual soil temperature was 23.9 ºC in JF3 and 21.5 ºC in JF8 (Fig. 4.1).

Table 4.1 Soil physico-chemical properties of forest and jhum fallows before the start of the experiment.

Site	pH	C (%)	N (%)	C/N ratio	AP (µg/g)	BD(g cm^3)
FF3	4.35±0.003	2.77±0.01	0.11±0.003	24.48±0.71	12.39±0.14	0.89±0.03
FF5	4.82±0.06	2.97±0.03	0.15±0.008	19.49±0.91	13. 46±0.14	0.95±0.01
JF3	4.37±0.02	1.17±0.01	0.056±0.003	20.9±18.6	11.8±0.14	1.11±0.03
JF8	4.47±0.01	1.19±0.008	0.07±0.01	17.6±3.35	15.7±0.4	1.3±0.02

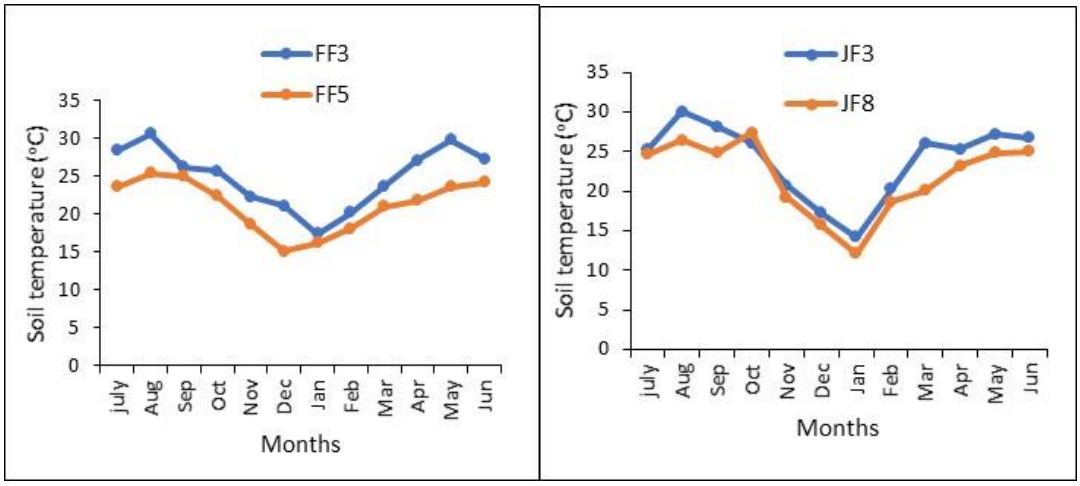

Fig. 4.1 Monthly variations in average temperature of soil in 3 years (FF3) and 8 years (FF8) forest fallows and 3 years (JF3) and 8 years (JF8) jhum fallows during study period (July 2019-June 2020).

4.2 Litter substrate quality

4.2.1 Initial litter nutrient concentrations

In Jhum fallows, litter quality (C, N, C/N, lignin and other nutrients) in different litters (TC, OS and TC+OS) varied considerably (Fig 4.2). The N concentration in different litter types varied from 1.56-4.18%. Carbon (41.6%) and lignin (21.9%) concentrations were significantly higher in OS litter as compared to TC litter (36.7% and 9.6%, respectively). Initial K concentration in TC+OS litter was significantly lower (0.26 mg/g) than OS litter (0.43 mg/g). However, P content ranged from 0.07 - 0.1 mg/g with maximum value in TC and minimum in OS. Maximum concentration of Mg and Ca was observed in TC (0.388 mg/g and 2.64 mg/g) and minimum in TC+OS (0.288 mg/g and 2.43 mg/g, respectively). Maximum C/N and lignin /N was found in OS (26.6 and 14.03) followed by TC (8.77 and 2.29, respectively).

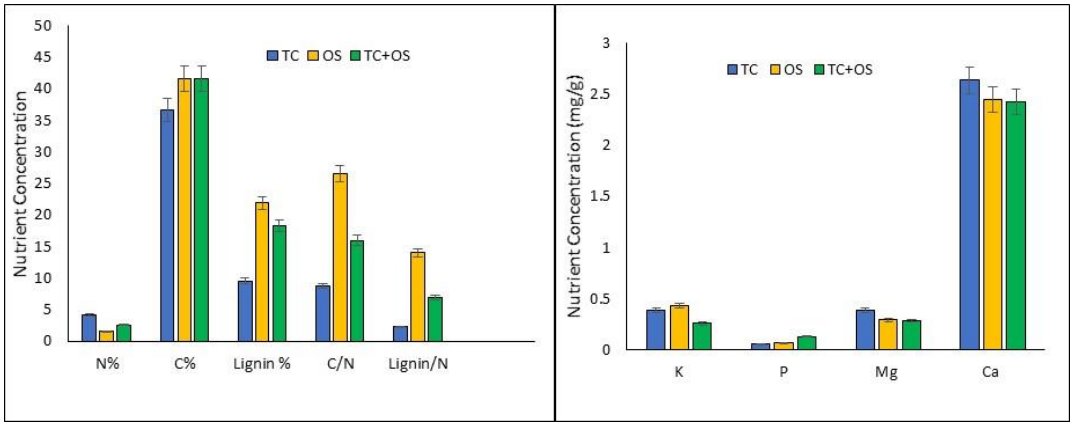

Fig. 4.2 Initial concentration of Nitrogen (N), Carbon (C), Potassium (K), Phosphorus (P), Magnesium (Mg), Calcium (Ca), Lignin, C/N and Lignin/N ratio in *Tephrosia candida* (TC), *Oryza sativa* (OS), *Tephrosia candida* + *Oryza sativa* (TC+OS) litters. Vertical lines on each bar show ±1SE.

4.2.2 Litter and elements mass remaining during litter decomposition under field experiment

Values of litter mass remaining in the field after every month till the end of the study is given in Fig. 4.3-4.5. In JF3 mass remaining for TC litters were 3.99%, however, TC litters decomposed completely at the end in JF8. Similarly, in JF8, OS litter also decomposed completely at the end, whereas, in JF3, mass remaining was 12.97%. For TC + OS litters 11.83% mass was remained in JF3 and 7.17% in JF8.

Mass remaining of elements (C, N, K, P, Mg and Ca) during the one-year decomposition period in two fields JF3 and JF8 are provided in Fig. 4.6-4.11. At the end of the study, element mass remaining in TC in JF3 ranged from 5-9% (Fig. 4.6-4.7). For example, C, N, K, P, Mg and Ca remains were: 9.29, 8.72, 5.50, 5.86, 8.99 and 8.76, respectively at the end the year. However, in OS litter decomposition, percent mass remaining for different elements at the end of the study were: 11.80, 12.93, 8.44, 9.63, 12.3 and 12.12, respectively for N, C, K, P, Mg and Ca (Fig. 4.8-4.9). In TC+OS, percent mass remaining for various elements during the study were 11.28 for N, 11.79 for C, 11.2 for K, 10.46 for P, 11.04 for Mg, 10.80 for Ca (Fig. 4.10-4.11).

In JF8, element mass remaining at the end of experiment in TC litter were: 4.9% for N, 1.54% for C, 1.54 for K, 1.4% for P, 5.08% for Mg and 4.9 for Ca (Fig. 4.6-

4.7). During the decomposition of OS litters, the element mass remaining for N, C, K, P, Mg and Ca were 5.02, 5.97, 1.96, 3.44, 5.45 and 5.31 percent respectively (Fig. 4.8-4.9). However, percent of element mass remaining at the end for TC+OS litter decomposition were: N (9.14), C (9.86), K (9.14), P (8.46), Mg (9.18) and Ca (8.97) (Fig. 4.10-4.11).

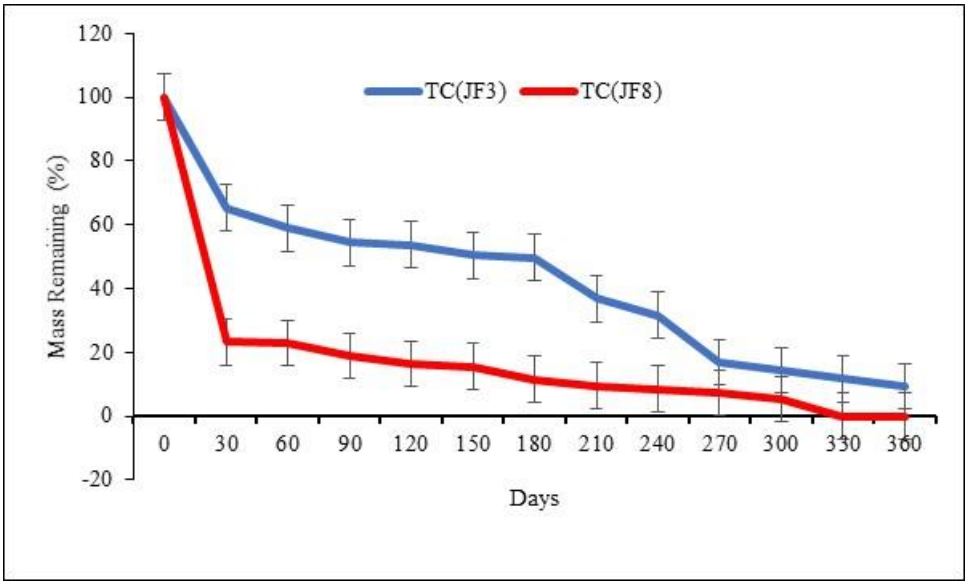

Fig.4.3 *Tephrosia candida* (TC) litter mass remaining in 3 years (JF3) and 8 years (JF8) jhum fallows. Vertical lines show ±1SE.

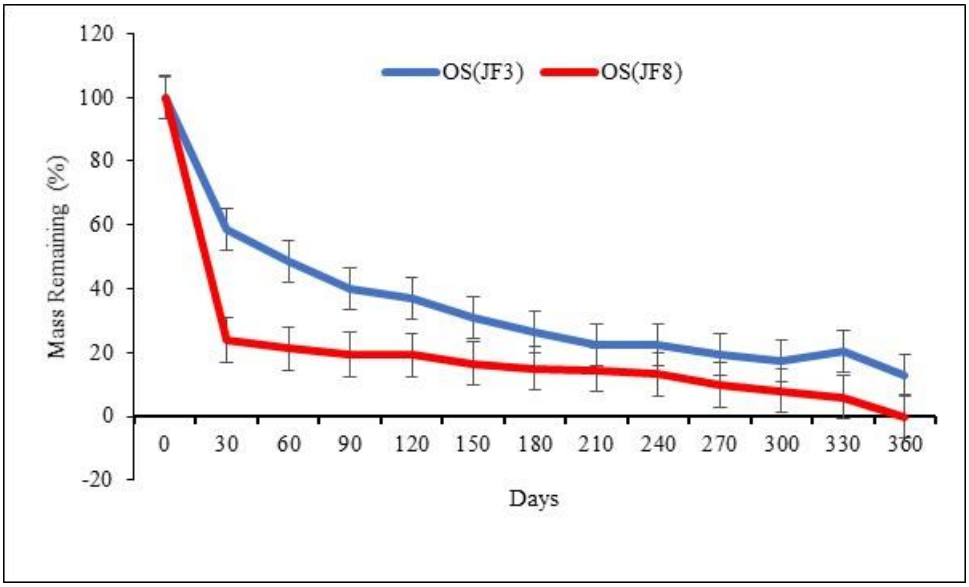

Fig. 4.4 *Oryza sativa* (OS) litter mass remaining in 3 years (JF3) and 8 years (JF8) jhum fallows. Vertical lines show ±1SE.

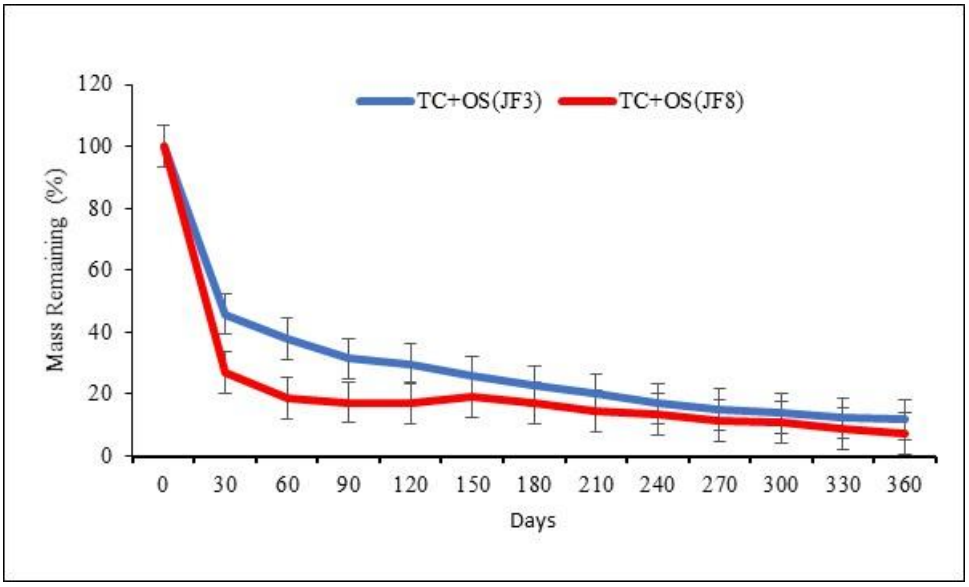

Fig. 4.5 *Tephrosia candida* + *Oryza sativa* (TC+OS) litter mass remaining in 3 years (JF3) and 8 years (JF8) jhum fallows. Vertical lines show ±1SE.

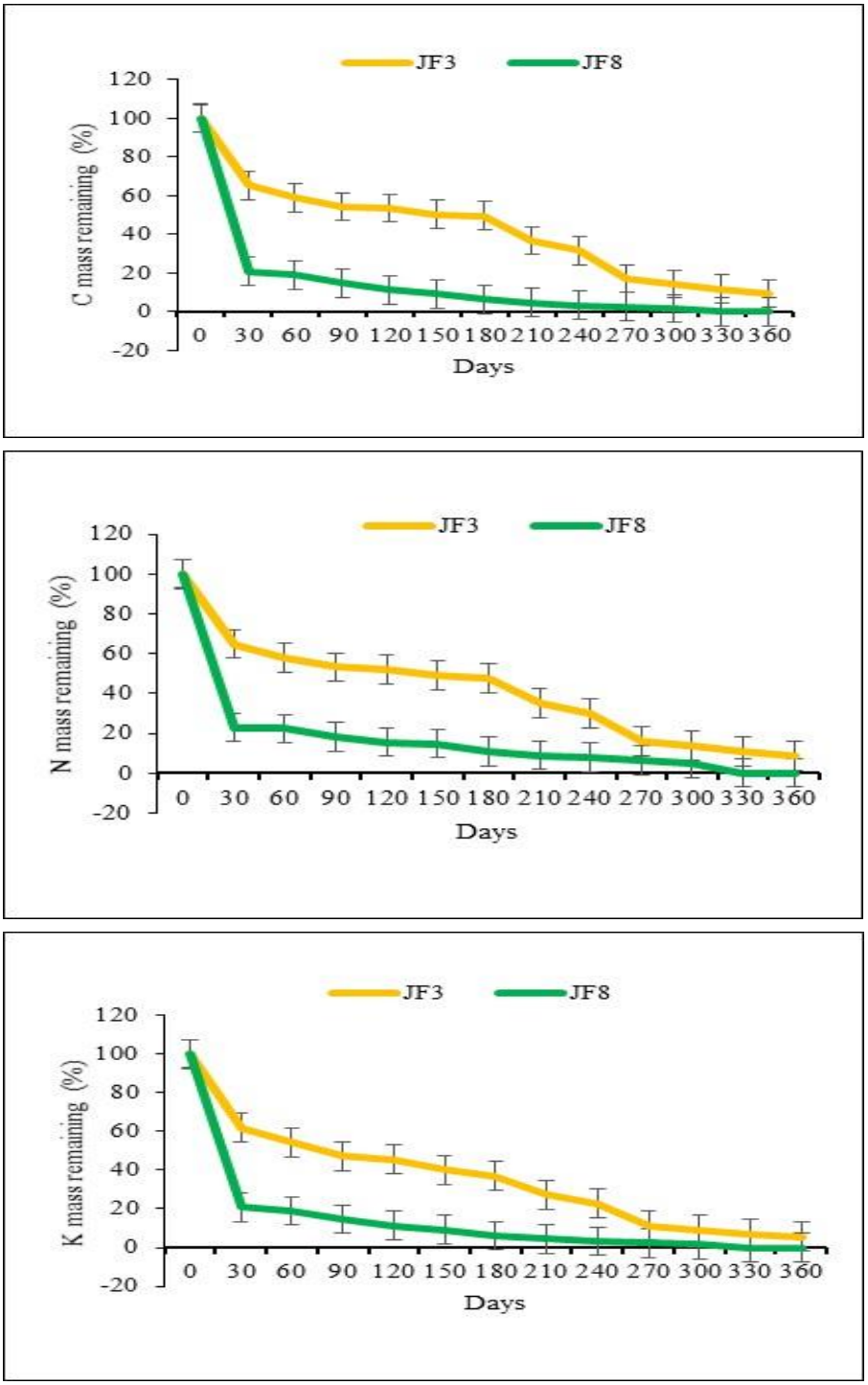

Fig.4.6 Changes in percent elements (C, N and K) mass remaining in *Tephrosia candida* litters during the course of decomposition in 3 years (JF3) and 8 years (JF8) jhum fallows. Vertical lines show ±1SE.

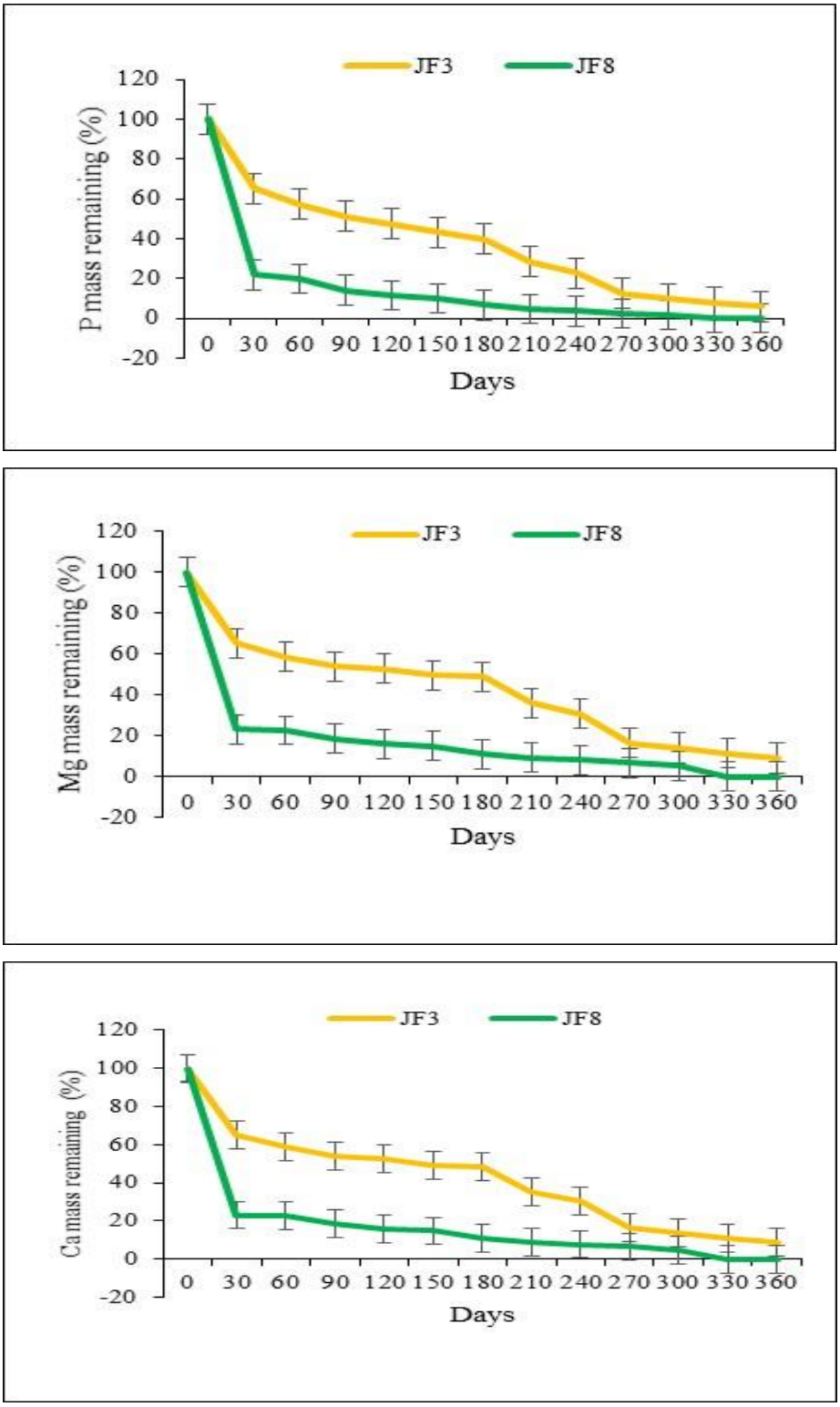

Fig.4.7 Changes in percent elements (P, Mg and Ca) mass remaining in *Tephrosia candida* litter during the course of decomposition in 3 years (JF3) and 8 years (JF8) jhum fallows. Vertical lines show ±1SE.

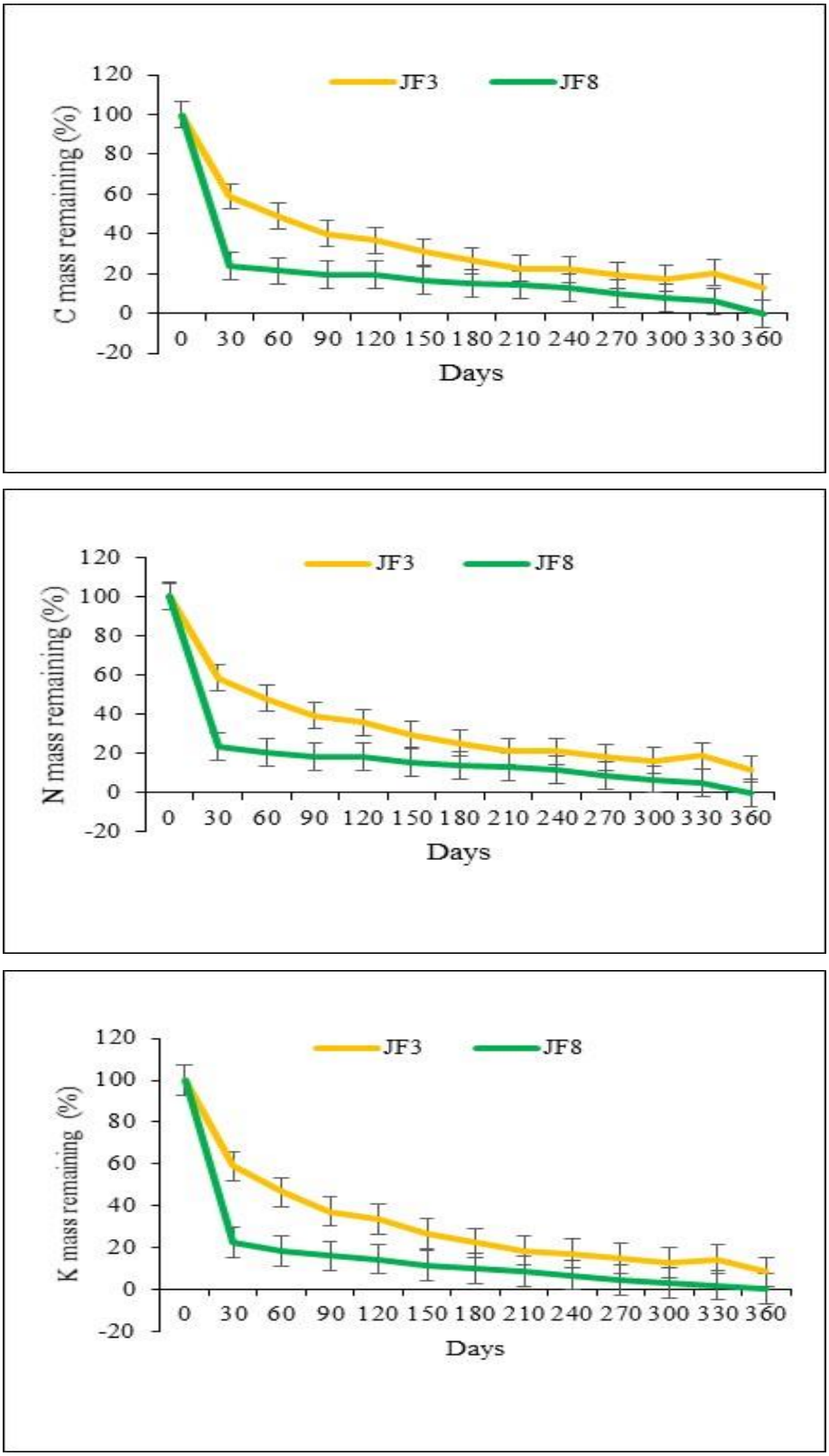

Fig.4.8 Changes in percent element (C, N and K) mass remaining in *Oryza sativa* litter during the course of decomposition in 3 years (JF3) and 8 years (JF8) jhum fallows. Vertical lines show ±1SE.

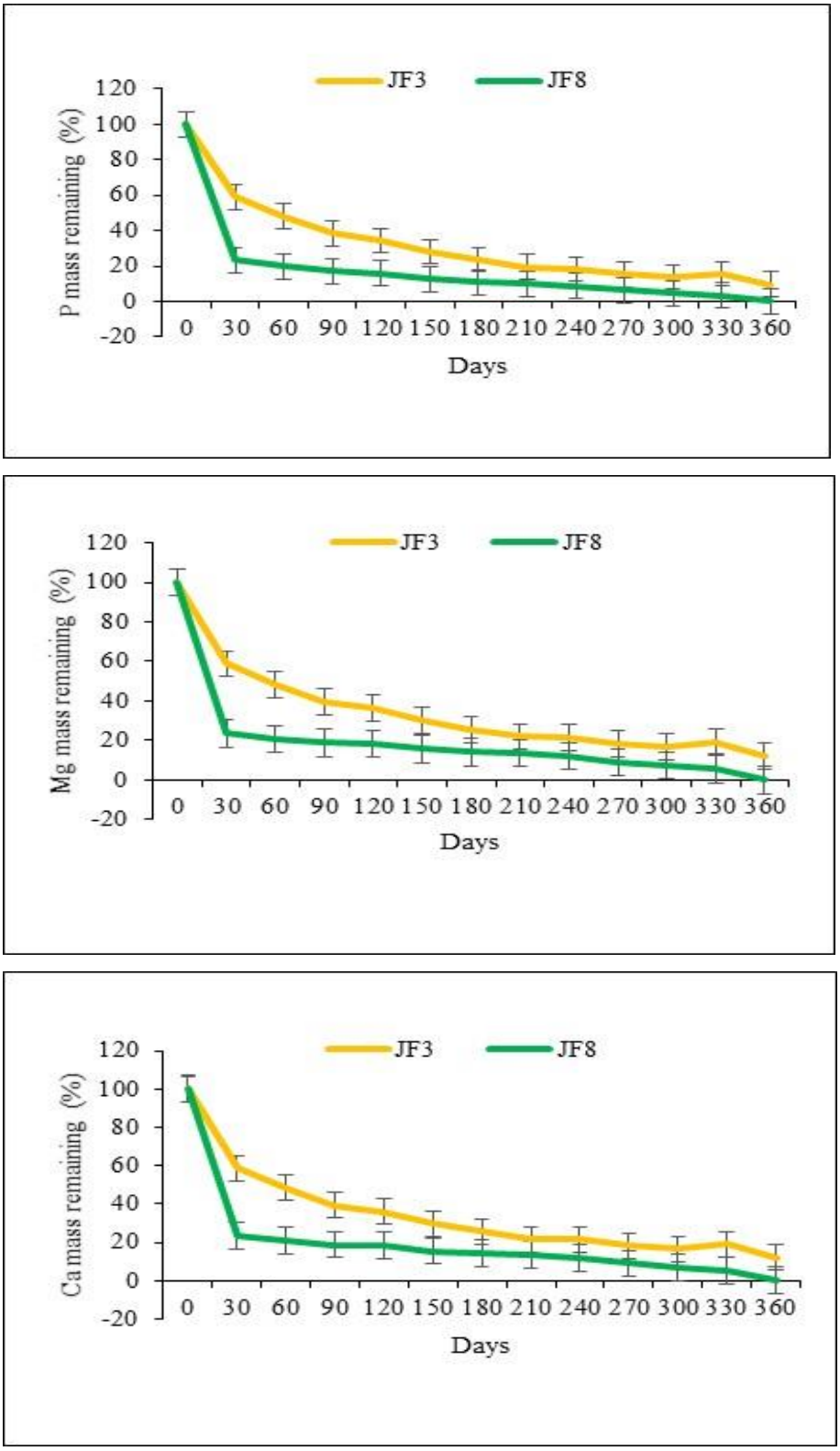

Fig.4.9 Changes in percent element (P, Mg and Ca) mass remaining in *Oryza sativa* litter during the course of decomposition in 3 years (JF3) and 8 years (JF8) jhum fallows. Vertical lines show ±1SE.

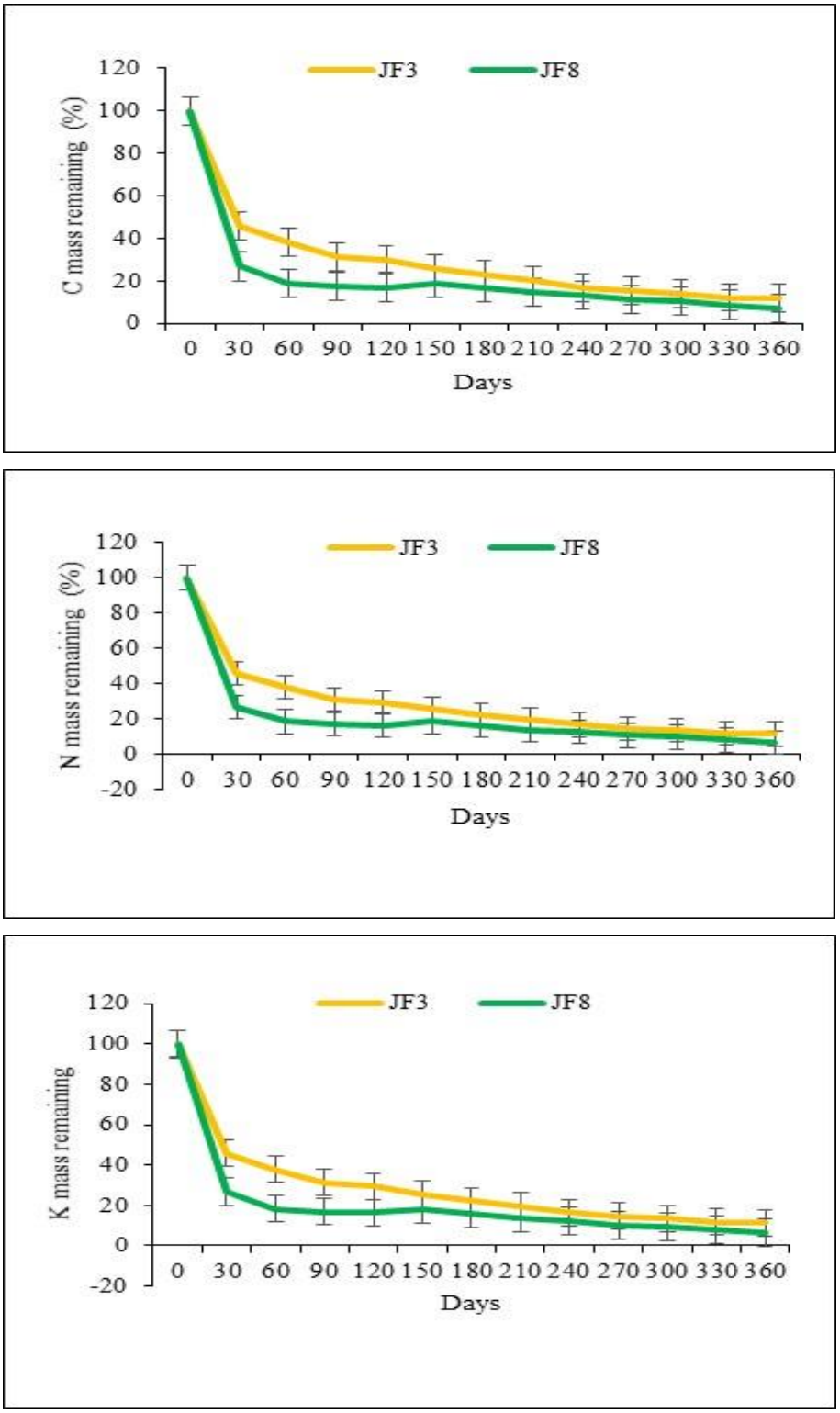

Fig.4.10 Changes in percent element (C, N and K) mass remaining in *Tephrosia candida +Oryza sativa* litter during the course of decomposition in 3 years (JF3) and 8 years (JF8) jhum fallows. Vertical lines show ±1SE.

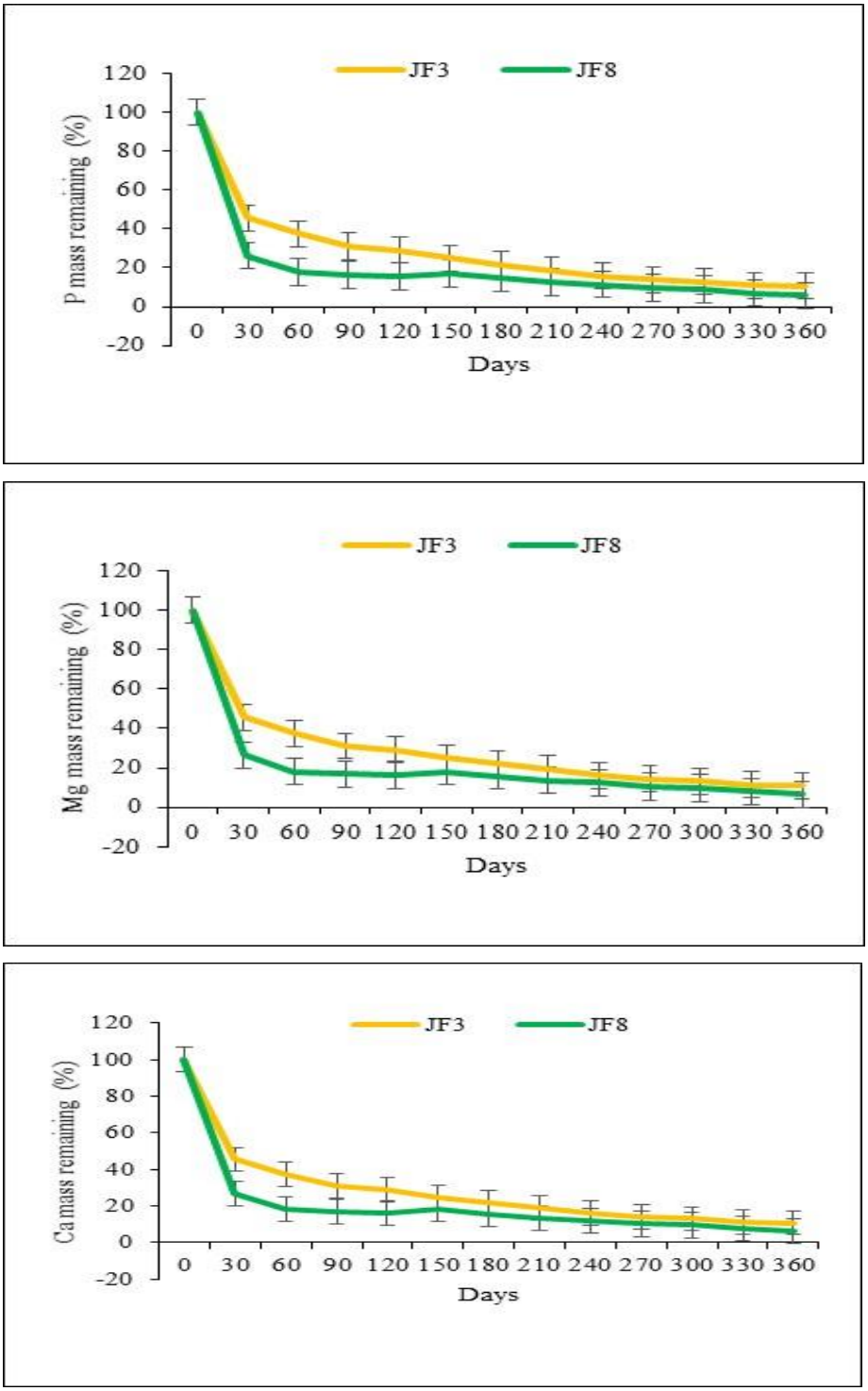

Fig.4.11 Changes in percent element (P, Mg and Ca) mass remaining in *Tephrosia candida* + *Oryza sativa* litter during the course of decomposition in 3 years (JF3) and 8 years (JF8) jhum fallows. Vertical lines show ±1SE.

4.2.3 Litter and element mass remaining during litter decomposition under microcosm

Litter mass remaining in pot experiment for all litter types is shown in Fig. 4.12 - 4.14. TC litter mass remaining was 33.7% in PJS3 and 15.8% in PJS8. OS litter mass remaining was 21.5% and 14.19%, respectively, in PJS3 and PJS8. However, mass remaining for TC + OS litters in PJS3 and PJS8 were 13.8% and 9.9%, respectively.

In PJS3, percent elements mass remaining after 12 months of decomposition in TC were: 32.15 for N, 33.63 for C, 22.51 for K, 20.26 for P, 32.64 for Mg and 31.59for Ca. Corresponding values for these elements in PJS8 were 14.61, 15.73, 8.51, 6.32, 14.92 and 14.62 (Fig. 4.15 - 4.16).

In PJS3, percent elements mass remaining for OS litters were: 19.34 for N, 21.45 for C, 12.03 for K, 17.24 for P, 20.22 for Mg and 20.05 for Ca. Corresponding values for these elements in PJS8 were: 12.56, 14.14, 5.94, 10.54, 13.18 and 12.63 (Fig. 4.17 – 4.18).

For TC+OS litter, percent element mass remaining after one year of decay were: 12.99 for N, 13.80 for C, 13.15 for K, 11.93 for P, 13.03 for Mg and 12.59 for Ca in PJS3. Corresponding values for these elements in PJS8 were: 9.14, 9.86, 9.14, 8.46, 9.18 and 8.97 (Fig. 4.19 – 4.20).

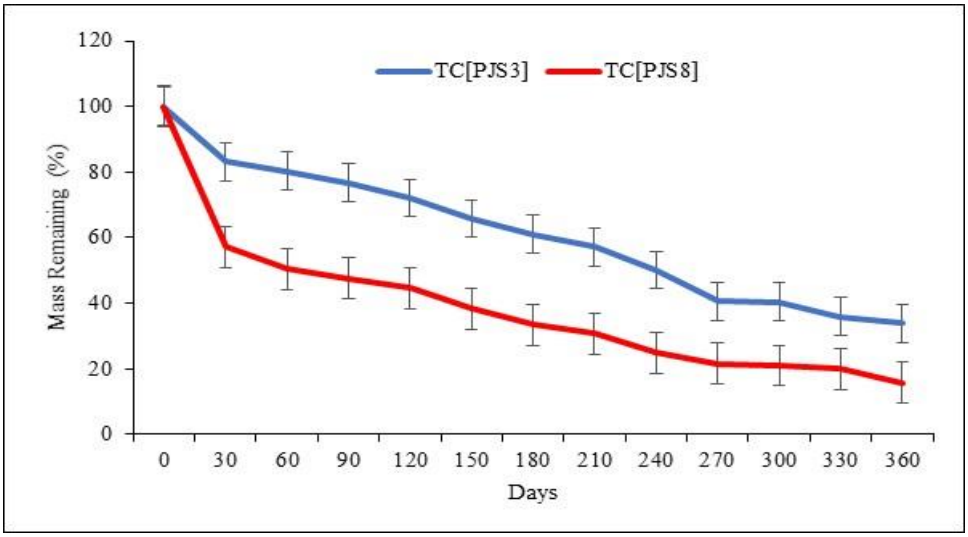

Fig. 4.12 *Tephrosia candida* (TC) litter mass remaining in 3 years (PJS3) and 8 years (PJS8) jhum fallow soil in microcosm. Vertical lines show ±1SE.

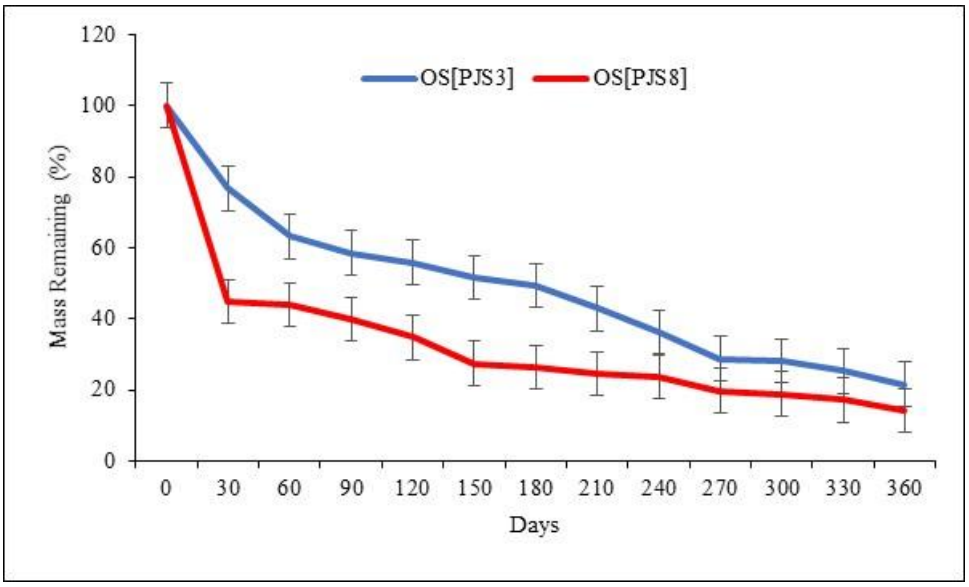

Fig. 4.13 *Oryza sativa* (OS) litter mass remaining in 3 years (PJS3) and 8 years (PJS8) jhum fallow soil in microcosm. Vertical lines show ±1SE.

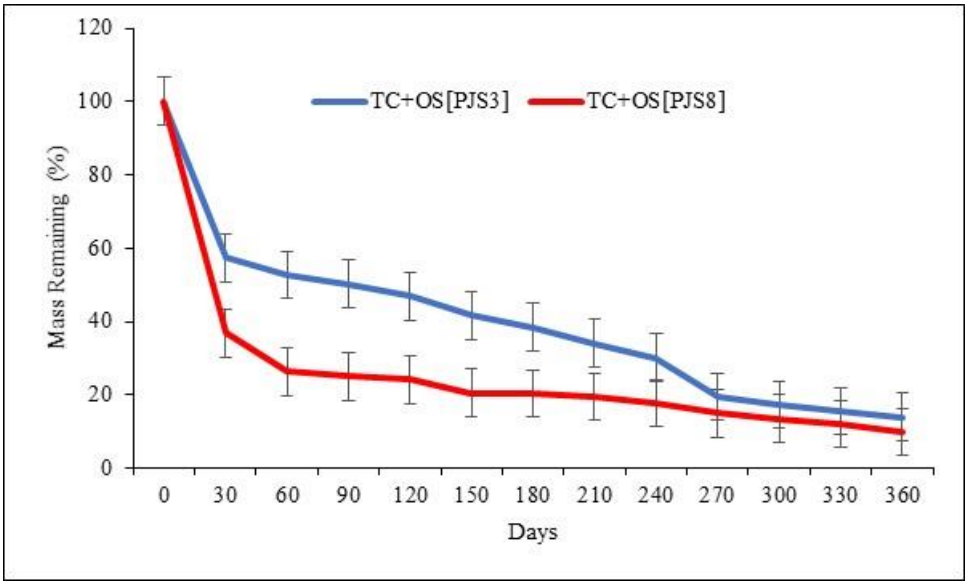

Fig. 4.14 *Tephrosia candida* + *Oryza sativa* (TC+OS) litter mass remaining in 3 years (PJS3) and 8 years (PJS8) jhum fallow soil in microcosm. Vertical lines show ±1SE.

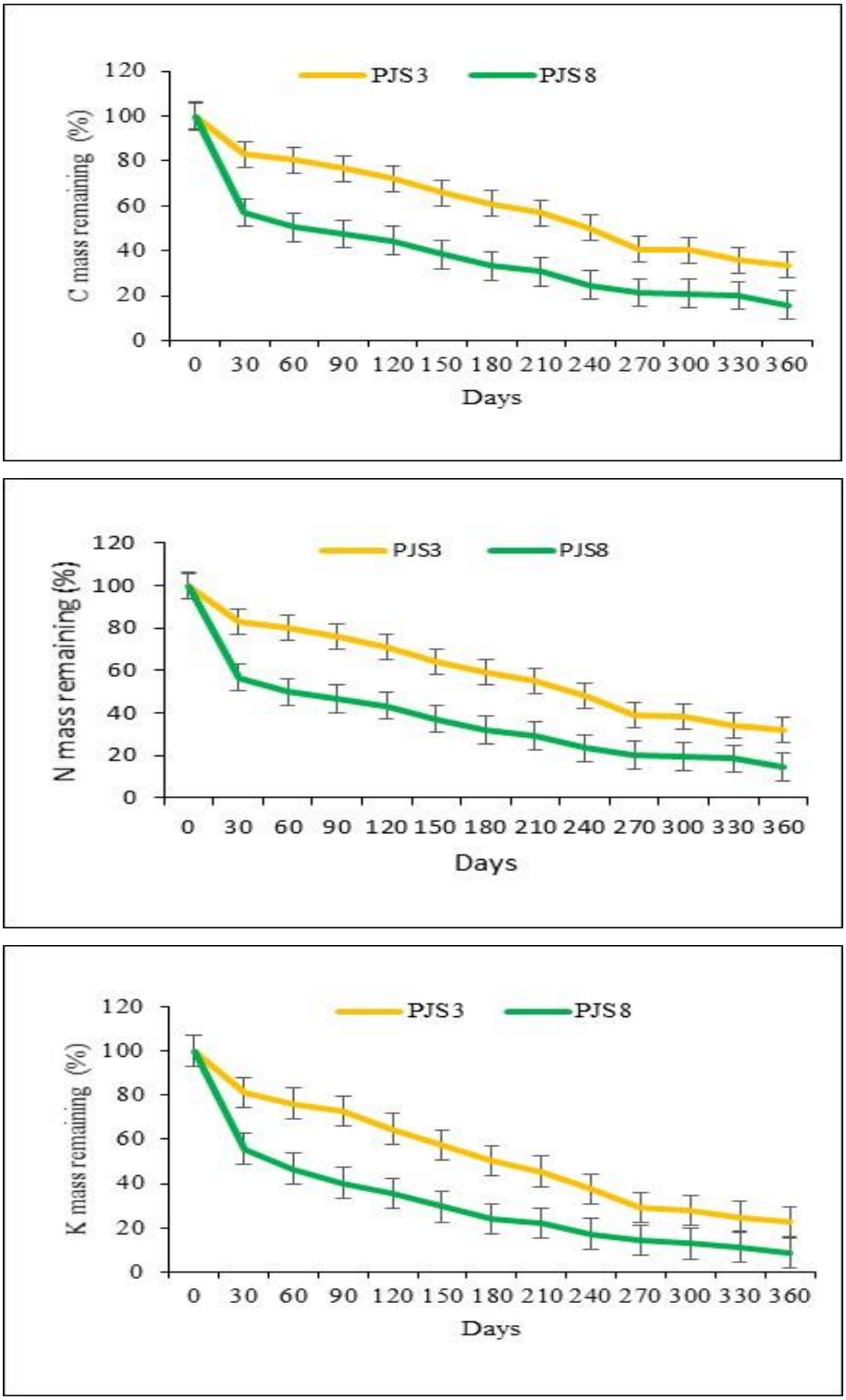

Fig.4.15 Changes in percent element (C, N and K) mass remaining in *Tephrosia candida* litter during the course of decomposition in 3 years (PJS3) and 8 years (PJS8) jhum fallow soil in microcosm. Vertical lines show ±1SE.

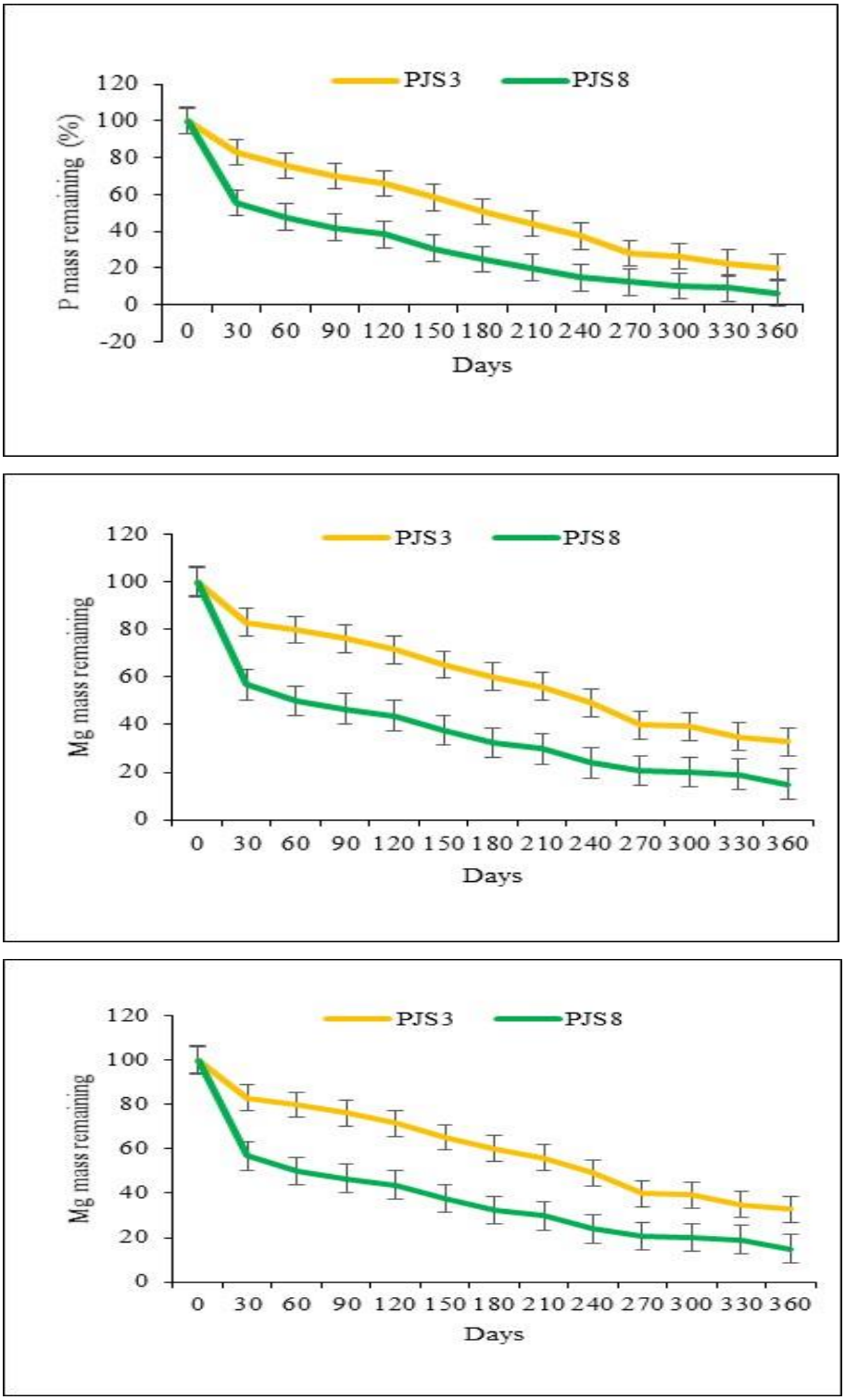

Fig.4.16 Changes in percent element (P, Mg and Ca) mass remaining in *Tephrosia candida* litter during the course of decomposition in 3 years (PJS3) and 8 years (PJS8) jhum fallow soil in microcosm. Vertical lines show ±1SE.

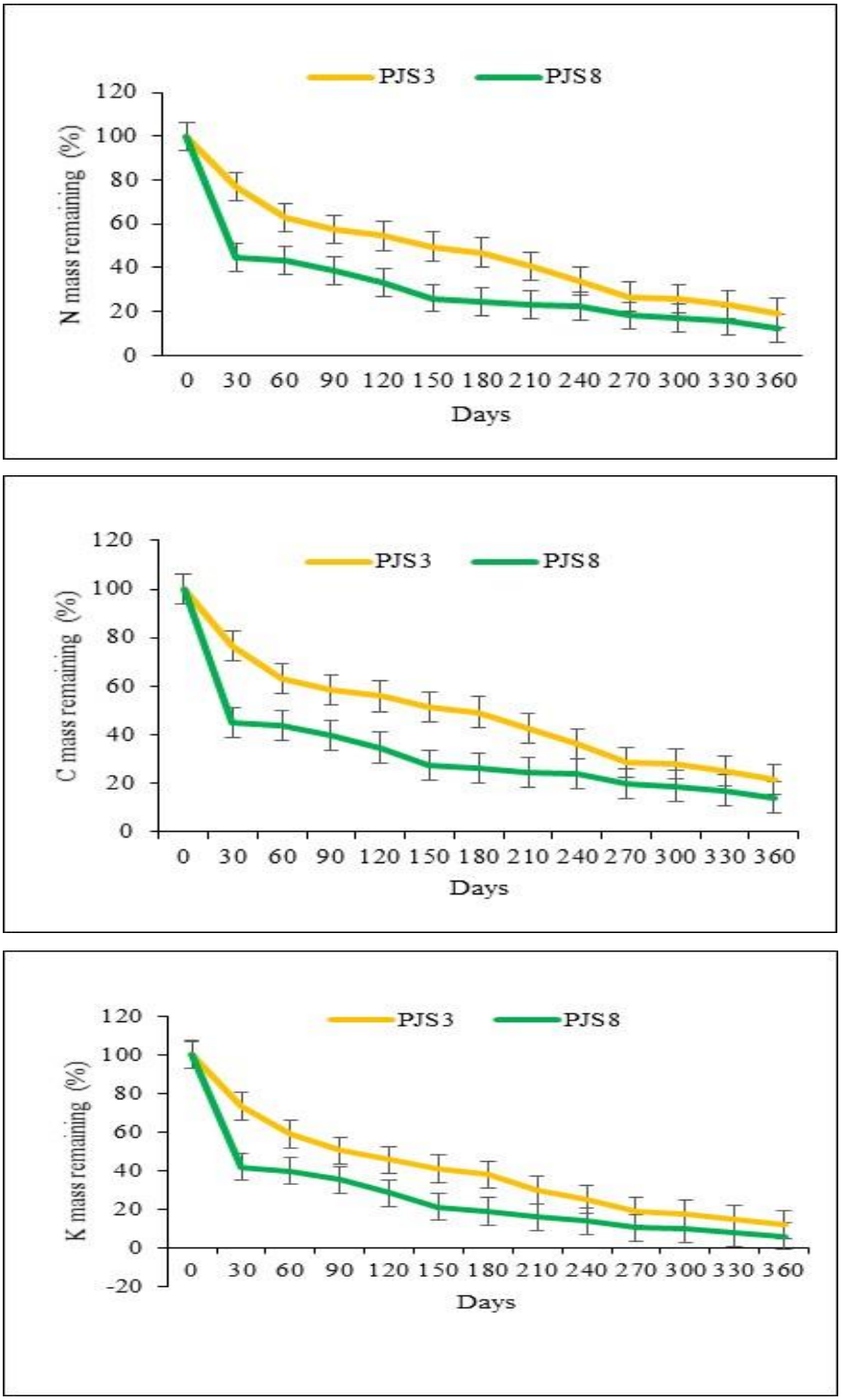

Fig.4.17 Changes in percent element (C, N and K) mass remaining in *Oryza sativa* litter during the course of decomposition in 3 years (PJS3) and 8 years (PJS8) jhum fallow soil in microcosm. Vertical lines show ±1SE.

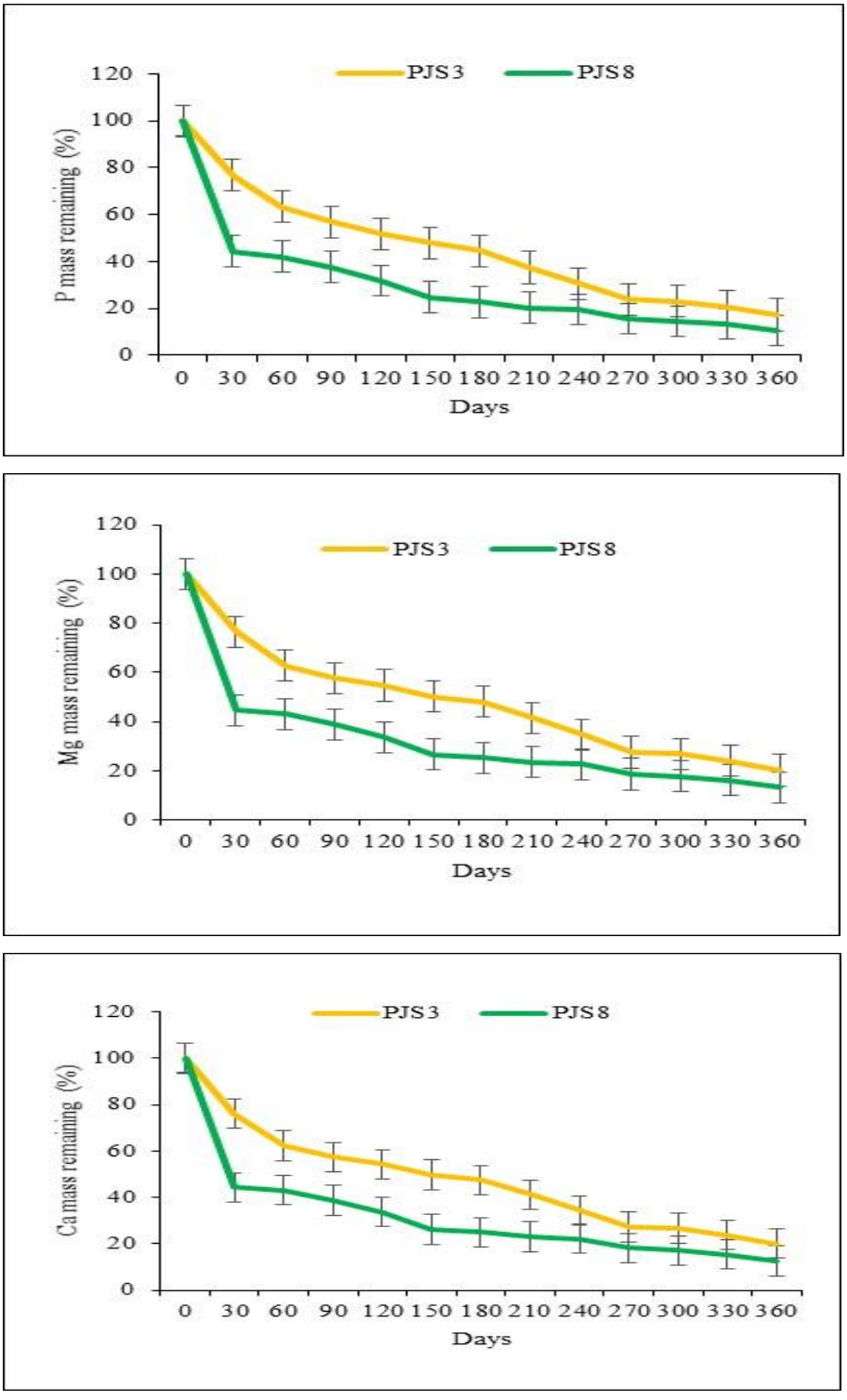

Fig.4.18 Changes in percent element (P, Mg and Ca) mass remaining in *Oryza sativa* litter during the course of decomposition in3 years (PJS3) and 8 years (PJS8) jhum fallow soil in microcosm. Vertical lines show ±1SE.

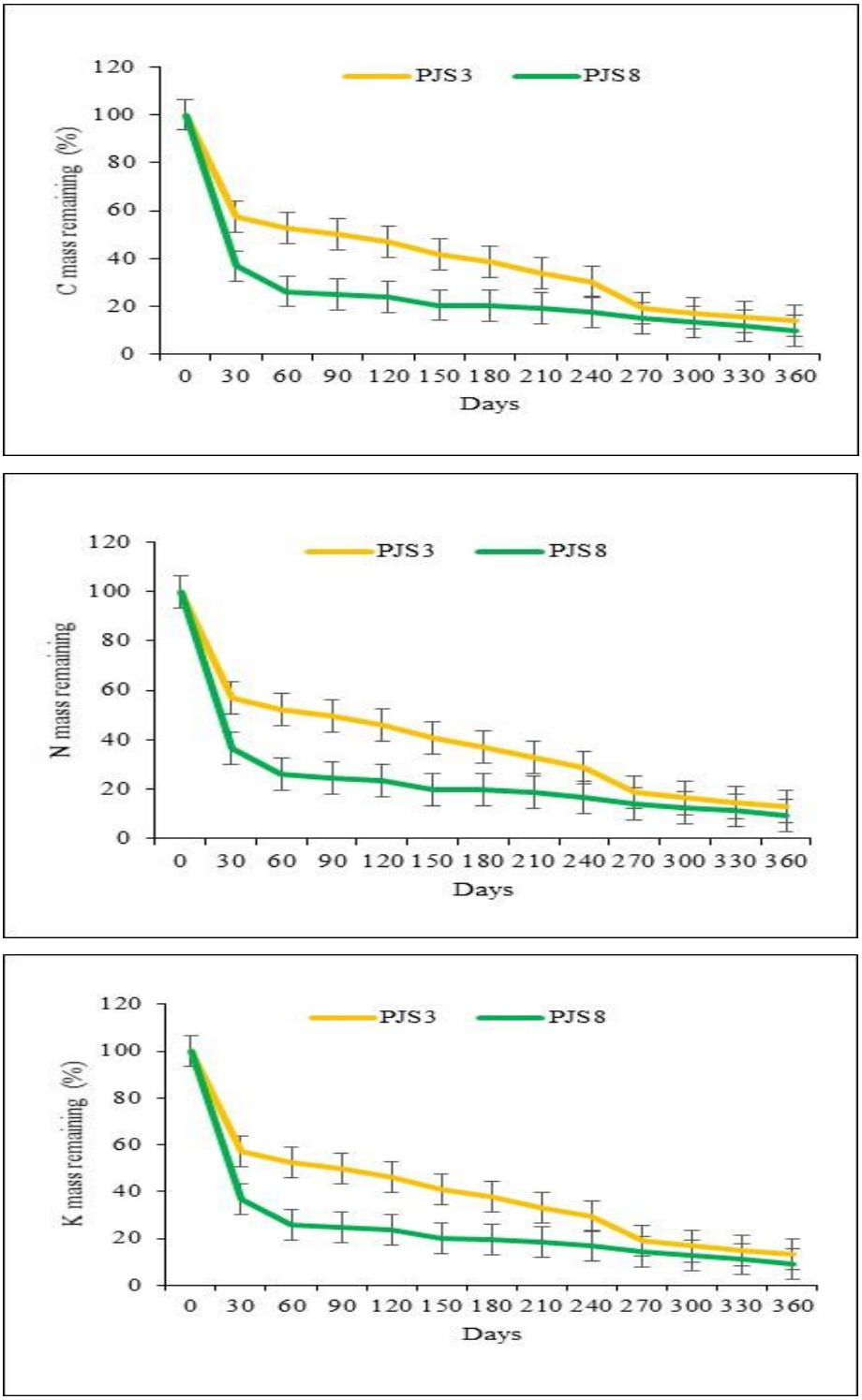

Fig.4.19 Changes in percent element (C, N and K) mass remaining in *Tephrosia candida* + *Oryza sativa* litter during the course of decomposition in 3 years (PJS3) and 8 years (PJS8) jhum fallow soil in microcosm. Vertical lines show ±1SE.

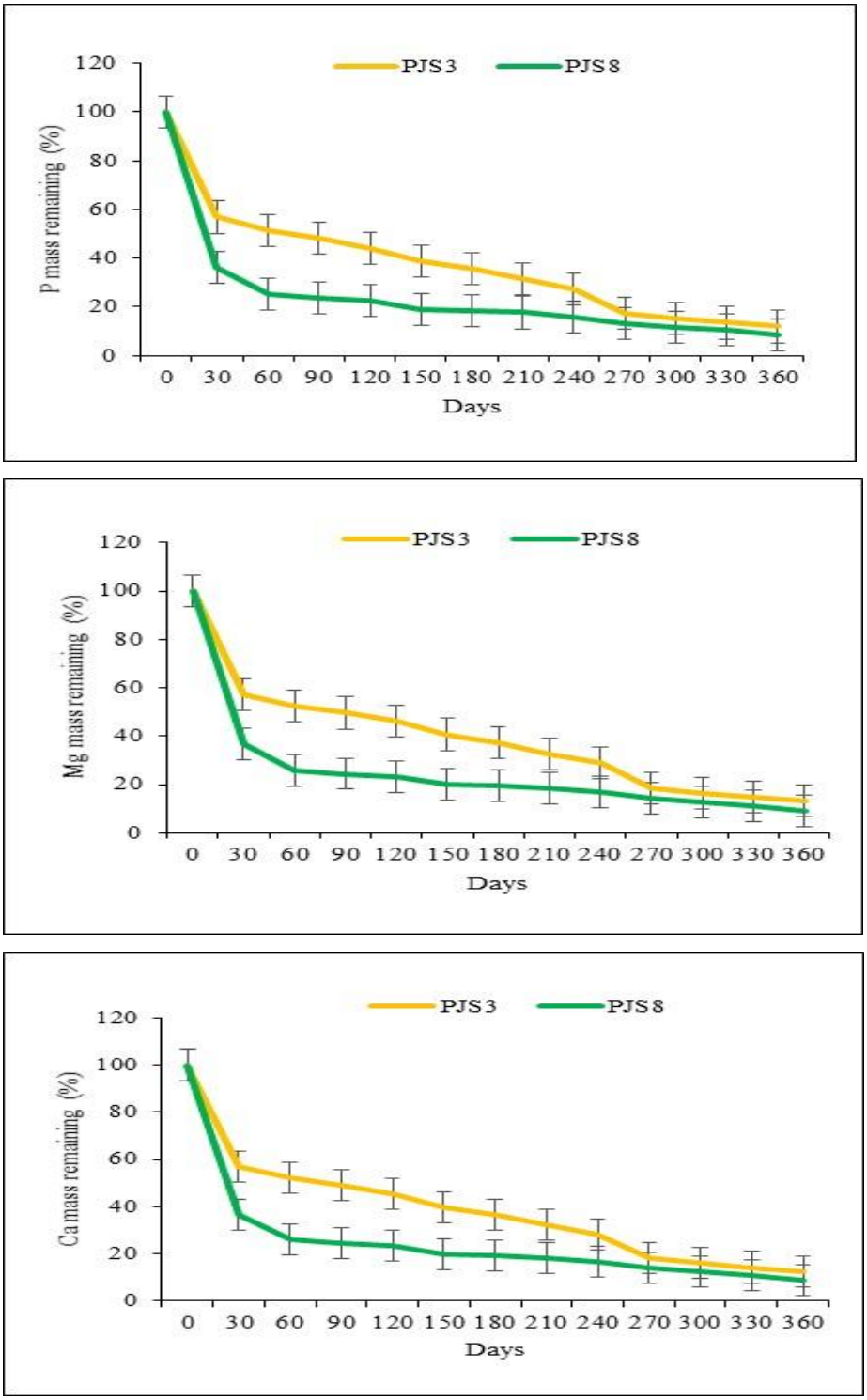

Fig.4.20 Changes in percent element (P, Mg and Ca) mass remaining in *Tephrosia candida* + *Oryza sativa* litter during the course of decomposition in 3 years (PJS3) and 8 years (PJS8) jhum fallow soil in microcosm. Vertical lines show ±1SE.

4.3 Leaf litter decomposition rate

4.3.1 Forest fallows (FF3 and FF5)

Litter mass remaining was significantly negatively correlated with days elapsed after litter placements in different fields (Fig. 4.21). In FF3 site, MGL decayed at a faster rate than BL and MFL in FF5. The daily instantaneous decay constant (k) in FF3 was 0.005946 for MGL. In FF5, it was 0.005908 for BL and 0.005946 for MFL. Days required for 50% decomposition of MGL were 126 days in FF3. However, in FF5, for BL and MFL the days required for 50% decomposition were 117 and 116, respectively. Similarly, time required for 95% decomposition in all three litter types varied from 504 – 540 days in two forest fallows (FF3 and FF5) (Table 4.2).

Table 4.2 Daily instantaneous decay rate (k) and time required for 50% and 95% decomposition of different leaf litters in Forest fallows

Forest fallows	**Leaf litter types**	**Daily instantaneous decay rate (k)**	**t_{50} (days)**	**t_{95} (days)**
FF5	BL	0.005908	117	507
	MFL	0.005946	116	504
FF3	MGL	0.005592	126	540

4.3.2 Jhum fallows (JF3 and JF8)

Mass remaining of different litter types was significantly negatively correlated with days. The daily instantaneous decay constant along with time essential for 50% and 95% decomposition of various litters in jhum fallows is given in Table 4.3.

However, mass remaining percent was higher in JF3 as compared to JF8 for all litter types. Litter mass remaining expressed in negative simple linear equation in JF3 and JF8 has been presented in Fig. 4.21. The daily instantaneous decay constant in JF3 were: 0.006001 for TC, 0.00587 for OS and 0.005933 for TC+OS. Corresponding values in JF8 were: 0.006116, 0.0068 and 0.006074. Similarly, in

JF3, the time required for 50% decomposition were: 115 days for TC, 118 days for OS and 117 days for TC+OS and time required for 95% decomposition were: 500 days for TC, 511 days for OS and 505 days for TC + OS). However, in JF8 time required for 50% decomposition were: 113 days for TC, 114 days for OS and 114 days for TC+OS days and 95% decomposition were 491 days for TC, 494 days for OS and 493 for days TC+OS (Table 4.3).

Table 4.3 Daily instantaneous decay rates (k) and time required for 50% and 95% decomposition of different leaf litters in Jhum fallows

Forest fallows	**Leaf litter types**	**Daily instantaneous decay rate (k)**	**t_{50} (days)**	**t_{95} (days)**
JF3	TC	0.006001	115	500
JF3	OS	0.00587	118	511
JF3	TC+OS	0.005933	117	505
JF8	TC	0.006116	113	491
JF8	OS	0.006074	114	494
JF8	TC+OS	0.00608	114	493

4.3.3 Microcosm (PJS3 and PJS8)

Litter mass remaining was significantly negatively correlated with days for all litter types in two pot experiments (PJS3 and PJS8) (Fig. 4.21). Litter mass remaining was higher in PJS3 as compared to PJS8. The daily instantaneous decay constant (k) for different litter types varied from 0.005585 – 0.00587 in PJS3 and 0.005798 – 0.005992 in PJS8 (Table 4.4). In PJS3, time required for 50% decay for different litter types varied from 118 – 124 days was comparatively higher (115 – 120 days) in PJS8 for different litter categories. Similarly, time required for 95% decay was 511 – 537 days in PJS3 and 500 – 514 days in PJS8 for different litter types.

Table 4.4 Daily instantaneous decay rate (k) and time required for 50% and 95% decomposition of different leaf litters in Microcosm

Forest fallows	**Leaf litter types**	**Daily instantaneous decay rate (k)**	**t_{50} (days)**	**t_{95} (days)**
PJS3	TC	0.005798	120	517
PJS3	OS	0.005585	124	537
PJS3	TC+OS	0.00587	118	511
PJS8	TC	0.005798	119	517
PJS8	OS	0.005813	118	514
PJS8	TC+OS	0.005992	115	500

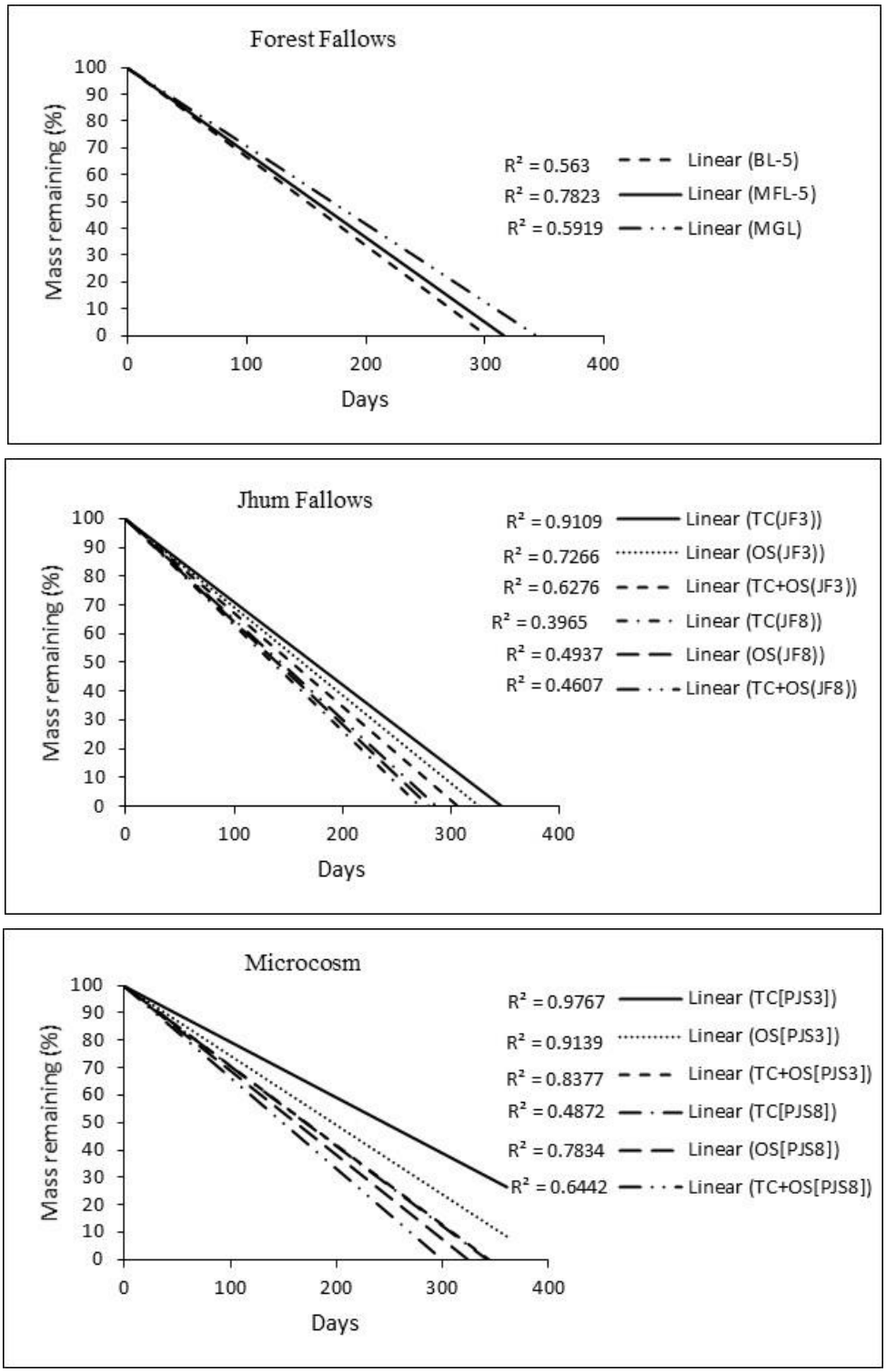

Fig 4.21 Litter mass remaining and time elapsed in days for bamboo litter (BL), mixed forest (MFL) and mixed grass litter (MGL) in forest fallows. Also shown are litter mass remaining and time elapsed in days for *Tephrosia candida* (TC), *Oryza sativa* (OS), *Tephrosia candida* + *Oryza sativa* (TC+OS) in 3 years (JF3) and 8 years (JF8) jhum fallows and 3 years (PJS3) and 8 years (PJS8) soil microcosms.

4.4 Relationship between decomposition rate and abiotic factors

Relationship between mass remaining of various litter types and abiotic factors was performed for Forest fallows (FF3 and FF5) and Jhum fallows (JF3 and JF8). Rainfall and humidity were negatively correlated with mass remaining of various litter types; however, temperature was positively correlated with mass remaining. In FF and JF rainfall was highly correlated with mass loss of various litter types (Table 4.5- Table 4.7).

Stepwise multiple correlation analysis investigating the integrated effect of nine factors (e.g., rainfall, humidity, air temperature, soil temperature, soil carbon, soil nitrogen and the different microbes) showed significant (R^2 97-98%) effect of rainfall and temperature in combination with soil carbon and nitrogen contents in JF3. In JF8 stand, soil carbon, soil temperature and actinomycetes together explained 98% variability in the decomposition of OS litter. However, in TC litter none of the variable entered the equation except soil carbon which explained 96% variability in mass loss alone. In case of TC+OS, rainfall and bacterial population together explained 79% variability in mass loss rate. In case of FF, in all litter types rainfall alone explained 39-59% variability in mass loss rates and none of the variables were selected in the equation. Equations for the various jhum fields and litter types are given below:

JF3:

TC=31.54 - 0.0697 Temperature - 18.416 Carbon; R^2=98.6%

OS=5.216 + 0.003226 Rainfall - 15.47 Nitrogen; R^2=97.3%

TC+OS=14.740 + 0.001809 Rainfall - 8.940 Carbon; R^2=98.4%

FF:

BL=-0.470 + 0.00783 Rainfall; R^2=59.36%

MFL=0.014 + 0.00441 Rainfall; R^2=38.64%

MGL=-0.061 + 0.00478 Rainfall; R^2=45.75%

JF8:

TC=7.319 - 3.193 Carbon; R^2=96.06%

OS=6.952 - 2.526 Carbon - 0.02143 Soil Temperature - 0.00719 Actinomycetes; R^2=98.7%

TC+OS=-1.549 + 0.002454 Rainfall + 0.00992 Bacteria; R^2=79.3%

Table 4.5 Correlation of mass remaining and abiotic and biotic factors for bamboo litter (BL), mixed forest litter (MFL) and mixed grass litter (MGL) types in 3 years (FF3) and 5 Years (FF5) forest fallows (n=11).

	BL	MFL	MGL
Rainfall	0.730*	0.600	0.658*
Humidity	0.420	0.137	0.364
Temperature	0.288	0.131	0.301
Bacteria	0.213	0.043	0.188
Fungi	0.438	0.258	0.325
Actinomycetes	0.116	0.163	0.187

* Correlation is significant at the 0.05 level.

Table 4.6 Correlation mass remaining and abiotic and biotic factors for *Tephrosia candida* (TC), OS-*Oryza sativa* (OS) and TC+OS-*Tephrosia candida* + *Oryza sativa* (TC + OS) litter types in 3 years jhum field, (n=11).

	TC litters	OS litters	TC + OS litters
Rainfall	0.676*	0.933**	0.894**
Humidity	0.097	0.190	0.138
Temperature	0.405	0.020	0.095
Bacteria	0.478	0.127	0.237
Fungi	0.102	0.488	0.378
Actinomycetes	0.146	0.103	0.084

* Correlation is significant at the 0.05 level.

** Correlation is significant at the 0.01 level.

Table 4.7 Correlation mass remaining and abiotic and biotic factors for *Tephrosia candida* (TC), OS-*Oryza sativa* (OS) and TC+OS-*Tephrosia candida* + *Oryza sativa* (TC + OS) litter types in 8 years jhum field, (n=11).

	TC litters	OS litters	TC + OS litters
Rainfall	0.984**	0.926**	0.800**
Humidity	0.444	0.420	0.248
Temperature	0.198	0.182	0.227
Bacteria	0.051	0.174	0.282
Fungi	0.123	0.498	0.351
Actinomycetes	0.0176	0.368	0.296

* Correlation is significant at 0.05 level.

**Correlation is significant at 0.02 level.

4.5 Changes in C, N and C/N ratio in soil during the course of decomposition

In field experiment, C concentration in the soil beneath the litter bag increased from 28% - 121% over initial in different litter categories during the course of study period. In JF3, the increase in soil C concentration beneath was 45.3%, 42.3% and 28% in TC, OS and TC+OS litters, respectively (Fig. 4.22). However, in JF8, increase in soil C concentration beneath TC, OS and TC+OS litters was 121%, 73% and 31.2%, respectively (Fig. 4.23). In JF3, increase in soil N concentration beneath TC, OS and TC+OS litters was 26.6%, 17.12% and 29.2% (Fig. 4.22) and correspondingly in JF8 was 25.2%, 19.1% and 18.9%, respectively (Fig. 4.23). And decrease in concentration of C/N ratio in soil of JF3 beneath TC, OS and TC+OS was 170%, 247% and 95% (Fig. 4.22) and similarly in JF8 was 480%, 382% and 163% (Fig. 4.23).

In pot experiment, increase in soil C concentration during the course of study ranged from 14% - 75.2%. In PJS3, increase in soil C concentration beneath TC, OS and TC+OS litters was 23.023%, 44% and 14% and increase in soil N concentration beneath TC, OS and TC+OS litters was 31% 14.2% and 14.3% (Fig. 4.24). Increase in soil C concentration in PJS8 beneath TC, OS and TC+OS litters was 75.2% 71.3% and 21.8% and increase soil N content was 22.3%, 9.93% and TC+OS 13.15% respectively (Fig. 4.25). Decrease in C/N ratio concentration of soils beneath TC, OS and TC+OS was 74%, 309% and 97% in PJS3 (Fig. 4.24) and 337%, 718% and 165% in PJS8 respectively (Fig. 4.25).

Changes in C, N, and C/N ratio during the path of decay in field experiment were significant ($p \leq 0.05$) mostly. Similarly changes in C, N and C/N ratio during decomposition in pot experiment were also mostly significant ($p \leq 0.05$).

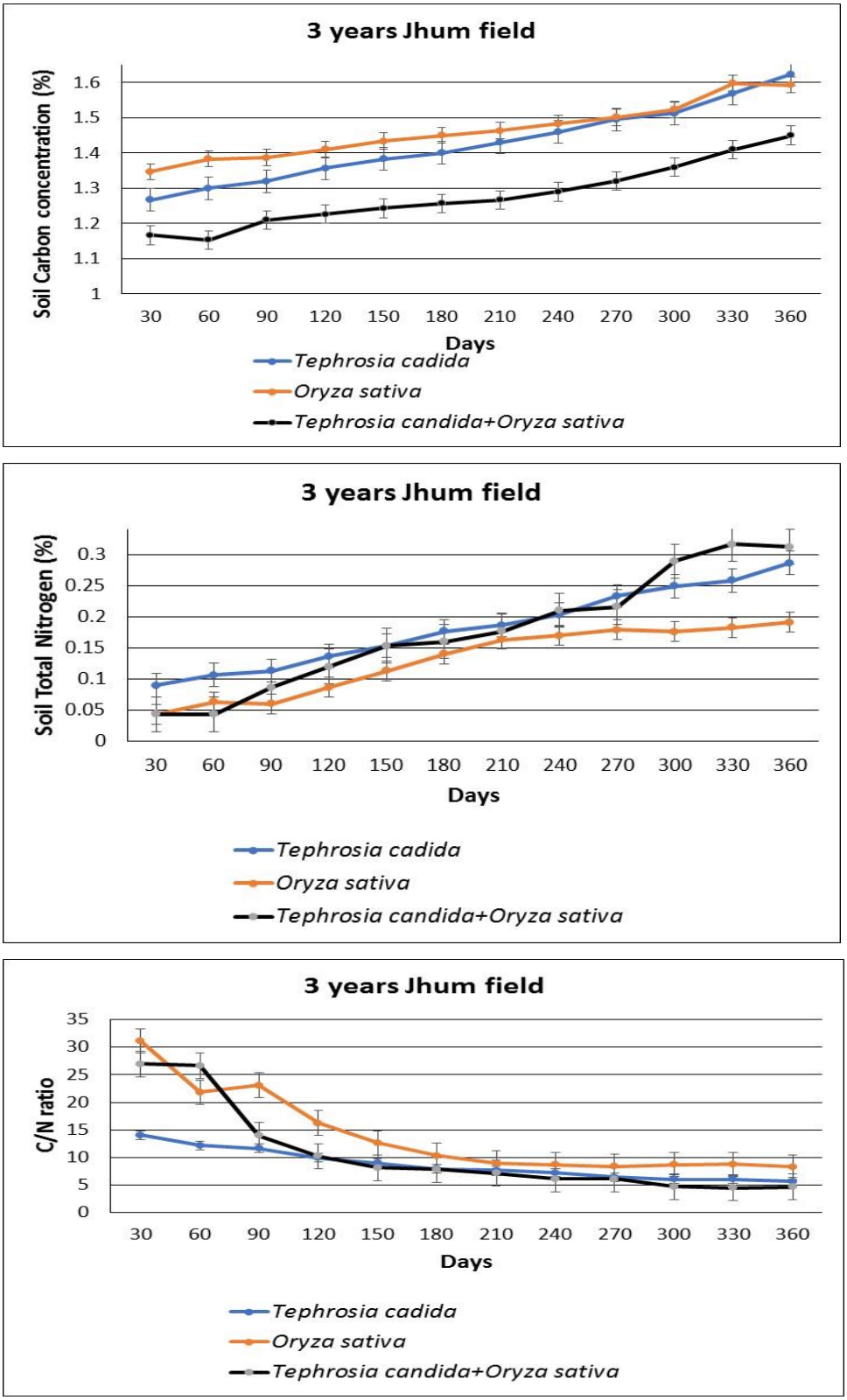

Fig 4.22 Changes in percent of C, N and C/N ratio in soil during decomposition in 3 years jhum field. Vertical lines show ±1SE.

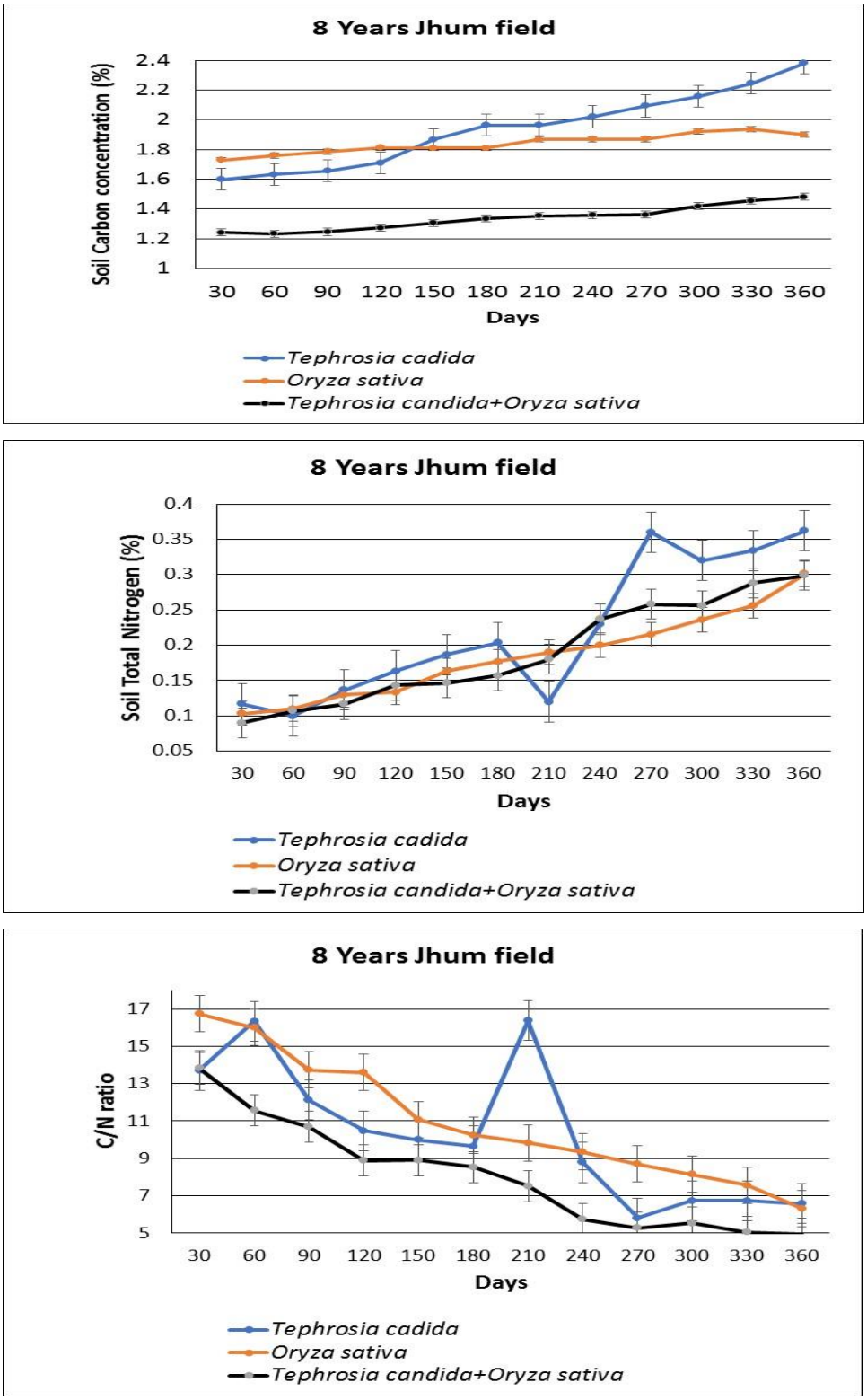

Fig. 4.23 Changes in percent of C, N and C/N ratio in soil during decomposition in 8 years jhum field. Vertical lines show ±1SE.

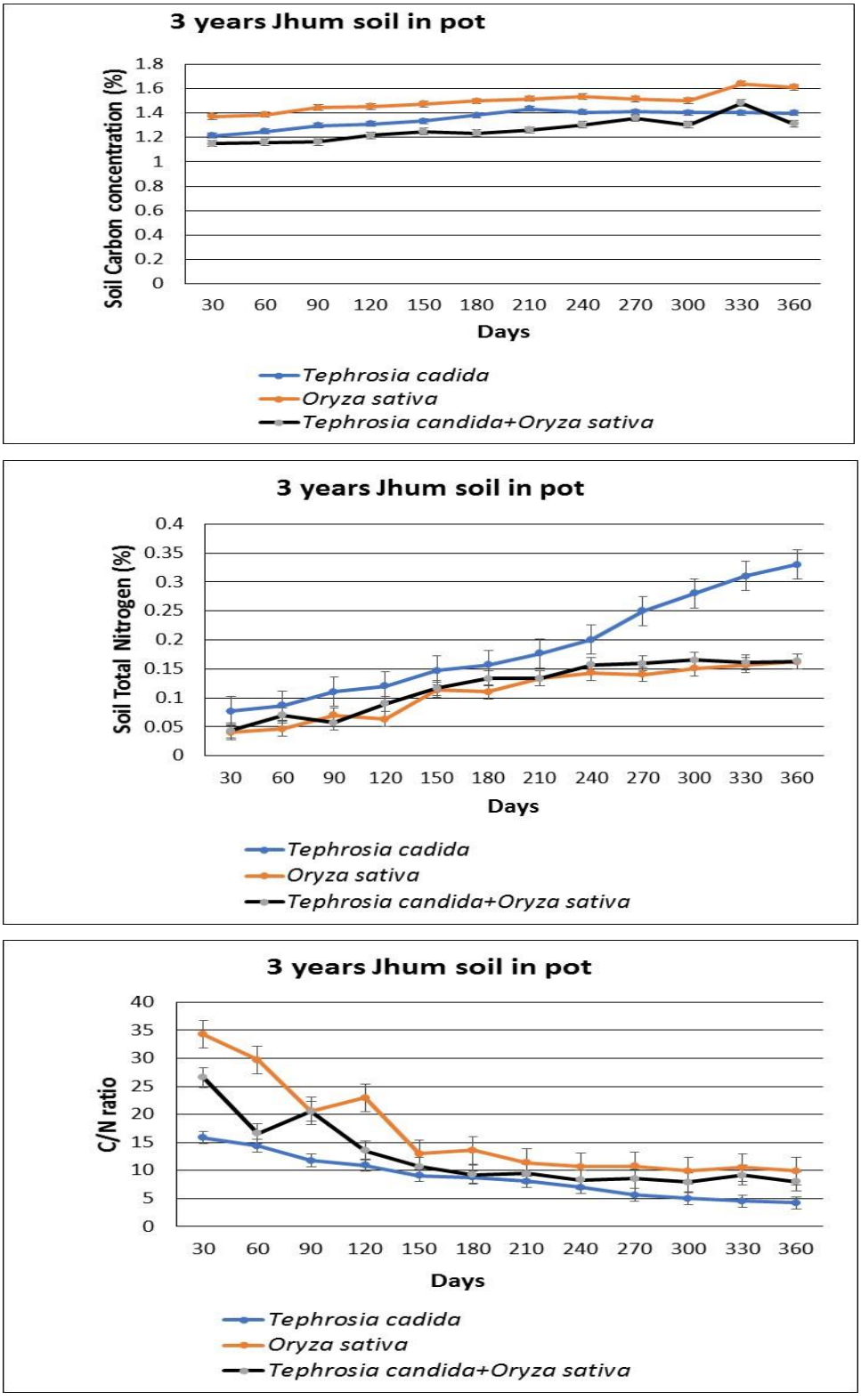

Fig. 4.24 Changes in percent of C, N and C/N ratio in 3 years jhum soil in pot during decomposition. Vertical lines show ±1SE.

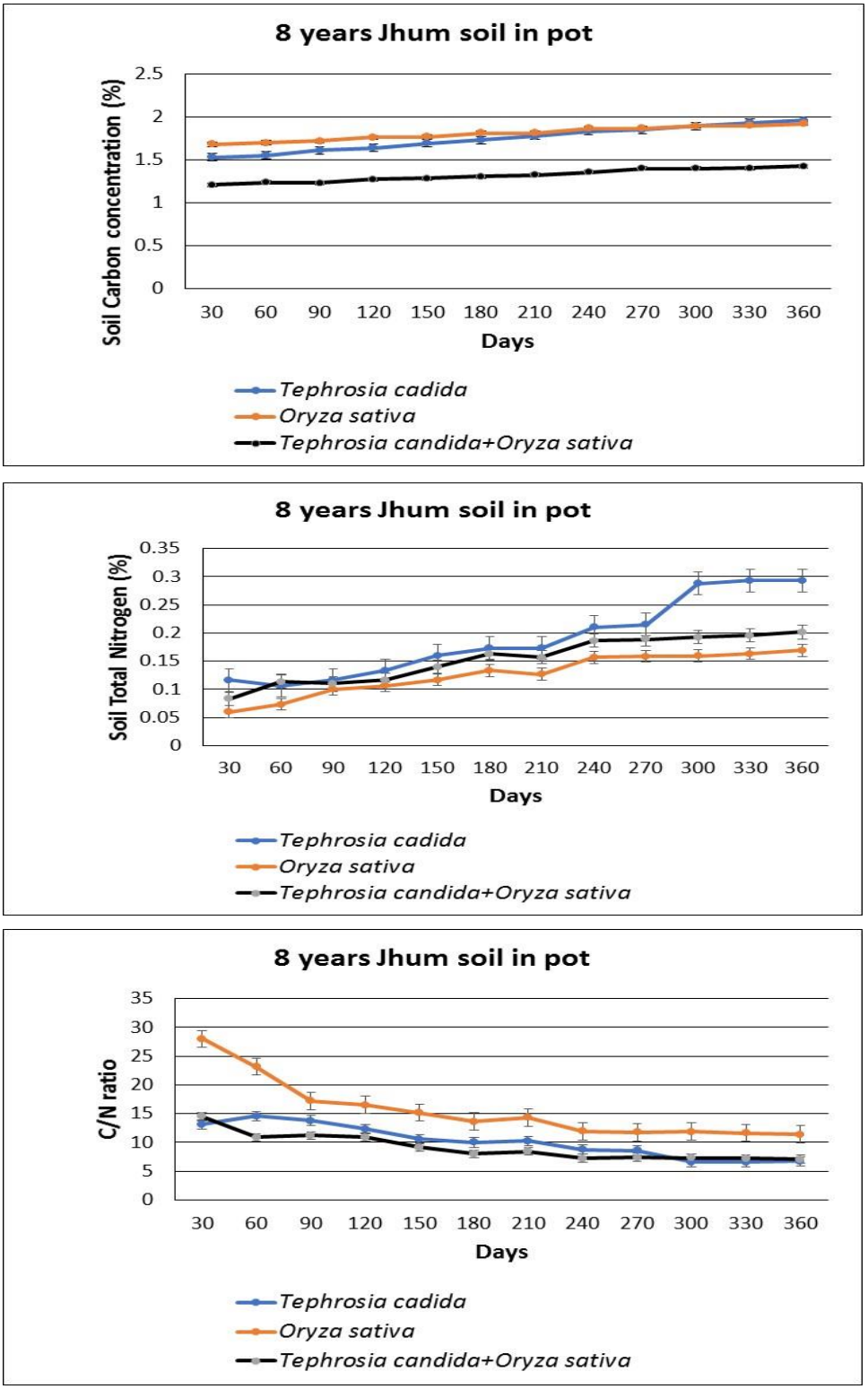

Fig. 4.25 Changes in percent of C, N and C/N ratio in 8 years jhum soil in pot during decomposition. Vertical lines show ±1SE.

4.6 Microbial population on decomposing litters

4.6.1 Forest fallows

In both the forest fallows (FF3 and FF5) microbial population showed an increasing trend up to seventh month of decomposition of different litter types. Microbial population was found to be less in FF3 (Fig. 4.26) as compared to FF5 (Fig.4.27). Maximum was observed for decomposing MFL ($2.73X10^6$ CFU/g) followed by decomposing BL ($2.62X10^6$ CFU/g) in FF5. However, in FF3 decomposing MGL showed ($2.42X10^6$ CFU/g) bacterial population. Similar trend was observed for fungal and actinomycetes population in both the fallows.

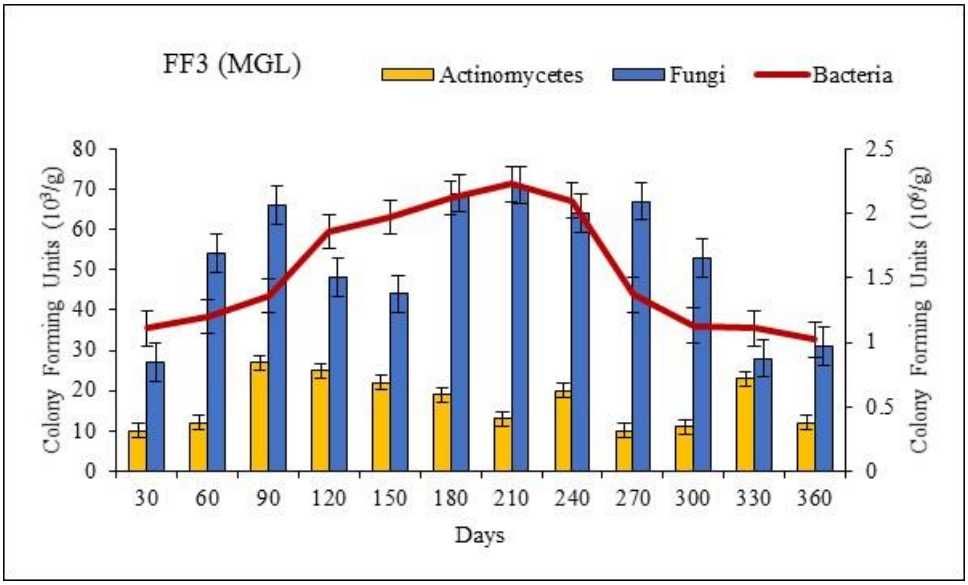

Fig.4.26 Colony Forming Units (CFUs) of decomposing mixed grass litters (MGL) in 3 years forest fallow (FF3). Vertical lines show ±1SE.

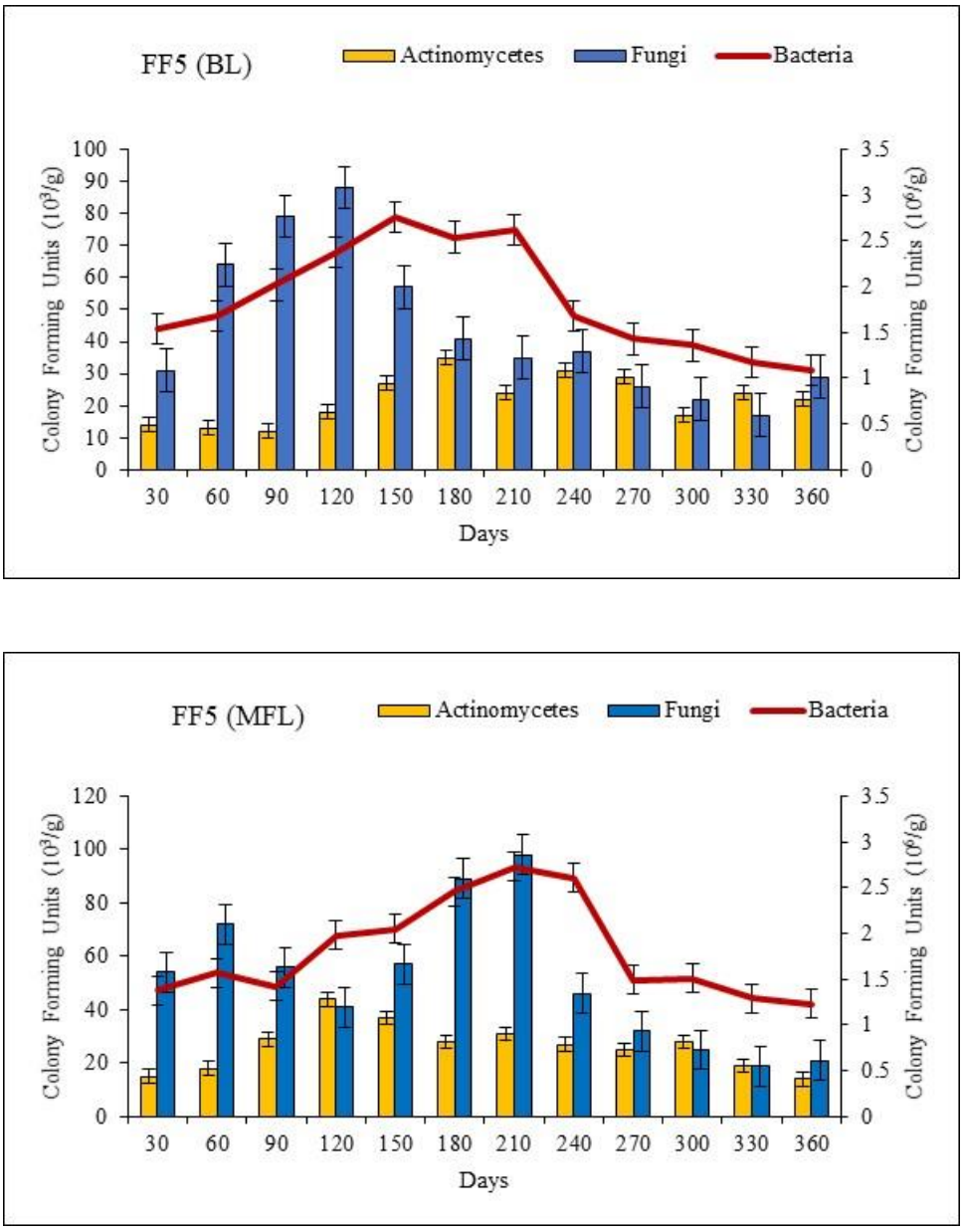

Fig.4.27 Colony Forming Units (CFUs) of decomposing mixed forest litters (MFL) and bamboo litters (BL) litters in 5 years forest fallow (FF5). Vertical lines show ±1SE.

4.6.2 Jhum fallows

Litter microbial population showed consistent increase in CFUs for different litter types in both the Jhum fallows (JF3 and JF8). After sixth month of decomposition of TC and TC+OS litters in JF3 (Fig. 4.28) fungal and bacterial population was found to be maximum, however, for OS decomposition only bacterial population was found to be maximum after third month and fungal after seventh months. In JF8 (Fig. 4.29), fungal population was maximum after third month of decomposition of all litter types. Actinomycetal population was less as compared to bacterial and fungal population. During the study, microbial population was found in order: Bacteria > Fungi > Actinomycetes.

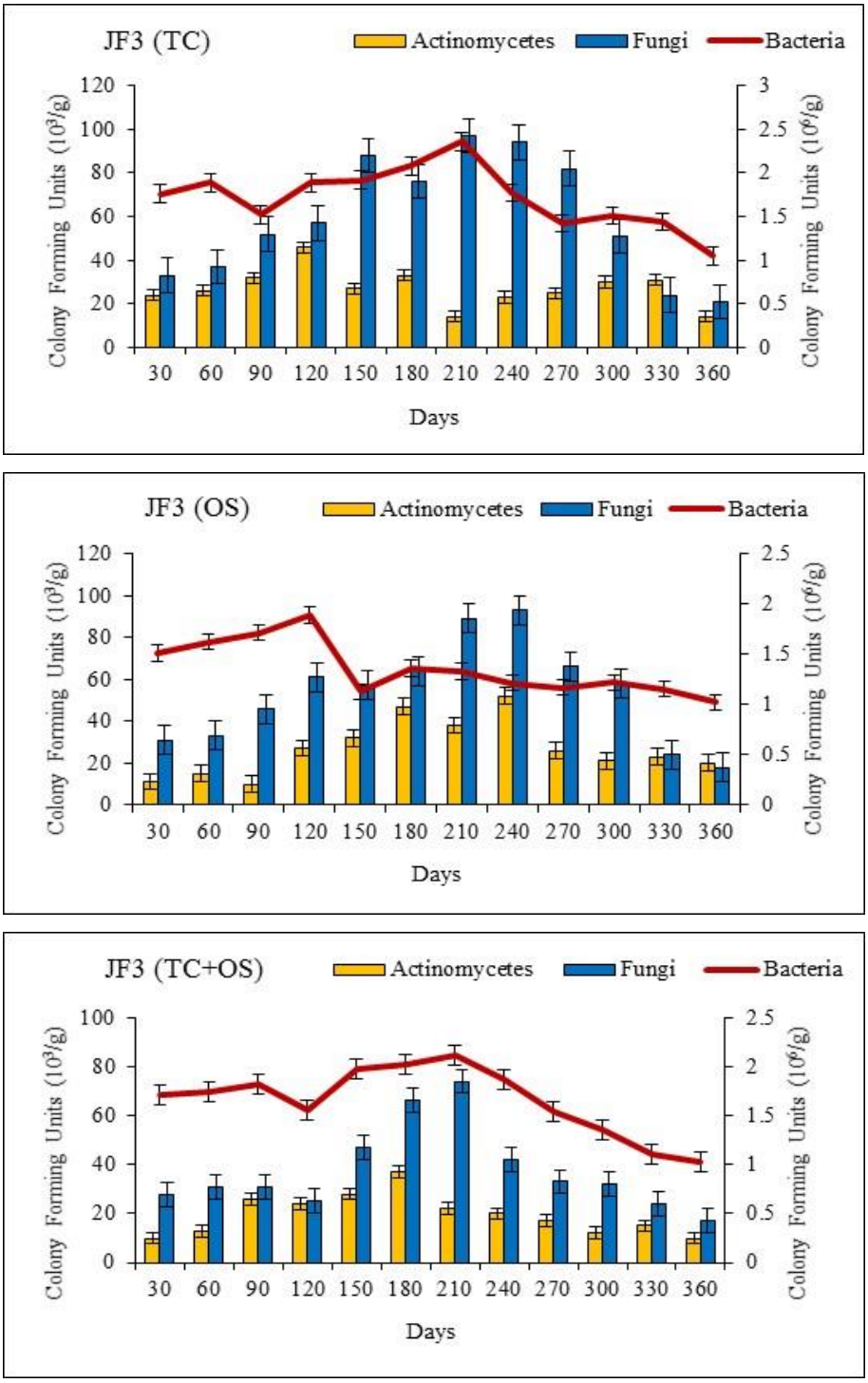

Fig.4.28 Colony Forming Units (CFUs) of decomposing *Tephrosia candida* (TC), *Oryza sativa* (OS) and *Tephrosia candida* + *Oryza sativa* (TC+OS) litters in 3 years jhum fallow (JF3). Vertical lines show ±1SE.

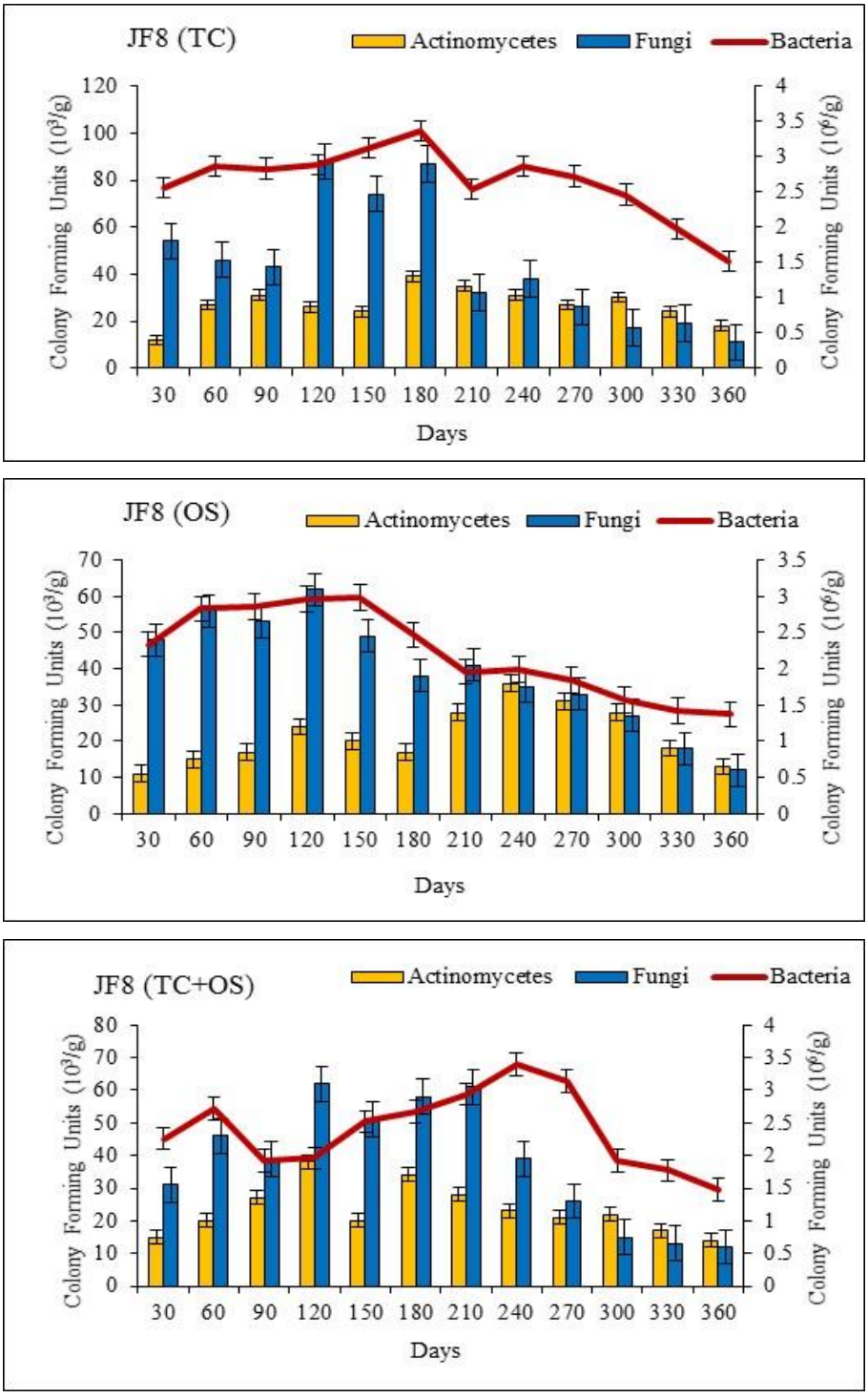

Fig.4.29 Colony Forming Units (CFUs) of decomposing *Tephrosia candida* (TC), *Oryza sativa* (OS) and *Tephrosia candida* + *Oryza sativa* (TC+OS) litters in 8 years jhum fallow (JF8). Vertical lines show ±1SE.

4.6.3 Microcosm

Similar periodical changes in microbial population were observed in microcosm in relation to Jhum fallows. In PJS3 (Fig.4.30) microbial population reaches its peak after forth month of decomposition. However, in PJS8 (Fig. 4.31) bacterial population increases in early days of decomposition followed by actinomycetes and fungal population. Maximum microbial population was observed for TC litters followed by TC+OS litters and minimum in OS litters.

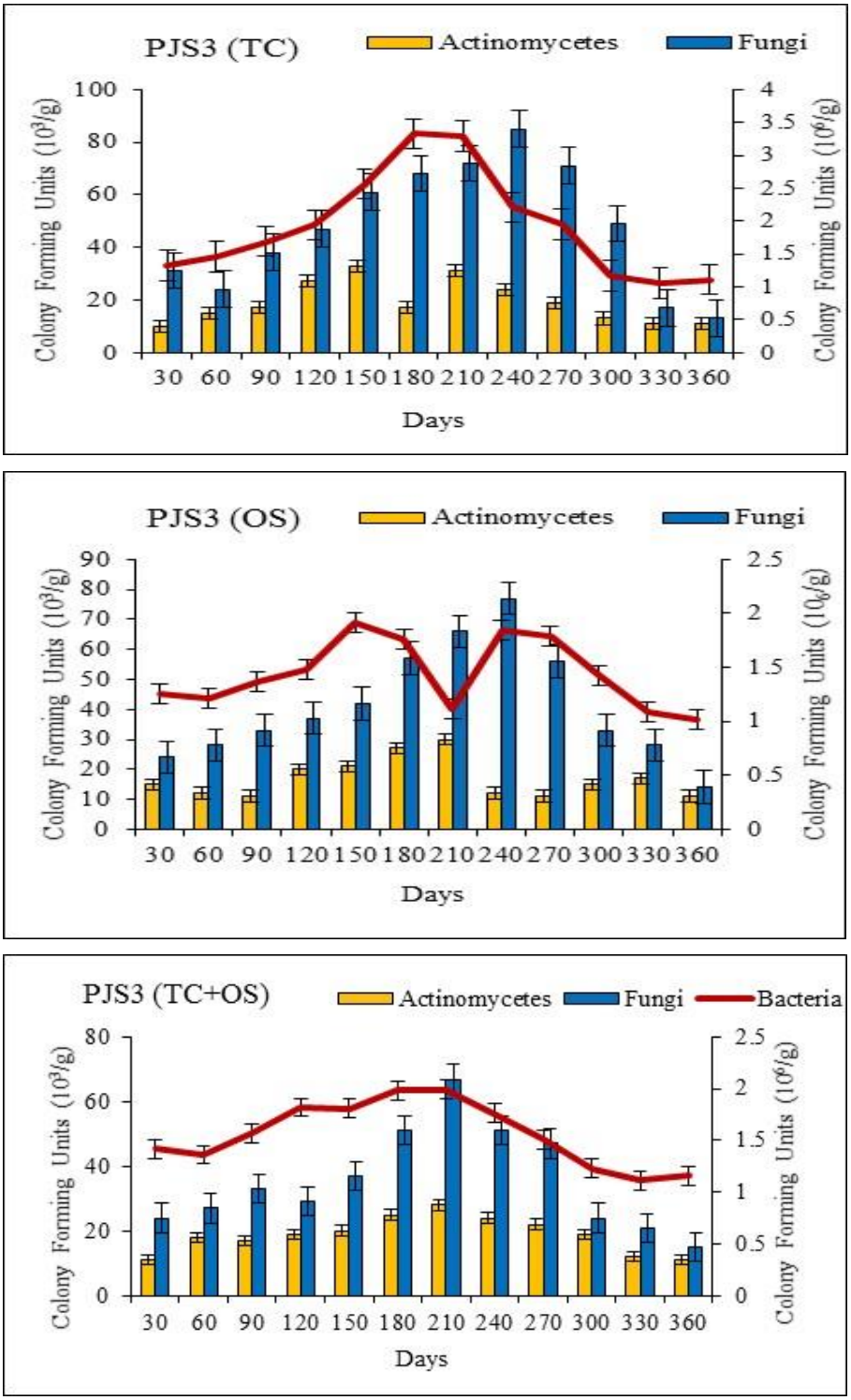

Fig.4.30 Colony Forming Units (CFUs) of decomposing *Tephrosia candida* (TC), *Oryza sativa* (OS) and *Tephrosia candida* + *Oryza sativa* (TC+OS) litters in 3 years jhum fallow soil (PJS3) in pot. Vertical lines show ±1SE.

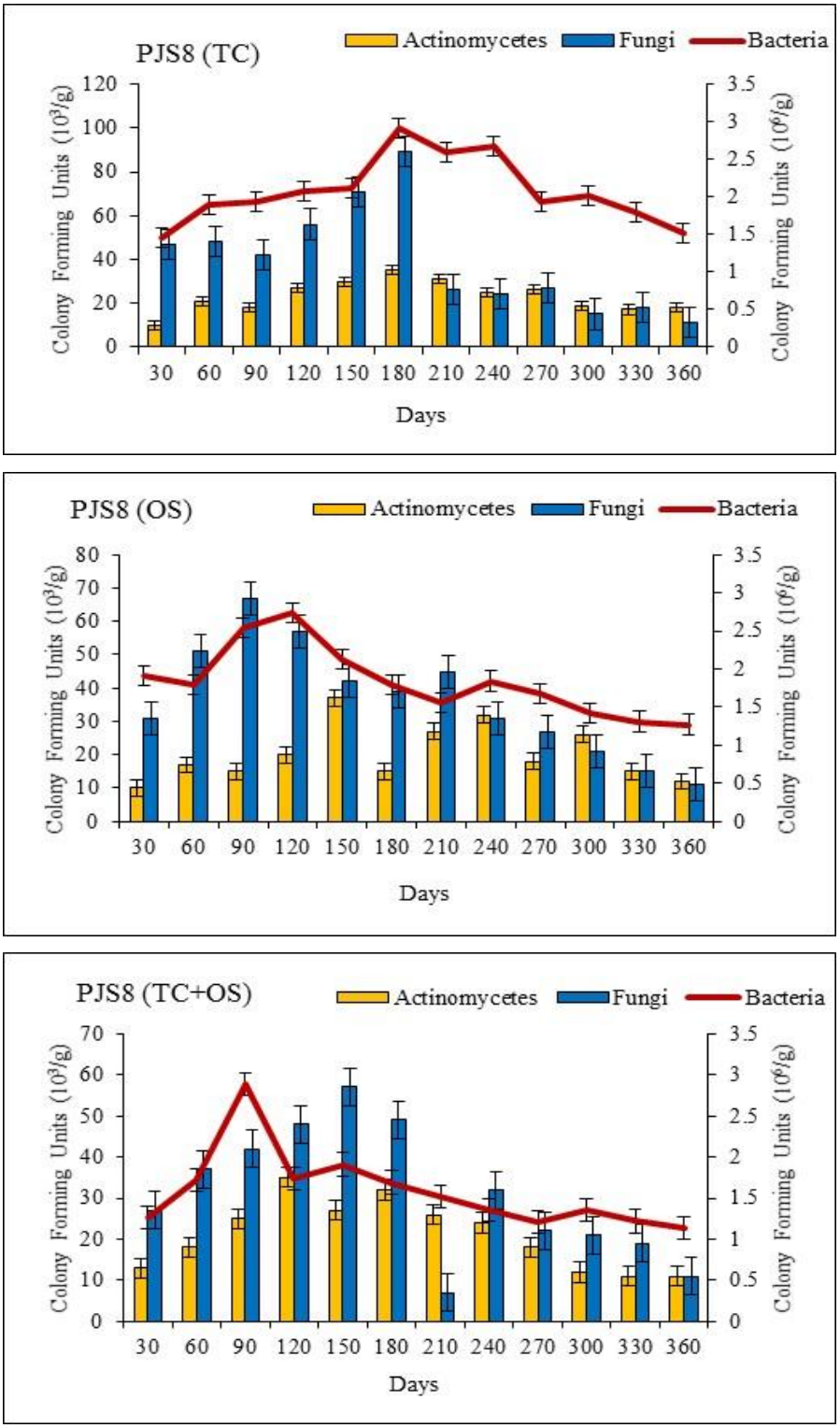

Fig.4.31 Colony Forming Units (CFUs) of decomposing *Tephrosia candida* (TC), *Oryza sativa* (OS) and *Tephrosia candida* + *Oryza sativa* (TC+OS) litters in 8 years jhum fallow soil (PJS8) in pot. Vertical lines show ±1SE.

4.7 Screening of microbial enzyme activity

Different representatives of bacteria, fungi and actinomycetes were isolated as single culture from microbial population plates. A total of sixty-six microbes were isolated from different litter types at different sites (FFs, JFs and PJSs). Out of total fifty-one microbes (Bacteria-20, Fungi-21 and Actinomycetes-10) were showing either one or more enzyme activity.

4.7.1 Catalase test

The ability of microbes to produce immediate or vigorous effervescences in presence of 3% H_2O_2 was tested (Fig. 4.32). Among sixty-six strains, fourteen bacterial strains (SKT060, SKT016, SKT020, SKT003, SKT061, SKT014, SKT008, SKT059, SKT004, SKT005, SKT010, SKT002, SKT009 and SKT017), eleven fungal strains (SKT034, SKT045, SKT033, SKT046, SKT043, SKT062, SKT042, SKT041, SKT036, SKT040 and SKT058) and eight actinomycetes strains (SKT059, SKT065, SKT050, SKT060, SKT052, SKT053, SKT55 and SKT030) strain were able to generate effervescences (Table 4.8).

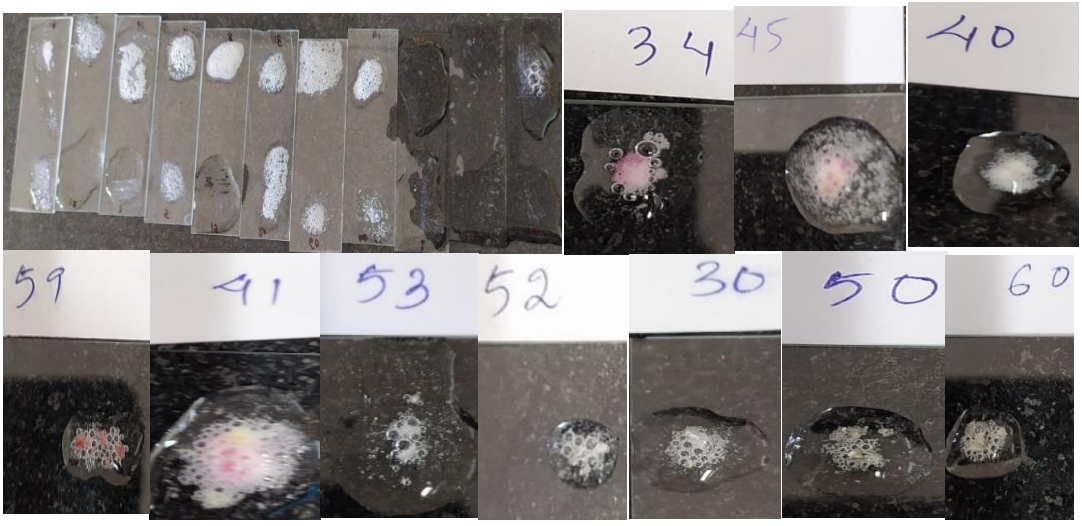

Fig 4.32 Catalase activity of isolates

4.7.2 Amylase test

The isolated microbes were tested for the production of amylase enzyme. The isolates were grown in Starch Agar plates and stained with Gram's iodine. Six bacterial strains (SKT020, SKT005, SKT010, SKT002, SKT009 and SKT019), nine fungal strains (SKT033, SKT043, SKT044, SKT042, SKT037, SKT040, SKT039, SKT045 and SKT035) and three actinomycetal strains (SKT051, SKT060 and SKT030) were capable of hydrolyzing the starch and produce clear zone (Fig. 4.33) on Starch Agar plates (Table 4.8)

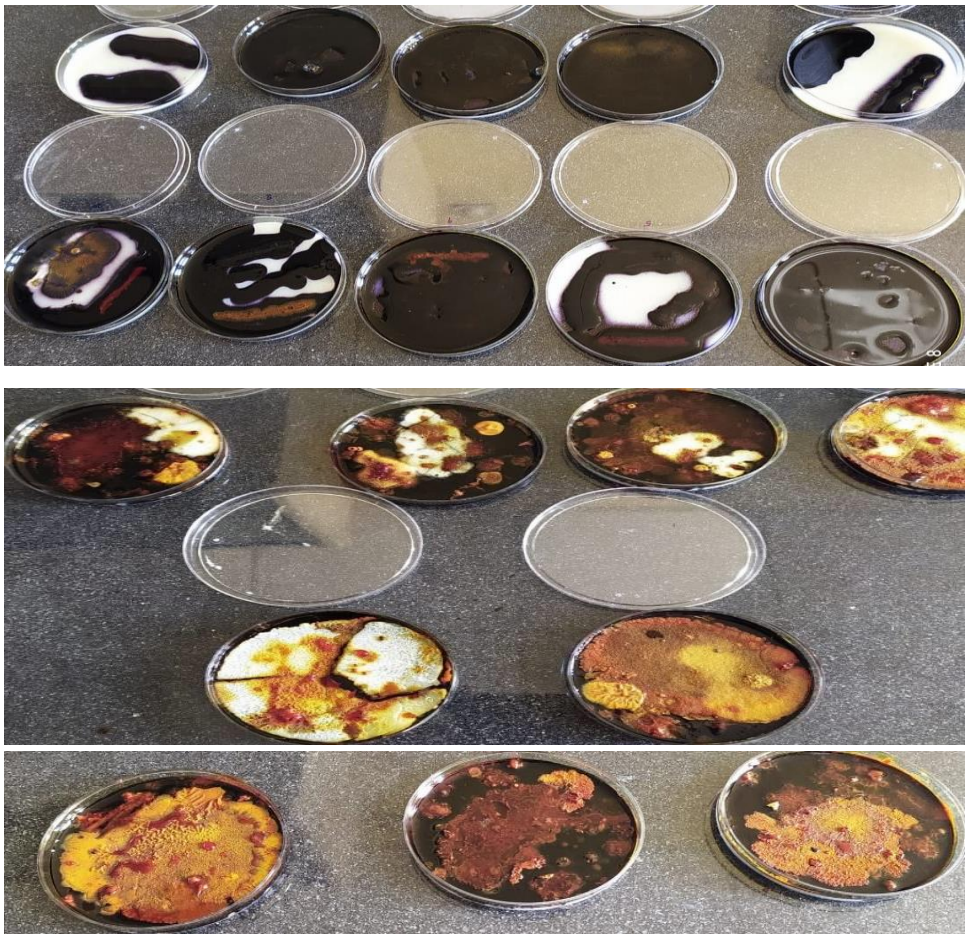

Fig.4.33 Amylase activity of isolates

4.7.3 Protease test

The production of protease was examined by growing the isolates on skim milk agar plates. A clear zone around the colony indicated the production of protease (Fig. 4.34). According to the study, eight bacterial strains (SKT016, SKT020, SKT061, SKT013, SKT005, SKT010, SKT002 and SKT009) eight fungal strain (SKT036, SKT037, SKT038, SKT040, SKT039, SKT064, SKT063 and SKT045) and four actinomycetal strains (SKT048, SKT052, SKT053 and SKT030) were found to produce protease (Table 4.8)

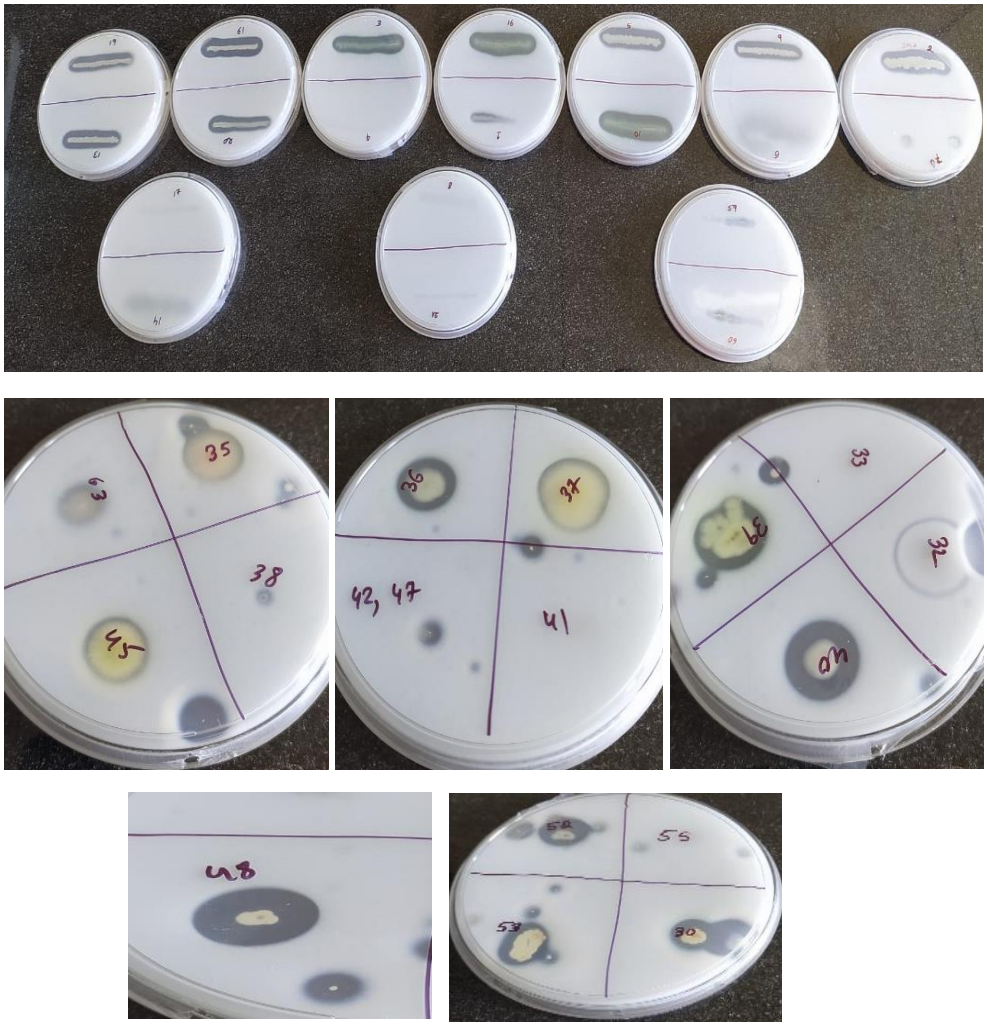

Fig.4.34 Protease activity of isolates

4.7.4 Lipase test

The isolates were grown on tributyrin agar plates. The production of lipase was observed by the formation of clear zone around the growing colony (Fig. 4.35). Out of sixty-six strains, only one bacterial strain (SKT008) was positive for lipase activity (Table 4.8).

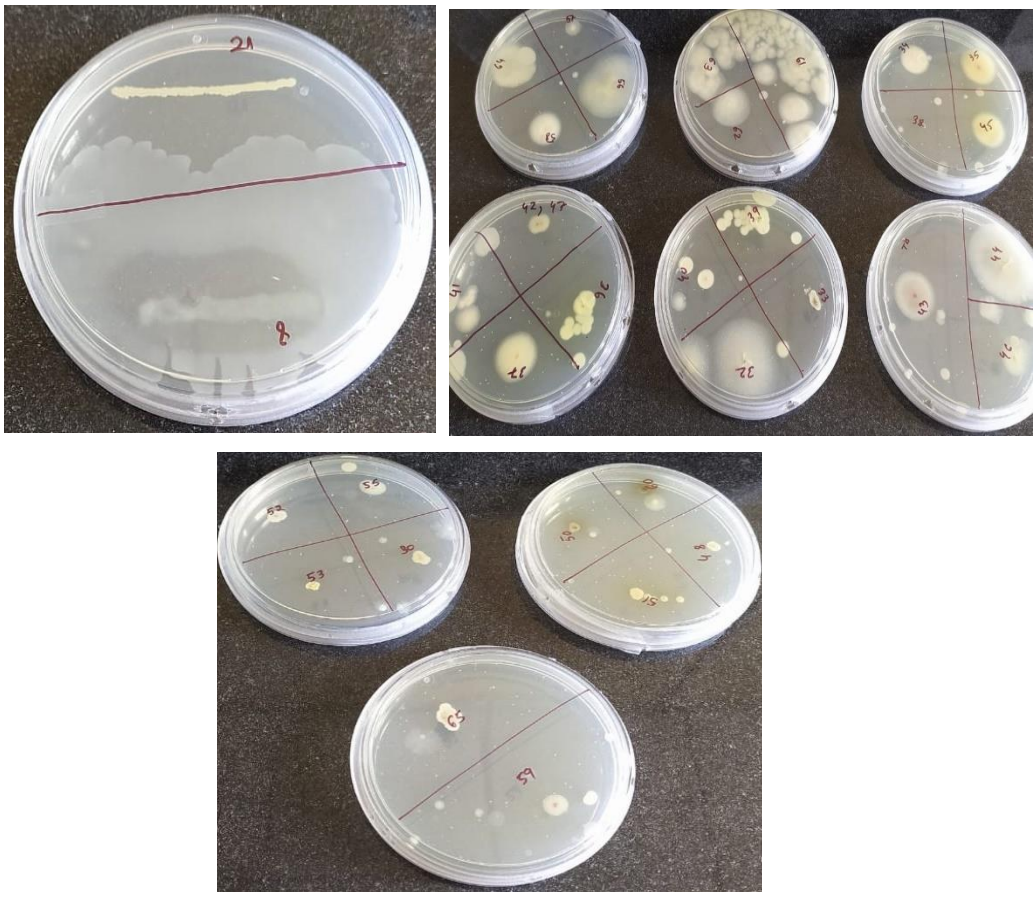

Fig.4.35 Lipase activity of isolates

4.7.5 Cellulase test

Among the total isolates, four bacterial strains (SKT061, SKT007, SKT002 and SKT009), six fungal strain (SKT033, SKT037, SKT063, SKT045, SKT035 and SKT034) and nine actinomycetal strains (SKT059, SKT065, SKT050, SKT048, SKT051, SKT060, SKT052, SKT053 and SKT055) (Fig. 4.32) were found to be the cellulase producer (Table 4.8)

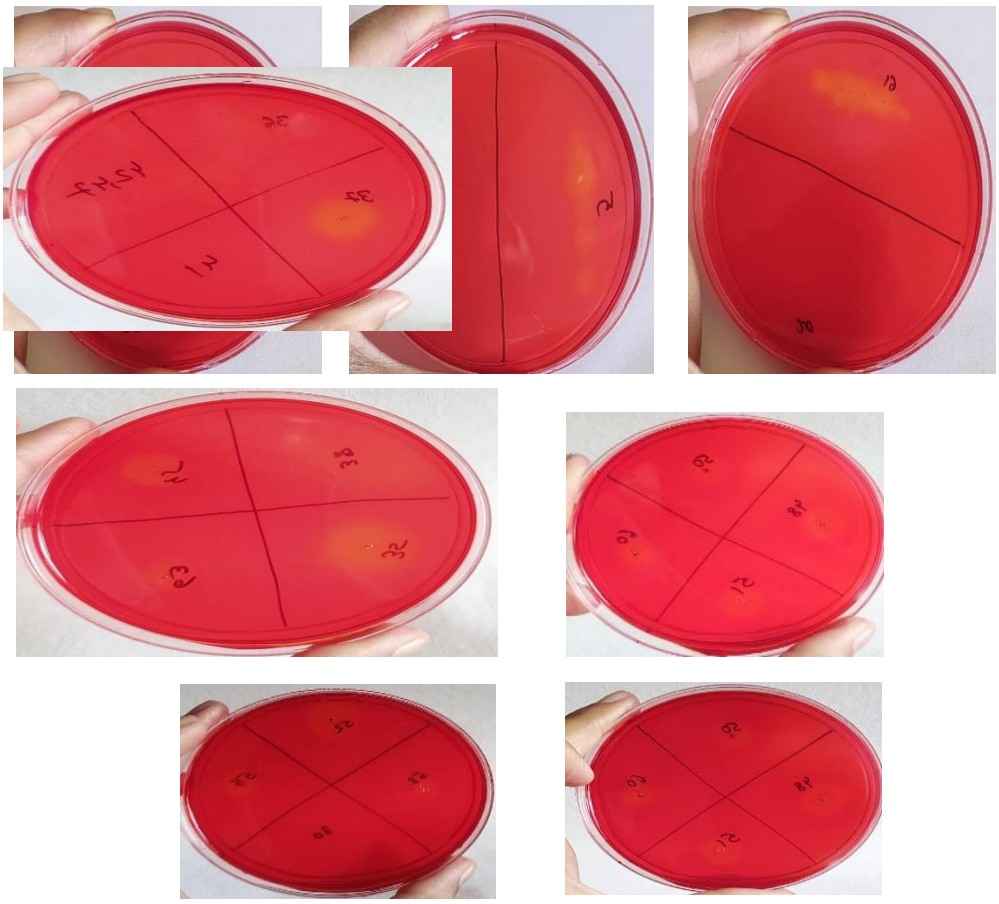

Fig.4.36 Cellulase activity of isolates

Table 4.8 Enzyme action of microbial isolates

Isolate code	Catalase	Amylase	Protease	Lipase	Cellulase
SKT060	+	-	-	-	-
SKT016	+	-	+	-	-
SKT020	+	+	+	-	-
SKT003	+	-	-	-	-
SKT061	+	-	+	-	+
SKT014	+	-	-	-	-
SKT008	+	-	-	+	-
SKT021	+	-	-	-	-
SKT059	+	-	-	-	-
SKT013	-	-	+	-	-
SKT004	+	-	-	-	-
SKT005	+	+	+	-	-
SKT010	+	+	+	-	-
SKT007	-	-	-	-	+
SKT002	+	+	+	-	+
SKT006	+	-	-	-	-
SKT019	-	+	+	-	-
SKT009	+	-	-	-	-
SKT017	+	-	-	-	-
SKT001	+	-	-	-	-
SKT033	+	+	-	-	+
SKT046	+	-	-	-	-
SKT043	+	+	-	-	-
SKT038	-	-	+	-	-
SKT044	-	+	-	-	-
SKT062	+	-	-	-	-
SKT042	+	+	-	-	-
SKT041	+	-	-	-	-
SKT037	-	+	+	-	+

SKT036	+	-	+	-	-
SKT040	+	+	+	-	-
SKT032	+	-	-	-	-
SKY039	-	+	+	-	-
SKT064	-	-	+	-	-
SKT057	+	-	-	-	-
SKT058	+	-	-	-	-
SKT056	+	-	-	-	-
SKT063	-	-	+	-	+
SKT045	+	+	+	-	+
SKT035	-	+	-	-	+
SKT034	+	-	-	-	+
SKT059	+	-	-	-	+
SKT065	+	-	-	-	+
SKT050	+	-	-	-	+
SKT048	-	-	+	-	+
SKT051	-	+	-	-	+
SKT060	+	+	-	-	+
SKT052	+	-	+	-	+
SKT053	+	-	+	-	+
SKT030	+	+	+	-	-
SKT055	+	-	-	-	+

4.8 Morphological and microscopical characterization of microbes

Microorganisms including bacteria, fungi and actinomycetes were classified by their colony appearances, pigment creation and development (Fig. 4.37). Bergey's Manual of Determinative Bacteriology was used for classification of bacteria and actinomycetes, however, Gliman's Manual of Soil Fungi was used for the identification of fungi. Microbial isolates showing three or more enzyme activities were examined morphologically with the help of their shape, size, margin, elevation, appearance, texture, pigmentation etc. (Table 4.9). In addition, microscopical

characteristic such as cellular morphology, shape, Gram staining was also observed in 100X magnification (Fig. 4.38). Based on colony and microscopical characteristics along with the enzyme activities, thirteen microorganisms were identified up to genus level. Among thirteen, four isolates were bacteria belonging to the genus *Streptobacillus* and *Streptococcus*, five were fungi belonging to the genus *Microsporum*, *Rhizopus* and *Aspergillus* and four were actinomycetes belonging to the genus *Streptomycetes*.

In the study, microorganisms were isolated from the decomposing litters of TC, OS and TC+OS in jhum fallows and microcosm. The microbial isolates were cultured on selective medium e.g., bacteria on NA, fungi on PDA and actinomycetes on SCA. Network of the colonies were observed when fully grown on agar surface.

Table 4.9 Morphological characterization of 13 isolated microbial strains

Isolate code	**Nature of colony**			**Color of colony**		**Days of growth**	**Media**	**Identified microbe**	**Litter type**
	Forms	Elevation	Margin	AM	SM				
SKT 002	Irregular	Umbonate	Undulate	Dark cream, rough, colony with 0.8 cm in dia.	Light cream	1 day	NA	*Streptobacillus* sp. (Gram -ve)	*T. candida*
SKT 005	Irregular	Raised	Undulate	Light cream, rough, colony with 0.5 cm in dia.	Light cream	1 day	NA	*Streptobacillus* sp. (Gram -ve)	*T. candida and O. sativa*
SKT 009	Circular	Flat	Undulate	Dark cream, rough, colony with 1.4cm in dia.	Cream	1 day	NA	*Streptobacillus* sp. (Gram -ve)	*T. candida + O. sativa*
SKT 020	Irregular	Raised	Undulate	Dark cream, smooth, colony with 1cm in dia.	Cream	1 day	NA	*Bacillus* sp. (Gram +ve)	*O. sativa*
SKT 033	Circular	Raised	Entire	Greenish grey, rough, colony with 0.1 cm in dia.	White	5 days	PDA	*Microsporum* sp.	*T. candida*
SKT 034	Circular	Umbonate	Filiform	White, wrinkled, colony with 2cm in dia	White	5days	PDA	*Rhizopus* sp.	*T. candida*
SKT 035	Circular	Umbonate	Filiform	White margin and light yellow in center, wrinkled, colony with 1.3 cm in dia.	White	5 days	PDA	*Rhizopus* sp.	*T. candida + O. sativa and O. sativa*
SKT 040	Circular	Undulate	Entire	White, Rough, colony with 2 cm in dia.	Cream	7 days	PDA	*Aspergillus* sp.	*T. candida*

SKT 045	Irregular	Umbonate	Curled	White, rough, colony with 1.5 cm in dia.	White	2 days	PDA	*Aspergillus* sp.	*T. candida* + *O. sativa*
SKT 030	Irregular	Raised	Curled	White, rough, colony with 0.3 cm in dia.	Light cream	3 days	SCA	*Streptomycetes* sp. (Gram +ve)	*T. candida* + *O. sativa*
SKT 052	Irregular	Flat	Undulate	Light grey, powdery, colony with 0.2cm in dia.	Light cream	2 days	SCA	*Streptomycetes* sp. (Gram +ve)	*O. sativa*
SKT 053	Irregular	Flat	Undulate	White, powdery, colony with 0.3 cm in dia.	Light orange	2 days	SCA	*Streptomycetes* sp. (Gram +ve)	*O. sativa*
SKT 060	Irregular	Flat	Undulate	Light grey, powdery, colony with 0.5 cm in dia.	Black	9 days	SCA	*Streptomycetes* sp. (Gram +ve)	*T. candida* + *O. sativa*

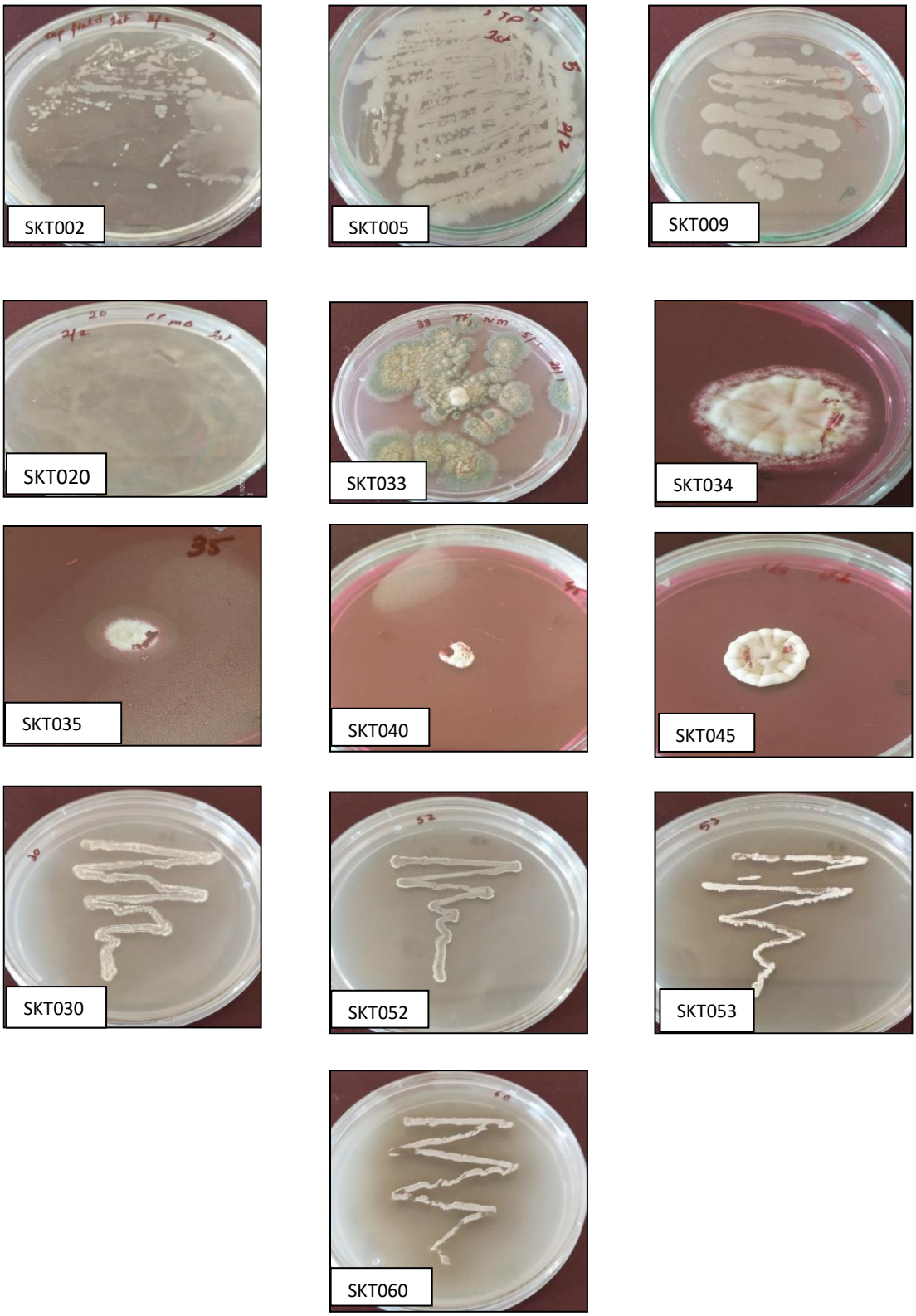

Fig 4.37 Total 13 isolated microbial strains

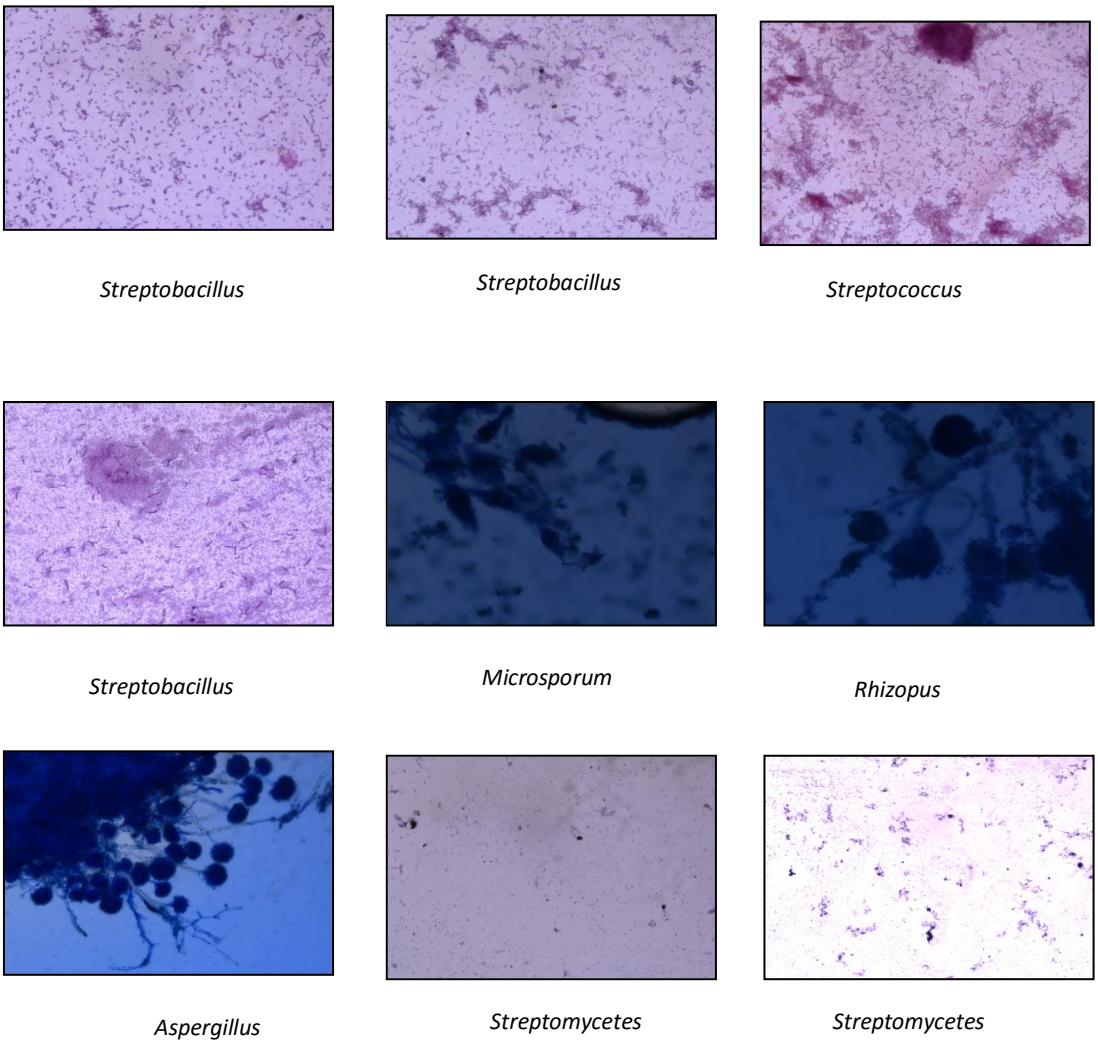

Fig 4.38 Microscopical morphology of microbial isolates

4.9 Bio-compost management

Microbial strain namely, SKT002, SKT005, SKT009, SKT020, SKT033, SKT034, SKT035, SKT040, SKT045, SKT030, SKT052, SKT053 and SKT060 isolated from different decomposing litters identified during the study. One ml of liquid microbial culture of above-mentioned microbial strains was mixed with 10 g of each litter types (TC, OS and TC+OS) in microcosm under controlled conditions. In addition to this, a mixture of all the microbial inoculum was made and added to the litters in a separate microcosm and decomposition rate was determined.

TC + OS litter with the mixture of microbial inoculum optimizes the process of litter decomposition and the pattern of nutrient release, and thus has better ability to produce compost as compared to TC and OS litter singly along with single microbial inoculum.

CHAPTER 5

Discussion

5.1 Soil fertility in shifting cultivation

Shifting cultivation has been reported to have negative consequences to the society, particularly because of the reduced fallow periods between two crop cycles as a result of increasing human population as did not allow enough time for the soil to recover fertility (Riessen, 2000). This has resulted in lowering crop productivity and caused a problem of food security for the farmers (Riessen, 2000). In the past, the fallow time was about 20-25 years which ensured enough time for the ecosystem to recover soil fertility and consequently greater crop yield. The decline in fallow length has resulted in a decrease in microbiological populations and increase in top soil loss and land degradation (Gafur, 2000). As a result, the Mizoram government has implemented number of land use policies in the past few decades to provide financial assistance to jhum farmers for their better livelihood options (Tripathi et al., 2017). Jhumias, on the other hand, are used to this practice and as soon as the financial assistance is terminated from the government, they return to continue shifting cultivation.

Shifting cultivation has been linked to land degradation, nutrient reduction, soil erosion, and ecosystem resilience in previous studies (Ewel et al., 1981; Kyuma et al., 1985; Andriesse et al., 1987; Ramakrishnan, 1992). According to studies, the primary cause of deforestation is the slash-and-burn farming technique (Sanchez, 1995), which results in higher soil loss due to increased erosion and as a result, lower agricultural yield (Al-Kaisi, 2001). One of the main initiatives to increase soil fertility and crop yield in shifting cultivated regions would be to develop bio-compost employing decomposing bacteria in left over litter after harvesting. As a result of this research, different decomposing microorganisms isolated from diverse litter types at shifting cultivated sites in Mizoram can be employed as bio-inoculants to generate bio-compost that can enhance soil fertility and crop productivity at shifting farming sites in Mizoram.

5.2 Litter decomposition and nutrient loss

Microorganisms are known to have a significant role in the breakdown of organic matter, nutrient cycling, maintaining structural and functional qualities of soil, and making nutrients available to plants through microbial succession during the decomposition process (Paul and Clark, 1989). Litter decomposition was shown to be faster in the first few months, then slower in the following months. Various authors from across the world have reported this phenomenon (Tripathi and Singh, 1992a and b; Pandey et al., 2007; Bohara et al., 2019). TC and corn litters have been shown to decompose quickly in the first few months (Wapongnungsang et al., 2017; Hou et al., 2019). Microbial degradation of easily analyzable labile compounds like sugar and starch, which provide sustenance for bacteria, regulates rapid disintegration in the early stages (Aerts and Chapin, 1999). The majority of studies have found that litter quality has a significant impact on the decomposition rate of leaf litter among species categories (Tripathi et al., 2006; Pandey et al., 2007). The high N and low lignin containing TC leaf litter degraded more quickly (k = 2.19 in JF3 and k = 2.23 in JF8) than the other two litter treatments. TC litter completely decomposed within six months. Similar results were found in *Sesbania* sp., where shoot degradation took only four months (Singh et al., 2007). For all leaf litter types, the daily instantaneous decay constant (k), the number of days essential for 50%, and 95% breakdown were in the following order: *T. candida* > *T. candida* + *O. sativa* > *O. sativa*. Quideau et al., (2005) found that leaf litters with increased N content decompose faster in the Chaparrel ecosystem in Southern California. However, the C:N ratio in OS litters could be partly to blame for the slow rate of decomposition (Bauder, 2000 and Fosu, et al., 2007). Different litter types decompose faster in longer fallow land in the study, which was comparable to Saplalrinliana et al., (2016), who found that adding forest litters to a jhum plot changes soil properties as the length of the fallow period increases, which is due to the faster rate of decomposition in longer fallows.

The breakdown rates of BL and MFL in FF5 and MGL in FF3 were not significantly different. Xuluc-Tolosa et al., (2003) and Oatertag et al., (2008) observed similar results for different litter successional (3, 13, >50 years) forests in Mexico. They also reported that litter decomposition rate did not vary with forest age. The annual decay constant of BL in FF5 was 2.18 at the end of the experiment.

When compared to dwarf bamboo leaf litters in young secondary forests in Northern Japan (Tripathi et al., 2006), the k value of the current study was significantly greater. Because rainfall and temperature are significant determinants for litter decomposition, higher decomposition rates were observed in the current study (Tripathi and Singh, 1992a and b).

In both fallow fields, the decomposition rate of TC, OS, and TC+OS leaf litters revealed a similar trend (JF3 and JF8). When comparing JF8 to JF3, the rate of breakdown was faster. When compared to JF3, litters in JF8 lost their weight faster. Previous research has found that the decomposition rate of litter varies substantially depending on the length of time that the area has been abandoned (Cornwell et al., 2008). In general, the rate of mass loss was much higher in the early months than in the later months. In JF8, differences in mass loss between litter types were not significant at first, but they became considerable as the decomposition of different litter types progressed. Similar results were found in the litter breakdown of two *Tephrosia* sp. species in Malawi, Africa (Munthali et al., 2013). 9–12% of litter mass remained in JF3 at the end of the study, but no litter mass remained in JF8 for TC litters since the majority of the litter mass was lost during the initial days of decomposition. Wapongnungsang and Tripathi (2017) found that 35–43 percent of TC litter mass remained after one year of decomposition in Mizoram. The rapid mass loss seen in this study could be attributed to large concentrations of labile compounds like sugars, amino acids, and soluble phenolics (Wang et al., 2004), as evidenced by the low C:N ratio in the litter (Wapongnungsang and Tripathi, 2017).

The first N content varied from 1.56 to 4.18 percent, which was greater than previous findings for wheat (0.61 percent) and TC (2.86 percent) respectively (Singh et al., 2007; Wapongnungsang and Tripathi, 2017). The C concentration of various initial litter components ranged from 36% to 41%, which was significantly greater than the report of TC litters (30–37%) in different Mizoram fallow periods (Wapongnungsang and Tripathi, 2017). However, initially lignin concentrations ranged from 9 to 21%, which was lesser than *Betula emanii* (26.5%) and *Sasa kurilensis* (27.5%) from Northern Japan's secondary forest (Tripathi et al., 2006). In tropical dryland agroecosystems, similar reports are known for *Sesbania* sp. (9.6%) and wheat straw (21.2%) (Singh et al., 2007). The average initial C:N ratio ranged

from 17 to 26%, but it was significantly lower in TC (8.77%) than in other litter types. The C:N ratio of TC litters in this study was lower than that of TC litters in Mizoram (13–17%) (Wapongnungsang and Tripathi, 2017). The initial lignin:N ratio ranged from 2 to 14 percent, with the lowest in TC litters and the highest in OS litters. In the tropical dryland, similar reports are known for *Sesbania* sp. (3.2%) (Singh et al., 2007). Lower C:N and lignin:N ratios in early litters have been shown to speed up the decomposition process (Tripathi and Singh, 1992a and b; Singh et al., 2007; Krishna and Mohan, 2017; Wapongnungsang and Tripathi, 2017). In the current investigation, a similar tendency was seen, with lower C:N and lignin:N ratios in TC litters showed faster breakdown than other litter treatments.

In the current study, only a small quantity of carbon and nutrient mass of different litters remained at the end of one year. The carbon and nutrient contained in the litter material lost considerably during the course of decomposition and enriching their abundances in the soil to be taken up by the plant roots (Tripathi and Singh 1992 a and b). The release of C and nutrients were faster in the first phase followed by their gradual release later (Wapongnungsang and Tripathi, 2017). The initial nutrient level has a significant impact on plant residue decomposition because it limits the proliferation and activity of soil microorganisms. The concentration of other key nutrients changes when litter decays, in addition to C and N (Osono and Takeda, 2004; Jacob et al., 2009; Manzoni et al., 2010). Depending on its mobility and microbial uptake, each nutrient has a unique set of dynamics. K mass loss was the fastest in this investigation, followed by P, Mg, and Ca. Similarly, faster K release was observed in leaf litters and rotting wood, which was attributed to microbial immobilization as well as leaching (Abbott and Crossley, 1982; Osono and Takeda, 2004; Jacob et al., 2009; Preston et al., 2009). Mg content is influenced by soil characteristics, and its dynamics revealed an early leaching phase followed by immobilization (Osono and Takeda, 2004; Preston et al., 2009). The behavior of P, as well as that of N, in decomposing litters had been extensively studied (Manzoni et al., 2010; Cleveland et al., 2013; He et al., 2016; Marklein et al., 2016). P mineralization is linked to N recycling and biomass. The nutrient release pattern of litters, like mass loss, was influenced by site circumstances and fallow age as longer fallows yield more nutrients than shorter fallows. According to Ostertag et al., (2008)

litter decomposition was faster in 60-year-old forests than in 10 and 30-year-old forests. Mayer (2008) also found that in old forests, litter decomposition and nutrient release are faster than in other successional phases. Nutrient mass loss was lower in the microcosm than in the field experiment, which could be attributable to less microbial activity because the experiment was conducted under controlled conditions.

The amount of organic carbon and total nitrogen in the soil grew dramatically as the length of the fallow periods increased in the study. The nutrient mass loss of distinct litter types during decomposition was synchronized with the increase in soil nutrients in jhum fallows. Leaf litter degradation produces a large amount of dissolved organic carbon molecules (Singh and Gupta, 1977). Higher initial N concentration in litters favors N release in soil (Hoorman et al., 2011), as TC litters were high in N and released more N to the soil after decomposition (Hoorman et al., 2011). The present study found that longer fallow land had higher soil N concentration than shorter fallow land, which was consistent with previous findings (Neff et al., 2005). The soil C/N ratio in mixed litter (TC+OS) type was seen to be less in a few months as a result of higher N release relative to C in the soil (Fig. 4.22 -4.25). Due to faster decomposition of litters, older fallows were able to add more organic matter and nutrients to the soil, which could be related to the decomposition research of TC components in Mizoram (Wapongnungsang et al., 2017). Increased nutrient input through organic residue addition may be linked to improved soil nutrients during fallow recovery (Jiang et al., 2009; Lungmuana et al., 2017). As a result, nutrient loss from decomposing litters accumulates in the soil, increasing soil nutrient concentration.

Leaf litter decomposition in microcosm experiment followed the similar pattern showing rapid decay during the first phase of decomposition followed by its slow release at later stages of decomposition. Decomposition of diverse litters has shown similar trends in other studies (Güsewell and Gessner, 2009; Munthali et al., 2013; Singh and Tripathi, 2020). The presence of a rapidly degradable labile material causes rapid mass loss in the early stages (Wang et al., 2004). Slow down of decomposition rates in the later stages has been reported to be the dominance of recalcitrant substances during the course of time, and it further slowdown at the stage

of completion of decomposition the significantly high dominance of recalcitrant substances such as lignin, pectin, polyphenols, and waxes (Pandey et al., 2007).

The mass remaining and days elapsed during decomposition yielded highest R^2 value found in this study (0.98) which corresponds to the R^2 value of decaying leaf litter in other tropical regions of India (Tripathi and Singh 1992 a and b). Mean annual decay constants (k values 2.09 and 2.13) in two pot experiments closely matched with the reports of microcosm study (2.09 – 2.13) carried out by Loria-Naranjo et al., (2018) in seagrass meadow. At the end of the pot trial experiment, the amount of nutrients in all litter types drastically reduced, whereas, the amount of C and N in the soil increased reflecting that the significant additions of C and nutrients from the litter to soil under pot experiment.

5.3 Abiotic factors and litter decomposition

Various abiotic factors like rainfall, humidity and temperature have been shown to have a substantial impact on litter decomposition and C and N release rates (Tripathi and Singh, 1992a and b, Pandey et al. 2007; Zhou et al., 2008; Paudel et al., 2015). Similarly, decomposition rates were shown to be strongly and positively associated with temperature in this study. In other studies, temperature was found to be the most important element in determining the rate of litter decomposition by Meentemeyer (1978) and Kirschbaum (2000). With a specific moisture content, soil microbial activity increases as the temperature rises (Kirschbaum, 1995). The influence of temperature on soil microbial development and activities has an effect on litter decomposition (Kravchenko et al., 2019). In the forest ecosystem, however, temperature and soil moisture predict decomposition rate (Berg and Staaf, 1980; Mc Claugherty and Berg, 1987; Taylor et al., 1989; Tripathi and Singh, 1992 a; Uma et al., 2014).

5.4 Microorganisms and litter decomposition

In the current study, microbial diversity on decomposing litters revealed that bacterial populations were followed by fungal and actinomycetal populations. In general, it is reported that bacteria are the primary microorganisms taking part in the litter decomposition followed by fungi and actinomycetes (Swift et al., 1979;

Tripathi et al., 1992 a and b; Kjoller and Struwe, 1992). Further, fungi and bacteria are thought to play a major role in litter and wood breakdown (Allmer et al., 2009; Bassler et al., 2010; Baldrin, 2016; Purahong et al., 2016; Tlaskal et al., 2016). The bacterial population was larger in both the jhum fallows and the microcosm than in other microorganisms (fungi and actinomycetes). Bridge and Spooner, (2001) found a similar trend in the microbial population. Bacteria were more active in breaking down labile chemicals in the early stages of decomposition; later, fungus play an important role in decaying complex molecules (Kjoller and Struwe, 1992). Research revealed that bacteria may degrade lignin and catabolize other metabolites resulting from partial fungal breakdown of litter (Bugg et al., 2011a and b). According to Hoorman, (2011), soil contains a considerable number of bacteria, one tenth of actinomycetes, and a tiny number of fungi, all of which are relevant to the study. According to the study, the microbial population was significantly greater in JF8 than in JF3 for all litter types. Lalnunzira and Tripathi, (2018), found that litter inputs on abandoned land increased soil organic matter, which in turn supported a large number of microorganisms through a feeding relationship.

Microbial population on decomposing litters in microcosm was lower than in the field experiment, which could be related to differences in abiotic conditions such as temperature and moisture from jhum fallows to microcosm (Xuluc – Tolosa et al., 2003; Pandey et al., 2007). The bigger section of interactable soil organic matter in the carbon pool of jhum fallows could explain the higher abundance of microbial groups in jhum fallow decomposing litters than in pots (Kramer and Gleixner, 2006, 2008). In the current study, the microbial population in degrading litters follows a clear order of bacteria> fungus> actinomycetes. This trend was strikingly similar in all Jhum fallows and microcosms (Heijboer, 2018).

5.5 Decomposing microbes and their microbial enzyme activity

In the present study, out of total 66 microbes isolated from different decomposing litter types (TC, OS and TC+OS) on field experiment (JF3 and JF8) following shifting cultivation and microcosm (PJS3 and PJS8), comprehensive enzyme test revealed 51 (83.3%) microbes responsible for decomposition. Out of that only 13 microbes were sorted based on their active enzyme action for further bio-

composting/bio-fertilizer development. Litter breakdown is influenced by microorganisms, which produce and optimize enzymatic dispersion (Ostaszewski and Nissen, 1988). Extracellular enzyme production is influenced by the type of microorganisms and their nutritional requirements, and it often follows litter decomposition dynamics. Catalase, amylase, protease, lipase, and cellulase enzyme tests were performed during the investigation because these enzymes are responsible for litter decomposition and are secreted by microorganisms. Various investigations have shown that bacterial populations can produce protease and cellulase enzymes during the early phases of decomposition (Purahong et al., 2016; Tlaskal et al., 2016). In the intermediate phase of decomposition, the fungal community grows (Voriskova and Baldrin, 2013; Purahong et al., 2016), with cellulase and endoxylanase being the most abundant enzymes (Fioretto et al., 2000; Snajdr et al., 2011). Lignin and humic acid degrading fungi become prevalent in the latter stages (Voriskova and Baldrin, 2013; Purahong et al., 2016). Finally, actinomycetes aid in the decomposition of cellulose, starch, and protein (Eida et al., 2012; Tiwari et al., 2019). Several actinomycetes were also found to have catalase and amylase activity in prior research (Momin and Tripathi, 2019). In the intermediate stage of the litter decomposition process, catalase-producing bacteria were the most prevalent. Out of 51 isolates, 13 isolates (SKT002, SKT005, SKT009, SKT020, SKT033, SKT034, SKT035, SKT040, SKT045, SKT030, SKT052, SKT053 and SKT060) were selected showing three or more enzyme activity. These isolates were grown separately as single culture. Bacteria were grown in NA plates (Sapkota, 2020), fungi in PDA plates (Aryal, 2015) and actinomycetes in SCA plates (Aryal, 2015). These isolates were further identified with respect to morphological characteristics and microscopic view. In the present study, maximum of the bacterial isolates were irregular in form with dark cream color aerial mycelium and light cream substrate mycelium. However, fungal isolates were mostly circular in form with white hairy aerial mycelium and white substrate mycelium. Actinomycetes isolates were mostly irregular with light grey to white in color with powdery aerial mycelium and black to orange substrate mycelium. The growth period of isolates varies from 1 day for bacteria followed by 5 – 7 days for fungi and 2 – 9 days for actinomycetes. Morphologically and microscopically 13 isolates were recognized up to genus level

following Bergey's Manual of Determinative Bacteriology (2000) for bacteria and actinomycetes and Gliman's Manual of Soil Fungi (1957) for fungi. In present study, mainly the bacterial genus was *Streptobacillus* sp. followed by *Bacillus* sp. Corresponding bacterial strains were also discovered in Northeast India's rhizospheric soil (Debi and Prakash, 2017). Previous enzymatic studies on these two bacterial taxa revealed the production of cellulase (Rajagopal et al., 2007; Sethi et al., 2013), catalase (Eisenberg et al., 2015; Babiker et al., 2016), and protease (Babiker et al., 2016; Pant et al., 2015). Some soil fungi are in charge of breaking down plant litter and releasing nutrients into the soil for plant growth because they decompose cellulose and lignin through enzymatic processes (Hoorman, 2011; Raaijmakers et al., 2009). Three separate genera of fungi were recovered from three different litter types in the study: *Microsporum* sp., *Rhizopus* sp., and *Aspergillus* sp. Giudice et al., (2012) and Debi & Prakash, (2017) found similar fungal findings in soil, indicating proteolytic enzyme activities that further break down the refractory components of leaf litters, resulting in decomposition. During the research, only one genus of actinomycetes, *Streptomycetes* sp., was isolated. Recent studies on actinomycetes have found salt tolerant activity in *Streptomycetes* sp. collected from Mizoram's shifting cultivated area (Momin and Tripathi, 2019), implying that the presence of this species as a decomposing actinomycetal strain indicates the salt acceptance ability in shifting cultivated soil. Actinomycetes are well known for their ability to degrade cellulose (Eida et al., 2012).

5.6 Bio-compost and soil fertility

Microbial inoculants were used in composting to boost the microbial population of decaying waste and speed up the decomposition process. The number of naturally occurring bacteria has been harmed as a result of modern-day pollution. Bio-composting is a waste-management biological process that involves microorganisms like bacteria, fungus, and actinomycetes, and results in the decomposition and recycling of organic matter into fertilizers. Bio-composting enhances soil quality by adding nutrients such as organic matter, nitrogen, phosphorus, and magnesium, which improve the water holding capacity, structure, and texture of soil, allowing it to retain more nutrients and moisture for plant growth.

Composting was done in this work using several litter kinds (TC, OS, and TC+OS) under controlled settings, with correct mesophilic, thermophilic, and maturation states (Wu et al., 2010). Composting was done by utilizing the inoculum of a microbial strain that had been identified. The decomposition process was shown to be very active and rapid during composting, which could be related to the presence of microbial inoculum. Recent research has shown that microbial inoculants can increase composting performance by speeding up the breakdown process and reducing odor (In, 2019). Certain bacteria inoculation has been proven to improve the fungal disease suppressive properties of compost supplemented soils (Hoitink, 2006). Different microbial inoculums were tested in this work, including bacterial inoculum (*Streptobacillus* sp. and *Bacillus* sp.), fungal inoculum (*Microsporum* sp., *Rhizopus* sp., and *Aspergillus* sp.), and actinomycetal inoculum (*Streptomycetes* sp.). These bacteria have been shown to produce extracellular cellulase, amylase, and protease, all of which are involved in the decomposition process (Umsakul et al., 2010). According to Trautmann and Olynciw (2000), the bacterial genus *Bacillus* sp. and the fungal genus *Aspergillus* sp. are primarily responsible for rapid decomposition in composting, while actinomycetes are vital in degrading complex organics such as cellulase, lignin, chitin, and pectin.

It was discovered that after 25 days, the texture of the litter types changes, followed by the appearance of black colored humus-like compounds after 70 days of decomposition. All of the litter categories in this study were converted to compost in under 80 days. A similar discovery was made on wheat straw that was transformed into compost in just 75 days (Pan et al., 2012). Controlled microbial composting (CMC), according to Ltibke (2016), can help with humus management and the faster and more efficient conversion of organic resources to compost. The CMC microbial inoculants produce high-quality compost with a large population of beneficial microorganisms that remain active after the compost is applied, improving soil quality and health, as well as crop development and production. Leguminous compost can improve the physico-chemical qualities of soil, particularly the availability of nutrients (Abdelfattah et al., 2021). Because TC is a member of the Leguminous family, it can be used alone or in combination with OS litters to generate high-quality compost. Furthermore, Leguminous organic materials in the

soil can help with microbial breakdown (Abdelfattah et al., 2021). According to Buragohain et al., (2017), bio-compost generates a significant number of microorganisms, which leads to an increase in soil organic matter and improved crop growth. According to Atkinson et al. (1996), adding microbial inoculum causes organic C to be reduced during composting. Composting results in a drop in the C:N ratio, which could be attributed to the transfer of organic C into CO_2 (Chefetz et al., 1998; Sanmanee et al., 2011).

5.7 Future perspectives

The state of Mizoram is characterized by various agro-climatic zones due to varying climate, soil and vegetation types. Therefore, this study needs to be further extended to the other agro-climatic regions of the Mizoram by studying litter decomposition and associate microbes along with their enzyme activities and ribotyping with proper replication from different regions for developing a more versatile bio-compost/bio-fertilizer for the state.

CHAPTER 6

Summary and Conclusion

Shifting cultivation in Northeast region of India including Mizoram is widely distributed agricultural system. Shifting cultivation practices can be linked with ecological, socio-economic and cultural life of the people. Efforts to address jhum as destructive remained challenging task due to its shortening cycles and ecological threats. The current practice of shifting cultivation in this region followed extravagant and unscientific land use pattern. The damaging effect of shifting cultivation are devastating and far reaching in degradation of environment. The decrease in cycle period of shifting cultivation in recent years has resulted in large scale of deforestation, soil and nutrient loss. Shifting cultivation has led to various soil related constraints- low inherent soil fertility, high susceptibility to soil erosion, severe soil physical degradation and nutrient imbalance. Soil fertility is crucial for agricultural productivity and therefore food security. Good management of soil fertility help to reduce soil, water and air pollution, regulate water resource availability, support a diverse and active biotic community and increase vegetation cover. Maximum population in this region depends on shifting cultivation for their livelihood which in turn deplete the soil fertility. Using of synthetic chemicals in present agricultural practice for enhancing crop yield has largely affect the soil fertility and yielding capacity. Farmers can improve soil fertility by optimizing various soil fertility management techniques by minimizing soil nutrients loss and maximizing net returns. In this respect, decomposition of various plant litters enables to enhance various soil ecosystem functions by converting litter components into humus. In addition, these litters also provide nutrients to the microorganisms in the soil and also improve soil health.

In the present study, three different litter types were used (TC, OS and TC+OS) with high, low and high + low substrate quality respectively, to improve soil fertility by the process of decomposition along with this active decomposing microbial strains were isolated and identified up to genus level. The results of the study revealed that TC litters decomposes faster followed by TC+OS and lastly OS litters in both field and microcosm. Additionally, the rate of decomposition was higher in longer fallow (JF8) as compared to the shorter fallow (JF3). In JF8 TC litters were

found to be fully decomposed by 240 days. The fast decomposition of TC litters may be due to high substrate quality. Initial substrate quality showed highest N content and lowest C:N and lignin:N ratio in TC, intermediate N content C:N and lignin:N ratio in TC+OS litters and lowest N content and highest C:N and lignin:N ratio in OS litters. This initial high N content favored the rapid release of N during initial stages of decomposition along with high concentration of labile substance as reflected by low C:N ratio in initial litter types. Slow decomposition of OS litters may be because of more stable polysaccharide which is reflected by high initial C content and high C:N ratio may also be responsible for this phenomenon. On the other hand, the rate of decomposition in microcosm was slower as compared to field experiment which may be due to various abiotic factors that influence decomposition process in field. The present study showed a positive correlation between temperature and decomposition days in field experiment (JF3 and JF8). Contrastingly, no such correlation was observed in microcosm as it was carried out under controlled conditions. The result suggested that the litter mass remaining at the end of the study followed the pattern of decomposition rate of different litter types. In JF8 no litter mass remained for TC litters at the end but about 7% mass was remaining for TC + OS litters and 11% for OS litters. However, in JF3 litter mass remaining was 9% (TC), 12% (OS) and 10% (TC+OS) respectively. In microcosm litter mass remaining was more for all litter types compared to field experiment. It was found to be about 9% (TC), 15% (OS) and 14% (TC+OS) in PJS8 and 13% (TC), 33% (OS) and 21% (TC+OS) in PJS3respectively. The variation in litter mass remaining among the litter types is due to the difference in rate of decomposition which further dependent on substrate quality.

The field experiment carried out in forest fallows (FF3 and FF5) during the study showed a slightly higher rate of decomposition than the rate of decomposition in microcosm but lower than Jhum fallows decomposition rate. In forest fallows, daily instantaneous decay rate varied from 2.04 – 2.17. However, in jhum fallows it varied from 2.14 – 2.23 and in microcosm it ranged from 2.03 to 2.18. The higher rate of decomposition in jhum fallows than the forest fallows may be due to the difference in fallow periods as longer fallows have more active decomposers as compared to shorter fallows and in the present study the forest fallows were 3 and 5 years where

as jhum fallows were 3 and 8 years. Along with the fallow period, substrate quality may also be responsible for slow rate of decomposition in forest fallows.

In field experiment, for jhum fallows N mass remaining at the end of the litter recovery ranged from 0 – 11%, and C mass remaining ranged from 0 – 12%. The K, P, Mg and Ca mass remaining in different litters at the end were 0 – 11%, 0 – 10%, 0 – 12% and 0 – 12% respectively. Accordingly, the C content in the soil increased up to 75% and N content up to 22% at the end of the study. It can be said that the major part of nutrient mass lost during the period of decomposition from various litter types were ultimately added to the soil and a minor portion is lost by leaching. However, litters of TC produce high biomass, dense vegetation cover and N_2 fixing ability and show high potential in supplying C and nutrients to the soil. OS is the major crop plant in Northeast India. OS litters can also release ~34% of organic C in soil during decomposition and also affect soil C and N cycle. So, the decomposition of litters resulted in increasing soil nutrients.

In present study, the microbial population in decomposing litters increased as the decomposition proceeded and reached its peak between fifth to seventh month of decomposition and gradually decreased during later stages. Microbial population was much greater in field experiment as compared to microcosm. Among the three groups of microorganisms, bacterial count was maximum throughout the study followed by fungal count and minimum was actinomycetal count for all litter types in field and microcosm. Tc litters in JF8 showed maximum bacterial count and actinomycetal count but maximum fungal count was observed in TC litters if JF3. In the present study, microbial population in different litter types were found to be in order TC > TC+OS > OS and maximum microbial population was observed in JF8. Results of microbial population in the study, revealed the similar pattern of decomposition rate with respect to litter types and fallow periods.

Total 66 microbial strains were isolated from decomposing litters during the entire study period. Out of total 51 microbial isolates were found to be positive towards decomposing enzymes activities. Depending on the activity towards the enzyme test, 13 microbial isolates were identified up to genus level, of which 4 (SKT002, SKT005, SKT009, SKT020) were bacteria, 5 (SKT033, SKT034, SKT035, SKT040, SKT045) were fungi and 4 (SKT030, SKT052, SKT053,

SKT060) were actinomycetes. Based on the colony morphology and microscopic view, bacteria were identified as *Streptobacillus* sp. and *Bacillus* sp., fungi as *Microsporum* sp., *Rhizopus* sp. and *Aspergillus* sp., and actinomycetes as *Streptomycetes* sp. These six identified microbial genera were further used as microbial inoculants in bio-compost preparation under controlled condition. The compost was prepared from the litters of TC, OS and TC +OS. It was observed that addition of microbial inoculants in litters boost up the decomposition process and ultimately resulted in fast compost formation.

In conclusion, the findings of the study demonstrated the pattern of leaf litter decomposition with varying substrate quality in two different forest fallows (FF3 and FF5) and jhum Fallows (JF3 and JF8). Through litter decomposition, C, N and other macro and micro nutrients were recycled in Jhum fallows. Apart from field experiment, microcosm information on changing pattern of litter nutrients and microbial population. Both field and laboratory microcosm showed faster decomposition in JF8 (field) and PJS8 (microcosm) for TC litters followed by TC + OS and OS litters. This revealed that litter substrate quality and microbial diversity are important factors responsible for litter decomposition. The mixture of TC and OS litters (high + low quality) would have significant effect in soil fertility as their rate of decomposition was intermediate and nutrient loss followed a slow and steady pattern which help the growing plants to utilize it in long run and avoiding their loss through leaching. Changes in bacterial, fungal and actinomycetal population during different stages of decomposition and their role in decomposition has significant potential in regulating soil nutrients in Jhum fields. Among various factors of litter decomposition, microbial enzymes play a major role in breaking down of various litter components. Rate of decomposition also depends on certain microbial strains as it was observed by using the same strain in bio-compost preparation. Thus, the combination of high and low quality litters in shorter fallow land along with microbial inoculants would be recommended for soil fertility management in shifting cultivation of Mizoram. These microbes can accelerate the process of decomposition in shorter fallow that may enhance the productivity and meet crop demand of growing population.

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ABSTRACT

Shifting cultivation in Northeast region of India including Mizoram is widely distributed agricultural system. Shifting cultivation practices can be linked with ecological, socio-economic and cultural life of the people. Efforts to address jhum as destructive remained challenging task due to its shortening cycles and ecological threats. The current practice of shifting cultivation in this region followed extravagant and unscientific land use pattern. The damaging effect of shifting cultivation are devastating and far reaching in degradation of environment. The decrease in cycle period of shifting cultivation in recent years has resulted in large scale of deforestation, soil and nutrient loss. Shifting cultivation has led to various soil related constraints- low inherent soil fertility, high susceptibility to soil erosion, severe soil physical degradation and nutrient imbalance. Soil fertility is crucial for agricultural productivity and therefore food security. Good management of soil fertility help to reduce soil, water and air pollution, regulate water resource availability, support a diverse and active biotic community and increase vegetation cover. Maximum population in this region depends on shifting cultivation for their livelihood which in turn deplete the soil fertility. Using of synthetic chemicals in present agricultural practice for enhancing crop yield has largely affect the soil fertility and yielding capacity. Farmers can improve soil fertility by optimizing various soil fertility management techniques by minimizing soil nutrients loss and maximizing net returns. In this respect, decomposition of various plant litters enables to enhance various soil ecosystem functions by converting litter components into humus. In addition, these litters also provide nutrients to the microorganisms in the soil and also improves soil health.

In the present study, three different litter types were used (TC, OS and TC+OS) with high, low and high + low substrate quality respectively, to improve soil fertility by the process of decomposition along with this active decomposing microbial strains were isolated and identified up to genus level. The results of the study revealed that TC litters decomposes faster followed by TC+OS and lastly OS litters in both field and microcosm. Additionally, the rate of decomposition was higher in longer fallow (JF8) as compared to the shorter fallow (JF3). In JF8 TC litters were found to be fully decomposed by 240 days. The fast decomposition of TC litters may be due to high

substrate quality. Initial substrate quality showed highest N content and lowest C:N and lignin:N ratio in TC, intermediate N content C:N and lignin:N ratio in TC+OS litters and lowest N content and highest C:N and lignin:N ratio in OS litters. This initial high N content favored the rapid release of N during initial stages of decomposition along with high concentration of labile substance as reflected by low C:N ratio in initial litter types. Slow decomposition of OS litters may be because of more stable polysaccharide which is reflected by high initial C content and high C:N ratio may also be responsible for this phenomenon. On the other hand, the rate of decomposition in microcosm was slower as compared to field experiment which may be due to various abiotic factors that influence decomposition process in field. The present study showed a positive correlation between temperature and decomposition days in field experiment (JF3 and JF8). Contrastingly, no such correlation was observed in microcosm as it was carried out under controlled conditions. The result suggested that the litter mass remaining at the end of the study followed the pattern of decomposition rate of different litter types. In JF8 no litter mass remained for TC litters at the end but about 7% mass was remaining for TC + OS litters and 11% for OS litters. However, in JF3 litter mass remaining was 9% (TC), 12% (OS) and 10% (TC+OS) respectively. In microcosm litter mass remaining was more for all litter types compared to field experiment. It was found to be about 9% (TC), 15% (OS) and 14% (TC+OS) in PJS8 and 13% (TC), 33% (OS) and 21% (TC+OS) in PJS3respectively. The variation in litter mass remaining among the litter types is due to the difference in rate of decomposition which further dependent on substrate quality.

The field experiment carried out in forest fallows (FF3 and FF5) during the study showed a slightly higher rate of decomposition than the rate of decomposition in microcosm but lower than Jhum fallows decomposition rate. In forest fallows, daily instantaneous decay rate varied from 2.04 – 2.17. However, in jhum fallows it varied from 2.14 – 2.23 and in microcosm it ranged from 2.03 to 2.18. The higher rate of decomposition in jhum fallows than the forest fallows may be due to the difference in fallow periods as longer fallows have more active decomposers as compared to shorter fallows and in the present study the forest fallows were 3 and 5 years where as jhum fallows were 3 and 8 years. Along with the fallow period, substrate quality may also be responsible for slow rate of decomposition in forest fallows.

In field experiment, for jhum fallows N mass remaining at the end of the litter recovery ranged from 0 – 11%, and C mass remaining ranged from 0 – 12%. The K, P, Mg and Ca mass remaining in different litters at the end were 0 – 11%, 0 – 10%, 0 – 12% and 0 – 12% respectively. Accordingly, the C content in the soil increased up to 75% and N content up to 22% at the end of the study. It can be said that the major part of nutrient mass lost during the period of decomposition from various litter types were ultimately added to the soil and a minor portion is lost by leaching. However, litters of TC produce high biomass, dense vegetation cover and N_2 fixing ability and show high potential in supplying C and nutrients to the soil. OS is the major crop plant in Northeast India. OS litters can also release ~34% of organic C in soil during decomposition and also affect soil C and N cycle. So, the decomposition of litters resulted in increasing soil nutrients.

In present study, the microbial population in decomposing litters increased as the decomposition proceeded and reached its peak between fifth to seventh month of decomposition and gradually decreased during later stages. Microbial population was much greater in field experiment as compared to microcosm. Among the three groups of microorganisms, bacterial count was maximum throughout the study followed by fungal count and minimum was actinomycetal count for all litter types in field and microcosm. Tc litters in JF8 showed maximum bacterial count (3.37×10^6 CFU/g) and actinomycetal count (46×10^3 CFU/g) but maximum fungal count was observed in TC litters if JF3. In the present study, microbial population in different litter types were found to be in order TC > TC+OS > OS and maximum microbial population was observed in JF8. Results of microbial population in the study, revealed the similar pattern of decomposition rate with respect to litter types and fallow periods.

Total 66 microbial strains were isolated from decomposing litters during the entire study period. Out of total 51 microbial isolates were found to be positive towards decomposing enzymes activities. Depending on the activity towards the enzyme test, 13 microbial isolates were identified up to genus level, of which 4 (SKT002, SKT005, SKT009, SKT020) were bacteria, 5 (SKT033, SKT034, SKT035, SKT040, SKT045) were fungi and 4 (SKT030, SKT052, SKT053, SKT060) were actinomycetes. Based on the colony morphology and microscopic view, bacteria were identified as *Streptobacillus sp.* and *Bacillus sp.*, fungi as *Microsporum sp.*, *Rhizopus sp.* and

Aspergillus sp., and actinomycetes as *Streptomycetes sp*. These six identified microbial genera were further used as microbial inoculants in bio-compost preparation under controlled condition. The compost was prepared from the litters of TC, OS and TC +OS. It was observed that addition of microbial inoculants in litters boost up the decomposition process and ultimately resulted in fast compost formation.

In conclusion, the findings of the study demonstrated the pattern of leaf litter decomposition with varying substrate quality in two different forest fallows (FF3 and FF5) and jhum Fallows (JF3 and JF8). Through litter decomposition, C, N and other macro and micro nutrients were recycled in Jhum fallows. Apart from field experiment, microcosm information on changing pattern of litter nutrients and microbial population. Both field and laboratory microcosm showed faster decomposition in JF8 (field) and PJS8 (microcosm) for TC litters followed by TC + OS and OS litters. This revealed that litter substrate quality and microbial diversity are important factors responsible for litter decomposition. The mixture of TC and OS litters (high + low quality) would have significant effect in soil fertility as their rate of decomposition was intermediate and nutrient loss followed a slow and steady pattern which help the growing plants to utilize it in long run and avoiding their loss through leaching. Changes in bacterial, fungal and actinomycetal population during different stages of decomposition and their role in decomposition has significant potential in regulating soil nutrients in Jhum fields. Among various factors of litter decomposition, microbial enzymes play a major role in breaking down of various litter components. Rate of decomposition also depends on certain microbial strains as it was observed by using the same strain in bio-compost preparation. Thus, the combination of high and low quality litters in shorter fallow land along with microbial inoculants would be recommended for soil fertility management in shifting cultivation of Mizoram. These microbes can accelerate the process of decomposition in shorter fallow that may enhance the productivity and meet crop demand of growing population.