LEAF LITTER DECOMPOSITION IN TROPICAL MOIST DECIDUOUS AND SUB-TROPICAL EVERGREEN FORESTS OF MIZORAM

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LEAF LITTER DECOMPOSITION IN TROPICAL MOIST DECIDUOUS AND SUB-TROPICAL EVERGREEN FORESTS OF MIZORAM

BY

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Submitted

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

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DECLARATION

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

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

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CERTIFICATE

This is to certify that the thesis entitled "**Leaf Litter Decomposition in Tropical Moist Deciduous and Sub-Tropical Evergreen Forests of Mizoram**" submitted to the Mizoram University, Aizawl for the award of the degree of Doctor of Philosophy in Forestry is the original work carried out by Mr. Ngangbam Somen Singh (Reg. No.MZU/Ph.D./696 of 30.10.2014) under my supervision. I further certified that the thesis is the result of his own investigation and neither the thesis as a whole nor any part of it was submitted earlier to any University or Institute for the award of any degree. The candidate has fulfilled all the requirements laid down in the Ph.D. regulations of the Mizoram University.

His passion oriented hard work for the completion of the research is to be duly appreciated.

Date: Prof. S.K. Tripathi

Place: (Supervisor)

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Place:

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List of abbreviation and symbols

%	Percentage	LSD	Least significant difference
^{0}C	Degree Celsius/ centigrade	m	Meter
1SE	Standard error	m^2	Meter square
Al	Alluminium	MBC	Microbial biomass carbon
amsl	Above the mean sea level	mg	Milligram
ANOVA	Analysis of variance	Mg	Magnesium
As	Arsenic	ml	Milliliter
C	Carbon	mm	Millimeter
C/N	Carbon: nitrogen ratio	Mn	Manganese
Ca	Calcium	N	Nitrogen
cm	Centimeter	Na	Sodium
cm^2	Centimeter square	Ni	Nikel
Cr	Chromium	nm	nanometer
Cu	Copper	P	Phosphorus
DW	Dry Weight	P<0.01	Significant level at 99 percent
dia	Diameter	P<0.05	Significant level at 95 percent
E	East	Pb	Lead
e.g.	*exemplia gratia*	ppm	Parts per million
et al	*et alia and others*	P_{avail}	Available phosphorus
etc	*et cetera*	r	Correlation coefficient
Fe	Iron	rpm	Rotation per minute
g	Gram	S	Sulphur
h	Hour	SOC	Soil Organic Carbon
ha	Hectare	SPSS	Statistical package for the social science
K	Potassium	wt	Weight
kg	Killogram	yr	Year
kg ha^{-1}	Kilogram per hectare	Zn	Zine

Chapter 1

1. Introduction

1.1 Global forests and their significance

Globally, forests cover about one-third (4.1 billion hectares) of the total area and regulate earth's atmosphere through number of ecosystems services, for example, purification of air and water, cycling of carbon and nutrients regeneration of soil fertility, and maintenance of biodiversity (Comín, 2010). Tropical forests play an important role in global terrestrial carbon pools by storing a total of ~1240 Pg C of which one-third stored in vegetation and two-thirds in the soil (Lalnunzira, et. al., 2019). Further, they harbours half of the world's biodiversity including sources of new medicines, and ecosystem services for rural populations, including clean and sustained water supplies, climate regulation and pollinators for crops (Lewis, et. al., 2015). However, these forests are regularly over-exploited leading to loss of forests cover (>11 million ha per year) thereby losing their entities to provide goods and services to society (Chazdon, 2014). Conversely, if wisely managed, tropical forests can provide economic benefits through ecotourism, non-timber forest products, a sustainable source of timber, and also through upcoming carbon financing mechanisms (Smith & Scherr, 2003).

1.2 Forests in India

In India, the total forest cover is 7,08,273 km^2 which is 21.5% of the total area of the country against the recommended 33% forest cover. As per Champion & Seth

(1936), forests in India are classified into Tropical, Sub-Tropical, Temperate, Sub-Alpine and Alpine forest. Tropical forests are the most abundant categories in the country which house proportionately large biodiversity and store huge amount of carbon in the vegetation and soil (Lalnunzira, et. al., 2019). Further, flora and fauna of these forests are facing high degree of threats due to human interferences. In terms of the area, the state of Madhya Pradesh covered largest forest area (>77, 000 km^2). However, in terms of percent forest cover the Mizoram stands second (86.3%) after Lakshadweep (90.3%) (ISFR, 2017).

1.3 The Forests of Mizoram

The total geographical area of the state under forest is ~18, 186 km^2 (ISFR, 2017) of which very dense forest constitutes (0.62%), moderate to dense forest (27.8%) and open forest (57.84%). The remaining area is under non-forest (13.74%).Champion & Seth, (1968) classified Mizoram forest under six types: 1) Cachar Tropical Semi-evergreen Forest; 2) Secondary Moist Bamboo Brakes; 3) Pioneer Euphorbiaceous Scrub; 4) East Himalayan Moist Mixed Deciduous Forest; 5) East Himalayan Sub-tropical Wet Hill Forest; and 6) Assam Sub-tropical Pine Forest. Later on, state forest has broadly classified the forests of the state into six categories; for example, 1) Tropical Wet Evergreen Forest, 2) Montane Sub-tropical Forest, 3) Temperate Forests, 4) Bamboo Forests, 5) Quercus Forests and 6) Jhumland. This classification was based on elevation, precipitation and species composition as per the details Singh, et. al., (2002).

Tropical wet evergreen forest is characterized by warm and humid climate and mostly occurs below 900 m amsl and is the dominant forest type in this region. This forest types can be seen in low lying area on steep slope river bank. Mean annual rainfall was 2000 – 2500 mm with average temperature of 20 – 22 ^{o}C. Bamboo and *Musa* are dominant species found on slope hill along river site.

Montane sub-tropical and semi-evergreen forests type occur between altitude of 900 and 1500 m amsl and are characterized by cool and less precipitation compared to tropical forest. These forests are also among major forest categories of this region. Some of the tree species found in these forests are Oak, *Pinus kesiya, Duabanga grandiflora, Schima wallichii,*etc.

Temperate forests are usually occurring at high elevation with altitude more than 1600 m amsl. The majority of the mountains in the state belong to this forest such as Lengteng, Phawngpui, Thaltlang etc. Some of the important species found in this forest are *Rhododendron arboretum, Lithocarpus dealbata, Elaeocarpus serratus, Garcinia anomala,* etc.

Bamboo forests can be seen in tropical and sub-tropical mixed forests below 1600 m amsl in mixed with other tree species or totally dominated by bamboo. Among the bamboo, *Melocanna baccifera* is the most dominant species some of the others tree species found in this region *Emblica officinalis, Syzygium* species, *Albizia procera,* etc.

Quercus forests are mostly found in higher altitude with cool climatic condition of sub-tropical and temperate areas. Some of the Oak species are: *Quercus griffithiana,*

Quercus floribunda and *Quercus glauca*. *Lithocarpus dealbata* is other main species associated with the *Quercus* forest in this region. Jhumland are common in entire state as the jhumming is extensively practiced in hilly areas of the state.

1.4 Litterfall and leaf litter decomposition in forest

In forest ecosystems, litter fall is the source of organic matter that provides nutrients tothe forest soil. Leaf litter fall accounts for $2/3^{th}$ of total above ground litter production in forests (Krishna & Mohan, 2017).Other part of total litterfall is consisting of small branch, twigs and reproductive structures (Singh, et. al., 1999; Lalnunzira & Tripathi 2018). After the litter reaches the forest floor it under goes into complex litter decomposition process that is strongly affected by the set of parameters like substrate quality (proportions of various carbon containing compounds and elemental C and N), biotic (group of microorganisms) and abiotic/environmental factors (Swift, et. al., 1979; McClaugherty & Berg, 1987; Tripathi & Singh, 1992 a & b; Pandey, et. al., 2007; Patricio, et. al., 2014; Boyero, et. al., 2014; Lalnunzira & Tripathi 2018; Bohara, et. al., 2019). Finally, the amount of litter on forest floor is influenced by forest composition (Olson, 1963). The nutrients contained in the litter are recycled through the process of decomposition and releases inorganic nutrients that helps in plant productivity and improving soil fertility by accumulating soil organic matter in forest ecosystems (Aponte, et. al., 2010 a & b; 2012).

When rate of decomposition become slower results in formation of organic matter (humus) on soil surface, whilst rapid litter decomposition helps in release nutrient

to the soil (Isaac & Nair, 2005). In tropical forest, litter decomposition is characterized by rapid mass loss and nutrient release (Swift, et. al., 1979; Promputtha, et. al., 2002; Thongkantha, et. al., 2008). The rate of litter mass loss is almost double in tropical forest than temperate forest (Hirobe, et. al., 2004; Kurokawa & Nakashizuka, 2008). Some nutrients such as N and P released rapidly from litter during decomposition (Ingram & Anderson, 1993; Arunachalam, et. al., 1997; Torreta & Takeda, 1999; Pandey, et. al., 2007). The rapid decay of litter in forest is due to dominance of cellulose and lignin degrading fungi and termite in the soil (Musvoto,et. al., 2000; Hirobe,et. al., 2004; Lodge,1997; Yamada,et. al., 2005). Litter decomposition in the forest is strongly influenced by three groups of factors, for example, aboitic variables, soil biota and litter quality (Swift et al. 1979, McClaugherty & Berg, 1987; Tripathi & Singh 1992 a & b, Lalnunzira & Tripathi 2018). These factors are described as under:

1.4.1 Role of abiotic factors in leaf litter decomposition

Number of abiotic factors that influences decomposition rate, among them role of temperature, humidity, precipitation, soil moisture are strongly associated with organic matter decay and mineralization of nutrients to the soil (Anderson, 1988), as these factors are responsible for the activity of decomposer organisms (Aber & Melillo, 1982).There are reports that the rate of decomposition depends on temperature in different ecosystems (Meentemeyer, 1978; Hobbie, 1996; Tripathi & Singh a & b; Lalnunzira & Tripathi 2018), and the rate of decay in the forests was sensitive to temperature during decomposition (Lloyd & Taylor, 1994; Kirschbaum, 2000).

Increased soil temperature and decreased soil moisture content significantly increased the rate of decomposition (Martius, et. al., 2004; Hobbie, et. al., 2010; Paudel, et. al., 2015) as a result of increased soil microbial population (Kirschbaum, 1995; Chapman & Koch, 2007). Apart from soil moisture and temperature, some soil features like bulk density and pH also affects litter decomposition (Cuevas & Medina, 1986). Nutrient cycles also differ with climate and topography feature along with soil types in tropical rain forest so temperature and moisture are also necessary factors in this process (Esperschutz, et. al., 2011).

1.4.2 Role of litter substrate quality on decomposition

Plants organic matters are main components of forest floor and deposited in soil which form humus layer (Klein & Dutrow, 2007; Santa Regina & Tarazona, 2001). The various litter constituents (i.e. C, N, lignin, cellulose, hemicelluloses and the ratios of C/N and Lignin/N etc.) in litter influenced mass loss rate and nutrients return in forest (Swift, et. al., 1979). Lignin content in litter varies from 4% - 50% depending on species (Esperschutz, et. al., 2013).

Litter decomposition vary broadly among species due to variation in leaf toughness, lignin, polyphenol contents and lignin/N ratio (Cornelissen, 1996; Wardle, et. al., 1997; Cadisch & Giller, 1997; Perez-Harguindeguy, et. al., 2000). Among them, N and lignin concentration is the most significant that regulate decomposition (Minderman, 1968; Fogel & Cromack, 1977; Gartner & Cardon, 2004;

Meentemeyer, 1978). Leaves of conifer species decomposes usually slower than deciduous species (Daubemire & Prusso, 1963; Gosz, et. al., 1973; Mikola, 1960; Ovington,1954). Decomposition rate are higher in species with higher ash content with lower lignin and C/N ratio content (Gonzalez & Seastedt, 2001). Few studies also reveal that variation in climatic and seasonal changes also affect rate of decomposition (Meentemeyer, 1978; Austin & Vitousek, 2000; Kumar, et. al., 2019). Other nutrients like potassium and magnesium also enhance activities of microorganism (Ingram & Anderson, 1993).

1.4.3 Role of soil microbes in leaf litter decomposition

The population and activities of soil microbial fauna strongly affects the process of litter decomposition (Schaefer & Schauermann, 1990; Dilly, et. al., 2004). The microbial population changes during the course of litter decomposition and these changes have been strongly related to the process of litter decomposition. In forest ecosystems, various role of soil organisms like earthworms, ants and termite are well understood but various types of other soil organism that associated in ecosystem needs to be explored more (Jones, et. al., 1994). In forest soil with rich nutrient, microbes are more active compare to stress condition (Pascoal & Cassio, 2004).There are number of microbes'present in the soil which enhance decomposition rate but still remains unnamed (Prosser, 2002). Among the soil microbes, groups of bacteria, fungi and actinomycetes were related with litter decomposition in different environment condition

(Frankland, 1992; Prescott, 1996; Chadwick, et. al., 1998). Several studies also reveal that 2/3 of the decomposition were influence by soil fungi (Kjoller & Struwe, 1992). And remaining 1/3 is mostly decomposing by bacteria and actenomycetes (Dilly & Munch, 1998; Kurihara & Kikkawa, 1986; Persson, 1980).

Among soil microbes, fungi are major litter decomposers but study on fungal identification in forest ecology still limited (Rayner & Boddy, 1988). In litter, lignin is among complex compounds found inlitters that help in rebuilt humus layer in forest (Guendehou, et. al., 2014). In initial phase of litter decomposition, degradation of labile substances and starch takes place which was followed by hemicellulose and cellulose in the second phase and lignin, pectin waxes and polyphenols in the later stages (Taylor, et. al., 1989; Tripathi & Singh, 1992 a & b). Fungi play a major role in breaking down of such lignin, hemi cellulose and cellulose (Osono & Takeda, 2002).

1.5 Scope of study

Mizoram is a hilly state with wide variations in altitude and topography that considerably affects vegetation types (species composition and their specific traits), environmental conditions and soil micro-biota. These three factors are the crucial determinants of litter decomposition rates. Therefore, this study has been designed to carry out litter decomposition in tropical moist deciduous forest (Sairang) at ~100m amsl and sub-tropical evergreen forest (Hmuifang) at ~1450m amsl with aim to test: 1) whether the magnitude of decay of two dominant and mixed species litters varies in two sites?; 2) whether the rates of litter decomposition of two dominant and mixed species litters decomposes differently under laboratory microcosm?; 3) whether the fungal population changes in two sites and do they affect the rate of decomposition of two dominant tree leaf litter in these forests? Since litter decomposition is an important process in forest nutrient cycling and is regulated by various factors. So this study will provide base line information in determining the important regulatory factors responsible for decomposition and nutrient release in two forests in Mizoram. The studies pertaining to the rate of decomposition in relation to various crucial factors affecting them are not available so far in this region and deserve immediate attention.

1.6 Objectives

This study aims to achieve following major objectives related to leaf litter decomposition in two forests (i.e. in the field), laboratory microcosm (control condition) and under controlled laboratory condition by inoculating the groups of dominant litter fungi from each forest to test the decomposing ability of dominant groups of fungi in litter decomposition;

1. To assess mass loss rates of important tree litters and their chemical constituents from the two forests.
2. To assess decomposition of important litters from the both forests under laboratory microcosm.
3. To assess litter decomposing fungi of two forests and decomposing ability of major filamentous fungi on important tree litters from two forests.

Chapter 2

2 Review of literature

2.1 Litterfall, decomposition and nutrient release during forest litter decomposition

In terrestrial ecosystem, litterfall and decomposition are the two important processes which are helpful in maintaining the soil fertility. Forest litter are comprises of different parts of the plants such as leaves, barks, stems, fruits, flowers, twigs etc. Accumulation of organic matter from the litter makes a complex structure in soil ecosystem (Grime, 1979) as a result of various abiotic and biotic factors. The rate of litter decomposition depends on quality of litter (tree species) and soil physico-chemical properties (Melillo, et. al., 1982). Several studies on litterfall and decomposition have been carried out in different parts of the world.

Carvalho, et. al., (2019) recently studied on litterfall and decomposition of *Pinus* speciesreveal that about 97% of litters are composed of leaves. Guendehou, et. al., (2014) used different tree leaves to determine decomposition rate and found that litter decomposition rates were influenced by litter substrate quality. In recent studies, Kumar, et. al., (2019) in Himalaya region, found that litter production was higher in the forest found at lower elevations compared to middle and higher elevation forests. They also reported significantly higher litter production during monsoon period compared to other season. Tripathi, et. al., (2009) have reported similar trends in dry topical bamboo ecosystems in India. Maximum litterfall in *Eucalyptus tereticornis* was observed in the month of November (Kumar, et. al., 2019).

Litter decomposition was higher in rainy season and lower in summer season (Singh, et. al., 1999; Tripathi & Tripathi, 2009). Tripathi, et. al., (2009) also reported that rate of decomposition was faster during monsoon compared to winter. Many researchers also found that decomposition was more rapid during rainy season due to suitable climatic condition for decomposer (Pant & Tiwari, 1992; Wedderburn & Carter, 1999; Devi & Yadav, 2007). Tripathi, et. al., (2006) studied in bamboo dominated forest and revealed that decomposition rate was faster during initial stages and decreases gradually in the later stages and also found that rainfall is an important abiotic factors affectinglitter decay.

Maximum litter production was observed during winter season and also found that nutrient decomposition was decreased in initial stage but N concentration was increased in three different agroforestry systems in northeast India (Tangjang, et. al., 2015). Upadhyaya, et. al., (2012) also revealed that decomposition and nutrient release patterns of selected multipurpose tree species from home garden showed higher nutrient retention in *Artocarpus heterophyllus* and *Mangifera indica*. Nutrient use efficiency and the tendency of nutrient immobilization of P and N were foundto be significantly higher in natural forest than plantation (Pandey, et. al., 2007) with exogenous supply of nutrients, and the same has been reported as the nutrient conservation strategy of the forest to cope up with nutrient limitations.

A recent study on leaf litter decomposition of *Leucaena leucocaephala* by Oladoye, et. al., (2019) stated that decomposition rate and nutrient release in below

ground was faster than above ground in leaf litters. Several factors were associated with increase in decomposition rate such as temperature, moisture, microbial activities and other litter chemical substrate quality (Gupta & Lekha, 1989; Pant & Tiwari, 1992).

The initial N and P concentration in litter showed positive correlation and C, lignin and C/N ratio observed negatively correlated with decomposition rate (Barbhuiya, et. al., 2008). The rate of decomposition was faster in wet tropical forest species with rapid nutrients returns (Barbhuiya, et. al., 2008). Soil nutrient release from litter decomposition are helpful for crop productivity and soil fertility especially in shifting cultivation area (Wapongnungsang, et. al.,2017).Nutrients like K concentration was higher during summer than other season whilst P concentration was lower in hot and humid season but higher in dry and cold season (Kumar, et. al., 2019). And also K concentration was increased with increase in altitude; there was no effect with altitude in P and N (Kumar, et. al., 2019).

Tree species type and its litter substrate quality are important as it returns to forest soil and influence quality of soil (Aerts, 1997).Chemical composition of litter depends upon the types of forest community that influenced the structure and dynamic of microbial activities in soil (Kutsch & Dilly, 1999; Heal & Dighton, 1986).Further, litter nutrient depends upon various factors like soil physico-chemical properties and others microbial activities (Kutsch & Dilly, 1999; Scholes & Walker, 1993).Generally, nutrient return was mainly due to litterfall, especially N in the soil (Muoghalu,

et.al.,1993; Hermansah, et. al., 2002). N concentration in tropical tree species was higher than temperate species (Reich, et. al., 1992).

2.2 Role of abiotic factors in litterfall and litter decomposition

Litterfall is a major pathway of returning nutrients from aboveground to the soil (Odiwe & Muoghalu, 2003). Litterfall and litter decomposition processes are important for maintaining the fertility of soil (Sundarpandian & Swamy, 1999; Pragasan & Parthasarathy, 2005). Abiotic factors, for example, rainfall, temperature and their associated variables have been reported to affect the pattern of leaf litterfall and nutrient return in the forest ecosystem (Tripathi & Singh 1995; Pandey, et. al., 2007; Bohara, et. al., 2019). Litterfall in tropical forests are highly seasonal and have been reported to be affected by the cool and dry period of the year as result of decreased temperature and precipitation (Tripathi & Singh 1995; Lalnunzira & Tripathi 2018).

The temperature has been reported as the prime factors determining the rates of litter decomposition (Meentemeyer, 1978; Hobbie, 1996; Lloyd & Taylor, 1994; Kirschbaum, 2000), due to increased soil microbial activity with rise in soil temperature and moisture (Kirschbaum, 1995). Further, the effect of temperature on litter decomposition and its influence on soil microbial growth and activities has also been reported by other studies (Swift, et. al., 1979; Berg, 2000; Kravchenko Irina, et. al., 2019). Similarly, soil temperature has also been found to affect the process of nutrient mineralization from the litter more strongly than humidity (Schlesinger, 1984).

Various climatic factors, soil physico-chemical properties, litter quality and biological properties have been reported to regulate decomposition rates (Fioretto, et. al., 2003). Soil temperature, soil moisture and litter chemical compounds have known to predict litter decomposition ratesin forest ecosystem (Berg & Staaf, 1980; Uma, et. al., 2014; McClaugherty & Berg, 1987; Taylor, et. al., 1989; Tripathi, 1992). Chen, et. al., (2014) revealed that changes in soil moisture and litter quality are among the major factors responsible for forest litter decomposition. Litter decomposition rate was higher during raining season than summer (Singh, et. al., 1999). Tripathi, et. al., (2005) revealed that rainfall was prime abiotic factor that affect mass loss rate in *Betula ermanii* forest. Seasonal variation in climate regulate decomposition rate in tropical forest by affecting the population of soil microbial diversity that promote decomposition of organic matter (Tripathi, 1992; Arunachalam, et. al., 1997).

2.3 Role of fungi in leaf litter decomposition

Litter decomposition in forest was affected by various soil fauna (e.g. number of invertebrate, bacteria and fungi) present in forest litter (Swift, et. al., 1979; Sridhar, et. al., 2013). Among them fungi play a more critical role because they can decompose complex compound derived from lignin-rich polymers and humus (Aponte, et. al., 2010 a, b; 2012). Fungi are capable of breaking down of lignin compounds from the litter. Studies have investigated and compared the difference in the decomposing abilities of various fungi. However, the profile and structure of decomposing fungal communities in

forest litter and the frequency of occurrence of fungi have been reported as key factors in the process litter decomposition (Osono & Takeda, 2002). Reports showed that fungi participate in different ways in the decomposition of litter (Fu-giang, et. al., 2004; Song, et. al., 2005). The quality of the leaves as a food source for microbial decomposers is another important factor. In the process of litter decomposition, there are three prominent phases have been described in the literature, for example; first phase as rapid loss of labile fractions of litter during; followed by the second phase, i.e. decomposition of cellulose and hemicelluloses and third phase, degradation of more complex and slow breakdown of substances like lignin resulting in exponential decay curve (McClaugherty & Berg, 1987). Arial part of plants supports numerous micro-organisms that may be unique or common to other microbes available on forest ground (Nadkarni, et. al.,2001).

Fungi enhanced litter decay, stuck inside node hole of tree trunk and help in improvement of nutrients for other organism living inside the hole (Nadkarni, et. al., 2001). Complex lignin compound present in litter were decomposed by fungi and bleaching of leaf in tropical and temperate forest was due to infestation of fungi (Osono & Takeda, 2001; Koide, et. al., 2005; Osono,et. al., 2008). Some species of saprobic fungi are more common in tropical than temperate region (Hudson, 1968).Some fungi are endophytic in nature and appear in fresh leaf than colonies initially before other saprobic fungi appear on forest ground. Such nature of fungi was observed in tropical as well as in temperate regions (Promputtha, et. al., 2002; Tokumasu & Aoki, 2002; Paulus, et. al., 2006; Kodsueb,et. al., 2008).

Broad body of literature reflected limitations ofstudies on decomposing ability of fungi in temperate region. In these studies the researchers have grouped the fungi based on their feeding habits, e.g. ligninolytic, cellulolytic, sugar fungietic and these group of fungi have been inoculated in the forest litter to assess their ability to decompose forest litter (Osono & Takeda, 2002, 2006; Osono,et. al., 2003, 2006). However, in tropical region, only few studies have been reported on fungi (Osono, et. al., 2008), and thereforesuch studies would be urgently required to assess the role of major fungal groups on forest litter decomposition in tropical and sub-tropical forest litter in this region.

2.4 Role of substrate quality in litter decomposition

Forest composition plays an important role in deposition of organic matter in soil. Maximum organic layer deposited in forest soil comes from vegetation composition (Santa Regina & Tarazona, 2001).Litters content various organic compounds such as sucrose, hydrocarbon, phenolic and glyceride. Such compounds are differed from plant parts (leaf, stem, roots, barks, etc.) and also differ among species. Litter quality are determined by amount of nutrient (C, N, P, K, etc.) and complex cell (lignin, hemicelluloses and cellulose) present in litter that influence decomposition and nutrient release in soil (Swift, et. al., 1979).Lignin content varies from 15 – 40% depend on species but in certain case lignin contain may be 5 – 50% due to variation in syringyl and guaiacyl content in litter (Esperschutz, et. al., 2013).The amount of hemicellulose and cellulose present in litter also differ among the species (Akpor, et. al., 2005; Fengel

& Wegener, 1983). Such alteration in organic and chemical compound within the species widely varied rate of litter decomposition (Cornelissen,1996; Wardle, et. al., 1997). Rate of litter decomposition differ due to litter quality such as N, C, lignin, cellulose, and ratios of C/N and lignin/N (Berg, et. al., 1993; Perez-Harguindeguy, et. al., 2000). Among litter quality, lignin and N are most crucial determinants of litter decomposition rates (Millar, et. al., 1936; Minderman, 1968; Fogel & Cromack, 1977; Meentemeyer, 1978; Gartner & Cardon, 2004).The litter substrate quality also affects decomposition process, as in initial stage degradation was higher due to labile substances and becomes slower as amount of recalcitrant compounds increases (Rosenbrock, et. al., 1995). The rate of cellulose decomposition in litter was higher between 35–37°C but also can be occur below 5°C and more 35°C (Kravchenko Irina, et. al., 2019).

Researchers found that decomposition of leaf litter can be predicted by the C: N ratio (Taylor, et. al., 1989), by the lignin content (Meentemeyer, 1978; Taylor, et. al., 1989; Tripathi, 1992; Tripathi & Singh, 1992 a), or by the lignin: nitrogen ratio (Melillo, et. al., 1982). Basically, high quality leaves (like nutrient-rich alder leaves) will decompose faster than low quality leaves (like nutrient-poor conifer needles). Several studies reveal variation in decomposition rates among species (Adams & Angradi, 1996; Cornelissen, 1996). However, litter quality and quantity can change population and activities of soil microbes (Sayer, et. al., 2006; Chen, et. al., 2014). In lignin, phenylpropanoid polymer has a highly complex and variable structure. Its chemical

determination is consequently not easy and different methods may lead to different results. Because it constitutes a barrier preventing decomposition of cellulose, lignin content of litter has been reported to control litter decomposition rate (Melillo, et. al., 1982; Stump & Binkley, 1993). Berg and co-workers (Berg & Staaf, 1980; McClaugherty & Berg, 1987) stated that in the initial stages (0 to 3 months) of leaf decay labile substances like starches and amino acids where recalcitrant substances like lignin and cellulose are leaving.

Chapter 3

3 Materials and Methods

3.1 Description of study site

3.1.1 About the state

Mizoram is one of the eighth states of Northeast India. It is situated at latitude 21° 57' - 24° 30' N and longitude 92° 15' - 93° 26' E, to extreme southern part of northeastern region. Mizoram is bounded by Assam in the north, Tripura in west and Manipur in the east and shares international boundary with Myanmar and Bangladesh in south and west. The state is part of the Indo-Burma biodiversity hotspots and is characterized by various climatic conditions due variation in altitude and vegetation. The annual temperature varies from 12^0C to 30^0C, mean annual precipitation was varied from 2160 mm to 3500mm. About 86.27% (ISFR, 2017) are under forest cover, some of the important tree species in the state are *Mesua ferrea*, *Schima wallichii* (Khiang), *Syzygium cumini* [Lenhmui (Hmui-pui)], *Toona ciliata syn. Cedrela toona* (Teipui), etc. *Musa* species are common along the hill slope and *Quercus* forests are located in higher altitude region.

3.1.2 About the study sites

The study sites were located at Hmuifang Reserve Forest (Sub-tropical Forest, STF) (Fig.3.1) 50 km from Aizawl towards south. This reserve forest was situated at latitude 23^0 27.2`N and longitude $92^0$45.0` E within Aizawl district with elevation of 1455mamsl. Climatic condition of the forest was cool and low to moderate type of temperature throughout the year. Sub-tropical semi-evergreen forest species of vegetation are found in this forest. Some of the important tree species are like *Dipterocarpus retusus, Quercus floribunda, Lithocarpus xylocarpus, Drypetes indica*, etc.

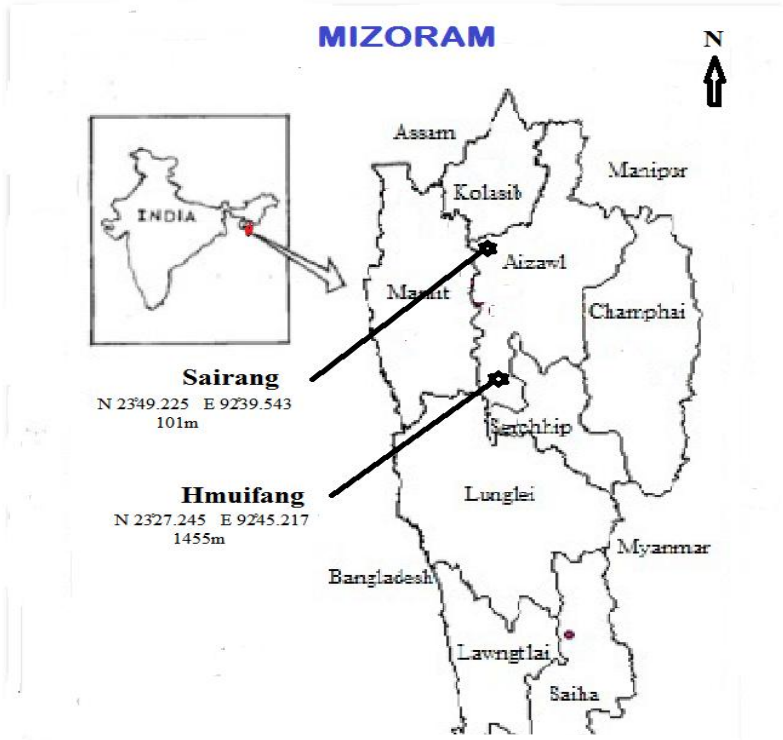

Fig. 3.1 Location of the study sites

Another forest site was selected at Sairang Forest (Tropical Forest, TF) (Fig 3.1) with contrast vegetation from the previous site. It was located at latitude $23^0$49.2`N and longitude $92^0$39.5` E with the elevation of 101 m amsl ~ 40 km from Aizawl toward northern. Climatic condition of the forest was humid and warm to hot type of temperature in whole year. Vegetation is mostly dominated with natural bamboo forest. *Melocanna bacciferа*is the common bamboo species found in this region. Other important vegetation is like *Actinodaphne angustifolia* (Keltebengthlep /Pa-khathnah sin), *Adina cordifolia, Haldina cordifolia* (Lungkhup), *Anogeissus acuminate*, etc. Both the forest sites has elevation difference of around ~1350 with different in vegetation.

3.1.3 Rainfall and soil temperature data

Daily rainfall (RF) data was collected from June 2015 to May 2016 from nearest Agriculture Department (crop husbandry) Sialsuk branch (~ 8 km apart from Sub-tropical Forest) and Lengpui (~ 5 km apart from Tropical forest). Soil temperature (ST) was recorded using mercury thermometer (ranges -10 to 110) at 10cm depth in soil.

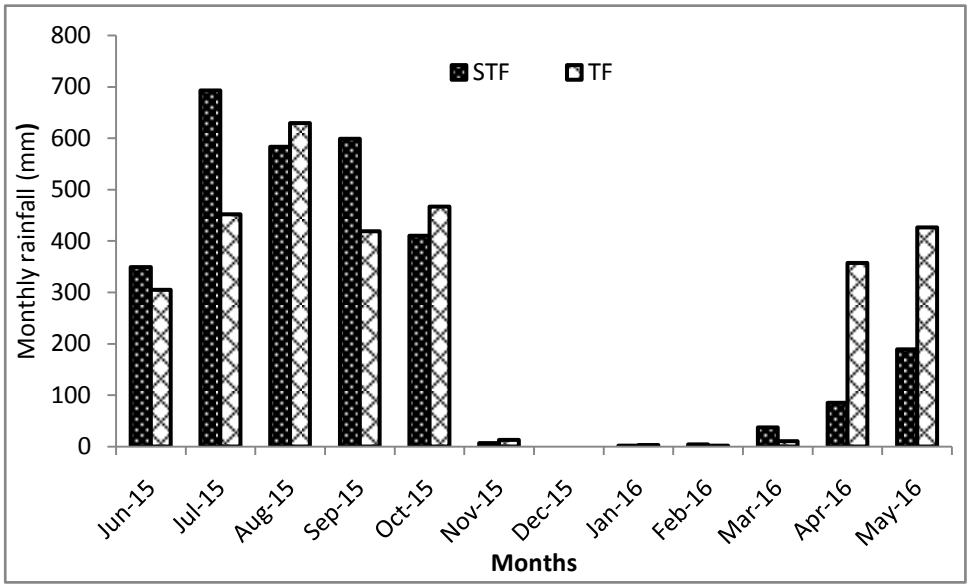

Fig. 3.2 Monthly variation in rainfalls during 12 months of studied (May 2015-May 2016) for both forest sites.

3.2 Soil sampling and analysis

Fresh soil was collected 3 random locations from each forest sites with depth (0 –15cm) using soil corer (4.2cm dia).

3.2.1 Analysis of soil texture, moisture, pH and bulk density

Soil texture was determined using hydrometer methods as described by Gee & Bauder, (1986). In brief, 50g of oven dry soil was taken in 250ml of lid beaker and mixed with distilled water than add 20 ml 30% hydrogenperoxide, 2g sodium hexametaphosphate and transfer to 1L measuring cylinder. The reading of hydrometer and temperature was recorded at 40 sec and 5h. Soil moisture content was determined gravimetrically by oven drying the soil samples at 105^0C for 48 h to constant weight

(Anderson & Ingram, 1993). Soil pH was using standard pH meter (Mettler Toledo, Switzerland) in 1:2.5 soil/water suspension. Bulk density (g cm^3) was analyzed using known volume of soil corer (4.2cm dia X 10cm height) and estimated volume of dry weight soil as per Brady, 1984.

3.2.2 Analysis of soil C, N and C/N ratio

Soil collected from sites were air dried, ground and sieved (1mm mesh) then used to determine C, N and C/N ratio contents using CHNS/O Elemental Analyzer with auto-sampler and TCD detector –Euro Vector, Model: EuroEA3000 at Central Instrumental Laboratory, Mizoram University.

3.2.3 Analysis of microbial biomass carbon (MBC)

Soil MBC was determined by chloroform fumigation extraction method (Brookes, et. al., 1985). Freshly collected soil samples were divided into two sub-samples (25g weight each). Half sub-sample was transfer to 50 ml beaker and fumigated with chloroform vapour and other half without fumigation (non-fumigation) for 24 h in desiccators. After 24 h, pressure was released from desiccators than added 100 ml of 0.5 M K_2SO_4 to samples and shook for 30min at 200 rpm in orbital shaker. Using Whatman No.42 filter paper, soil suspension was filtered and 10ml of supernatant was used for determination of C using wet oxidation method of SOC by Walkley & Black, (1947). The difference in C content between fumigated and non-fumigated was determined. Then MBC was calculated using conversion factor, K_{EC} = 0.38 (Vance, et. al., 1987;

Wu, et. al., 1990; Dilly & Munch, 1998). MBC content was expressed in µg g^{-1} (DW) soil.

3.2.4 Determination of available phosphorus (P_{avail})

P_{avail} in soil was determined by stannous chloride blue colour method (Bray & Kurtz, 1945). In brief, 0.5g of finely grinded air dried soil was extracted with 50ml of 0.03N NH_4F in 0.025N HCL and kept for 5 min in shaker. After that, using Whatmann No. 42 filter paper soil suspension was filtered. Then 5 ml of filtrate was taken 50ml beaker, 5 ml of Dickman Bray's reagent and 1 ml stannous chloride was added to develop blue colour. Using spectrophotometer, intensity of blue colour was measured at 660 nm and concentration of P was obtained from the standard curve.

3.2.5 Determining soil microbial population

Freshly collected soil was bought to laboratory, 1 g of homogenized and clean (removed pieces of stone, root, leaves, etc.) soil sample was added to the first test tube containing 10 ml (dilution factor 10^{-1}) of distilled water and remaining test tube containing 9 ml of distilled water. Then after mixing thoroughly, 1 ml from the first test tube was transfer to next test tube containing 9 ml of distilled water (dilution factor 10^{-2}) then same serial dilution process continue to obtained 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} (Serial dilution technique by Martin, 1950). For colony forming units (CFUs), experiment was based on Dilution Plate Method (Waksman, 1922). Different agar media were prepared separately for fungi, actinomycetes and bacteria. For fungi, potato dextrose agar (PTA) added with antibiotic 0.08% of penicillin, chloramphenicol and

rose bengalat the time of preparing. Starch casein agar (SCA) mixed with nystatin (0.08%) used for isolation of actinomycetes. For bacterial counts, media prepare from nutrient agar added with around 0.08% of nystatin and actidione. Dilution of 10^{-3} to 10^{-5} use for isolation of fungi, 10^{-4} to 10^{-6} actinomycetes and 10^{-5} to 10^{-7} were used for bacterial isolation. 1ml of each dilution was added into petri-plates containing solid media (triplicates). Then media plates were incubated at 28 ± 1^0C for fungal growth and 25 ± 1^0C for both actinomycetec and bacterial growth. After 24 h of incubation actinomycetes and bacteria population started counted and for fungi after 72 h of incubation. The microbial population was expressed in CFU/g of soil. [Note: all glass wares and media used in this experiment were sterile at 120^0C for 20 min in autoclave]

3.3 Sampling and field experimental design for litterfall and decomposition

3.3.1 Measuring litterfall

Litter input in each sites of the forest were recorded through 10 randomly placed (permanent plots 15 – 25m away from each plot) nylon net litter traps (each 50cm x 50m, 15cm deep, 1.5mm mesh) kept above the ground (~50cm) within the sites. Litter from the traps was collected at monthly interval for 12 months from June 2015 to May 2016. Collected litter samples were over dried and weighed and litter mass expresses as g m^2.

3.3.2 Setting up of litter decomposition experiment

Freshly fallen (senescence) leaves were collected from randomly selected plots in each forest sites. Two dominant trees leaf litters (based on species composition)along with a mixed litter category were collected from each forest site. From STF, *Quercus floribunda* (QF) *Drypetes indica* (DI) and a mixed leaf litter component (M_1) and from TF site, *Melocanna baccifera* (MB), *Tectona grandis* (TG) and a mixed leaf litter (M_2) were collected and used for decomposition experiment.

About 10± 0.01g of air dried leaf litter was enclosed in nylon litter bags (15 × 15cm with 1-mm mesh). For each forest site, a total of about 220 bags (two dominant trees leaves and a mixed leaves) was prepared and placed randomly at six locations (15 – 25 m distances between plots). This way a total of440 bags were prepared for both forest sites and randomly placed on the forest floor for periodical retrieval. Monthly, 18 litter bags (6 replicates each for 3 litter category) were retrieved from each site. Litter bags were bought to the laboratory and wash carefully in running water to remove adhere soil particles. Litter was dried separately in hot air oven at 70^0C for 24 h to constant weight. Weight of litter was recorded. Dried litter samples were ground and sieved in 1.5 mm mesh for further analysis.

3.3.3 Computation of litter decay

The mean relative decomposition rate (RDR) was calculated by using the formula:

RDR (mg g^{-1} d^{-1}) = In $(W_1 - W_0)/(t_1 - t_0)$, where W_0 = weight of litter present at time t_0; W_1 = weight of litter present at time t_1, and $t_1 - t_0$ = sampling interval (days). The equation gives the relative weight loss rate; the negative sign of the value, however, is ignored.

The annual decay constant (k) was calculated using negative exponential decay model of Olson, (1963): $W_t/W_o = \exp^{(-kt)}$, where W_o = initial weight and W_t = weight remaining after time t. As suggested by Olson, (1963), the time required for 50% and 95% weight loss was calculated as $t_{50} = 0.693/k$ and $t_{95} = 3/k$.

3.4 Laboratory microcosm experiment

Leaf litter decomposition through this method was based on modified methods of Taylor & Parkinson, (1988). A laboratory microcosm was set up under controlled laboratory condition as described below (Fig. 3.3).

3.4.1 Setting up of microcosm experiment

Top soil (organic layer without humus) was collected randomly from forest floor and mixed thoroughly. The leaf litter sample was used as described in section 3.3.2.

Microcosms (Fig. 3.3) was made from polyvinyl chloride (PVC) pipe (12cm in diameter and 16cm in height) fitted with a screw lid with a sealed bottom and a bottom pierced with l-cm diameter hole allowing excess water to be drained off. Soil mixture consisting of mineral soil collected from MZU campus and surface organic horizon (3: 1) collected from the two forest site was placed at the bottom of the microcosm. Above the organic horizon, about 5g of air dried yellowish senescent

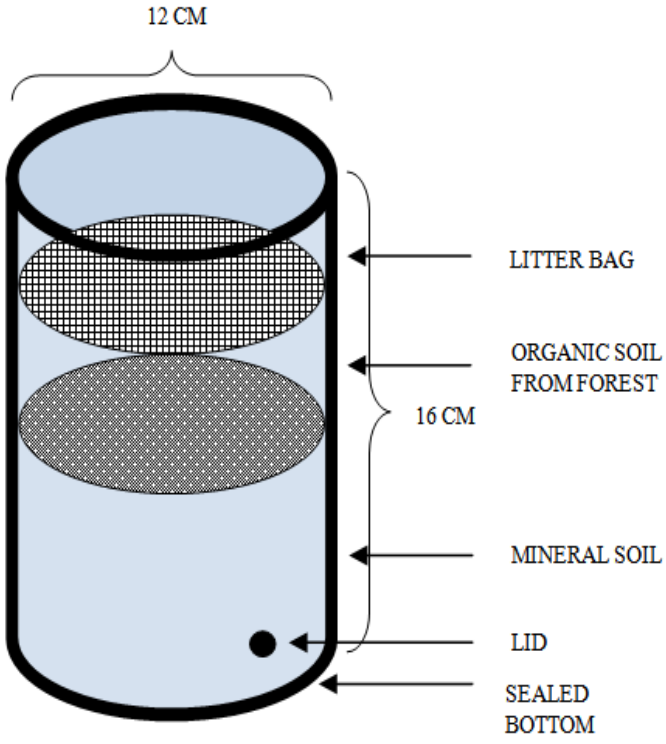

Fig. 3.3 Microcosm design

leaves of two dominant species along with mixed leaf litter sample was rewetted for 1h in distilled water and place above the organic layer (top) of the microcosm. Then hand held mist sprayer used for maintaining soil moisture once every day with known amount of distilled water. The replicated samples were recovered on 15, 40, 75, 120 and 180 days after experimental setup.

3.4.2 Measuring litter decay

The samples were recovered on 15, 40, 75, 120 and 180 days after experimental setup. The decay constants rate and mass loss were analyzed as detailed in section 3.3.3.

3.4.3 Analysis of soil nutrient

After recovery litter bags, soil (top layer) sample was collected from laboratory microcosm. Samples were dried in hot air oven at 80^0C for 36 h until constant weight. Then samples were ground and sieved (0.05 mm mesh) and sealed in zip lock bag for nutrient analysis. Soil nutrient was determined using Leco Truspec TM CN analyser and Thermo Scientific ICP spectrometer (iCAP 6000 series), at Environmental and Geographical Sciences Research Laboratory, Manchester Metropolitan University (UK).

3.5 Decomposing ability of major filamentous fungi

This experiment was based on modified method of Fu-giang, et. al., (2004), in this experiment, major filamentous fungi isolated from forest litter were used to investigate its decomposing ability on leaf of four tree species viz. two tree leaf species

from tropical forest (*Melocanna baccifera* and *Tectona grandis*) and other two from sub-tropical forest (*Quercus floribunda* and *Drypetes indica***)**.

3.5.1 Collection and preparation of litter samples

Freshly fallen (senescence) leaves of the tree species were collected from two forest (section 3.3.2) sites and kept for air dried in shade.

3.5.2 Fungal isolation and identification

Fermented leaf litter collected from the forest floor were cut into small pieces, 5 pieces were placed on PDA (Potato Dextrose Agar) plates containing 0.08% of penicillin, chloramphenicol and rose bengal. A total of 200 pieces were deposited in 40 PDA plates from each forest site. The plates were incubated at 25^0C in the dark and check every day for fungi growth (total fungi). Samplings of 5 (major fungi from each site) based on highest frequencies of occurrence were sub-culture onto other petridishs (PDA media) for pure culture and its identification. All the 5 pure culture fungi were transfers to one petridish (PDA media) and mark as major fungi. Spores of pure cultures of all 5 fungi were identified to genus and species level by microscopic examination (10x, 40x magnification, Olympus CX41, Japan) and culture characteristics. Identification was based on the published descriptions of (Gilman, 1957; Ellis, 1976; Nelso, et. al., 1983; Barnett & Hunter, 1972).

3.5.3 Experiment setup

Leaf samples were disked (2.0 cm in diameter) with a metal borer. Disks were dried 36 h at 60^0 C to constant weight and weighed to obtain the original weight of the leaf disks. Then leaf disks were kept in moistened paper towels between base and lid of petridish, then autoclaved (120^0C for 20 min). The sterilized leaf disks were place to inoculated petidish (with the major fungal colonies) and other disks to petidish (inoculated to total fungal colonies which were placed on petridishs) and uninoculated dishes served as control. A total of 100 leaf disks (25 disks from each tree species including control) were incubated at 27^0 C and 90% humidity in the dark (Osono & Takeda, 2002).

3.5.4 Decomposition kinetics and Mass loss

After incubation, leaf litter was removed carefully than cleaned with a small brush and sterilized water, after that it was oven dried for 36 h at 60^0 C and weighed. The original mass was estimated with the same method before inoculation. Decomposing ability of the group of major filamentous fungi was evaluated according to weight loss of litter (%). Samples (decomposing material) were harvested after 5^{th} week (day 35 -marked as early stage) and 10^{th} week (day 70 -marked as later stage) of litter decomposition.

3.6 Analysis of litter samples

Finely grinded (sieved 1mm) oven dried litter samples were analyzed nutrient concentrations using Thermo Scientific ICP spectrometer (iCAP 6000 series), at Manchester Metropolitan University (UK).

3.6.1 Determination of lignin and cellulose/crude fibre

Oven dried leaf samples were ground in the laboratory for analysis of lignin ADF) and cellulose/Crude fibre (CF) using Fibrotron Automatic Fibre Analysis (Model: FRB 6, Version 1), Tulin Equipments, Chennai, India. Lignin in litter sample was determined by using 1 N H_2SO_4 and Cetyl Trimethyl Ammonium Bromide (CTAB). At the beginning, 0.5g of sample was placed in glass crucible (sample + crucible weight) and boiled with 100 ml of Acid Detergent Solution (ADS) at 350^0C after that temperature was reduced to 250^0C for 30 min. Liquid was drained from crucible and make sample dried in hot oven and record weight. Percent lignin content in samples was calculated as: (crucible weight + sample) – (weight of crucible) x 100/ weight of original sample.

Cellulose/ crude fibre (CF) content was determined by boiling 0.5g of samples along with Neutral Detergent Solution (NDS) and ethoxy ethanol and Na_2SO_4 at 350^0C for 45 min in fibrotron. Then crucible with sample was oven dried and recorded weight (crucible + sample weight). After that entire crucible was placed in muffle furnace at 500^0C for 4h and thereafter recorded the weight of crucible (crucible with ash content).

Cellulose content was calculated as: (crucible + sample weight) – (crucible with ash content) x 100/ weight of original sample.

3.7 Statistical analysis

To analyze seasonal variation between litter mass and corresponding abiotic factors, total monthly litter mass loss was correlated with various abiotic parameters (e.g. total monthly rainfall, mean monthly soil temperature and soil parameters) for all sites separately. Correlation coefficient was performed to examine relationship between various litter nutrients content and mass loss.

For microcosm experiment, one-way analysis of variance (ANOVA) was used to find least significant different (LSD, $p \leq 0.05$) between number of days (i.e. 0, 40, 75, 120 and 180 days) of litter decomposition and their respective nutrient (C, N and C/N ratio) concentrations along with changes in microbial population counts (fungi, actinomycetes and bacteria) in the soil. Further, LSD (at $p \leq 0.05$) was performed using MS (7)-excel to understand the difference between litter mass remaining and litter nutrient concentrations. Stepwise multiple forward regression analysis was carried out using minitab-18 software to evaluate the effect of various factors (soil microbes and soil and litter nutrients) on mass loss.

To understand the effect of major and total group of fungi on various leaf litters samples at different stages (e.g. early and late), LSD was performed following one way ANOVA using MS-Excel 7.

Chapter 4

4 Results

4. 1 Soil physico-chemical and biological characteristics of two forests

The periodical changes in soil temperature ranged from 14.8 ^{0}C – 25.1 ^{0}C in STF site and from 15.8 ^{0}C –28.33 ^{0}C in TF site (Fig. 4.1). Maximum monthly temperature was recorded during the month of September (25.8^{0}C) in STF and August (28.33 ^{0}C) in TF. The periodical changes in soil temperature values for two forest sites were highly correlated (r = 0.87) with each other. Mean annual soil temperature was 19.3 ^{0}C in STF and 23.17 in TF site (Fig. 4.1). Total monthly rainfall and mean monthly soil temperature of the two sites were also strongly correlated (i.e. STF, r = 0.94; TF, r = 0.89) with each other.

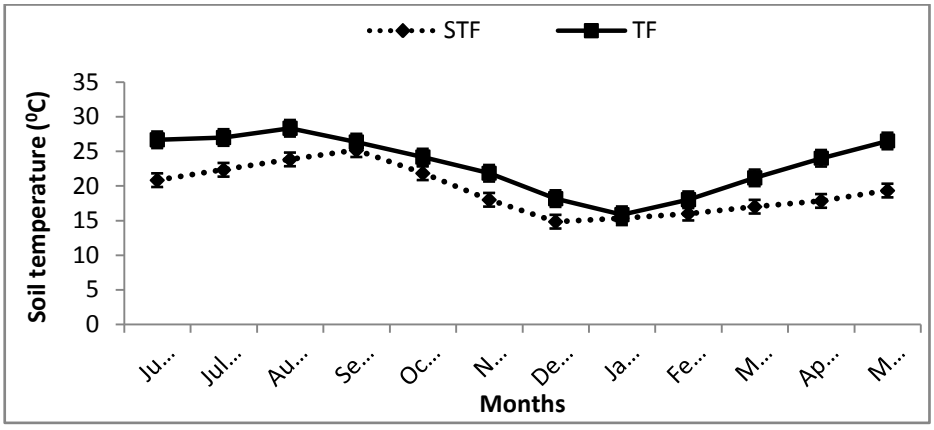

Fig. 4.1 Monthly variations in mean soil temperature in two forest sites (STF and TF) during 12 months of studied (May 2015-May 2016). For soil temperature was reported as mean ± 1SE, n=3. Soil temperature data was collected using thermometer during the field visit.

Percent C and N, C/N ratio, P and MBC concentration was higher in STF site (3.76%, 0.29%, 12.73, 117.98 and 31.03 respectively) compared to TF (2.4%, 0.21%, 11.24 and 26.1 respectively). Soil pH was found to be more acidic in STF (4.77) than TF (4.83). Bulk density was higher in TF (0.92) compared to STF site (0.82). The percent clay was 14% in soils of both forest sites. However, silt and sand percentage varied from 16.6-18.4% and 67.6-69.3% in two forest sites (Table 4.1).

Table 4.1 Soil physico-chemical properties (Carbon, C; Nitrogen, N; C/N ratio; Phosphorus pentaoxide, P_2O_5; soil pH; Microbial Biomass Carbon, MBC; Bulk Density, BDand Soil texture) of two forest sites before the start of experiment. The value was analyzed from forest top soil layer (0 – 15 cm depth).

Site	%C	%N	C/N ratio	P_2O_5 (kg/ha)	pH	MBC µg g^{-1}	BD g cm^3	Soil texture (%)		
								Clay	Sand	Silt
STF	3.768	0.296 ±	12.73	117.98	4.77	31.03	0.822±0.14	14.0	67.6	18.4

	± 0.04	0.005		± 13.57	± 0.2	± 0.48		± 2	± 2.4	± 1
TF	2.417 ± 0.01	0.215 ± 0.007	11.24	99.17 ± 4.52	4.83 ± 0.18	26.11 ± 0.26	0.928±0.1	14.0 ± 1.1	69.3 ± 1.3	16.6 ± 0.6

*(mean ± 1SE,n=3).

The fungal population count varied from 9 x 10^{-3} in TF to 5 x 10^{-3} in STF site. Actinomycetes population count ranged from 49 x 10^{-6} in TF to 38 x 10^{-6} in STF site. Soil bacterial count varied from128 x 10^{-6} in STF to 174 x 10^{-5} in TF site (Table 4.2).

Table 4.2 Colony Forming Units (CFUs) of soil microbes from initially collected soil samples of the two forest sites.

Site	Microbial population Counts (CFUs/g)		
	Fungi	Actinomycetes	Bacteria
STF	5 x 10^{-3}	38 x 10^{-6}	128 x 10^{-6}
TF	9 x 10^{-3}	49 x 10^{-6}	174 x 10^{-5}

* Colony count based on triplicate.

4.2 Annual litter production in two forest sites

Total annual litter production was 6718.5 kg $ha^{-1}yr^{-1}$ in STF which is higher than litter production at TF (2114.01 kg $ha^{-1}yr^{-1}$) sites. The litter production in these forests were highly seasonal reflecting significant differences (df = 55, $p < 0.05$) with months and the value of total litterfall (both leaf and non-leaf).The total leaf litter production at STF site was 4327.5 (kg $ha^{-1}yr^{-1}$) which accounts for about 64.4% of the total litterfall and the remaining was non-leaf litter. The total leaf litter production at TF site was

2114.01 (kg $ha^{-1}yr^{-1}$) which accounts for about 59 % of the total litterfall and the remaining was non-leaf litter. Litterfall was highest during the months of Mar-16 to May-16 in both sites and the litterfall in the remaining months were distributed almost equally (Fig. 4.2).

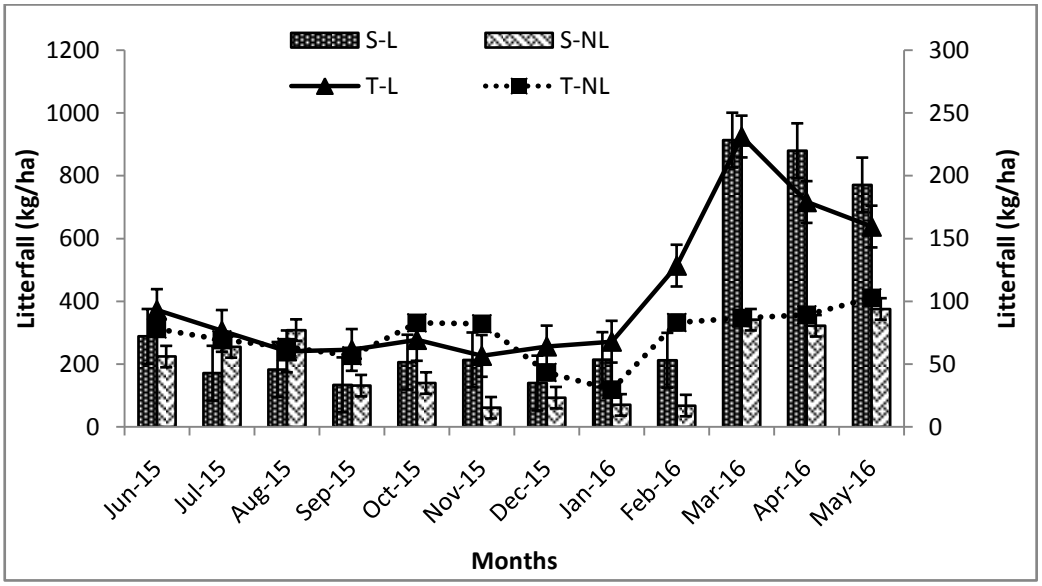

Fig. 4.2 Monthly variation of litter (leaf and non-leaf) input in both forest sites (kg $ha^{-1}yr^{-1}$) during course of decomposition. Maximum leaf litterfall was observed in Mar-16 (both STF and TF sites) whereas minimum litter was observed in the month of Sep-15 (STF) and Jun-15 (TF). Maximum non-leaf litter input was observed in the month ofMay-16 (STF) and Apr-16 (TF), minimum in the month of Nov-15 (STF) and Jan-16 (TF). There are significance differences between periodic months and litterfall (both leaf and non-leaf) at $p < 0.05$, $df = 55$.

4.3 Mass loss of dominant and mixed species leaf litters

Initial mass loss (two months) in all litter components was higher compared to later stage in STF site, about 35 – 45% mass loss was recorded during first two retrievals. But in case of TF site, decomposition was faster compared to STF site and complete litter decomposition occurred within a period of five months. In STF site, about 55 – 67% of litter was decomposed at the end of retrieval period (Fig. 4.3). DI recorded maximum mass (66.24%) compared to the QF (56.7%) and M1 (64.3%). In TF site, 80 – 90 % litter was decayed with five months of litter deposited. During second monthly retrieval, about 63% MB litter mass lost, ~35% mass loss occurred in both TG and M2 (Fig. 4.3).

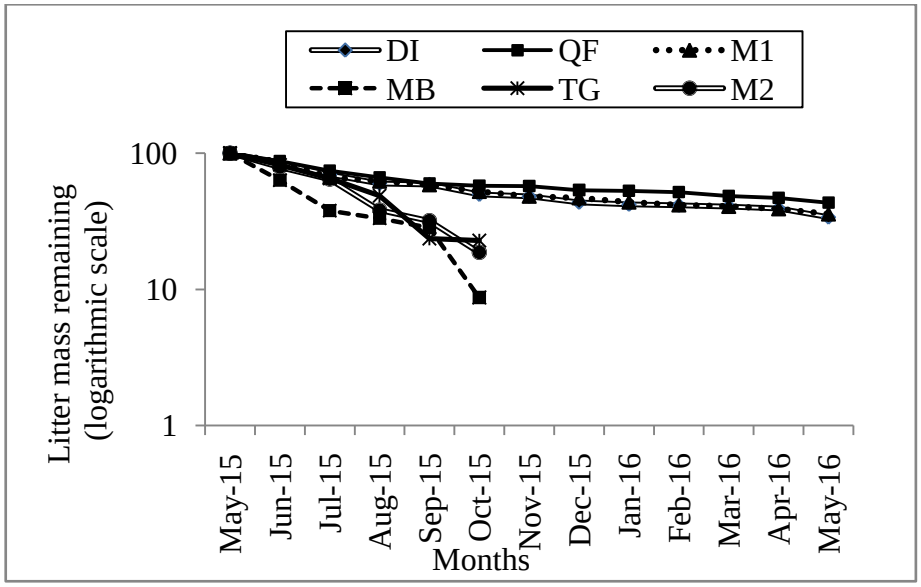

Fig. 4.3 Monthly variation in litter mass loss (logarithmic scale) of different leaf litter components in both sites. At the end of the study, about 30-45 % of litter mass

was remained in STF site, whereas, in TF site only about 10-20% of the litter mass was remaining after 4 months of decomposition. All values are means $\pm$ 1SE, n = 6.

In STF site, the value of RDR for different litter components ranged from 1.4 – 9.5 mg^{-1} g^{-1} day^{-1} during initial stage and 5.2 – 6.1mg^{-1} g^{-1} day^{-1} in later stages of decomposition. For TF site, RDR value for different component ranged from 1.8 – 6 mg^{-1} g^{-1} day^{-1} in early stages and 6.2 – 7.1 mg^{-1} g^{-1} day^{-1} in later stages. Mass remaining after 365 days of decomposition in STF site was 33.7 – 43.2 % and no mass was remained for site TF (Table 4.3). Annual decayed constant k ranged from 1.6 – 1.8 in STF and 2.2 – 2.23 in TF site. In STF site, time required for 50% mass loss ranged from 137 – 150 days and 113– 115 in TF site. However, time required for 95% mass loss for different components would range from 595 – 651 days in STF and 491 – 498 in TF site (Table 4.3).

Table 4.3 Mass loss and time required for different litter component at various level of decay (t_{50}50% and t_{95} 95% mass loss). Percent mass remaining after 365 days in STF and 150 days in TF site.

Sites	Litter types	Mass remaining (%)	Annual decay rate (K)	t_{50} (days)	t_{95} (days)
STF	DI	33.76	1.84	137.44	594.97
	QF	43.26	1.68	150.33	650.79
	M1	35.69	1.80	140.67	608.97
TF	MB	8.68	2.20	114.97	497.69
	TG	22.7	2.23	113.41	490.94
	M2	18.6	2.23	113.65	491.99

4.4 Liter substrate quality

4.4.1 Concentrations of CF, ADF, C, N and C/N ratio in litter samples

Considerable variations occurred in initial concentrations of CF, lignin and other nutrients in the litter samples of two forest stands (Fig 4.4). In different litter components the amount of CF ranged from 18 - 30 % and the amount of lignin content ranged from 3 - 7%. Maximum CF and lignin contents were noticed in M2 litter (i.e. 29% and 6.76%) and minimum in TG litter (i.e. 18% and 3.31%). However, C concentration ranged from 37 – 48%, which was maximum in TG (47.6%) and minimum in MB litter (37.01%). N content ranged from 0.9 – 2.2% in different litter categories in two forest sites with maximum N concentration in MB (2.2%) and minimum in DI (0.9%). Highest C/N ratio was observed in TG (45.7) followed by DI (43.4) and the lowest (16.7) occurred in MB (Fig. 4.4).

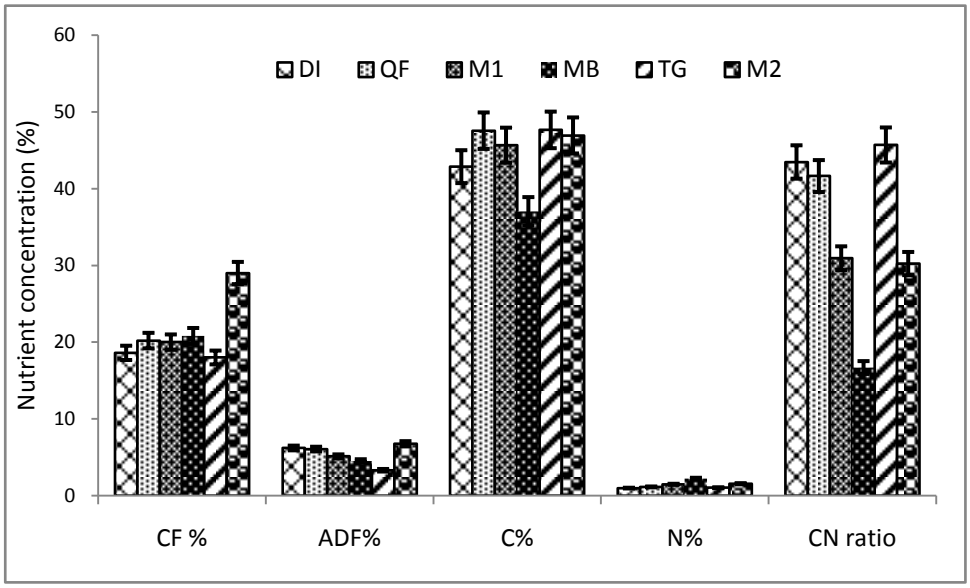

Fig. 4.4 Various concentration of Crude fibre (CF), Lignin (ADF), Carbon (C), Nitrogen (N) and C/N ratio indifferent initial litter components. (Mean ±1SE, n=3)

4.4.2 Carbon and nutrient concentrations during litter decomposition

In forest ecosystem, nutrients are recycled through different above ground plant parts, among which leaf litter contributed major part. Various nutrients (C, N, P, Na, Mg, Ca and S) mass remaining from different leaf litter during 12 months of decomposition in STF and TF (150 days) site are provided in figures (Fig. 4.5 – 4.10).

For STF site, the nutrient mass remaining after 12 months in DI litter was ranged between 19 and 58%. Various nutrient concentrations at the end of experiment were C (35.2%), N (48.8%), P (55.2%), Na (19.4%), Mg (57.8%), Ca (46.2%) and S (49.5%)

respectively. Maximum nutrient concentration at the end of the study was observed in Mg (57.8%) while minimum was recorded in Na (19.4%) (Fig. 4.5).

Nutrient mass remaining at the end of decomposition in QF leaf litter ranged from 41.9 – 401% for different nutrients (Fig 4.6).Percent carbon and nutrients mass remaining after 12 months of litter decay were:C (41.9%), N (163.4%), P (245%), Na (183.9%), Mg (97.3%), Ca (61.1%) and S (401%) for QF litter. The nutrient contents in S (401%) and P (245%) was considerably higher compare to others nutrients and minimum concentration at the end of the study was observed in case of C (41.9%) (Fig. 4.6).

In M1 litter, concentration of carbon and nutrient varied (34 to 284%) considerably. After the end of 1 year period, carbon and nutrients concentrations in the litter material were: C (34.5%), N (108%), P (167%), Na (156%), Mg (108%), Ca (56.2%) and S (284.4%) (Fig. 4.7).

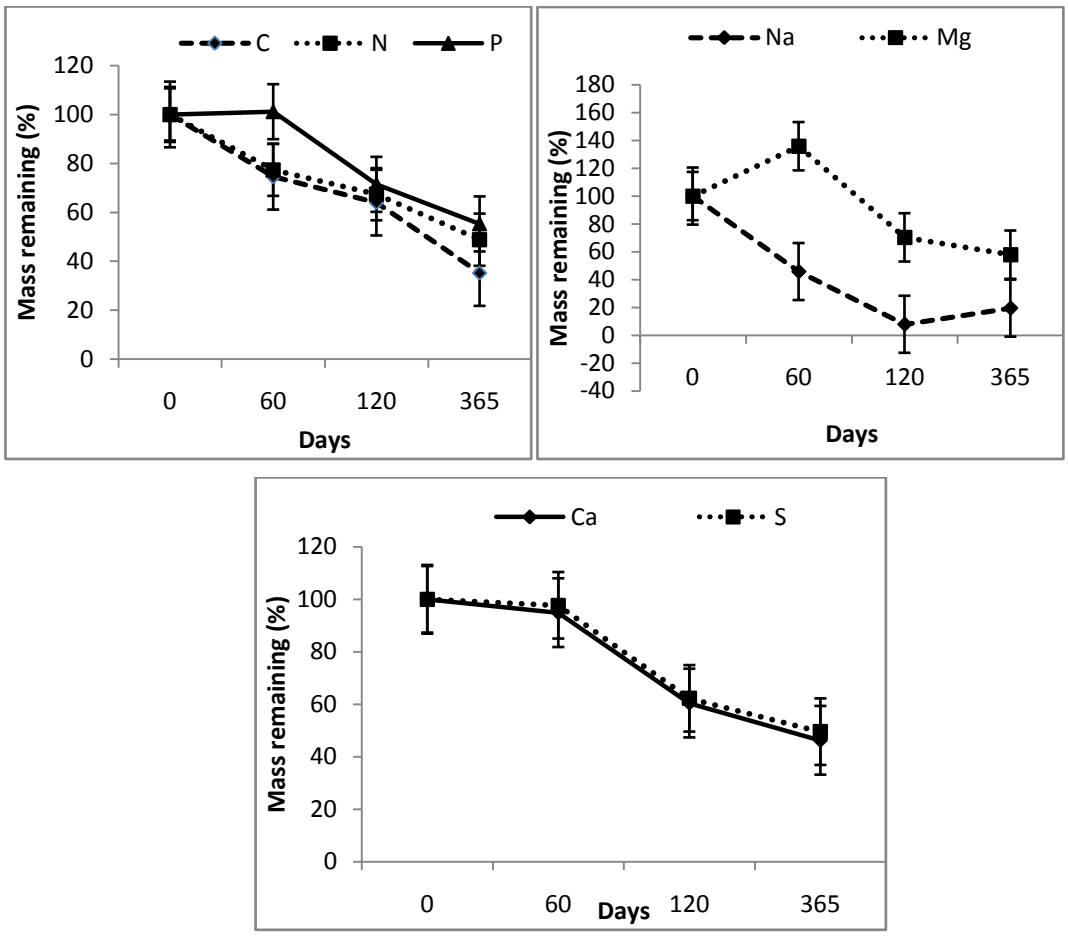

Fig. 4.5 Temporal changes in nutrients contents in litter (DI) components during course of decomposition in STF site.

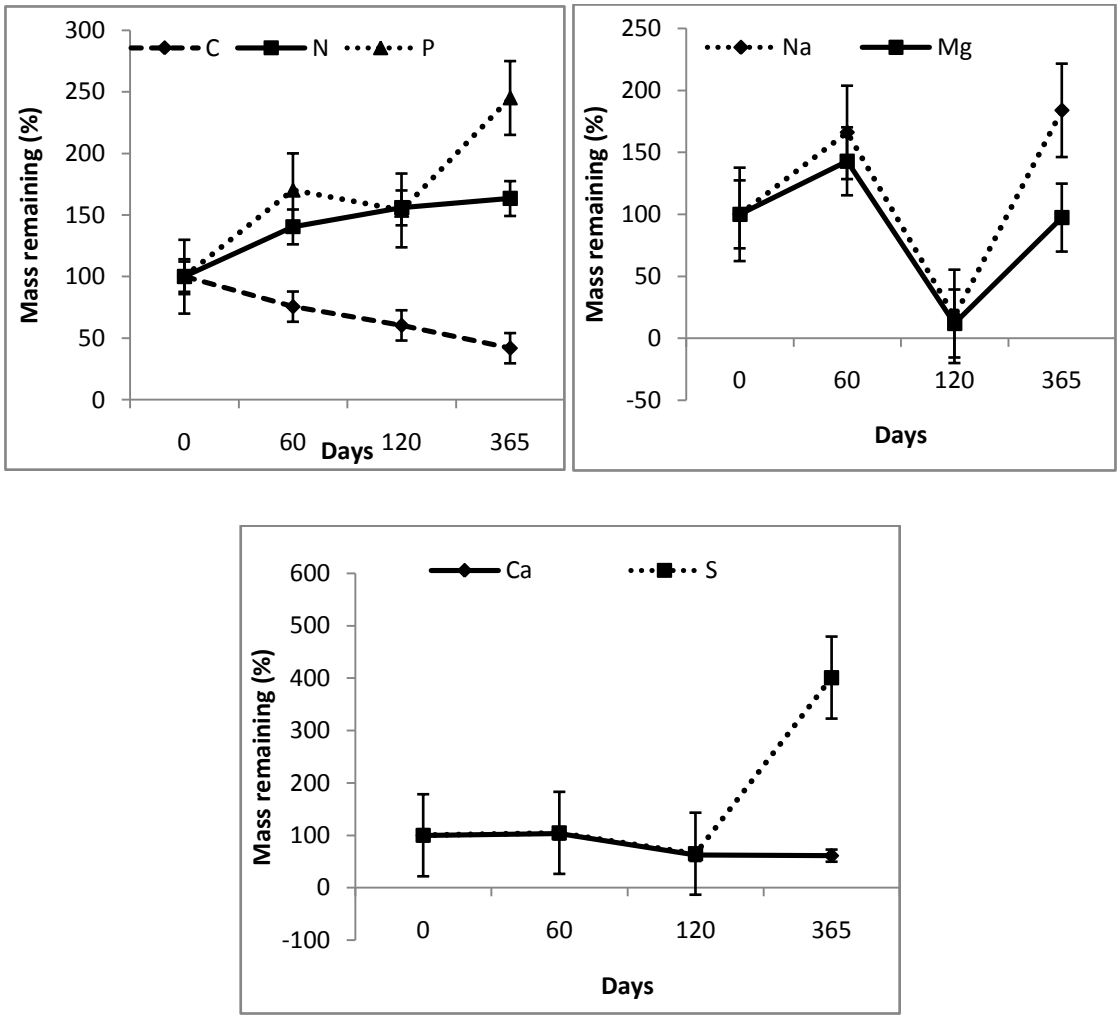

Fig. 4.6 Temporal changes in nutrients contents in litter (QF) components during course of decomposition in STF site.

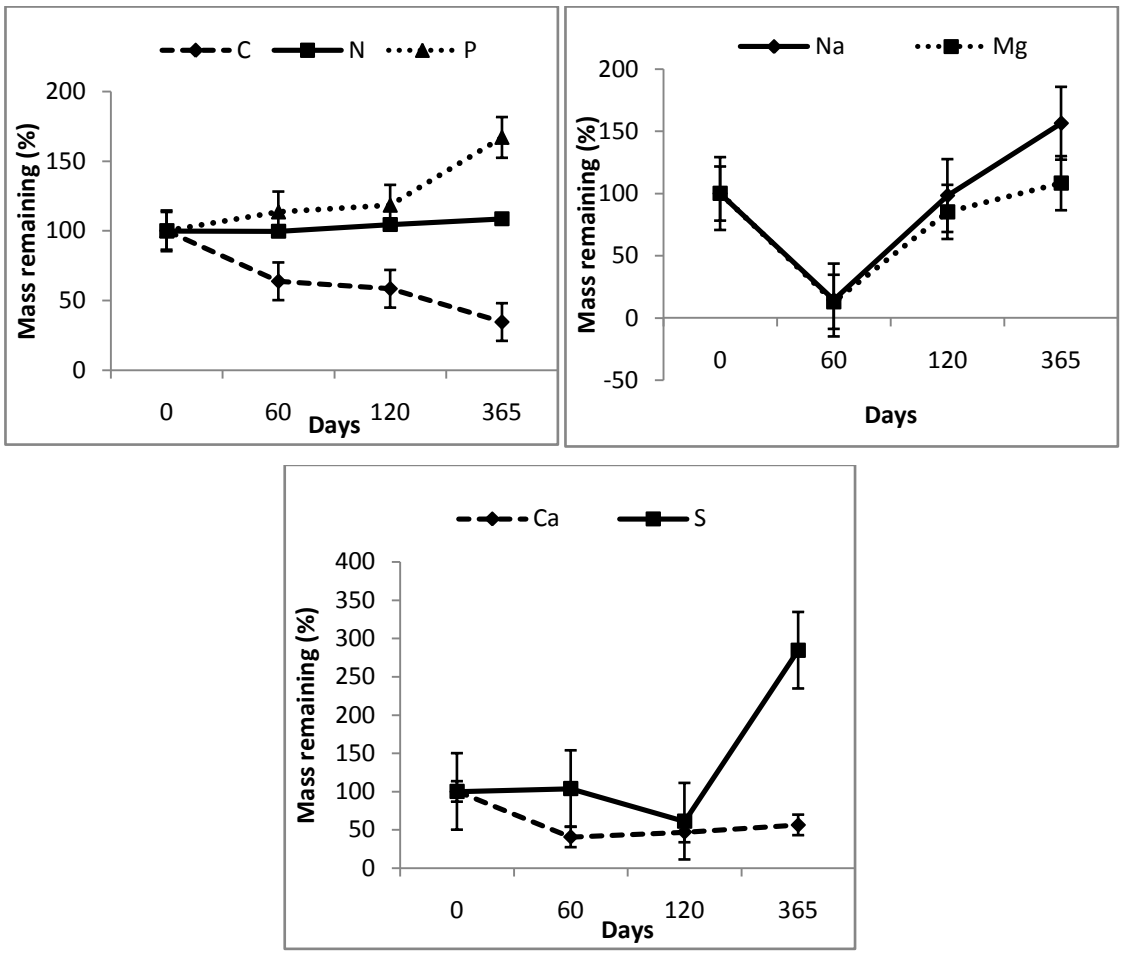

Fig. 4.7 Temporal changes in nutrients contents in litter (M1) components during course of decomposition in STF site.

For TF site, decomposition was faster and most of the litter was almost decayed during the initial 5 months (150 days) of litter placement. The variation in nutrients content of different litter component after end of 150 days were given in Fig. 4.8 – 4.10. In MB leaf litter, nutrient mass remain was between 10 to 230% of the original. Some of the nutrients contents after 150 days retrieval were: C (27.5%), N (16.8%), P (10.1%), Na (19.9%), Mg (33.2%), Ca (10.2%) and S (229%) (Fig. 4.8).

In TG litter, after 150 days of retrieval days, carbon and nutrient concentration ranged between 19 and 45%. The nutrient concentration in litter was C (19.4%), N (36.8%), P (19.3%), Na (43.1%), Mg (30.5%), Ca (44.5%) and S (31.3%). Maximum nutrient remains were observed in Ca (44.5%) and minimum in P (19.3%) as given in Fig. 4.9.

For M2 mixed leaf litter, carbon and nutrient mass remaining was ranged from 3 – 33%. After 150 days carbon and nutrient concentrations were: C (31.7%), N (32.6%), P (13.7%), Na (14.4%), Mg (22.2%), Ca (3.9%) and S (19.3%).Maximum nutrient concentration was observed in N (32.6%) and minimum in Ca (3.9%) (Fig. 4.10).

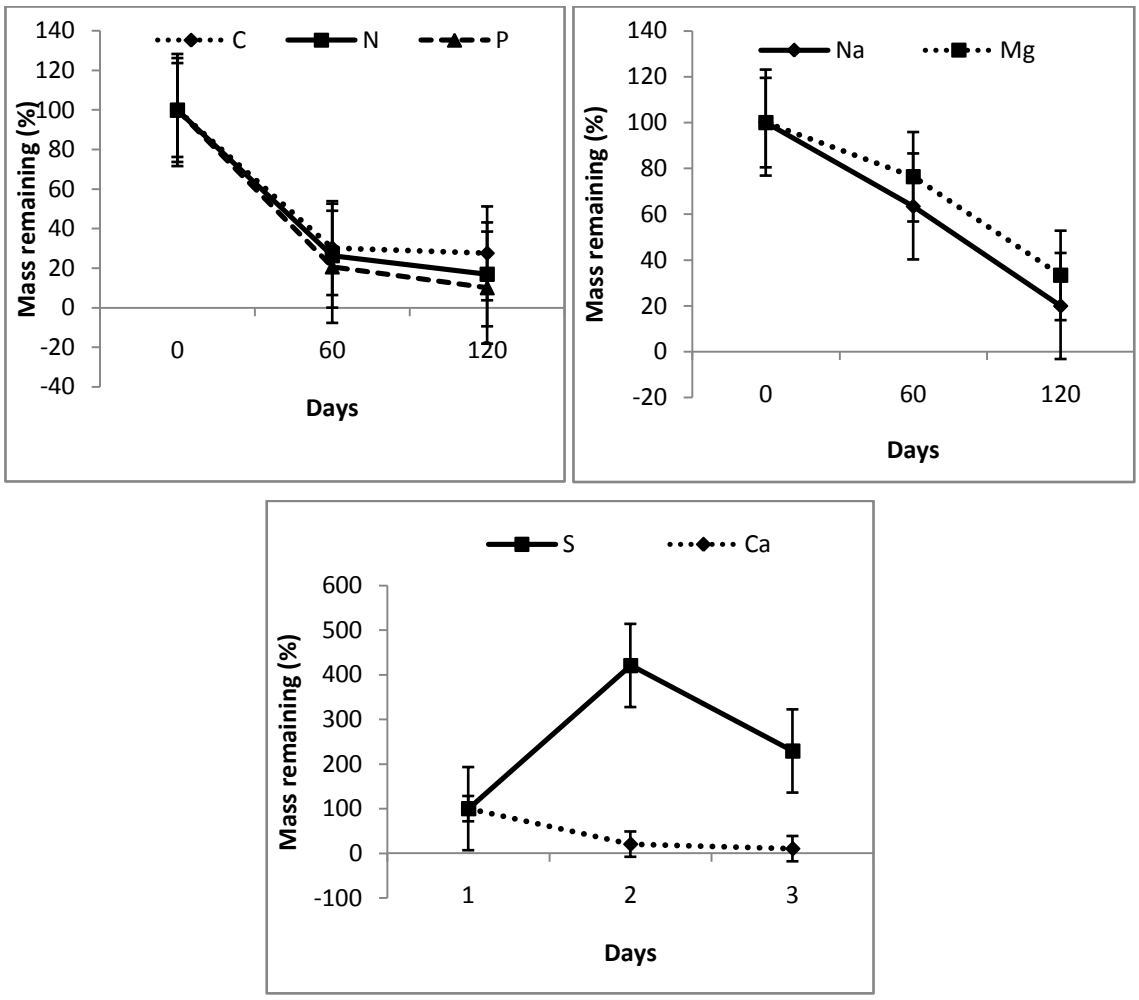

Fig. 4.8 Temporal changes in nutrients contents in litter (MB) components during course of decomposition in TF site.

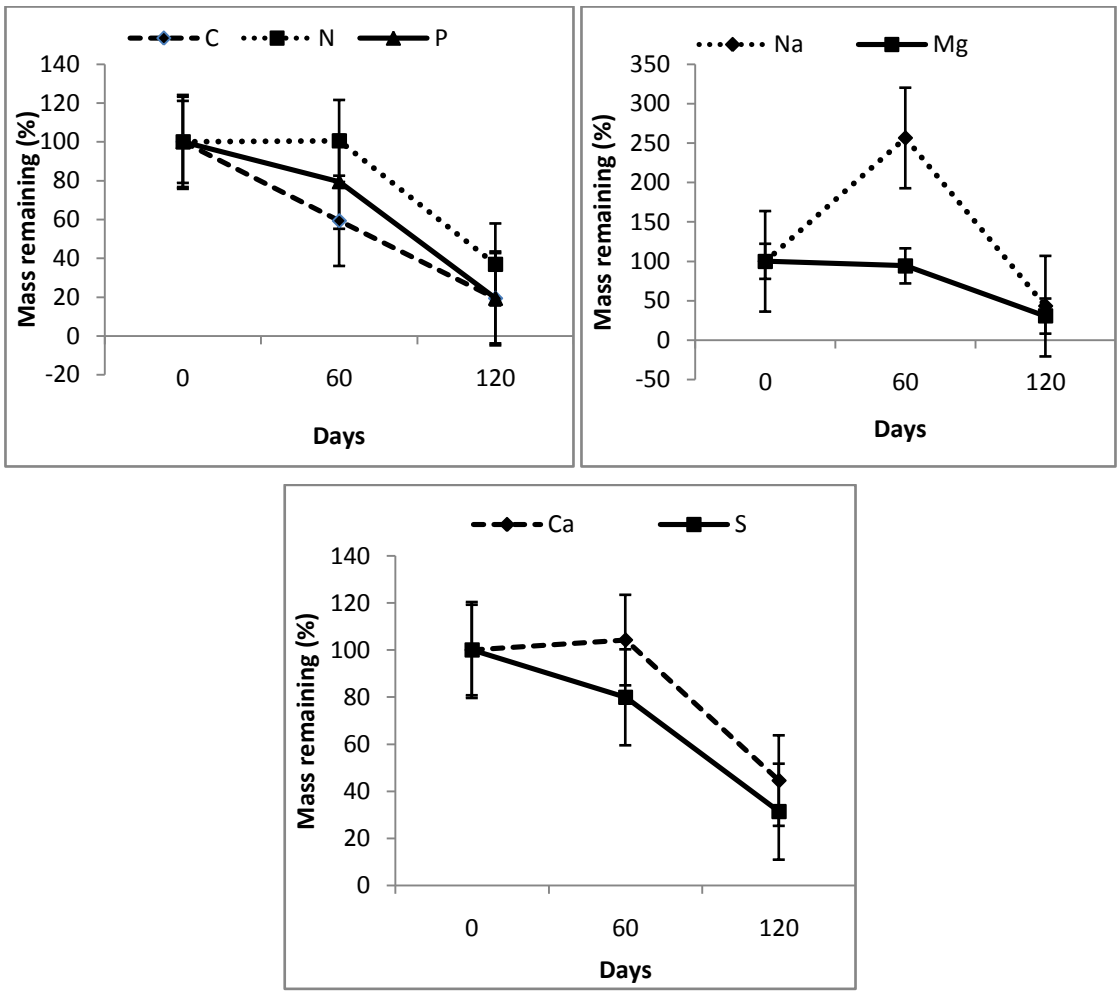

Fig. 4.9 Temporal changes nutrients contents in litter (TG) components during course of decomposition in TF site.

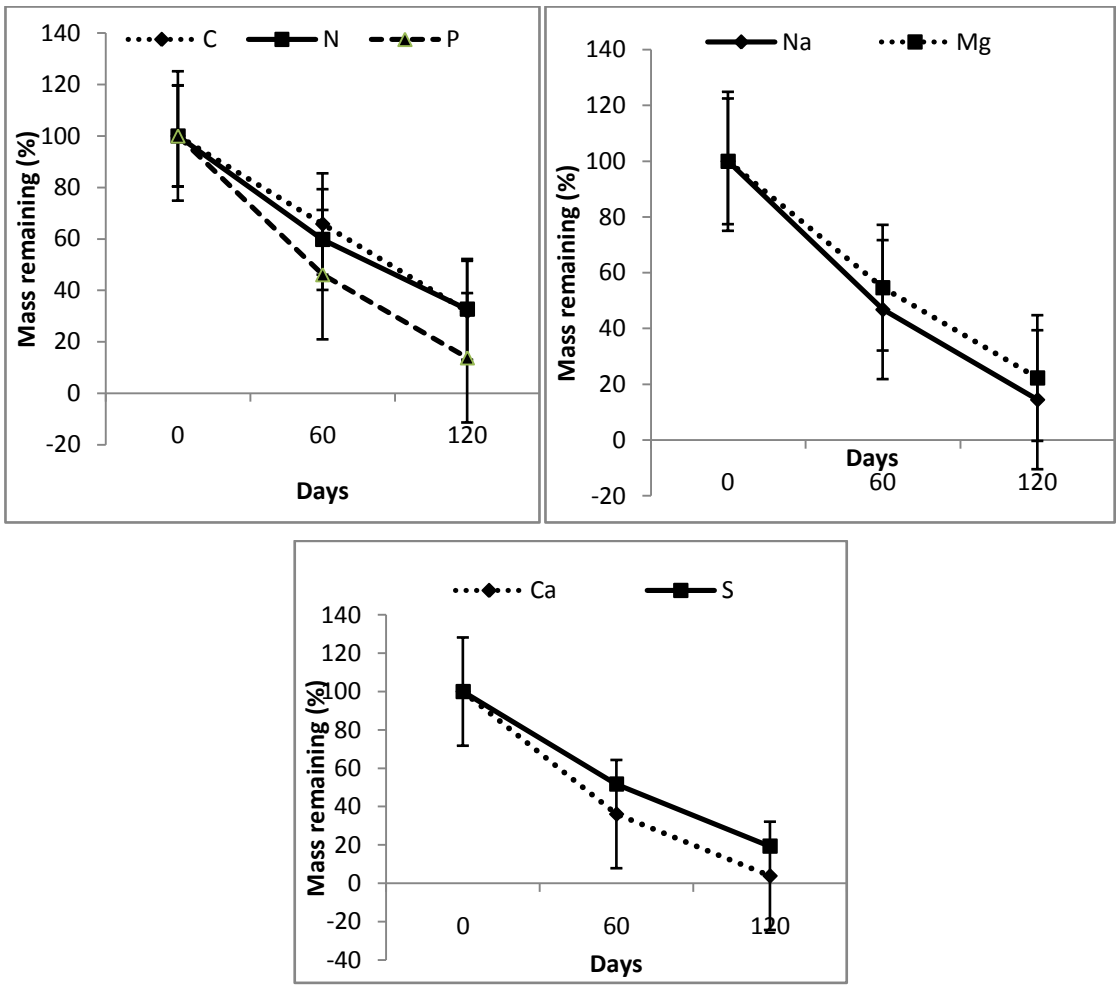

Fig. 4.10 Temporal changes in nutrients contents in litter (M2) components during course of decomposition in TF site.

4.5 Relationship between leaf litter mass remaining and abiotic factors

In the present study, rainfall and soil temperature were significantly negatively correlated with litter mass remaining in both forest sites. In STF, RF was significantly negatively correlated ($R = -0.72$, $p < 0.01$) with mass remaining (Table 4.4). In STF, significant negative correlation was observed in RF and various mass remaining (i.e. DI, QF and M1) with decomposition day.

In TF site, ST was negative correlated ($R = -0.64$) with decomposition days (Table 4.4). Mass remaining in different litter components (e.g. MB, TG and M2) was negatively correlated with decomposition days (Table 4.5).

Table 4.4 Various correlations between decomposition days, abiotic factor (RF-rainfall and ST-soil temperature) and leaf litter (DI-*Drypetes indica*, QF-*Quercus floribunda* and M1-mixed leaf).

Correlations

		Days	RF	ST	DI	QF	M1
Days	Pearson Correlation	1	-.722**	-.640*	-.913**	-.911**	-.915**
	Sig. (2-tailed)		.008	.025	.000	.000	.000
	N	13	12	12	13	13	13
RF	Pearson Correlation	-.722**	1	.938**	.682*	.607*	.659*
	Sig. (2-tailed)	.008		.000	.015	.036	.020
	N	12	12	12	12	12	12
ST	Pearson Correlation	-.640*	.938**	1	.609*	.512	.592*
	Sig. (2-tailed)	.025	.000		.036	.089	.043
	N	12	12	12	12	12	12
DI	Pearson Correlation	-.913**	.682*	.609*	1	.994**	.997**
	Sig. (2-tailed)	.000	.015	.036		.000	.000
	N	13	12	12	13	13	13
QF	Pearson Correlation	-.911**	.607*	.512	.994**	1	.993**
	Sig. (2-tailed)	.000	.036	.089	.000		.000
	N	13	12	12	13	13	13
M1	Pearson Correlation	-.915**	.659*	.592*	.997**	.993**	1
	Sig. (2-tailed)	.000	.020	.043	.000	.000	
	N	13	12	12	13	13	13

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 4.5 Correlations between abiotic factor (RF-rainfall and ST-soil temperature) and leaf litter mass remaining (MB-*Melocanna baccifera*, TG-*Tectona grandis* and M2-mixed leaf).

Correlations

		Days	RF	ST	MB	TG	M2
Days	Pearson Correlation	1	-.397	-.458	-.845**	-.890**	-.887**
	Sig. (2-tailed)		.202	.135	.000	.000	.000
	N	13	12	12	13	13	13
RF	Pearson Correlation	-.397	1	.892**	.533	.572	.546
	Sig. (2-tailed)	.202		.000	.074	.052	.066
	N	12	12	12	12	12	12
ST	Pearson Correlation	-.458	.892**	1	.659*	.662*	.657*
	Sig. (2-tailed)	.135	.000		.020	.019	.020
	N	12	12	12	12	12	12
MB	Pearson Correlation	-.845**	.533	.659*	1	.966**	.976**
	Sig. (2-tailed)	.000	.074	.020		.000	.000
	N	13	12	12	13	13	13
TG	Pearson Correlation	-.890**	.572	.662*	.966**	1	.994**
	Sig. (2-tailed)	.000	.052	.019	.000		.000
	N	13	12	12	13	13	13
M2	Pearson Correlation	-.887**	.546	.657*	.976**	.994**	1
	Sig. (2-tailed)	.000	.066	.020	.000	.000	
	N	13	12	12	13	13	13

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

4.6 Leaf litter decomposition in laboratory microcosm

Litter mass remaining over time has been shown in Fig.4.11. Mass remaining followed a simple linear trend in various leaf litter components. However, the litter selected from TF site (MB, TG and M2) showed faster decomposition rate than litter from STF (DI, QF and M1). Litter mass remaining was significantly correlated with litter decomposition time elapsed in days. Different litter components reflect that 96-98% variability the litter mass remaining was accounted by the time elapsed since the litter was placed (Fig.4.11).

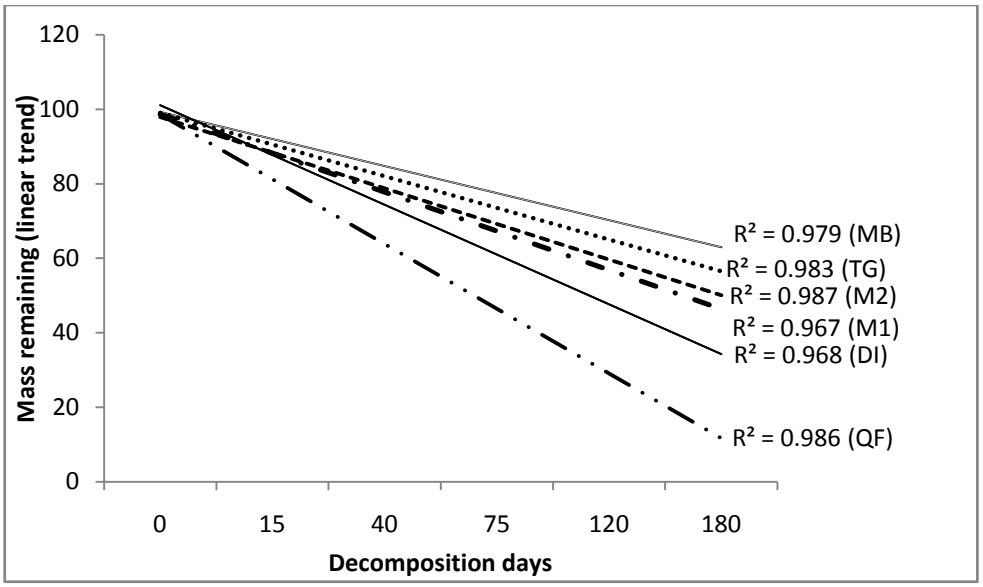

Fig. 4.11 Litter mass loss expressed in simple linear equation with R^2 value for various leaf litter (DI, QF, M1, MB, TG and M2) components in microcosm. Trend lines showed differences in the rate of mass loss among the litter types.

Mean annual decay constant (k) for various litter components ranged from 1.2 – 1.7 in STF and 1.9 – 2.8 in TF. Maximum decay constant (k) was observed in MB (2.8) followed with TG (2.4) and minimum in QF (1.2) as shown in Table 4.6. Projected mass loss for 50% decay would require 87 to 208 days for all litter types. Average days required for 50% decomposition was 142 – 208 in STF which was significantly higher compared to 87 – 128 in TF site. For 95% decay, 616 – 901 days would be required in for the decomposition of various components in STF site which requires distinctly higher time compare to 379 – 557 days in TF site (Table 4.6).

Table 4.6 Annual decay constants (k) value for both forest sites along with t_{50} and t_{95}. t_{50} and t_{95} are time required for 50% and 95% decomposition of litter.

Site	Litter types	K	t_{50}	t_{95}
STF	DI	1.5	167.058	723.194
	QF	1.2	208.304	901.749
	M1	1.7	142.484	616.812
TF	MB	2.8	87.653	379.451
	TG	2.4	105.176	455.307
	M2	1.9	128.864	557.853

4.7 Changes in C, N and C/N ratio in soil and litter materials

The soil C concentration at the end of litter recovery ranged from 40.6 – 230%. In STF site, the concentration of litter C was DI (46.6%), QF (41.2%), M1 (40.6%), MB (230%), TG (201%) and M2 (187%). N concentration was DI (42%), QF (46%) and M1 (42%) whereas for TF site MB (185%), TG (162%) and M2 (165%). And C/N ratio in various soil was DI (111.3%), QF (89.9%), M1 (97%), MB (123.4%), TG (123.2%) and M2 (112.3%) as given in Fig. 4.12.

The C remaining at the end of the experiment was DI (56.7%), QF (60.5%), M1 (47.3%), MB (11.2%), TG (27.9%) and M2 (41.9%). And N concentration was DI (78.5%), QF (98.7%), M1 (44.8%), MB (11.8%), TG (46.7%) and M2 (48.4%) (Fig 4.13). C/N ratio was ranged from 12.3 – 51.9, C/N ratio in various leaf litter are DI (40.2), QF (37.5), M1 (51.9), MB (12.3), TG (18.2) and M2 (38.1) as shown in Fig. 4.14.

Changes in litter N and C/N ratio during the course of decomposition was significant ($p \leq 0.05$), whereas, the variations in litter C was not significant. Litter C/N ratio declining after 15 days till end of the experiment (Fig. 4.14).

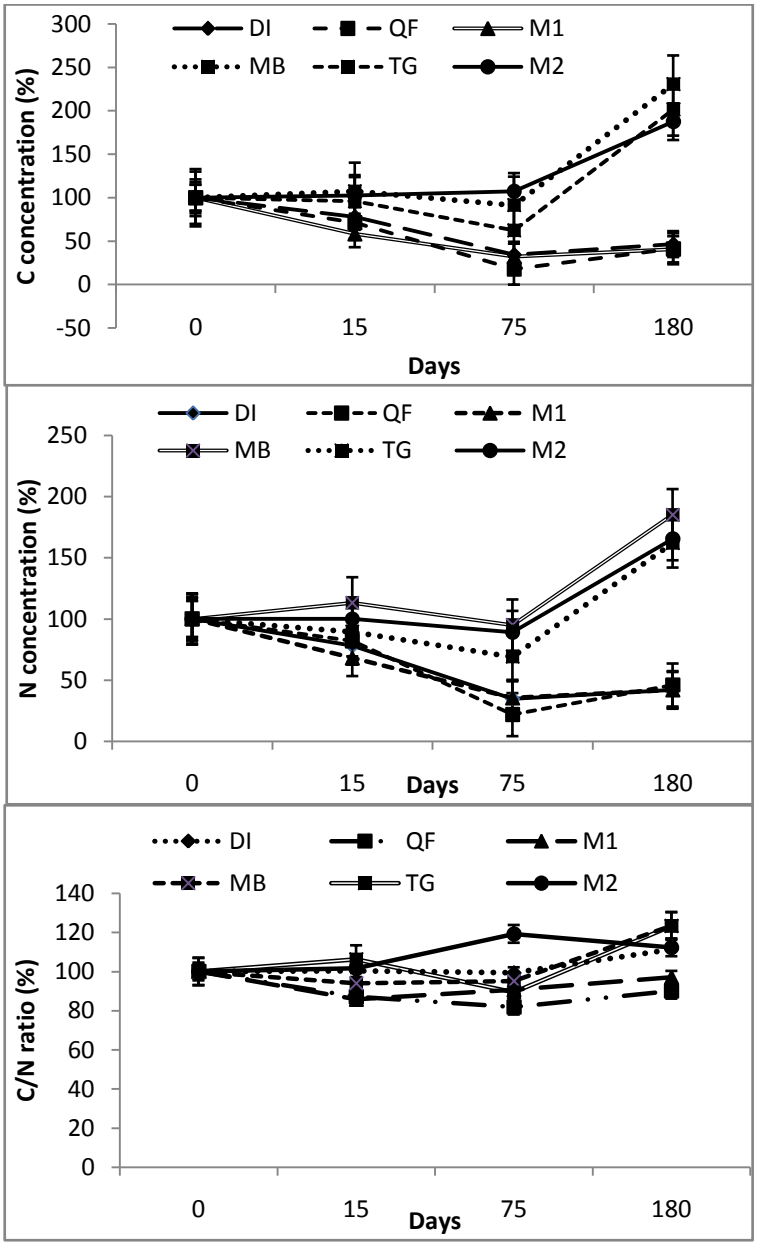

Fig 4.12 Changes in per cent C, N and C/N ratio in soil (cf. original amount in the soil) during the course of decomposition laboratory microcosm. MB, TG and M2 are leaves of *Melocanna baccifera*, *Tectona grandis* and mixed litter from subtropical forest. DI, QF and M1 are *Drypetes indica*, *Quercus floribunda* and mixed tropical forest litter placed in the laboratory microcosm.

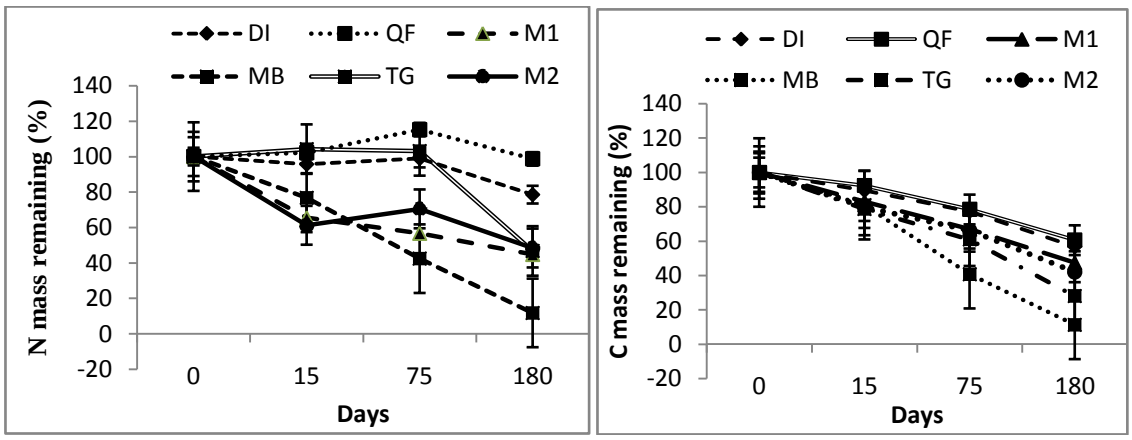

Fig. 4.13 Carbon and nitrogen mass remaining in microcosm experiment in different litter component of TF than STF site.

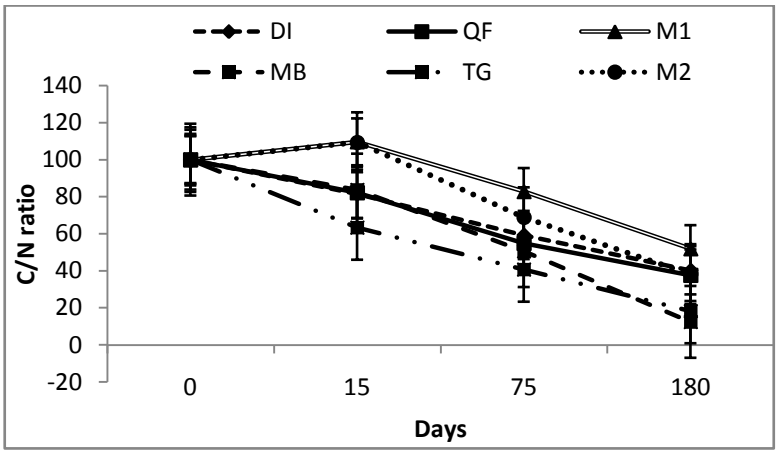

Fig.4.14 Temporal changes in C/N ratios of various leaf litter components during decomposition.

4.8 Mass remaining of various nutrients in leaf litter under laboratory microcosm

Marked changes in the concentration of different nutrient (P, Na, Mg, K, Ca, Mn, Cr, Ni, Cu, Zn, Pb, As, Al, S and Fe) occurred in different leaf litter categories during the course of decomposition (0, 15, 75 and 180 days) in laboratory microcosm (Fig. 4.15 – 4.26). The nutrient mass remaining after 180 days of decomposition in DI litter are P

(101.3%), Na (55.5%), Mg (85.7%), K (47.8%), Ca (97.8%), Mn (80.7%), Cr (38.7%), Ni (58%), Cu (61.4%), Zn (360%), Pb (1460%), As (23.7%), Al (33.8%), S (102%) and Fe (23.5%). Higher value was observed considerably in Pb while minimum value was observed in Fe (23.5%) (Fig. 4.15).

In QF litter, nutrient mass remaining of various nutrients are given in Fig. 4.16. After 180 days of decomposition, mass remaining for different nutrients were: P (130.3%), Na (86.5%), Mg (85%), K (43.6%), Ca (119.3%), Mn (111.4%), Cr (50.1%), Ni (57.8%), Cu (89.9%), Zn (331.4%), Pb (744.2%), As (52.6%), Al (95.5%), S (136.5%) and Fe (71%). Notably, Zn, Pb and S showed considerably higher incraese in the concentrations after 6 months compared to initial nutrient contents (Fig. 4.16).

In case of M1 (mixed leaf litter), after 180 days retrieval periods, mass reaming of various nutrients were: P(51.6%), Na (33.8%), Mg (79.2%), K (29.7%), Ca (55.4%), Mn (26.5%), Cr (45.3%), Ni (42.4%), Cu (48.5%), Zn (279.3%), Pb (1070.5%), As (53.5%), Al (63.2%), S (66.3%) and Fe (83.1%). Zn and Pb have considerably higher concentrations at the end of decomposition (Fig. 4.17).

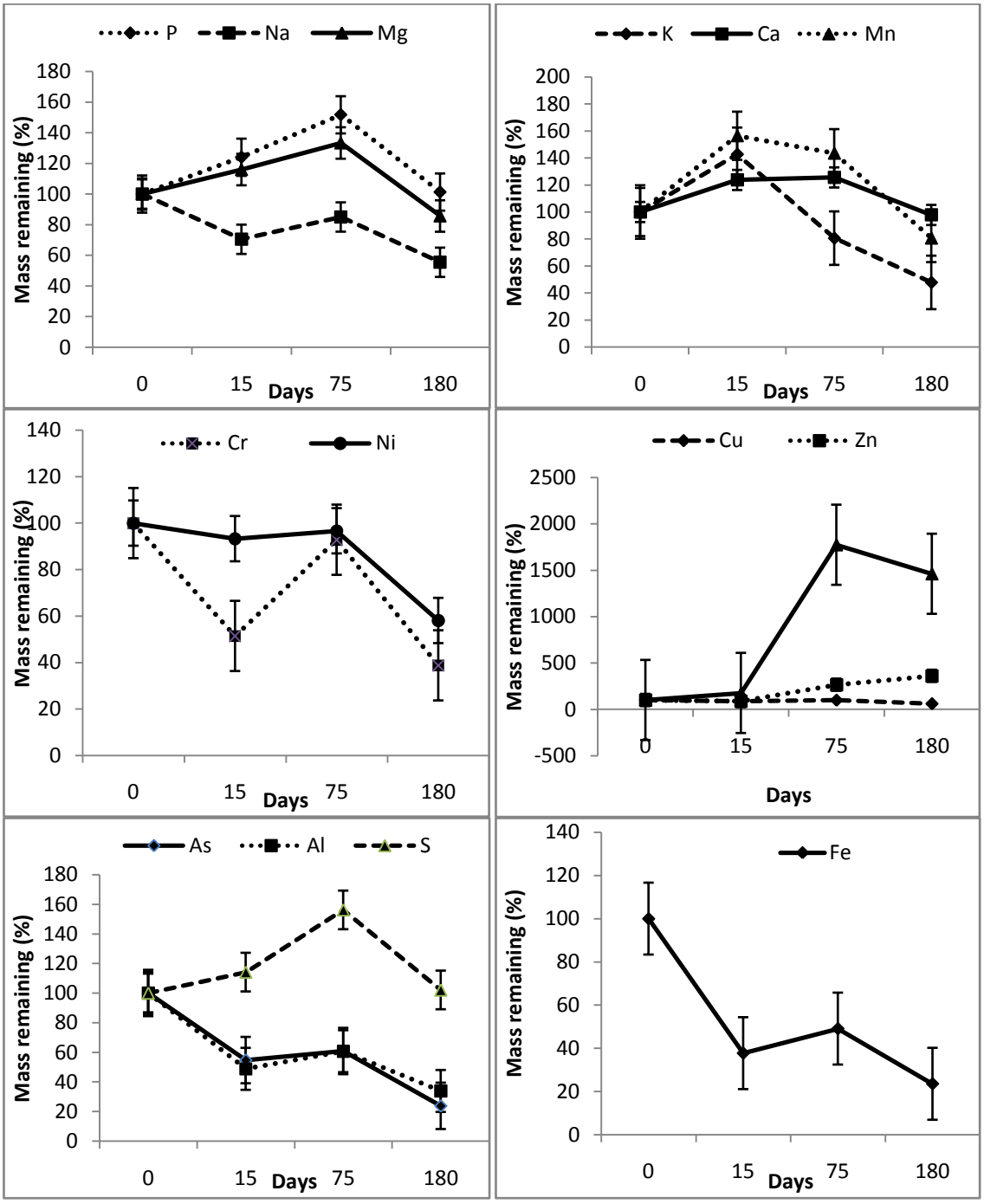

Fig.4.15 Mass remaining of various litter nutrients (P, Na, Mg, K, Ca, Mn, Cr, Ni, Cu, Zn, Pb, As, Al, S and Fe) concentration in DI during the course of litter decomposition in microcosm.

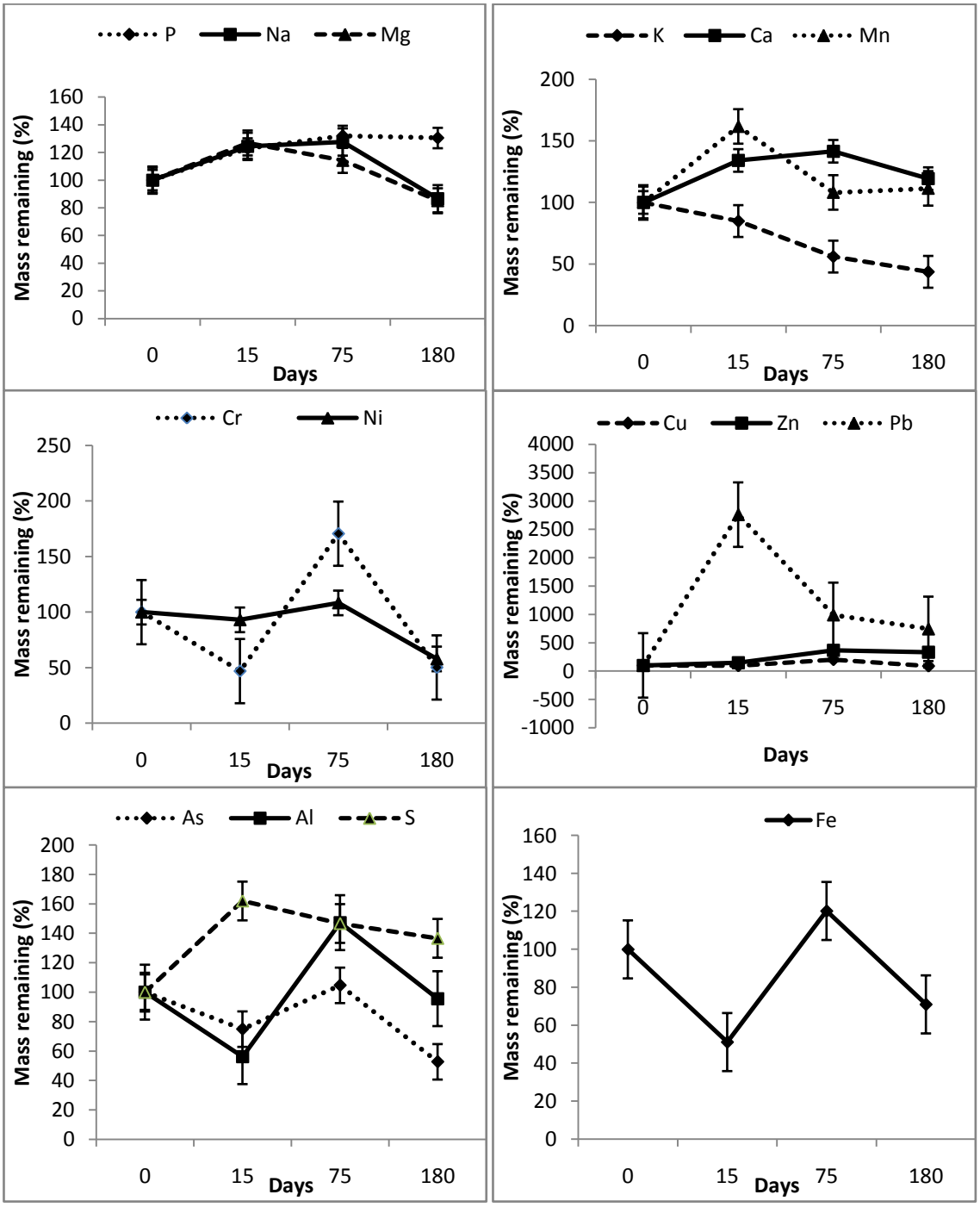

Fig.4.16 Mass remaining of various litter nutrients (P, Na, Mg, K, Ca, Mn, Cr, Ni, Cu, Zn, Pb, As, Al, S and Fe) concentration in QF (litter) during course of litter decomposition in microcosm.

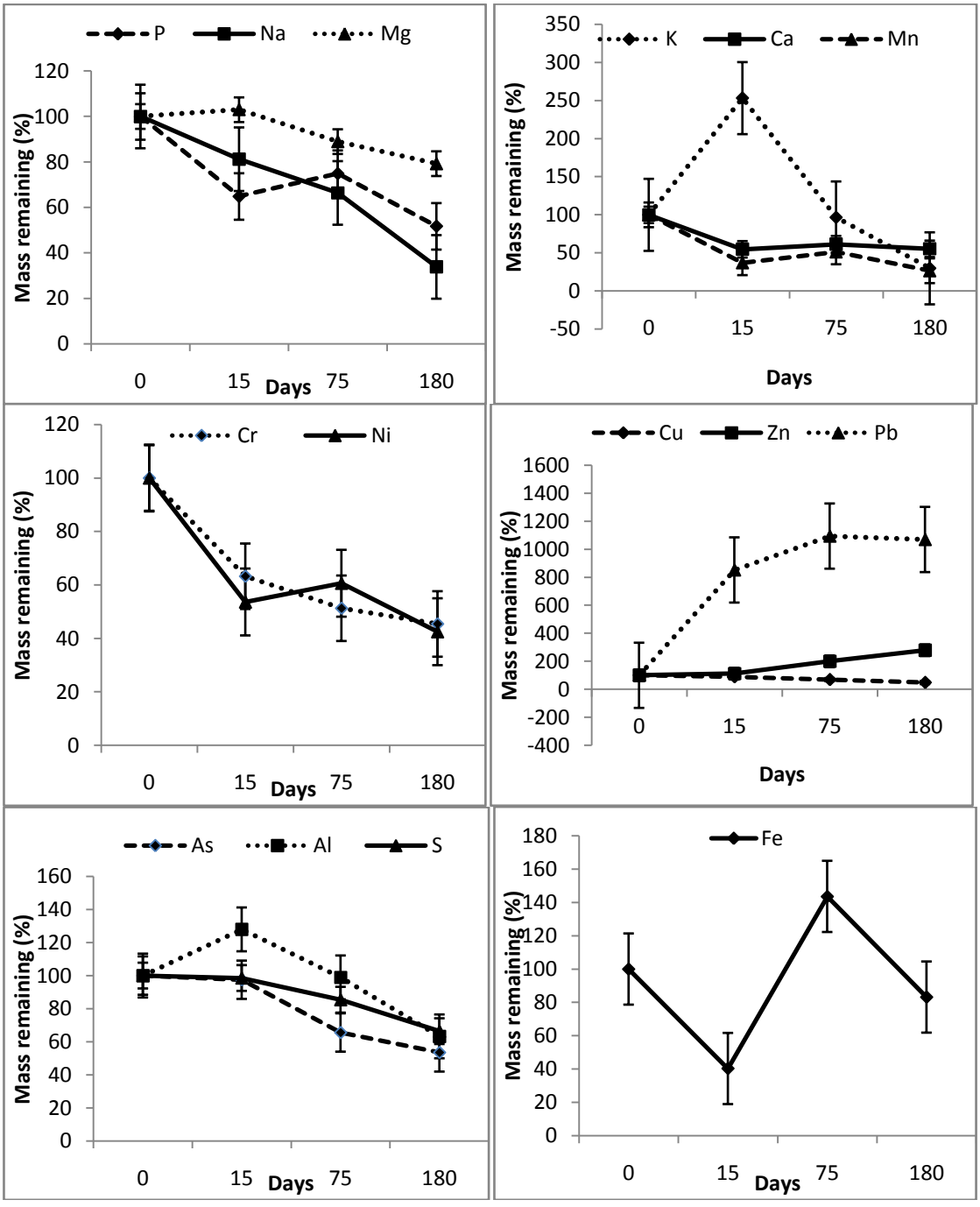

Fig.4.17 Mass remaining of various litter nutrients (P, Na, Mg, K, Ca, Mn, Cr, Ni, Cu, Zn, Pb, As, Al, S and Fe) in M1 (litter) during course of litter decomposition in microcosm.

Mass remaining of various leaf litter nutrient (P, Na, Mg, K, Ca, Mn, Cr, Ni, Cu, Zn, Pb, As, Al, S and Fe) in MB were given in Fig. 4.18. Percent mass remaining of nutrients after 180 days of decomposition in litter were: P (16.8%), Na (13.5%), Mg (12.7%), K (2.4%), Ca (19.7%), Mn (25.5%), Cr (25.3%), Ni (10.4%), Cu (22.9%), Zn (87.9%), Pb (264.4%), As (12.3%), Al (47.1%), S (13.4%) and Fe (49.3%). Highest increase in the mass remaining was observed in Pb (264.4%) and lowest in K (2.4%).

In TG litter, nutrient mass remaining ranged from 30 – 70% (Fig. 4.19). Mass remaining of various nutrients at the end of the experiment was: P (48.8%), Na (47.6%), Mg (45%), K (5.1%), Ca (78.6%), Mn (49.1%), Cr (50.5%), Ni (35.9%), Cu (38.7%), Zn (178.9%), Pb (890.5%), As (68.1%), Al (117.1%), S (55.6%) and Fe (87.8%). Nutrient content in Pb was considerably higher as compared to other nutrients..

In case of M2 (mixed leaf litter), nutrient remaining ranged between 28 and 70%. Mass remaining of various nutrients after 6 months of decomposition were: P (42.8%), Na (33.4%), Mg (68.2%), K (30.9%), Ca (40.2%), Mn (28.9%), Cr (64.2%), Ni (30.1%), Cu (36.3%), Zn (172%), Pb (1526%), As (51.7%), Al (60.9%), S (53.9%) and Fe (58.7%). The value in Pb was considerably higher compared to others nutrients (Fig. 4.20).

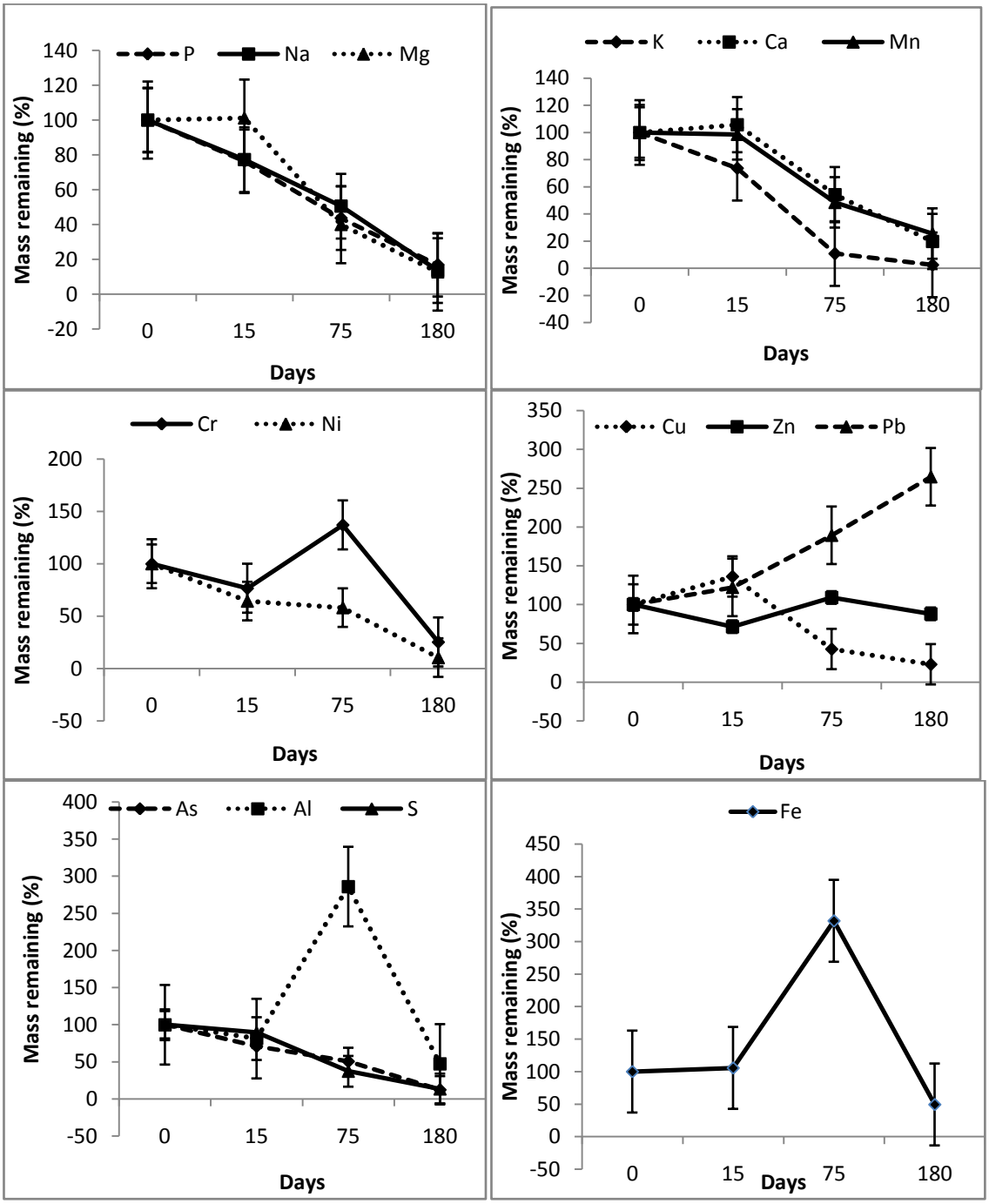

Fig.4.18 Mass remaining of various nutrients (P, Na, Mg, K, Ca, Mn, Cr, Ni, Cu, Zn, Pb, As, Al, S and Fe) in MB (litter) during decomposition in microcosm.

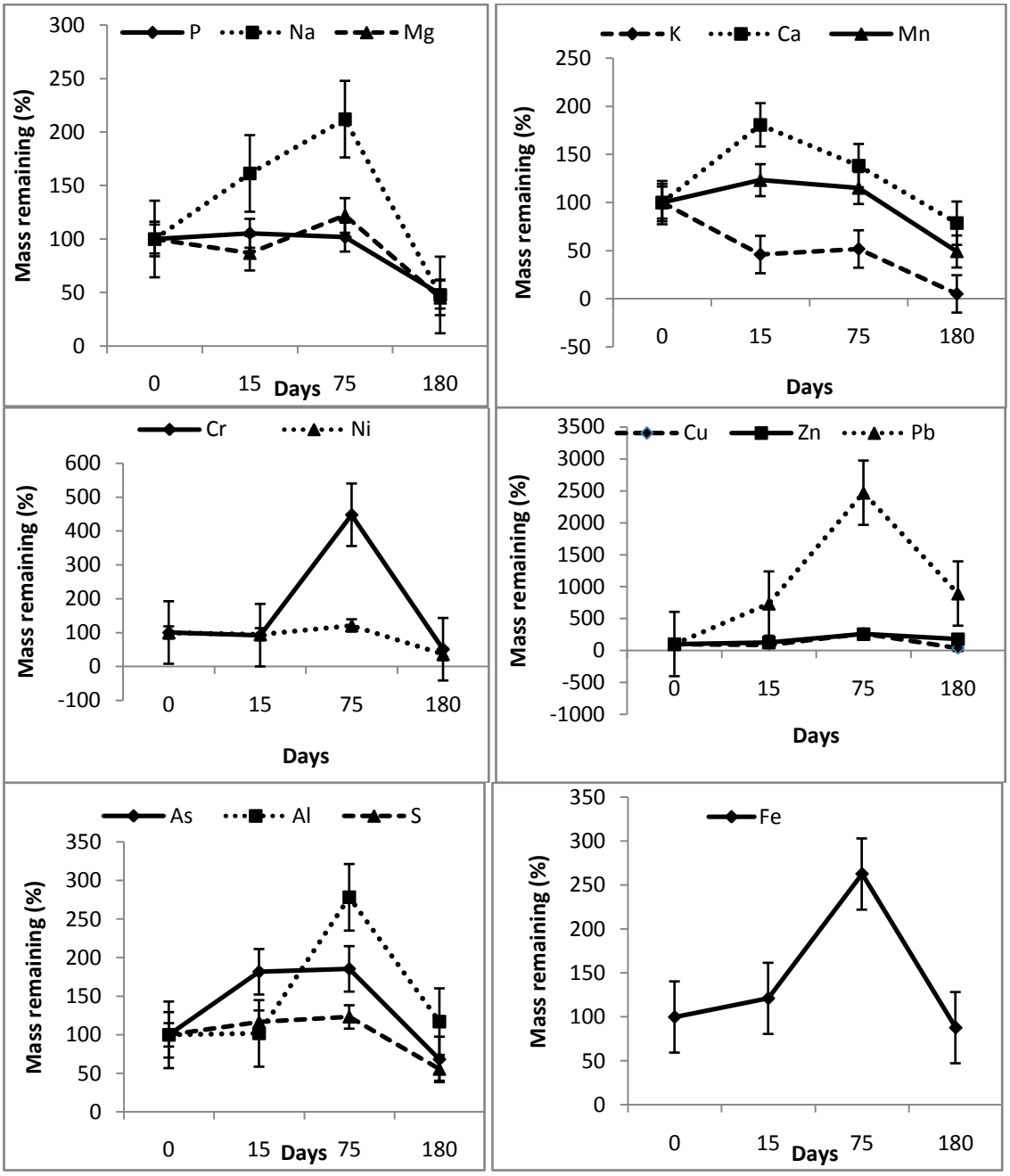

Fig.4.19 Mass remaining of various nutrients (P, Na, Mg, K, Ca, Mn, Cr, Ni, Cu, Zn, Pb, As, Al, S and Fe) in TG (litter) during course of decomposition microcosm.

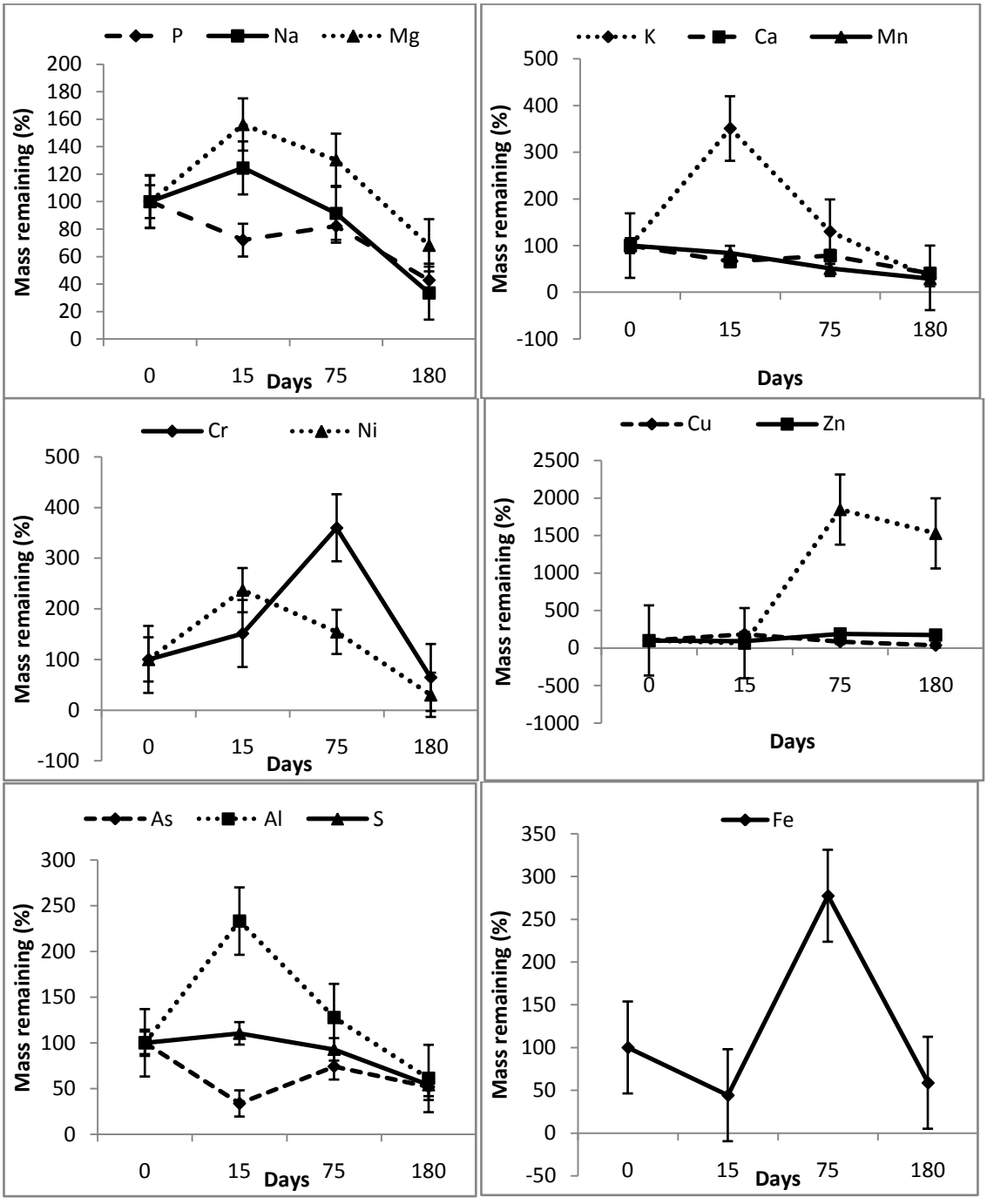

Fig.4.20 Mass remaining of various nutrients (P, Na, Mg, K, Ca, Mn, Cr, Ni, Cu, Zn, Pb, As, Al, S and Fe) in M2 (litter) during course of decomposition in microcosm.

4.9 Mass remaining of various soil nutrients under different litter treatment in laboratory microcosm

After recovery of various litter components from laboratory microcosm, soil nutrient status was assessed. Variation of soil nutrient content after recovery of litter at the end of 180 days were given in Fig. 4.21 – 4.26. In most cases, nutrient contents after the end of recovery was increased as compared to initial nutrient concentration. The concentration of Ca was considerably higher in most treatments while S concentration was lower compared to others nutrients in the soil. Majority of nutrients in soil of STF (DI, QF and M1) were considerably higher compared to nutrients in TF (MB, TG and M2) as given in Fig. 4.21 – 4.26.

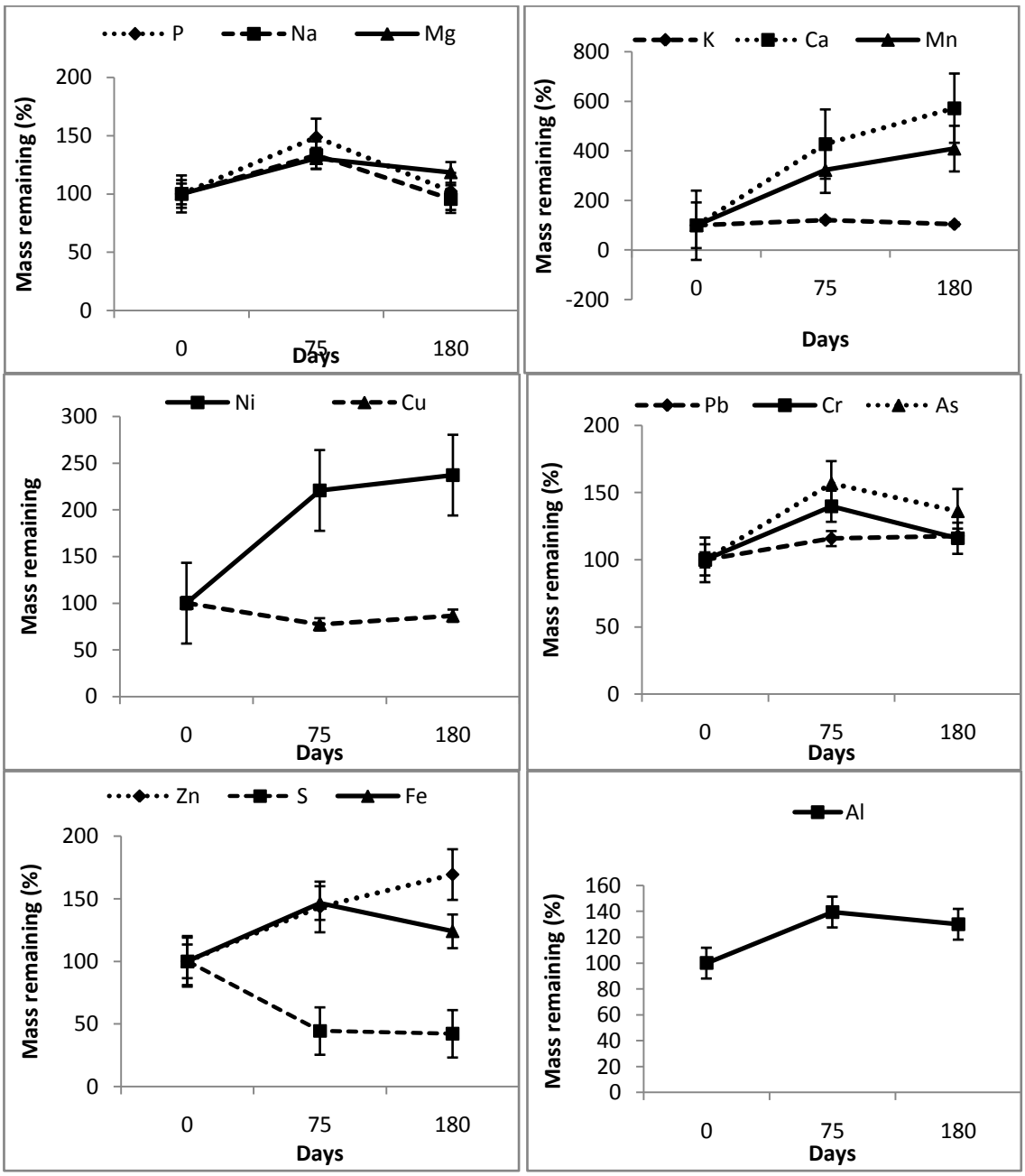

Fig.4.21 Mass remaining of various soil nutrients (P, Na, Mg, K, Ca, Mn, Cr, Ni, Cu, Zn, Pb, As, Al, S and Fe) in DI litter deposited soil during course of decomposition in laboratory microcosm.

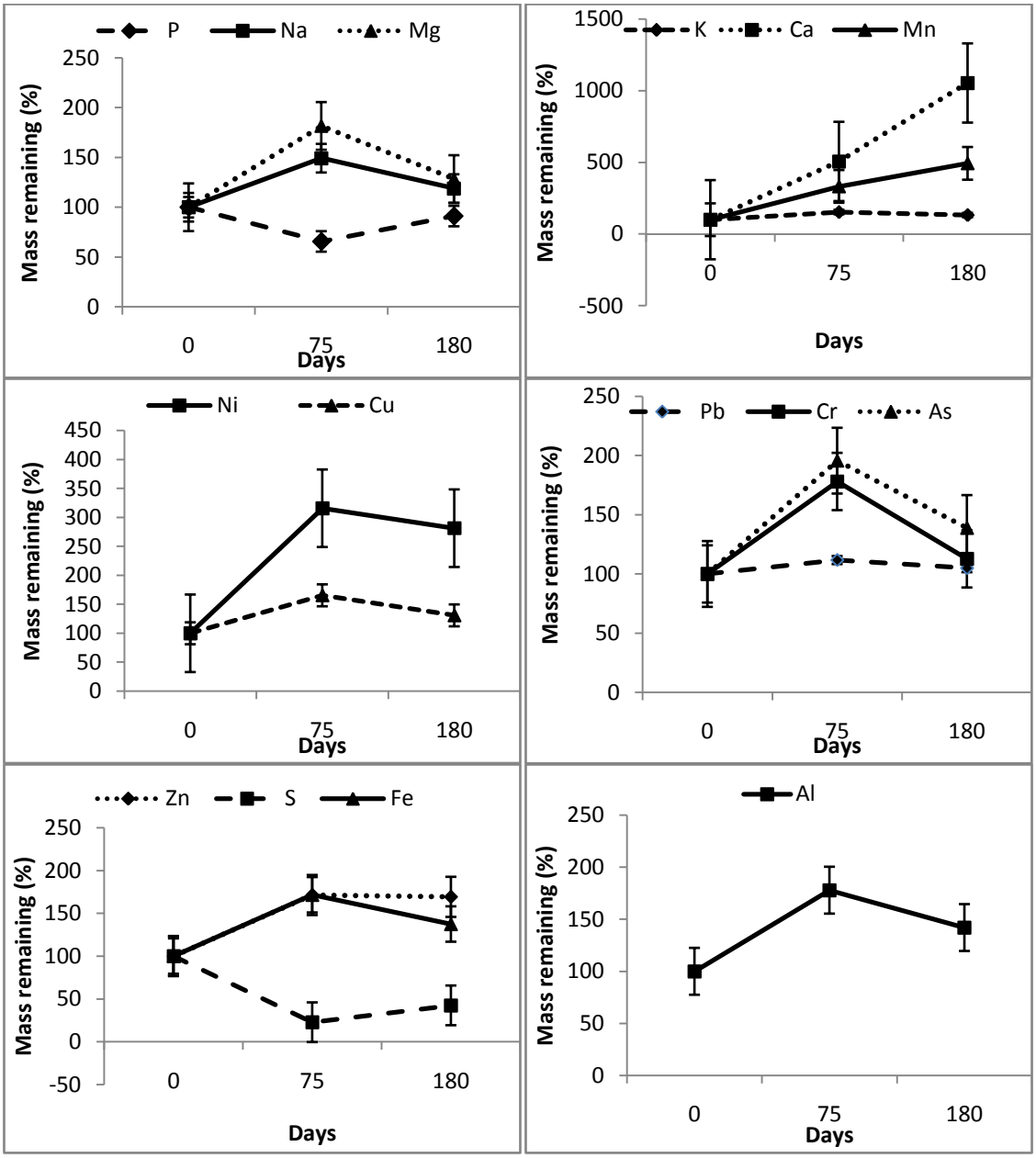

Fig.4.22 Mass remaining of various soil nutrients (P, Na, Mg, K, Ca, Mn, Cr, Ni, Cu, Zn, Pb, As, Al, S and Fe) in QF litter deposited soil during course of decomposition.

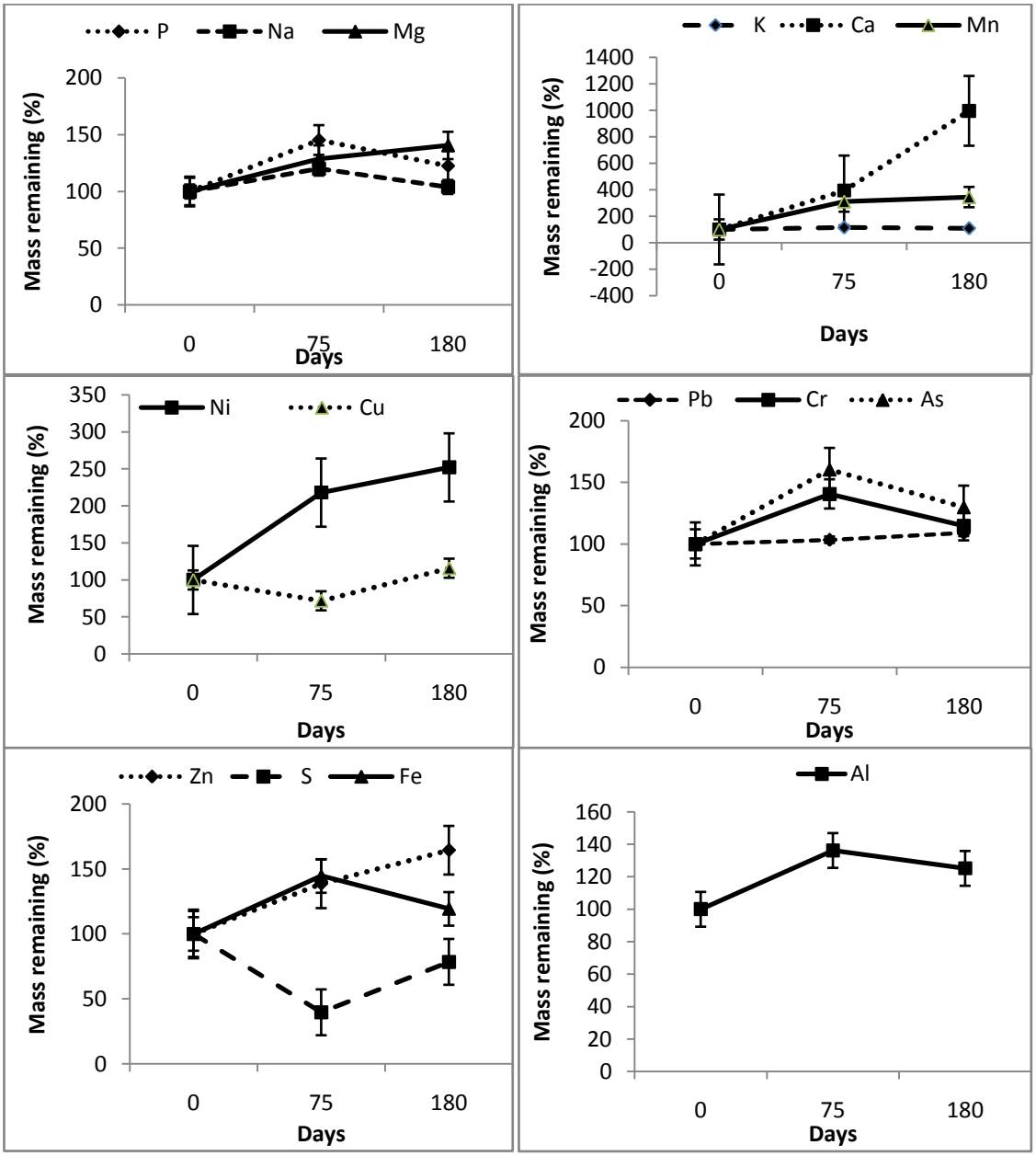

Fig.4.23 Mass remaining of various soil nutrients (P, Na, Mg, K, Ca, Mn, Cr, Ni, Cu, Zn, Pb, As, Al, S and Fe) in M1 litter deposited soil during course of decomposition.

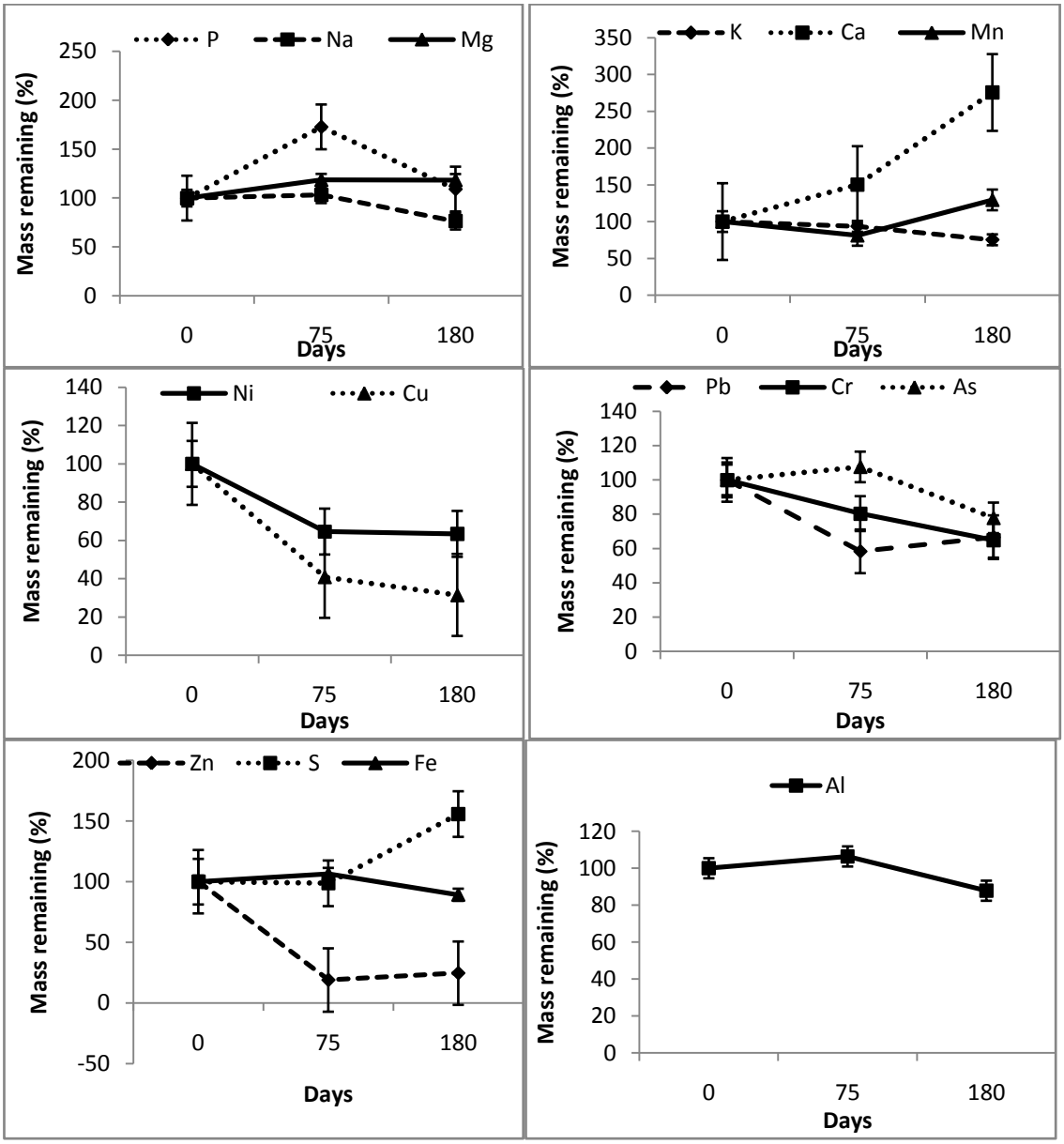

Fig.4.24 Mass remaining of various soil nutrients (P, Na, Mg, K, Ca, Mn, Cr, Ni, Cu, Zn, Pb, As, Al, S and Fe) in MB litter deposited soil during course of decomposition.

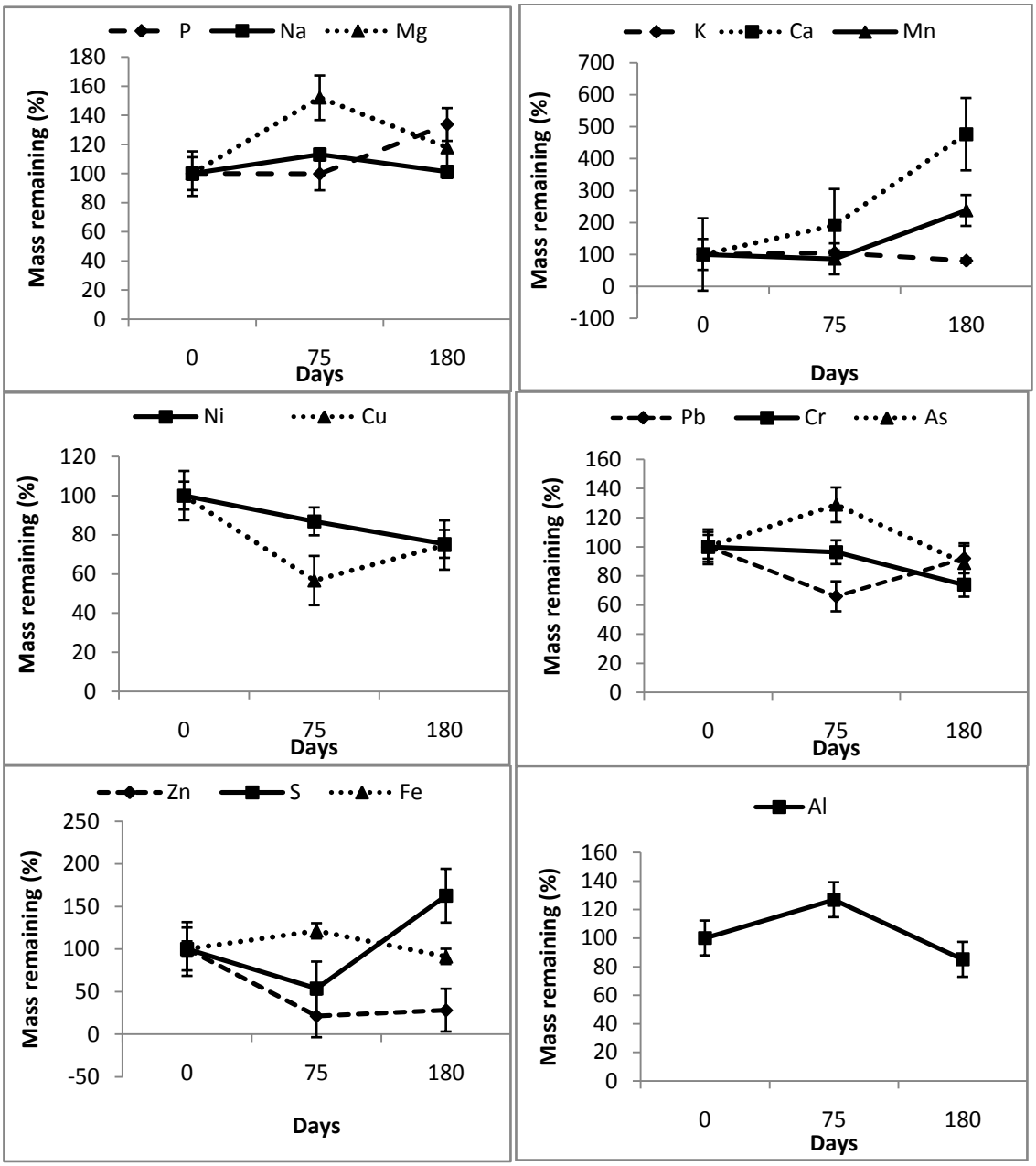

Fig.4.25 Mass remaining of various soil nutrients (P, Na, Mg, K, Ca, Mn, Cr, Ni, Cu, Zn, Pb, As, Al, S and Fe) in TG litter deposited soil during course of decomposition.

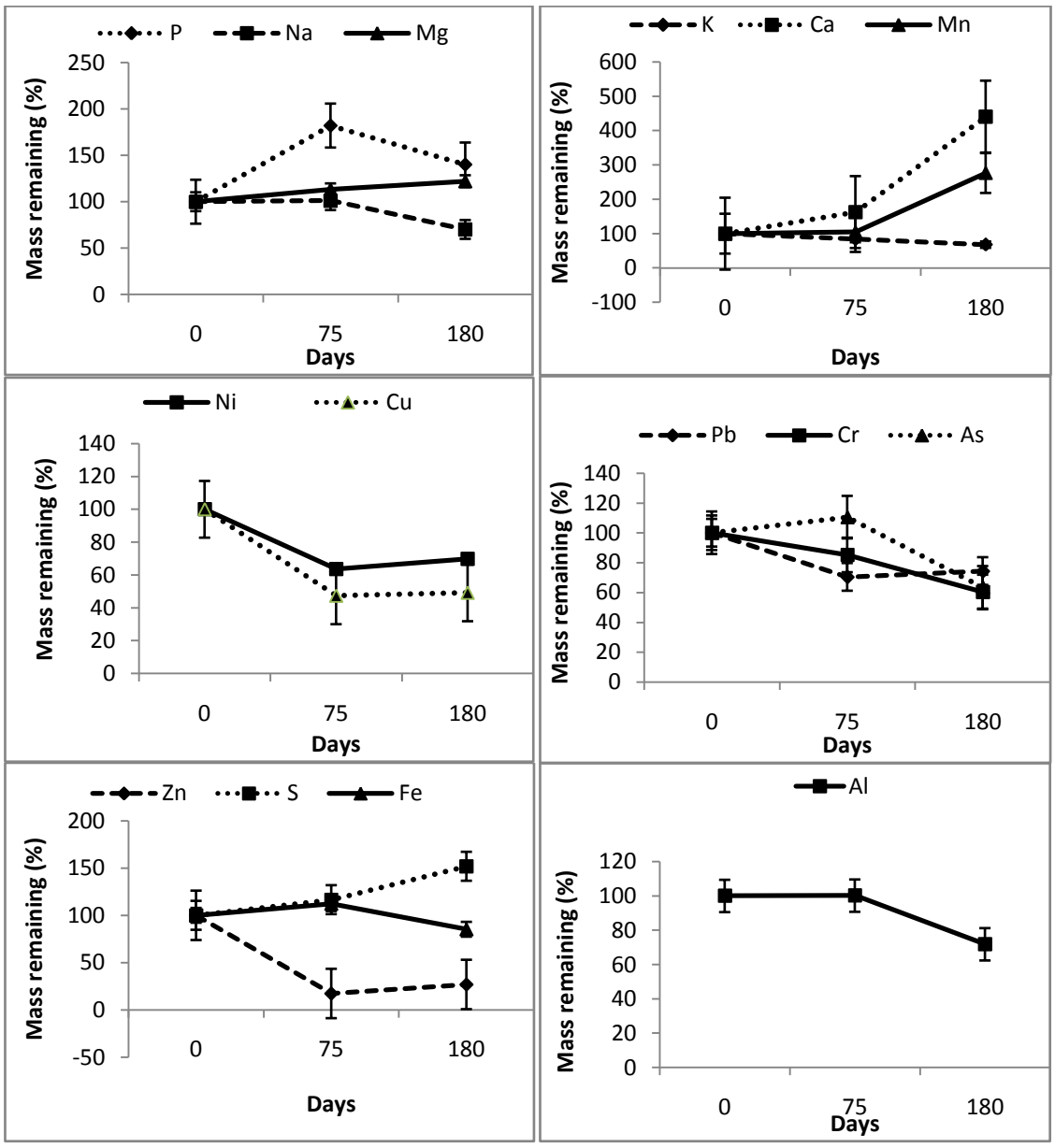

Fig.4.26 Mass remaining of various soil nutrients (P, Na, Mg, K, Ca, Mn, Cr, Ni, Cu, Zn, Pb, As, Al, S and Fe) in M2 litter deposited soil during course of decomposition.

4.10 Changes in soil microbial population in mixed litter decomposition under laboratory microcosm

Soil microbial population (fungi, actinomycetes and bacteria) showed consistent increase in CFUs of fungi in STF. However, after 40 days of decomposition CFUs of fungi was maximum in TF and minimum in STF (Fig. 4.27). In both sites, actinomycetes show rapid decreases in initial 40 days followed by a constant population till final retrieval dates. Bacterial counts decreases from initial to 40 days then increased gradually at the end of retrieval period in STF (Fig. 4.27). The overall results of soil microbial population count (i.e. CFUs) reveal that microbial population was in order: bacterial >actinomycetes > fungi during the study (Fig. 4.27).

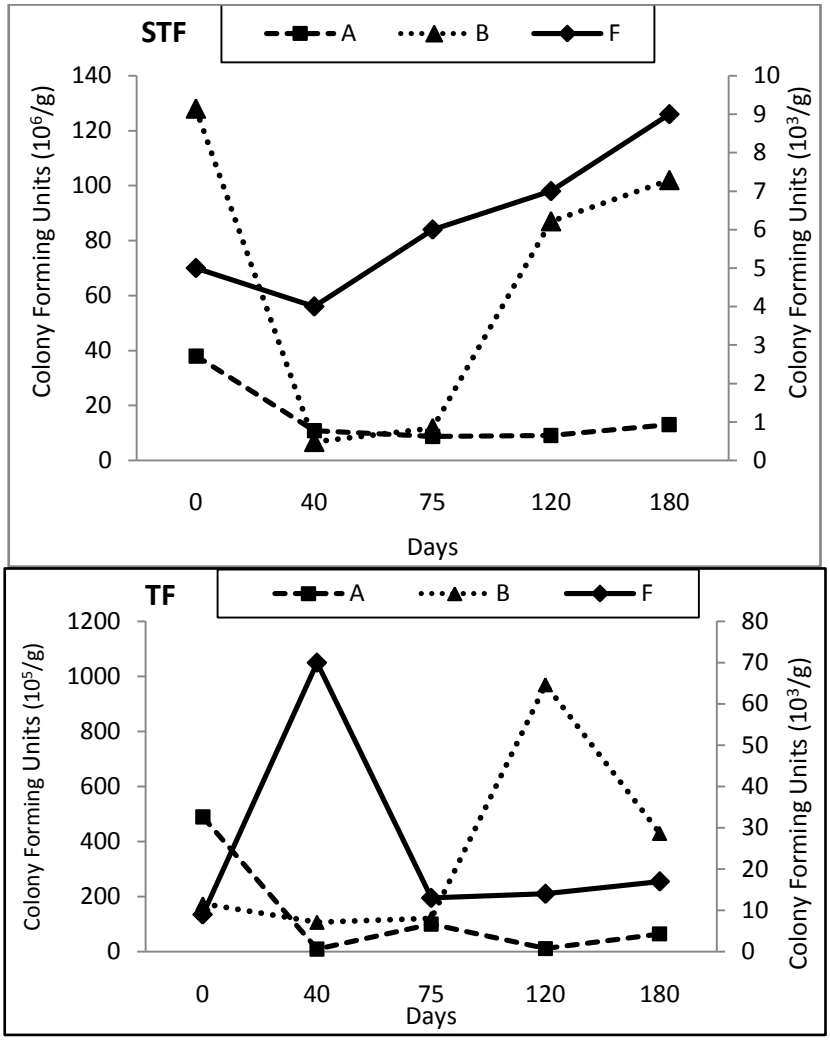

Fig.4.27 Colony forming units (CFUs) of soil microbes (F=Fungi, A= Actinomycetes and B= Bacteria) from two forest (STF & TF).

4.11 Correlation between mass loss and soil microbes and nutrients (soil and litter)

To assess effect of biotic and abiotic variables (soil microbes, soil nutrients and litter nutrients) on litter mass loss rates, best fit regression model was applied to predict litter mass rate in mixed leaf litter. In STF site, N in both soil and litter accounted for about 95.97% variability in mass loss. In TF site, soil microbes (fungi and actinomycetes) along with litter C/N ratio were selected as stronger predictor variables accounting 99.84% variability in mass loss rates. Equations are as follows:

Sub-tropical forest (STF), mass loss rate (%)

$$= 10.76 + 10.94\ (N_{soil}) - 7.71\ (N_{litter})$$

$$R^2 = 95.97\ \%$$

Tropical forest (TF), mass loss rate (%)

$$= -27.26 + 0.8361\ (F) + 0.2886\ (A) + 0.6018\ (C/N_{litter}\ \text{ratio})$$

$$R^2 = 99.84\ \%$$

4.12 Decomposing ability of major filamentous fungi

The results on decomposing ability of groups of filamentous fungi (5 dominant fungal groups and total fungal groups from each site) in four leaf litter categories were presented under different subheads.

4.13 Chemical composition of initial leaf litter

Chemical compositions of all four leaf litter categories showed wide variations among various litter components. Acid detergent fibre (ADF%) ranged from 49.6 % – 52% in these litter categories. Maximum ADF%, C% and CN ratio was observed in *Tectona grandis* while minimum in *Melocanna baccifera.* Among other chemical composition, N, P, Mg, Ca and Mn were higher in *Melocanna baccifera* compared to other leaf litter categories. Na contents in all the leaf species were almost constant (~ 0.01%) as given in Table 4.7.

Table 4.7 Initial chemical composition of four leaf litter types used for fungal inoculations to assess the decomposing ability of fungi.

Plant species	ADF %	C%	N%	CN ratio	P	Na	Mg	K	Ca	Mn
MB	49.6 ± 0.23	37.04 ± 0.02	2.22 ± 0.02	16.69	0.09 ± 0.003	0.01 ± 0.003	0.19 ± 0.006	0.36 ± 0.004	0.75 ± 0.003	0.04 ± 0.001
TG	52 ± 0.05	47.64 ± 0.03	1.04 ± 0.04	45.68	0.05 ± 0.001	0.01 ± 0.00	0.13 ± 0.003	0.45 ± 0.007	0.58 ± 0.004	0.01 ± 0.00
DI	51.6 ± 0.3	42.85 ± 0.09	0.99 ± 0.09	43.46	0.02 ± 0.00	0.01 ± 0.00	0.06 ± 0.001	0.09 ± 0.004	1.02 ± 0.017	0.03 ± 0.002

QF	50.39 ± 0.2	47.54 ± 0.15	1.14 ± 0.02	41.63	0.02 ± 0.003	0.01 ± 0.00	0.10 ±0.002	0.14 ± 0.004	0.55 ± 0.01	0.06 ± 0.005

* (mean ± 1SE, n=3).

4.14 Major fungi isolated from two forest sites

The major fungal groups isolated from two forest sites of Mizoram belong to Ascomycota (*Phoma spp., Cladosporium spp., Aspergillus niger, Myrothecium verrucaria, Tricoderma viride, Periconia macrospinosa, Penicilium spp. and Chaetomium spp.)* and zygomycota (*Rhizopus spp.* And *Mucos spp.*) (Table 4.8). After 2 weeks of fungi inoculation, 2 – 3 (dominant) fungal colonies begin to occupy the whole petridish. This showed a strong competition within the fungi colonies for nutrient utilization during decomposition in the ecosystem.

Table 4.8 Major filamentous fungi (10 numbers, 5 from each site) isolated from two sites.

Sites	**Fungal species**	**Taxa**
Tropical forest		
	Phoma spp.	Ascomycota
	Cladosporium spp.	Ascomycota
	Aspergillus niger	Ascomycota
	Myrothecium verrucaria	Ascomycota
	Tricoderma viride	Ascomycota
Sub-tropical forest		
	Rhizopus spp.	zygomycota
	Mucos spp.	zygomycota
	Periconia macrospinosa	Ascomycota
	Penicilium spp.	Ascomycota
	Chaetomium spp.	Ascomycota

4.15 Effect of fungal inoculation on mass loss and changes in litter chemistry

Though higher mass loss occurred in TG with respect to total fungal inoculation as compared to the inoculation by dominant fungal groups, but the differences were marginal and not significant. In case of MB and DI the mass loss was almost equal in both treatment (i.e. inoculation dominant fungi and total fungi). Effect of total fungal inoculation was more pronounced in QF than inoculation by major fungal groups (Table 4.9).

At the end of 10 weeks study, inoculation of total fungi recorded maximum weight loss in QF and minimum in MB. However, inoculation by major fungi reflected maximum mass loss in TG followed with DI and minimum in MB (Table 4.9). The group of major fungi belongs to Ascomycota and Zycomycota had more ability to decompose selected leaf litter in both early and later stages (Table 4.9).

Table 4.9. Decomposition ability of different group and total fungi on various leaf litters. Litter dry weight in during initial stage (g), weight after 5 weeks (early stage) and weight after 10 weeks (later stages).

	Early Stage				Later Stage			
	Major Fungi		Total Fungi		Major Fungi		Total Fungi	
	IW (g)	FW (g)	IW (g)	FW (g)	IW (g)	FW (g)	IW (g)	FW (g)
Tropical Forest								
MB	0.107 ± 0.001	0.058 ± 0.001	0.105 ± 0.002	0.06 ± 0.001	0.085 ± 0.002	0.041 ± 0.001	0.09 ± 0.002	0.047 ± 0.002
TG	0.219 ± 0.001	0.14 ± 0.005	0.235 ± 0.002	0.12 ± 0.002	0.236 ± 0.002	0.123 ± 0.002	0.23 ± 0.002	0.016 ± 0.002
LSD	0.006	0.017	0.004	0.004	0.007	0.006	0.006	0.008
Sub-Tropical Forest								
DI	0.283 ± 0.001	0.219 ± 0.002	0.26 ± 0.001	0.21 ± 0.001	0.256 ± 0.003	0.176 ± 0.002	0.295 ± 0.003	0.2 ± 0.001
QF	0.286 ± 0.002	0.258 ± 0.002	0.33 ± 0.002	0.29 ± 0.003	0.317 ± 0.003	0.267 ± 0.003	0.335 ± 0.002	0.032 ± 0.001
LSD	0.014	0.007	0.007	0.011	0.010	0.009	0.009	0.006

Mean ± 1SE (n=5); IW = Initial weight (g) of leaf litter; FW = Final weight (g) after decomposition. (LSD = $p < 0.05$)

After inoculation by major fungal group, per cent mass remaining of TG was found to significantly higher in first 5 weeks followed by MB and TG. However, after 10 weeks of inoculation by total fungi MB had higher mass remaining and the TG had lowest mass remaining. Inoculation by major fungal groups for 10 weeks in TG showed

highest mass remaining. Inoculation by major fungi in QF showed higher mass remaining than inoculation by total fungi (Fig.4.28). During the study, the overall mass loss was range from 10 % – 90 %, maximum mass loss was observed in later stage. The group of Ascomycota on MB and TG showed maximum decomposition compare to mix group of Ascomycota and Zygomycota (Fig.4.28).

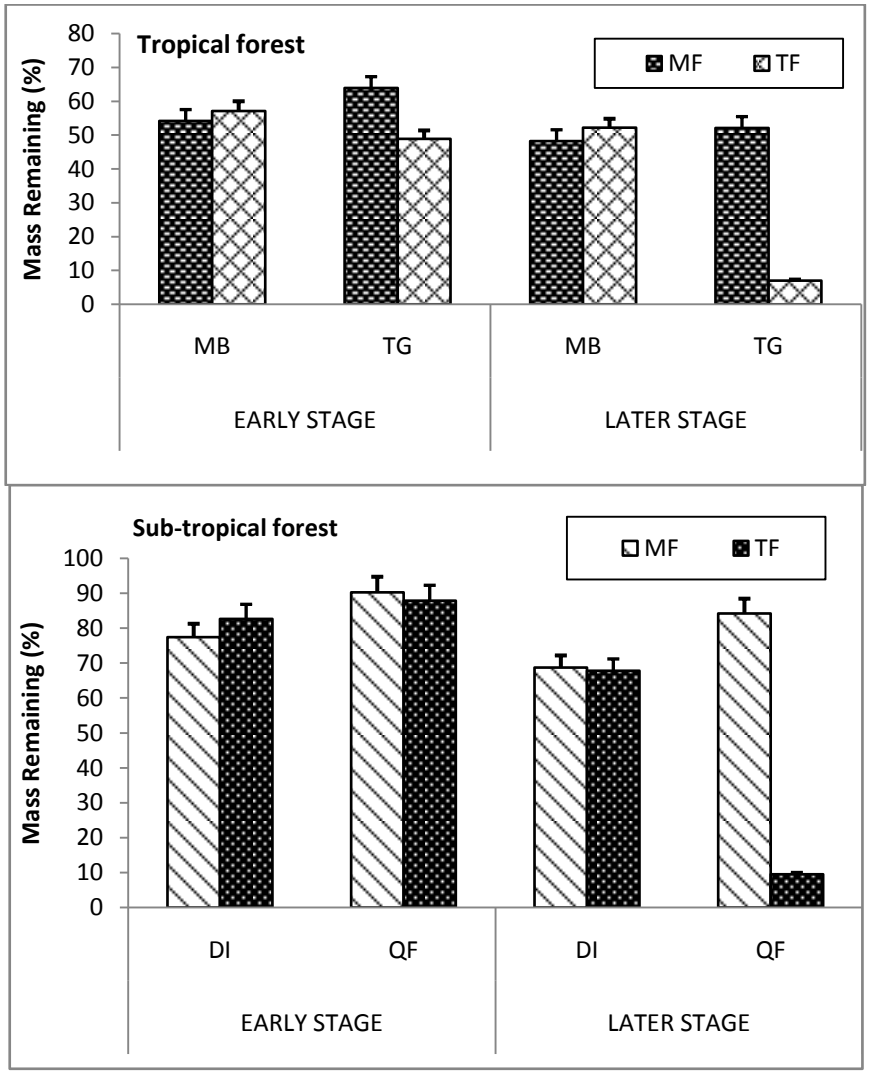

Fig.4.28 Mass remaining (%) of litter (MB and TG) in tropical forest and sub-tropical forest (DI and QF) after 5 weeks (early stages) and 10 weeks (later stages). MF= Major fungi; TF= Total fungi; Mean $\pm$ 1SE, n=5

Mass remaining of ADF ranged from 2.23 – 32.02 at the end of 10 weeks, whereas after 5 weeks the values ranged from 11.35 – 33.19 (Table 4.10). The inoculation of major fungi showed maximum ADF remaining in QF and minimum in TG during the end of 5 weeks and 10 weeks. Inoculation of total fungi causes maximum

loss of ADF in QF (23.59%) followed by TG (9.13%). However, the lowest mass remaining was observed in QF (1.17%) after inoculation by major fungal groups (Table 4.10).

Table 4.10 Acid Detergent Fibre (ADF) remain after 5 weeks (early stage), 10 weeks (later stage) and loss during 5-10 weeks of different leaf discs from two sites inoculation with major fungi and total fungi.

Leaf Species	Incubation type	After 10 weeks (%)	Upto 5 weeks (%)	Loss during 5-10 (%)
MB	Major Fungi	11.19	14.55	3.36
	Total Fungi	12.53	14.63	2.10
TG	Major Fungi	13.34	19.77	6.42
	Total Fungi	2.23	11.35	9.13
DI	Major Fungi	22.55	26.93	4.38
	Total Fungi	20.07	26.46	6.39
QF	Major Fungi	32.02	33.19	1.17
	Total Fungi	3.82	27.41	23.59

Chapter 5

5. Discussions

In forest ecosystems, litter production and decomposition are two important processes which are affecting the overall health of forest ecosystems (Lalnunzira & Tripathi, 2018) and their ability to provide goods and services to the society (Comín, 2010 Chazdon, 2014, Lewis et al 2015). These two processes are profoundly affected by number of factors, for example, environment factors (i.e. precipitation, temperature), forest composition, soil physico-chemical properties, and micro-biota. Variations in forest composition within a specific region due to topographic condition are strongly linked to decomposition rate, soil properties and microbial compositions in forest soil (Chowlani et al 2019). Tropical forest are mostly comprises of deciduous type of tree species while broadleaf semi-evergreen tree species confine to sub-tropical forest. Such variation in forest composition affects litterfall and nutrient turnover in forest. Litter production and decomposition have been studied in natural and different age forest group in Mizoram (Lalnunzira & Tripathi, 2018). However, information of litterfall and litter decomposition in two contrasting forest sites is limited and no work has been done so far in this region. This study analyzed annual above ground litter production and leaf litter decomposition (e.g. in two forests under filed conditions and laboratory microcosm) of dominant tree species litter. Further, assessed the pattern of litter nutrient

loss during the process of decomposition and studied the decomposing ability of certain major fungi in litter decomposition. The experiment findings of this study are discussed in the following major heads:

5.1 Litterfall and litter decomposition and factors affecting them in forests

The total annual litterfall was 6718.5 kg $ha^{-1}yr^{-1}$ in STF and 2114.01 kg $ha^{-1}yr^{-1}$ in TF in the present study. The amount of litter production in STF was averaged but in TF was towards the lower side of the range reported (2660 – 9640 kg $ha^{-1}yr^{-1}$) for natural and other age group of forest in tropical moist forest of Mizoram (Lalnunzira & Tripathi, 2018). Pandey, et. al., (2007) also reported 5477 kg $ha^{-1}yr^{-1}$ of annual litterfall in oak dominated forest in Manipur. Recent study on four different agroforestry tree species in western Himalaya has reported an average litterfall of 2190 kg $ha^{-1}yr^{-1}$ (Singhal, et. al., 2019) which is slightly more litter accumulation from TF site. Litter production in TF site was considerably low compare to finding from different forest reported by various researchers. The site (TF) was dominated by natural bamboo *Melocanna baccifera*, from last decade local villager extract young bamboo shoot from this site for selling as vegetable, due to heavy extraction of young shoots, bamboo culms reducing day by day as mature and old culms are getting die. Extremely low litterfall in the TF is related to extraction of bamboo for the regular consumption for fulfilling the dietary demand of the local population which does not allow the regeneration of young

shoots. At present situation this is the major threat to bamboo dominated forest in this region.

Decomposition was faster in initial few months (June – Aug) followed by slower decomposition in remaining months. Similar trends have been reported by the different authors from different forest ecosystems over the world (Tripathi and Singh 1992 a & b; Pandey et al. 2007; Bohara, et. al., 2019). Recently, decomposition of early stage maize litter in semi-arid cropland has been reported as rapid decomposition during initial period (Hou, et. al., 2019). Similarly, in *Tephrosia candida* litter rapid mass losss was reported during initial two months in shifting cultivation area of Mizoram (Wapongnungsang et. al., 2017). Such rapid mass loss in initial stage of litter decomposition is mainly the result of microbial degradation of easily decomposable labile substances like sugar, starch which are the preferred source of food for the microorganisms (Aerts & Chapin, 2000).

At the end of the study, about 30 – 45% of litter mass was remaining in STF whereas, no litter mass was remains in TF site at the end of the experiment as the most of the litter mass lost in the initial 120 days of litter decomposition. Similar finding of STF site was reported in *Tephrosia candida* leaf litter where 35-43% litter mass remain at the end of 1 yr decomposition in shifting area of Mizoram (Wapongnungsang et. al., 2017). Recent study in western Himalaya (agroforestry species) for 1 yr leaf litter decomposition in four species finds that about 13% (*Grewia optiva*), 35% (*Ficus roxburghii*) and 11% (C. *australis*) litter mass was remained (Singhal, et. al., 2019). In

tropical environments the decomposition has been reported to high compared to the subtropical environments because of favorable climatic conditions (Tripathi and Singh 1992 a&b; Wapongnungsang et. al., 2017; Hou, et. al., 2019).

At the end of the experiment, annual decay constant (k) rate ranged from 1.6 – 1.8 in STF and 2.2 – 2.23 in TF. The k value of the present study was considerably higher compared to understory dwarf bamboo (*Sasa kurilensis*) leaf litter decomposition in young secondary forest of northern Japan (Tripathi, et. al., 2006). Recent finding in four agroforestry tree species in western Himalaya reported k value in *C. australis* (2.3) and *G. optiva* (2.12) (Singhal, et. al., 2019), which were similar to those of the k value obtained for TF site in the present study. The authors have further reported k value of two other species like *Bauhinia variegate* (1.64) and *Ficus roxburghii* (1.05) which were either similar or little less the k value obtained in STF site. Variation in litter mass loss was affected by litter species, vegetation composition in forest and soil 'microbes' activities (Anderson, et. al., 1983).

Initial crude fibre concentration in various litter components was ranged between 18 to 29%, and initial lignin (ADF) concentration ranged from 3.3 – 6.7% which was lower than *Betula emanii* (26.5%) and *Sasa kurilensis* (27.5%) from young secondary forest in Northern Japan (Tripathi, et. al., 2006). Carbon concentration in different initial litter components was between 37 and 48% which was considerably higher compared to report of *Tephrosia candida* (30 – 37%) in different fallow period of Mizoram (Wapongnungsang, et. al., 2017). Similar reports are available from the *Betula emanii*

(45.1%) and *Sasa kurilensis* (38.3%) young secondary forest of Northern Japan (Tripathi, et. al., 2006). The value of initial N ranged between 0.9 and 2.2% which is similar to finding of Tripathi, et. al., (2006) in *Betula emanii* (1.5%) and *Sasa kurilensis* (0.9%). However, the same was lower compared to report of *Tephrosia candida* (1.8 – 2.8%) in Mizoram (Wapongnungsang, et. al., 2017). Average C/N ratio ranged from 35 – 45, whereas, it was remarkably less in MB (16) compared to the others litter. The average C/N ratio in the present study is higher compared to the reports of *Tephrosia candida* (13 – 17) in Mizoram (Wapongnungsang, et. al., 2017). Lower C/N ratio in initial litter has been reported to speed up the process of litter decomposition (Tripathi and Sing 1992 a& b; Krishna, & Mohan, 2017). Similar trend was observed in the present study as lower C/N ratio in MB (16) is much faster compared to other litter component.

In TF, litter mass lost within 12 months and so the nutrient mass lost. However, in STF, carbon and nutrient mass remaining at the end of 1 year were: C (34 – 49%), N (48 – 163%), P (55 – 255%), Na (19 – 189%), Mg (57 – 108%), Ca (46 – 61%). S mass remaining at the end of the study varied significantly with species. Increase in the amount of various nutrients during the process of decomposition suggests microbial immobilization of these nutrients in these forest ecosystems which may related to microbial metabolism that may regulated the process of nutrient cycling in these forest ecosystems (Tripathi and Singh 1992b). Majority of the nutrient contents were differ mainly due to species differ and microbial activities. The C and N remains in litter from

the present finding were considerably higher compared to value reported in *Tephrosia candida* (22 – 24% for C, 6 – 13 % for N) in Mizoram (Wapongnungsang, et. al., 2017. Among various nutrients Mg is an important micro nutrient in plant litter that can be lost easily through decomposition (Anderson & Ingram, 1983). Depends on factors like soil types, environment and topography and others soil microbes, nutrients recycling in the forest ecosystems differ considerably (Esperschutz, et. al., 2011).

In the present study, majority of soil physico-chemical properties such as C, N, C/N ratio, P_2O_2 and MBC in STF site have higher value than TF. As it plays important role during litter decomposition, among them soil pH and bulk density affects nutrients permeability and other microbial activities in litter decays (Cuevas & Medina, 1986). Other physico-chemical properties (MBC) affect microbial activities in soil (Akpor, et. al., 2006).

Soil microbial diversity in present study show that fungi and actinomycetes population are more in TF but bacterial population are considerably higher in STF site. Soil microbes (fungi, actenomycetes and bacteria) are known for breaking down of litter (Dilly, et. al., 2004). In both sites, bacterial counts were higher compared to other microbes (fungi and actenomycetes). Similar trends have been reported by (Bridge & Spooner, 2001). In initial stage of decomposition, bacteria are more active in breaking labile substances, later fungi help in decaying complex substances (Kjoller&Struwe, 1992). Soil microbes' activities depend on various factors like temperature, moisture and litter substrate quality (Hobbie, 1996).

5.2 Litter decomposition under laboratory microcosm

In present laboratory microcosm study on decomposition of various leaf litter showed rapid decay in initial stages (up to 40 days) then slowly decreases till end of the recovery period. Similar trends have been reported in the decomposition of various litter (Güsewell & Gessner, 2009; Munthali, et. al., 2013). Rapid mass loss in initial stage is mainly presence of labile substance (e.g, amino acid, sugar and soluble phenolic) which are easily degraded by microbes (Wang, et. al., 2004). Decomposition becomes slower after completion of labile breakdown which mainly remains of cellulose and others recalcitrant substances (e.g. lignin, pectin, polyphenols and waxes) which takes times to break down (Pandey, et. al., 2007).

The annual decay constant (k) value (1.7– 1.9) was within value of present field study (1.6 – 2.2) but higher as compare to reports from field (0.012) sites (Loría-Naranjo, et. al., (2019). Torreta& Takeda, (1999) reported lower k value (0.99–1.05) while higher value (2.32 – 2.88) was reported from shifting cultivation of Mizoram area, compared to the present finding by Wapongnungsang, et. al., (2017).

In litter decomposition, among various factors, soil temperature and moisture was significantly important, higher soil temperature with low moisture content generally slow in litter decomposition (Martius, et. al., 2004; Hobbie, et. al., 2010, Paudel, et. al., 2015).

In present study, there is increase mass remaining of N at the end of recovery days in some litter types, reflect microbial immobilization of N which has been reported

in *Quercus serrata* and *Pinus densiflora* by Salamanca, et. al., (1997). According to Albers, et. al., (2004) initial content of low N in litter has significant effect on litter during decomposition. At present study, C content decreases in soil as well as litter in both sites except in TF site where soil C content increases progressively till the end as a result of slow release of carbon to the soil during the process of litter decomposition by by microbes.

In initial, soil microbial population counts was significantly low in microcosm compared to field experiments which could be due to changes in temperature and moisture from natural forest floor to that in microcosm. As temperature and moisture are important factors for microbial activities (Pandey, et. al., 2007; Xuluc-Tolosa, et. al., 2003). Soil C/N ratio in both STF sites fluctuated from time to time. During decomposition, soil microbes also depend on C/N ratio of litter. As bacterial count noticed low litter C/N while higher fungi reported in litter having high C/N ratio (Hodge, et. al., 2000). Bacterial CFUs were numerously higher with high litter C/N ratio study of litter in microcosm (Güsewell & Gessner, 2009). Litter decomposition is mainly depending upon litter quality and soil moisture with optimum temperature present in forest soil.

5.3 Role of abiotic factors in leaf litter decomposition

In forest ecosystems, many reports indicated that litter decomposition process is strongly affected by factors like abiotic, biotic, litter substrate quality (Tripathi & Singh, 1992 a & b; Pandey, et. al., 2007; Zhang, et. al., 2008; Zhou, et. al., 2008; Paudel, et. al., 2015). Among the abiotic factors, temperature is key factors that determine the rates of litter decomposition (Meentemeyer, 1978; Kirschbaum, 2000). As temperature rises, soil microbial activity increases with certain moisture content (Kirschbaum, 1995). The effect of temperature in litter decomposition is due to its influence on soil microbial growth and activities (Kravchenko Irina, et. al., 2019). Cellulose decomposition in litter was higher between 35–37°C but can also occur below 5°C and more 35°C (Kravchenko Irina, et. al., 2019). Soil temperature also affects the rate of mineralization in litter more than the humidity (Schlesinger, 1984). However, impact of long-term effects of global warming on soil nutrients and organic matter are not fully understood (Kirschbaum, 2006; Hartley, et. al., 2008), which needs to be taken care through well planned experiments.

In addition, other factors such as soil properties, litter quality and microbial activities also regulate the process of decomposition (Fioretto, et. al., 2003). During litter decomposition, soil physico-chemical characteristics significantly affect the decomposition, for example, soil texture is one of the important determinant which stimulates nutrient, water dynamics and other properties such as soil pH, permeability, porosity (Coleman, et. al., 1999). However, temperature and soil moisture along with

litter quality predict decomposition rate in forest ecosystem (Berg & Staaf, 1980; Uma, et. al., 2014; Mc Claugherty & Berg, 1987; Taylor, et. al., 1989; Tripathi, 1992). From seasonal variation it was reported that litter decomposition rate was higher during raining season than summer (Singh, et. al., 1999). Seasonal variation in climate regulate the rate of decomposition that affect the population of soil microbial diversity in tropical forest (Tripathi, 1992; Arunachalam, et. al., 1997).

5.4 Impact of soil microbes on litter decomposition

In forest, litter decomposition is affected by various microbes (bacteria and fungi) present in forest floor (Swift, et. al., 1979; Sridhar, et. al., 2013). Among them fungi play a critical role as 70% of litter in the forest litter have been reported to be degraded by this group of microbes (Aponte, et. al., 2010 a, b; 2012). Studies have investigated and compared the difference in the decomposition abilities of various fungi. However, the profile and structure of decomposing fungal communities in forest litter and the frequency of occurrence of fungi have been reported as key factors in the process (Osono & Takeda, 2002). In the present study, a group of fungi were inoculated in the litter under laboratory condition to examine the rate of litter decomposition. Many researcher have reported importance of fungi in litter decomposition but a very few have study on particular fungi for specific litter types. More study was needed to understand role of individual target fungi for the decomposition of particular litter species.

5.5 Importance of litter substrate quality in decomposition

The quality of the leaves as a food source for microbial decomposers is another important factors which responsible for the decomposition of forest litter. Reports show that fungi participate in different ways in the decomposition of litter (Fu-giang, et. al., 2004; Song, et. al., 2005). Researchers found that decomposition of leaf litter can be predicted by the C: N ratio (Taylor, et. al., 1989), by initial lignin content (Meentemeyer, 1978; Taylor, et. al., 1989; Tripathi, 1992; Tripathi & Singh, 1992), or initial lignin:nitrogen ratio (Melillo, et. al., 1982). Several studies reveal variation in decomposition rates among species (Adams &Angradi, 1996; Cornelissen, 1996). However, litter quality and quantity can change population and activities of soil microbes (Sayer, et. al., 2006; Chen, et. al., 2014). In lignin, phenylpropanoid polymer has a highly complex and variable structure. Its chemical determination is consequently not easy and different methods may lead to different results. Because it constitutes a barrier preventing decomposition of cellulose, lignin content of litter has been reported to control litter decomposition rate (Melillo, et. al., 1982; Stump & Binkley, 1993.The present studies shows fluctuation of nutrient concentration with decomposition time. C and N mass remaining was decreased at the end of decomposition in both field and laboratory microcosm whilst other nutrient concentration differed with litter species and decomposition days.

Chapter 6

6. Summary and conclusions

In forest ecosystem, litter production and decomposition represents a major pathway of nutrient cycling, which are affected by group of factors such as climate, edaphic and microbes. Among the various factors, temperature, precipitation and soil moisture have been found to significantly affect litterfall and litter decay. These factors activate microbial activities that help in decomposition of forest litter. In addition, litter quality and soil property have also been found to strongly influence the rate of litter decomposition. Litter quality such as carbon, nitrogen and C/N ratio helps in providing energy for microbes during different stages of decomposition. Generally, litter containing higher nitrogen in warm and humid condition shows higher decomposition rate than litter with less nitrogen content in cold sites.

Mizoram is a hilly state with wide variations in altitude and topography that considerably affects vegetation types (species composition and their specific traits), environmental conditions and soil micro-biota. The present study provides information on the important regulatory factors responsible for decomposition and nutrient release in two contrasting forests in Mizoram. Laboratory microcosm gives information on various factors related to litter decomposition of the two forest sites under control environment.

And also, the decomposing ability of filamentous major fungi on dominant tree species litter of two sites.

During the present study, annual rainfall was 2958.3 mm in STF and 3083.7 mm in TF with mean annual soil temperature of 19.3 ^{0}C in STF) and 23.17^{0}C in TF site. In STF, RF has significantly strong negative correlation (R= - 0.72) with decomposition time at $p < 0.01$ level. And also significantly ($p < 0.05$) strong negative correlation was observed between ST and decomposition days. On other TF site, both RF and ST moderately negative correlation with decomposition days.

Result of field experiment reveals that soil physico-chemical properties from both forest site shows majority of soil properties (i.e. % C, % N, C/N ratio, P_2O_2 and MBC) in STF site have higher values compared to TF site. The value of C and N, C/N ratio, P and MBC concentration was higher in STF site compared to TF. Soil pH was found to be more acidic in STF than TF. Soil microbial population count (CFUs) reveals that fungi and actinomycetes diversity higher in TF compare to STF but bacterial count was higher in STF sites.

The total annual litter production recorded on monthly basis for a year was 6718.53 kg $ha^{-1}yr^{-1}$ in STF which was more higher than the litter production of TF (2114.01 kg $ha^{-1}yr^{-1}$) sites. In both forest litter production was average compare to the finding of other tropical and sub-tropical forest litter production. In leaf litter decomposition, about 33 – 45 % of litter mass remains after end of 12 months retrieval in STF site but in TF site litter mass were decomposed after 6 months of litter placed on

the site, this might be due to temperature, humidity and microbial activities in TF site which was significantly higher compared to other site. The annual decay rate was higher at TF site (2.2 – 2.23) compared to STF (1.6 – 1.8).

Various concentration of CF, Lignin compound and other nutrient present in initial content influences leaf litter mass loss. Amount of CF and lignin contents ranged between 18 to 30 % and 3 to 7% respectively in different leaf litters. A maximum CF and lignin content was observed in M2 and minimum in TG. C concentration was ranged from 37 – 48%, maximum C content was observed in TG and minimum in MB. For N content, value varies from 0.9 – 2.2% and maximum N concentration observed in MB while minimum in DI. Highest C/N ratio was observed in TG followed by DI and minimum in MB with the value of 16.7. Highly significant correlation was observed between various initial litter properties and different litter components at $p < 0.01$.

In laboratory microcosm, the litters selected from TF site (MB, TG and M2) shows faster decomposition than STF (DI, QF and M1). Annual decay constant (k) rate was ranged from 1.2 – 1.7 (STF) and 1.9 – 2.8 (TF). Maximum decay constant (k) rate was observed in MB followed with TG and minimum was observed in QF. The soil N content at the end of litter recovery ranged from 42 – 185%. In STF site, N concentration was 42 - 44% whereas for TF site was 162 - 185%. The concentration of C in the soil was 40 - 47% and that of TF was 187 - 230%. Both N and C in STF soil was considerably lower compare to TF site, and C/N ratio was considerably higher in all litter deposited soil. In litter, N and C/N ratio changes during the course of

decomposition which were significantly positively correlated ($p \leq 0.05$). C/N ratio in litter decreases from 15 days till 180 days in all litter component. In soil, C and N decreases till 75 days after that increase gradually till the end of the experiment. C/N ratio in litter decreases when decomposition days increase. Others nutrients (P, Na, Mg, K, Ca, Mn, Cr, Ni, Cu, Zn, Pb, As, Al, S and Fe) differ among species and recovery days in both soil and litters.

Major finding from decomposition ability of filamentous fungi was that most of the major fungi selected from two sites of Mizoram belongs to Ascomycota (*Phoma spp., Cladosporium spp., Aspergillus niger, Myrothecium verrucaria, Tricodermaviride, Periconiamacrospinosa, Penicilium spp.* and *Chaetomium spp.)* and zygomycota (*Rhizopus spp.* and*Mucos spp.*). Mass remain were higher in both DI and QF in the first 5 weeks later there was changes after early stage. The remaining mass of QF (major fungi) was higher than total fungi of the same leaf species. This shows that the total fungi in later stage decomposed more rapidly after 5 weeks. During the study, the overall mass loss was range from 10 % – 90 %, maximum mass loss was observed in later stage. The remaining of ADF was range from 2.23 – 32.02 at the end of 10 weeks, after 5 weeks the value was range from 11.35 – 33.19. The maximum ADF in major fungi and lower in total fungi may be due to insist of leaf litter by more fungi in total fungi group. The uses of ADF in leaf litter by fungi diversity depends on availability of soluble matters such as the amount of nitrogen and nutrient content differ on leaf that influences the ability of the fungi to decompose in forest floor

In conclusion, the finding of the study demonstrates the pattern of litter production and decomposition of leaf litter in two different forest environments. Litter decomposition is important in the performance of forest ecosystems. Through litter decomposition, carbon and nutrients like C, N, P and others macro- and micro- nutrients were recycled in forest ecosystem. As litter decomposition is a complex process, various soil physico-chemical, environment, plant composition and microbial factors are responsible for decomposition that varies from ecosystem to ecosystem. Apart from natural forest ecosystem, microcosm provides information on changing pattern of litter quality and decomposer organisms on the mass loss rates in two forest litter types under controlled laboratory conditions. Both field and laboratory microcosm showed that litters from TF (MB, TG and M2) sites decompose faster as compare to STF sites. This reveals that litter substrate quality and soil microbes are important factors responsible for litter decomposition. Changes in microbial population bacteria and fungi during the different stages of decomposition and their role in litter decomposition has significant potential in regulating the soil carbon stock in tropical forest ecosystems. Among various factors of litter decomposition, fungi play a major role in breaking down of lignin and holocellulose. Rate of decomposition also depends on fungal species and types of litter as we can observe it from the use of same fungi for decomposition of different leaf litter in tropical forest which will help in the study of soil nutrients dynamic in forest ecosystem.

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PHOTOPLATES

a) Leaf litter sample collection

b) Litter sample weight

c) Nylon litter bag

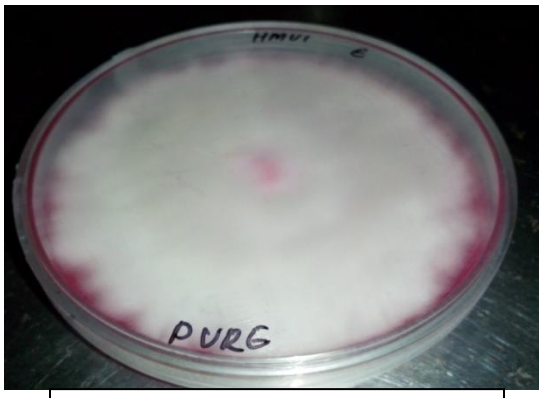
d) Microcosm experiment setup

e) Litter trap and litterfall collection

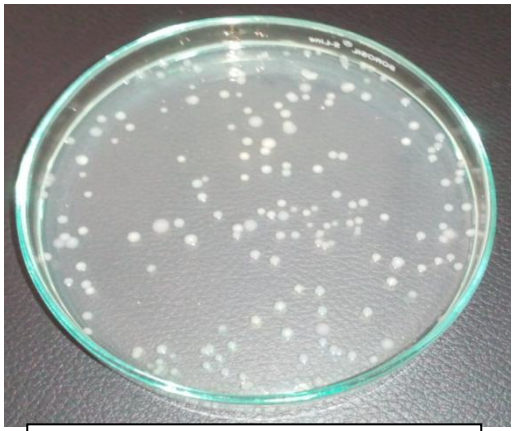
f) Litter after retrieved

g) Litter disks preparation for autoclave

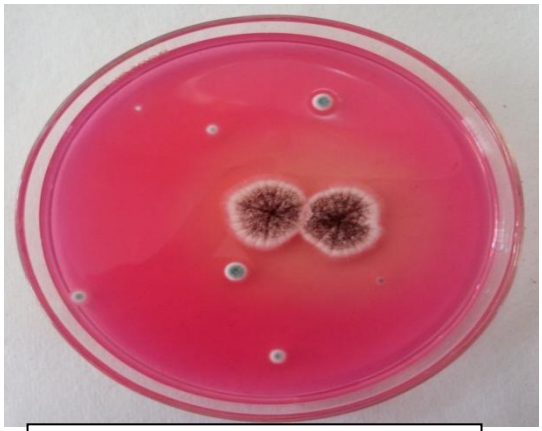
h) PDA media with litter disks



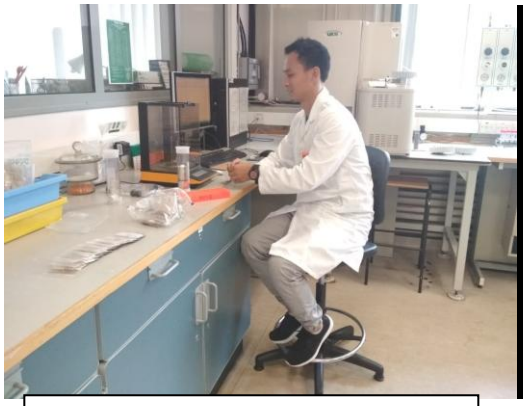
i) Sub-culture of major fungi



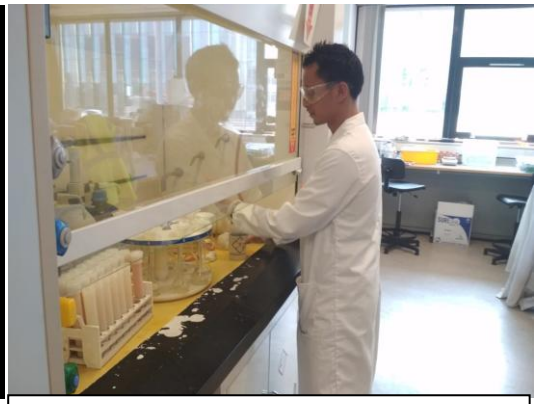
j) Bacterial population counts



k) Fungi population counts



l) Preparation of samples



m) Extraction and digestion of samples

n) MMU Staff (UK)

o) Visiting field site with supervisors

PARTICULARS

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DEGREE	: Doctor of Philosophy
DEPARTMENT	: Forestry
TITLE OF THESIS	: Leaf Litter Decomposition in Tropical Moist Deciduous and Sub-Tropical Evergreen Forests of Mizoram
DATE OF ADMISSION	: 10.8.2013

APPROVAL OF RESEARCH PROPOSAL:

1. BOS	: 16.10.2014
2. SCHOOL BOARD	: 30.10.2014
REGISTRATION NO & DATE	: MZU/Ph.D./696 of 30.10.2014
EXTENTION (IF ANY)	: NO

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ABSTRACT

LEAF LITTER DECOMPOSITION IN TROPICAL MOIST DECIDUOUS AND SUB-TROPICAL EVERGREEN FORESTS OF MIZORAM

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ABSTRACT

In forest ecosystem, litter production and decomposition represents a major pathway of nutrient cycling, which are affected by group of factors such as climate, edaphic and microbes. Among the various factors, temperature, precipitation and soil moisture have been found to significantly affect litterfall and litter decay. These factors activate microbial activities that help in decomposition of forest litter. In addition, litter quality and soil property have also been found to strongly influence the rate of litter decomposition. Litter quality such as carbon, nitrogen and C/N ratio helps in providing energy for microbes during different stages of decomposition. Generally, litter containing higher nitrogen in warm and humid condition shows higher decomposition rate than litter with less nitrogen content in cold sites.

Mizoram is a hilly state with wide variations in altitude and topography that considerably affects vegetation types (species composition and their specific traits), environmental conditions and soil micro-biota. The present study provides information on the important regulatory factors responsible for decomposition and nutrient release in two contrasting forests in Mizoram. Laboratory microcosm gives information on various factors related to litter decomposition of the two forest sites under control environment. And also, the decomposing ability of filamentous major fungi on dominant tree species litter of two sites.

To understand leaf litter decomposition among different elevation sites, two forests (Hmuifang and Sairang) were selected in Mizoram which have elevation

difference of ~1350 m amsl. Leaf litter comprises of two dominant tree leaf species and a mixed leaf litter from each forest site was used to determine mass loss and various nutrients release in forest soil. Nylon litter bag technique was used for performing litter decomposition in forest field as well as in laboratory microcosm. 10 major fungi (5 from each forest site) were isolated based on frequency to understand the ability of these fungi on leaf litter (dominant tree leaf species) decomposition.

For litterfall, litter traps are made of nylon mess (50cm x 50cm length with 15cm height), a total of 20 litter traps (10 traps in each forest site) were placed randomly at a distances of 15 to 25 m away from each plots. Nylon litter bag technique was used for performing litter decomposition in forest field as well as in laboratory microcosm. About 10 g of air dried leaf litter was enclosed in nylon litter bags (15 × 15cm with 1-mm mesh). For each forest site, a total of about 220 bags (two dominant trees leaves and a mixed leaves) was prepared and placed randomly at six locations (15 – 25 m distances between plots).

For laboratory microcosm experiment, microcosm was made from polyvinyl chloride (PVC) pipe (12cm in diameter and 16cm in height) fitted with a screw lid and a sealed bottom and a bottom pierced with l-cm diameter hole allowing excess water to be drained off. Soil mixture consisting of mineral soil collected from MZU campus and surface organic horizon (3: 1) collected from the two forest site. About 5g of air dried yellowish senescent mixed leaves and two leaf species litter (90 bags from each site) rewetted for 1h in distilled water before enclose to microcosm. Then hand held mist

sprayer used for maintaining soil moisture. The replicated samples were recovered on 15, 40, 75, 120 and 180 days after experimental setup.

To study decomposing ability of major filamentous fungi, fermented leaf litter collected from the forest floor were cut into small pieces, 5 pieces were placed on PDA (Potato Dextrose Agar) plates containing 0.08% of penicillin and chloramphenicol. A total of 200 pieces were deposited in 40 PDA plates from each forest site. The plates were incubated at 25^0C in the dark and check every day for fungi growth (total fungi group). Five dominant fungi (i.e. each site) were selected based on highest frequencies of occurrence from litter and sub-culture onto other petridishs (PDA media) for pure culture and its identification. These five dominant fungi were inoculated in a petridish and marked as major fungi. Leaf samples were disked (2.0 cm in diameter) with a metal borer. Disks were dried 36 h at 60^0 C to constant weight and weighed to obtain the original weight of the leaf disks. The sterilized leaf (autoclave) disks were place to inoculated petridish (with the major fungal colonies) and other disks to petidish (inoculated to total fungal colonies which were placed on petridishs) and uninoculated dishes served as control. A total of 100 leaf disks (25 disks from each tree species including control) were incubated at 27^0 C and 90% humidity in the dark. After incubation, leaf litter was removed carefully than cleaned with a small brush and sterilized water, after that it was oven dried for 36 h at 60^0 C and weighed. The original mass was estimated with the same method before inoculation. Decomposing ability of the group of major filamentous fungi was evaluated according to weight loss of litter

(%). Samples (decomposing material) were harvested after 5^{th} week (day 35 - marked as early stage) and 10^{th} week (day 70 - marked as later stage) of litter decomposition.

During the present study, annual rainfall was 2958.3 mm in STF and 3083.7 mm in TF with mean annual soil temperature of 19.3 ^{0}C in STF) and 23.17^{0}C in TF site. In STF, RF has significantly strong negative correlation (R= - 0.72) with decomposition time at $p < 0.01$ level. And also significantly ($p < 0.05$) strong negative correlation was observed between ST and decomposition days. On other TF site, both RF and ST moderately negative correlation with decomposition days.

Result of field experiment reveals that soil physico-chemical properties from both forest site shows majority of soil properties (i.e. % C, % N, C/N ratio, P_2O_2 and MBC) in STF site have higher values compared to TF site. The value of C and N, C/N ratio, P and MBC concentration was higher in STF site compared to TF. Soil pH was found to be more acidic in STF than TF. Soil microbial population count (CFUs) reveals that fungi and actinomycetes diversity higher in TF compare to STF but bacterial count was higher in STF sites.

The total annual litter production recorded on monthly basis for a year was 6718.5 kg $ha^{-1}yr^{-1}$ in STF which was more than five times litter production of TF (2114.01kg $ha^{-1}yr^{-1}$) sites. In both forest litter production was average as compare to the finding of other tropical and sub-tropical forest litter production. In leaf litter decomposition, about 33 – 45 % of litter mass remains after end of 12 months retrieval in STF site but in TF site litter mass were decomposed after 6 months of litter placed on the site, this might be due to temperature, humidity and microbial activities in TF site

which was significantly higher compared to other site. The annual decay rate was higher at TF site (2.2 – 2.23) compared to STF (1.6 – 1.8).

Various concentration of CF, Lignin compound and other nutrient present in initial content influences leaf litter mass loss. Amount of CF and lignin contents ranged between 18 to 30 % and 3 to 7% respectively in different leaf litters. A maximum CF and lignin content was observed in M2 and minimum in TG. C concentration was ranged from 37 – 48%, maximum C content was observed in TG and minimum in MB. For N content, value varies from 0.9 – 2.2% and maximum N concentration observed in MB while minimum in DI. Highest C/N ratio was observed in TG followed by DI and minimum in MB with the value of 16.7. Highly significant correlation was observed between various initial litter properties and different litter components at $p < 0.01$.

In laboratory microcosm, the litters selected from TF site (MB, TG and M2) shows faster decomposition than STF (DI, QF and M1). Annual decay constant (k) rate was ranged from 1.2 – 1.7 (STF) and 1.9 – 2.8 (TF). Maximum decay constant (k) rate was observed in MB followed with TG and minimum was observed in QF. The soil N content at the end of litter recovery ranged from 42 – 185%. In STF site, N concentration was 42 - 44% whereas for TF site was 162 - 185%. The concentration of C in the soil was 40 - 47% and that of TF was 187 - 230%. Both N and C in STF soil was considerably lower compare to TF site, and C/N ratio was considerably higher in all litter deposited soil. In litter, N and C/N ratio changes during the course of decomposition which was significantly positively correlated ($p \leq 0.05$). C/N ratio in litter decreases from 15 days till 180 days in all litter components. In soil, C and N decreases

till 75 days after that increase gradually till the end of the experiment. C/N ratio in litter decreases when decomposition days increase. Others nutrients (P, Na, Mg, K, Ca, Mn, Cr, Ni, Cu, Zn, Pb, As, Al, S and Fe) differ among species and recovery days in both soil and litters.

Major finding from decomposition ability of filamentous fungi was that most of the major fungi selected from two sites of Mizoram belongs to Ascomycota (*Phoma spp., Cladosporium spp., Aspergillus niger, Myrothecium verrucaria, Tricoderma viride, Periconia macrospinosa, Penicilium spp.* and *Chaetomium spp.)* and zygomycota (*Rhizopus spp.* and *Mucos spp.*). Mass remain were higher in both DI and QF in the first 5 weeks later there was changes after early stage. The remaining mass of QF (major fungi) was higher than total fungi of the same leaf species. This shows that the total fungi in later stage decomposed more rapidly after 5 weeks. During the study, the overall mass loss was range from 10 % – 90 %, maximum mass loss was observed in later stage. The remaining of ADF was range from 2.23 – 32.02 at the end of 10 weeks, after 5 weeks the value was range from 11.35 – 33.19. The maximum ADF in major fungi and lower in total fungi may be due to insist of leaf litter by more fungi in total fungi group. The uses of ADF in leaf litter by fungi diversity depends on availability of soluble matters such as the amount of nitrogen and nutrient content differ on leaf that influences the ability of the fungi to decompose in forest floor

In conclusion, the finding of the study demonstrates the pattern of litter production and decomposition of leaf litter in two different forest environments. Litter decomposition is important in the performance of forest ecosystems. Through litter

decomposition, carbon and nutrients like C, N, P and others macro- and micro- nutrients were recycled in forest ecosystem. As litter decomposition is a complex process, various soil physico-chemical, environment, plant composition and microbial factors are responsible for decomposition that varies from ecosystem to ecosystem. Apart from natural forest ecosystem, microcosm provides information on changing pattern of litter quality and decomposer organisms on the mass loss rates in two forest litter types under controlled laboratory conditions. Both field and laboratory microcosm showed that litters from TF (MB, TG and M2) sites decompose faster as compare to STF sites. This reveals that litter substrate quality and soil microbes are important factors responsible for litter decomposition. Changes in microbial population bacteria and fungi during the different stages of decomposition and their role in litter decomposition has significant potential in regulating the soil carbon stock in tropical forest ecosystems. Among various factors of litter decomposition, fungi play a major role in breaking down of lignin and holocellulose. Rate of decomposition also depends on fungal species and types of litter as we can observe it from the use of same fungi for decomposition of different leaf litter in tropical forest which will help in the study of soil nutrients dynamic in forest ecosystem.