IMPACT OF DIFFERENT FALLOW PERIODS AND TREATMENTS WITH NATIVE LITTER-SOIL-MICROBES ON RHIZOSPHERIC MICROBIAL DIVERSITY AND SOIL CARBON AND NITROGEN DYNAMICS IN SHIFTING CULTIVATION SITES IN MIZORAM

THESIS

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BY:

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DECLARATION

I, **H. Ramchhanliana**, hereby declared that the subject matter of this thesis entitled, "***Impact of different fallow periods and treatments with native litter-soil-microbes on rhizospheric microbial diversity and soil carbon and nitrogen dynamics in shifting cultivation sites in Mizoram***" is the record of work done by me and that the content of the thesis did not form basis for the award of any previous degree to me or anybody else, and that the thesis has not been submitted by me for any research degree in any other University/Institute.

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

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This is to certify that the thesis entitled **"Impact of different fallow periods and treatments with native litter-soil-microbes on rhizospheric microbial diversity and soil carbon and nitrogen dynamics in shifting cultivation sites in Mizoram"** submitted by **H. Ramchhanliana (Ph.D Regn. No. MZU/ Ph.D./578 of 13.05.2013)** in partial fulfillments of the requirements for the award of degree of **Doctor of Philosophy** in Forestry Department of the Mizoram University, Aizawl, embodies the record of original investigations carried by him under my supervision. He has been duly registered and the thesis presented is worthy of being considered for the award of the Doctor of Philosophy (Ph. D) Degree. The thesis or part thereof has not been submitted by him for any degree to this or any other university**.**

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List of abbreviations and symbols

%	Per cent	Mg	Magnesium
µg	Microgram	MBC	Microbial Biomass Carbon
ANOVA	Analysis of variance	MBN	Microbial Biomass Nitrogen
APA	Acid Phosphatase Activity	mg	Milligram
BS	Bulk Soil	mL	Milli Litre
BD	Bulk Density	mg kg^{-1}	Milligram per kilogram
C	Carbon	mM	Milli Mole
CFU	Colony Forming Units	N	Nitrogen
cm	Centimeter	N_{-min}	Nitrogen mineralization
cm^2	Centimeter square	NH_4^+- N	Ammonium nitrogen
CO_2	Carbon dioxide	NO_3^- -N	Nitrate nitrogen
DHA	Dehydrogenase Activity	°C	Degree Celsius
DS	Dry Season	P	Phosphorus
dw	Dry weight	PGP	Plant Growth Promoting
e.g.	*exemplia gratia*	RS	Rhizosphere Soil
FAO	Food Agriculture Organizations	RE	Rhizosphere Effect
FL	Fallow Land	SE	Standard Error
GBPHIED	Govind Ballabh Pant National Institute of Himalayan Environment and Sustainable Development	SOC	Soil Organic Carbon
g	Gram	SMC	Soil Moisture Content
h	Hour	SPSS	Statistical Package for the Social Science
HSD	Honest Significant Difference	T_L	Litter treatment
IBM	International Business Machines	T_M	Microbial inocula treatment
IVI	Importance Value Index	T_S	Soil treatment
K	Potassium	WS	Wet Season
kg	Kilogram		
LSD	Least Significant Difference		
m	Meter		

Abstract

This study assessed the effect of fallow periods and treatments (e.g. litter-T_L, soil-$T_S$and microbial inocula-T_M) on rhizosphere microbial populations, soil carbon and nitrogen dynamics and quantification of root exudates of annual plants in different fallow ages. Fallow lands of 2 years (FL-2), 5 years (FL-5) and 10 years (Fl-10) were selected from Muallungthu, Mizoram. Dominant early colonizing annual plants e.g. *Crassocephalum crepidiodes*, *Ageratum conyzoides* and *Bidens Pilosa* were selected to study variations in rhizosphere soil (RS) and bulk soil (BS).Length of fallow period had significant impact on soil physico-chemical (moisture content, pH, soil organic carbon, total nitrogen, NH_4^+ -N, NO_3^- -N, nitrification and N-mineralization rate), biochemical properties (microbial biomass C and N, acid phosphatase activity and dehydrogenase activity) and microbial populations (Bacterial and fungal) in the order FL-10>Fl-5>FL-2 (P>0.05, Kruskal-Wallis test).In contrast, the rhizosphere effect of annual plants on soil chemical and biochemical properties significantlydecreased with fallow periods. In general, soil nutrients e.g. SOC, TN, NH_4^+ -N, NO_3^- -N, nitrification and N-mineralization rate, MBC, MBN, APA, DHA, bacterial and fungal populations were considerably increased in treatment (T_L, $T_S$and T_M) compared to control. However, significant increased (P<0.05, LSD) was noted in T_L but not in T_S and T_M relative to control. The rhizosphere effect of annual plants was highest in control compared to different treatments. The rate of exudation (c) ranged from 0.32-0.97 mg C fine $root^{-1}$ day^{-1}. In terms of fallow periods, the root exudates from annual plants were highest in FL-2 followed by FL-5 and FL-10. Among the annual plants, rate of root exudation significantly increased in dominant plants of each selected site. The present study also indicates that soil nutrients were significantly (P<0.05, HSD) enhanced in RS compared to BS except in NO_3^- -N.In conclusion, T_L had significant influence on soil attributes that enhanced the microbial activity for sustainable agriculture farming mainly in nutrient deficient soil short fallow (FL-2). The rhizosphere nutrient cycling of annual plants (rhizosphere effect) in short fallow had significant impact on soil variables that speed up the re-establishment of vegetation in different fallow. The present result also indicates that root exudates play central role for enhanced RS properties and shows that plant-soil-microbe interaction had significantly influenced the rhizosphere effect on annual plants on soil variables.It can be concluded that litter treatment (T_L) and rhizosphere microbes of annual plants play major roleby altering the soil structure and nutrient availability for sustainable shifting agricultural systems.

Chapter 1

Introduction

1.1 Shifting cultivation: an overview

Shifting cultivation, popularly known as *Jhum* agriculture in Northeast India, is one of the most ancient system of farming supposed to have originated in the Neolithic period around 7000 B.C. (Borthakur, 1992; Subhramanyam and Sambamurty, 2000). It is also alternatively called as "Slash and Burn" system of cultivation. This system of cultivation is considered as the first step in transition from food gathering and hunting to food production system and is still widely practiced in moist tropical forests of the world like Africa, Latin America, Pacific, Caribbean and South East Asia. The practice is locally called as *jhum*, Bukma, Taungya, Kaingin, Lading, Poddu, Suddo Mannu, Swidden, Milpa or Tavya (Eastmond and Faust, 2006; Thomaz, 2009; Thakuria and Sharma, 2014) in different parts of the world. This is aprevalent form of agriculture commonly practiced in Northeast India. It involves slashing a piece of forest land during winter and burning the biomass *in situ* after drying and cropping for 1-3 years depending on the level of soil fertility, followed by land abandonment as "fallow" to recover the soil fertility through natural regeneration (Tripathi *et al.,* 2017). Previously fallow period was about 20-30 years which was reported to be sufficient to recover the soil fertility (Singh *et al.,* 2003; Grogan *et al.,* 2012). As long as the human population density was low and fallow cycles were long enough to restore soil fertility, this system of cultivation was ecologically balanced and economically viable (Tanaka *et al.,* 2001; Brunn *et al.,* 2009; Grogan *et al.,* 2012). However, fallow period has been considerably decreased due to increased human population that has adversely impacted the ecology and economics of the system.

1.2 Indian scenario of shifting cultivation

Shifting cultivation is a common farming system in the tropical forests of southwestern, central, and eastern India, where around 2 million people involved in this farming system approximately on 11 million hectares of land. At present, this system of farming is pre-dominant in northeast India, especially in the hill tracts. The north eastern region situated in the eastern Himalayan region is one of the 'biodiversity hotspots' of the world. However, increasing human interference and over-exploitation of natural resources are responsible for accelerated biodiversity loss (Yadav *et al*., 2013). Although it is one of the richest regions of biodiversity resources in the world, it is also the habitat of the poorest people, whose livelihoods heavily depend on those resources. In Northeast India, over 100 tribal ethnic minorities are still following this system and in certain parts of this region, it is not only practiced by tribal minorities but also by the landless people and lowland migrants. In Northeast India, out of 4.0 million hectares of net sown areas, about 1.6 million hectares area is under *jhumming* with very low average productivity. Shifting cultivation, being a labour intensive and low subsidy based farming system, provides an assured source of food security to poor people of the hills region. Therefore, in northeast India due to increase human population density and demand of livelihood security, people in this region still practice the most unsustainable form of agriculture that includes shortening of the fallow period as well as conversion of forest to permanent agricultural expansions (Yadav *et al*., 2012). Perhaps, main impact of slashing and felling in regard to sustainability is the disruption of natural nutrient cycling and acceleration of nutrient flow out of the agro ecosystem. Arunachalam, (2002) has indicated that slash and burn is a major agricultural system and expects that this system would reduce soil fertility in the long run in northeast India. While slashing and burning have been the fundamentals for *jhum* cultivation as it has

also been reported to increase faunal and microbial deterioration, soil erosion and land degradation (Nair, 1993; Marafa and Chau, 1999).

Shortening of fallow cycles in northeast India has affected the plant succession; annual species were not succeeded by long life span woody species and during a course of time, the soil seed bank was replaced with seeds of weedy shrubs. Thus, bamboo and other annual and bi-annual species dominated the abandoned site in this region (Rao and Ramkrishnan, 1989; Raman *et al*., 1998; Hauchhum and Tripathi, 2017a).

A report published by United Nations Development Programme (UNDP), (2013) indicate a gradual decline in the length of fallow periods in Nagaland which has reduced time period for recovery of its fertility ultimately affect the cultivator's annual yield who were once able to produce enough food grains for their livelihood per year. Further, this reduced fallow cycle has also been reported to increase soil erosion and disrupted the watershed area. However, plantation of *Alnus nepalensis* along with crops has long been practice in *jhum* lands of Nagaland to suffice the need for nitrogen (N) (Rathore *et al*., 2010). The mixed cropping pattern of this agroecosystem increases soil fertility and protects the crops from pest and insect attacks (Ramakrishnan, 1984). Tangjang, (2009) showed that in Arunachal Pradesh, the fallow length was reduced from 15-20 years to 8-10 years due to increasing necessity for cultivation of land.

Table 1.1 Area of fallow land in northeast India.

State	Fallow land other than current fallow (ha)	Current fallow land (ha)	Total fallow land (ha)
Arunachal Pradesh	70	40	110
Assam	50	79	129
Manipur	0	0	0
Meghalaya	155	58	213
Mizoram	181	66	247
Nagaland	101	59	160
Sikkim	4	5	9
Tripura	1	1	2
NEI total	562	308	870
All India	10,321	14,267	14588

Note: '000 hectare.
Source: Basic Statistics of North Eastern Region, 2015.

In Mizoram, slash and burn agricultural practices is common and widespread and is the foundation of livelihood support for the poor rural people. Maithani, (2005) observed that *jhumming* is the main occupation of the populace and a major source of economy. According to Maithani, (2005), there were about 58,000 families (approximately 25% population) involved in *jhum* farming; on the other hand, study carried out by GBPIHED, (2006) reported that there were 50,000 families involved in this system. However, there has been an ambiguous report on the area under *jhumming*. A report given by GBPIHED, (2008) reveals that annual area under shifting cultivation in Mizoram was 63,000 ha. On the other hand, Kumar, (2012) reported that during 2004-2005, area under shifting cultivation was 64536 ha whereas Pachuau, (2009) reported as 40,969 ha land (50% of the total cropped area) under *jhumming* during the same period (2004-05).

Table 1.2 Area under fallow land in Mizoram during the last 10 years.

Year	Fallow land other than current fallow (ha)	Current fallow land (ha)
2005-2006	1,97,192	40,969
2006-2007	1,66,078	41,465
2007-2008	1,65,981	44,947
2008-2009	1,70,850	40,089
2009-2010	1,80,800	66,023
2010-2011	1,82,262	66,607
2011-2012	1,83,115	61,188
2012-2013	1,94,031	50,380
2013-2014	1,61,132	47,073
2014-2015	1,65,368	48,151

Source: Statistical Abstract of Mizoram, 2015 published by Directorate of Economics and Statistics, Govt. of Mizoram.

1.3. Ecology of shifting cultivation

Shifting cultivation is probably one of the most misunderstood and a controversial land use system. In 1957, Food Agriculture Organization (FAO) declared this practice as the most serious land-use problem in tropical region. Arguments brought forward that this form of land use is an ecologically harmful and economically inefficient with low output-input ratio, have been inaccurate or outright wrong. Earlier, this system has been found to be the best solution for agriculture as long as human population density was low and fallow cycles were long enough to recover soil fertility. However, this argument has been vital to land use management and policy development in its affected region (Mertz, 2002, Maithani, 2005). Stability of *jhumming* primarily depends upon the length of fallow cycle that allows adequate time to revegetate abandoned land through secondary succession. Thus, the length of fallow period plays a central role in secondary forest biomass build up leading to quantitative differences in releasing soil nutrients during burning (Ramakrishnan, 1998).

In Northeast India, due to population pressure, limited land resources, land tenure system and several other anthropogenic activities, fallow cycle has substantially reduced to 2-3 years compared from 15-25 years earlier (Ramakrishnan, 1992). Reduced fallow period has

led to decreased soil fertility, increased soil erosion and reduced crop yields (Ziegler *et al.,* 2009), and posed a serious concern about food security for the farmers. Therefore, this system of cultivation with short fallow cycle (2-3 years) is considered as most unsustainable farming system resulting in large scale environmental degradation and ecological imbalance (Grogan *et al.,* 2012).

Shifting cultivation includes a continuous cycle of clear-felling and natural regeneration and thus these sites provide information on understanding vegetation succession patterns. Shifting cultivation fallows have been shown to recover faster than other abandoned agricultural lands such as pastures, agroforestry, and monoculture plantations (Ferguson *et al.,* 2001). Although fallow land may not completely return to its original state i.e. natural forest even if the disturbance is completed, but several changes have been reported to occur to reestablish fallow lands nearly equal to forest ecosystems which of course required long periods (Guariguata and Ostertag, 2001; Peña-Claros, 2003; Finegan and Nasi, 2004; Delang and Li, 2013). Earlier reports revealed that basal area of trees has been shown to increase over time and reach half the value in the adjoining mature forest within three decades in shifting cultivation sites adjoining dry forests (Kennard, 2002; Ruiz *et al.*, 2005) as well as semi-deciduous forests (Kammesheidt, 1999; Toledo and Salick, 2006). Tree density has been reported to increase with stabilization of the ecosystem at later stages of succession (Kammesheidt, 1999; Lebrija-Trejos *et al.,* 2010; Van Do *et al.,* 2010; Aweto, 2013). Plant species richness has been found to recover to mature forest in 20–40 years; however, full recovery of species composition, mainly rare species and species endemic to the region has been reported to take longer than 100 years (Saldarriaga *et al.,* 1988; Vankat and Snyder, 1991; Finegan, 1996; Van Gemerden *et al.,* 2003). On contrary, in an African tropical site where traditional agriculture practices were carried out in fallow periods of 5 to 15 years without burning, species composition and abundance in comparison with adjoining forest

recovered within 30 years (N'Dja *et al.,* 2008). It is expected that forest recovery is faster in traditional shifting cultivation landscapes than in sites with more recent types of the practice as shown by some studies (N'Dja *et al.,* 2008). The period of forest recovery following shifting cultivation is also affected by the number of cultivation cycles in a site (Lawrence *et al.,* 2005).

1.4. Changes in soil fertility during shifting cultivation

Most of the tropical soils are highly weathered, leached and often nutrient impoverished and phosphorus (P) limited. Burning of biomass during shifting cultivation is believed to be the easiest method to add and increase soil nutrient which is locked in plant biomass (e.g. P, which is often highly limited in upland humid tropical soils) and increase soil pH and cation-exchange capacity that ultimately enhance the ability of soil to retain nutrients. Certini, (2005) reported that burning leads to changes in the chemical properties of soil with regard to changes in quantity and quality of soil organic matter, soil moisture content (SMC), availability of nutrients, exchange capacity, and base saturation. On contrary, a number of soil organisms have been believed to be destroyed due to burning of vegetation (Buddle *et al*., 2006; Malmström *et al*., 2008, 2009). The fundamental principle for sustainable shifting cultivation is that nutrient removal within harvested crop and nutrient losses due to leaching and fire volatilization should not surpass plant-available nutrient inputs from nitrogen fixation and *in situ* soil biogeochemical transformations from non-available forms (e.g., P-containing compounds) (Lawrence and Schlesinger, 2001).

The biochemical and microbial properties of the soil is often suggested as an early and sensitive indicator of soil ecological pressure or restoration processes in both native and secondary ecosystems (Martyniuk and Wagner, 1978; Dick, 1994). Generally, it is supposed that alterations in microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), basal soil respiration, and metabolic quotient can explain most of the soil ecological

processes. Short-term changes in microbial biomass reveal a long-term trend of organic matter accumulation (Powlson *et al*., 1987). These parameters are closely related to primary productivity of an ecosystem (Zak and Pregitzer, 1990), responsible for functioning of the soil system. Numerous studies have been published on potential use of microbial properties as indicator of soil productivity or microbial activity (Sparling, 1997; Klose *et al*., 2004). Biochemical parameters include the soil enzyme activities, which play a vital role in regulating soil nutrient cycling. Soil enzyme activities result from activities of accumulated enzymes and from enzyme activities of proliferating microorganisms (Kiss *et al*., 1975), which are very responsive and provide immediate and precise information on small changes occurring in soil (Dick and Tabatabai, 1993). There are increasing evidences that such parameters are also sensitive indicators of ecology stress suffered by soil and its recovery period, because microbial activity has a direct influence on stability and fertility of ecosystems (Dick *et al*., 1996).

Biological input from forest litter and root exudates are thought to be the modulators of diversity and population of soil microbiota. Soil microorganisms regulate soil processes by their role in decomposing soil organic matter, nutrient cycling and stabilization of plant ecosystems (Wardle, 2002). At ecosystem level, it has been reported that the above-ground biomass and below-ground microorganisms are related through soil as a medium (Bardgett *el al.*, 2008). Thus, soil restoration in shifting cultivation must consider functioning and interactions of above-ground vegetation and below-ground microorganisms which affect the soil fertility. Further, changes in below-ground microbiota tightly coupled with the processing of soil carbon (C) and N through their control over enzyme activities (Harper *et al.*, 2005). These parameters are still poorly understood in relation to the accumulation of forest biomass, nutrient cycling and role of microbes that varies with fallow length following shifting cultivation.

Conversion of forest to shifting agriculture site results in regional climate change, loss of biodiversity, changes in species abundances, soil degradation, change in forest vegetation from primary to secondary succession and eventually to grassland (Holden, 2001). Further, conversion of land leads to soil infertility and nutrient impoverishment because of changes occur in soil processes (Grogan *et al.,* 2012). Thus, there is an urgent need to understand the mechanism by which shifting cultivation system could be transformed into sustainable way of cultivation with less environmental consequences for the future. Therefore, understanding the plant-soil-microbe interactions with different treatment under different fallow period would provide essential information for sustainable agriculture system.

1.5. Rhizosphere: an Overview

Plant covers a large area of soil in a close vicinity of the roots affected by physical, chemical and biological properties of roots. This specific zone, termed "Rhizosphere" is a narrow zone of soil around the living roots. Ever since Lorentz Hiltner, coined the term *rhizosphere* in 1904 to described the zone of bacterial activity around legume roots, there have been numerous reports of enhanced level of microbial biomass and activity in the rhizosphere soil (RS) relative to bulk soil (BS) (Lynch, 1987; Grayston *et al.,* 1996; Cardon and Whitbeck, 2007). Root induced changes are called as rhizosphere effect. These result from release of labile C from roots to soil through sloughed cells and exudates which has been regarded as important to mineral nutrition of herbaceous plants (Marschner, 1995). Rhizosphere effects are also caused by the inputs of root-derived substrate (rhizodeposition) accounting around 17% of the C fixed by photosynthesis (Nguyen, 2003; Jones *et al.,* 2004). Furthermore, uptake of water and nutrients by plant roots change SMC and nutrient availability in the rhizosphere (Marschner *et al.,* 1986). The rhizosphere zone thus functions as a hotspot of microbial activity and biochemical cycling in soils (Griffiths, 1994; Pinton *et al.,* 2007). It is a habitat for an immense interactive community of rhizotrophic

microorganisms whose activities mainly determine the physico-chemical properties of the RS. Root exudates have long been recognized as key source of energy for many microorganisms in soil and there is large number of evidence suggesting that release of soluble organic substances from roots is significantly stimulated by the presence of microbes around them (Barber and Lynch, 1977). However, information on the magnitude of rhizosphere effect and associated microorganisms in various plants and ecosystems are least studied.

Rhizosphere has been reported as poorly defined zone of soil with a microbiological gradient in which maximum changes in the population of microflora in soil is evident neighboring to root and decline with distance away from it (Newman, 1978; Bowen, 1991; Pinton *et al.*, 2001; Mukerji, 2002). The immediate surface of plant root together with any closely adhering particles of soil or debris is differentiated from it and has been called rhizoplane. Higher microbial activity in rhizosphere and rhizoplane regions is reported to be fueled by exudates released from plant roots (Bansal and Mukerji, 1996). These exudates are typically carbohydrate monomers (sugars), amino acids and organic acids, which are suitable substrates for a number of microorganisms. This makes plant roots an unstable habitat for microorganisms due to interactions of roots, soil and microbes which are frequently changing their characteristics. The compounds released by plant roots increases nutrient availability in the rhizosphere by mobilizing poor soluble mineral nutrient and changing the soil pH (Jones and Darrah, 1994), providing substrates for fast-turnover of rhizosphere microbes (Phillips *et al.*, 2012).

1.6. Changes in root exudation and soil nutrients in the rhizosphere

The quality of root exudates released by specific plants may provide a niche for occurrence of microorganisms colonizing the rhizosphere, and thus alter composition and diversity of microorganisms in the rhizosphere. These rhizosphere microbes play an

important role in ecological fitness of their host plant (Drake *et al.,* 2013). Important microbial processes that are expected to occur in the rhizosphere include pathogenesis and its counterpart, plant growth promotion as well as production of antibiotics, geochemical cycling and plant colonization (Kent and Triplett, 2012). Therefore, plant-microbe interactions may be beneficial, harmful or neutral to the plant depending on the microorganisms and plants within prevailing soil and environmental conditions (Bais *et al.,* 2006).

In forest ecosystems, it has been presumed that labile C pools are spatially and temporally variable that changes the rhizosphere effect on soil microbial properties (Grayston *et al.,* 1996; van Hees *et al.,* 2005). Enhanced microbial activity in the rhizosphere of oak and birch seedlings have been reported (Netti, 1995; Ivarson and Katznelson, 1960). Since then, there have been number of reports of enhanced rhizosphere microbial activity for tree seedlings (Norton and firestone, 1991; Bradley and Fyles, 1995; Priha *et al.,* 1999). Recently, studies on rhizosphere effects for mature trees in forest soil have been reported (Harnesmaa *et al.,* 2005, Phillips and Fahey, 2008; Zhao *et al.,* 2010). However, there is no information available in India till date on the rhizosphere effect of annual plants especially under different fallow periods following shifting cultivation. Recently, Zhang *et al.,* (2012) reported enhance microbial properties in RS of *Artemisia capillaries* and *A. sacrorum* with increased age of abandoned land in Loess plateau, China. However, there is ample scope for identifying the composition of microbial community structure and improving our understanding on soil microbial mechanisms in RS of different annual plants under different soil conditions depending on fallow length.

Conversion of primary forest to secondary forest leads to changes in biotic and abiotic factors in the rhizosphere which ultimately affect soil fertility and plant growth in these ecosystems. Further, during secondary succession, the modules for proliferation of long life-cycle species have been destroyed due to agricultural activities and stable seed pool of annual

and bi-annual plants have occupied the fallow land and developed into dominant species in these ecosystems. Therefore, changes in the biotic composition alter the abiotic condition of the soil; particularly in rhizosphere because of higher microbial activity in this region (Hartmann *et al.,* 2009; Kent and Triplett, 2012) that may lead to have profound effects on the structure and the function of derived ecosystem especially in fallows. The studies on shifting cultivation are mainly based on anthropological and social aspect, scientific studies centered on the rhizosphere effect of dominant and co-dominant annual plants in different fallow periods are not available so far. Thus, this is an ideal time for comprehensive studies on changes in key rhizosphere and BS in fallow lands following shifting agriculture in different parts of Northeast India, particularly in Mizoram where such studies are lacking. The Outcomes of the study may lead to synchronization of soil nutrient availability with that of crop nutrient demand, to enhance the crop productivity with less environmental consequence. Further, maintaining proper microbial process and activity may speed up the vegetation succession in developing the forest ecosystem under different fallow length.

1.7. Major objectives of the study

This study aims to understand the role of rhizosphere root induced changes or rhizosphere effect of different annual plants with native litter-soil-microbes amendment on different fallow lands following shifting cultivation in Muallungthu, Mizoram. Study is design to achieve the following major objectives:

i) To determine periodic changes in the rhizospheric microbial composition and diversity in different shifting cultivation sites in relation to fallow period and treatments.

ii) To quantify periodic changes in rhizospheric carbon fluxes (Carbon compounds) from dominant plant species in different shifting cultivation sites in relation to fallow periods and treatments.

iii) To determine the changes in the rhizospheric and BS carbon (organic C, microbial biomass C), nitrogen (total N, microbial biomass N, NO_3^-, NH_4^+, N-mineralization rate), acid phosphatase activity (APA) and enzyme activity in shifting cultivation sites of different allow periods with various treatments.

Chapter 2

Review of Literature

2.1 Ecological studies on shifting cultivation in the World

Shifting cultivation, a traditional farming system, adopted historically in tropical hilly regions of the World (Central America, Central Africa and South East Asia), which is commonly known as "Slash and Burn" or "Swidden agriculture" (Thakuria and Sharma, 2014). Globally, shifting cultivation is a type of land use practice with different regional names, known as *roça* agriculture in Brazil, as *Chitimene* in Zaire and Zambia, as *Ladang* in Malay and in as *Taungya* in Myanmar (Thakuria and Sharma, 2014). There are 40-50 countries worldwide (Schuck *et al*., 2002) with ~300-500 million people directly or indirectly involved in this system (Goldammer, 1988; Sanchez, 1996; Kleinman *et al*., 1996). It has been estimated that about 200 million people i.e. 7% of the world population is still practicing this system in about 300 million hectare i.e. 5% of cultivated land throughout the world (Kumar, 2008). Recent reports suggest that cultivators mostly settle in mountainous and hilly parts of Latin America, Central Africa and South East Asia (Van Vliet *et al*., 2012). Li *et al*. (2014) review that shifting agriculture is still practiced by indigenous people in SEA countries like Cambodia, Laos, Malaysia, Myanmar, Indonesia, Thailand and Vietnam. Fox and Vogler, (2005) also concluded that shifting cultivation is still a predominant agricultural system in the past half century based on studied conducted in eight locale area in montane mainland of South East Asia. Cramp *et al*. (2009) emphasized that *jhum* farming plays a key role in guaranteeing livelihood security for local cultivators against market fluctuations in South East Asia. An empirical research carried out by Vien *et al*. (2006) in Vietnam's upland highlighted that cultural practice of shifting cultivation was perceived as eco-friendly and sustainable farming as it increase the crop yield.

This system of cultivation was initially started long back (~7000 years) involving clearing and burning of forest vegetation followed by cropping for about 2-3 years and abandoned the land for ~20-30 years to recover soil fertility through natural regeneration, and the practice was considered sustainable i.e. ecologically balanced and economically productive (Brady, 1996; Inoue *et al*., 2010; Comte *et al*., 2012; Toky and Ramakrishnan, 1981; Grogan *et al*., 2012). The main philosophy of sustainable shifting cultivation in the past based on losses of plant-available nutrients correspond to new supply over the full rotational cycle by conserving all other components of soil quality (e.g., texture and organic matter content that together determine water holding capacity) (Lawrence and Schlesinger 2001; Grogan *et al*., 2012). However, in recent years, practice of shifting cultivation is characterized by shortening of fallow period (< 5 years) and as a result this practice is posing problem of degradation of natural forest, soil fertility and environment, and food security to the rural population (Grogan *et al*., 2012). The practice of shifting cultivation is reported to account for ~60 % forest losses worldwide each year (Lele *et al*., 2008).

This type of agricultural practices, in essence, is a form of exploitation of land with long term rotations, being the secondary forest one of the elements of the rotation. Boserup, (1981) classified the influence of population density on shifting cultivation (inhabitants per km^2) as: forest fallow (very sparsely population density - 0-4); bush fallow (sparsely populated - 4-16); short fallow (medium population density - 16-64) and continuous cultivation (dense population density - >64). Population density is the driving force in renovating shifting cultivation in forms of agriculture closer to permanent cultivation, and therefore, shifting cultivation require adoption of new technologies with modern inputs to sustain soil productivity (Jong *et al*., 2001). Shortening fallow in the state of Mizoram is because of medium population density of ~48 inhabitants per km^2. In a wider view, the types of shifting agriculture differ according to primary vegetation (primary forest, secondary

forest, bushes, agro-forest, meadows, pastures and savannahs), type of cultivators (indigenous communities, colonists, and settlers), final vegetation (secondary forest, pastures, permanent crops and agro-forestry, plantation crops), and the length of fallow (no fallow or continuous cycle, short fallow – 1 to 2 years; medium fallow – 3 to 8 years; long fallow - more than 8 years) (Fujsaka and Escobar, 1997).

Shifting cultivation is supposed to originate in Asian agricultural systems (Meine *et al.*, 2008). Sanchez, (1996) reported that global pioneer study of slash and burn agriculture started during the 1930's. The review published by Li *et al*. (2014) indicated that 1950 -1960 was a period for important works of anthropology with other subjects like soil science, agronomy and geography. While during 1970 to 1980, analysis of shifting cultivation expanded globally with many other disciplines like ecology and evolution, and slowly it has become a scientific debate in the humid tropical regions and many scholars highlighted the drawback of this system of cultivation than the benefits (Li *et al*. 2014). However, it still remains as a dominant agricultural system practiced in humid tropics (Van Vliet *et al*., 2012).

2.2 Ecological studies on the effect of burning on soil and vegetation

Forest fire plays an important role in tropical region during land conversion and burning of vegetation (Malingreau and Tucker, 1988, Eva and Lambin, 2000; Aragãoand et al., 2008). From the past two decades, extensive forest clearing for agricultural activities have made the current level of fire incidence in the tropical region a matter of international concern (Cochrane, 2003). Fire is a dominant manmade disturbance in slash and burn agriculture or *jhum* areas in the north eastern region. Yadav, (2013) reported that uses of fire in slash and burn agriculture system allows them to manage soil fertility and suppressed weed and insect attack. The benefits of clearing of bush debris and reduction of weed invasion that would compete with the crops for sunlight, soil nutrients and water has been reported by Babalola, (2000). The ash deposit after burning helps to increase the soil fertility by

immediate release of the locked mineral nutrients (e.g. Mg, Ca, and available P) for crops (Scheuner *et al.,* 2004; Niemeyer *et al.,* 2005). Increased soil temperatures after burning has been reported to stimulate biological activity, enhance organic matter and mineralization to increase nutrient availability (Ojima *et al.* 1994; Brye, 2006). Filho *et al*. (2013) has stated that after slash-and-burn operations all soil moisture is not lost due to the latent heat of vaporization which prevents soil temperature from exceeding 95°C where water completely vaporizes and ultimately decreases its negative impacts on the physical properties of the soil. In addition, Tripathi and Barik, (2003) has underlined that *jhum* farming helps in protecting the rich cultural diversity that exist among the *jhum* farmers of the north east India consisting of more than 200 tribes, as it is linked into their culture and tradition.

Pandey *et al.* (2011) reported that bacterial population (*B. clausii, B. licheniformis, B. megaterium, B. subtilis, B. thuringiensis, P. aeruginosa* and *P. stutzeri*) and actinomycetes population were significantly higher in soils of fired plots after 4 weeks of burning event in shifting cultivation in Papumpare district of Arunachal Pradesh, which indicated enhanced recovery of soil microbial properties. This shows that burning operations in *jhum* plots have microbiological benefits as *Bacillus* and *Pseudomonas* species play a crucial role in plant growth promotion and bio-control. Further, studies reported increase soil fertility after conversion, with an increase of P, calcium (Ca), and magnesium (Mg) that indicates that the stocks of these macronutrients are not affected (Zarin *et al.,* 1998; Frizano *et al.,* 2003; Oliveira, 2008).

Clearing forests for slash and burn farming contribute to climate change, biodiversity loss, reduced timber supply, flooding, siltation, soil degradation and change of forest vegetation from primary to secondary and eventually to grassland (Holden, 2001).Soil degradation is accountable for changing 0.3-0.8% of the world's arable land unsuitable for agricultural activities every year and an additional 200 million hectares of cultivated area

would be required over next 30 years to feed the increasing population (Den Bigelaar *et al.,* 2004; Lafond *et al.,* 2006). Clearing of forests and burning for shifting agriculture are the main reasons of deforestation (Monela and Abdallah, 2007; Zahabu, 2008), which has led to increased soil erosion and run-off mostly in hilly areas, thus the soil has become susceptible to erosion (Shoaib *et al.*, 1998; Sfeir- Younis and Dragun, 1993).

The adverse effects of slash and burn farming on the environment are well recognized scientific facts. Different scholars (biologist, foresters and conservationists) have observed the impact of shifting cultivation on biodiversity in the tropical forests (Raman, 2001). FAO (1957) discloses that shifting agriculture was identified long time ago as a threat to tropical forests. Many scholars accepted that shifting agriculture effects are very destructive (Lal and Prajapatu, 1990; Tiwari, 1991; Dwivedi, 1993). These assumptions are based on the scientific research and data conducted globally (Tawnenga *et al.*, 1997).

Tripathi and Barik, (2003) stated that the most important harmful impact of shifting agriculture is the damage it causes to soil system as it lowers soil acidity, organic matter and total C and N but it enhanced P and cations. Impacts on soil physical properties due to slash-and-burn have been reported to increase leaching process and results in decrease quantity of organic matter (McDonald *et al*., 2002; Frizano *et al*., 2003). Mamede and Araújo, (2008) has also mentioned about the decrease in diversity of seed bank due to use of fire in the conversion phase of shifting cultivation where this phenomenon could be responsible for decrease in biodiversity in these forest ecosystems. Earlier studies have reported negative effects on soil fertility and an increase in the occurrence of erosion (Brand and Pfund, 1998; McDonald *et al*., 2002).

2.3 Ecological studies on the effect of fallow period on soil and vegetation

Slash and burn farming was an economically efficient and ecologically balance agricultural practice in earlier period when village population densities were low, and the

fallow cycle was long enough to restore soil fertility (Tanaka *et al*., 2001; Bruun *et al*., 2009). However, fallow cycles have been shortened from about two decades down to 2-3 years across South East Asia and other parts of the world (Cairns and Garrity 1999; Kato *et al*., 1999; Eastmond and Faust, 2006; Schmidt-Vogt *et al*., 2009) because of increased rural population. Shortened fallow length has led to decrease soil fertility, increased soil erosional losses, and reduce crop yields (Ramakrishnan 1992; Bruun *et al*., 2009; Thomaz 2009; Ziegler *et al*., 2009).

The length of fallow period over many centuries has been found to positively affect soil fertility after cropping and thereby increase accumulation of soil nutrients, water, and organic matter (Doran *et al*., 1998; Sarmiento, 2000). Miranda *et al*. (2009) reported that 5 years fallow land improved porosity, macroporosity, and water management compared to 2 years fallow. Further, Samake´ *et al*. (2005) reported that soil organic carbon and soil nutrient (N, P, and K) concentrations significantly increased with increasing fallow period up to 7 years. Increased in soil nutrients have been attributed to decay of above-ground and root biomass of fallow vegetation and occurrence of leguminous species among the vegetation (Wapongnungsang *et al*., 2017). Previous studies also revealed gradual accumulation of soil organic matter and enhancement in other soil properties at 10 years of fallow (Areola *et al*., 1982).

The length of fallow period helps to restore soil nutrient lost during the previous cropping stage (Liang *et al*., 2009) and the fallow phase assures sufficient buildup of nutritional elements into the forest vegetation (Richter *et al*., 2000). Borggaard *et al*. (2003) also reported considerable reduction in fallow length (~3–5 years) in the Chittagong Hill Tracts area in Bangladesh bordering northeast India due to population pressure and the land has become unsustainable for cultivation of crops from recent decades. Shortening of fallow would suppress forest vegetation to recover its soil fertility and control of weeds (Rasmussen

and Jensen, 1999). Further, destabilization of the nutrient cycles of soil/plant systems, based on increase fallowing cycles, has also been presented to have negative impact on the soil system (Hölscher *et al*., 1997; Gafur *et al*., 2003; Davidson *et al*., 2007).

Soil microbes play a central role in proper functioning of agroecosystems because they serve as facilitator of essential nutrient cycling processes and are an important for the labile C and N pools in soil (Paul, 2007). Steenwerth *et al.* (2002) reported that soil microbial biomass, measured by phospholipid fatty acid content, declined sharply in soils in fallow phase of 2 years compared with adjacent cropped and grassland soils in California coastal valleys. Enwall *et al.* (2007) illustrated that bare fallow land had low potential ammonia oxidation and a different ammonia oxidizer diversity compared to cropped soils, possibly due to the reduced N-mineralization (N_{-min}) and lack of N fertilizer in the fallow soil, these results indicate that bare fallow can affect soil microbial activity indirectly through altering the soil properties such as N availability and pH. Fallow period improves the buildup of nitrate concentration through mineralization of organic matter (Smika, 1983; Campbell *et al*., 1990). Cochran *et al*. (2006) stated that during the early years of crop production in the northern Great Plains, reasonably increase supply of organic matter enhance N_{-min} by aeration with tillage and greater soil moisture content during these fallow periods.

In northeast India, it has been reported that the recovery of soil fertility is unsuccessful when fallow cycle is shorter than 10 years (Ramakrishnan, 2006). Tripathi and Barik, (2003) has also illustrated that due to shortening of fallow period, secondary succession do not acquire enough time period to regenerate and eventually gets converted into degraded wastelands. Toky and Ramakrishnan, 1981 reported that mean annual losses of major soluble nutrients due to run-off did not differ between fields of varying fallow period, but surface run-off sediment losses were 40% higher in the short fallow period. In addition, soil erosional loss, watershed siltation, and smoke problems have become serious concerns

relating to mature forest habitat destruction, biodiversity losses, and conservation issues in these regions (Singh *et al*., 2010).

2.4 Studies on vegetation succession during forest fallows

Vegetation succession during shifting cultivation was significantly affected by existing seed bank disruption and poor seed dispersal rates from surrounding forests (Kammesheidt, 1999; Vieira and Proctor, 2007; Del Castillo and Rios, 2008). In general, succession of pioneer vegetation occurred rapidly in the early stages of regeneration followed by delayed retrieval of woody biomass (Toky and Ramakrishnan, 1983; Uhl, 1987; Raman *et al*., 1998). Recovery of plant species richness has been reported to be comparable to mature forest in 20-40 years; however, recovery of forest structure and functioning has been reported to take several decades (Finegan, 1996; Van Gemerden *et al*., 2003; N'Dja *et al*., 2008).Van Gemerden *et al*. (2003) showed that endemic plant species have not fully recovered even after a fallow period of 50-60 years. On the other hand, Kennard, (2002) reported that fallow land in tropical dry forests of lowland Bolivia accumulated 75 percent of mature forest species within 5 years of active cultivation, though basal area was similar with that in mature forests only in 50-years old fallows.

Dominant plants in an ecosystem are often responsible for changes in soil properties which lead to intricate local interactions between vegetation and soil in semiarid ecosystems (Wilson and Agnew, 1992). In hilly region of the semiarid Loess Plateau, vegetation destruction caused by removal of firewood, overgrazing, and unsuitable agricultural practices together with adverse environmental and extreme climate conditions have led to permanent degradation of land, loss of soil fertility and deterioration of environment (Wang, 2002; Jiang *et al*., 2007). The main causes of vegetation destruction in this region were unsuitable agriculture practice, collection of firewood and fodder which lead to permanent land degradation (Jiang *et al*., 2007). Primarily, soil degradation is caused by in vegetation cover

due land abandonment, allowing increased erosion and decreased soil quality (Jia *et al*., 2004). The restoration of native vegetation cover in abandoned land play key role in decreasing soil erosion, mainly important for those lands with low productivity or over 20ºslope angle (Wang, 2002).

Lawrence, (2004) reported that cultivation and fallow period also affected the recovery process of vegetation succession in shifting cultivation. Author has further reported that species density and evenness waned considerably with increase in number of fallow cycle in shifting cultivation sites in Indonesia. In northeast India, shortening of fallow cycles to 2–3 years resulted in suppression of vegetation succession, since pioneer woody species were succeeded by weed species, and over time the soil seed bank was replaced with seeds of weedy shrubs (Saxena and Ramakrishnan, 1984). Fallow period with 10 years in these regions were dominated by bamboo cover (Rao and Ramakrishnan, 1989; Raman*et al*., 1998). On the other hand, bamboo being dominant and an early colonizer species may assist in soil-nutrient recovery and provide microclimatic conditions for regeneration to its relatively faster growth rates in contrast to woody tree species (Rao and Ramakrishnan, 1989; Raman*et al*., 1998; Hauchhum and Tripathi, 2017a). Vegetation succession has been interrelated with degraded soil properties (Aweto, 1981), and fallow land might have the potential to recover its soil quality by re-establishing the natural vegetation (Wang, 2002; Jia *et al*., 2004). Wang, (2002) showed that restoration of abandoned land with native vegetation regeneration through secondary succession can reinstate the soil quality, maintain fertility of soil, and improve stability of ecosystem.

2.5 Studies on plant rhizosphere

2.5.1 Rhizosphere dynamics

Rhizosphere, a thin film of soil around the roots, was first defined over a century ago by Lorentz Hiltner (Hiltner, 1904; Hartmann *et al.,* 2008) and was redefined by Pinton as the

narrow zone that includes the soil influenced by roots along with the root tissues colonized by microorganisms (Pinton *et al.,* 2001; Morgan *et al.,* 2005). The term rhizosphere is now being used in a more general sense to describe soil influenced physically and or chemically by any root system (Chanway, 2002). The rhizosphere zone is thus a unique hot spot where soil microbes are considerably stimulated around the roots, as a result of release of C-compounds by roots (Jones *et al*., 2004; Hinsinger *et al*., 2006). Depletion occurring as a consequence of the sink-effect of absorbing roots of higher plants has been observed for P (Hinsinger, 2001) and for other major nutrients such as K and NH_4^+-N, which are substantially more mobile than P in the soil (Jungk, 2002; Hinsinger *et al*., 2005). Thus, the region of soil surrounding and including the plant root is of crucial importance for plant health and nutrition (Marschner, 1995). Further, the rhizosphere zone has been broadly classified into three distinct zones by (Lynch, 1987; Pinton *et al*., 2001): endorhizosphere, rhizoplane and ectorhizosphere. Endorhizosphere may be defined as a narrow zone that consists of the root tissue including endodermis and the cortical layers. Rhizoplane is the root surface where soil particles and microbes adhere. It consists of epidermis, cortex and mucilaginous polysaccharide layer. Ectorhizosphere is a zone consists of soil immediately adjacent to the root.

Apart from these three basic zones, certain other layers may be defined in some cases e.g. in plants with mycorrhizal association, there is a zone termed as the mycorrhizosphere (Linderman, 1988) while in some other plants, strongly adhering dense layer termed as ‘‘rhizosheath’’, is found and consists of root hairs, mucoid material, microbes and soil particles (Curl and Truelove, 1986). The root itself is a part of the rhizosphere as endophytic microorganisms colonize the inner root tissues as well (Bowen and Rovira, 1999). The volume of soil which is not a part of the rhizosphere, i.e. which is not influenced by the roots is known as BS (Gobat *et al*., 2004). The dead roots are transformed in soil by rhizospheric

activity but it is different from the BS, thus, rhizosphere may be considered as a unique region distinct from BS (Gobat *et al*., 2004).

In rhizosphere, interactions of plant-microbe-soil significantly change the physical and chemical properties of RS which in turn change the microbial properties of the rhizosphere (Nihorimbere *et al*., 2011). In addition, root exudates play an important role in mediating the relationships between plant roots and microbial activity in the rhizosphere (Badri *et al*., 2009; 2013a; Chaparro *et al*., 2013). Plant roots discharge 5%-21% of their photosynthetically fixed C as soluble sugars, amino acids or as secondary metabolites (Badri and Vivanco, 2009; Badri *et al*., 2013b; Chaparro *et al*., 2013) and these provides favourable microclimatic conditions for microorganisms and stimulate its activity in the rhizosphere.

2.5.2 Changes in root exudation and rhizodeposition

The term "rhizodeposition" was first defined by Whipps and Lynch (1985) as ''the material lost from plant roots, including water-soluble exudates, secretions of insoluble materials, lysates, dead fine roots and gases like carbon dioxide and ethylene''. It was redefined as ''the organic compounds released by living plant roots into their surrounding environment'' (Whipps, 1990; Nguyen, 2003) and may also comprise the inorganic ions (Uren, 2001). Corresponding to almost 15–60 % of the total photosynthetic production of plant and results in deposition of substantial carbon and energy reserves in the rhizosphere for microorganisms (Curl and Truelove 1986; Lynch and Whipps 1990).

Rhizodeposition play a significant role in determining the rhizosphere C fluxes. Rhizodeposit is subdivided into different parts i.e. root cap cells and root tissues (sloughed root hairs and epidermal cells) (Rovira, 1956), mucilage and root exudates (Nguyen, 2003). Root exudates are the most important part of rhizodeposit and are classified into two types depending on their molecular weight (Nguyen, 2003). Plant root exudates have been grouped into two classes: low molecular weight compounds, such as amino acids, organic acids,

sugars, phenolic compounds, and other secondary metabolites, and high molecular weight compounds, such as polysaccharides and proteins (Uren, 2001; Farrar *et al*., 2003; Cheng and Gershenson, 2007). High molecular weight exudates form the second class and these are generally enzymes, proteins and mucilage (polysaccharides) (Narasimhan *et al*., 2003; Bais *et al*., 2006; Badri and Vivanco, 2009). High molecular weight exudates are more significant in terms of total mass of the root exudates but have comparatively lesser variety than the first class (Bais *et al*., 2006). In addition, quality and quantity of rhizodeposition is also affected by a number of biotic and abiotic factors associated with plant and soil (Rovira, 1956; Lynch and Whipps, 1990; Nguyen, 2003; Jones *et al*., 2004). The amount and type of root exudates is regulated by plant species, age of plants, and abiotic factors like soil type, pH, temperature, and the occurrence of microorganisms (Badri and Vivanco, 2009; Uren, 2000).

Exudates may also be divided as active and passive exudates on the basis of their role and mode of secretion from the roots (Rougier and Chaboud, 1989; Bais *et al*., 2006). The functions of passive exudates have been least understood and are diffused from the roots as basal exudation depending on the gradient (Bais *et al*., 2006). They constitute about 3–5 % of the total carbon fixed during photosynthesis (Pinton *et al*., 2001). Exudates secreted through open membrane pores of the plants is known as active exudates and they have a specific function such as lubrication and defense (Jones *et al*., 2004; Bais *et al*., 2006). Furthermore, exudates can be classified on the basis of their biological activity such as signaling molecules, phytoalexins, phytohormones, enzymes or allelochemicals (Nannipieri *et al*., 2007).

The release of soluble carbon C from plant roots play enormous importance for plant-microbe interactions in the rhizosphere for C sequestration, nutrient cycling in agriculture and forest systems and function of ecosystem (Singh *et al.,* 2004). Continual release of C from root may represent up to half of fixed C allotted to below ground (Nguyen, 2003) and

promotes substantial release of nutrients from at the ecosystem level (Finzi *et al.*, 2015). Hence, plant root becomes a major source of C in soil and a considerable amount of C fixed during photosynthesis (20-60%) can be translocated into the rhizosphere zone (Kuzyakov and Domanski, 2000). Transfer of C from plant roots to bulk soil is of importance, but is poorly understood process relative to other terrestrial C cycling system (Jones *et al.*, 2004).

Cheng and Gershenson, (2007) reported that chemical composition of the rhizodeposit is the most important factor for functioning rhizodeposition. The structure, amount and level of exudations depend on genetic factors and differ widely among different plant species and environmental conditions (Kochian *et al*., 2005). Determination of root exudates in the rhizosphere is governed by their chemical properties, their stability and the soil volume through which they diffuse (Nannipieri *et al*., 2007). They may lose their structure and properties and hence get disabled as a result of processes like adsorption, biodegradation, volatilization, chemical degradation, etc. (Nannipieri *et al*., 2007).

Exudation and plant health are mutually associated. The amount and type of exudates affects the microbial diversity including beneficial and harmful microorganisms as well as several ecological processes in the rhizosphere (Bolton *et al*., 1993; Jaeger *et al*., 1999; Paterson *et al*., 2007) which in turn influence the plant processes like the rooting patterns, nutrient availability and pathogen persistence in the rhizosphere (Bolton *et al*., 1993; Bowen and Rovira, 1999; Barea, 2000). At the same time microbial activities in the rhizosphere alter the root exudation process and pattern thus, rhizodeposition intensely influence the structural and functional aspects of microbial communities in the rhizosphere (Prashar *et al*., 2013).

2.5.3 Process of plant root exudation

Root exudation is a process by which organic compounds are released from living plant roots into the surrounding soil; it is a ubiquitous phenomenon (Jones and Darrah, 1995). Root exudation process occurs via at least two potential mechanisms, and the exudation rates

vary widely among species and environmental conditions (Kochian *et al*., 2005). Exudates are transported across the cellular membrane and released into the surrounding rhizosphere zone (Nihorimbere *et al*., 2011). Plant products are also released from roots border cells and root border like cells which separate from border as they grow (Bais *et al*., 2006). However, identification of root exudates with respect to chemical composition and concentration in the soil is very complicated due to methodological difficulties (Stolp, 1988). Efficiency of exudation process may thus be enhanced by stress factors affecting membrane integrity such as nutrient deficiency, temperature extremes, or exudation stress (Ratnayale *et al*., 1978). The proportion of C released from roots has been assessed to be as much as 50% in the young plants (Whipps, 1990) that decrease in mature plants in the field conditions (Jensen, 1993).

Plant roots release low molecular weight organic compounds to soil known as exudates mainly due to the concentration gradient between root cells and soil solution or which actively secreted in response to nutrient stress and the presence or absence of plant and microbial community (Jones *et al*., 2004; Bais *et al*., 2006;). Recent studies indicate that most exudates contained sugars, amino acid and organic acids (Badri and Vivanco, 2009) and this flux account between 5%-21% of net assimilated C (Badri *et al*., 2013b; Chaparro *et al*., 2013). Regardless the relatively minor magnitude of this flux, root exudates are considered to play vital role in regulating the soil nutrient availability in plant ecosystem due to their chelating properties that helps in stimulating microbial activity (Marschner, 1995). Further, assimilated photosynthate is the main source of deriving the exudates (Neumann and Romheld, 2001) and thus, represent a semi-continuous input of labile C to soil in contrast to transient inputs of C resulting from litter inputs (Kuzyakov and Cheng, 2001).

Plant have different strategies to release and secrete different compounds to the rhizosphere zone (Badri and Vivanco, 2009; Weston *et al*., 2012). In general, exudates from plant roots may be released via either passive (diffusates) or active (secretions) mechanisms.

Low molecular weight organic compounds are mainly secreted through passive process whereas polar and uncharged molecules are transported by direct passive diffusion, a process that relies on membrane permeability, polarity of exudates and cytosolic pH (Badri and Vivanco, 2009). Furthermore, other compounds such as secondary metabolites, polysaccharides and proteins are released with the help of membrane–bound proteins (Weston *et al*., 2012).

2.5.4 Effect of rhizosphere on soil nutrients

The impact of rhizosphere processes on plant nutrient cycling is one of the most important factors which is rather intricate to understand (Hobbie, 1992; Grayston *et al*., 1996). The rhizosphere processes have been well studied for agriculture crops and grasses under controlled environments; however, evidence on rhizosphere processes of tree species in natural conditions is inadequate (Parmelee *et al*., 1993; Kuzyakov *et al*., 2000; Jones *et al*., 2004). Plant roots may affect rhizosphere nutrient cycling through nutrient uptake, rhizodeposition and microbial which play an important role for sustaining ecosystem development mainly in nutrient-limited soil conditions (Gobran *et al*., 1998; Wang and Zabowski, 1998). Rhizosphere nutrient cycling of trees in comparison to annual plants may vary significantly owing to variation on their nutrient requirement and uptake, soil conditions as well as developmental stage (Grayston *et al*., 1996; Gobran *et al*., 1998).

Rhizodeposition is the main cause of root-induced changes (Kuzyakov, 2002; Paterson, 2003) and may also control the magnitude of rhizosphere effect on soil nutrients (Jones *et al*., 2004). Increased soil fertility due to addition of N fertilization leads to reduction of belowground C accumulation (Giardina *et al*., 2004; Phillips and Fahey, 2007) and thus reduces rhizosphere effects (Fontaine *et al*., 2011; Ai *et al*., 2012; Blagodatskaya *et al*., 2014). On the other hand, no changes (Cheng *et al*., 2003) and even positive (Phillips and Fahey, 2008) responses of rhizosphere effects to N fertilization have also been reported.

Thus, to have better predictions on rhizosphere effects and to include them into ecosystem process, better studies of rhizosphere effects under different environmental conditions is required (Perveen *et al*., 2014).

Previous studies indicated soil nutrients i.e. available N and P increased (Turpault *et al*., 2005), decreased (Wang *et al*., 2001; Chen *et al*., 2002) and remained unaffected (Parmelee *et al*., 1993; Ehrenfeld *et al*., 1997) in RS relative to BS. Plant rhizosphere generally increased C deposits (Cheng *et al*., 1996) and higher soil microbial population (Griffiths, 1994) in comparison to BS. Changes in soil pH were observed by a number of studies (Zhao *et al*., 2010; Zhang *et al*., 2012; Zhu *et al*., 2014). Changes in microbial community and biomass lead to additional alteration of microbial activity in the rhizosphere (Schimel and Schaeffer, 2012). The enzyme activities on soil organic matter decomposition and nutrient are significantly higher in RS compared to BS (Priha *et al*., 1999; Phillips and Fahey, 2006). Earlier studies suggest that microbial respiration and $N_{\text{-min}}$ are likely to be higher in RS (Kuzyakov, 2002; Phillips and Fahey, 2008; Zhu and Cheng, 2011).

2.5.5 Carbon flux in rhizosphere

The amount of C in soils is 4.5 times greater than in terrestrial biomass (Jobbagy and Jackson, 2000), and changes of C in soil is one of the unknown mystery in global C budget (Ciais *et al.,* 2014). The amount of soil organic carbon represents the net balance between C inputs from leaf, stem, and root litter and C outputs including decomposition of C caused by soil microorganisms (Davidson and Janssens, 2006; Regnier *et al.,* 2013; Tian *et al.,* 2015). Influence of various factors such as vegetation type and productivity (Ren *et al.,* 2012), temperature (Davidson and Janssens, 2006), soil moisture (Ryan and Law, 2005), soil properties and nutrient (Tian *et al.,* 2010), and disturbance regimes such as land use change (Post and Kwon, 2000) and fire (Harden *et al.,* 2000) can affect the size of soil organic C pool.

The change in photoassimilate from trees to soil is one of the least understood and most poorly quantified aspect of terrestrial C cycle (Grayston *et al.,* 1996; Cheng, 1999; Kuzyakov and Domanski, 2000). Rhizosphere C flux is mainly due to sloughing of cells, and release of low molecular weight exudates and organic exudations from fine roots and mycorrhizal hyphae to soil. Rhizosphere C flux is extremely difficult to quantify because the released C occurs in a narrow zone around roots (Norton *et al.,* 1990; Rygiewicz and Andersen, 1994) and is rapidly consumed by rhizosphere microflora because of its high quality as a microbial substrate (Leyval and Berthelin, 1993). Estimates of rhizosphere C flux in trees range from 1% to 12% of assimilated C (Grayston *et al.,* 1996), although this wide range of estimates may result as much from the methodologies employed as from actual differences between tree species and forest ecosystems (Meharg, 1994).

Despite uncertainty in the magnitude of rhizosphere C flux, this process is regarded as a potentially responsive component to global change because it is sensitive to elevated CO_2 and temperature (Grayston *et al.,* 1996), and intimately linked to soil microbial activity, organic matter decomposition and nutrient release (Cheng, 1999; Hütsch *et al.,* 2002; Paterson, 2003). Moreover, changes in rhizosphere C flux may result in feedbacks to ecosystem C storage (Hu *et al.,* 1999). Numerous studies suggest rhizosphere C is likely to be stimulated under elevated CO_2 across a wide range of plant taxa (Norby *et al.,* 1987; Zak *et al.,* 1993; Rouhier *et al.,* 1996; Paterson *et al.,* 1997; Hungate *et al.,* 1999; Pendall *et al.,* 2004). Increases in rhizosphere C flux may limit C storage in soil if microbial breakdown of SOM is enhanced through rhizosphere priming effects i.e. changes in rate of soil organic matter decomposition by microbes as influenced by root exudates (Kuzyakov, 2002). However, increases in rhizosphere C flux may also result in greater ecosystem C storage if microbial activity in the rhizosphere increases nutrient availability to plants, and results in greater net C assimilation rates and C storage in plant biomass (Hu *et al.,* 1999). In northern

hardwood forests of the northeastern US, acidic deposition and climate change are influencing the distribution and abundance of many tree species (Iverson and Prasad, 2002; Bailey *et al.,* 2004). Because rhizosphere C flux may differ several-fold within and between plant taxa (Kuzyakov and Domanski, 2000), shifts in species composition could have profound implications for C storage and nutrient availability.

2.5.6 Plant-microbe interactions in rhizosphere

Rhizosphere microflora includes bacteria, fungi, nematodes, protozoa, algae and microarthropods (Raaijmakers *et al.,* 2001).These soil microorganisms including bacteria and fungi occupied the rhizosphere and have great influence on plant root exudation (Jones *et al.,* 2004; Matilla *et al.,* 2010). Consequently, the rhizosphere zone become a hot spot of microbial activities due increased nutrient supply and root exudation (Brimecombe *et al.,* 2007; Barriuso *et al*., 2008). The number and diversity of microorganisms are related to the quantity and quality of exudates but also to the outcome of the microbial interactions that occur in the rhizosphere (Somers *et al.,* 2004). An important feedback of high microbial activity in the rhizosphere is root development and plant growth in general. Thus, rhizosphere microorganisms are responsible for a critical relationship between plant and soil (Lynch, 1990). For example, Morgan *et al.* (2005) reported that fungal abundance is 10-20 times and bacterial abundance is 2-20 times higher in RS compared to BS. Competition among microorganisms is very high for nutrient sources in the rhizosphere; therefore, microorganisms have different strategies, giving rise to a range of antagonistic to synergistic interactions, both among themselves and with the plant (Perotto and Bonfante, 1997).

The microorganisms in RS contest each other for water, nutrients and space and sometimes improve their competitiveness by developing an intimate association with plant. This process can be regarded as an ongoing process of micro-evolution in low-nutrient environments, which are quite common in natural ecosystems (Schloter *et al.,* 2000). Plant-

microbe interactions may thus be considered beneficial, neutral, or harmful to the plant, depending on the specific microorganisms and plants involved and on the prevailing environmental conditions (Bais *et al.,* 2006).

Plant species, plant developmental stage and soil type have thus been indicated as major factors determining the composition of rhizosphere microbial communities (Broeckling *et al.,* 2008). A number of studies have revealed that plants determine and form the selection of microbes by releasing specific root exudates compounds that changes the rhizosphere microbial community (Bakker *et al.,* 2012; Berendsen *et al.,* 2012; Chaparro *et al.,* 2012). Further, microbial communities in the rhizosphere greatly depend on the plant species and its growth period (Bulgarelli *et al.,* 2012; Lundberg *et al.,* 2012). For example, comparison of fungal community in wheat, oat and pea indicate that fungi population count increased significantly in pea RS compared to other crops (Turner *et al.,* 2013). Wang *et al.* (2009a) and Hannula *et al*. (2010) also reported that the fungal community is affected by the plant species and growth period.

Peiffer *et al.* (2013) found that bacterial communities were significantly varied with relative abundances, richness and diversity in the rhizosphere of 27 maize inbred lines, attributed to host genetics. Xu *et al.* (2009) also demonstrate the impact of soil type; plant genotype and growth phase on bacterial communities in the RS of Soybean and found that bacterial communities changed considerably with plant growth period. Plant-microbe interactions in rhizosphere have significant impact on soil C and nutrient availability to plants (Kuzyakov, 2002; Cheng *et al.,* 2014; Sun *et al.,* 2014). These interactions have been described to have beneficial effect on plants like disease suppression (Weller *et al.,* 2002; Haas and Defago, 2005; Mendes *et al.,* 2011), increased nutrient accumulation and uptake (Lugtenberg *et al.,* 2002; Morrisey *et al.,* 2004) and higher immunity to abiotic condition (Selvakumar *et al.*2012; Zolla *et al.,* 2013) and biotic pressure (Badri *et al.,* 2013b,

Zamioudis and Pieterse, 2012), each of these leads to increased plant development (Berg, 2009).

Chapter 3

Materials and Methods

3.1 Site Description

The study was conducted in three shifting cultivation stands of different fallow ages (2 years fallow land, FL-2; 5 years fallow land, FL-5; 10 years fallow land, FL-10) in Muallungthu, Mizoram. The ages of fallow lands were identified through interviewing the land owner. Slashing of vegetation was carried out in January, 2013 which was dried and burnt during first week of March, 2014. The geographical position of the study sites are given in Table 3.1.

Table 3.1. The geographical position of the study site.

Fallow	Area (acre)	Latitude	Logitude	Altitude(m)
2 year	1.5	23º36’43.2” N	92º43’11.9”E	850
5 years	2.0	23º36’40.8” N	92º43’13.4"E	853
10 years	2.0	23º36’43.8” N	92º43’14.4”E	810

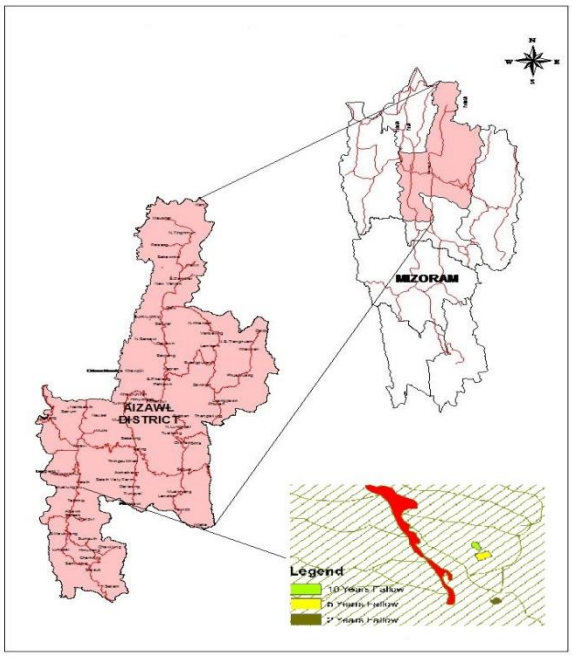
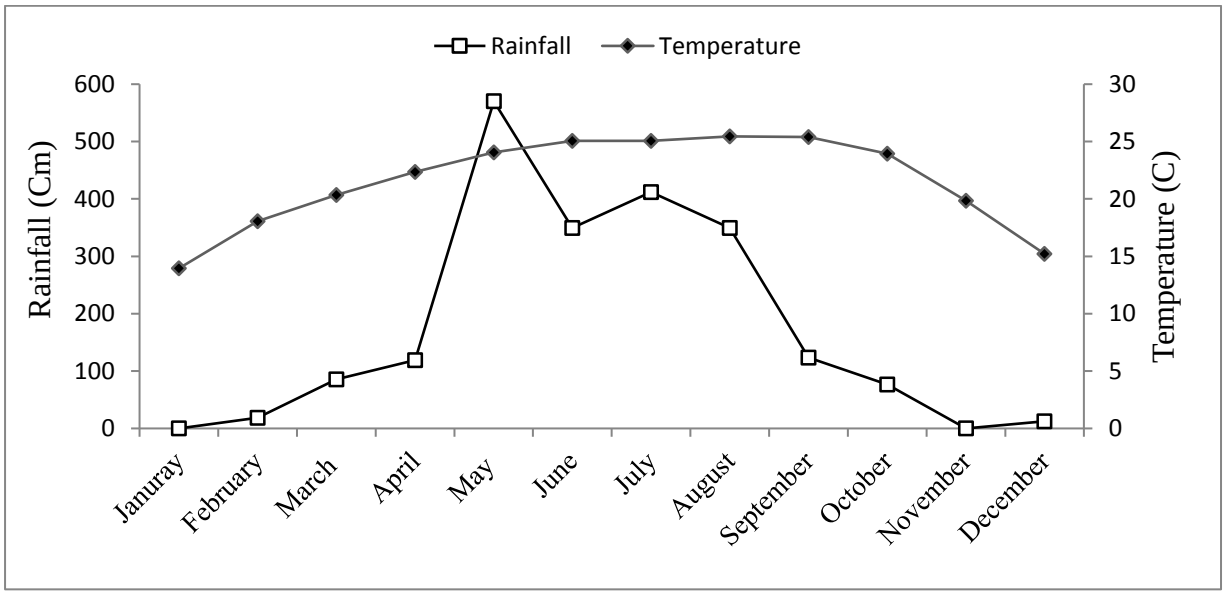


Fig.3.1. Map of the study site.

3.2 Climate and Soil of the study site

Aizawl district of Mizoram experiences warm humid tropical climate. Muallungthu and its adjoining places like Falkawn, Tachhip and Aibawk located on the southern part of Aizawl district experiences hot-humid weather during the summer months with an average temperature of 25°C during May to August. Winter months usually remains cold with moderate summer months. Monthly temperature (ºC) and rainfall of the study site is given in Fig. 3.2.

Fig. 3.2. Monthly rainfall (cm) and temperature (°C) data of Muallungthu in 2014.



Source: Department of agriculture (Research and Education), Govt. of Mizoram.

Soils of the study sites falls under red soil group and the soil order is inceptisols. Soils are mostly light to medium texture (sandy loam and clay loam) with depth ranging from deep to very deep. Soils of more than 90% of the geographical area are acidic (pH below 5.4). Soil bulk density (BD) of the study site ranged from 9.7-1.3 mg m^{-3}.

3.3 Experimental Design

In all fallow sites (FL-2, FL-5, FL-10) about 1 ha land was selected for conducting experiments. The selected site was divided into four plots (10m × 30m) perpendicular to the

slope. Each plot was further divided into 3 sub plots of 10 x 10 m perpendicular to the slope. Layout of the plots is described in Table 3.2. Soil and litter from the adjoining forest was amended in to plots after burning of the site. The third plot was treated with microbial inoculation and the fourth one was considered as control. Microbial inoculants was developed from rhizospheric and endophytic bacteria isolated from roots of *Thysanolaena maxima* one of most abundant early successional plant collected from the burn sites in the second fortnight of March 2013. Isolates were screened for acidity and heat tolerance ability and multifaceted plant growth promoting properties *viz*. ability of pectinase, cellulose, and IAA production, nitrogen fixation; phosphate solubilization [P in $Ca_3(PO_4)_2$, $AlPO_4$, $FePO_4$ and phytate amended media]. Based on the results of the PGP attributes of early colonizers, 5 best strains were selected on the basis of their maximum scores achieved for multifaceted PGP properties and were used to formulate microbial consortium in finely grinded dry compost (passed through 1 mm sieve). A 130 ml volume broth of each strain (total broth volume 650 ml for five strains) was added to 1 kg sterile compost and mixed aseptically. Then the mixture was packed and sealed aseptically and stored in cool dry place. The cfu counts of each gram of compost were 1.25×108 up to 3 months from the date of manufacture.

Table 3.2. Basic layout of experimental design (10m × 10m plot) with control and different treatment of selected sites (2, 5 and 10 years fallow ages) in Muallungthu, Mizoram.

T_S **(Plot 1)**	**T_L** **(Plot 2)**	**T_M** **(Plot 3)**	**Control** **(Plot 4)**
Top soil amendment from nearby forest @ 1 ton per hectare	**Litter amendment from adjacent forest @ 5 ton per hectare**	**Microbial inocula of dominant soil microbes**	**Without any amendment**

3.4 Soil Sampling

Soil sampling was carried out in the month of June, 2014 (wet season-WS) and December (dry season-DS), 2014, from BS and RS of three dominant annual plants (e.g. *Crassocephalum crepidioides*, *Ageratum conyzoides* and *Bidens pilosa*). Plants from different locations were excavated at 20 cm depth from each plot (10m × 10m) with the help of soil corer (5 cm diameter). The above annual plants were selected due to their dominance and abundance than other annual plant species. RS was collected from soil firmly attached to live roots after gentle shaking and BS was the soil remaining in core after removal of RS. One composite soil (~500g) consists of 12 soil cores randomly taken within each plot. Composite soil was thoroughly mixed and divided into three replicates. Live fine roots, debris and stones were removed at the site. Soil samples were brought to the laboratory and sieved through 1 mm mesh and divided into two parts, one part was air dried for analysis of soil pH, soil organic carbon (SOC) and total nitrogen (TN) and the other half was kept in deep freezer (-20ºC) as fresh soil for measuring SMC), MBC and MBN, NH_4^+ -N and NO_3^- -N, microbial properties (fungal and bacterial population) and enzyme activities (e.g. dehydrogenase activity-DHA and acid phosphatase activity-APA). Microbial properties, enzyme activities, MBC and MBN were analyzed within two weeks to avoid effect of freezing that can alter the microbial properties.

3.5 Laboratory analysis

3.5.1 Soil physical and chemical properties

The amount of SMC was estimated gravimetrically as difference between fresh and oven dry samples at 105ºC for 48 hrs. Soil pH was measured with a glass electrode using pH meter at 1:2.5 soil water suspension ratio**.** Air dried soil was used to determine SOC by following potassium dichromate wet oxidation method described by Walkley (1947). Soil

sample (0.5 g) was mixed with 10 mL of 1N $K_2Cr_2O_7$ and 20 ml of conc. H_2SO_4 in a 500 mL conical flask and kept for 30 minutes. After oxidation, 200 mL distilled water and 5 mL H_3PO_4 was added to the flask. The residual $K_2Cr_2O_7$ was titrated with freshly prepared 0.5 N ferrous ammonium sulphate in presence of diphenylamine indicator. SOC content in soil was expressed in mg g^{-1} soil. Air dried soil sample was used to determine TN using CHN analyzer (CHNS-O Elemental Analyzer EUROEA, 3000).

Freshly collected soil sampleswere used to determine NH_4^+ -N following Indophenol Blue method outlined by Rowland, (1983). Fresh soil weighing 20g was suspended in 100 mL deionized water and shaken for 15 mins. The soil suspension was filtered with whatmann No. 1 filter paper. 5ml of extract solution was taken and 8ml of Rochelle's reagent, 1ml of sodium nitroprusside, 2mL sodium phenate and 0.5mL of sodium hypochlorite were added to developed blue colour. The intensity of blue colour was measured at 625nm using spectrophotometer and the concentration was obtained from a standard curve. The aliquot solution obtained for determining NH_4^+ -N was also used to determine the concentration of NO_3^- -N.Extract solution (10ml) was taken in a 100 ml beaker and kept in hot water bath for drying. Phenol disulphonic acid (2 mL) and 20 mL distilled water were added. Then, ammonium hydroxide was added until yellow colour persisted. The intensity of yellow colour was measured at 410nm using spectrophotometer and concentration was obtained from a standard curve. Nitrification rate was calculated as a change in NO_3^--N concentration during incubation period i.e. NO_3^--N after incubation - NO_3^--N before incubation. The rate of N_{-min} was measured *in situ* by buried bag technique (Eno, 1960).It was calculated as the change in soil mineral nitrogen i.e. (NH_4^+-N + NO_3^- -N after incubation) – (NH_4^+-N + NO_3^- -N before incubation) concentration within 30 days incubation period.

3.5.2. Soil biochemical properties

Freshly collected soil samples were used for MBC determination by the procedure chloroform – fumigation – extraction method (Brookes and Joergensen, 2006). Root debris and organic residues were removed from the sample and the sample was divided into two sub samples (25 g each for fumigate and non-fumigate). The sub sample was taken into 50 ml beaker. One sub-sample was fumigated with chloroform vapour and other sub-sample without fumigation was kept in a desiccator for 24 h. After 24 h incubation, the remaining chloroform in the fumigated sample (desiccator) was removed by releasing the pressure maintaining valve. To each sub-sample (fumigate and non-fumigate), 100 mL 0.5 M K_2SO_4 was added and the samples were shaken for 30 mins. The soil suspensions were filtered through a Whatmann No. 42 filter paper and 10 mL of the supernatants were used for determination of MBC by wet oxidation method similar to determination of SOC described in section 3.5.1.3. The difference in C content between fumigated and non-fumigated sub-samples were determined and then, MBC was calculated using a conversion factor, KEC = 0.38 (Vance *et al*., 1987; Wu *et al*., 1990; Dilly and Munch, 1998). The value of MBC was expressed in mg kg^{-1}.

The supernatants obtained from fumigated and non-fumigated samples for determination of MBC were also used for analyzing MBN. 10 mL of the aliquot was taken in a digestion tube where 10 mL conc. H_2SO_4 and 2.0 g digestion mixture were added and then the suspension was digested in an aluminum heating block (KEL Plus, Pelican Equipment, India) at 360°C for5 hrs. After digestion, N content was determined by following Kjeldahl method. Change in N content between fumigated and non-fumigated sub-samples were determined, the value of MBN was calculated by using the conversion factor, KEN = 0.45 (Gijsman *et al*., 1997; Jenkinson, 1988; Ross and Tate, 1993). The value of MBN was expressed as mg kg^{-1}.

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

Fresh soil samples were used to determine the APA following a protocol described by Tabatabai and Bremner (1969). Soil sample (1 g) was taken in an Erlenmeyer flask and to this 4 mL of MUB (Modified Universal Buffer, pH 6.5), 0.25 mL of toluene and 1 mL of *p*-NPP (*p*-nitrophenyl phosphate) were added and incubate at 37°C for 1 h. After incubation, 1 mL of 0.5 M $CaCl_2$ and 4 mL 0.5M NaOH were added to the soil suspension and filtered using funnel fitted with cotton plug. Intensity of yellow colour was measured in the filtrate at 400 nm using spectrophotometer. The concentration of *p*-nitrophenol in the filtrate was determined against a standard curve prepared by using *p*-nitrophenol standard solution. The value of APA was expressed as μg (PNP) g^{-1} (dw) soil h^{-1}.

Serial dilution method was employed to analyze the fungal and bacterial population in RS and BS (Johson and Curl, 1972). Fresh soil sample was used to determine the fungal population. 1g fresh sample was suspended in 9 ml distilled water and thoroughly shaken for 15 minutes in a horizontal shaker. 1 ml of the suspension was again diluted in 9 mL distilled

water to get a dilution factor of 10^{-2}. The same procedure was followed till dilution factor of 10^{-4} was obtained. 0.5 mL of dilution factor 10^{-4} was plated in sterilized Petridishes containing 20 mL of solidified potato dextrose agar medium supplemented with Rose Bengal (33 mg/L). The inoculated petri plates were incubated at 25±1ºC for 4 days. The fungal population was counted and calculated in terms of colony forming units (CFU) expressed in CFU per g soil.

Fresh soil sample was used to determine the bacterial population. One g of fresh sample was suspended in 9 ml distilled water and thoroughly shaken for 15 minutes in a horizontal shaker. One ml of the suspension was again diluted in 9 mL distilled water to get a dilution factor of 10^{-2}. The same procedure was followed till dilution factor of 10^{-6} is obtained. 0.5 ml of dilution factor 10^{-6} was plated in sterilized petri dishes containing 20 mL of solidified nutrient agar supplemented with *Nistidine* medium. The inoculated petri plates were incubated at 25±1ºC for 4 days. The bacterial population was counted and expressed in CFU per gram soil.

3.6 Vegetation analysis

The vegetation analysis was carried out at the month of June, 2014 by laying five quadrates (1m×1m) randomly within each plot. Species composition, relative abundances and importance value index (IVI) were calculated as the procedure outlined by Mueller-Dombois and Ellenberg, (1974).

Diversity indices by Shannon-Wiener and Margalef were calculated using the following formulae: $H' = -\Sigma\, pi Ln pi$ Shannon and Wiener, (1963)

Where pi is proportion of individuals of species

$R = (S-1)/Ln N$ Margalef, (1958)

Where R is Margalef index. S is the species number in each quadrant and N is the number of all individual species.

3.7. Analysis of root exudates

Analysis of root exudates was conducted in three selected sites i.e. 2 years, 5 years and 10 years fallow following the method described by Phillips *et al*. (2008). From these selected sites, three dominant annual plants like *A. conyzoides, C. Crepidioides and B. pilosa* were selected to measure their root exudates (C compounds) for a period of six months (June-November), 2014. Root exudates were collected during June-August were considered as wet season (WS) and September-November as dry season (DS).

3.7.1 Excavation of roots

The terminal fine roots (<2 mm diameter with laterals) was carefully excavated from soil (Lucash *et al*., 2005). In order to ensure that the roots were selected species, the root system was traced back to its parent. Once unearthed, fine root (10-15 cm length) was wash/rinse with a C-free nutrient solution (0.5 mM NH_4NO_3, 0.1 mM KH_2PO_4, 0.2 mM K_2SO_4, 0.2 mM $MgSO_4$, 0.3 mM $CaCl_2$) to minimize osmotic stress of the roots and adhering soilwas removed. The intact root was placed into a soil-sand mixture (1:1 ratio) and re-buried for two days. The re-burial was intended to allow additional time for roots to recover any potential injury or stress sustained during the excavation and washing process. The soil attached to roots was removed again and rinse with nutrient solution before it was placed into the cuvette.

3.7.2 Cuvette assembly

A syringe (20 mL) was used to collect the root exudates. Each syringe was back-filled with sterile glass beads. At the narrow end of each syringe, a mesh cloth with plastic filter

was folded into a cone shape that support and prevent them from clogging the syringe outlet during collection of solution (exudates). At the top of each syringe, a rubber septum with a small slit cut to accommodate the protruding root was use to seal off the cuvette. To protect the exposed portion of the root from drying out, a moist cotton cloth was placed around the upper root segment and secured with parafilm. A small volume of dilute nutrient solution was added to the cuvette to maintain humid conditions during the incubation. The cuvettes was covered with aluminum foil, placed to the excavated area and covered with several layers of litter to allow the root system to equilibrate with the cuvette environment.

3.7.3 Collection of exudates

After 2 days of equilibration period, a dilute nutrient solution was added to each cuvette to facilitate removal of accumulated C exudates. To ensure complete removal of C from the cuvette, each cuvette was filled with and flushed with C-free nutrient solution. This process was repeated three or four times for complete removal of exudates. The root inserted inside the cuvette was taken for determination of its biomass. Two control cuvettes filled with glass beads and nutrient solution were similarly covered and buried in soil in each plot. All solutions collected was kept in refrigerator at 4ºC until analyses (<48hrs). All samples were analyzed for C exudate in TOC – Vcph Total Organic Analyzer, Schimadzu, Japan.

3.8 Statistical analysis

Paired sample *t*-test was performed to test significant differences among soil variables between RS and BS. Variation in fallow phases was analyzed by the non-parametric Kruskal–Wallis H test, incorporating 1000 randomizations at a Monte-Carlo test of significance with a 99% confidence limit. Tukey's HSD was performed to test the rhizosphere effects on soil variables of different fallows. The magnitude of the rhizosphere effect was calculated as the

percentage difference between paired RS and BS samples for each soil variable. Statistical analysis was carried out in software package IBM SPSS Statistics 20.0 for Windows.

Chapter 4

Results

The findings in present study, rhizosphere effect of three annual plants (i.e. *C. crepidioides*, *A. conyzoide*s and *B. pilosa)* in relation to different treatments and fallow ages on soil physico-chemical properties, microbial, biochemical and root exudation were presented by comparing the changes in RS and BS.

4.1 Impact of fallow length on vegetation composition

Considerable changes in annual plant community were noticed with greater abundances in older fallows (Table 4.1). In general, three dominant annual plant species (*A. conyzoides*, *C. crepidioides* and *B. pilosa*) were recorded from the three selected sites. However, the species dominance changed with fallow age. For example, *A. conyzoides* was dominant species in FL-2 (IVI, 97) followed by two co-dominant species (*B. pilosa*, 61 and *C. crepidioides*, 42). In FL-5, dominance was almost equally shared by all three species (IVI, 59-61). However, in FL-10, *B. pilosa* was the most dominant species (IVI, 63) followed by *C. crepidioides* (38) and *A. conyzoides* (28). Plant species diversity and richness increases with fallow age. The other co-dominant species include: *Chromolaena odorata*, *Knoxia corymbosa* and *Cantella asiatica* in FL-2. In FL-5 and FL-10, other associates plants species were: *Scoperia dulcis*, *Oxalis corniculata* and *Spilanthes acamella*.

Table 4.1. Floristic composition of different fallow lands in Muallungthu, Mizoram.

Fallow	Plant	Density	IVI (%)	Shanon-Wiener index (H)	Species richness
2 years	*A. conyzoides*	15.4	97.12	1.68	3.86
	B. pilosa	8.3	62.41		
	C. crepidioides	4.6	41.91		
5 years	*A. conyzoides*	35.4	61.12	1.78	4.28
	B. pilosa	34.6	58.81		
	C. crepidioides	31.8	60.12		
10 years	*A. conyzoides*	9.2	27.59	1.91	5.13
	B. pilosa	29.3	62.64		
	C. crepidioides	14.2	38.66		

4.2. Impact of various treatment on soil characteristic

4.2.1 Soil physico-chemical properties

No significant variation (P<0.05) on SMC between treatment and control in both the sampling season. During WS, the highest amount of SMC was marked in RS of *B. pilosa* in FL-10 (30.34%) and lowest was recorded in BS of FL-2 (13.4%). On the other hand, the highest and lowest amount of SMC in DS was 23.9% and 10.3% respectively. Similarly, no significant variations were recorded in soil pH between treatments and control in both sampling seasons; the soils were highly acidic with soil pH ranging between 4.91-5.5 in WS and 5.09-5.6 in DS (Table 4.3). The amount of SOC and TN were considerably higher in treatments (T_S, T_M and T_L) compared to control, however, statistically significant (P<0.05) differences were noted in T_L but not in T_S and T_M in both WS and DS. The amount of SOC and TN ranged from 2.05-4.82 mg g^{-1} and 0.20-0.47 mg g^{-1}, respectively in WS and 1.31-4.19 mg g^{-1} and 0.15-0.40 mg g^{-1}, respectively in DS (Table 4.4 and 4.5). The percent increase from control to T_L in the SOC content was highest in RS of *C. crepidioides* (76%). Cooresponding values were (55%) for TN in RS of *B. pilosa*.

Table 4.2. Changes in soil moisture content (%) in bulk soil (BS) and rhizosphere soil of *Crassocephalum crepidioides* (Cc), *Ageratum conyzoides* (Ac) and *Bidens pilosa* (Bp) in different treatments (T_S- Soil, T_M – Microbial inocula and T_L – Litter) and a control in different fallow ages during wet season (WS) and dry season (DS).

		BS		Cc		Ac		Bp	
Fallow	Treatment	WS	DS	WS	DS	WS	DS	WS	DS
2 yrs	Control	13.3±1.65	10.3±2.46	17.1±2.13	14.6±1.57	20.9±2.38	16.3±2.02	18.6±0.91	14.9±1.21
	T_S	14.4±2.20	12.7±1.57	19.1±1.50	19.1±1.51	23.5±2.36	19.2±2.71	20.4±1.88	15.9±2.50
	T_M	16.7±2.57	14.3±2.03	21.6±2.45	21.6±2.45	24.8±2.20	19.2±1.04	23.1±1.65	18.2±1.01
	T_L	18.7±2.68	15.1±1.12	21.9±1.99	18.2±1.11	23.6±2.95	19.9±2.68	23.3±2.61	19.5±2.61
	LSD	**7.10**	**5.75**	**9.95**	**9.00**	**10.65**	**8.16**	**10.65**	**7.45**
5 yrs	Control	15.9±1.48	12.1±1.28	20.6±2.44	17.1±2.13	22.5±2.85	18.7±1.95	20.2±2.54	16.7±2.04
	T_S	18.1±2.24	14.5±2.25	22.2±2.42	18.5±2.94	26.1±1.57	21.1±2.64	21.4±2.76	17.8±2.83
	T_M	18.2±2.67	15.1±2.90	22.4±2.25	18.8±2.09	24.3±2.30	20.7±2.03	20.7±1.19	17.4±2.49
	T_L	19.4±2.12	15.6±0.86	22.8±2.64	19.3±2.29	26.4±2.02	21.6±1.96	25.4±2.69	21.8±1.73
	LSD	**11.8**	**10.65**	**10.6**	**10.2**	**9.15**	**9.95**	**7.35**	**9.55**
10 yrs	Control	18.6±1.38	14.9±1.20	20.1±1.55	16.4±2.47	21.2±1.70	18.5±2.56	23.5±2.10	19.6±2.98
	T_S	19.1±2.68	15.1±2.34	23.1±1.87	18.9±2.25	25.2±2.51	20.9±2.39	26.1±1.57	22.5±1.92
	T_M	20.3±2.05	17.0±2.03	23.8±2.71	19.6±1.02	24.1±2.15	20.3±2.11	27.9±2.43	23.6±1.78
	T_L	22.5±2.28	16.6±2.09	25.7±2.50	21.4±2.09	26.7±2.32	22.8±2.75	30.3±1.67	23.9±2.37
	LSD	**9.40**	**8.80**	**6.30**	**9.20**	**7.05**	**10.45**	**6.95**	**6.15**

LSD (P<0.05).

Table 4.3. Changes in **s**oil pH in bulk soil (BS) and rhizosphere soil of *Crassocephalum crepidioides* (Cc), *Ageratum conyzoides* (Ac) and *Bidens pilosa* (Bp) in different treatments (T_S- Soil, T_M – Microbial inocula and T_L – Litter) and a control in different fallow ages during wet season (WS) and dry season (DS).

		BS		Cc		Ac		BP	
Fallow	Treatment	WS	DS	WS	DS	WS	DS	WS	DS
2 yrs	Control	5.32±0.05	5.42±0.04	5.17±0.06	5.31±0.05	5.06±0.04	5.16±0.04	5.12±0.08	5.24±0.15
	T_S	5.31±0.04	5.41±0.03	5.18±0.11	5.32±0.12	5.05±0.16	5.09±0.16	5.16±0.06	5.26±0.13
	T_M	5.32±0.09	5.42±0.11	5.11±0.19	5.21±0.22	4.91±0.15	5.11±0.15	5.06±0.12	5.18±0.21
	T_L	5.34±0.07	5.42±0.04	5.04±0.06	5.26±0.15	4.92±0.13	5.04±0.19	4.97±0.13	5.12±0.18
	LSD	**0.205**	**0.19**	**0.37**	**0.465**	**0.041**	**0.47**	**0.59**	**0.535**
5 yrs	Control	5.42±0.04	5.41±0.05	5.27±0.09	5.27±0.12	5.21±0.24	5.31±0.20	5.23±0.11	5.33±0.06
	T_S	5.39±0.08	5.39±0.07	5.15±0.12	5.22±0.03	5.14±0.06	5.21±0.05	5.16±0.12	5.26±0.09
	T_M	5.41±0.10	5.41±0.08	5.25±0.07	5.33±0.07	5.26±0.14	5.36±0.08	5.31±0.15	5.29±0.02
	T_L	5.34±0.05	5.34±0.04	5.18±0.06	5.31±0.06	5.19±0.09	5.23±0.08	5.21±0.08	5.33±0.07
	LSD	**0.225**	**0.185**	**0.265**	**0.235**	**0.465**	**0.36**	**0.37**	**0.21**
10 yrs	Control	5.44±0.04	5.44±0.05	5.14±0.06	5.21±0.05	5.21±0.15	5.55±0.15	5.12±0.09	5.12±0.09
	T_S	5.49±0.12	5.49±0.07	5.28±0.12	5.34±0.12	5.25±0.05	5.33±0.07	5.21±0.06	5.31±0.05
	T_M	5.55±0.15	5.55±0.07	5.26±0.7	5.41±0.08	5.32±0.05	5.47±0.08	5.34±0.03	5.39±0.04
	T_L	5.46±0.08	5.46±0.06	5.29±0.05	5.34±0.02	5.32±0.06	5.32±0.06	5.24±0.06	5.27±0.09
	LSD	**0.345**	**0.34**	**0.245**	**0.235**	**0.275**	**0.255**	**0.195**	**0.22**

LSD (P<0.05).

Table 4.4. Changes in **s**oil organic carbon (mg g^{-1}) in bulk soil (BS) and rhizosphere soil of *Crassocephalum crepidioides* (Cc), *Ageratum conyzoides* (Ac) and *Bidens pilosa* (Bp) in different treatments (T_S-Soil, T_M–Microbial inocula and T_L–Litter) and a control in different fallow ages during wet season (WS) and dry season (DS).

		BS		Cc		Ac		Bp	
Fallow	Treatment	WS	DS	WS	DS	WS	DS	WS	DS
2 yrs	Control	2.05±0.27	1.31±0.21	2.91±0.21	2.11±0.13	3.22±0.35	2.28±0.24	3.17±0.22	2.18±0.19
	T_S	2.15±0.25	1.35±0.17	2.83±0.076	2.07±0.16	3.13±0.12	2.41±0.15	2.97±0.13	2.30±0.15
	T_M	2.31±0.49	1.64±0.21	3.04±0.13	2.28±0.16	3.37±0.16	2.63±0.17	3.12±0.08	2.45±0.35
	T_L	2.73±0.13	2.09±0.09	3.48±0.17	2.71±0.22	3.87±0.18	3.12±0.13	3.59±0.13	2.86±0.16
	LSD	**0.605**	**0.056**	**0.485**	**0.52**	**0.068**	**0.555**	**0.455**	**0.71**
5 yrs	Control	2.62±0.08	1.71±0.11	3.39±0.23	2.62±0.15	3.36±0.13	2.51±0.196	3.57±0.13	2.48±0.30
	T_S	2.74±0.04	2.16±0.09	3.55±0.22	3.13±0.12	3.43±0.12	2.82±0.22	3.46±0.12	2.94±0.14
	T_M	3.06±0.16	2.39±0.23	3.84±0.27	3.36±0.16	3.65±0.13	3.23±0.16	3.67±0.11	3.11±0.21
	T_L	3.18±0.11	2.72±0.14	3.83±0.11	3.73±0.14	4.21±0.19	3.65±0.14	4.04±0.11	3.42±0.05
	LSD	**0.325**	**0.470**	**0.065**	**0.505**	**0.445**	**0.315**	**0.36**	**0.46**
10 yrs	Control	3.03±0.23	2.18±0.22	3.65±0.07	2.97±0.24	3.56±0.29	2.93±0.20	3.95±0.08	3.18±0.07
	T_S	3.12±0.09	2.56±0.19	3.72±0.19	3.35±0.14	3.65±0.15	3.26±0.17	4.06±0.18	3.65±0.12
	T_M	3.41±0.22	2.79±0.06	3.96±0.12	3.39±0.19	3.87±0.18	3.47±0.18	4.18±0.19	3.82±0.14
	T_L	3.84±0.11	3.21±0.13	4.43±0.13	3.73±0.22	4.55±0.13	3.96±0.17	4.86±0.05	4.19±0.21
	LSD	**0.540**	**0.500**	**0.425**	**0.625**	**0.615**	**0.06**	**0.0435**	**0.445**

LSD (P<0.05).

Table 4.5. Changes insoil total nitrogen (mg g^{-1}) in bulk soil (BS) and rhizosphere soil of *Crassocephalum crepidioides* (Cc), *Ageratum conyzoides* (Ac) and *Bidens pilosa* (Bp) in different treatments (T_S-Soil, T_M–Microbial inoculaand T_L–Litter) and a control in different fallow ages during wet season (WS) and dry season (DS).

		BS		Cc		Ac		Bp	
Fallow	Treatment	WS	DS	WS	DS	WS	DS	WS	DS
2 yrs	Control	0.20±0.012	0.15±0.007	0.21±0.013	0.21±0.008	0.24±0.024	0.24±0.015	0.27±0.021	0.23±0.007
	T_S	0.27±0.010	0.16±0.013	0.28±0.014	0.23±0.024	0.30±0.026	0.24±0.012	0.33±0.015	0.25±0.012
	T_M	0.30±0.017	0.18±0.013	0.27±0.019	0.26±0.013	0.32±0.018	0.29±0.008	0.36±0.101	0.27±0.018
	T_L	0.29±0.004	0.22±0.012	0.31±0.17	0.27±0.017	0.32±0.013	0.30±0.007	0.35±0.035	0.30±0.009
	LSD	**0.035**	**0.035**	**0.05**	**0.055**	**0.0065**	**0.035**	**0.004**	**0.04**
5 yrs	Control	0.24±0.021	0.18±0.008	0.27±0.014	0.26±0.023	0.29±0.011	0.25±0.009	0.31±0.003	0.22±0.012
	T_S	0.33±0.022	0.20±0.014	0.35±0.012	0.26±0.013	0.36±0.023	0.27±0.016	0.38±0.016	0.28±0.016
	T_M	0.31±0.011	0.22±0.012	0.33±0.013	0.31±0.004	0.38±0.019	0.32±0.015	0.41±0.012	0.31±0.013
	T_L	0.31±0.018	0.26±0.014	0.32±0.012	0.33±0.023	0.35±0.011	0.36±0.015	0.38±0.008	0.34±0.017
	LSD	**0.06**	**0.045**	**0.04**	**0.055**	**0.05**	**0.045**	**0.035**	**0.045**
10 yrs	Control	0.29±0.013	0.22±0.015	0.28±0.017	0.25±0.016	0.32±0.020	0.26±0.011	0.35±0.021	0.29±0.006
	T_S	0.34±0.009	0.24±0.013	0.35±0.005	0.31±0.020	0.39±0.016	0.30±0.009	0.41±0.023	0.32±0.011
	T_M	0.34±0.024	0.29±0.011	0.32±0.011	0.33±0.023	0.38±0.016	0.35±0.008	0.43±0.019	0.37±0.003
	T_L	0.36±0.019	0.30±0.010	0.37±0.02	0.36±0.021	0.43±0.018	0.38±0.019	0.47±0.024	0.40±0.008
	LSD	**0.055**	**0.035**	**0.045**	**0.065**	**0.055**	**0.04**	**0.065**	**0.02**

LSD (P<0.05).

The level of NH_4^+ -N, NO_3^- –N and the rates of nitrification and N_{-min} were relatively higher in all the treatments (T_S, T_M and T_L) as compared to control but could not meet the level of significance in some treatment. However, variations were significant with the sampling season i.e. WS and DS. The concentration of NO_3^- –N significantly increase in T_L and T_M compared to control in BS of all the three fallow periods, however, in RS, significant difference was marked between T_M and T_L with control only in *A. conyzoides* (FL-5) and *B. pilosa* (FL-10) in WS. In DS, significant difference (P<0.05) was recorded in all treatments and plants compared to control except Ts. The concentration of NO_3^- –N ranged from 0.262-0.528 mg kg^{-1} (Table 4.7). The mean increment from control plot to T_M and T_L in NO_3^- –N was 37% and 46%, respectively, in WS and 94% and 59% in DS. However, significant changes in NH_4^+ -N were not recorded between treatments and control in WS except in T_M and T_L in DS. The amount of NH_4^+ -N varies from 0.227-1.024 mg kg^{-1} (Table 4.6). The rate of mean increment in DS from control to T_M and T_L was 53% and 34%, respectively. The rate of nitrification increased significantly in T_L over control in both season but significant change was not noted T_S and T_M. The nitrification rates ranged from 0.121-0.661 mg kg^{-1} and 0.085-0.535 mg kg^{-1}, respectively in WS and DS. The average increment in nitrification rate from control to T_L during was 37% and 56% in WS and DS (Table 4.8). Similarly, N_{-min} rate was significantly higher in T_M and T_L relative to control in all the sites as well as in the RS of all the three plants studied. The rate of N_{-min} ranged from 0.244-1.341 mg kg^{-1} in WS and 0.136-1.003 mg kg^{-1} in DS (Table 4.9).

Table 4.6. Changes inconcentration of NH_4^+ -N (mg kg^{-1}) in bulk soil (BS) and rhizosphere soil of *Crassocephalum crepidioides* (Cc), *Ageratum conyzoides* (Ac) and *Bidens pilosa* (Bp) in different treatments (T_S-Soil, T_M–Microbial inocula and T_L–Litter) and a control in different fallow ages during wet season (WS) and dry season (DS).

		BS		Cc		Ac		Bp	
Fallow	Treatment	WS	DS	WS	DS	WS	DS	WS	DS
2 yrs	Control	0.23±0.05	0.12±0.03	0.39±0.04	0.24±0.02	0.48±0.03	0.29±0.02	0.43±0.01	0.26±0.01
	T_S	0.26±0.05	0.16±0.02	0.38±0.05	0.26±0.03	0.47±0.09	0.32±0.06	0.42±0.07	0.28±0.05
	T_M	0.32±0.05	0.18±0.03	0.47±0.02	0.31±0.01	0.53±0.05	0.35±0.03	0.46±0.05	0.31±0.03
	T_L	0.39±0.06	0.22±0.03	0.54±0.05	0.34±0.03	0.62±0.09	0.45±0.03	0.59±0.05	0.40±0.03
	LSD	**0.066**	**0.095**	**0.129**	**0.054**	**0.079**	**0.076**	**0.064**	**0.0675**
5 yrs	Control	0.34±0.10	0.20±0.04	0.58±0.08	0.35±0.05	0.59±0.06	0.36±0.04	0.52±0.09	0.32±0.05
	T_S	0.42±0.09	0.25±0.05	0.62±0.12	0.41±0.08	0.62±0.06	0.41±0.04	0.54±0.07	0.35±0.05
	T_M	0.52±0.10	0.31±0.05	0.71±0.07	0.49±0.06	0.75±0.13	0.52±0.06	0.67±0.09	0.45±0.05
	T_L	0.61±0.12	0.35±0.07	0.82±0.12	0.53±0.07	0.88±0.11	0.56±0.04	0.84±0.09	0.47±0.08
	LSD	**0.076**	**0.185**	**0.126**	**0.206**	**0.247**	**0.096**	**0.177**	**0.193**
10 yrs	Control	0.45±0.08	0.25±0.03	0.67±0.06	0.41±0.04	0.70±0.02	0.42±0.01	0.81±0.07	0.46±0.03
	T_S	0.55±0.07	0.33±0.04	0.73±0.08	0.46±0.05	0.71±0.08	0.45±0.05	0.86±0.12	0.53±0.06
	T_M	0.64±0.11	0.38±0.06	0.64±0.11	0.51±0.07	0.79±0.08	0.49±0.05	0.96±0.13	0.56±0.07
	T_L	0.74±0.09	0.43±0.05	0.94±0.10	0.56±0.06	0.91±0.12	0.54±0.07	1.02±0.14	0.62±0.07
	LSD	**0.126**	**0.15**	**0.138**	**0.178**	**0.164**	**0.163**	**0.221**	**0.197**

LSD (P<0.05).

Table 4.7. Changes inconcentration of NO_3^- –N (mg kg^{-1}) in bulk soil (BS) and rhizosphere soil of *Crassocephalum crepidioides* (Cc), *Ageratum conyzoides* (Ac) and *Bidens pilosa* (Bp) in different treatments (T_S-Soil, T_M–Microbial inocula and T_L–Litter) and a control in different fallow ages during wet season (WS) and dry season (DS).

		BS		Cc		Ac		Bp	
Fallow	Treatment	WS	DS	WS	DS	WS	DS	WS	DS
2 yrs	Control	0.26±0.04	0.17±0.07	0.11±0.02	0.05±0.02	0.13±0.01	0.08±0.02	0.13±0.03	0.07±0.02
	T_S	0.35±0.03	0.21±0.08	0.14±0.04	0.07±0.02	0.16±0.03	0.11±0.03	0.15±0.03	0.09±0.03
	T_M	0.41±0.02	0.24±0.08	0.12±0.04	0.11±0.03	0.18±0.01	0.13±0.03	0.17±0.03	0.11±0.03
	T_L	0.48±0.03	0.26±0.02	0.17±0.04	0.16±0.01	0.18±0.02	0.17±0.04	0.17±0.03	0.13±0.04
	LSD	**0.098**	**0.057**	**0.093**	**0.048**	**0.057**	**0.053**	**0.044**	**0.072**
5 yrs	Control	0.38±0.03	0.28±0.03	0.19±0.02	0.11±0.01	0.23±0.01	0.13±0.02	0.19±0.03	0.11±0.02
	T_S	0.44±0.04	0.32±0.04	0.23±0.03	0.18±0.01	0.28±0.02	0.14±0.01	0.22±0.03	0.15±0.01
	T_M	0.57±0.02	0.37±0.04	0.26±0.03	0.23±0.05	0.31±0.02	0.19±0.03	0.25±0.01	0.18±0.04
	T_L	0.59±0.02	0.42±0.05	0.27±0.03	0.27±0.07	0.32±0.02	0.24±0.03	0.26±0.02	0.2±10.03
	LSD	**0.096**	**0.083**	**0.086**	**0.109**	**0.112**	**0.036**	**0.078**	**0.083**
10 yrs	Control	0.54±0.05	0.38±0.06	0.32±0.03	0.18±0.04	0.28±0.04	0.16±0.04	0.35±0.03	0.22±0.05
	T_S	0.63±0.04	0.46±0.05	0.37±0.04	0.21±0.07	0.33±0.04	0.18±0.06	0.42±0.03	0.26±0.05
	T_M	0.76±0.04	0.51±0.07	0.41±0.04	0.27±0.05	0.39±0.03	0.24±0.05	0.52±0.03	0.31±0.02
	T_L	0.83±0.05	0.58±0.05	0.43±0.04	0.31±0.08	0.39±0.04	0.34±0.05	0.53±0.03	0.38±0.02
	LSD	**0.121**	**0.204**	**0.064**	**0.104**	**0.093**	**0.101**	**0.054**	**0.097**

LSD (P<0.05).

Table 4.8. Changes innitrification rate (mg kg^{-1} $month^{-1}$) in bulk soil (BS) and rhizosphere soil of *Crassocephalum crepidioides* (Cc), *Ageratum conyzoides* (Ac) and *Bidens pilosa* (Bp) in different (T_S-Soil, T_M–Microbial inocula and T_L–Litter) and a control in different fallow ages during wet season (WS) and dry season (DS).

		BS		Cc		Ac		Bp	
Fallow	Treatment	WS	DS	WS	DS	WS	DS	WS	DS
2 yrs	Control	0.12±0.03	0.08±0.00	0.22±0.01	0.15±0.01	0.25±0.02	0.17±0.01	0.22±0.03	0.14±0.01
	T_S	0.14±0.06	0.11±0.01	0.22±0.03	0.17±0.01	0.22±0.02	0.20±0.01	0.25±0.02	0.16±0.00
	T_M	0.18±0.01	0.15±0.01	0.26±0.01	0.24±0.01	0.29±0.02	0.22±0.00	0.26±0.03	0.23±0.01
	T_L	0.23±0.03	0.18±0.00	0.31±0.03	0.27±0.01	0.31±0.03	0.27±0.00	0.35±0.02	0.29±0.01
	LSD	**0.131**	**0.062**	**0.081**	**0.056**	**0.078**	**0.054**	**0.077**	**0.043**
5 yrs	Control	0.24±0.02	0.19±0.02	0.36±0.03	0.24±0.01	0.40±0.03	0.28±0.01	0.33±0.03	0.26±0.01
	T_S	0.27±0.03	0.22±0.01	0.36±0.03	0.29±0.00	0.34±0.04	0.27±0.01	0.38±0.03	0.30±0.01
	T_M	0.31±0.03	0.24±0.00	0.41±0.03	0.33±0.01	0.42±0.02	0.34±0.01	0.38±0.02	0.29±0.02
	T_L	0.37±0.03	0.27±0.03	0.50±0.03	0.36±0.02	0.46±0.03	0.33±0.02	0.46±0.03	0.33±0.01
	LSD	**0.061**	**0.092**	**0.096**	**0.096**	**0.102**	**0.102**	**0.072**	**0.067**
10 yrs	Control	0.35±0.03	0.27±0.01	0.47±0.04	0.35±0.00	0.50±0.05	0.33±0.01	0.54±0.03	0.36±0.01
	T_S	0.39±0.01	0.32±0.01	0.52±0.03	0.39±0.01	0.53±0.03	0.39±0.02	0.57±0.02	0.43±0.01
	T_M	0.43±0.03	0.35±0.01	0.54±0.03	0.45±0.01	0.51±0.03	0.45±0.01	0.63±0.06	0.51±0.01
	T_L	0.48±0.01	0.40±0.01	0.52±0.05	0.51±0.02	0.59±0.04	0.48±0.02	0.66±0.02	0.53±0.02
	LSD	**0.073**	**0.071**	**0.123**	**0.121**	**0.127**	**0.125**	**0.115**	**0.115**

LSD (P<0.05).

Table 4.9. Changes inN-mineralization rate in (mg kg^{-1} $month^{-1}$) in bulk soil (BS) and rhizosphere soil of *Crassocephalum crepidioides* (Cc), *Ageratum conyzoides* (Ac) and *Bidens pilosa* (Bp) in different treatments (T_S-Soil, T_M–Microbial inocula and T_L–Litter) and a control in different fallows during wet season (WS) and dry season (DS).

		BS		Cc		Ac		Bp	
Fallow	Treatment	WS	DS	WS	DS	WS	DS	WS	DS
2 yrs	Control	0.24±0.03	0.13±0.02	0.41±0.04	0.29±0.03	0.48±0.03	0.32±0.01	0.43±0.03	0.30±0.01
	T_S	0.33±0.03	0.22±0.02	0.49±0.304	0.41±0.03	0.52±0.05	0.38±0.02	0.54±0.04	0.36±0.05
	T_M	0.43±0.04	0.31±0.03	0.52±0.04	0.47±0.02	0.68±0.03	0.47±0.04	0.66±0.04	0.45±0.03
	T_L	0.55±0.04	0.35±0.01	0.54±0.07	0.51±0.04	0.81±0.05	0.56±0.05	0.84±0.05	0.55±0.02
	LSD	**0.111**	**0.087**	**0.015**	**0.117**	**0.13**	**0.122**	**0.125**	**0.121**
5 yrs	Control	0.45±0.06	0.32±0.02	0.65±0.07	0.48±0.03	0.69±0.02	0.56±0.03	0.66±0.10	0.51±0.01
	T_S	0.51±0.06	0.41±0.04	0.67±0.01	0.57±0.02	0.7±0.06	0.60±0.05	0.69±0.06	0.59±0.05
	T_M	0.65±0.04	0.48±0.02	0.83±0.07	0.63±0.03	0.88±0.06	0.64±0.01	0.83±0.08	0.61±0.06
	T_L	0.84±0.05	0.55±0.04	1.07±0.08	0.73±0.04	1.09±0.08	0.71±0.03	1.02±0.05	0.72±0.04
	LSD	**0.183**	**0.105**	**0.209**	**0.102**	**0.198**	**0.114**	**0.233**	**0.146**
10 yrs	Control	0.64±0.03	0.49±0.03	0.83±0.07	0.67±0.02	0.82±0.11	0.63±0.02	0.94±0.06	0.67±0.02
	T_S	0.69±0.06	0.57±0.05	0.89±0.08	0.75±0.03	0.86±0.06	0.72±0.01	0.97±0.08	0.79±0.04
	T_M	0.83±0.05	0.65±0.03	1.04±0.04	0.81±0.02	1.02±0.05	0.83±0.02	1.19±0.07	0.94±0.02
	T_L	0.97±0.05	0.76±0.01	1.16±0.06	0.95±0.01	1.21±0.14	0.89±0.02	1.34±0.06	1.00±0.07
	LSD	**0.168**	**0.112**	**0.206**	**0.076**	**0.319**	**0.093**	**0.237**	**0.144**

LSD (P<0.05).

4.2.2 Variations on soil biochemical properties

In general, the microbial properties (e.g. MBC, MBN, DHA and APA) in BS and RS were considerably higher for all the treatments (T_S, T_M and T_L) relative to control and significant difference in microbial properties were marked between the sampling season i.e. WS and DS. The amount of MBC and DHA were significantly (P<0.05) enhanced in T_M and T_L compared to control but no significant change in T_S. On the other hand, the amount of MBN and APA were significantly increased in T_L but no significant difference in T_S and T_M compared with control was noted in WS. Similarly, in DS, changes in the amount of MBC, APA and DHA were significant in T_L compared to control. The values of MBC and MBN ranged from 123-479 mg kg^{-1} and 7-49 mg kg^{-1}, respectively. The amount of MBC and MBN was highest in *B. pilosa* in FL-10 and lowest in BS of FL-2 (Table 4.10 and 4.11). In addition, the amount of APA and DHA were 11-79 µg PNP g^{-1} soil h^{-1} and 2.49-18.76 µg TPF g^{-1} soil h^{-1} respectively between species and seasons (Table 4.12 and 4.13). The percent increase in MBC compared to control was: 16% in T_M and 25% in T_L. Corresponding values for MBN, APA, and DHA were: 24% in T_M and 35% in T_L, 20% in T_M and 36% in T_L and 20% in T_M and 33% in T_L, respectively, in WS. Percent increase in DS in T_M and T_L treatment was: 17-24% for MBC, 27-44% for MBN, 34-60% for APA and 15-25% for DHA.

Table 4.10. Changes in microbial biomass carbon (mg kg^{-1}) in bulk soil (BS) and rhizosphere soil of *Crassocephalum crepidioides* (Cc), *Ageratum conyzoides* (Ac) and *Bidens pilosa* (Bp) with different treatments (T_S-Soil, T_M–Microbial inocula and T_L–Litter) and a control in different fallows during wet season (WS) and dry season (DS).

		BS		Cc		Ac		Bp	
Fallow	Treatment	WS	DS	WS	DS	WS	DS	WS	DS
2 yrs	Control	168±11	123±08	257±12	210±09	303±08	221±15	277±20	226±13
	T_S	184±14	135±12	264±17	224±15	312±13	252±12	296±14	232±12
	T_M	208±19	160±14	291±21	253±10	334±15	284±16	325±19	262±16
	T_L	247±10	166±18	349±15	265±19	363±19	279±17	332±13	271±10
	LSD	**11**	**20**	**18**	**14**	**17.5**	**22.5**	**13.5**	**14**
5 yrs	Control	219±25	158±10	302±24	249±15	342±09	260±12	331±12	274±17
	T_S	239±21	170±16	345±12	240±17	349±14	266±13	352±15	276±09
	T_M	275±16	213±15	365±20	309±11	384±22	263±20	372±21	284±14
	T_L	294±17	225±15	386±11	298±12	405±19	282±17	393±12	335±16
	LSD	**13**	**12**	**24.5**	**17.5**	**29**	**19**	**32**	**20**
10 yrs	Control	292±19	239±23	393±18	299±18	384±15	307±11	423±20	322±11
	T_S	307±13	233±16	414±16	319±07	409±11	293±18	429±18	334±18
	T_M	349±11	272±12	448±19	345±16	445±15	350±12	466±19	357±15
	T_L	371±23	301±21	460±22	370±25	460±13	365±14	479±21	391±21
	LSD	**24.5**	**34**	**34.5**	**28.5**	**35.5**	**24.5**	**28.5**	**28**

LSD (P<0.05).

Table 4.11. Changes in microbial biomass nitrogen (mg kg^{-1}) in bulk soil (BS)and rhizosphere soil of *Crassocephalum crepidioides* (Cc), *Ageratum conyzoides* (Ac) and *Bidens pilosa* (Bp) with different treatments (T_S-Soil, T_M–Microbial inocula and T_L–Litter) and a control in different fallows during wet season (WS) and dry season (DS).

		BS		Cc		Ac		Bp	
Fallow	Treatment	WS	DS	WS	DS	WS	DS	WS	DS
2 yrs	Control	13±1.48	7±1.36	21±2.01	13±1.41	23±2.46	17±2.01	19±3.18	15±2.25
	T_S	14±2.36	9±1.06	21±1.58	14±1.56	26±1.88	17±1.84	23±3.53	17±2.07
	T_M	18±1.24	12±2.62	25±1.61	18±2.13	31±3.17	21±1.61	28±2.08	19±2.61
	T_L	21±1.97	15±2.02	28±3.45	21±3.78	32±3.27	25±2.13	31±1.96	24±3.51
	LSD	**5.0**	**5.7**	**7.05**	**7.3**	**8.95**	**5.85**	**8.55**	**8.25**
5 yrs	Control	19±1.91	12±1.18	29±2.75	21±2.65	27±3.58	22±2.82	26±1.99	19±3.01
	T_S	20±2.23	15±2.18	28±3.31	20±3.39	27±1.01	23±2.87	29±1.73	25±1.15
	T_M	23±2.81	18±1.07	31±3.36	28±1.31	33±2.64	24±1.32	28±2.05	25±1.97
	T_L	26±1.61	20±1.41	37±3.61	30±2.92	35±4.76	27±1.76	32±2.01	27±1.79
	LSD	**6.7**	**4.725**	**10.05**	**8.25**	**10.15**	**7.1**	**5.95**	**6.4**
10 yrs	Control	28±3.31	19±1.42	35±1.94	28±2.75	34±3.24	25±2.41	39±2.88	31±2.82
	T_S	31±2.78	22±2.27	40±2.87	29±2.68	37±1.97	26±1.97	43±3.79	32±2.84
	T_M	35±1.97	23±2.53	43±2.35	30±2.13	41±3.15	28±2.05	46±3.76	32±1.31
	T_L	35±2.31	25±2.17	41±2.92	30±1.24	44±1.43	31±1.79	49±1.93	37±2.79
	LSD	**8.1**	**6.6**	**7.85**	**7.1**	**7.9**	**6.35**	**9.8**	**7.75**

LSD (P<0.05).

Table 4.12. Changes in acid phosphatase activity (µg PNP g^{-1} soil h^{-1}) in bulk soil (BS)and rhizosphere soil of *Crassocephalum crepidioides* (Cc), *Ageratum conyzoides* (Ac) and *Bidens pilosa* (Bp) with different treatments (T_S-Soil, T_M–Microbial inocula and T_L–Litter) and a control in different fallow ages during wet season (WS) and dry season (DS).

		BS		Cc		Ac		Bp	
Fallow	Treatment	WS	DS	WS	DS	WS	DS	WS	DS
2 yrs	Control	17±1.06	11±0.92	29±2.01	18±2.31	37±1.84	26±1.96	33±2.34	21±2.74
	T_S	19±1.62	12±3.83	32±2.11	25±2.24	37±3.92	30±3.05	34±2.92	25±2.81
	T_M	22±2.74	14±1.96	37±2.31	27±3.79	40±3.19	35±3.14	39±3.19	29±2.48
	T_L	26±3.34	19±2.11	41±3.05	32±2.81	44±1.98	39±2.95	45±1.35	34±3.34
	LSD	**8.45**	**7.3**	**8.1**	**14.85**	**9.35**	**8.85**	**9.0**	**6.7**
5 yrs	Control	26±4.45	14±2.34	42±3.09	25±2.05	38±1.36	29±2.33	40±4.07	26±3.48
	T_S	30±1.05	17±3.43	49±2.23	28±3.48	42±2.40	33±3.07	45±3.55	29±3.78
	T_M	35±3.91	23±3.41	48±3.52	31±3.61	44±4.52	34±2.52	50±1.98	35±2.05
	T_L	38±4.04	27±3.07	49±2.90	37±1.91	48±2.46	44±4.15	53±3.73	39±4.45
	LSD	**9.55**	**7.7**	**8.85**	**9.15**	**9.55**	**9.0**	**10.95**	**10.55**
10 yrs	Control	37±3.62	20±2.59	52±3.54	30±1.64	55±2.81	37±3.19	57±2.14	43±2.28
	T_S	39±2.13	25±3.61	56±3.15	37±4.79	53±2.43	39±2.92	61±3.36	45±1.66
	T_M	46±4.42	30±2.47	59±4.00	45±3.46	63±3.61	42±3.44	69±3.84	50±2.82
	T_L	59±3.79	37±4.42	70±3.17	53±3.68	74±3.90	48±2.97	79±0.94	58±2.56
	LSD	**10.35**	**11.05**	**11.15**	**10.7**	**9.65**	**9.95**	**7.3**	**8.65**

LSD (P<0.05).

Table 4.13. Changes in dehydrogenase activity (µg TPF g^{-1} soil h^{-1}) in BS (BS)and RS of *Crassocephalum crepidioides* (Cc), *Ageratum conyzoides* (Ac) and *Bidens pilosa* (Bp) with different treatments (T_S-Soil, T_M–Microbial inocula and T_L–Litter) and a control in different fallow ages during wet season (WS) and dry season (DS).

		BS		Cc		Ac		Bp	
Fallow	Treatment	WS	DS	WS	DS	WS	DS	WS	DS
2 yrs	Control	4.56±0.37	2.49±0.12	7.59±0.43	4.93±0.21	8.56±0.14	5.98±0.23	7.93±0.31	5.06±0.15
	T_S	5.06±0.39	2.86±0.34	7.76±0.29	4.99±0.26	8.95±0.26	6.52±0.26	8.29±0.67	5.64±0.39
	T_M	6.24±0.29	3.61±0.24	9.29±0.46	5.81±0.43	10.13±0.53	6.79±0.35	9.64±0.31	5.78±0.19
	T_L	7.93±0.64	4.35±0.59	10.31±0.38	6.18±0.26	10.53±0.52	6.65±0.17	11.03±0.41	6.62±0.37
	LSD	**1.375**	**0.62**	**1.235**	**0.93**	**1.245**	**0.85**	**1.115**	**0.905**
5 yrs	Control	7.59±0.51	4.55±0.31	11.09±0.56	7.84±0.19	11.83±0.58	7.53±0.26	10.19±0.24	6.96±0.28
	T_S	8.27±0.28	4.66±0.21	11.37±0.47	7.51±0.29	12.51±0.47	7.16±0.47	11.54±0.53	6.92±0.34
	T_M	9.29±0.24	5.23±0.45	12.39±0.37	7.43±0.22	13.74±0.52	8.240.36±	12.33±0.27	7.41±0.37
	T_L	10.71±0.43	6.08±0.36	13.91±0.23	8.35±0.14	15.21±0.34	9.12±0.43	12.61±0.48	7.57±0.58
	LSD	**1.175**	**0.055**	**1.31**	**0.66**	**1.46**	**1.17**	**1.345**	**1.245**
10 yrs	Control	10.81±0.69	6.14±0.25	14.82±0.87	9.05±0.38	14.14±0.81	8.45±0.51	16.05±0.53	10.24±0.18
	T_S	11.94±0.43	6.48±0.45	15.21±0.41	9.13±0.67	14.53±0.33	7.72±0.20	16.39±0.61	9.49±0.43
	T_M	13.57±0.76	7.84±0.59	16.95±0.89	10.18±0.56	16.33±0.56	9.81±0.34	18.59±0.64	10.88±0.35
	T_L	15.72±0.28	8.42±0.39	17.91±0.73	10.75±0.44	17.69±0.74	10.61±0.56	18.76±0.72	11.25±0.43
	LSD	**1.77**	**1.36**	**2.31**	**1.16**	**1.965**	**1.32**	**1.94**	**1.075**

LSD (P<0.05).

4.2.3 Variations in microbial populations

The number of fungal and bacterial colonies enumerated in RS and BS in WS and DS was depicted in Tables 4.14 and 4.15. The number of colonies of microbial population i.e. fungal and bacterial population significantly increased (P<0.05) in T_L and T_M compared to control however the differences were not significant in T_S in BS and RS in both the sampling season. In addition, the number of colonies formed by bacterial population considerably increased compared to fungal population. Comparing the sampling season, the number of microbial populations were significantly increased in WS compared to DS. The number of colonies counted in fungal population ranged from 14-50 CFU 10^3 g^{-1} soil in WS and 9-48 CFU 10^3 g^{-1} soil in DS. Further, the number of bacterial colonies counted in WS was 25-85 CFU 10^6 g^{-1} soil and 16-67 CFU 10^6 g^{-1} soil in DS. The average percent increase in T_M and T_L for fungal population was 27% and 41% in WS and 47% and 59% in DS relative to control. Similarly, the average increment from control to T_M and T_L for bacterial population is 24% and 35% in wet period and 29% and 43% in dry period. The bacterial colonies enumerated in BS and RS were significantly enhanced as compared to fungal population in both the sampling season.

Table 4.14. Changes in fungal population (CFU 10^3 g^{-1} soil) in bulk soil (BS) and rhizosphere soil of *Crassocephalum crepidioides* (Cc), *Ageratum conyzoides* (Ac) and *Bidens pilosa* (Bp) with different treatments (T_S-Soil, T_M–Microbial inocula and T_L–Litter) and a control in different fallow ages during wet season (WS) and dry season (DS).

		BS		Cc		Ac		Bp	
Fallow	Treatment	WS	DS	WS	DS	WS	DS	WS	DS
2 yrs	Control	13.58±1.81	8.55±1.91	24.85±1.58	19.85±2.36	26.91±2.53	22.21±3.04	29.41±1.40	17.35±1.95
	T_S	17.83±2.19	12.35±2.96	27.64±2.82	22.35±2.01	31.61±2.11	25.88±1.82	32.64±2.37	21.17±3.26
	T_M	21.07±2.37	16.71±2.96	31.01±3.29	26.08±2.01	35.95±2.61	29.78±1.82	34.91±2.31	31.09±3.26
	T_L	23.91±2.89	19.13±2.77	34.04±3.85	27.13±2.37	40.29±0.94	33.71±2.18	38.81±1.95	34.37±2.34
	LSD	**6.6**	**7.75**	**9.05**	**7.7**	**7.75**	**7.1**	**6.9**	**7.6**
5 yrs	Control	18.67±1.08	14.18±2.69	32.46±2.08	26.98±3.14	32.77±1.91	22.11±3.29	34.29±1.74	26.06±4.65
	T_S	22.91±1.04	17.71±3.41	36.392.23±	29.27±6.10	34.49±1.81	18.21±4.91	35.75±1.40	32.12±3.67
	T_M	27.88±2.14	22.75±3.41	42.39±3.61	38.34±6.10	36.01±2.77	33.05±4.91	40.54±2.72	36.65±3.67
	T_L	32.83±4.03	26.16±3.63	45.48±4.95	39.75±2.52	39.66±1.91	25.16±1.28	43.75±1.79	37.15±3.34
	LSD	**9.55**	**7.95**	**6.8**	**10.6**	**5.45**	**5.2**	**8.45**	**5.3**
10 yrs	Control	23.49±1.61	18.37±2.27	37.97±1.91	30.22±2.04	34.01±1.99	27.68±3.82	40.18±1.95	35.12±2.85
	T_S	27.11±1.57	22.24±2.04	38.85±2.38	32.31±1.86	36.82±1.94	31.26±1.89	41.38±3.29	36.36±2.63
	T_M	32.44±2.77	28.45±4.49	40.86±2.55	37.83±2.98	41.37±1.43	37.67±1.73	46.21±3.03	41.21±2.64
	T_L	35.73±3.18	33.83±2.81	45.28±3.43	43.57±2.22	44.74±1.92	42.88±1.84	50.36±3.38	47.56±3.47
	LSD	**7.35**	**9.4**	**8.1**	**7.1**	**5.6**	**7.6**	**9.15**	**8.95**

LSD (P<0.05).

Table 4.15. Changes in bacterial population (CFU 10^6 g^{-1}soil)in bulk soil (BS) and rhizosphere soil of *Crassocephalum crepidioides* (Cc), *Ageratum conyzoides* (Ac) and *Bidens pilosa* (Bp) with treatments (T_S-Soil, T_M–Microbial inocula and T_L–Litter) and a control in different fallows during wet season (WS) and dry season (DS).

		BS		Cc		Ac		Bp	
Fallow	Treatment	WS	DS	WS	DS	WS	DS	WS	DS
2 yrs	Control	24.73±2.23	16.44±2.88	44.97±3.17	34.48±2.08	49.56±1.51	31.75±2.71	46.41±1.49	35.77±2.68
	T_S	33.81±1.78	23.48±2.99	49.55±2.24	36.49±2.53	53.77±2.66	36.63±2.82	48.19±1.58	39.22±2.81
	T_M	41.61±1.85	28.67±2.63	56.09±3.23	43.75±3.62	61.87±2.81	44.21±3.48	52.97±3.29	41.25±2.96
	T_L	47.12±2.98	34.29±2.24	51.85±2.23	47.41±3.63	68.67±2.78	49.67±2.57	63.79±1.54	47.89±1.66
	LSD	**9.05**	**8.30**	**8.50**	**9.35**	**7.70**	**8.95**	**6.55**	**7.95**
5 yrs	Control	35.39±3.59	25.42±2.11	56.11±3.41	40.24±1.45	54.12±2.36	39.32±2.82	53.21±3.13	42.53±3.12
	T_S	38.54±0.67	29.68±2.35	50.26±3.09	42.46±4.85	61.54±3.10	41.41±3.51	56.25±0.91	45.33±2.69
	T_M	48.01±1.12	37.95±3.06	64.17±2.79	52.67±2.71	66.16±3.55	49.66±2.34	61.51±1.86	54.65±3.21
	T_L	53.75±2.87	42.34±2.94	67.88±2.95	54.68±2.31	69.44±1.69	53.81±3.26	65.45±2.68	57.11±2.79
	LSD	**7.35**	**8.25**	**9.45**	**9.55**	**8.55**	**9.30**	**7.10**	**9.15**
10 yrs	Control	47.59±3.01	39.3±4.12	65.51±3.62	51.89±3.23	63.13±2.45	53.01±2.88	68.51±3.72	55.37±2.09
	T_S	51.75±0.98	45.27±3.02	68.24±2.79	52.24±3.52	66.38±3.60	54.32±2.69	73.47±3.15	55.76±3.36
	T_M	62.66±2.37	49.17±4.45	74.12±3.21	57.04±2.71	73.23±1.92	55.63±3.11	78.01±3.17	63.20±2.46
	T_L	67.06±0.69	57.09±3.41	80.14±1.53	63.78±2.17	80.88±2.49	62.13±2.59	84.74±3.65	67.27±2.01
	LSD	**6.20**	**11.65**	**8.90**	**9.05**	**8.25**	**8.70**	**7.80**	**7.80**

LSD (P<0.05).

4.3 Changes on rhizosphere soil properties with fallow periods

4.3.1 Changes in RS physico-chemical properties

The SMC in RS significantly increased with fallow period. Significant variation with fallow period was marked in RS of *B. pilosa* but no significant changed (P<0.05) with fallow period in RS of *C. crepidioides* and *A. conyzoides* (Fig. 4.1a). The value of soil pH was significantly differed (P<0.05) with fallow periods in RS of *A. conyzoides* but no significant difference in RS of *C. crepidioides* and *B. pilosa* (Fig. 4.1b). The amount of SOC significantly varied (P<0.05) with fallow periods in RS of *C. crepidioides* in both seasons. However, no significant changed in RS of *A. conyzoides* during WS and *B. pilosa* in DS (Fig. 4.1c). Similarly, the amount of TN significantly differed with fallow periods in RS of *C. crepidioides* but no significant difference (P<0.05) in RS of *A. conyzoides* as well as *B. pilosa* in DS (Fig. 4.1d).

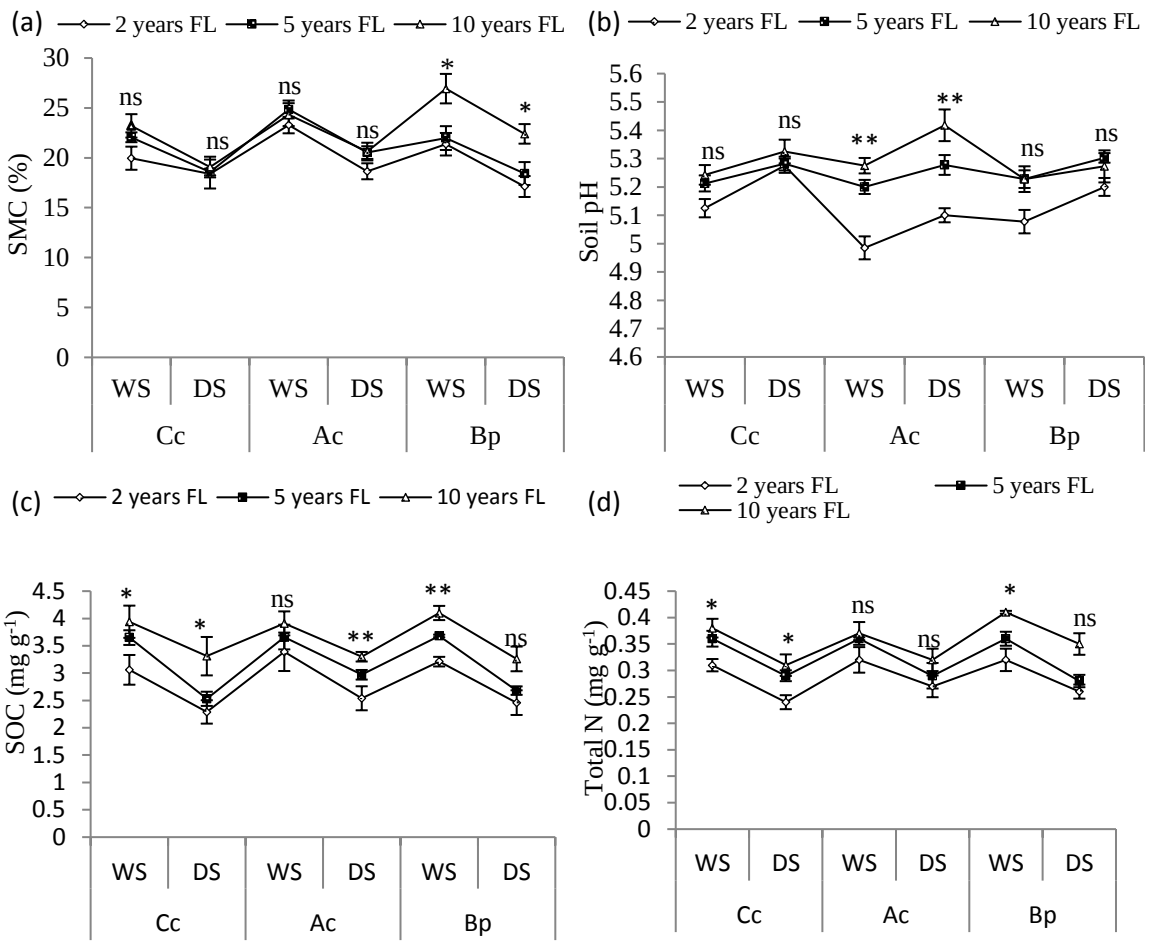


Fig.4.1. (a) Soil moisture content (SMC), (b) soil pH, (c) Soil organic carbon (SOC) and (d) total nitrogen (N) value in rhizosphere soil of *Crassocephalum crepidioides* (Cc), *Ageratum conyzoides* (Ac) and *Bidens pilosa* (Bp) in different fallow lands (FL) i.e. 2 years, 5 years and 10 years during wet season (WS and dry season (DS). Values are mean ±1SE. ns indicate non- significant, * and ** indicate significant difference at $P>0.01$ and $P>0.05$ by non-parametric Kruskal-Wallis H test (1000 randomization) incorporating Monte Carlo significance at 99% confidence limit.

The level of NH_4^+ –N and NO_3^- –N concentration significantly enhanced ($P<0.05$) with fallow periods in RS of *C. crepidioides* and *B. pilosa* in both the sampling season. However, a significant change with fallow periods was not recorded in RS of *A. conyzoides* in DS. Concentration of NH_4^+ –N and NO_3^- –N ranged from 0.45-0.91 mg kg^{-1} and 0.13-0.45 mg kg^{-1} respectively in WS. On the other hand, the concentration of NH_4^+ –N and NO_3^- –N in DS ranged from 0.289-0.546 mg kg^{-1} and 0.099-0.296 mg kg^{-1} (Figs. 4.2 a and b). The mean

increment in NH_4^+–N from FL-2 to FL-5 and FL-10 was 39% and 63% respectively. Similarly, the average increment percent in NO_3^- –N from FL-2 to FL-5 and FL-10 was 68% and 145% respectively. The rate of nitrification and N_{-min} significantly enhanced (P<0.05) with fallow period in RS of all the annual plants studied as well as in all the sites. The rate of nitrification and N_{-min} varied between 0.25-0.60 mg kg^{-1} and 0.494-1.11 mg kg^{-1} in wet period; it ranges from 0.212-0.458 mg kg^{-1} and 0.420-0.855 mg kg^{-1} in dry period (Figs. 4.2 c and d). The increment in nitrification rate from FL-2 to FL-10 was highest in RS of *B. pilosa* (123%) and lowest in RS of *A. conyzoides* (92%). Similarly, the increment percentage from FL-2 to FL-10 in N_{-min} was greatest *B. pilosa* (105%) and *A. conyzoides* (56%).

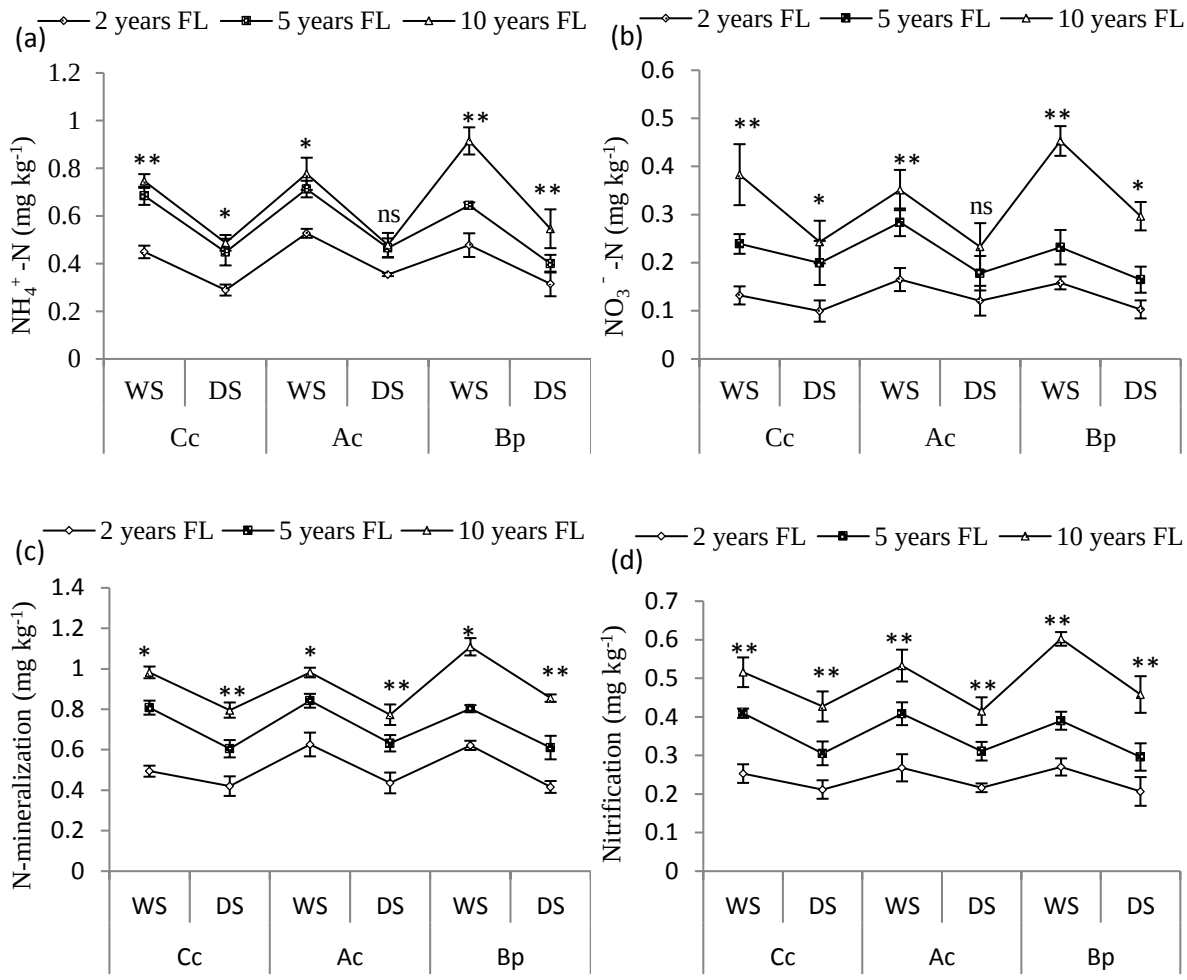


Fig.4.2. (a) Concentration of NH_4^+ -N, (b) NO_3^- –N (c) N-mineralization (d) Nitrification rate in rhizosphere soil of *Crassocephalum crepidioides* (Cc), *Ageratum conyzoides* (Ac) and *Bidens pilosa* (Bp) in different fallow lands (FL) i.e. 2 years, 5 years and 10 years during wet season (WS) and dry season (DS).Values are mean ±1SE. ns indicate non- significant, * and ** indicate significant difference at P>0.01 and P>0.05 by non-parametric Kruskal-Wallis H test (1000 randomization) incorporating Monte Carlo significance at 99% confidence limit.

4.3.2 Changes in RS biochemical properties

The biochemical properties (e.g. MBC, MBN, APA and DHA) of RS significantly increased (P<0.05) with fallow periods. The amount of MBC and MBN in RS of all the studied plants significantly varied with fallow periods for both seasons. The amount of MBC and MBN were highest in RS of *B. pilosa* in FL-10 and lowest in RS of *C. crepidioides* in DS of Fl-2. The average increment in MBC from FL-2 to FL-10 in RS of *C. crepidioides, A.*

conyzoides and *B. pilosa* was 43.75%, 27.5% and 43.49%, respectively (Fig. 4.3. a). Similarly, the amount of MBN for three species increases from FL-2 to FL-10 by 71%, 38% and 75% in RS (Fig. 4.3b). The amount of DHA significantly ($P<0.05$) increased with fallow periods for all the annual plants studied. Similarly, the value of APA significantly increased in RS of *B. pilosa*, but not in RS of *C. crepidioides* and *A. conyzoides* during DS (Fig. 4.3d). The highest increment in APA from FL-2 to FL-10 was recorded in *B. pilosa* (78%) followed by *C. crepidioides* (65%) and *A. conyzoides* (41%). Similarly, the increase in DHA from FL-2 to FL-10 was greatest in *B. pilosa* (85%) followed *C. crepidioides* (82%) and *A. conyzoides* (53%).

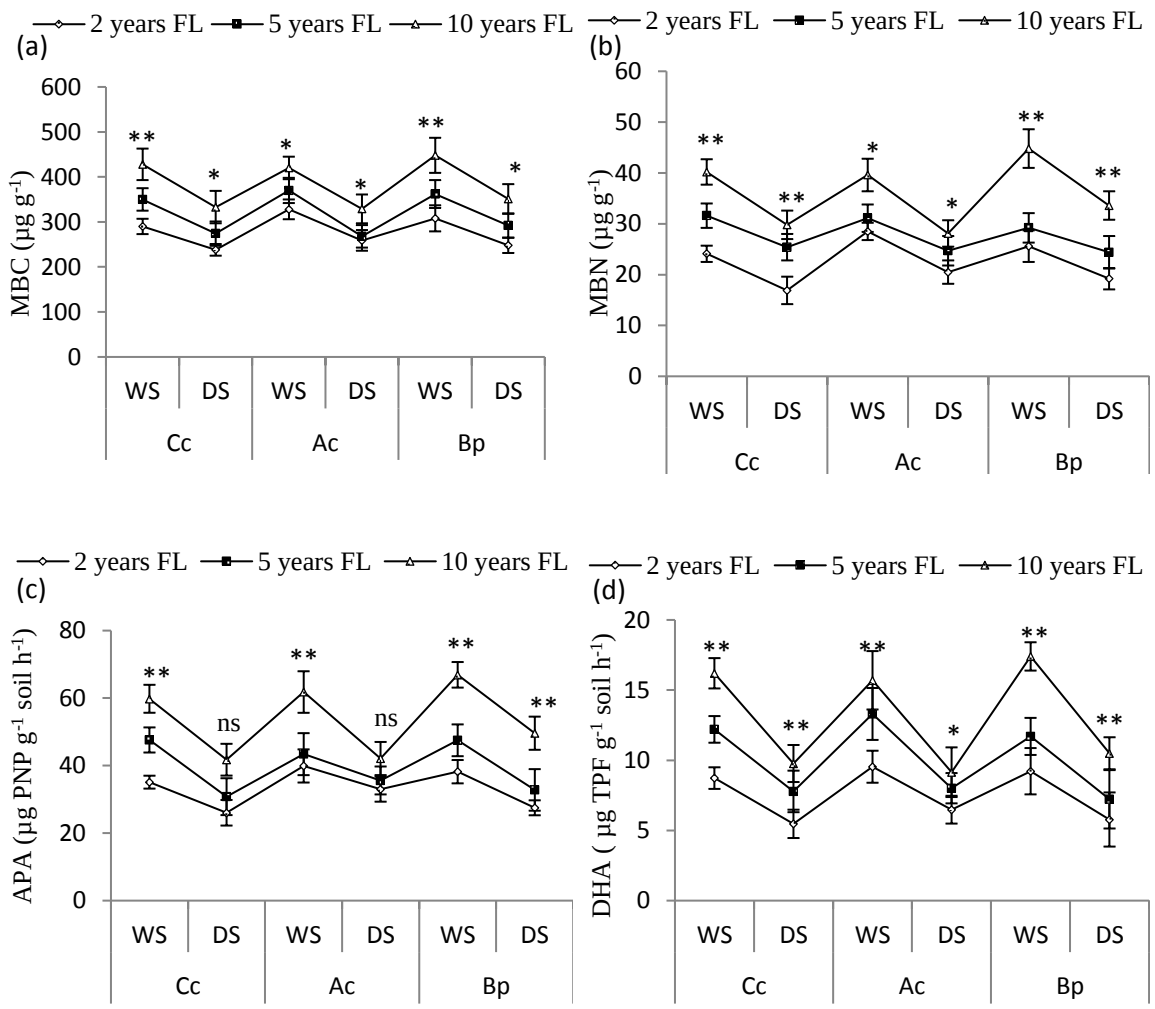


Fig.4.3. Biochemical properties (a) microbial biomass carbon (MBC), (b) microbial biomass nitrogen (MBN), (c) acid phosphatase activity (APA) and (d) dehydrogenase activity (DHA) in rhizosphere soil of *Crassocephalum crepidioides* (Cc), *Ageratum conyzoides* (Ac) and *Bidens pilosa* (Bp) in different fallow lands (FL) i.e. 2 years, 5 years and 10 years during wet season (WS) and dry season (DS).Values are mean ±1SE. ns indicate non- significant, * and ** indicate significant difference at $P>0.01$ and $P>0.05$ by non-parametric Kruskal-Wallis H test (1000 randomization) incorporating Monte Carlo significance at 99% confidence limit.

4.3.3 Changes in RS microbial populations

The colonies of fungal and bacterial population in RS of the three annual plants significantly enhanced ($P<0.05$) with fallow periods. Fig. 4.4a shows that fungal population in RS of *C. crepidioides* and *B. pilosa* significantly differed ($P<0.05$) with fallow periods but the same did not differ significantly in RS of *A. conyzoides* in both the sampling season.

Similarly, Fig. 4.4b indicates that the bacterial population enumerated from RS of *B. pilosa* significant varied with fallow periods in both the season. On the other hand, no significant variation in bacterial population was marked in RS of *C. crepidioides* during DS and in RS of *A. conyzoides* during WS. The average increment in fungal population from FL-2 to FL-10 was highest in RS of *C. crepidioides* (45%) followed by *B. pilosa* (43%) and *A. conyzoides* (21%). Likewise, the increases in bacterial population from FL-2 to FL-10 were greatest in *B. pilosa* (45%) and lowest in *A. conyzoides* (30%).

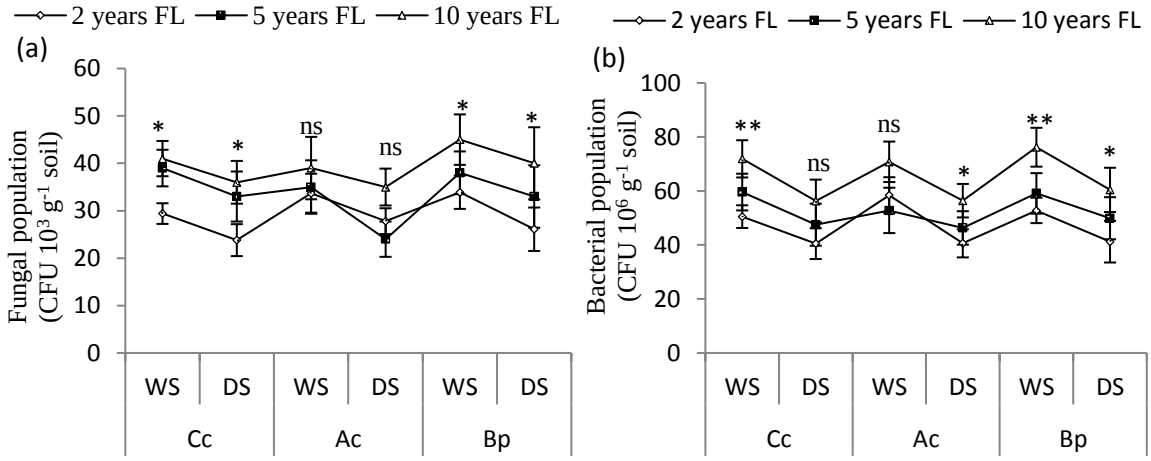


Fig.4.4. Microbial populations: (a) fungal population (b) bacterial population in rhizosphere soil of *Crassocephalum crepidioides* (Cc), *Ageratum conyzoides* (Ac) and *Bidens pilosa* (Bp) in different fallow lands (FL) i.e. 2 years, 5 years and 10 years during wet season (WS) and dry season (DS).Values are mean ±1SE. ns indicates not significant, * and ** indicate significant difference at $P>0.01$ and $P>0.05$ by non-parametric Kruskal-Wallis H test (1000 randomization) incorporating Monte Carlo significance at 99% confidence limit.

4.4 Changes in soil variables between rhizosphere and bulk soil

The amount of SMC significantly increased ($P<0.05$) in RS compared to BS and the value of soil pH showed significant difference between RS and BS. The soil pH value significantly ($P<0.05$) decreased in RS as compared to BS for all the annual plants studied in FL-2 during wet and dry periods. However, no significant change showed in soil pH between BS and RS of *C. crepsidioides* and *A. conyzoides* in FL-5. The highest significant difference

in soil pH between RS and BS was recorded in *A. conyzoides* (6.77% decreased) in FL-2. The total amount of SOC and TN also significantly increased (P<0.05) in RS than BS for all the plants as well as in all the study sites (Table 4.16). Similarly, the concentration of NH_4^+ -N, rate of nitrification and N_{-min} significantly increased (P<0.05) in RS of the annual plants as compared to BS but on contrary to these results, the concentration of NO_3^- –N significantly decreased (P<0.05) in RS than BS in all the sites (Table 4.17). The decrease in NO_3^- –N from BS to RS was highest in *C. crepidioides* of FL-2 with 182% and 122% in both the sampling season. On the other hand, the decrease in NO_3^- –N from BS to RS was lowest in *B. pilosa* of FL-10 with 53% and 62% in both the sampling season.

Table 4.16. Soil moisture content, soil pH, soil organic carbon (SOC) and total nitrogen (TN) in bulk soil (BS) and rhizosphere soil of *Crassocephalum crepidioides* (Cc), *Ageratum conyzoides* (Ac) and *Bidens pilosa* (Bp) in different fallow site in wet season (WS) and dry season (DS).

Fallow period	Soil	SMC (%)		Soil pH		SOC (mg g^{-1})		TN (mg g^{-1})	
		WS	**DS**	**WS**	**DS**	**WS**	**DS**	**WS**	**DS**
2 years	BS	15.8	13.1	5.23	5.42	2.31	1.63	0.23	0.17
	Cc	19.9**	18.3*	5.12*	5.27*	3.06**	2.29**	0.31**	0.24**
	Ac	23.3**	18.7**	4.98**	5.12**	3.39**	2.54**	0.32**	0.27**
	Bp	21.3**	17.1**	5.07*	5.31**	3.21**	2.46**	0.32**	0.26**
5 years	BS	17.9	14.5	5.21	5.39	2.9	2.32	0.27	0.21
	Cc	22.1**	18.6**	5.20ns	5.28*	3.65**	2.53**	0.36**	0.29**
	Ac	24.9**	20.5**	5.23ns	5.27*	3.66**	2.9**	0.36**	0.21**
	Bp	21.9*	18.4**	4.91*	5.31ns	3.68**	2.68**	0.34**	0.28*
10 Years	BS	20.2	16.1	5.24	5.48	3.35	2.71	0.31	0.26
	Cc	23.0*	19.1*	5.27**	5.32**	3.94**	3.31**	0.38**	0.31**
	Ac	24.3*	20.6**	5.23*	5.42ns	3.91**	3.33**	0.37**	0.32**
	Bp	26.9**	22.4**	5.10**	5.27*	4.10**	3.26**	0.41**	0.35**

ns indicate non-significant variation between rhizosphere and BS by paired sample *t*-test.
* Significant variations between rhizosphere and BS at $P<0.05$.
** Significant variations between rhizosphere and BS at $P<0.01$.

Table 4.17. NO_3^+ –N, NH_4^- –N, Nitrification rate and N-mineralization rate in bulk soil (BS) and rhizosphere soil of *Crassocephalum crepidioides* (Cc), *Ageratum conyzoides* (Ac) and *Bidens pilosa* (Bp) in different fallow site in wet season (WS) and dry season (DS).

Fallow	Soil	NO_3^--N (mg kg^{-1})		NH_4^+-N (mg kg^{-1})		Nitrification (mg kg^{-1})		N-mineralization (mg kg^{-1})	
		WS	DS	WS	DS	WS	DS	WS	DS
2 years	BS	0.375	0.221	0.299	0.172	0.169	0.132	0.387	0.259
	Cc	0.132**	0.099**	0.449**	0.289**	0.253**	0.211**	0.494**	0.420**
	Ac	0.165**	0.121**	0.527**	0.354**	0.268**	0.216**	0.626**	0.436**
	Bp	0.158**	0.103**	0.477**	0.314**	0.270**	0.206*	0.622**	0.416**
5 years	BS	0.489	0.347	0.478	0.281	0.296	0.230	0.612	0.442
	Cc	0.239**	0.199**	0.684**	0.448**	0.409**	0.305**	0.808**	0.605**
	Ac	0.284**	0.178**	0.712**	0.466**	0.408**	0.311**	0.842**	0.632**
	Bp	0.232**	0.164**	0.644**	0.399**	0.390**	0.296**	0.803**	0.611**
10 years	BS	0.694	0.481	0.598	0.351	0.416	0.336	0.785	0.618
	Cc	0.383**	0.243**	0.746**	0.487**	0.515**	0.427**	0.982**	0.796**
	Ac	0.351**	0.233**	0.776**	0.478**	0.533**	0.415**	0.981**	0.773**
	Bp	0.451**	0.296**	0.915**	0.546**	0.602**	0.458**	1.108**	0.855**

ns indicate non-significant variation between rhizosphere and BS by paired sample *t*-test.
* Significant variations between rhizosphere and BS at $P<0.05$.
** Significant variations between rhizosphere and BS at $P<0.01$.

The amount of microbial properties in RS and BS for all the annual plants studied during two sampling season were listed in Table 4.18. Marked changes were recorded in microbial properties (e.g. microbial biomass and enzyme activities) between RS and BS for all the sites in both the season. The amount of MBC and MBN significantly enhance (P<0.05) in RS compared to BS for all the annual plants studied in the entire selected sites. Similarly, the value of enzyme activities (i.e. APA and DHA) showed significant increase (P<0.05) in RS than BS. Furthermore, the number of fungal and bacterial population showed significant increased (P<0.05) in RS compared to BS for all the plants studied.

Table 4.18. Microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), dehydrogenase activity (DHA), acid phosphatase activity (APA), fungal population and bacterial population in bulk soil (BS) and rhizosphere soil of *Crassocephalum crepidioides* (Cc), *Ageratum conyzoides* (Ac) and *Bidens pilosa* (Bp) in different fallow site in wet season (WS) and dry season (DS).

		MBC (mg kg^{-1})		MBN (mg kg^{-1})		DHA (µg TPF g^{-1} soil h^{-1})		APA (µg PNP g^{-1} soil h^{-1})		Fungi (CFU 10^3 g^{-1} soil		Bacteria (CFU $10^6 g^{-1}$soil)	
Fallow	Soil	WS	DS	WS	DS	WS	DS	WS	DS	WS	DS	WS	DS
2 years	BS	201	146	16.9	11.2	5.95	3.32	21.3	14.35	19.1	14.2	36.8	25.7
	Cc	290**	238**	24.1**	16.9**	8.73**	5.47**	35.1**	26.0**	29.4*	23.8**	50.5*	40.5**
	Ac	328**	259**	28.5**	20.5**	9.54**	6.48**	39.9**	33.0**	33.7**	27.8**	58.4	40.6**
	Bp	308**	248**	25.6**	19.2**	9.22**	5.78**	38.2**	27.5**	33.9**	26.1**	52.8	41.2**
5 years	BS	257	192	22.5	16.6	8.97	5.13	32.6	20.8	25.5	20.2	43.9	33.9
	Cc	350**	274**	31.6**	25.4**	12.2**	7.78**	47.6**	30.8**	39.1**	33.5**	59.6	47.5**
	Ac	370**	268*	31.1**	24.7**	13.3**	8.01**	43.4**	35.6**	35.7**	24.6*	52.8	46.3**
	Bp	362**	292**	29.2**	24.4**	11.7**	7.22**	47.5**	32.8**	38.5**	33.1**	59.1	49.9**
10 years	BS	330	261	32.8	22.7	13.1	7.23	45.7	28.5	29.7	25.7	57.4	47.7
	Cc	428**	333**	40.2**	29.8**	16.2**	9.77**	59.8**	41.7**	40.6**	35.9**	71.9	56.3**
	Ac	420**	329**	39.6**	28.1**	15.7**	9.15**	61.8**	42.1**	39.2**	34.8**	70.8	56.4*
	Bp	449**	351**	44.8**	33.6**	17.4**	10.5**	66.9**	49.6**	44.5**	40.1**	76.2	60.4**

ns indicate non-significant variation between rhizosphere and BS by paired sample *t*-test.
* Significant variation between rhizosphere and BS at P<0.05.
** Significant variation between rhizosphere and BS at P<0.01.

4.5 Rhizosphere effect of annual plants in different fallows and treatments

Contrary to enhanced soil nutrients with fallow periods, the rhizosphere effect of annual plants with respect to all nutrients decreased considerably. The highest rhizosphere effect was noticed in FL-2 followed by FL-5 and Fl-10. The rhizosphere effect of annual plants on SOC and TN were given in Fig. 4.5(a) and (b). Rhizosphere effect on SOC significantly differed with fallow periods in both *C. crepidioides* and *A. conyzoides* except in *B. pilosa* between FL-5 and FL-10. However, rhizosphere effect on TN was not significant between FL-2 and FL-5 in *C. crepidioides* but significant in *A. conyzoides* among all fallow ages. The rhizosphere effect of SOC was greatest in *A. conyzoides* (62.75%) in FL-2 in dry period in FL-10 and lowest in *A. conyzoides* (15.75) of FL-10 during WS (Fig. 4.5a). Similarly, the rhizosphere effect on TN was greatest in *A. conyzoides* (51.75%) of 2 years fallow and lowest in *C. crepidioides* (18%) in FL-10 (Fig. 4.5b).

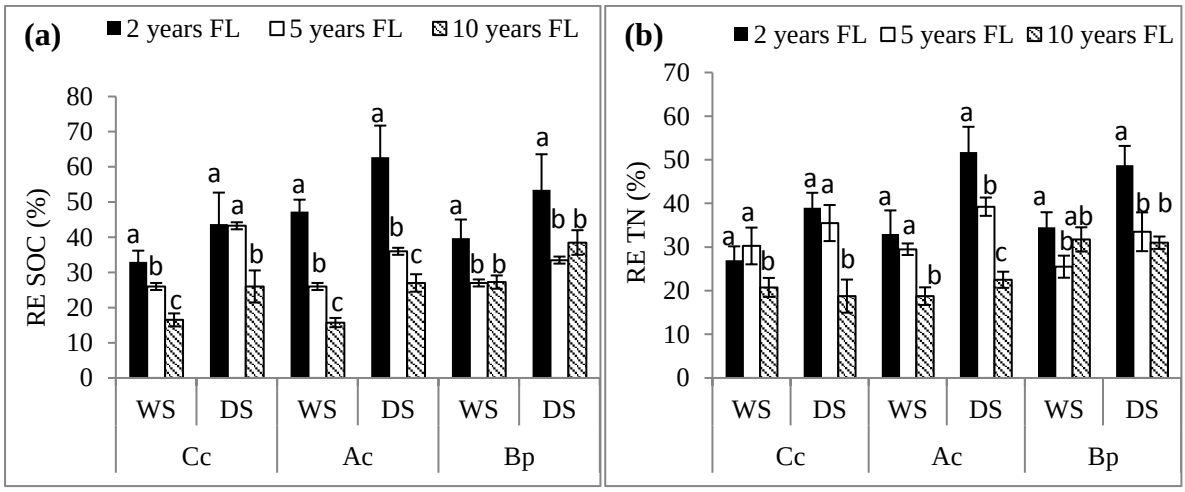


Fig.4.5. Magnitude of rhizosphere effect (RE) of *Crassocephalum crepidioides* (Cc), *Ageratum conyzoides* (Ac) and *Bidens pilosa* (Bp) on (a) SOC: soil organic carbon and (b) TN-total nitrogen in wet (WS) and dry (DS) season. Different letters indicate significant difference at $P<0.05$ in different fallow lands (FL).

In general, the rhizosphere effect of annual plants on MBC showed significant difference with fallow periods except in *B. pilosa* between FL-5 and FL-10 in WS (Fig. 4.6a).

The rhizosphere effect of *C. crepidioides* on MBN marked significant difference between FL-2 and FL-10 but not between FL-2 and FL-5. The highest (78%) and lowest (27%) rhizosphere effect on MBC was recorded in *A. conyzoides* of FL-2 and FL-10 respectively. In addition, significant difference in rhizosphere effect of *A. conyzoides* on MBN was recorded with fallow periods but no significant changed between FL-5 and FL-10 in *B. pilosa* (Fig. 4.6b). The rhizosphere effect of annual plants on MBN ranged from 20% -90% under different fallow periods.

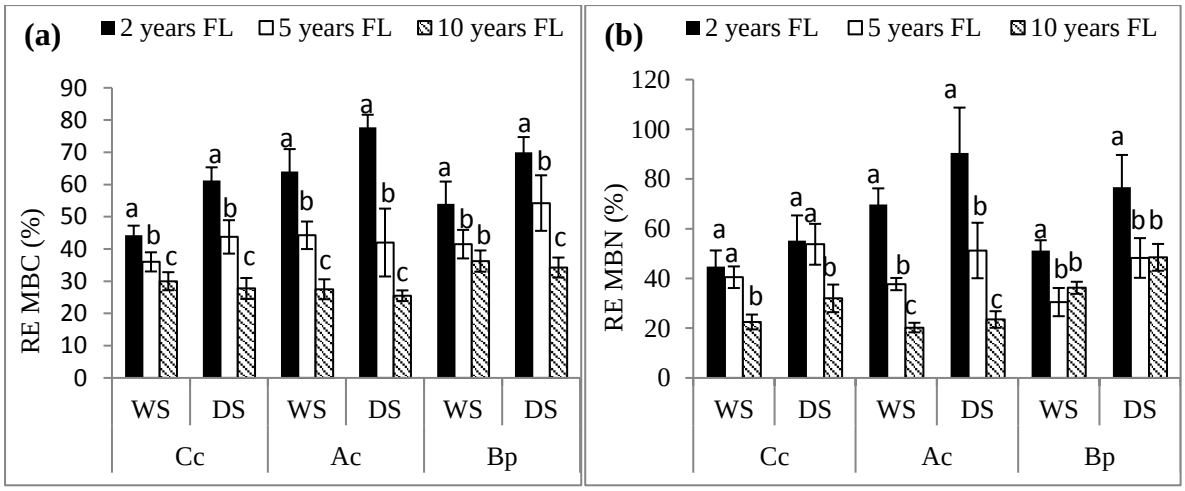


Fig.4.6. Magnitude of rhizosphere effect (RE) of *Crassocephalum crepidioides* (Cc), *Ageratum conyzoides* (Ac) and *Bidens pilosa* (Bp) on (a) MBC: microbial biomass carbon and (b) MBN: microbial biomass nitrogen in wet (WS) and dry (DS) season. Different letters indicate significant difference at $P<0.05$ in different fallow lands (FL).

The rhizosphere effect of *C. crepidioides* and *A. conyzoides* on DHA varied significantly with fallow periods but did not vary in *B. pilosa* between FL-5 and FL-10. The rhizosphere effect of annual plants on DHA ranged from 21% to 102% in FL-2 and FL-10. Similarly, no significant rhizosphere effect of *B. pilosa* on APA was recorded for FL-5 and FL-10 but significant variation in *C. crepidioides* in wet season and *A. conyzoides* in dry season was recorded (Fig. 4.7a). The rhizosphere effect on APA was significantly high (138%) in FL-2 in *A. conyzoides* and significantly less (32%) in FL-10 (Fig. 4.7b).

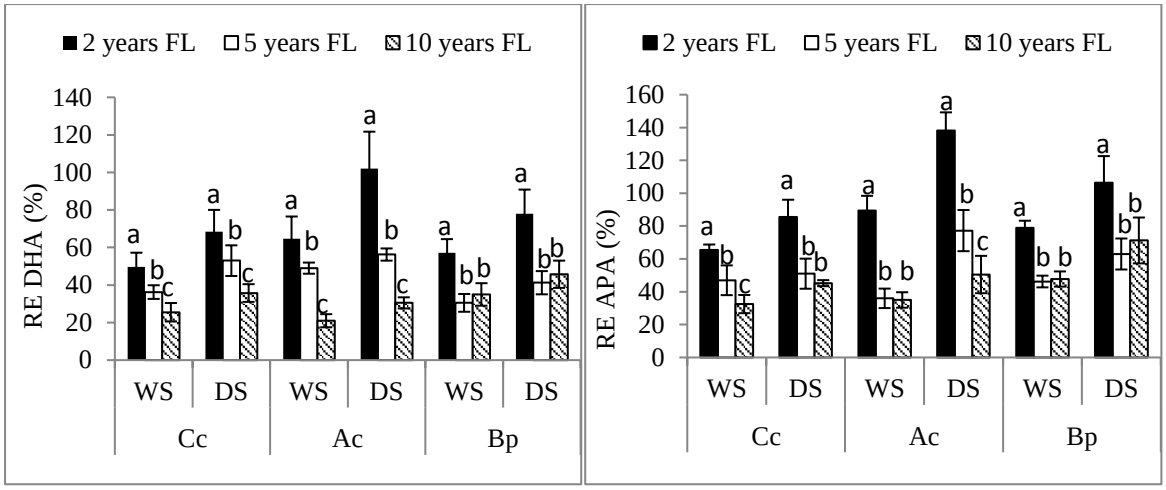


Fig.4.7. Magnitude of rhizosphere effect (RE) of *Crassocephalum crepidioides* (Cc), *Ageratum conyzoides* (Ac) and *Bidens pilosa* (Bp) on (a) DHA: dehydrogenase activity and (b) APA: acid phosphatase activity in wet (WS) and dry (DS) season. Different letters indicate significant difference at $P<0.05$ in different fallow lands (FL).

The rhizosphere effect of the three annual plants on NH_4^+ -N significantly differed with fallow periods. The magnitude of rhizosphere effect on NH_4^+ -N varied between 32%-109% in different fallow periods (Fig. 4.8a). Similarly, rhizosphere effect on $N_{\text{-min}}$ rate did not differ significantly between FP-5 and FP-10 in *C.crepidioides* and *B. pilosa* whereas significant difference was noticed in *A. conyzoides*. The percent rhizosphere effect on $N_{\text{-min}}$ ranged from 25% -78% in different fallow periods (Fig. 4.8b).

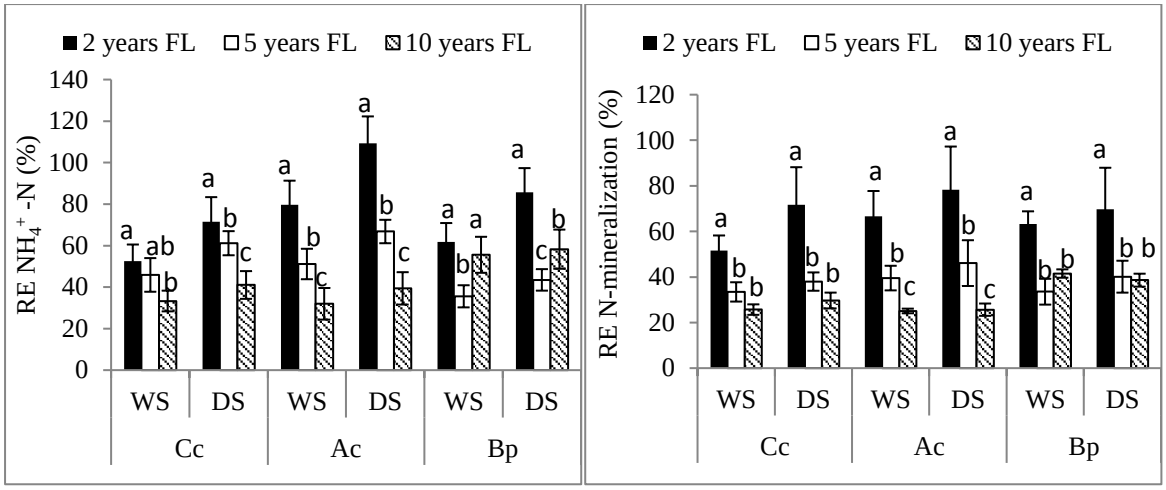


Fig.4.8. Magnitude of rhizosphere effect (RE) of *Crassocephalum crepidioides* (Cc), *Ageratum conyzoides* (Ac) and *Bidens pilosa* (Bp) on (a) NH_4^+ -N and (b) N-mineralization rate in wet (WS) and dry (DS) season. Different letters indicate significant difference at $P<0.05$ in different fallow lands (FL).

The rhizosphere effect of annual plants on soil variables under different treatments (T_S, T_M and T_L) in WS and DS were given in Table 4.19 and Table 4.20. In general, the rhizosphere effect of control was significantly greater than treatment plots in WS and DS. The magnitude of rhizosphere effect of annual plants ranged: 15-57% for SOC, 17-48% for TN, 19-90% for MBC, 15-82% for MBN, 18-112% for APA, 13-87% for DHA and 12-119% for N_{-min} in WS. Corresponding values were: 16-79% for SOC,13-61% for TN, 21-87% for MBC, 19-143% for MBN, 28-154% for APA, 24-140% DHA and 15-197% for N-min in DS. The rhizophere effect was highest for N_{-min} rates in *A. conyzoides* in FL-2 site in DS and lowest in *C. crepidioides* in WS. In addition, the rhizosphere effect of annual plants on soil microbial properties were significantly high in DS compared to WS.

Table 4.19. Magnitude of rhizosphere effect (%) of *Crassocephalum crepidioides* (Cc), *Ageratum conyzoides* (Ac) and *Bidens pilosa* (Bp) on soil biochemical properties in different treatments (T_S-Soil, T_M–Microbial inocula and T_L–Litter) and a control in different fallow ages during wet season.

		2 years Fallow			5 years Fallow			10 years Fallow		
Properties	Treatment	Cc	Ac	Bp	Cc	Ac	Bp	Cc	Ac	Bp
SOC	Control	42^{a}	57^{a}	55^{a}	29^{a}	28^{ab}	36^{a}	20^{a}	17^{a}	30^{a}
	T_S	31^{b}	46^{b}	38^{b}	30^{a}	25^{ab}	26^{ab}	19^{a}	16^{a}	30^{a}
	T_M	32^{b}	45^{b}	35^{b}	25^{a}	19^{b}	19^{b}	12^{a}	12^{a}	22^{a}
	T_L	27^{b}	41^{b}	31^{b}	20^{a}	32^{a}	27^{ab}	15^{a}	18^{a}	27^{a}
TN	Control	33^{a}	48^{a}	38^{a}	37^{a}	29^{a}	33^{a}	17^{a}	17^{a}	24^{a}
	T_S	31^{a}	23^{b}	41^{a}	38^{a}	26^{a}	23^{a}	25^{a}	14^{a}	35^{a}
	T_M	25^{ab}	33^{b}	34^{ab}	24^{ab}	31^{a}	24^{a}	24^{a}	21^{a}	34^{a}
	T_L	19^{b}	28^{b}	25^{b}	22^{b}	32^{a}	22^{a}	17^{a}	23^{a}	34^{a}
MBC	Control	53^{a}	80^{a}	65^{a}	37^{ab}	56^{a}	51^{a}	34^{a}	31^{a}	44^{a}
	T_S	43^{a}	69^{ab}	61^{a}	44^{a}	45^{ab}	47^{ab}	35^{a}	33^{a}	39^{ab}
	T_M	40^{a}	60^{bc}	56^{a}	32^{ab}	39^{b}	35^{b}	28^{ab}	27^{ab}	33^{ab}
	T_L	41^{a}	47^{c}	34^{b}	31^{b}	37^{b}	33^{b}	23^{b}	19^{b}	29^{b}
MBN	Control	60^{a}	76^{a}	46^{b}	52^{a}	42^{a}	38^{ab}	25^{a}	22^{a}	39^{a}
	T_S	51^{a}	82^{a}	63^{a}	36^{bc}	33^{a}	42^{a}	29^{a}	20^{a}	38^{a}
	T_M	36^{b}	69^{b}	51^{ab}	32^{c}	42^{a}	18^{c}	21^{a}	15^{a}	29^{a}
	T_L	32^{b}	52^{b}	45^{b}	42^{ab}	34^{a}	24^{bc}	15^{a}	24^{a}	39^{a}
DHA	Control	66^{a}	87^{a}	73^{a}	46^{a}	56^{a}	34^{a}	37^{a}	30^{a}	48^{a}
	T_S	54^{ab}	77^{ab}	63^{ab}	37^{ab}	51^{a}	39^{a}	27^{ab}	21^{ab}	37^{a}
	T_M	49^{b}	62^{b}	54^{bc}	33^{ab}	47^{a}	32^{a}	25^{ab}	20^{ab}	36^{a}
	T_L	30^{c}	33^{c}	39^{c}	29^{b}	42^{a}	17^{b}	13^{b}	13^{b}	19^{b}
APA	Control	71^{a}	112^{a}	91^{a}	65^{a}	51^{a}	56^{a}	39^{a}	46^{a}	52^{a}
	T_S	68^{a}	95^{b}	79^{b}	60^{a}	40^{a}	47^{ab}	43^{a}	35^{a}	54^{a}
	T_M	67^{a}	79^{c}	74^{b}	34^{b}	26^{b}	42^{ab}	30^{a}	36^{a}	51^{a}
	T_L	56^{a}	72^{c}	72^{b}	29^{b}	27^{b}	40^{b}	18^{b}	23^{b}	34^{b}
N-mineralization	Control	89^{a}	81^{a}	49^{b}	75^{a}	102^{a}	58^{ab}	95^{a}	82^{a}	119^{a}
	T_S	41^{b}	56^{b}	31^{b}	43^{b}	77^{b}	46^{b}	66^{bc}	38^{c}	73^{b}
	T_M	25^{c}	74^{a}	64^{a}	70^{a}	107^{a}	63^{a}	75^{b}	57^{b}	80^{b}
	T_L	12^{c}	35^{c}	10^{c}	45^{b}	54^{c}	20^{c}	51^{c}	42^{c}	78^{b}

SOC: soil organic carbon, total: total nitrogen, MBC: microbial biomass carbon, MBN: microbial biomass nitrogen, DHA: dehydrogenase activity, APA: acid phosphatase activity and N-mineralization rate. Different letters in columns indicate significant differences with treatments.

Table 4.20. Magnitude of rhizosphere effect (%) of *Crassocephalum crepidioides* (Cc), *Ageratum conyzoides* (Ac) and *Bidens pilosa* (Bp) on biochemical properties in different treatments (T_S-Soil, T_M–Microbial inocula and T_L–Litter) and a control in different fallow ages during dry season.

		2 years Fallow			5 years Fallow			10 years Fallow		
Properties	Treatment	Cc	Ac	Bp	Cc	Ac	Bp	Cc	Ac	Bp
SOC	Control	62^{a}	74^{ab}	66^{a}	52^{a}	46^{a}	44^{a}	36^{a}	34^{a}	46^{a}
	T_S	53^{ab}	79^{a}	72^{a}	44^{ab}	31^{b}	36^{ab}	31^{a}	27^{a}	42^{a}
	T_M	39^{b}	59^{b}	49^{b}	40^{ab}	34^{b}	29^{ab}	21^{a}	24^{a}	36^{a}
	T_L	21^{c}	39^{c}	27^{c}	37^{b}	33^{b}	25^{b}	16^{a}	23^{a}	30^{a}
TN	Control	40^{a}	60^{a}	53^{a}	44^{a}	39^{a}	22^{a}	13^{a}	18^{a}	31^{a}
	T_S	43^{a}	50^{ab}	56^{a}	30^{ab}	35^{a}	40^{a}	29^{a}	25^{a}	33^{a}
	T_M	44^{a}	61^{a}	50^{a}	41^{a}	45^{a}	41^{a}	13^{a}	21^{a}	27^{a}
	T_L	29^{a}	36^{b}	36^{a}	27^{b}	38	31^{a}	20^{a}	26^{a}	33^{a}
MBC	Control	70^{a}	79^{a}	83^{a}	57^{a}	64^{a}	73^{a}	25^{a}	28	34
	T_S	65^{ab}	87^{a}	71^{ab}	41^{ab}	56^{a}	62^{a}	37^{a}	25	43
	T_M	51^{b}	77^{a}	63^{b}	45^{ab}	23^{b}	33^{b}	27^{a}	28	31
	T_L	59^{ab}	68^{a}	61^{b}	32^{b}	25^{b}	49^{b}	22^{a}	21	29
MBN	T_C	83^{a}	143^{a}	108^{a}	75^{a}	82^{a}	60^{a}	46^{a}	33^{a}	64^{a}
	T_S	56^{b}	86^{b}	88^{b}	35^{c}	53^{b}	64^{a}	33^{a}	18^{a}	45^{b}
	T_M	46^{bc}	72^{bc}	56^{c}	54^{b}	32^{c}	35^{b}	30^{a}	20^{a}	39^{b}
	T_L	36^{c}	61^{c}	55^{c}	51^{b}	38^{c}	34^{b}	19^{b}	23^{a}	46^{b}
DHA	Control	97^{a}	140^{a}	103^{a}	72^{a}	65^{a}	52^{a}	47^{a}	37^{a}	66^{a}
	T_S	74^{b}	127^{a}	97^{a}	61^{a}	53^{a}	48^{a}	40^{ab}	34^{a}	46^{a}
	T_M	61^{b}	88^{b}	60^{b}	42^{b}	57^{a}	41^{ab}	29^{b}	25^{a}	38^{a}
	T_L	42^{c}	53^{c}	52^{b}	37^{b}	50^{a}	24^{b}	27^{b}	26^{a}	33^{a}
APA	Control	72^{b}	150^{a}	99^{b}	71^{a}	104^{a}	84^{a}	45^{a}	81^{a}	109^{a}
	T_S	109^{a}	143^{a}	102^{b}	62^{a}	93^{a}	72^{ab}	45^{a}	53^{b}	75^{b}
	T_M	97^{a}	154^{a}	151^{a}	37^{b}	53^{b}	55^{bc}	50^{a}	40^{bc}	46^{c}
	T_L	64^{b}	106^{b}	74^{c}	34^{b}	59^{b}	41^{c}	41^{a}	28^{c}	55^{c}
N-mineralization	Control	137^{a}	197^{a}	186^{a}	34^{c}	62^{b}	24^{b}	53^{b}	15^{c}	87^{b}
	T_S	46^{b}	124^{b}	79^{c}	63^{b}	97^{a}	23^{b}	55^{b}	23^{c}	73^{b}
	T_M	52^{b}	120^{b}	100^{b}	91^{a}	103^{a}	52^{a}	131^{a}	127^{a}	174^{a}
	T_L	43^{b}	98^{c}	72^{c}	67^{b}	79^{b}	42^{a}	46^{b}	35^{b}	70^{b}

SOC: soil organic carbon, total N: total nitrogen, MBC: microbial biomass carbon, MBN: microbial biomass niitrogen, DHA: dehydrogenase activity, APA: acid phosphatase activity and N-mineralization rate. Different letters in columns indicate significant differences with treatments.

4.6. Changes in root exudation rates in three annual plants with fallows

Root exudates were collected for a period of six month from June to November, 2014 to evaluate the monthly variation on rate of exudation among annual plants. Over six sampling months, no significant monthly variations were recorded from annual plants. However, the rate of exudation was higher in post-monsoon season compared to monsoon season (Fig. 4.9). Greatest exudation rate was observed in *A. conyzoides* in FL-2. The exudation rates ranged from 0.32-0.97 mg C fine root^{-1} day^{-1} and were strongly influenced by plant species as well as soil condition. No significant difference in root exudation rate between *A. conyzoides* (0.84 mg C fine root^{-1} day^{-1}) and *B. pilosa* (0.76 mg C fine root^{-1}day^{-1}) in FL-2 and FL-5 was observed. Further, the rate of exudation did not vary in *C. crepidioides* and *A. conyzoides* in FL-10 (Fig. 4.10a). Among fallow periods, root exudation was highest in FL-2 followed by FL-5 and FL-10. Significant changes were recorded among fallow periods in *C. crepidioides* and *A. conyzoides* but not in 5 and 10 years fallow in *B. pilosa* (Fig. 10b). Root exudation rate in annual plants during DS was significantly higher compared to WS. The highest exudation rate was noted in DS in *A. conyzoides* of FL-2 (0.916 mg C fine root^{-1} day^{-1}) and lowest in WS of *C. crepidioides* in FL-10 (0.416 mg C fine root^{-1}day^{-1}) (Fig. 11).

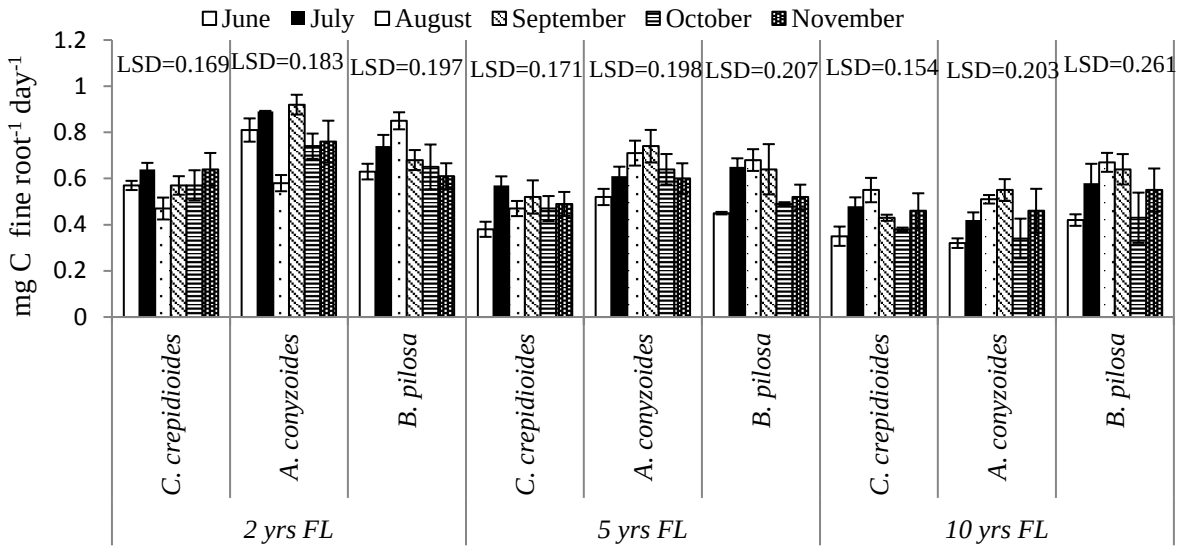


Fig.4.9. Monthly variation in root exudation rate among annual plants in different fallow lands (FL).

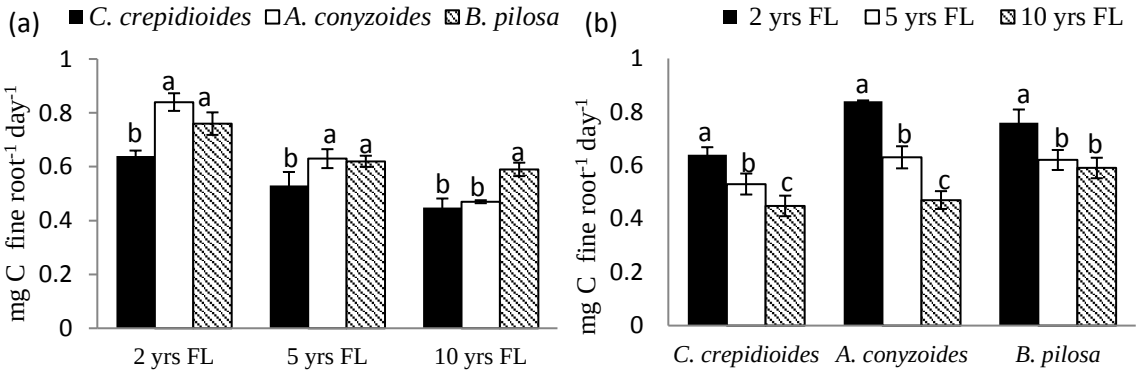


Fig.4.10. Changes in root exudation rate (average of six sampling dates) (a) among annual plants (b) among different fallow lands (FL).

4.7. Correlation and multivaraite analysis of rhizosphere soil variables

The correlation coefficient values (r) among different soil nutrients in RS of annual plants for WS and DS were presented (Table 4.21a and b). The soil variables are significantly positively correlated with each other in WS except soil pH, which showed negative correlation with MBC, MBN, DHA, nitrification and NO_3^- -N. SMC in WS was also significantly negatively correlated with TN. Generally, in DS, soil variables showed

significantly positively correlated with each other except in few soil variables like SMC and soil pH. The amount of MBC showed no positive correlation with SOC, DHA, APA, NO_3^--N and nitrification rate.

Result of multivariate analysis (3-way ANOVA) of different soil variables of RS during WS and DS were shown (Table 4.22a and b). The RS soil nutrients showed significant variations with fallow periods and treatments except in SMC and soil PH both in WS and DS. Among the RS of annual plants, significant variation was marked in SOC, MBC, APA, DHA and fugal population during WS. On the other hand, significant variation was recorded in TN, MBC, APA, DHA and bacterial population during DS.

Table 4.21a. Pearson's correlation coefficient among soil variables of RS of annual plants in wet season.

Soil variables	SMC	pH	SOC	TN	MBC	MBN	DHA	APA	Fungi	Bacteria	NO_3^--N	NH_4^+-N	Nitrification	N-min
SMC	1													
pH	-0.59	1												
SOC	0.79*	0.04	1											
TN	0.55	0.05	0.85**	1										
MBC	0.85**	-0.06	0.92**	0.80**	1									
MBN	0.90**	-0.04	0.86**	0.77**	0.91**	1								
DHA	0.84**	-0.08	0.87**	0.82**	0.91**	0.94**	1							
APA	0.84**	0.01	0.91**	0.86**	0.93**	0.91**	0.96**	1						
Fungi	0.81**	0.06	0.89**	0.83**	0.87**	0.90**	0.90**	0.90**	1					
Bacteria	0.81**	0.13	0.85**	0.75**	0.77**	0.91**	0.85**	0.86**	0.86**	1				
NO_3^--N	0.82**	-0.11	0.83**	0.69**	0.89**	0.84**	0.79**	0.81**	0.87**	0.68**	1			
NH_4^+-N	0.76**	0.02	0.95**	0.84**	0.91**	0.88**	0.93**	0.95**	0.85**	0.88**	0.73**	1		
Nitrification	0.64**	-0.02	0.86**	0.92**	0.81**	0.85**	0.92**	0.91**	0.84**	0.79**	0.71**	0.89**	1	
N_{-min}	0.75**	0.01	0.87**	0.88**	0.87**	0.91**	0.96**	0.93**	0.91**	0.84**	0.77**	0.89**	0.95**	1

SMC: soil moisture content, SOC: soil organic carbon, TN: total nitrogen, MBC: microbial biomass carbon, MBN: microbial biomass niitrogen, DHA: dehydrogenase activity, APA: acid phosphatase activity, N_{-min}: N-mineralization rate.

* indicate significant varaition at $P<0.05$.

** indicate significant variation at $P<0.01$.

Table 4.21b. Pearson's (2-tailed) correlation coefficient among soil variables of RS of annual plants in dry season.

Soil variables	SMC	pH	SOC	TN	MBC	MBN	DHA	APA	Fungi	Bacteria	NO_3^--N	NH_4^+-N	Nitrification	N-min
SMC	1													
pH	-0.09	1												
SOC	0.56	0.04	1											
TN	0.67*	0.11	0.91**	1										
MBC	0.36	0.44	0.57	0.61*	1									
MBN	0.47	0.18	0.85**	0.85**	0.73**	1								
DHA	0.57	-0.02	0.64*	0.69*	0.05	0.47	1							
APA	0.75	-0.03	0.81**	0.90**	0.56	0.73**	0.67*	1						
Fungi	-0.08	0.55	0.62**	0.52	0.62*	0.61*	0.09	0.24	1					
Bacteria	0.41	0.33	0.85**	0.88**	0.81**	0.82**	0.43	0.79**	0.75**	1				
NO_3^--N	0.49	0.17	0.96**	0.86**	0.61*	0.89**	0.62*	0.76**	0.64*	0.84**	1			
NH_4^+-N	0.67*	0.12	0.92**	0.92**	0.45	0.79**	0.78**	0.85**	0.45	0.79**	0.91**	1		
Nitrification	0.58*	0.32	0.89**	0.85**	0.56	0.85**	0.58*	0.75**	0.61*	0.79**	0.92**	0.88**	1	
N_{-min}	0.72**	0.13	0.91**	0.90**	0.695*	0.87**	0.56	0.91**	0.48	0.84**	0.89**	0.87**	0.91**	1

SMC: soil moisture content, SOC: soil organic carbon, TN: total nitrogen, MBC: microbial biomass carbon, MBN: microbial biomass niitrogen, DHA: dehydrogenase activity, APA: acid phosphatase activity, N_{-min}: N-mineralization rate.

* indicate significant varaition at $P<0.05$.

** indicate significant variation at $P<0.01$.

Table 4.22a. Results of multivariate analysis (3-way ANOVA) among plants, fallow periods and treatments for soil characteristics in wet season.

Soil parameters	Plants	Fallow	Treatment	Plants × fallow	Plants × Treatment	Fallow × Treatment	Plants × Fallow × treatment
Soil pH	ns	*	ns	ns	ns	ns	ns
SMC	ns	ns	ns	ns	ns	ns	ns
SOC	*	**	**	*	ns	ns	ns
TN	ns	**	**	*	ns	ns	ns
MBC	**	**	**	**	ns	ns	ns
MBN	ns	**	**	*	ns	ns	ns
APA	**	**	**	ns	ns	*	ns
DHA	*	**	**	**	ns	ns	ns
Fungi	*	**	**	*	ns	ns	ns
Bacteria	ns	**	**	**	ns	ns	ns
NH_4^+ -N	ns	**	**	ns	ns	ns	ns
NO_3^- -N	ns	**	**	**	ns	ns	ns
N_{-min}	ns	**	**	ns	ns	ns	ns
Nitrification	ns	**	**	ns	ns	ns	ns

SMC: soil moisture content, SOC: soil organic carbon, TN: total nitrogen, MBC: microbial biomass carbon, MBN: microbial biomass niitrogen, DHA: dehydrogenase activity, APA: acid phosphatase activity, N_{-min}: N-mineralization rate. * indicate significant varaition at $P<0.05$, ** indicate significant variation at $P<0.01$ and ns indicate no-significant vaariation.

Table 4.22b. Results of multivariate analysis (3-way ANOVA) among plants. Fallow periods anmd different treatments for soil characteristics in dry season.

Soil parameters	Plants	Fallow	Treatment	Plants × fallow	Plants × Treatment	Fallow × Treatment	Plants × Fallow × treatment
Soil pH	ns	ns	ns	ns	ns	ns	ns
SMC	ns	ns	ns	ns	ns	ns	ns
SOC	ns	**	**	*	ns	ns	ns
TN	*	**	**	*	ns	ns	ns
MBC	**	**	**	**	ns	*	*
MBN	ns	**	**	*	ns	ns	ns
APA	*	**	**	**	ns	ns	ns
DHA	*	**	**	**	ns	ns	ns
Fungi	ns	**	**	ns	ns	ns	ns
Bacteria	*	**	**	ns	ns	ns	ns
NH_4^+ -N	ns	**	**	ns	ns	ns	ns
NO_3^- -N	ns	**	**	ns	ns	*	ns
N_{-min}	ns	**	**	*	ns	ns	ns
Nitrification	ns	**	**	*	ns	**	ns

SMC: soil moisture content, SOC: soil organic carbon, TN: total nitrogen, MBC: microbial biomass carbon, MBN: microbial biomass niitrogen, DHA: dehydrogenase activity, APA: acid phosphatase activity, N_{-min}: N-mineralization rate. * indicate significant varaition at $P<0.05$, ** indicate significant variation at $P<0.01$ and ns indicate no-significant vaariation.

Chapter 5

Discussion

Fallow length play significant role in soil fertility and crop productivity in the shifting cultivation because of quantity and quality of regenerating plant biomass of secondary vegetation and associated microbes over time. The relationship between litter mass and its impact on soil fertility and associated microbes under different fallows has still been poorly understood. Scientific studies suggest that litter and soil microbes may have profound effects for sustainable shifting agricultural practices. Further, rhizosphere processes, particularly root exudation and rhizodeposition along with associated microbes have been reported to play a vital role in maintaining plant growth and ecosystem stability especially in nutrient-limited soil condition by critically affecting the soil nutrient cycling. Rhizosphere processes has been least studied relative to its importance on vegetation establishment and forest growth. Therefore, rhizosphere may serve as potential factor for improving soil fertility and crop productivity in agricultural ecosystem, especially in shifting agriculture.

This chapter deals with analysis of the effect of addition of litter-soil-microbial consortium on soil biochemical and microbial properties in different fallow lands. It also discusses the impact of fallow length as well as rhizosphere effect of early generating annual plants on soil microbial properties in different fallow ages. Finally, this study explains the seasonal variation in root exudation of annual plants in different fallow ages. The experimental findings of the present study are discussed hereunder as follows:

5.1 Changes in vegetation composition with fallow periods

Vegetation survey in the present study indicates that the annual plant species *A. conyzoides* (IVI-97%) is the pioneer and dominant species during first young fallow land (Fl-2 and Fl-5). However, in old fallow land (FL-10), due to increase in plant diversity (1.91) and

richness (5.13), competition among annual plants upsurge and *A. conyzoides* (IVI-28%) and *C. crepidioides* (IVI-39%) were inhibited and the site was dominated by *B. pilosa* with IVI-63% (Hauchhum and Tripathi, 2017b). The dominance of annual plants in fallow land from early stage to mid-stage may be due to variation in the past agriculture activities that might have inhibited the proliferation of plants with long-life cycle. On the other hand, annual and bi-annual plants with stable soil seed pool occupied the fallow land and become dominant during early and mid-stages of succession. These results clearly revealed that the plant community in shorter fallow phase (FL-2 and FL-5) was dominated by single species and multiple species with competitive plant community in longer fallow (FL-10) phase (Hauchhum and Tripathi, 2017b). These findings supports that species richness in abandoned land is usually relatively lower during the earlier stage of succession which is in consistent with previous findings (El-Sheikh, 2005; Wang *et al.*, 2009). Zhang *et al.* (2012) also reported that species richness gradually increases from 1-year abandoned cropland (1.71) to 15-years abandoned cropland (3.07) and then rapidly decreased thereafter. Therefore, the present study supports that species composition and richness of annual plants gradually increased from younger fallow land (FL-2 and Fl-5) to older fallow land (FL-10).

5.2 Impact of fallow length on soil properties in RS and BS

The inclusion of fallow period in shifting cultivation is an agricultural management practices to restore the soil productivity after cropping phase, mainly by accumulation of organic matter, soil nutrients and water (Sarmiento, 2000). The fallow period caused changes in the soil physico-chemical as well as biochemical properties in both the sampling season except SMC (Table 4.22 a and b). Previous findings highlighted variation in soil properties under different fallow period (Giardina *et al.*, 2000; Kavvadias *et al.*, 2001). In the present finding, the physico-chemical and microbial properties in BS and RS were enhanced significantly with increase in fallow length. Increase BS pH with increasing length of fallow

phase as well as after burning was more prominent in acidic soils than in alkaline soils and was reported to be the result of loss of OH, oxide formation and release of alkaline cations like Ca, Mg, and K (Certini, 2005). Increasing soil pH with fallow period was recently reported by Terefe *et al.,* 2008; Granged *et al.,* 2011; Kulmala *et al.,* 2014. In contrast to increased pH in BS, RS significantly decrease with increase fallow length which may be due greater root exudates of annual plants in different fallow land (Hauchhum and Tripathi, 2017b).

Present findings indicate that TN and SOC in BS were significantly enhanced with increased fallow period which may be due to increase in C with increase length of forest succession and that the site could regain its original status (Tinker *et al.,* 1996). On the other hand, TN and SOC in RS increase with fallow period and the difference was significant. This may be the result of greater enhancement of SOC and TN by the rhizosphere processes of annual plants in young fallow land compared to annual plants in old fallow land. The rhizosphere of the three annual plants showed increased MBC and MBN with increase length of fallow period indicating enhances metabolic abilities of rhizosphere microbes on nutrient substrate in old fallow land. Similarly, the amount of MBC and MBN in BS significantly enhanced in longer fallow length suggesting improvement of soil fertility status through secondary succession in fallow land (Hauchhum and Tripathi, 2017b). This indicates that the age of fallow land had positive effect on soil microbial biomass build up. Increase in size of biological pool with fallow period was thought to be due to greater substrate input from diverse vegetation in older fallows (Haripal and Sahoo, 2013).

Soil enzymes are an important indicator of microbial activity and always depend on soil types, vegetation cover, microbial biomass and microbial diversity during vegetation succession (Taylor *et al*., 2002). In the present study, the enzyme activities (DHA and APA) in RS and BS marked significant increase with increasing fallow age. Increasing fallow age

caused gradual addition of organic matter to the soil through decomposition of litter and root biomass of vegetation developed on surface layer. Further, increased organic matter content supply and nutrient substrates act as energy source for microorganisms and increase the microbial population, hence, higher enzyme activities. Our results corroborate the previous finding by Cheng and Yue, (2012) and Haripal and Sahoo, (2013) The present finding indicates that the RS showed increase APA and DHA with fallow period, a possible reason for this finding is less nutrient cycling in young fallow due to abiotic stress in young fallow, whereas, in subsequent successional stage, abiotic stress is benign and nutrient substrate utilization by RS microbes is maximum that is stimulated by the plant root exudates. The present result is in conformity with earlier results reported by Miethling *et al*. (2000), Chen *et al*. (2002) and Knauff *et al*. (2002).

Soil microorganisms are responsible for most biological transformations that result to the development of nutrients in the soil (Shculz *et al*., 2013). These microbes have significant impact on the soil function and are considered key indicator of soil quality. The current study revealed that the microbial populations (fungal and bacterial) in BS and RS were significantly influenced by fallow age. The significant increase in microbial population in BS of longer fallow length may be the result of greater SMC and accumulation of organic matter due to increase in species richness and abundance in old fallow. The larger number of bacterial population than fungal population is not surprising as soil usually contains greater number of bacteria than fungi (Sylvia *et al*., 2005). The possible reason for this is that bacteria are less vulnerable to changes in soil and environmental conditions unlike fungi which are easily controlled by changes in soil pH, nutrient and harsh environmental conditions (Jin *et al*., 2010, Sui *et al*., 2012). The increased amount of microbial population in RS with fallow period suggest that the rhizosphere microbes are favoured by the greater nutrient substrate

supply in older fallow land and plant root exudates also play vital role in determining the microbial population. It indicates that the microbes found in RS are greatly influenced by the presence of organic matter, soil and environmental conditions.

Increased vegetation cover and plant richness in older fallow land could have retain the available nutrients (e.g. N and P) which was an important factor for increase in NH_4^+ -N and NO_3^- -N with fallow period. Enhanced nitrification and $N_{\text{-min}}$ in older fallow than young fallow may be attributable to increased organic matter decomposition and greater microbial activity (Singh *et al.,* 2001). Greater concentration of ammonium than nitrate may be the result of potential loss of nitrate through leaching in sloping agriculture field where surface runoff is high. Furthermore, the acidic nature of the soil also might have inhibited the activity of autotroph nitrifiers in the soil to some extent that reduced the rate of nitrification (Chao *et al*., 1993).

5.3 Changes in soil properties in RS and BS

Rhizosphere, a narrow zone ~2 mm around roots, has been reported greater physico-chemical and biological properties than BS because of presence of the presence of higher microbes and their activities (Koranda *et al.,* 2011), which are fueled by the continuous release of number of low molecular weight organic compounds by the roots. The current investigation revealed significant difference between RS and BS and indicate that soil biochemical and microbial properties were significantly higher in RS compared to BS. The soil pH significantly decrease in RS compared to BS may be due to exudation of organic anions by plant roots as well as respiration in alkaline soils, cation-anion exchange balance by roots, and redox-coupled processes. The change in rhizosphere pH has been demonstrated by several researchers (Zhao *et al.,* 2010; Calvaruso *et al.,* 2014). The changes in soil pH in

the present study were similar to that in rhizosphere of silver birch by Rosenvald *et al.* (2010) and in rhizosphere of annual plants by Zhang *et al.* (2012).

Cycling in plant roots under different soil and environmental conditions may significantly vary due to soil condition, nutrient requirement, growth period and type of vegetation (Grayston *et al.,* 1996). The present study demonstrates significant increase of SOC and TN in the rhizosphere of annual plants compared to BS. This result may be due to accelerated C exudation by plant roots and greater microbial activities in the RS. Jones *et al.* (2009) reported that rhizodeposits account for as much as 25% of the total C allocated belowground or 10% of the net C fixed through photosynthesis. Enhance MBC and MBN in RS may be the resultant of higher microbial population and activity in this zone and the release of metabolites through root exudation that stimulate the growth and reproduction of microbes here in. In Loess plateau, Zhang *et al*. (2012) have reported that increase microbial properties in the RS compared to BS was facilitated by dominant annual species at the abandoned land. Consequently, higher enzyme activities of APA and DHA (Martinez-alcala *et al*., 2010,Tscherko *et al*., 2004; Gardner *et al*.,2011) and population of bacteria and fungi (Broeckling *et al*., 2008; Khanikar and Singh, 2015) in rhizosphere indicate dominating role of microbes by supplying sugars, acids, hormones, sloughed root cells and mucilage through root exudation that serves as a source of energy for microorganisms to enhance their activity.

Increase in NH_4^+ -N, nitrification rate and $N_{\text{-min}}$ rate RS compared to BS have been reported by most previous studies on tree seedlings and trees in forest ecosystem (Singh *et al.,* 2001). The observation in the present study showed enhance concentration of NH_4^+ -N, nitrification rate and $N_{\text{-min}}$ rate in RS. Gradual increase in potential $N_{\text{-min}}$ and nitrification rate in RS indicate that plant roots stimulated gross $N_{\text{-min}}$ in the present study, as net $N_{\text{-min}}$ rate is the result of gross $N_{\text{-min}}$ and N-mobilization. Generally, the rhizosphere priming effects on soil organic matter is the primary result for enhance $N_{\text{-min}}$ in the rhizosphere (Colin- Belgrand

et al., 2003; Phillips and Fahey, 2006). Nitrogen transformation is strongly related to rate of organic matter decomposition and may be strongly augmented by the rhizodeposition which in turn activated the microbial growth (Jackson *et al*., 2008). The greater net nitrification rate in RS relative to BS did not result in increased accumulation of NO_3^- -N in rhizosphere. On contrary to increase NH_4^+ -N and N_{-min} in RS, the concentration of NO_3^- -N significantly decrease in RS compared to BS indicating the uptake of NO_3^- -N by the plant roots and microbes in rhizosphere exceeded the nitrification rate. Since, the study site soil is N-limited, and thus it is suggested that the microbial growth is N-limited rather than C-limitation, and that rhizosphere C-flux may reduce the N-availability if nutrient – limited rhizosphere microbes immobilize N (Cheng *et al*., 1996; Phillips and Fahey, 2006). Changes in N-concentration in plant rhizosphere varied among different studies. For example, significantly higher NH_4^+ and unchanged NO_3^- concentration in rhizosphere relative to BS were reported by Colin- Belgrand *et al.* (2003) in three forest sites and in mature Douglas–fir (*Pseudosutga menziesii*) stand by Turpault *et al.* 2005. On the other hand, Wang et al. (2005) observed great depletion of NH_4^+ and NO_3^- in rhizosphere of Norway spruce (*Picea abies*) and European beech (*Fagus sylvatica*) seedlings. Erhenfield *et al.* (1997) found that NH_4^+ and NO_3^- were not influence by live plant roots in mineral soil.

The present study indicates that the microbial populations (fungal and bacterial) were significantly greater in RS compared to BS. These findings are in agreement with other works reported higher fungal and bacterial population in RS compared to BS of different plants (Broeckling *et al*., 2008; Khanikar and Singh, 2015). Significant changes in distribution of microbial population as influence by different plants have been reported by Koeberl *et al*. (2013). Higher bacterial population than fungal population in the present study revealed that the rhizosphere of the plant studied favours the growth of bacteria which is similar to earlier reports (Tamilarasi *et al*., 2006; Karthikeyan *et al*., 2008). Broeckling *et al*. 2008 reported

that population richness of microbes in particular rhizosphere of plant species is the result of influence of root exudates released by plant roots. It shows that plant exudates play an important role in the microbial activity that gradually increase the microbial population in RS compared to BS. The seasonal changes in microbial population in rhizosphere could be due climate and soil variables that affect the growth of microbes. In addition, higher fungal and bacterial populations during WS may be the result of increase SMC and exudation of palnt roots in the rhizosphere zone (Rigobelo and Nahas, 2004). From the result of analysis of correlation coefficient, it indicates that variation in microbial population is greatly influence by seasons where fungal and bacterial population positively significantly correlated with SMC in WS but not in DS. Seasonal influence on rhizosphere microbes had also been reported by Collado *et al.* (1999) and Gao *et al.* (2005).

5.4 Seasonal variation on soil properties

In the current investigation, changes in soil properties between WS and DS were tested. It shows that the soil properties were significantly influence by seasonal variation where WS has higher biochemical and microbial properties compared to DS. Earlier studies highlight increase soil chemical and microbial properties during WS relative to DS (Hauchhuma and Tripathi, 2017b). Seasonally, the TN, SOC, MBC and MBN show peaked amount during WS and significantly low during DS. The high amount of MBC and MBN during WS may be the result of increased SMC content and relative humidity which is ideal for microbial activity. Earlier studies reported a close relationship between SMC and microbial biomass (Ravina et al., 1995; Devi and Yadava, 2006). Lower amount of microbial biomass during DS may be due to low SMC that suppressed the activity of soil microbes. In addition, higher mobilization of soil nutrients by microbes during the composition of organic matter results in increase microbial biomass during WS relative to DS. Yang *et al.* (2010) reported that an increase of soil microbial biomass results in immobilization of nutrients,

whereas decrease in microbial biomass results in mineralization of nutrients. During the cold and dry winter i.e DS, a slow rate of decomposition, attributable to low microbial activity, might have resulted in greater immobilization of inorganic N by microorganisms resulting in reduced N_{-min} (Maithani *et al*., 1996).

In the present study, the enzyme activities showed peak value during WS relative to DS. During DS, due to moisture stress, the microbes were in dormant state and their activities were greatly reduced resulting in lower value of enzyme activities but increased SMC during WS season stimulate the microbial activity that enhance the plant growth as well as the microbial population. This may be responsible for increased microbial population in WS than DS. Analysis of correlation coefficient between soil SMC and microbial properties reveal significant positive correlation during WS (Table 4.21a) but not in DS (Table 4.21b). These results indicate that SMC had significant influence on soil microbial properties. The present results are in conformity with precious findings by Binarani and Yadava, (2010) and Haripal and Sahoo, (2014).

5.5 Variations in the magnitude of rhizosphere effects of annual plants

Dominant species among the three annual plants studied in different fallow period showed greater magnitude of rhizosphere effect on soil microbial properties. The magnitude of rhizosphere effect of annual plants is poorly understood and there is limited information especially in annual plants of different fallow period. Such information on rhizosphere effect of annual plant in different fallow land is crucial for improving our understanding on how annual plants contribute to soil fertility under environmental stress condition depending on the fallow age. Soil condition and plant species have been reported to play an important role in determining the rhizosphere effect (Hinsinger *et al*., 2009). Present study suggest that the recovery soil fertility in fallow land was accelerated by the rhizosphere effect of dominant

annual plant species and the magnitude of this effect depends on the level of soil fertility as reflected by fallow ages. Vegetation succession results in competition for soil nutrients and abiotic resources between different species in plant ecosystem. Usually different plant species show variation in soil microbial properties in the rhizosphere due to differences in microbial population as well as soil changeable conditions (Tscherko *et al.,* 2004). The present study revealed that the rhizosphere microbial populations were higher in dominant species like *A. conizoides* in FL-2 and FL-5 and *B. pilosa* in FL-10 compared to other species; this may be responsible for greater rhizosphere effect in dominant species and indicate that microbes in rhizosphere play an important role in the magnitude of rhizosphere effect. In addition, the current investigation revealed that the amount of C exudates collected from dominant annual plant showed significant increase compared to other companion species. These results demonstrated that dominant species utilize higher amount of C and N substrate than other companion species. Dominant species in plant ecosystem usually has higher root system and exhibit stronger competiveness for substrate resources than other species. Wang *et al.* (2009b) observed that plant aboveground biomass and coverage are positively correlated with belowground biomass, fine root length density and fine root area density. However, aboveground and belowground biomass as well as fine root density and area were not analyzed in this study.

The successional stage in plant ecosystem can significantly affect the soil microbial properties in plant rhizosphere. On contrary to enhanced rhizosphere microbial properties with fallow period, the rhizosphere effect of annual plants significantly decreased with fallow periods. Increased rhizosphere effect in young fallow with oligotrophic soil conditions was possibly the result of higher exudation rates per unit of roots as a result of greater root-microbe interaction, which led to rapid growth of early regenerating plants. However, in longer fallow phase, RS fertility slowly enriches the BS and thereby decreases the

rhizosphere effect due to increased area of rhizosphere as a result of root proliferation (Hauchhum and Tripathi, 2017b). In addition, variation in rhizosphere effect of plants is largely influence by the amount and types of exudates from plant roots. In addition, the C-exudation was higher in shorter fallow land compared to longer fallow land. The amount of root exudates regulates the magnitude of rhizosphere effect in annual plant species found in different fallow land. This may be responsible for greater rhizosphere effect in shorter fallow land. Further, as species richness and abundance gradually increase from shorter fallow phase to longer fallow phase, it shows that competition for soil nutrient substrate and abiotic resources would be less in shorter fallow phase and in turn, facilitate the plant species to absorb more nutrient in the rhizosphere zone thereby increasing the rhizosphere effect in shorter fallow phase.

The rhizosphere effect of annual plants studied has significantly increased soil nutrients in RS of control plots compared to treated (T_S, T_M and T_L). This shows that soil-litter-microbial inocula alter the rhizosphere effect of annual plants on soil properties. The rhizosphere effect of annual plants on SOC ranged from 34%-74% in T_C whereas in T_L it ranged from 16%-39%. Similarly, in other soil variables like TN, MBC, MBN, APA, DHA and $N_{\text{-min}}$, the rhizosphere effect was significantly enhanced in control compared to amended soils. The present findings are in agreements with previous works by Colin-Belgrand *et al*. (2003) where it was observed that rhizosphere effect of *Psudosutga* and *Picea* on NH_4^+ and $N_{\text{-min}}$ considerably reduced in fertilized plot compared to unfertilized plot. Similarly, Phillips and Fahey, (2008) found that fertilization had substantially reduced the rhizosphere effect of red oak and sugar maple in MBC and MBN. These findings suggest that decreased belowground C allocation and decreased C flux from roots to soil microbes in response to increase in soil nutrients. In addition, the increased rhizosphere effects in control may have resulted from the stimulatory effects of nutrients on the rhizosphere microbes.

5.6 Variation in root exudates of annual plants with fallow periods

Root-microbe interactions play an important role in coupling C and nutrient cycles (Cheng *et al.,* 2014), and knowledge of how plants root-microbe interactions influence ecosystem processes is critical for predicting the biogeochemical activities and shifts in forest composition. Rhizodeposition may represent a key biogeochemical process determining the amount and sink strength for C in plant ecosystem, this information on root C exudation in mature forest trees has been quantified but estimation of the root C exudates of annual plants is limited and rare. In this study, we quantify the amount of C exudation of annual plants like *A. conyzoides*, *C. crepidioides* and *B. pilosa* over six sampling months in different fallow phases. The magnitude of rhizosphere effect as well as the C exudation rate significantly varied among the plants studied which may the result of differences in ability of roots to acquire soil nutrients and structure of the plant roots. In the present study, we found that magnitude of rhizosphere effect and mass-specific exudation rate was greatest in dominant plants (~10-20%) of each fallow site compared to other companion species. The greater mass-specific exudation in dominant plants in each site may be related to the root morphology, root densities and fine root biomass. The result of root C exudation by annual plants suggests the timing of exudation measurements can contribute to variable estimates among ecosystems. Seasonal variation have significant influence on the root C exudates depending on temperature, rainfall and soil temperature with the highest exudation rates occurring in July and September compared to lower exudation rates in June and October. Thus, soil temperature play significant role in belowground C flux (Urbanski *et al.,* 2007; Yin et al., 2014; Tückmantel *et al.,* 2017). However, the soil temperature of the study site was not measured in this study. These shows that abiotic factors have significant influenced the C in root exudates and the source-sink C relationships may also be responsible for variation in C

flux (Kaiser *et al.,* 2010). Hence, collecting root exudates during only one or two season may result in relatively low or high estimates (Brzostek *et al.,* 2013).

Variation in exudation rates among different plants is thought to be driven by site-specific factors such as nutrient availability, rooting densities, and abiotic factors of the study sites. In the present investigation, it was recorded that the root C exudation rate was greater in shorter fallow with nutrient deficient site compared to longer fallow with fertile soil. In general, root exudation is increased in response to nutrient deficiency in the top soil (Neumann and Romheld, 1999; Phillips *et al.,* 2011; Yin *et al.,* 2014) due to the active secretion of specific carboxylates via anion channels (Jones *et al.,* 2004), the same is observed in the present study. The current investigation demonstrates that annual plants in shorter fallow has mineralized soil organic matter decomposition and $N_{\text{-min}}$ in the rhizosphere, a process which would couple rhizosphere C fluxes with nutrient return (i.e. greater need to exude and prime, to get nutrients out of soil organic matter) due to low nutrient availability. In contrast, due to greater addition of nutrient substrates and faster decomposition rates leading to higher mineral nutrients in longer fallow presumably results in decreased utilization of nutrients from soil organic matter that in turn may drastically reduce the root exudation. Thus, enhanced exudation may be an evolutionarily stable strategy for increasing nutrient availability if plant release more energy-rich C in stimulating microbial activity (i.e., a greater C cost) in exchange for the greater nutrient return of soil organic matter-degrading microbes access (Cheng *et al.,* 2014). These results indicate that the root exudation rate of different plants depends on the soil nutrient availability where co-occurring plant species have both scavenging and mining the nutrient acquisition.

5.7 Impact of soil-litter-microbial inocula amendment on soil properties

Soil-litter-microbial inocula amendment was conducted after slashing and burning of the study sites. This study was conducted to analyze the impact of different amendment on soil physico-chemical and microbial properties of different fallow phase. Amendment showed slight changed in the soil properties as compared to field soil i.e. soil without any amendment. The findings in this study revealed that soil biochemical and microbial properties were higher in amended soil compared to unamend soil in the order T_L>T_M>T_S>control. Significant change in soil physico-chemical and microbial properties were observed in T_L as compared to other amendment i.e. T_S and T_M. Addition of litter from natural forest showed significant increase in soil microbial properties. Increase in SOC from 1.22% to 2.53% after litter amendment was recorded in Bangladesh (Sarkar *et al*., 2010). Similarly, Novara *et al*. (2015) recently found that litter addition from secondary forest successions in abandoned agricultural land increases the content of SOC from 1.5% to 1.9% in the top soil layer and from 1.4% to 1.7% up to a depth of 15 cm. Isaac and Nair, (2002) also observed that the addition of jack leaf litter significantly increase SOC. Fascinatingly, the types of litter differentially impacted SOC content in amended soils. Increase in SOC content was significantly higher in FL-10 compared to other site i.e. FL-2 and FL-5 amended soil. Similarly, the addition of litter show varying effect on the availability of N (NH_4 and NO_3) as well as nitrification and N_{-min} rate in different fallow land. The amendment of litter significantly increase the available N compared with unamend soil. However, the amendment of forest soils and microbial inocula did not significantly change the available N as compared with unamended soil. It is well recognized that addition of litters activates the soil microbial populations and elicit the rate of decomposition process (Sarkar *et al.*, 2010). Consequently, the rate of nitrification and N_{-min} significantly enhanced due to greater microbial activity and this may be a probable cause of increasing trend of soil N in litter amendment soil.

The present study revealed that microbial biomass (MBC and MBN) were significantly enhanced in T_M and T_L compared to control. However, no significant difference was recorded between T_S and control in microbial biomass. Increase in MBC and MBN with litter amendment with increasing fallow length supports the findings by Chen*et al.,* (2006), Jiang *et al*., (2009) and Li *et al.*, (2014). In the current investigation, the amendment of litter and microbial inocula considerably increases the amount of biomass utilized by soil microbes as nutrient substrate that significantly enhances the value of MBC and MBN. The amount of MBC and MBN largely depend on the availability of soil organic matter (Chen *et al.,* 2006). Increased amount of MBC and MBN with the types and amount of litter incorporated could also be correlated with the soil organic matter contributed by leaf litters and the microbes working on it that determines the rate of litter decomposition.

Findings in the present study demonstrate an increase in enzyme activity i.e. APA and DHA in T_M and T_L compared to control during fallow phase. On the other hand, comparison of T_S and control did not show significant variation. This indicate that forest soil amendment did not influence the microbial community which may be due to less addition of forest soil that will support changes in microbial properties and enzyme activities of *jhum* soil. The amount of DHA and APA significantly increased after litter amendment in a microcosm experiment conducted by Li *et al*. (2014) in Southern China. Similar results were obtained in the present investigation, for example, longer fallow length support higher activity of DHA and APA. These results corroborate the findings of Haripal and Sahoo, (2013) who reported an increase in DHA and APA activity while studying impact of conversion of forest land to agricultural lands in Odisha, India in a chronosequence of 2 year, 4 year, 6 year, 11 year and 15 year forest fallow. Similarly, the current finding indicate that microbial populations (Fungal and bacterial) in T_L and T_M may due to the increase in organic biomass from litter

and microbial inocula that provided nutrient resources for growth and development of soil microbes.

Chapter 6

Summary and Conclusions

The rhizosphere processes of annual plants (early regenerating plants) on soil variables play an important role in vegetation development during secondary succession. Understanding the interaction of soil-microbes and plant root become vital for proper functioning of the ecological processes. On the other hand, vegetation succession and successional stage (age of fallow land) following shifting cultivation had significant influence on the growth and progress of plant ecosystem. Further, slashing of forest vegetation and burning in *jhum* farming is considered as a major source of soil nutrient loss in hill tropical region. Therefore, amendment of soil-litter from natural forest and microbial inocula would be an important technique to enhance soil fertility for sustainable agricultural practices. In addition, knowledge on exudation rate of C from the plant roots and its impact on RS properties as well as its effect on re-establishment of vegetation in fallow land would be fascinating to understand the root exudates of annual plants. Thus, studies on the impact of fallow periods and soil-litter-microbial amendment on soil variables in different fallow period would be important to sustain soil fertility level in shifting cultivation. Further, exudation rate of C and impact of rhizosphere effect of annual plants on soil variables would improve our understanding on the relationship between soil and associated root microbes that can be manipulated to increase soil fertility and crop productivity in shifting agriculture.

Three shifting cultivation sites with different fallow age (FL-2, FL-5 and FL-10) were selected from Muallungthu village where most of the people commonly practice shifting agriculture system. After slash and burn activities, early regenerating annual plants were analyzed using quadrat method to determine species richness and abundance of the regenerating plants. The three sites were divided into four plots (10m × 30m) for different amendment and control. The first plot consists of soil amendment with forest soils from

adjacent forest, the second amendment include application of litter collected from adjacent forest; third amendment was carried out with microbial consortium and fourth plot was without any amendment i.e. control plot. Amendment was carried out after burning i.e. the month of March. From each plot, three annual species like *Ageratum conyzoides*, *Crassocephalum crepidioides* and *Bidens pilosa* were selected to study the RS properties in relation to BS. The magnitude of rhizosphere effect of annual plants was calculated as the percent difference between RS and BS sample for each soil variable. Soil sampling was carried out at the month of June (wet season) and December (dry season), 2014, of annual plants like *Crassocephalum crepidioides*, *Ageratum conyzoides* and *Bidens pilosa* were excavated at 20 cm depth from each plot (10m × 10m) with the help of soil corer (5 cm diameter). One composite soil (~500g) consists of 12 soil cores randomly taken within each plot. Composite soil was thoroughly mixed and divided into three replicates. The soil physico-chemical and microbial properties were analyzed to examine the impact of different amendment and rhizosphere effect. Root exudates (C) was collected from roots of annual plants selected for a period of six months i.e. June –November, 2014. The method employed for collecting root exudates was given by Phillips *et al.* (2008).

The major findings under different objectives of the present study are summarized below:

Growth and development of early regenerating plant species were influenced by the age of fallow land but dominance of annual plant species changed with fallow length. For example, *A. conyzoides* was the dominant species in FL-2 (IVI, 97) followed by *B. pilosa* (IVI-61) and *C. crepidioides* (IVI-42). However, the dominance was almost equally shared by all three species in FL-5 and IVI ranged from 59-61. *B. pilosa* was the most dominant species (IVI-63) in FL-10 followed by *C. crepidioides* (IVI-38) and *A. conyzoides* (IVI-28). Thus, plant species diversity and richness increases with fallow ages.

Soil variables in the present study showed significant variation with respect to fallow period, treatment and annual plants except in SMC and pH.Highest amount of SMC was marked in RS of *B. pilosa* in FL-10 (30.34%) and lowest was recorded in BS of FL-2 (13.4%). Similarly, significant variations were not recorded in soil pH between treatments and control in both sampling seasons; soils were highly acidic with soil pH ranged from 4.91-5.6. The amount of SOC and TN were considerably higher in all the treatments (T_S, T_M and T_L) compared to control with significant variation (P<0.05) in T_L compared to control whereas no significant changed in T_S and T_M. The amount of SOC and TN ranged from 2.05-4.82 mg g^{-1} and 0.20-0.47 mg g^{-1}, respectively in WS and 1.31-4.19 mg g^{-1} and 0.15-0.40 mg g^{-1} respectively in DS. The highest percent increment from T_C to T_L in SOC was recorded in RS of *C. crepidioides* (76%), similarly, in TN, the highest increment was marked in RS of *B. pilosa* (55%).

The amount of NH_4^+ -N, NO_3^- –N, nitrification and N_{-min} rates were relatively higher in all the treatments (T_S, T_M and T_L) as compared to control and significantly differed with the sampling season i.e. WS and DS. The concentration of NO_3^- –N ranged from 0.262-0.528 mg kg^{-1}. There was an average increment from control to T_M and T_L in NO_3^- –N is 37% and 46% respectively in WS and 94% and 59% in DS. No significant change was observed in NH_4^+ -N between treatments and control in WS, however, significant variation was marked between T_M and T_L in DS. The amount of NH_4^+ -N varies from 0.227-1.024 mg kg^{-1}. Rate of nitrification was significantly increased in T_L compared to control both in the season but no significant change was seen in case of T_S and T_M. Rate of nitrification in WS was 0.121-0.661 mg kg^{-1} and 0.085-0.535 mg kg^{-1} in DS. The average increment in nitrification rate from control to T_L during WS and DS is 37% and 56%. Similarly, N_{-min} rate was significantly higher in $T_M$and T_L relative to control in all the sites as well as in the RS of all the three

plants. The value of N_{-min} rate ranged from 0.244-1.341 mg kg^{-1} in WS and 0.136-1.003 mg kg^{-1}in DS.

The microbial properties (e.g. MBC, MBN, DHA and APA) were considerably higher for all the treatments (T_S, T_M and T_L) relative to control and significant difference in microbial properties were marked between each sampling season i.e. WS and DS. The value of MBC and MBN ranged from 123-479 mg kg^{-1} and 7-49 mg kg^{-1} respectively and the highest amount of MBC and MBN was recorded in *B. pilosa* in FL-10 and lowest in BS of FL-2. In addition, the amount of APA and DHA varied from 11-79 µg PNP g^{-1} soil h^{-1} and 2.49-18.76 µg TPF g^{-1} soil h^{-1} respectively. The mean percent increment recorded from control to T_M and T_L was: MBC (T_M-16% and T_L-25%), MBN (T_M-24% and T_L-35%), APA (T_M-20% and T_L-36%) and DHA (T_M-20% and T_L-33%) in WS. Further, the increment in DS was: MBC (T_M-17% and T_L-24%), MBN (T_M-27% and T_L-44%), APA (T_M-34% and T_L-60%) and DHA (T_M-15% and T_L-25%). Number of colonies of microbial population i.e. fungal and bacterial population significantly increased in T_L and T_M compared to control however no significant change was observed in T_S. In additionto it, the number of colonies formed by bacterial population considerably increased compared to fungal population. Number of colonies counted in fungal population ranged from 14-50 CFU 10^3 g^{-1} soil in WS and 9-48 CFU 10^3 g^{-1} soil in DS. Moreover, number of bacterial colonies counted in WS was 25-85 CFU 10^6 g^{-1} soil and in WS was 16-67 CFU 10^6 g^{-1} soil. The average increment percent from control to T_M and T_L for fungal population was 27% and 41% in WS and 47% and 59% in DS. Similarly, the average increment from control to T_M and T_L for bacterial population was 24% and 35% in WS and 29% and 43% in DS.

The amount of SMC and soil pH significantly did not change with fallow period. The amount of SOC significantly varied with fallow periods in RS of *C. crepidioides* in both the sampling seasons. However, significant change was not noted in RS of *A. conyzoides* during

WS and *B. pilosa* in DS. Similarly, the amount of TN significantly differed with fallow periods in RS of *C. crepidioides* but no significant difference was observed in RS of *A. conyzoides* as well as *B. pilosa* in DS. The concentration of NH_4^+ –N and NO_3^- –N marked significant variation with fallow periods. The mean increment in NH_4^+ –N from FL-2 to FL-5 and FL-10 was 39% and 63% respectively. Similarly, the average increment percent in NO_3^- –N from FL-2 to FL-5 and FL-10 was 68% and 145% respectively. The rate of nitrification and $N_{\text{-min}}$ significantly enhanced (P<0.05) with fallow period. The increment from FL-2 to FL-10 in nitrification rate was highest in RS of *B. pilosa* (123%) and lowest in RS of *A. conyzoides* (92%). Similarly, the increment percentage from FL-2 to FL-10 in $N_{\text{-min}}$ was greatest *B. pilosa* (105%) and *A. conyzoides* (56%).

The microbial properties (e.g. MBC, MBN, APA and DHA) of RS significant increased (P<0.05) with fallow periods. The mean increment in MBC from FL-2 to FL-10 in RS of *C. crepidioides, A. conyzoides* and *B. pilosa* was 43.75%, 27.5% and 43.49% respectively. Similarly, the amount of MBN increased from FL-2 to FL-10 by 71%, 38% and 75% in RS of *C. crepidioides, A. conyzoides* and *B. pilosa* respectively. The highest increment in APA from FL-2 to FL-10 was recorded in *B. pilosa* (78%) followed by *C. crepidioides* (65%) and *A. conyzoides* (41%). Similarly, increase in DHA from FL-2 to FL-10 was greatest in *B. pilosa* (85%) followed *C. crepidioides* (82%) and *A. conyzoides* (53%). Colonies of fungal and bacterial population in RS of the three annual plants significantly enhanced with fallow periods. The average increment in fungal population from FL-2 to FL-10 was highest in RS of *C. crepidioides* (45%) followed by *B. pilosa* (43%) and *A. conyzoides* (21%). Likewise, increase in bacterial population from FL-2 to FL-10 was higher in *B. pilosa* (45%) and lowest in *A. conyzoides* (30%).

Root exudates were collected for a period of six month from June to November, 2014 to evaluate rate of exudation (C) among annual plants. The exudation rates ranged from 0.32-

0.97 mg C fine root^{-1} day^{-1}. Rate of root exudation was highest in *A. conyzoides* both in FL-2 and FL-5. However, in FL-10, root exudation was highest in *B. pilosa*. Among fallow periods, root exudation was highest in FL-2 period followed by FL-5 and FL-10. Root exudation rate in annual plants during DS was significantly high compared to WS and the highest exudation rate DS was marked in *A. conyzoides* of FL-2 (0.916 mg C fine root^{-1} day^{-1}) and lowest in recorded in WS of *C. crepidioides* in FL-10 (0.416 mg C fine root^{-1}day^{-1}).

Fallow periods had significant influence on the rhizosphere effects annual plants and rhizosphere effects considerably increased in shorter fallow phase as compared to longer fallow phase. Similarly, the effects of different amendment alter the rhizosphere effect. The rhizosphere effect of annual plants significantly increases in control as compared to amendment.

The amount of SMC content significantly increase in RS compared to BS. In general, the soil biochemical and microbial properties were significantly enhanced in RS compared to BS. However, the concentration of NO_3 significantly reduced in RS with respect to BS which may be due to greater uptake of NO_3 by the plant root.

In conclusion, the present study demonstrates that litter amendment (T_L) had significant influence on soil variables that stimulate microbial activity for sustainable agricultural practices especially in nutrient deficient soil of shifting cultivation. The present investigation also revealed that rhizosphere nutrient cycling of annual plants in young fallow land had significant impact on soil properties that accelerate re-establishment of vegetation in fallow land. Enhancement of soil microbial properties in RS compared to BS indicates that microbial activity in RS was stimulated by the nutrient substrates that act as a source of energy by microbes. Root exudates from plant roots also play an important role for increase microbial properties in RS. This result shows soil-microbe interaction with associated plant

root had significantly influenced the rhizosphere effect of different plant species on soil variables. The greater rhizosphere effect of dominant annual plants strongly indicates that the RS microbes could be an important factor for sustainable agriculture farming by inoculating with the seeds of different crops. It can be concluded litter treatment and rhizosphere microbes of annual plants play significant role for sustaining shifting cultivation by altering the soil quality for better growth and development of crops.

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Photo Plates

Crassocephalum crepidioides ***Ageratum conyzoides***

Forest fire and after burning in Muallunghtu

Bidens pilosa **Soil Collection**