

**INFLUENCE OF RHIZOSPHERE ON SOIL FERTILITY IN
DIFFERENT LAND USE SYSTEMS OF MIZORAM**

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**INFLUENCE OF RHIZOSPHERE ON SOIL FERTILITY IN
DIFFERENT LAND USE SYSTEMS OF MIZORAM**

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

MIZORAM UNIVERSITY

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DECLARATION

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

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

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CERTIFICATE

This is to certify that the thesis entitled “**Influence of Rhizosphere on Soil Fertility in Different Land Use Systems of Mizoram**” submitted to the Mizoram University, Aizawl for the award of the degree of Doctor of Philosophy in Forestry is the original work carried out by Mr. Chowlani Manpoong (Regd. No.MZU/Ph.D/777 of 19.05.2015) under my supervision. I further certify that the thesis is the result of his own investigation and neither the thesis as a whole nor any part of it was submitted earlier to any University or Institute for the award of any degree. The candidate has fulfilled all the requirements laid down in the Ph.D. regulations of the Mizoram University.

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

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LIST OF NOTATIONS AND ABBREVIATIONS

Notations		Abbreviations	
%	Percent	Al	Aluminum
e.g.	Exempli gratia	BF	Bamboo Forest
et al	Et alia	C	Carbon
etc	Et cetera	Ca	Calcium
°C	Degree Celsius	CEC	Cation exchange capacity
h	Hour	FL	Fallow Land
p <0.01	Significant level at 1 percent	K	Potassium
p <0.05	Significant level at 5 percent	LSD	Least significant difference
R	Correlation coefficient	MBC	Microbial biomass carbon
ha	Hectare	Mg	Magnesium
kg	Kilogram	MWD	Mean weight diameter
g	Gram	N	Nitrogen
mg	Milligram	Na	Sodium
mg g⁻¹	Milligram per gram	NH₄ – N	Ammonium nitrogen
mg kg⁻¹	Milligram per kilogram	NF	Natural Forest
µg g⁻¹	Microgram per gram	NO₃ – N	Nitrate nitrogen
mm	Millimetre	OPP	Oil Palm Plantation
m	Metre	OTUs	Operational taxonomical units
cm	Centimeter	P_{avail}	Available phosphorous
m²	Metre square	PD	Phylogenetic diversity
cm²	Centimeter square	RP	Rubber Plantation
meq	Milli equivalent	SE	Standard error
Cmol kg⁻¹	Centi mole per kilogram	SEF	Soil evaluation factor
		SFI	Soil fertility Index
		SOC	Soil organic carbon
		sp	Species
		SR	Soil respiration
		TN	Nitrogen
		yr	Year

CHAPTER 1

INTRODUCTION

1.1 Preamble

Land use is a physical entity of topography and spatial arrangement of natural resources including soil, minerals, water and biota. Landscape transformations from natural forest ecosystems to multitude of land use types (e.g. secondary forests and plantations) are linked with noticeable changes in structure and functioning of ecosystem at various spatio-temporal scales. Landscape changes are occurring very rapidly in to the ecosystems which lead to land degradation and decline in level of soil fertility. These changes are most profound in North-East India as result of increasing population densities which adversely affect the soil productivity of natural and modified ecosystems. The soils in this hill region are prone to heavy erosion and degradation mostly due to shifting cultivation practices and heavy rainfall during the rainy seasons. The cultivation practices along the slope have caused a rapid loss in tree cover that eventually led to change in soil physico-chemical and biological properties, which further affects process of soil aggregation and C sequestration in the soil. Shifting cultivation locally known as *Jhum* is a traditional means of agriculture practice which include clearing and burning of forests after which the cultivation of multiple crops takes place. Recently, due to increase in population needs and suitability of the soil and climatic conditions, vast area has been developed into Oil Palm and Rubber plantation.

Land use change is ecologically sensitive component of the tropical forest ecosystems affecting all components of the ecosystems. Changes in the ecosystem components significantly affect the fertility of soil by altering rhizosphere structure, function and interactions. Therefore, it is important to understand the effect of land use change on rhizosphere properties and to effectively manage the soil properties as a result of changing land use patterns in the region.

1.2 Land use change in Mizoram

Mizoram is a hilly region covering a geographical area of 21, 087 km² with 85% forest cover having the second largest forest cover with respect to its geographical area in the country (Forest Survey Report, 2017). It is a region with rich biodiversity where the land use change has been a widespread phenomenon besides shifting agriculture (Tripathi *et al.*, 2016; Grogan *et al.*, 2012). Tropical and subtropical forests of Mizoram are significantly affected by land use change, particularly shifting cultivation and plantations which profoundly affects soil fertility and crop productivity. The monoculture practice of rubber and oil palm plantations has occurred widely across the region (Economic survey report, Mizoram 2016-2017) to sustain productivity and economic growth of the state.

As per the Forest Survey Report, (2017), the forest area of the state has increased significantly which was due to increased regeneration of bamboo species and plantations. Further, the large extent of natural forest has been converted to Oil Palm and Rubber plantations as per land capability, suitability and sloppy terrain of the region. The shifting cultivation has been practiced since time immemorial and is considered as a predominant land use system covering 19 to 45 % forest area. Recent

decline in forest cover have occurred due to increased settled agriculture systems and decline in area under jhum cultivation. This is probably due the introduction of the new land use policy (NLUP) by the Government of Mizoram.

The significant decrease in the area of shifting cultivation is primarily due to increase in the plantations of Oil Palm and Rubber. Out of the total area (i.e. 21, 08, 700 hectares) of the state, total potential area of Oil palm plantation is 1, 01, 000 hectares with 1,000 hectares of Rubber plantations in different districts (Economic survey Mizoram, 2016-2017). In the year 2013-2014 the Oil palm plantation in Aizawl district was expanded to 331 hectares. In addition, 57 percent of the geographical area is covered with bamboo forest showing positive impact in terms of forest cover. The distribution of land use and land cover of six districts of Mizoram is shown in table 1.1. The maximum proportion of area has been covered by open forest, bamboo, dense forest and abandoned *jhum*. Besides, these land use category, agriculture and horticulture plantations have played a main role in uplifting the livelihood and food security of the people. Land use and land cover of Aizawl district is shown in figure 1.1.

1.3 Land use change and soil properties

Land use change is considered as one of the major factors that affect the nutrient distribution pattern in the soil. Exponential increase in anthropogenic activities particularly, shifting cultivation has profoundly affect the soil fertility by causing substantial loss in soil nutrient availability (Wapongnungsang *et al.*, 2018). Land use modifications in tropical region have led significant changes in soil properties by strongly affecting the soil organic matter content. The factors such as

vegetation cover, quality and quantity of litter fall and root distribution pattern may cause a significant difference in the distribution of soil organic carbon in different land uses. The change in soil organic matter due to conversion of natural forest to various land uses may have crucial effects on soil physico-chemical and biological properties.

Table 1.1 Distribution of land use categories (km²) in different districts of Mizoram

Land Use Categories	Districts					
	Aizawl	Champhai	Lawngtlai	Lunglei	Mamit	Saiha
Built-up lands	66.8	23.3	22.6	28.1	16.7	12.6
Wet rice cultivation	16.5	46.5	21.5	8.1	7.09	12.1
Agri/Horti plantations	24.3	19.7	8.4	9.1	8.93	1.05
Dense forest	482.2	715.8	495.1	639.1	406.3	448.7
Open forest	1320.1	1374.4	658.9	1422.2	727.4	384.9
Forest plantation	19.4	10.6	18.2	18.8	13.01	5.69
Bamboo	1134.4	457.3	844.9	1871.1	1563.1	362.0
Scrubland	12.8	9.8	19.4	18.9	21.4	12.8
Water body	18.7	11.3	24.1	36.6	17.7	9.05
Current Jhum	177.5	187.7	93.6	157.6	72.9	46.4
Abandoned Jhum	303.2	328.4	270.1	326.4	170.3	103.6

(Source: Lallianthanga *et al.*, 2014a; Lallianthanga *et al.*, 2014b; Lallianthanga & Hmingthanpuii, 2013; Lallianthanga & Sailo, 2013a; Lallianthanga & Sailo, 2013b; Lallianthanga & Sailo, 2013c).

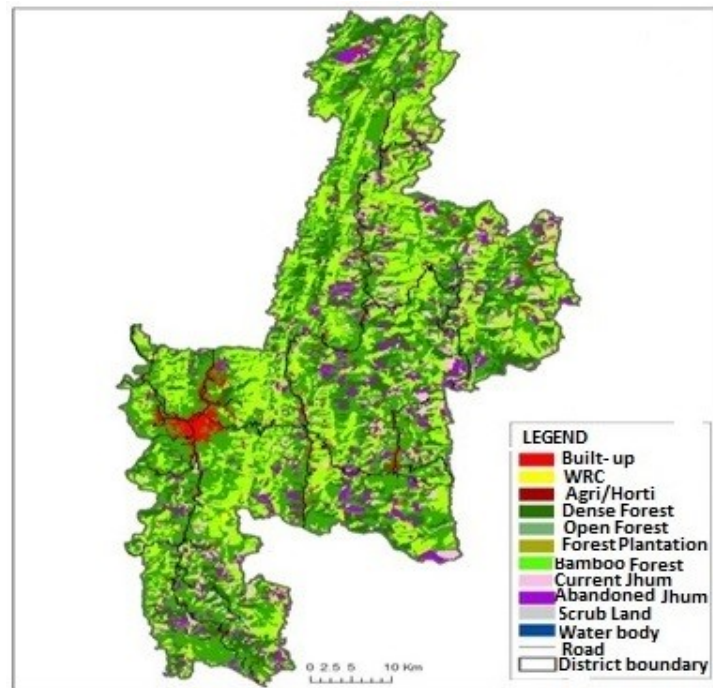


Figure 1.1 Land use and land cover of Aizawl district of Mizoram (Source: Lallianthanga & Hmingthanpuii, 2013).

Soil pH influences the physical, chemical and biological properties of the soil. A little change in soil pH induces significant alterations in the rhizosphere region by significantly affecting the microbial communities and their activities in the rhizosphere. Soil organic carbon is an important indicator of soil quality. It acts as one of the major components of the global C cycle and varies significantly with relation to land use change (Ali *et al.*, 2017). Changes in vegetation type may impair C cycling, decrease SOM content, and increase CO₂ emissions depending on the addition of plant litter and root exudates (Murty *et al.*, 2002). The presence of high C/N ratio in plant litters decreases the decomposition rates due to which the litters accumulates on the soil surface (Tejada *et al.*, 2009), at the same time plant litter having low C/N ratio (<25) degrades faster which eventually enhances the nutrient

availability in the soil. SOM mineralization is reduced in acidic soils due to less abundance of bacterial populations and their activity.

Soil microorganisms play an important role in organic matter decomposition. The soil microbial properties like microbial biomass, metabolic quotient and enzymes activity regulate the soil nutrient availability by affecting the soil ecological processes (Jiang *et al.*, 2009). These properties are mainly responsible for the change in primary productivity of any ecosystem and thus it can be considered as an indicator to ecological stress (Dick *et al.*, 1997).

Microorganisms through their enzymatic activities help in maintaining the soil ecosystem function by degrading soil organic matter, catalyzing the biochemical reactions involved in nutrient cycling and energy transfer (Sinsabaugh *et al.*, 1991). Therefore, microbial activities are recognized as one of the important indicators of the changes in soil management and indicate early responses to ecosystem change as a result of any disturbance.

The CO₂ released from the soil is the product of autotrophic and heterotrophic respiration. CO₂ released during the processes of decomposition of SOM by microbes referred to as heterotrophic respiration. In addition, roots respiration also contributes to the CO₂ efflux from soil indicating autotrophic respiration. Soil microbial biomass can be estimated by soil respiration rates which can further be related to nutrient cycling in the soil (Park *et al.*, 2012). Soil respiration rate is generally low when SOM declines indicating less microbial activity in the soil. Physical factors such as soil temperature, moisture, aeration including the availability of nitrogen also influences soil respiration rates. Further, a

magnitude of nutrient turnover in an ecosystem could be indicated by the soil respiration rate.

1.4 Land use change and rhizosphere

The structural changes in tree species composition and biomass accumulation during ecosystem development have been well documented (Singh *et al.*, 2015), very few investigations have been carried out on the functioning of secondary forests (Chazdon, 2014). Studies are highly limited on changes in the soil microbial communities in plantation soils (i.e. Rubber and Oil Palm). Aboveground changes in vegetation composition and soil characteristics may profoundly alter the microbial composition in the rhizosphere soil, and thus investigating the microbial composition and soil properties in forests and plantations would be an important effort to recommend restoration practices for landowners and public agencies.

The term rhizosphere, coined for the first time by Hiltner, a German Scientist in 1904, who reported intense bacterial activity in the soil surrounding roots. More recently, the term has been broadened to describe the narrow zone of soil adjacent to the plant roots where the microbial population and their activities are significantly more than that of the bulk soil (Darrah, 1993; Lynch, 1990). The rhizosphere zone is highly sensitive to small changes in plant species, habitat, climate, soil type etc. (Jones, 2004; Hartmann *et al.*, 2009) because of changes in delicately balanced relationships in root-microbe interactions. Land use change has been reported to prominently affect rhizosphere properties by altering the native bacterial community structure and functioning (Jangid *et al.*, 2013). The conversion of natural forests to

various land use practices may alter ecological patterns through modified rhizosphere characteristics.

It is a unique environment with coexistence of soil and roots where the soil physico-chemical and biological properties changes due to the effect of root biomass and associated activities. This results into a unique atmosphere in the rhizosphere region different from that of bulk soil (Hinsinger *et al.*, 2009; Bais *et al.*, 2006). This region is endowed with rich microbial diversity and is reported 10-100 times larger than in the bulk soil (Holland *et al.*, 2016). Thus the microbial communities present in the rhizosphere may regulate the nutrient cycling and distribution pattern in the soil.

Rhizosphere is the primary link between soil-plant systems and has a vital role in maintaining the ecological balance within land use systems. Plant roots are well known to secrete a variety of primary metabolites (organic acids, carbohydrates, and amino acids) and secondary metabolites (alkaloids, terpenoids, and phenolics) which are believed to shape, signal and interfere with rhizosphere microflora. In addition, the impact of the rhizosphere microbiome rely upon the chemical exudates, which mediates the interactions via signaling molecules which are produced and secreted by plants and microbes (Bai *et al.*, 2015; Bulgarelli *et al.*, 2012; Lundberg *et al.*, 2012).

Rhizosphere effect has been found to stimulate the soil microbial populations through the release of root exudation by plants (Phillips *et al.*, 2011). The various processes involved in rhizosphere region has a major role in C sequestration and nutrient distribution pattern in terrestrial ecosystems (van Veen *et al.*, 1991) and thus the region is identified as a key component in C management (Coleman *et al.*, 1992).

Rhizosphere effects have widely been conducted for individual plants due to which the ecological importance of rhizosphere effect has poorly been understood. The effect of rhizosphere processes on net primary productivity, decomposition rates and C fluxes and storage may play a key role in mediating ecosystem response to climate change (Phillips, 2007). This has led to understand the magnitude of ecosystem consequences on C release as root exudates and to understand the rhizosphere effects.

Bacterial community in the rhizosphere soil exhibits high concentration and dynamism because of readily available C released by the roots to fuel microbial metabolism (Hiltner, 1904). Therefore, rhizosphere is considered as complex and highly dynamic microenvironment associated with diverse microbial communities which are responsible for influencing soil properties (Philippot *et al.*, 2013) in a number of ways. For instance, they are involved in plant growth promotion by stimulating growth (Mendes *et al.*, 2011), nutrient acquisition (Campbell & Greaves, 1990) and helping plants to tolerate abiotic stress (Perez-Jaramillo *et al.*, 2016). Reports have revealed that rhizosphere harbors thousands of different bacteria specific to the plant species and root zone (Peiffer *et al.*, 2013; Marschner *et al.*, 2011). This influence the soil physico-chemical and biological processes that drive the terrestrial ecosystems (Bulgarelli *et al.*, 2013). The native microbial composition and diversity tend to change according to the soil edaphic factors and the nature of substrates produced by the existing plant communities as rhizodeposits.

It is evident that different land use practices modify their rhizosphere microbiome, with distinct microbial community composition mainly through the stimulatory effect of root exudates on the microbial communities (Berendsen *et al.*,

2012). Reports have shown the impact of agricultural practices on soil bacterial community structure (Jorquera *et al.*, 2014; Chen *et al.*, 2013). The changes in underlying rhizosphere microbiome responsible for the sustainable productivity of land uses are important to understand the rhizosphere effect. Studies are highly limited on bacterial diversity with the change in land use, particularly fallow lands and plantations soil.

1.5 Land use change and root exudation

The change in plant species composition after the conversion of natural forest to different land uses may lead to change in nature and magnitude of C released as root exudates. The study of Yin *et al.* (2014) indicated that root exudates drives the nutrient cycling in forests ecosystems, mostly when the mineralization is accelerated by the root-derived C.

Rhizodeposition is defined as the material released by the plant roots and mainly includes the water-soluble compounds and secretions of insoluble materials. Root exudates are soluble sugars, amino acids, organic acids, fatty acids, sterols and proteins (Dennis *et al.*, 2010; Badri & Vivanco, 2009). Root exudates vary both in terms of quantity and quality between plant species. The composition and quantity of root exudates are often affected by the soil physico-chemical characteristics such as soil pH, soil moisture, soil temperature, soil texture and nutrient availability (Hartmann *et al.*, 2009). The dense populations of microorganisms in the rhizosphere region fuelled by root exudates may directly affect the nutrient availability (Suriyagoda *et al.*, 2012). Soil microorganisms also influence root exudation by

degrading exudate compounds and secreting their metabolites into the rhizosphere soil simultaneously.

Root exudates act as a key determinant of microbial community distribution in the rhizosphere. The wide variety of compounds released as exudates may regulate the soil microbial community structure which may further change the soil properties (Nardi *et al.*, 2000). As the C availability in soil limits the microbial growth, the rhizosphere effect may primarily be due to the release of C-containing compounds from the roots.

In addition, exudates act as primers for soil organic matter decomposition (Dormaar, 1990). Root exudates are also well known for containing compounds that exerts stimulatory and inhibitory effect on microbial community (Hartmann *et al.*, 2009). Studies have suggested that root exudates may regulate the rhizomicrobiomes which in turn may affect the quality and quantity of exudates. Since, rhizodeposition could be the most important factor that influences the microbial biomass and activity in the rhizosphere, the understanding of nature and contribution of the exudates in the nutrient availability in different land use systems is of primary importance. Overall, this study directly links root-derived C to soil microbial activities and nutrient distribution at the ecosystem scale.

1.6 Land use change and soil aggregation

The type of land uses affects soil aggregate size fractions and their distribution patterns as result of changing quality of soil organic matter, soil microbiota and their byproducts, magnitude of exudation etc. Various management operations involved in agriculture and plantations (tillage, irrigation, addition of

organic matter, fertilizer application etc) influence the structural stability of the soil. The changes in root biomass, architecture and density in different land use may also cause disturbance of soil aggregates.

Soil aggregate is one of the basic units of soil structural stability and can be substantially modified by soil management practices (Chen *et al.*, 2017; Chivenge *et al.*, 2011). The quality and quantity of soil organic matter influences the aggregate formation and its continuing loss may decrease soil structural stability (Liu *et al.*, 2014). Soil aggregates can be categorized as microaggregate (<0.250 mm), mesoaggregate (0.250-1.00 mm), and macroaggregate (>1.00 mm) depending upon the sizes (Tisdall & Oades, 1982). Studies have reported that land use change reduces the aggregate stability (Singh *et al.*, 2015; Tripathi *et al.*, 2012; Golchin & Asgari, 2008; Tripathi *et al.*, 2008). In addition, type of roots and their density and architecture also influences aggregate size distribution (Miller & Jastrow, 1990) in the soil. Soil aggregate stability is the physical properties that may act as an indicator of soil quality and is considered as important factor regulating soil erosion, nutrient availability and physical properties such as infiltration, aeration, water holding capacity and water use efficiency (Six *et al.*, 2004; Arshad & Coen, 1992). Therefore, maintaining aggregate stability is a prerequisite for preserving soil productivity, minimizing soil erosion and land degradation (Erktan *et al.*, 2016).

Tisdall & Oades, (1982) stated that mineral particles binds together to form microaggregates which further get converted to meso- and macroaggregates due to the temporal organic binding agents such as polysaccharides, roots, and fungal hyphae. The large macroaggregates are more sensitive to land use change due to which they are considered as important indicator to affect the changes in soil quality.

The stability of soil aggregate reflects the behavior of aggregates with the land use change and is generally determined by soil texture and organic-inorganic binding agents, which in turn determine the severity of soil degradation (Gupta *et al.*, 2009).

SOC plays an important role in the formation and stabilization of soil aggregates (Mishra *et al.*, 2014). Generally, higher SOC promotes soil aggregation due to the binding nature of the humic substances present in the soil organic matter. Soil aggregation and SOC are strongly influenced by land use change (Kumar *et al.*, 2013) which may further alter the soil physico-chemical properties, soil microbial composition and functioning of rhizosphere. These changes may affect soil structural stability, soil aggregation thereby affecting the SOC storage and nutrient turnover in soils.

Soil aggregation and SOC dynamics in different land use system have different potentials for C sequestration (Dulazi *et al.*, 2016). The land use with least soil disturbances increases SOC due to better physical protection by the soil aggregates. On the other hand, land use exerting high soil disturbance for instance, a change from forest to cropland, usually leads to severe loss in SOC due to rapid decomposition caused by disruption of soil aggregates (Murty *et al.*, 2002). Soils under natural forest and grasslands have greater aggregate stability and SOC concentration due to the continuous input of carbon from aboveground and belowground biomass (Spohn & Giani, 2011). However, less report is available on the impact of shifting cultivation and plantations on soil aggregation which is more sensitive land use change in the region. Although there are number of reports on changes in soil aggregation due changing land use (Chen *et al.*, 2017; Liu *et al.*, 2014; Gupta *et al.*, 2009), however, information on soil aggregate stability in the

hilly region of northeast India is very limited. Therefore, such study deserves attention to understand the change in aggregate stability with land use change in the region and may come with solutions to manage such land uses.

1.7 Rationale of the study

Assessing soil quality of various land use systems is an important component of research for the suitability of the sites for the sustained production system to the society for their ability to provide goods and services. Aizawl is situated in the northern part of Mizoram and can be considered as one of the important sites to study the different land use systems because of rapidly changing land uses from natural vegetation to the various land use types including shifting cultivation as the prominent one. Bamboo forms an important natural and modified ecosystem in the region as secondary growth in forest fallow due to widespread slash and burn agriculture and under plantations because of its multiple uses.

Therefore, assessment of soil properties and the rhizosphere effects upon different land use systems are important to identify the changes in soil quality. Hence, the study focuses on the following issues: soil fertility; bacterial communities; soil aggregate stability and magnitude of root exudates in different land uses of Mizoram. The main objective of this thesis is to identify the change in soil physico-chemical and biological properties including the bacterial communities in different land use systems of Mizoram.

1.8. Objectives

This study focuses on different land use systems pertaining to rhizosphere.

1. To assess the role of rhizospheric activities on microbial properties and the soil fertility status.
2. To estimate water-stable soil aggregates and their chemical constituents.
3. To estimate the type and magnitude of the root exudates in the rhizosphere of key species.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Overview on land use change

In tropical regions, conversions of natural forest to various land uses as a result of intense anthropogenic activities are common phenomenon. Such conversions are mainly because of exponential increase in human population and over-utilization of resources for food, livestock and other utility products (Barot *et al.*, 2017; Choudhary *et al.*, 2016; Guillaume *et al.*, 2016). The conversion of natural forest ecosystems into a multitude of secondary forests and plantations for livelihood options are linked with noticeable changes in structural and functioning of the ecosystem (De Mandal *et al.*, 2017; Lewis *et al.*, 2015; Singh *et al.*, 2015; Tripathi *et al.*, 2008). Major land use changes are characterized by loss of biodiversity, soil fertility and ecosystem stability which has raised concern on food security and environmental degradation (Tripathi *et al.*, 2015; Grogan *et al.*, 2012; Verburg *et al.*, 2011). Changing ecosystem structure and functioning has led to alter many essential soil properties (Tao *et al.*, 2018; Yu *et al.*, 2015) which often leads to erosion, loss of soil organic matter and soil nutrients, and decline in soil structure (An *et al.*, 2010; Khuswaha *et al.*, 2001).

There are various studies reporting different soil fertility parameters in various land uses of the world (Laganriere *et al.*, 2010; Toriyama *et al.*, 2007). In a series of reports on rhizosphere investigations in soils along fertility gradient, it has been reported that nutrient concentration in the soil changes the interactions between

roots, soil and microorganisms (Merckx *et al.*, 1987). Islam & Weil, (2000) reported that land use alterations allow the soil biological characteristics to respond rapidly to physico-chemical changes in soils. Several studies (Guariguata *et al.*, 2001; Reiners *et al.*, 1994) have shown that land use change often leads to loss in biodiversity, decrease soil nutrients, and physical changes such as rates of infiltration, percolation, aeration and erodability.

In northeastern part of India, the state of Mizoram is characterized by steeply sloped topography and has adopted a diverse range of land use types (Lallianthanga *et al.*, 2014b; Lallianthanga & Hmingthanpuii, 2013). The majority of the population (>60%) is involved in the shifting agricultural practices for their livelihood that leads to degradation of forest areas. The efforts have been made by the forest department to raise variety of plantations like teak, bamboo, rubber and oil palm that has created a landscape dominated by mosaic of ecosystem types including natural forest, fallow lands and plantations. Such ecosystem types have been created to improve the livelihood of the local population which has significantly altered the ecosystem structure and functioning of the region. There are studies that describe changes in plant species composition and diversity in different land use systems (Devi *et al.*, 2018; Tripathi *et al.*, 2016). Singh *et al.* (2015) have studied the changes in plant species composition and diversity in the areas affected by stone mining. Further, studies have documented the changes in leaf and root production and decomposition in natural forests in relation to regenerating forest following stone mining activity (Lalnunzira *et al.*, 2019; Lalnunzira & Tripathi, 2018). The other investigators reported changes in plant species composition, diversity and fine root dynamics in

natural forest and fallow lands (Wapongnungsang *et al.*, 2019; Singha & Tripathi, 2017).

2.2 Impact of land use on soil properties

Several researchers have studied the impact of land use changes on soil properties (Lalnunzira & Tripathi, 2018; Hauchhum & Tripathi, 2017; Singha & Tripathi, 2017; Wapongnungsang *et al.*, 2017; Suma *et al.*, 2011; Joshi, 2002). Studies have confirmed that land use changes in tropical areas cause considerable change in soil physico-chemical and biological properties (Shepherd *et al.*, 2000; Lal, 1996). Offiong *et al.* (2009) reported higher levels of SOC, TN and CEC in undisturbed secondary forest than in disturbed forest. Recently, the length of fallow period after the shifting cultivation has been found to regulate the accumulation of SOM and nutrient availability (Wapongnungsang *et al.*, 2017; Sarkar *et al.*, 2015). Tawnenga *et al.* (1997) found a decrease in soil fertility level in second year cropping than in first year cropping across the Jhum land in Mizoram.

Soil organic matter (SOM) is an important indicator for soil quality assessment and soil fertility evaluation in the tropical regions (Paniagua *et al.*, 1999). Gol, (2009) found a significant decrease in SOC and TN due to conversion of natural forest to continuous cultivation land. Jamaluddin *et al.* (2013) reported that rehabilitated forest has higher SOM content than secondary forests which further declines rapidly due to deforestation and clearing activities (Akbar *et al.*, 2010). Similarly, Nadporozhskaya *et al.* (2006) found higher SOM with time due to the recovery of carbon pools. Vitousek & Howarth, (1991) reported that nitrogen is a limiting nutrient in many ecosystems.

About 14% of the terrestrial carbon is stored in tropical soils (IPCC, 2000), however, the population rise has rapidly changed the land use practices in tropical forests to meet their demand of food and timber. Guo & Gifford, (2002) found a significant increase in soil carbon stocks with conversion of cropland to pasture, tree plantation and secondary forest. Losses in soil organic carbon due to conversion of natural forest to secondary vegetation is well recognized (Yan *et al.*, 2012). Globally, the conversion of forest to cropland has caused a decline of 24 % in soil carbon (Murty *et al.*, 2002).

Merckx *et al.* (1987) stated that in soils poor in nutrients, less carbon is lost by the roots, either because of less plant production or because of physiological differences in root exudation. He also reported that decomposition processes which continues even at a low rate of activity, leads to the degradation of low C/N ratio stable compounds, thus increasing mineralization rates as compared to nutrient rich soils.

Higher exchangeable aluminum (Al) is reported in acidic soils (Brown *et al.*, 1989) that increases with increase in depth (Akbar *et al.*, 2010). Al inhibits calcium and magnesium uptake which further reduces the growth of fine roots and causes imbalance in soil nutrient distribution (Angelica *et al.*, 2012). Higher value of exchangeable Al has been reported in rehabilitated forest than in secondary forest (Jamaluddin *et al.*, 2013).

2.3. Impact of land use on rhizosphere and bacterial community

After the coining of the word rhizosphere by Lorenz Hiltner in 1904 and report that the region of soil around the plant roots are characterized by intense

biological activities where the physical, chemical, and biological parameters are directly influenced by root secretions and associated soil microorganisms. Number of studies have been conducted to test the hypothesis of Hiltner (1904) and supported the view. Guo *et al.* (2015) distinguished the rhizosphere soil from the bulk soil by high microbial activity based on readily available carbon derived from root exudations and other root deposits. The rhizomicrobiome communities are diversely and abundantly distributed in terrestrial ecosystems (Philippot *et al.*, 2013). The microbial ecologists have enormously studied on the diversity and functioning of the rhizomicrobiome by using next-generation sequencing (Philippot *et al.*, 2013; Mendes *et al.*, 2015). The bacterial communities and its diversity in the cave sediments of Indo-Burma hotspot has been well explored (De Mandal *et al.*, 2017; De Mandal *et al.*, 2016).

Reports have revealed that rhizosphere harbors thousands of different bacterial species with different nature of distribution related to plant species and root zones (Peiffer *et al.*, 2013; Marschner *et al.*, 2011) which influences the physico-chemical and biological processes that drive the terrestrial ecosystems (Bulgarelli *et al.*, 2013). The bacterial abundance in the rhizospheric zone is greater than in bulk zone (Guo *et al.*, 2015). The type of vegetation affects the soil bacterial community in various ways such as biomass production, quality and quantity of litter, belowground carbon distribution and nutrient cycling (Dennis *et al.*, 2010). Plant-microbe interactions positively influence the plant growth through various mechanisms. Proteobacteria are involved in fixation of atmospheric nitrogen (Moulin *et al.*, 2001) whereas an endophytic microbe induces intolerance of biotic and abiotic stresses (Schardl *et al.*, 2004). Plant growth-promoting rhizobacteria has a vital role

in the overall plant growth mechanism through the production of essential hormones and enzymes (Gray & Smith, 2005). Significant increase in microbial biomass and its activity with increasing plant diversity has also been reported by Zak *et al.* (2003).

The microbial diversity is influenced by the physical and chemical properties of the rhizosphere soil. Microbial composition in the rhizosphere is strongly linked with changes in forest composition, soil pH, temperature and soil moisture (Fierer & Jackson, 2006). Mendes *et al.* (2015) argued that members under the phyla *Acidobacteria* varied with soil properties and land use change, whereas *Proteobacteria* is regarded as free-living bacteria present in different habitats (Yang *et al.*, 2015). It has also been reported that *Acidobacteria* is mostly abundant in forest soils, while *Proteobacteria* occurs in disturbed soils (Chakraborty *et al.*, 2015). *Verrucomicrobia* is reported to be found under diverse soil management practices and may act as bio-indicator of tropical soil (Navarrete *et al.*, 2015). In Brazilian Amazonia, the abundance of *Verrucomicrobia* was reported to be less in agriculture and pasture land than in natural forest (Mendes *et al.*, 2015).

Zelenev *et al.* (2005) found greater fraction of cultivable bacteria in rhizosphere soil (2-7%) than in bulk soil (1%). Studies have demonstrated a series of growth rates for different bacterial species by using root exudates as the sole carbon source (Dennis *et al.*, 2010). The structure of microbial communities is influenced by the relative ability of the bacteria to metabolize the available carbon sources. Consequently, it can be assumed that the key components of root exudates may affect the distribution of rhizospheric bacterial communities.

2.4 Root exudation as a driver of rhizomicrobiome

Plant roots releases an array of compounds as exudates in the form of sugars, amino acids, organic acids, hormones, and enzymes, most of which are available to the microbial community in the soil (Nguyen, 2003; Grayston *et al.*, 1996). These rhizodeposits are soluble organic compounds with low molecular weight and are passively released to soil due to difference in concentration between root cells and soil solution (Bais *et al.*, 2006). They are actively secreted during nutrient stress and change in plant and microbial communities (Jones *et al.*, 2004). Marschner, (1995) & Lynch, (1990) stated that root exudate mediates the availability of soil nutrients due to chelating property and their role in stimulating microbial activities. Grayston *et al.* (1996) found that tree species differ in the quantity and quality of rhizodeposits which consequently influences the response of tree species to the change in soil fertility. Grayston *et al.* (1996) found that the quantity and chemical quality of rhizodeposition by different tree species influences the soil fertility.

Root exudate act as a source of energy supply and biomass production for soil microorganisms (Dennis *et al.*, 2010) and is considered as a main source of carbon in the soil (Marschner, 1995). The qualitative and quantitative composition of root exudates differs with plant species and age (Hertenberger *et al.*, 2002), edaphic factors such as soil moisture (Yin *et al.*, 2014) and soil type (Neumann *et al.*, 2014). Phillips *et al.* (2011) & Sorensen *et al.* (2009) found that different plant species releases various types of organic compounds that modify rhizosphere conditions by affecting the structure and activity of microbial community. Further, a diverse plant composition may lead to diverse nature of root exudates and greater bacterial diversity in the soil (Philippot *et al.*, 2013). Root exudates may signify the

mechanistic link between the plant communities and the composition and functioning of bacterial communities (Lange *et al.*, 2015; Eisenhauer *et al.*, 2013).

Phillips & Fahey, (2006) reported a negative correlation between magnitude of rhizosphere effects on soil microbes and soil pH, suggesting a close link between rhizosphere carbon flux and soil fertility. A recent study of Keiluweit *et al.* (2015) confirmed that exudate stimulates the mineralization of soil organic matter which affects nutrient cycling in rhizosphere. As root exudate enhances nutrient availability, it also influences the root-root interactions in the rhizosphere (Bais *et al.*, 2006). Moreover, respiration in the rhizosphere is governed by the combination of roots and microorganisms (Kuzyakov, 2006; Kuzyakov *et al.*, 2001).

However, greater priming effects have been reported in both fertile soils (Cheng, 1999; Hungate *et al.*, 1997) and infertile soils (Kuzyakov, 2002). Studies of Cheng *et al.* (2014) & Yin *et al.* (2014) suggested an important linkage between fluxes of root carbon and the fraction of nutrients present in soil organic matter. Recently de Graaff *et al.* (2013) indicated that rhizosphere priming is induced by a high ratio of labile carbon to stable carbon in soil due to enhanced microbial biomass.

Exudation rates are reported to correlate positively with soil nutrients and organic matter decomposition in rhizosphere of ~80 years old forest of south Indiana (Yin *et al.*, 2014). Approximately, root exudate has been reported to contribute 18% of the total net mineralization. Studies also showed that different tree species drives the magnitude of root exudates on nutrient availability through the presence of labile carbon (Kuzyakov, 2010). Yin *et al.* (2014) estimated that root exudation contributes 21% of the labile carbon whereas microbial activities affected by root exudates

contributes to around 20% of the mineralized carbon in Oak, sugar maple and tulip poplar forest.

2.5 Effect of land use on soil aggregate stability

Land use change is among the key factors affecting the soil aggregate stability. Studies have reported that stable soil aggregates are one of the major factors of soil fertility that affect plant productivity (Tripathi *et al.*, 2012; Tripathi *et al.*, 2008; Dick *et al.*, 1997). It indicates the soil organic matter content of an ecosystem (Angers & Carter, 1996). According to the model of aggregate hierarchy proposed by Tisdall & Oades, (1982) & Hassink, (1997), microaggregates binds together to form macroaggregates by the presence of binding agents such as polysaccharides, roots and fungal hyphae (Six *et al.*, 2004). Natural forest and reforested soils have greater aggregate stability compared to cultivated soils (Islam & Weil, 2000).

Land use change significantly affected soil organic carbon and nitrogen concentrations in soil aggregate fractions (Liu *et al.*, 2014; Wick *et al.*, 2009). Studies of Chen *et al.* (2017) found higher carbon content within macroaggregate (>0.25 mm) fractions than in microaggregate (0.25-0.053 mm) fractions. In contrast, John *et al.* (2005), reported lowest carbon concentration in macroaggregates fractions. In general, higher C/N ratios in macroaggregate fractions than finer-sized fractions have been reported (Chen *et al.*, 2017).

Somasundaram *et al.* (2013) found that soil under *Leucaena leucocephala* have larger mean weight diameter than soil under mixed forest. This was related to fast growth, N-fixation and high litter decomposition ability of the species. Elliott,

(1986) reported that rapidly growing legume dominated forests enhances the soil aggregate stability in successional forest due to the addition of labile carbon through litter fall and rhizodeposits. Tripathi *et al.* (2008) reported that soil aggregation pattern and distribution in dry tropical forest is affected by the addition of nitrogen and phosphorous depending on the presence of soil organic matter. Greater MWD in rubber-based agroforestry system compared to other agroforestry systems was reported by Chen *et al.* (2017).

SOM improve soil aggregation, particularly macroaggregates which promotes the stability of aggregates (Elliott, 1986) and the proportion of macroaggregates declines with soil depth (Chen *et al.*, 2017). SOC is the main factors affecting the aggregate stability of soils (Elliot *et al.*, 1986; Chaney & Swift, 1984). It has also been found that agroforestry systems promote the stability of soil aggregates due to plant residues present on the soil surface (Six *et al.*, 2004; Elliott, 1986).

Macroaggregates are more susceptible to cultivation techniques and addition of organic matter (Chen *et al.*, 2017). Higher proportion of macroaggregates has been reported in various ecosystems such as grassland, cardamom plantation (Kyung *et al.*, 2010) whereas greater proportions of microaggregates have been reported in pine forest (Kyung *et al.*, 2010). Chen *et al.* (2017) reported increased in the proportion of >5 mm and 2-5 mm fractions and reduced in the proportion of 0.5-1 mm, 0.25-0.5 mm, and 0.053-0.25 mm fractions under rubber-based agroforestry system.

Enhanced organic matter accumulation increases the population of soil microbe and earthworm which further increases the proportion of macroaggregate

(Zhang *et al.*, 2010). The arrangement and distribution of fine roots in soil has also been reported to regulate the aggregates stability (Erktan *et al.*, 2016; Pohl *et al.*, 2009). Thus, land uses with profuse growth of fine roots may improve the distribution of macroaggregate. Greater carbon stocks in soils of rubber agroforestry systems have led to increase in macroaggregates and aggregation indices (Chen *et al.*, 2017).

Clay is considered as the major binding agent for the formation of soil aggregate and stability (Wick *et al.*, 2009). Clay content of 24% - 56% in rubber-based agroforestry systems have been shown to contribute to greater soil aggregation (Chen *et al.*, 2017). Gupta *et al.* (2009) reported high clay content in poplar agroforestry system which resulted in higher aggregate stability which further increases mean weight diameter (MWD).

2.6 Effect of land use on soil organic carbon

Studies have reported a drastic decline in soil organic carbon content following the forest conversions to various plantations (Guillaume *et al.*, 2015; van Straaten *et al.*, 2015; de Blécourt *et al.*, 2013). Conversion of natural forest to cropland has been reported to decrease 25 % to 30 % soil organic carbon (Don *et al.*, 2011). Berndes *et al.* (2011) argued that land use change contributes about 15% of greenhouse gas emissions. Soils in humid tropical region stores 30% of the soil organic carbon (692 Gt C) (Jobbágy & Jackson, 2000), which is equivalent to the carbon present in the atmosphere (589 Gt C) (Ciais *et al.*, 2013). The greatest fluxes in soil carbon occur due to natural forest being converted to open forest (Houghton & Goodale, 2004). Thus, a small change in climate or land use pattern may

contribute to a significant amount of carbon fluxes in the atmosphere (Guo & Gifford, 2002; Post & Kwon, (2000). It has been estimated that changes in land uses in the tropics leads to a net release of 0.6 to 1.2 Gt carbons per year (Achard *et al.*, 2014).

In the last 20 years the demands for tree cash crops have increased globally. Oil palm plantation areas have extended to 15.9 Mha and rubber to 9.4 Mha (Guillaume *et al.*, 2016). Globally, the deforestation in tropical areas for various tree cash crop plantations has caused a significant change in soil carbon. van Straaten *et al.* (2015) reported the loss of one-half of stored soil carbon due to the conversion of forests to plantations like oil palm (*Elaeis guineensis*), rubber (*Hevea brasiliensis*), and cacao (*Theobroma cacao*). Soils under oil palm plantation are reported to degrade more than under rubber plantation due to the loss in SOC and TN content and increased bulk density (Guillaume *et al.*, 2016).

Oil Palm (*Elaeis guineensis*) is the most rapidly expanding equatorial food and bio fuel crops in the world. Euler *et al.* (2015a) reported that small land holding farmers in Indonesia are mostly engaged in land modification for rubber and palm oil production. Besides, the positive impact on livelihood of small land holding farmers (Euler *et al.*, 2015b), these plantations also leads to ecosystem degradation (Barnes *et al.*, 2014). Soil erosion has been identified as the factor affecting the soil carbon losses in oil palm plantations (Guillaume *et al.*, 2015; Gharibreza *et al.*, 2013).

In general, soil carbon content in rubber plantation is medium to high, however, depletion of soil carbon by continuous rubber plantation compared to adjoining natural forest was observed by Ulaganathan *et al.* (2013).

CHAPTER 3

MATERIALS AND METHODS

3.1 General description of the study area

The study sites were located within Aizawl district, northern part of Mizoram, Northeast India. The geographical coordinates ranges between 24°25' and 23°18' N latitude and 92°37' and 93°11' E longitude (Fig 3.1). Aizawl district is bordered by Champhai district and Manipur in the east, Mamit district and Kolasib district in the west, Assam in the north and Serchhip district in the south. The temperature during summer period ranges from 20°C to 34°C and during winter from 8°C to 17°C. The total annual rainfall is 2500 mm with relative humidity upto 90%. The study sites experience moderate humid tropical climate and receive high rainfall. The climatic conditions range from tropical, sub-tropical to temperate. About 85% of total geographical area of the state is under forest cover (Forest Survey Report, 2017). The forest type is mainly consists of tropical wet evergreen forest. Other dominant forest categories are semi evergreen forest and tropical moist deciduous forest including bamboo forest of different species with domination of *Melocanna baccifera* (MIRSAC, 2007). Most of the area falls under the category of class-II to class-IV of land use capability, and requires appropriate soil management practices (Mizoram SAPCC, 2012-17). The soil order of the land uses belongs to Inceptisols (Colney & Nautiyal, 2013) and primarily consisting sand-loamy and clay-loamy soil rich in organic carbon. The fertility of soils is mostly affected by the change in land use, shortening of Jhum cycle, steep slopes, soil erosion and landslides associated with high intensity rainfall.

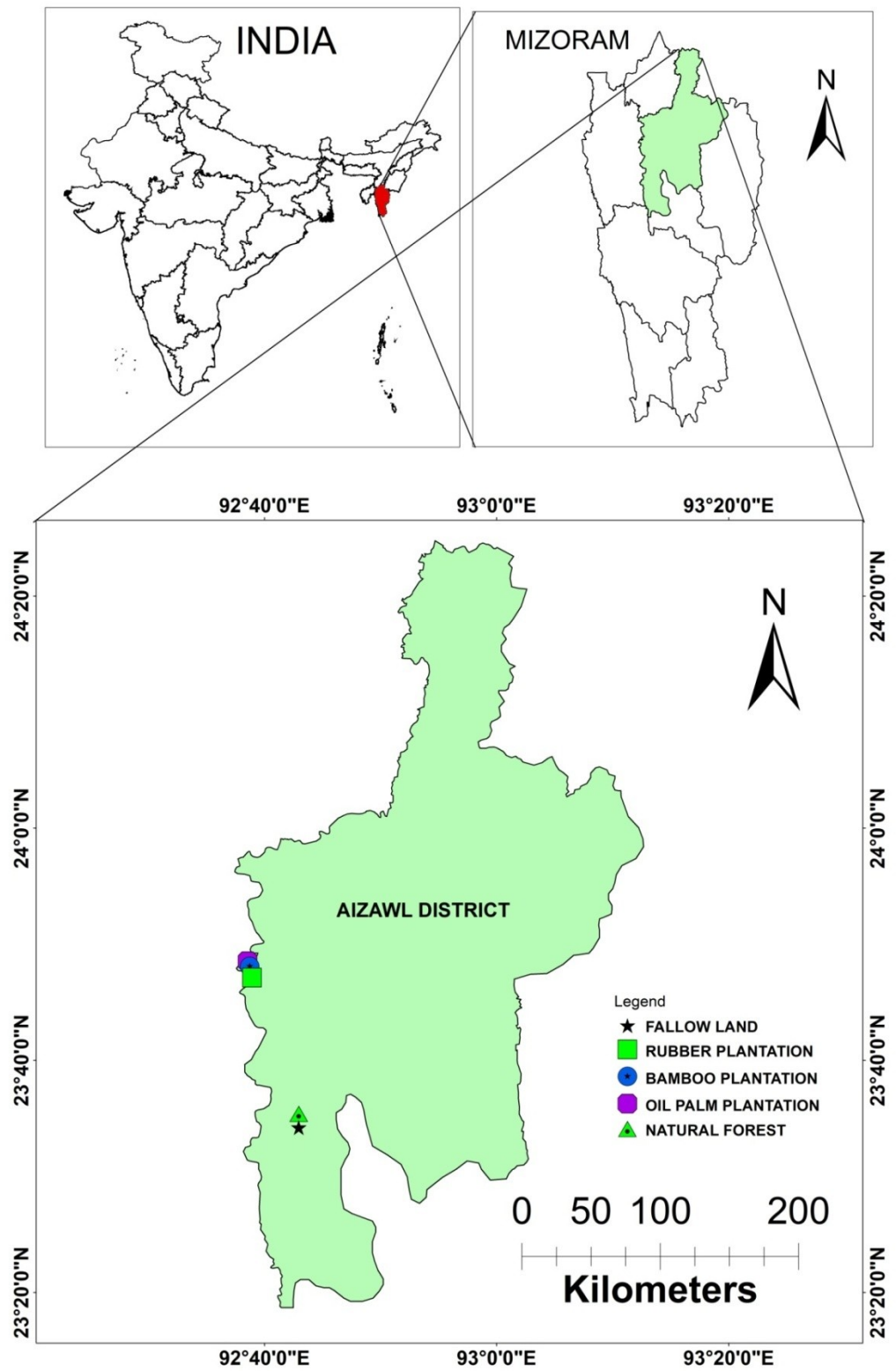


Figure 3.1 Map of study sites

3.2 Description of study sites and land uses

The study was carried out in five dominant land uses; RP (Rubber Plantation), BF (Bamboo Forest), OPP (Oil Palm Plantation), FL (Fallow Land) and NF (Natural forest). The geographical coordinates for different sites are: 23°47.123' N lat and 92°36.831' E long for RP, 23°47.559' N lat and 92°36.492' E long for BF, 23°47.771' N lat and 92°36.080' E long for OPP, 23°35.392' N lat and 92°42.952' E long for FL and 23°35.207' N lat and 92°43.016' E long for NF. The monoculture of RP (*Hevea brasiliensis*) and OPP (*Elaies guineensis*) were 10 years old with plantation area of ~3.5 ha and ~5 ha respectively. BF (*Melocanna baccifera*), FL, and NF were 12, 20 and >100 years old, respectively. The vegetation of FL was secondary successional type with the dominant species of Broom grass (*Thysanolaena maxima*) and small patches of bamboo along with other woody species. The NF was a mixed forest dominated by *Schima wallichii* tree species along with other woody associates like *Albizzia chinensis*, *Callicarpa arborea*, *Castanopsis tribuloides*, *Duabanga grandiflora*, *Macaranga peltata*, *Sterculia villosa*, *Toona ciliata* etc.

3.3 Methodology

3.3.1 Experimental design and soil sampling

A sample area representing about 1 hectare land was selected in each land use system and within each 1 hectare, five replicated plots (100 m²) were established as site replicates by maintaining the distance of 20 m between the plots.

3.3.1.1 *Soil physico-chemical and biological properties*

A total of 30 soil cores were collected following random sampling technique from each land use which was further composited into 5 samples (1 sample represent each replicated plot) representing each land use system for soil analysis. The replicated soil cores from each land use were collected as soil samples. Soil adhered to fine roots was considered as rhizosphere soil and the soils devoid of roots were considered as bulk soil. It was sieved by a 2 mm mesh and further divided into two subsamples. One of which was air dried and the other was stored at -20° C for further analysis. The soil sampling for the analysis of physico-chemical and biological properties were carried out for three seasons; Pre-Monsoon, Mid-Monsoon and Post-Monsoon.

3.3.1.2 *Soil bacterial diversity*

The rhizosphere soil samples for DNA extraction was collected from 5-6 random locations (ca. 3-5 m away from each other) from each permanent plots and were pooled into sterile tubes, kept to frozen in dry ice and transported to the laboratory for further analysis.

3.3.1.3 *Soil aggregate sizes*

Soils were randomly collected from 5 replicated plots (each 100 m²) within a sample area of about 1 hectare land in different land use systems. Each replicated plot was established at a distance of about 20 m apart from each other to express true replication. A total of 10 soil samples representing each land use were obtained using a shovel to avoid compression and disturbance of the sample and ensured minimum wall surface area to volume ratio to decrease the risk of compaction. The parts of the soil not touched by the shovel were labeled and placed in rigid large sampling boxes.

3.3.2 Analysis of soil physico-chemical properties

3.3.2.1 Soil physical properties

Bulk density was estimated by core method (Blake, 1965). The gravimetric method was followed to determine the soil moisture content. Soil sample were weighed before and after the sample was oven dried at 105°C until the constant weight was attained. Total porosity was calculated using dry bulk density assuming a particle density of 2.65 g cm⁻³ (Danielson and Sutherland, 1986). Soil texture was determined by Hydrometer method (Bouyoucos, 1926) using the USDA textural classification chart.

3.3.2.2 Soil chemical and biological properties

Soil pH was measured in a soil-water suspension (1:2.5 soil-water ratios) with pH analyzer. Soil organic carbon (SOC) and total nitrogen (TN) were determined on finely grounded air-dried soils by dry combustion in a CHNS/O Elemental Analyzer with autos ampler and TCD detector –Euro Vector, Model: EuroEA3000. Available phosphorous (P_{avail}) was analyzed following Allen *et al.* (1974) method. Nitrate nitrogen (NO₃-N) was estimated by phenol disulphonic acid method (Harper, 1924) and ammonium nitrogen (NH₄-N) by indophenol-blue method (Rowland, 1983). Exchangeable cations [calcium (Ca), potassium (K), magnesium (Mg), aluminum (Al) and sodium (Na)] were determined using the Agilent 4100 Microwave Plasma-Atomic Emission Spectrometer (MP-AES).

Soil Fertility Index (SFI) (Moran *et al.*, 2000) and Soil Evaluation Factor (SEF) (Lu *et al.*, 2000) was evaluated using the following equations:

Soil Fertility Index (SFI) = pH + organic matter + P (mg kg⁻¹ dry soil) + K (cmol kg⁻¹) + Ca (cmolkg⁻¹) + Mg (cmolkg⁻¹) - Al (cmolkg⁻¹).

Soil Evaluation Factor (SEF) = [K (cmolkg⁻¹) + Ca (cmolkg⁻¹) + Mg (cmol kg⁻¹) - log (1 + Al (cmolkg⁻¹))] x organic matter + 5

Soil respiration (SR) rate was determined by alkali absorption method described by Kirita, (1971). Soil microbial biomass carbon (MBC) was analyzed by dichromate digestion method as described by Vance *et al.* (1987).

3.3.3 Estimation of bacterial communities

3.3.3.1 DNA extraction

Bacterial DNA was extracted from the composites of rhizosphere soil samples of different land use systems using the Fast DNA spin kit (MP Biomedical, Solon, OH, USA). The V4 hyper variable region of the 16S rRNA gene was amplified using 10 pmol/μl of each forward and reverse primer. PCR Master Mix contained 2 μL of each primers, 0.5 μL of 40 mM dNTP (NEB, USA), 5 μL of 5X Phusion HF reaction buffer (NEB, USA), 0.2 μL of 2U/μL F-540 Special Phusion HS DNA Polymerase (NEB, USA), 5 ng input DNA and water to make up the total volume to 25 μL. The PCR conditions were 98°C for 30 sec followed by 30 cycles of 98°C for 10 sec; 72°C for 30 sec and a final extension at 72°C for 5 sec followed by 4°C hold (De Mandal *et al.*, 2016).

3.3.3.2 Pre-processing and sequence analysis

Paired-end Mi-seq (Illumina) sequencing (2 X 250 bp) was carried out at Scigenome Lab, Cochin, India and the raw data was submitted to NCBI- Sequence

Read Archive (SRA): SUB4657919 with accession number PRJNA514616. All the raw fastq sequences were analyzed using the QIIME software package v.1.8.0 (Caporaso *et al.*, 2010). The raw paired end reads obtained were assembled using PEAR: a Paired-End read merger tool (Zhang *et al.*, 2014). Poor quality (quality score <25) and smaller reads (read length <100 bp) were filtered out using the `split_libraries` command. Pre-processed sequence reads were clustered to operational taxonomic units (OTU's) using UCLUST method with similarity threshold of 97% and were taxonomically classified using Green genes database (Edgar, 2010). Alpha and beta diversity plots were also generated using QIIME (De Mandal *et al.*, 2017).

3.3.3.3 Diversity comparisons

The bacterial diversity changes were measured using the alpha diversity metrics: PD (Phylogenetic Diversity) whole tree, Chao 1 and observed species. Distance matrixes were generated using the phylogenetic un-weighted and weighted UniFrac for 16S rRNA gene sequences (Lozupone & Knight, 2005) and the beta-diversity distance matrices were plotted using a Principal Coordinates Analysis (PCoA). The alpha diversity indexes used in this experiment represent species richness. Treatment's effects over bacterial community was compared using the estimators Faith's PD, phylogenetic measure of diversity based on total branch length of phylogeny captured by a sample, proposed by Faith, (1992), the Chao-1 (estimator of total species richness proposed by Chao, 1984), and observed species (number of species detected). Rarefaction curves were used to determine whether sampling depth was sufficient to characterize the bacterial community present in different land uses. To build rarefaction curves, each community was randomly sub-sampled

without replacement at different intervals, and the average number of OTUs at each interval was plotted against the size of the subsample (Gotelli *et al.*, 2001). The metric calculation was performed using QIIME software.

3.3.4 Estimation of soil aggregate size fractions

The collected soil samples were air-dried at room temperature for few hours and then the large clods (>5 cm) were gently broken along natural planes of weakness into natural aggregates. The soils were then air-dried for 2 weeks before being passed through an 8 mm sieve to remove coarse plant residues, roots and any stones >8 mm. Further a sub-sample (thoroughly mixed the sample before taking the sub-sample to ensure a representative sub-sample) of 100 g were considered for further analysis.

Aggregate size distribution was determined by mechanical sieving (Kemper & Rosenau, 1986) into different size fractions (4.75–8 mm; 2–4.75 mm; 1–2 mm; 0.5–1 mm; 0.25–0.5 mm and <0.25 mm). The sieves were placed in a stack (i.e., 4.75, 2.00, 1.00, 0.50, 0.25 and 0.053 mm) with the largest mesh size on top and a closed recipient at the bottom. The sample was poured onto the top sieve after which the stack was placed within the machine with the top sieve covered by a lid. The stack of sieves was secured tightly in the machine and shook them at a speed of 210 cycles min⁻¹ for 5 minutes (Diaz-Zorita *et al.*, 2007). The sieves were then emptied onto their corresponding metal trays ensuring that all soil was collected on the trays and no soil remains on the sieves. Lastly the trays were weighed with soil and recorded the weight. Each aggregate size fraction was calculated as a percent relative

to total dry sample and mean weight diameter (MWD) for aggregate stability was determined according to the following formula (Kemper & Chepil, 1965).

$$MWD = \sum_{i=1}^n XiWi \quad (1)$$

where MWD is the mean weight diameter of soil aggregates, Xi is the mean diameter of each aggregate size fraction (mm) and Wi is the proportion of total sample weight occurring in each size fraction.

3.3.4.1 Estimation of aggregate associated OC stock

An aggregate-associated OC stock (g m^{-2}) in different land uses was estimated using the following formulae (Wei *et al.*, 2013).

$$\text{Aggregate-associated OC stocks} = M_i \times OC_i \quad (2)$$

$$M_i = \frac{D \times BD \times w_i}{10} \quad (3)$$

where M_i is the amount of soil in the i th size fraction (kg m^{-2}) and OC_i is the soil organic carbon concentration of the i th size fraction (g kg^{-1}), D is the thickness (cm) of the soil layer, BD is the bulk density (g cm^{-3}) and w_i is the proportion of the total soil in the i th size fraction (%).

3.3.5 Estimation of root exudates

The collection and analysis of root exudates was done following the modified culture-based cuvette system developed especially for field-based exudate collections (Phillips *et al.*, 2008). Root exudation was measured in three plantation sites. Efforts were made to estimate root exudation in NF and FL, however, it could not be possible to trace out the roots of specific tree species. Therefore, the root exudation was measured in RP, OPP and BF only. The key plants of each land use

systems were considered for estimation of root exudation. The important plant species were selected on the basis of their abundances considering the role of species on the soil fertility as well as the carbon stock of these sites.

3.3.5.1 Excavation and cuvette assembly

Terminal fine roots (<2 mm diameter) were excavated at the interface between O and A soil horizons within the distance of 0-3 m from the bole of targeted tree (Lucash *et al.*, 2005). The root systems were traced back to the targeted tree to ensure that the roots excavated belongs to the selected tree species. The root segments of 15-20 cm length were then rinsed with a nutrient solution in order to remove the adhered soil particles. The intact fine roots was then placed in a soil-sand mixture (1:1 ratio) and re-buried into the soil for 24 – 48 h.

Soil-free fine roots were then placed into a 30 ml glass syringe from which the plunger was removed, and each glass syringe was filled with sterile acid-washed glass beads. A 30 µm mesh cloth was folded into a cone shape at the tapered end of each syringe to prevent the glass beads from clogging the syringe outlet during the removal of the solution. A small volume of diluted nutrient solution was then added in the cuvette to maintain humid conditions during the incubation period. The cuvette was then covered with aluminum foil, returned to the excavated area, and covered with several layers of litter to allow the fine root system to equilibrate with the cuvette environment. Three control cuvettes filled with glass beads were similarly covered and buried into the soil.

3.3.5.2 Collection and analysis of root exudation

After 2-3 days of equilibration period, a diluted nutrient solution was again added to each glass cuvette in order to facilitate the removal of accumulated root exudates. Each cuvette was filled with 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) and flushed to ensure the complete removal of C from the cuvette before the experimental incubation period. The process was consecutively repeated. Cuvettes were flushed three times with a C-free nutrient solution to remove the accumulated exudates using the procedure described above. All the cuvettes were reopened after the incubation period and the collected solutions were refrigerated at 4°C until analysis (<48 h). All the samples were analyzed for total organic carbon using TOC- V_{CPH} Total Organic Carbon Analyser, Shimadzu.

3.3.6 Statistical analysis

All the data are presented in the form of mean and standard error (1SE). Significant differences between soil variables, aggregate size fractions and bacterial diversity across different land use systems were determined using one-way analysis of variance. Least significant difference (LSD) test was performed to compare between means at $p \leq 0.05$. Pearson correlation analysis was carried out between the soil nutrients, soil aggregate size fractions and microbial diversity indices. Stepwise multiple regression analysis was also performed among soil variables, microbial diversity and different aggregate size classes to develop the regression equations. All the statistical analysis were performed by using SPSS version 18.0 (SPSS Inc., Chicago, IL) and Minitab version 18 (Minitab Inc., State College, PA).

CHAPTER 4

RESULTS

4.1 Changes in soil physico-chemical and biological properties across land uses

4.1.1 Soil physical properties

The studied soil physical properties were significantly different ($p < 0.05$) across the land use systems (Table 4.1). Bulk density (BD) values ranged from 1.06 g cm^{-3} – 1.27 g cm^{-3} with maximum density in RP and minimum in BF. Soil porosity level across the land uses ranged from 51.6 % - 58.8 %. Porosity was higher in BF, FL and NF compared to RP and OPP. The soil texture of all the land use was sandy loam with percent of sand, silt and clay ranging from 62.5 % - 71.2 %, 17.0 % - 20.6 % and 11.8 % - 16.9% respectively. The clay and silt percent was greater in plantations soils compared to other land uses whereas the sand percent was high in BF soils.

Table 4.1 Physical properties of soil in different land use systems. Values are means $\pm 1\text{SE}$. LSD is shown at $p < 0.05$. (RP-rubber plantation, BF-bamboo forest, OPP-oil palm plantation, FL-fallow land and NF-natural forest).

Land Uses	Bulk Density (g cm^{-3})	Porosity (%)	Sand (%)	Silt (%)	Clay (%)
RP	1.27 ± 0.04	51.6 ± 1.5	62.5 ± 0.10	20.6 ± 0.04	16.9 ± 0.05
BF	1.06 ± 0.01	58.8 ± 0.5	71.2 ± 0.99	17.0 ± 0.94	11.8 ± 1.94
OPP	1.16 ± 0.02	55.8 ± 0.7	63.7 ± 2.08	19.6 ± 1.63	16.7 ± 0.45
FL	1.09 ± 0.01	58.5 ± 0.4	68.6 ± 0.05	17.8 ± 0.55	13.6 ± 0.6
NF	1.10 ± 0.004	58.2 ± 0.2	69.2 ± 1.91	18.8 ± 0.78	12.0 ± 1.13
LSD	0.18	7.09	4.23	2.97	3.34

4.1.2 Soil chemical properties

The soil moisture (SM) content in rhizosphere soil ranged from 17.5 % to 26.9 % with highest SM content in NF during mid-monsoon and was followed in decreasing order by BF>FL>RP>OPP, whereas, in bulk soil it ranged from 16.3 % to 26.3 %. However, the SM content in plantations (RP and OPP) varied significantly with other land uses. The soil pH values in rhizosphere soil ranged from 4.4 to 5.6 during pre-monsoon, 4.1 to 4.9 during mid-monsoon and 4.5 to 5.0 during post-monsoon (Table 4.2). Bulk soil was more acidic than rhizosphere soil and the values ranged from 4.1- 5.3 during pre-monsoon, 4.4 to 4.8 during mid-monsoon and 3.8 to 4.7 during post-monsoon. FL was more acidic during pre-monsoon compared to other land uses whereas NF was more acidic during mid-monsoon and post-monsoon. However, greater soil acidity was observed during mid monsoon season.

Soil organic carbon (SOC) concentration was highest during mid-monsoon season and were significantly different ($p < 0.05$) across the land uses. The values ranged from 1.4 % to 2.7 % and 1.1 % to 2.9 % in rhizosphere soil and bulk soil respectively (Table 4.2). Seasonal variation in SOC concentrations in rhizosphere soils was significant ($p < 0.05$) and the values ranged from 1.7 % to 2.5 % during pre-monsoon, 2.1 % to 2.7 % during mid-monsoon and 1.4 % to 2.6 % during post-monsoon. The maximum concentration was found in rhizosphere soil of NF and lowest in RP. Similar trend to SOC was observed in TN concentrations during seasons with higher concentration in NF and less concentration in plantation soils. The TN percent in rhizosphere soil ranged from 0.19 % to 0.28 % during pre-monsoon, 0.22 % to 0.31 % during mid-monsoon and 0.15 % to 0.26 % during post-monsoon. However, TN concentration in bulk soils ranged from 0.14 % to 0.28 % in

all the seasons. In general, rhizosphere soils showed higher SOC and TN concentrations than bulk soils across all the land uses.

The P_{avail} in the rhizosphere soil across the land use systems ranged from 4.5 mg g⁻¹ to 5.4 mg g⁻¹ during pre-monsoon, 4.8 mg g⁻¹ to 6.4 mg g⁻¹ during mid-monsoon and 2.2 mg g⁻¹ to 4.3 mg g⁻¹ during post-monsoon (Table 4.2). The values were significantly different ($p < 0.05$) between the seasons and the maximum concentration was obtained during mid-monsoon which decreased abruptly during post-monsoon. RP and OPP showed higher P_{avail} content during pre-monsoon and mid-monsoon compared to other land uses with abrupt increase in NF during post-monsoon. The P_{avail} content was consistently low in FL during all the season. The values in the bulk soils were comparatively lower across the land uses and the values during all the seasons ranged from 2.1 mg g⁻¹ to 5.9 mg g⁻¹.

4.1.3 Soil biological properties

NH₄-N concentration in rhizosphere soil ranged from 5.1 mg g⁻¹ to 8.5 mg g⁻¹ during pre-monsoon, 8.9 mg g⁻¹ to 11.6 mg g⁻¹ during mid-monsoon and 6.0 mg g⁻¹ to 8.1 mg g⁻¹ during post-monsoon (Table 4.3). The NH₄-N concentration in bulk soil ranged from 4.7 mg g⁻¹ to 7.1 mg g⁻¹ during pre-monsoon, 8.1 mg g⁻¹ to 10.5 mg g⁻¹ during mid-monsoon and 4.4 mg g⁻¹ to 11.1 mg g⁻¹ during post monsoon. The rhizosphere soil of RP showed higher concentrations of NH₄-N than other land uses during pre-monsoon whereas BF showed the maximum NH₄-N concentration during mid-monsoon and post-monsoon. On the other hand, bulk soil of BF showed high NH₄-N concentration during pre-monsoon and OPP showed high NH₄-N concentration during both mid-monsoon and post-monsoon.

Table 4.2 Seasonal variation in chemical properties of rhizosphere (RS) and bulk (BS) soil under different land use systems. Values are mean \pm 1SE. LSD is shown at $p < 0.05$. (RP-rubber plantation, BF-bamboo forest, OPP-oil palm plantation, FL-fallow land, NF-natural forest).

Season/Land Use		Soil chemical characteristics									
		Soil Moisture (%)		Soil pH		Soil Organic Carbon (%)		Total Nitrogen (%)		Available Phosphorous (mg g^{-1})	
		RS	BS	RS	BS	RS	BS	RS	BS	RS	BS
Pre-Monsoon	RP	22.4 \pm 0.3	20.9 \pm 0.3	4.9 \pm 0.1	4.8 \pm 0.07	1.7 \pm 0.2	1.9 \pm 0.7	0.20 \pm 0.01	0.21 \pm 0.05	5.5 \pm 0.09	5.4 \pm 0.6
	BF	23.6 \pm 2.0	22.3 \pm 0.5	5.6 \pm 0.2	5.3 \pm 0.05	1.8 \pm 0.1	1.1 \pm 0.3	0.19 \pm 0.02	0.14 \pm 0.03	5.4 \pm 0.02	5.3 \pm 0.05
	OPP	17.5 \pm 2.8	16.3 \pm 3.6	5.3 \pm 0.1	4.9 \pm 0.1	2.0 \pm 0.3	2.0 \pm 0.1	0.24 \pm 0.01	0.22 \pm 0.01	5.3 \pm 0.1	5.4 \pm 0.01
	FL	22.1 \pm 1.4	21.9 \pm 1.7	4.4 \pm 0.1	4.3 \pm 0.04	2.3 \pm 0.3	2.1 \pm 0.1	0.25 \pm 0.02	0.23 \pm 0.003	4.5 \pm 0.6	2.7 \pm 0.5
	NF	23.5 \pm 2.0	22.4 \pm 2.0	4.7 \pm 0.3	4.1 \pm 0.07	2.5 \pm 0.2	2.5 \pm 0.4	0.28 \pm 0.02	0.27 \pm 0.01	5.2 \pm 0.2	4.9 \pm 0.4
	LSD	6.08	6.47	0.67	0.27	0.81	1.30	0.05	0.08	0.98	1.21
Mid-Monsoon	RP	24.6 \pm 0.6	19.8 \pm 0.2	4.7 \pm 0.1	4.5 \pm 0.09	2.1 \pm 0.08	1.7 \pm 0.04	0.23 \pm 0.01	0.21 \pm 0.008	6.4 \pm 0.7	5.2 \pm 0.5
	BF	25.7 \pm 0.6	24.7 \pm 1.2	4.9 \pm 0.09	4.8 \pm 0.07	2.3 \pm 0.07	2.3 \pm 0.08	0.25 \pm 0.02	0.22 \pm 0.01	4.9 \pm 0.7	5.6 \pm 0.3
	OPP	23.9 \pm 2.6	19.7 \pm 1.3	4.5 \pm 0.2	4.4 \pm 0.1	2.2 \pm 0.1	2.1 \pm 0.05	0.22 \pm 0.01	0.24 \pm 0.009	5.9 \pm 0.3	5.9 \pm 0.5
	FL	26.6 \pm 0.7	26.3 \pm 1.3	4.7 \pm 0.2	4.5 \pm 0.2	2.6 \pm 0.1	2.3 \pm 0.2	0.27 \pm 0.01	0.25 \pm 0.006	4.8 \pm 0.6	4.1 \pm 0.1
	NF	26.9 \pm 1.7	24.6 \pm 1.4	4.1 \pm 0.1	4.4 \pm 0.1	2.7 \pm 0.05	2.9 \pm 0.1	0.31 \pm 0.01	0.28 \pm 0.009	5.6 \pm 0.5	4.1 \pm 0.5
	LSD	4.80	3.85	0.62	0.42	0.28	0.44	0.04	0.02	3.37	1.35
Post-Monsoon	RP	20.2 \pm 0.9	20.2 \pm 1.0	4.7 \pm 0.03	4.6 \pm 0.1	1.4 \pm 0.1	1.2 \pm 0.1	0.15 \pm 0.01	0.12 \pm 0.008	2.9 \pm 0.4	2.7 \pm 0.5
	BF	22.7 \pm 0.5	20.9 \pm 1.8	5.0 \pm 0.09	4.7 \pm 0.09	1.7 \pm 0.7	1.4 \pm 0.2	0.17 \pm 0.04	0.16 \pm 0.008	2.2 \pm 0.4	2.1 \pm 0.4
	OPP	17.7 \pm 1.6	18.3 \pm 1.8	4.8 \pm 0.2	4.5 \pm 0.2	1.8 \pm 0.1	1.6 \pm 0.2	0.18 \pm 0.03	0.18 \pm 0.01	3.4 \pm 0.1	2.2 \pm 0.2
	FL	23.7 \pm 0.8	22.6 \pm 1.2	4.8 \pm 0.1	4.7 \pm 0.06	2.1 \pm 0.7	1.6 \pm 0.5	0.19 \pm 0.03	0.15 \pm 0.08	3.1 \pm 0.7	2.7 \pm 0.4
	NF	25.3 \pm 0.6	20.6 \pm 0.3	4.5 \pm 0.04	3.8 \pm 0.1	2.6 \pm 0.8	2.4 \pm 0.02	0.26 \pm 0.04	0.26 \pm 0.005	4.3 \pm 0.4	3.0 \pm 0.7
	LSD	3.13	4.33	0.34	0.39	1.96	0.88	0.1	0.11	1.50	1.57

Comparatively bulk soil of NF and FL showed the lowest $\text{NH}_4\text{-N}$ concentration during pre-monsoon. Mid-monsoon favored high $\text{NH}_4\text{-N}$ in both rhizosphere and bulk soil compared to other two seasons across all the land uses. Subsequently higher $\text{NO}_3\text{-N}$ was observed in rhizosphere soil of NF during all the seasons whereas lowest $\text{NO}_3\text{-N}$ was obtained in BF. The values of $\text{NO}_3\text{-N}$ in rhizosphere soil ranged from 0.7 mg g^{-1} to 1.2 mg g^{-1} during pre-monsoon, 1.5 mg g^{-1} to 3.04 mg g^{-1} during mid-monsoon and 0.96 mg g^{-1} to 2.69 mg g^{-1} during post-monsoon. The $\text{NO}_3\text{-N}$ concentrations were significantly different ($p < 0.05$) between the seasons and the values were greater during mid-monsoon and lowest during pre-monsoon.

MBC in rhizosphere soil ranged from $334 \text{ } \mu\text{g g}^{-1}$ to $353 \text{ } \mu\text{g g}^{-1}$ during pre-monsoon, $354 \text{ } \mu\text{g g}^{-1}$ to $436 \text{ } \mu\text{g g}^{-1}$ during mid-monsoon and $348 \text{ } \mu\text{g g}^{-1}$ to $403 \text{ } \mu\text{g g}^{-1}$ during post-monsoon. Bulk soil showed less MBC than rhizosphere soil across the land uses and the values ranged from $184 \text{ } \mu\text{g g}^{-1}$ to $231 \text{ } \mu\text{g g}^{-1}$ during pre-monsoon, $223 \text{ } \mu\text{g g}^{-1}$ to $265 \text{ } \mu\text{g g}^{-1}$ during mid-monsoon and $212 \text{ } \mu\text{g g}^{-1}$ to $280 \text{ } \mu\text{g g}^{-1}$ during post-monsoon. MBC was lowest during pre-monsoon across all the land uses. The values varied significantly ($p < 0.05$) between the seasons and the highest MBC was shown during mid-monsoon season in both rhizosphere and bulk soils across all the land uses. FL and NF showed the maximum values compared to other land uses during all the seasons in both rhizosphere soil and bulk soil. OPP showed the lowest MBC during all the seasons. The variations between plantation soils (RP and OPP) and FL, BF and NF were significant ($p < 0.05$) during all the seasons. The values also varied significantly between rhizosphere soil and bulk soil across the land uses.

The soil respiration in rhizosphere soil ranged from 109 mg CO₂ m⁻² h⁻¹ to 200 mg CO₂ m⁻² h⁻¹ during pre-monsoon, 278 mg CO₂ m⁻² h⁻¹ to 430 mg CO₂ m⁻² h⁻¹ during mid-monsoon and 183 mg CO₂ m⁻² h⁻¹ to 240 mg CO₂ m⁻² h⁻¹ during post-monsoon (Table 4.3). The values between both soil types and seasons varied significantly ($p < 0.05$) across the land uses. The soil respiration rate was significantly greater in rhizosphere soils than in bulk soils with greater values during mid monsoon across all the land uses in both the soils. Values in bulk soils across the land uses ranged from 73 mg CO₂ m⁻² h⁻¹ to 133 mg CO₂ m⁻² h⁻¹ during pre-monsoon, 145 mg CO₂ m⁻² h⁻¹ to 300 mg CO₂ m⁻² h⁻¹ during mid-monsoon and 109 mg CO₂ m⁻² h⁻¹ to 173 mg CO₂ m⁻² h⁻¹ during post-monsoon. RP showed the maximum soil respiration rate and lowest in BF. The soil respiration rates increased sharply from pre-monsoon to mid-monsoon which further decreased during post-monsoon.

4.1.4 Soil cation exchange capacity

The seasonal variations in exchangeable cations concentrations are shown in Table 4.4. The values varied significantly across the land use systems ($p < 0.05$) during all the seasons. The Ca values ranged from 0.05 cmol kg⁻¹ to 0.32 cmol kg⁻¹ during pre-monsoon, 0.06 cmol kg⁻¹ to 0.34 cmol kg⁻¹ during mid-monsoon and 0.06 cmol kg⁻¹ to 0.22 cmol kg⁻¹ during post-monsoon. The Mg values ranged from 0.05 cmol kg⁻¹ to 0.24 cmol kg⁻¹ during pre-monsoon, 0.08 cmol kg⁻¹ to 0.22 cmol kg⁻¹ during mid-monsoon and 0.12 cmol kg⁻¹ to 0.29 cmol kg⁻¹ during post-monsoon. Both Ca and Mg concentration was highest in RP during pre-monsoon and post-monsoon whereas BF showed highest value during mid-monsoon. However, least concentration variations were recorded in FL and NF stands during all the seasons.

Table 4.3 Seasonal variations in biochemical properties of rhizosphere (RS) and bulk (BS) soil under different land use systems. Values are mean \pm 1SE. LSD is shown at $p < 0.05$. (RP-rubber plantation, BF-bamboo forest, OPP-oil palm plantation, FL-fallow land, NF-natural forest; NH₄-N-ammonium nitrogen, NO₃-N-nitrate nitrogen, MBC-microbial biomass carbon).

Season/Land Use		Soil biological characteristics							
		NH ₄ -N (mg g ⁻¹)		NO ₃ -N (mg g ⁻¹)		MBC (μg g ⁻¹)		Soil respiration (mg CO ₂ m ⁻² h ⁻¹)	
		RS	BS	RS	BS	RS	BS	RS	BS
Pre-Monsoon	RP	8.5 \pm 1.2	5.8 \pm 1.0	1.1 \pm 0.1	0.82 \pm 0.06	343 \pm 1.4	198 \pm 1.8	200 \pm 7.2	133 \pm 3.2
	BF	5.2 \pm 0.1	7.1 \pm 1.4	0.7 \pm 0.05	0.71 \pm 0.08	341 \pm 2.4	184 \pm 2.0	109 \pm 5.7	73 \pm 5.8
	OPP	6.4 \pm 1.5	6.7 \pm 0.8	1.1 \pm 0.1	1.04 \pm 0.05	334 \pm 3.1	225 \pm 2.3	135 \pm 5.2	101 \pm 2.3
	FL	5.1 \pm 0.3	5.5 \pm 0.7	1.2 \pm 0.3	0.83 \pm 0.2	351 \pm 1.5	231 \pm 2.6	117 \pm 5.2	94 \pm 4.0
	NF	7.8 \pm 1.2	4.7 \pm 0.6	1.2 \pm 0.2	1.23 \pm 0.4	353 \pm 2.1	227 \pm 2.7	126 \pm 3.9	97 \pm 3.4
	LSD	3.32	3.11	0.65	2.79	4.2	6.9	8.41	17.4
Mid-Monsoon	RP	10.6 \pm 0.3	9.6 \pm 0.2	1.5 \pm 0.3	1.44 \pm 0.2	388 \pm 2.7	260 \pm 2.6	430 \pm 3.6	300 \pm 8.4
	BF	11.6 \pm 2.1	8.1 \pm 0.3	1.75 \pm 0.6	3.63 \pm 0.6	373 \pm 1.5	223 \pm 1.7	278 \pm 4.2	145 \pm 3.6
	OPP	10.3 \pm 1.1	10.5 \pm 1.5	2.75 \pm 0.2	2.67 \pm 0.7	354 \pm 3.3	251 \pm 2.5	421 \pm 7.5	295 \pm 6.4
	FL	8.9 \pm 0.5	9.9 \pm 0.4	1.91 \pm 0.2	1.44 \pm 0.3	436 \pm 4.8	265 \pm 2.9	395 \pm 5.1	275 \pm 2.1
	NF	9.6 \pm 0.3	9.1 \pm 0.3	3.04 \pm 0.5	4.04 \pm 0.3	418 \pm 2.7	263 \pm 1.7	400 \pm 2.6	256 \pm 5.1
	LSD	2.62	2.38	1.55	1.29	6.0	7.6	26.0	21.5
Post-Monsoon	RP	7.2 \pm 1.5	7.1 \pm 0.4	1.41 \pm 0.6	1.01 \pm 0.1	381 \pm 3.3	212 \pm 3.5	240 \pm 2.6	173 \pm 3.6
	BF	8.1 \pm 1.3	6.8 \pm 1.4	0.96 \pm 0.2	3.21 \pm 0.2	370 \pm 2.4	214 \pm 3.4	183 \pm 1.4	109 \pm 2.9
	OPP	6.0 \pm 0.2	11.1 \pm 1.9	1.61 \pm 0.3	2.44 \pm 0.4	348 \pm 1.5	219 \pm 2.4	204 \pm 3.6	138 \pm 2.3
	FL	6.3 \pm 0.3	4.4 \pm 0.1	1.19 \pm 0.2	1.41 \pm 0.3	403 \pm 3.1	266 \pm 1.6	216 \pm 1.7	149 \pm 3.4
	NF	7.7 \pm 0.2	10.8 \pm 0.3	2.69 \pm 0.5	2.65 \pm 0.2	400 \pm 2.9	280 \pm 2.4	205 \pm 2.6	145 \pm 4.8
	LSD	3.87	3.57	1.07	2.42	8.6	7.8	13.1	9.6

The concentrations also varied significantly between the rhizosphere soil and the bulk soil with maximum concentration in rhizosphere soil. Simultaneously, higher concentration of K, Al and Na was observed in FL and NF compared to soil of plantations (RP, OPP). The values of K ranged from 0.044 cmol kg⁻¹ to 0.243 cmol kg⁻¹ during pre-monsoon, 0.052 cmol kg⁻¹ to 0.126 cmol kg⁻¹ during mid-monsoon and 0.018 cmol kg⁻¹ to 0.116 cmol kg⁻¹ during post-monsoon, whereas Al values ranged from 0.004 cmol kg⁻¹ to 0.019 cmol kg⁻¹ during pre-monsoon, 0.001 cmol kg⁻¹ to 0.005 cmol kg⁻¹ during mid-monsoon and 0.003 cmol kg⁻¹ to 0.017 cmol kg⁻¹ during post-monsoon. NF showed the highest Na concentration among the land use and the values ranged from 0.005 cmol kg⁻¹ to 0.036 cmol kg⁻¹ during pre-monsoon, 0.02 cmol kg⁻¹ to 0.052 cmol kg⁻¹ during mid-monsoon and 0.012 cmol kg⁻¹ to 0.018 cmol kg⁻¹ during post-monsoon.

The cation exchange capacity (CEC) varied significantly across the land use systems (Table 4.5). Rhizosphere soil showed higher CEC (100 g⁻¹ soil) than bulk soil in all the land use system. CEC was comparatively higher in RP than BF, OPP, FL and NF. The values during pre-monsoon ranged from 2.64 meq to 3.02 meq in rhizosphere soil and 2.49 meq to 2.90 meq in bulk soil with highest value in RP. FL and NF showed low CEC compared to BF, RP and OPP during all the seasons. BF showed highest CEC in both rhizosphere and bulk soil during mid-monsoon and the values ranged from 2.68 meq to 3.00 meq and 2.54 meq to 2.92 meq in rhizosphere and bulk soils, respectively. The CEC values ranged from 2.68 meq to 2.98 meq in the rhizosphere soil and 2.65 meq to 2.96 meq in bulk soil, respectively during post monsoon.

Table 4.4 Seasonal variations in exchangeable cations concentration in rhizosphere (RS) and bulk (BS) soil under different land use systems. Values are mean \pm 1SE. LSD is shown at $p < 0.05$. (RP-rubber plantation, BF-bamboo forest, OPP-oil palm plantation, FL- fallow land, NF- natural forest; Ca-calcium, Mg-magnesium, K-potassium, Al-aluminum, Na-sodium).

Season/Land Use		Exchangeable cations (cmol kg ⁻¹)									
		Ca		Mg		K		Al		Na	
		RS	BS	RS	BS	RS	BS	RS	BS	RS	BS
Pre-Monsoon	RP	0.32±0.05	0.20±0.06	0.24±0.03	0.24±0.02	0.054±0.005	0.045±0.01	0.005±0.001	0.004±0.001	0.005±0.001	0.008±0.004
	BF	0.15±0.02	0.19±0.04	0.14±0.008	0.15±0.03	0.054±0.03	0.022±0.008	0.004±0.001	0.004±0.001	0.004±0.001	0.003±0.001
	OPP	0.09±0.009	0.06±0.005	0.13±0.01	0.12±0.02	0.044±0.006	0.023±0.005	0.006±0.001	0.010±0.004	0.003±0.001	0.006±0.001
	FL	0.03±0.006	0.04±0.008	0.10±0.007	0.06±0.002	0.243±0.07	0.053±0.008	0.019±0.003	0.018±0.005	0.025±0.011	0.006±0.002
	NF	0.05±0.01	0.01 ±0.001	0.05±0.011	0.02±0.004	0.217±0.08	0.047±0.02	0.013±0.002	0.016±0.004	0.036±0.010	0.016±0.004
	LSD	0.16	0.11	0.09	0.06	0.16	0.04	0.005	0.01	0.02	0.008
Mid-Monsoon	RP	0.26±0.004	0.18±0.012	0.21±0.014	0.20±0.014	0.052±0.002	0.045±0.001	0.003±0.000	0.002±0.000	0.03±0.002	0.012±0.002
	BF	0.34±0.010	0.23±0.010	0.22±0.012	0.25±0.012	0.064±0.006	0.037±0.006	0.001±0.000	0.004±0.000	0.02±0.001	0.013±0.000
	OPP	0.13±0.009	0.12±0.005	0.15±0.011	0.13±0.010	0.065±0.006	0.036±0.036	0.001±0.000	0.004±0.000	0.033±0.001	0.018±0.001
	FL	0.06±0.004	0.08±0.007	0.19±0.008	0.17±0.006	0.126±0.010	0.102±0.001	0.005±0.000	0.006±0.000	0.022±0.002	0.018±0.001
	NF	0.07±0.007	0.03±0.005	0.08±0.006	0.06±0.008	0.081±0.004	0.026±0.002	0.002±0.000	0.004±0.000	0.052±0.005	0.018±0.001
	LSD	0.04	0.02	0.03	0.03	0.02	0.008	0.0006	0.001	0.007	0.003
Post-Monsoon	RP	0.22±0.006	0.21±0.007	0.29±0.008	0.19±0.015	0.062±0.002	0.034±0.002	0.003±0.000	0.004±0.000	0.015±0.001	0.018±0.001
	BF	0.14±0.006	0.20±0.009	0.13±0.10	0.21±0.006	0.018±0.001	0.032±0.001	0.003±0.000	0.001±0.000	0.018±0.000	0.012±0.001
	OPP	0.12±0.010	0.17±0.012	0.22±0.014	0.17±0.010	0.039±0.001	0.031±0.001	0.003±0.000	0.001±0.000	0.016±0.001	0.010±0.001
	FL	0.12±0.009	0.17±0.008	0.28±0.010	0.29±0.018	0.116±0.002	0.088±0.003	0.017±0.013	0.004±0.000	0.012±0.001	0.009±0.001
	NF	0.06±0.004	0.08±0.003	0.12±0.006	0.12±0.006	0.083±0.001	0.048±0.001	0.006±0.000	0.002±0.000	0.018±0.001	0.012±0.000
	LSD	0.02	0.02	0.03	0.03	0.004	0.005	0.01	0.0004	0.002	0.001

4.1.5 Soil fertility index and soil evaluation factor

The Soil Fertility Index (SFI) and Soil Evaluation Factor (SEF) are shown in Table 4.6 and Table 4.7, respectively. SFI in rhizosphere soil ranged from 12.3 to 15.4 during pre-monsoon, 13.0 to 13.8 during mid-monsoon and 11.5 to 13.3 during post-monsoon across the land uses. The SFI of bulk soil was less compared to rhizosphere soil. SFI values of bulk soil ranged from 10.5 to 13.9 during pre-monsoon, 11.2 to 13.5 during mid-monsoon and 10.6 to 12.2 during post-monsoon. SFI was highest in RP and FL during pre-monsoon and post-monsoon, respectively, whereas the highest SFI value was recorded in NF during post monsoon. The SFI values were lowest in FL and NF during pre-monsoon and mid-monsoon, respectively.

SEF values in rhizosphere soil ranged from 5.3 to 5.6 during pre-monsoon, 5.2 to 5.6 during both mid-monsoon and post-monsoon whereas the SEF values for bulk soil ranged from 5.0 to 5.4 during pre-monsoon, 5.1 to 5.4 during mid-monsoon and 5.2 to 5.5 during post-monsoon. Highest SEF was observed in RP and lowest in NF, however, the differences were not significant with respect to land use and seasons.

Table 4.5 Seasonal variations in Cation Exchange Capacity of different land use systems. Values are means \pm 1SE. LSD is shown at $p < 0.05$. (RP-rubber plantation, BF-bamboo forest, OPP-oil palm plantation, FL-fallow land, NF-natural forest).

Land Uses	Cation Exchange Capacity (meq 100g ⁻¹ soil)					
	RP	BF	OPP	FL	NF	LSD
Pre-monsoon						
Rhizosphere soil	3.02 \pm 0.07	2.75 \pm 0.04	2.67 \pm 0.03	2.64 \pm 0.04	2.75 \pm 0.11	0.2
Bulk soil	2.90 \pm 0.1	2.77 \pm 0.08	2.63 \pm 0.01	2.56 \pm 0.01	2.49 \pm 0.005	0.19
Mid-monsoon						
Rhizosphere soil	2.95 \pm 0.01	3.00 \pm 0.02	2.78 \pm 0.02	2.80 \pm 0.02	2.68 \pm 0.02	0.07
Bulk soil	2.83 \pm 0.02	2.92 \pm 0.02	2.70 \pm 0.01	2.77 \pm 0.01	2.54 \pm 0.02	0.06
Post-monsoon						
Rhizosphere soil	2.98 \pm 0.01	2.70 \pm 0.02	2.80 \pm 0.02	2.93 \pm 0.01	2.68 \pm 0.01	0.05
Bulk soil	2.86 \pm 0.02	2.85 \pm 0.01	2.79 \pm 0.02	2.96 \pm 0.02	2.65 \pm 0.01	0.06

Table 4.6 Seasonal variations in Soil Fertility Index of different land use systems. Values are means \pm 1SE. LSD is shown at $p < 0.05$. (RP-rubber plantation, BF-bamboo forest, OPP-oil palm plantation, FL-fallow land, NF - natural forest).

Land uses	Soil Fertility Index					
	Pre-monsoon		Mid-monsoon		Post-monsoon	
	RS	BS	RS	BS	RS	BS
RP	15.4 \pm 0.6	13.4 \pm 0.5	13.8 \pm 0.7	12.2 \pm 0.4	12.1 \pm 0.3	10.6 \pm 0.3
BF	13.9 \pm 0.1	12.9 \pm 0.6	13.2 \pm 0.6	13.3 \pm 0.6	11.5 \pm 0.6	11.1 \pm 0.2
OPP	14.8 \pm 0.5	13.9 \pm 0.3	13.8 \pm 0.7	13.5 \pm 0.5	12.4 \pm 0.2	11.1 \pm 0.7
FL	12.3 \pm 0.9	10.5 \pm 1.7	14.3 \pm 2.1	13.3 \pm 0.04	12.9 \pm 0.6	11.5 \pm 0.4
NF	14.8 \pm 0.5	13.5 \pm 0.8	13.0 \pm 0.6	11.2 \pm 0.5	13.3 \pm 0.6	12.2 \pm 0.5
LSD	1.81	3.0	3.53	1.50	1.52	1.56

Table 4.7 Seasonal variations in Soil Evaluation Factor of different land use systems. Values are means \pm 1SE. LSD is shown at $p < 0.05$. (RP-rubber plantation, BF-bamboo forest, OPP-oil palm plantation, FL-fallow land, NF - natural forest).

Land uses	Soil Evaluation Factor					
	Pre-monsoon		Mid-monsoon		Post-monsoon	
	RS	BS	RS	BS	RS	BS
RP	5.6 \pm 0.08	5.4 \pm 0.1	5.5 \pm 0.02	5.4 \pm 0.02	5.6 \pm 0.02	5.4 \pm 0.02
BF	5.3 \pm 0.04	5.3 \pm 0.08	5.6 \pm 0.02	5.4 \pm 0.01	5.3 \pm 0.02	5.4 \pm 0.01
OPP	5.3 \pm 0.03	5.1 \pm 0.02	5.4 \pm 0.03	5.2 \pm 0.01	5.4 \pm 0.02	5.3 \pm 0.02
FL	5.4 \pm 0.08	5.1 \pm 0.01	5.3 \pm 0.01	5.4 \pm 0.01	5.5 \pm 0.02	5.5 \pm 0.02
NF	5.3 \pm 0.11	5.0 \pm 0.01	5.2 \pm 0.02	5.1 \pm 0.01	5.2 \pm 0.02	5.2 \pm 0.009
LSD	0.22	0.18	0.06	0.06	0.06	0.06

4.1.6 Correlation and regression analysis of soil parameters

The investigated soil properties were subjected to a correlation analysis against soil moisture (SM) and soil organic carbon (SOC) and the Pearson's correlation coefficients were determined. SM was positively correlated ($p < 0.05$) with SOC, TN, P_{avail} , NH_4-N , NO_3-N , MBC and SR across all the land uses (Table 4.8). Soil pH was negatively correlated with SOC whereas TN, P_{avail} , NH_4-N , NO_3-N , MBC and SR showed a significant positive correlation ($p < 0.05$) with SOC in all the land uses (Table 4.9). The efforts were made to develop a multiple correlation model to predict the value of soil CO_2 with that of soil properties (i.e. soil pH, SOC, TN, MBC) but none of the soil property variables entered the equation except SOC and MBC alone in predicting the amount of soil CO_2 efflux.

Table 4.8 Pearson correlation coefficient (r) of soil moisture (SM) versus soil variables in different land use systems (SOC-Soil organic carbon, TN-Total nitrogen, P_{avail}- Available phosphorous, NH₄-Ammonium, NO₃-Nitrate, MBC-Microbial biomass carbon, SR-Soil respiration)

	Land uses	pH	SOC	TN	P _{avail}	NH ₄	NO ₃	MBC	SR
SM	Rubber Plantation	0.050	0.924	0.972	0.963	0.993	0.214	0.142	0.773
	Bamboo Forest	-0.352	0.99	0.999	0.620	0.721	0.859	0.307	0.733
	Oil Palm Plantation	-0.801	0.899	0.087	0.689	0.994	0.961	0.75	0.979
	Fallow Land	0.598	0.717	0.455	0.375	1.000	0.939	0.951	1.000
	Natural Forest	-0.975	0.999	0.568	0.268	0.823	0.952	0.974	0.963

Table 4.9 Pearson correlation coefficient (r) of SOC versus soil variables in different land use systems (SOC-Soil organic carbon, TN-Total nitrogen, P_{avail}- Available phosphorous, NH₄-Ammonium, NO₃-Nitrate, MBC-Microbial biomass carbon, SR-Soil respiration)

	Land uses	pH	TN	P _{avail}	NH ₄	NO ₃	MBC	SR
SOC	Rubber Plantation	-0.334	0.808	0.787	0.963	0.571	0.509	0.956
	Bamboo Forest	-0.479	0.996	0.504	0.811	0.923	0.437	0.821
	Oil Palm Plantation	-0.459	0.514	0.937	0.942	0.744	0.385	0.791
	Fallow Land	-0.13	0.947	0.915	0.738	0.913	0.468	0.704
	Natural Forest	-0.982	0.596	0.300	0.842	0.942	0.966	0.971

Therefore, regression equations were developed for predicting CO₂ efflux against SOC (Table 4.10) and MBC (Table 4.11) in different land use systems. The equation to predict CO₂ efflux with SOC and MBC alone was significant at $p < 0.05$ with a varied coefficient of determination R² across the land use systems.

Table 4.10 Regression equation to predict CO₂ efflux (mg CO₂ m⁻² h⁻¹) with SOC (%) as a predictor variable across different land use systems

Land use	Equation	R-sq	R-sq (adj)
Rubber Plantation	CO ₂ efflux = 171.7 + 54.05 SOC	87.52%	75.03%
Bamboo Forest	CO ₂ efflux = - 184.2 + 201.3 SOC	99.52%	99.04%
Oil Palm Plantation	CO ₂ efflux = - 449.8 + 320.8 SOC	96.97%	93.95%
Fallow Land	CO ₂ efflux = - 1125 + 588.3 SOC	99.44%	98.88%
Natural Forest	CO ₂ efflux = - 1292 + 570.0 SOC	95.27%	90.54%

Table 4.11 Regression equation to predict CO₂ efflux (mg CO₂ m⁻² h⁻¹) with MBC (µg g⁻¹) as a predictor variable across different land use systems

Land use	Equation	R-sq	R-sq (adj)
Rubber Plantation	CO ₂ efflux = - 44.4 + 0.8254 MBC	84.02%	68.03%
Bamboo Forest	CO ₂ efflux = - 1191 + 3.913 MBC	93.29%	86.58%
Oil Palm Plantation	CO ₂ efflux = - 1830 + 5.876 MBC	97.74%	95.48%
Fallow Land	CO ₂ efflux = - 1003 + 3.140 MBC	91.62%	83.25%
Natural Forest	CO ₂ efflux = - 486.7 + 1.734 MBC	99.81%	99.62%

4.2 Changes in bacterial communities across land uses

4.2.1 *Bacterial diversity in land use systems*

Illumina paired end sequencing was carried out to assess the bacterial community composition in the soils of OPP, BF, RP, FL and NF. Species diversity estimator viz., Chao 1, Phylogenetic diversity, Simpson, Shannon-Wiener and observed species matrices were used to estimate the total number of bacterial species present in the community. A total of 2076313 sequencing reads were obtained in the present study. The results of alpha diversity indices showed that bacterial diversity was highest in BF compared to other land uses except that Shannon-Wiener index was marginally higher in RP (Table 4.12).

Operational Taxonomic Units (Bacterial groupings based on 97% similarity between the species within the group) was highest in BF across all the land use system. Phylogenetic diversity was higher in BF and least in FL. Similarly, Chao 1 diversity index and OTU were found to be highest in BF and least in FL. In general, the diversity of FL was lower than the other land use, indicating the ecological environment of FL to be more fragile. The rarefaction curves (Fig. 4.1 – 4.3) represent the OTUs vs sequences in each sample. It was found that with the increase in the number of sequences the observed OTUs were also found to increase and reach saturation plateau. Rarefaction curve for Shannon metric indicated that the sample has reached near saturation for higher taxonomic levels.

The beta diversity analysis was performed using Unweighted (non-phylogenetic) and Weighted (phylogenetic) Unifrac methods showing distinct clusters separating the bacterial communities in five different land uses (Fig. 4.4 and

4.5). NF had unique bacterial community which was totally different from other land uses (i.e. RP, OPP, BF and FL).

Table 4.12 Number of sequencing reads and estimates of diversity indices in different land use systems.

Land uses	Total reads	Chao 1 index	Phylogenetic Diversity	Simpson index	Shannon index
Rubber Plantation	479142	75753	2846	0.998	13.28
Bamboo Forest	404224	93993	3054	0.998	13.27
Oil Palm Plantation	439548	76255	2848	0.998	12.94
Fallow Land	329879	73013	2485	0.997	12.63
Natural Forest	423520	79119	2779	0.998	12.92

The taxonomic distributions of Bacterial phyla of OPP, BF, RP, FL and NF are presented in Fig. 6. It exhibits that 61.71% and 0.97% of the OTU were classified under Bacteria and Archaea, respectively, whereas 37.31% of the OTU was not taxonomically classified. OTU percentage varied with the different land use systems. Higher OTUs of *Acidobacteria*, *Bacteroidetes* were observed in NF whereas lowest was found in RP. Results also depicted that the % of OTUs of FL and NF were nearly similar i.e. the % of OTUs in FL was in the recovery stage to NF. This depicts that the bacterial community in other plantations has deviated compared to NF.

4.2.2 Bacterial community composition at the phylum level

For all the soil samples, *Acidobacteria*, *Proteobacteria* and *Verrucomicrobia* were the three most predominant phyla, totally accounting for more than 50% of all the sequences (Fig 4.6). NF showed the maximum abundance of *Acidobacteria* and

Proteobacteria which decreased with the change in land use whereas OPP showed the least distribution of both *Acidobacteria* and *Proteobacteria*. *Acidobacteria* and *Proteobacteria* was dominant in NF which decreased with land use change and showed the phyla distribution in a decreasing order as: FL>BF>RP>OPP. *Verrucomicrobia* was dominant in FL and was found in decreasing order as: OPP>RP>NF>BF. The relative abundance was found strongly correlated with the soil pH, with *Acidobacteria* representing larger portion of soil bacterial communities at low pH soils. Results also showed that *Verrucomicrobia* and *Acidobacteria* decreased with the increase in organic C, N and P concentration.

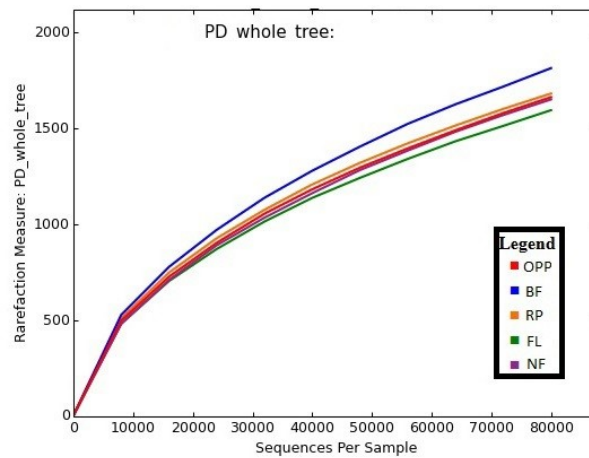


Figure 4.1 Rarefaction analysis of alpha diversity (Phylogenetic Diversity whole tree). (OPP-oil palm plantation, BF-bamboo forest, RP-rubber plantation, FL-fallow land, NF -natural forest).

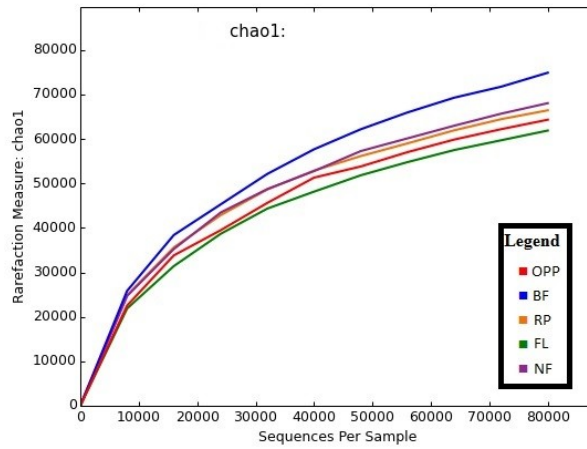


Figure 4.2 Rarefaction analysis of alpha diversity (Chao 1). OPP-oil palm plantation, BF-bamboo forest, RP-rubber plantation, FL-fallow land, NF - natural forest.

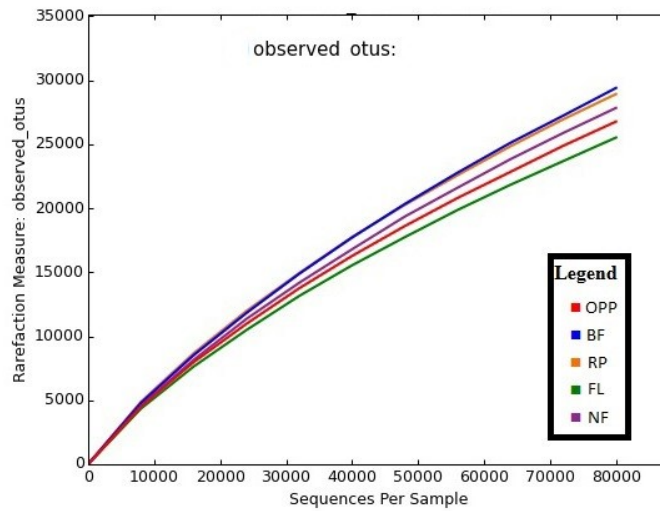


Figure 4.3 Rarefaction analysis of alpha diversity (Observed OTUs). (OPP-oil palm plantation, BF-bamboo forest, RP-rubber plantation, FL-fallow land, NF - natural forest).

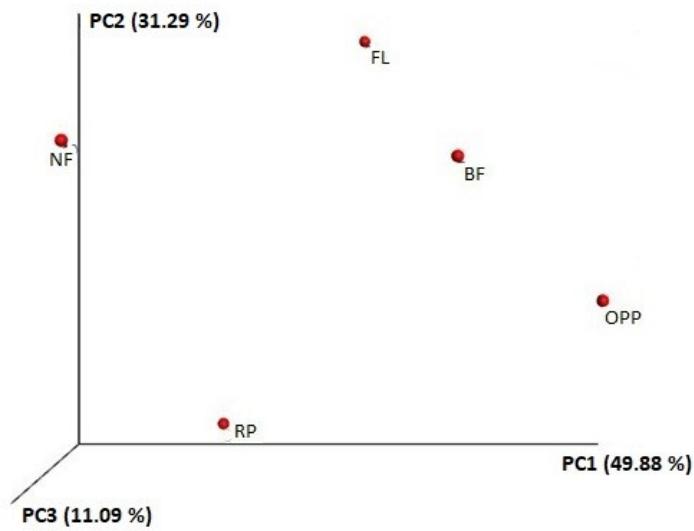


Figure 4.4 Unweighted PcoA plot among different land use systems (OPP-oil palm plantation, BF-bamboo forest, RP-rubber plantation, FL-fallow land, NF - natural forest).

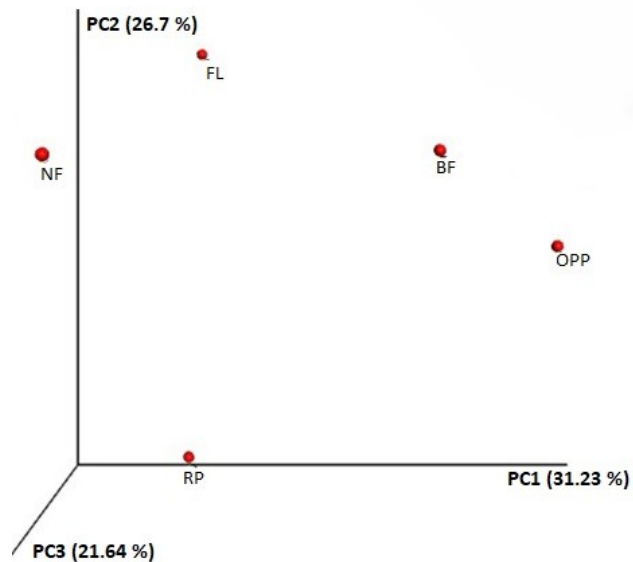


Figure 4.5 Weighted PcoA plot among different land use systems (OPP-oil palm plantation, BF-bamboo forest, RP-rubber plantation, FL-fallow land, NF - natural forest).

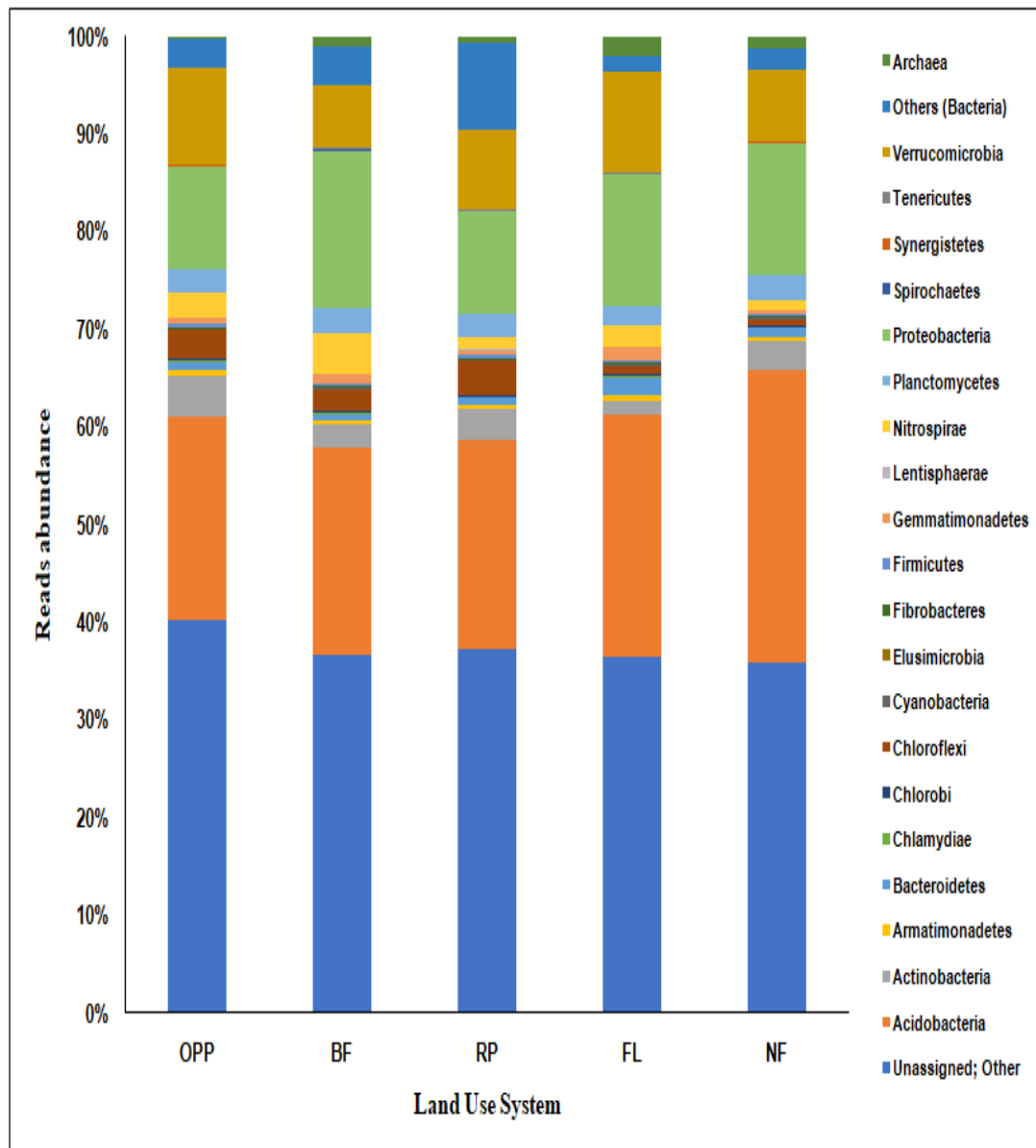


Figure 4.6 Taxonomy classifications of reads at phylum level in different land use systems (OPP- Oil Palm Plantation, BF- Bamboo Forest, RP- Rubber Plantation, FL- Fallow Land, NF- Natural Forest).

The present dataset also contains a considerable number of sequences affiliated to phylum *Lentisphaerae*, *Chloroflexi*, *Planctomycetes*, *Nitrospirae* and *Actinobacteria*. Within our dataset, we also identified a number of *Actinobacterial* sequences in all the soil types. In OPP sample, however, highest numbers of *Actinobacterial* sequences were identified. In addition, other low abundances candidate phyla were also detected which is as follows: *Chlorobi*, *Firmicutes*, *Bacteroidetes*, *Crenarchaeota*, *Gemmatimonadetes*, *Armatimonadetes*, *Thermi*, *Elusimicrobia*, *Cyanobacteria*, *Synergistetes*, *Euryarchaeota*, *Fibrobacteres*, *Chlamydiae*, *Tenericutes*, *Parvarchaeota*, *WS3*, *WPS-2*, *GAL15*, *FCPU426*, *AD3*, *TM7*, *OP3*, *OD1*, *TM6*, *BRC1*, *BHI80-139*, *NKB19*, *WS2*, *OP11*, *NC10*, and *GN02*.

4.2.3 Correlation and multiple regression analysis

Various soil properties were correlated with microbial diversity parameters. Soil pH was significantly positively correlated with PD, Chao1 index, Simpson index and Shannon index across different land uses ($r=0.76-0.98$, $p < 0.05$, Table 4.13). However, other rhizosphere soil variables had low correlation. Further, stepwise multiple regression analysis was performed to understand the effects of different soil characteristics on microbial diversity. Analysis result indicated that PD was strongly positively correlated (adjusted $R^2=95\%$ and predicted $R^2=78\%$) with pH (Table 4.14). However, the pH along with TN and P significantly improved the R^2 values (99-100%) in predicting PD. Shannon index was also correlated with soil pH but with a low coefficient value.

The PCA plot with PC1 and PC2 showed a cumulative variance of 57.15 % and 22.18 % respectively (Fig. 4.7). These PCA model with two components captures over 79.33% of the variance. NF and BF were closely related in terms of their abundant bacterial phyla and soil characteristics among different land uses. RP and OPP showed positive PC1 score and contrastingly NF, FL and BF showed negative PC1 scores. The variables that are responsible for this contrast are: AD3, *Chloroflexi*, *Actinobacteria*, P_{avail}, BR, TOC, TN, *Acidobacteria*, *Proteobacteria*, *Gemmatimonadetes*, *Bacteroidetes*, APA and *Crenarchaeota* and they have high contributions in PC1 built-up the process. Variables such as AD3, *Chloroflexi*, *Actinobacteria*, P, SR, TOC and TN have positive loadings for PC1 the rest has negative loadings for PC1.

Table 4.13 Pearson correlation coefficient (r) between soil biochemical properties and microbial diversity indices (PD- Phylogenetic Diversity; SI-Simpson index; SWI-Shannon-Wiener index; TOC-Total Organic Carbon; TN- Total Nitrogen; P_{avail}- Available Phosphorous; NH₄-N-Ammonium Nitrogen; NO₃-N-Nitrate Nitrogen; MBC-Microbial Biomass Carbon; SR- Soil Respiration) (*p* <0.05).

	Chao1	PD	SI	SWI	pH	TOC	TN	P _{avail}	NH ₄ -N	NO ₃ -N	MBC	SR	Ammonification
PD	0.791	1											
SI	0.444	0.864	1										
SWI	0.604	0.878	0.774	1									
pH	0.873	0.984	0.761	0.846	1								
TOC	-0.588	-0.120	0.394	-0.080	-0.295	1							
TN	-0.384	-0.193	0.266	-0.274	-0.328	0.852	1						
P_{avail}	0.496	0.897	0.997	0.813	0.803	0.329	0.200	1					
NH₄-N	0.880	0.871	0.750	0.657	0.871	-0.137	0.057	0.774	1				
NO₃-N	0.876	0.780	0.410	0.518	0.867	-0.604	-0.563	0.460	0.701	1			
MBC	0.744	0.371	0.137	-0.027	0.457	-0.429	-0.010	0.155	0.690	0.651	1		
BR	-0.413	0.075	0.314	0.467	-0.041	0.493	0.071	0.305	-0.254	-0.420	-0.875	1	
Ammonification	0.792	0.350	-0.006	-0.002	0.475	-0.668	-0.289	0.029	0.603	0.744	0.958	-0.881	1
Nitrification	0.851	0.485	0.176	0.120	0.585	-0.557	-0.187	0.207	0.737	0.776	0.979	-0.819	0.982

Table 4.14 Stepwise multiple regression between microbial diversity and soil properties (PD-Phylogenetic diversity; TOC-Total organic carbon; TN-Total nitrogen; P_{-avail}- Available phosphorous)

Variables	R-sq	R-sq(adjusted)	R-sq(predicted)
PD versus pH	96.74%	95.66%	77.98%
PD versus pH, TOC	99.91%	99.83%	78.89%
PD versus pH , TN, P _{-avail}	100.00%	100.00%	99.92%
Shannon index versus pH	71.51%	62.02%	28.41%

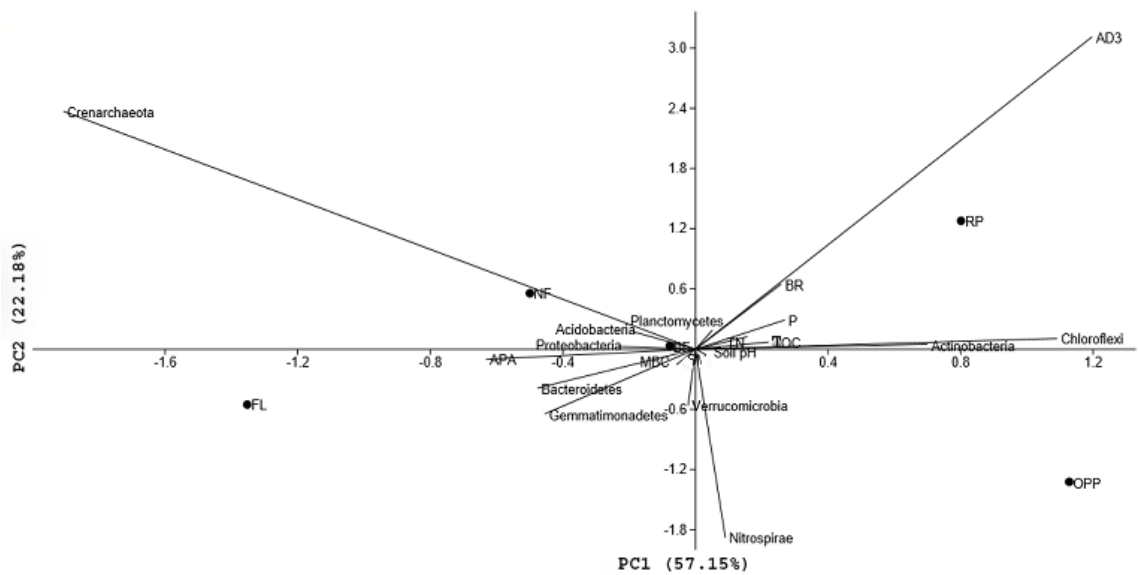


Figure 4.7 Principle component analysis (PCA) of dominant bacterial phyla with soil properties (TOC- Total Organic Carbon, TN- Total Nitrogen, P- Phosphorus, BR- Basal respiration, MBC- Microbial Biomass Carbon, OPP-Oil Palm Plantation, BF-Bamboo Forest, RP-Rubber Plantation, FL-Fallow Land, NF-Natural Forest).

4.3 Soil aggregation under different land use systems

4.3.1 Aggregate size distribution and mean weight diameter

The variations in macroaggregate and microaggregate size distributions was significant ($p < 0.05$), whereas, the variations in mesoaggregates were narrow and not significant across land uses. The percent variations in macro- ($>2000 \mu\text{m}$), meso- ($250\mu\text{m} - 2000 \mu\text{m}$) and micro-aggregates ($<250\mu\text{m}$) ranged from 51- 64%, 30.3- 39.2% and 5.6-11.5%, respectively (Fig. 4.8). The percent contribution of macroaggregates and mesoaggregates were considerably higher than microaggregates. The proportion of macroaggregates was maximum in plantation soils (RP and OPP) and minimum in BF. There were 18% and 19% increase in macroaggregate size fractions in RP and OPP compared to NF, whereas a marginal increase of 5% was recorded in FL. However, mesoaggregates was maximum in BF and minimum in RP. Proportions of microaggregate size fractions were maximum in NF with a proportionate decrease of 44%, 52%, 16% and 24% in RP, OPP, BF and FL, respectively.

The MWD ranged from 2.57 mm - 3.16 mm in different land uses. The variation was significantly ($p < 0.01$) different in various land uses (Table 4.15). The maximum MWD was obtained in plantation soils (RP and OPP) and minimum in BF.

4.3.2 Aggregate fractions associated SOC and TN

Variation in aggregate-associated SOC and TN content was significant ($p < 0.05$) across the land uses. The values of aggregate-associated SOC and TN ranged from 1.31%– 2.84% and 0.14% – 0.31%, respectively (Fig. 4.9 and Fig. 4.10) in

these land uses. The concentration of both SOC and TN associated with the macroaggregates was noticeably higher than mesoaggregates and microaggregates. The NF had significantly high SOC and TN associated with various aggregate sizes. However, the minimum amount of SOC and TN associated with macro-, meso- and micro-aggregates were recorded in OPP, BF and RP, respectively.

Table 4.15 Aggregate size classes distributions and their mean weight diameter (MWD) in different land use systems. Values are means \pm 1SE. LSD is shown at $p < 0.05$. (RP-rubber plantation, BF-bamboo forest, OPP-oil palm plantation, FL-fallow land, NF-natural forest)

Land Uses	Aggregate size fractions (mm)						MWD (mm)
	>4.75	4.75-2	2-0.5	0.25-0.5	0.053-0.25	<0.053	
RP	32.9 \pm 1.1	30.5 \pm 1.5	15.5 \pm 0.5	10.2 \pm 0.6	4.6 \pm 0.7	6.4 \pm 0.8	3.16
BF	20.3 \pm 2.0	30.9 \pm 1.4	18.5 \pm 0.5	14.3 \pm 1.3	6.3 \pm 0.2	9.8 \pm 1.4	2.57
OPP	36.4 \pm 3.3	26.4 \pm 1.5	14.2 \pm 1.5	12.3 \pm 2.2	5.3 \pm 1.0	5.5 \pm 1.1	3.09
FL	29.6 \pm 2.4	25.9 \pm 0.3	16.6 \pm 1.0	12.7 \pm 0.6	6.6 \pm 0.3	8.7 \pm 0.8	2.93
NF	27.7 \pm 0.5	25.4 \pm 0.4	16.1 \pm 0.6	11.0 \pm 0.7	8.3 \pm 1.1	11.5 \pm 0.6	2.80
LSD	6.30	3.48	2.77	3.74	2.32	3.07	0.37

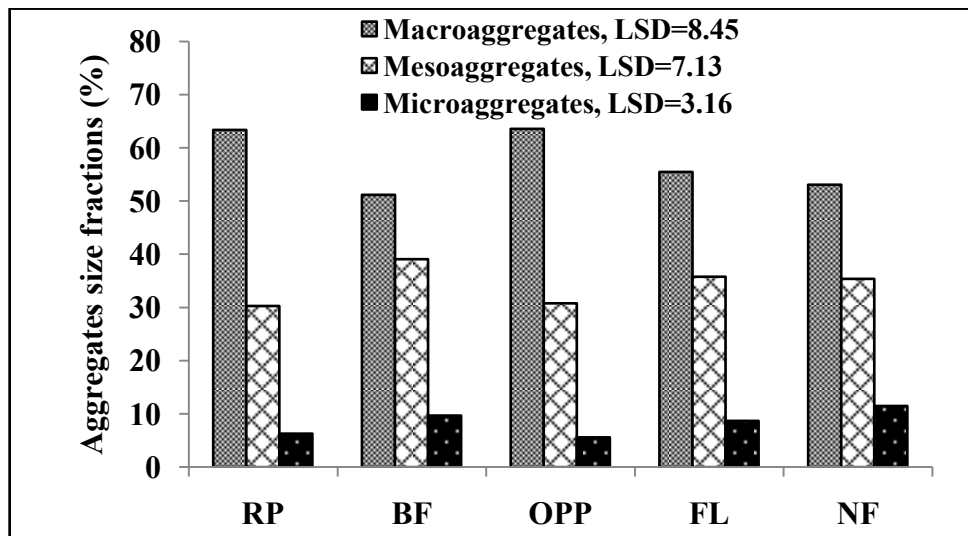


Figure 4.8 Variation in distribution of soil macroaggregates (>2.00 mm), mesoaggregates (0.250-2mm) and microaggregates (<0.250 mm) in different land use systems. Values are means. LSD is shown at $p < 0.05$. (RP-rubber plantation, BF-bamboo forest, OPP-oil palm plantation, FL-fallow land, NF - natural forest).

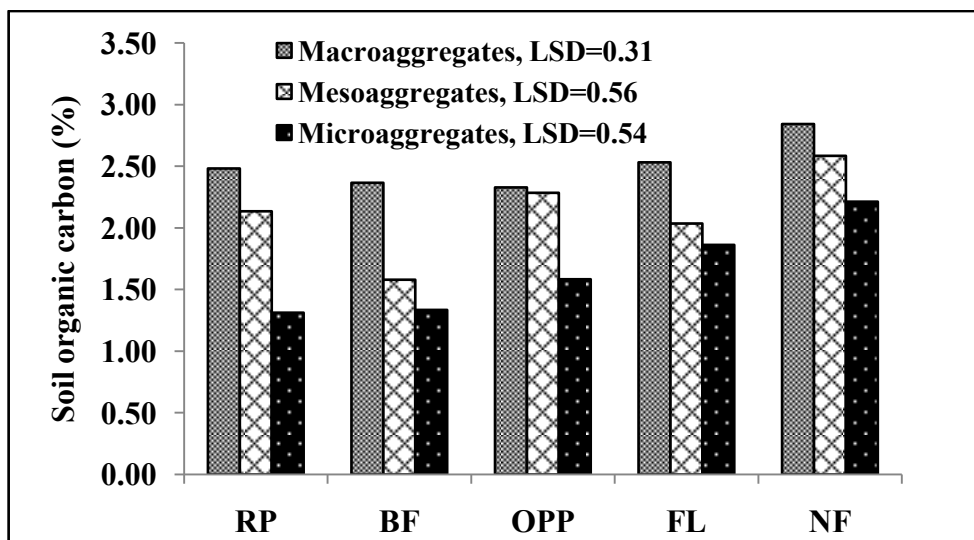


Figure 4.9 Amount of soil organic carbon in macro-, meso- and micro-aggregates fractions in different land use systems. Values are means. LSD is shown at $p < 0.05$. (RP-rubber plantation, BF-bamboo forest, OPP-oil palm plantation, FL-fallow land, NF -natural forest).

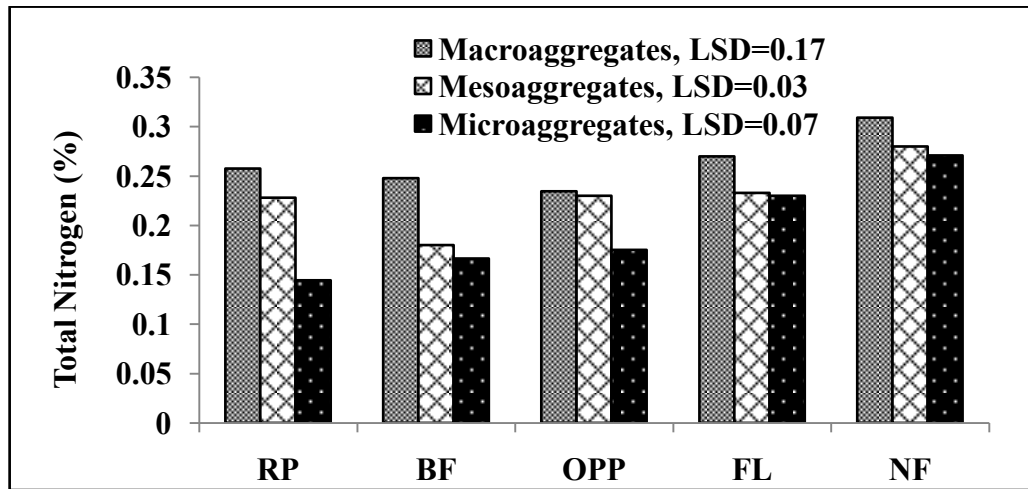


Figure 4.10 Amount of total nitrogen in macro-, meso- and micro- aggregates in different land use systems. Values are means. LSD is shown at $p < 0.05$. (RP-rubber plantation, BF-bamboo forest, OPP-oil palm plantation, FL-fallow land, NF -natural forest).

4.3.3. C/N ratio in aggregate size fractions

The C/N ratio of the aggregate size fractions was not significant and the ratio ranged from 8.26 – 9.91 across the land uses (Table 4.16). However, macroaggregate fractions showed wider C/N ratio and was followed by mesoaggregates and microaggregates fractions across the land use systems.

Table 4.16 C/N ratios in macro-, meso- and micro- aggregates size fractions in different land use systems. Values are means $\pm 1SE$.

Land uses	C/N ratio		
	Macroaggregates	Mesoaggregates	Microaggregates
Rubber Plantation	9.79 \pm 0.8	9.35 \pm 0.4	9.32 \pm 1.1
Bamboo Forest	9.61 \pm 0.4	8.77 \pm 0.3	8.61 \pm 1.3
Oil Palm Plantation	9.91 \pm 0.3	9.86 \pm 0.8	9.49 \pm 1.6
Fallow Land	9.38 \pm 0.1	8.73 \pm 0.8	8.26 \pm 0.5
Natural Forest	9.23 \pm 0.5	9.24 \pm 0.4	8.70 \pm 1.5

4.3.4 Aggregate-associated OC stocks

The changes in aggregate-associated OC stocks varied greatly among aggregate size fractions and land use systems (Fig. 4.11). There was a significant decline ($p < 0.05$) in aggregate-associated OC stock within aggregate. OC stocks were significantly higher in the macroaggregate fractions than in mesoaggregate and microaggregate. The amount of OC in macro-, meso- and microaggregate ranged from 1551 g m⁻² to 2013 g m⁻², 789 g m⁻² to 1171 and 106 g m⁻² to 325 g m⁻² respectively. The OC stocks in aggregate fractions decreased with the conversion of NF to fallow land and plantations except in RP, the OC stocks in macroaggregates was greater than NF (Fig. 4.11).

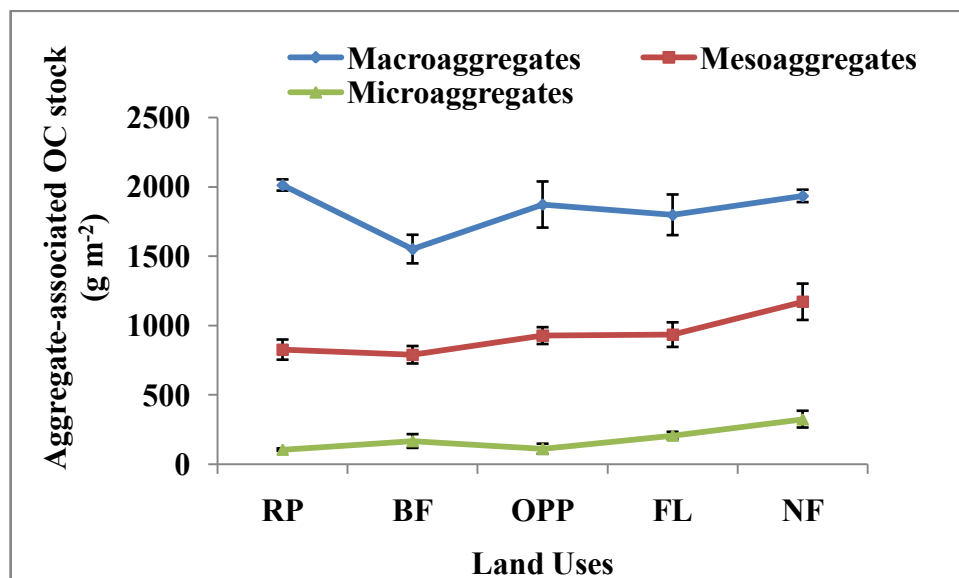


Figure 4.11 Aggregate-associated OC stock in different land uses. Values are means \pm 1SE. (RP-rubber plantation, BF-bamboo forest, OPP-oil palm plantation, FL-fallow land, NF -natural forest).

4.3.5 Multiple regression analysis

Stepwise multiple regression analysis was performed between different aggregate classes and soil properties to develop the regression equations (Table 4.17). Different soil properties variables were tried to predict the MWD and aggregate classes. The silt and clay contents entered the equation and significantly ($R^2=86\%$ and $R^2_{(adj)}=84\%$, $p <0.05$) predicted the MWD. Macroaggregates were significantly ($R^2_{(adj)}=96\%$, $p <0.01$) predicted by MWD, TN and BD. However, meso- and micro-aggregates were significantly ($R^2_{(adj)}=78-88\%$, $p <0.01$) positively predicted by SM, silt and clay contents and MWD and TN, respectively.

Table 4.17 Stepwise multiple regression equation relating mean weight diameter (MWD) and aggregate sizes versus soil properties in different land uses (TN- Total nitrogen, BD-Bulk density, SM-Soil moisture)

Variable	Regression Equation	R-sq	R-sq (adj)
MWD	1.989 + 0.0615 Silt + 0.0407 Clay	86.22%	83.92%
Macroaggregate	2.26 + 11.32 MWD - 34.2 TN + 26.35 BD	96.48%	95.52%
Mesoaggregate	68.44 - 0.619 SM - 1.571 Silt - 0.615 Clay	90.58%	88.01%
Microaggregate	6.10 - 4.60 MWD + 60.3 TN	81.16%	78.02%

4.4 Root exudation rates in different land use systems

Rate of root exudation varied significantly ($p < 0.05$) between different land use systems. The root exudation rates were measured for four times a year (April-May, July-August, October-November and January-February) for two years during 2015 and 2016 (Fig. 4.12). The monthly exudation rates of RP (*Hevea brasiliensis*) during the year 2015 ranged from 0.39 – 0.63 mg C g⁻¹ root day⁻¹ whereas it ranged from 0.35 – 0.54 mg C g⁻¹ root day⁻¹ during 2016. In BF (*Melocanna baccifera*) exudation rates decreased compared to RP and the value ranged from 0.40 – 0.49 mg C g⁻¹ root day⁻¹ during 2015 and 0.34 – 0.44 mg C g⁻¹ root day⁻¹ during 2016. The exudation rate in OPP (*Elaeis guineensis*) was lowest among the species studied. The values ranged from 0.31 – 0.41 mg C g⁻¹ root day⁻¹ during both 2015 and 2016. Root exudation rate was highest during the summer season in all the land use systems.

The annual root exudation rate varied significantly across the land uses (Fig. 4.13). RP showed the maximum annual root exudation rate among the land use systems and the values ranged from 137 – 204 mg C g⁻¹ root yr⁻¹. The values in BF ranged from 135 – 158 mg C g⁻¹ root yr⁻¹. The annual root exudation rate was lowest in OPP and the values ranged from 114 – 141 mg C g⁻¹ root yr⁻¹.

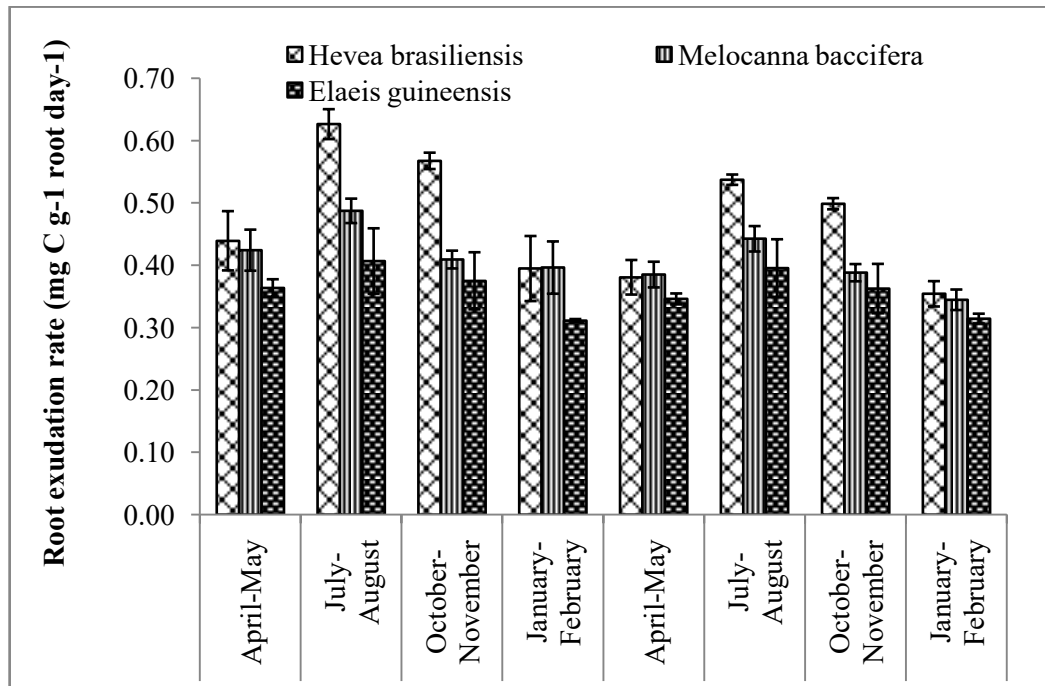


Figure 4.12 Monthly variations in root exudation rate of rubber (*Hevea brasiliensis*), bamboo (*Melocanna baccifera*) and oil palm (*Elaeis guineensis*) during 2015 and 2016. Values are mean \pm 1SE.

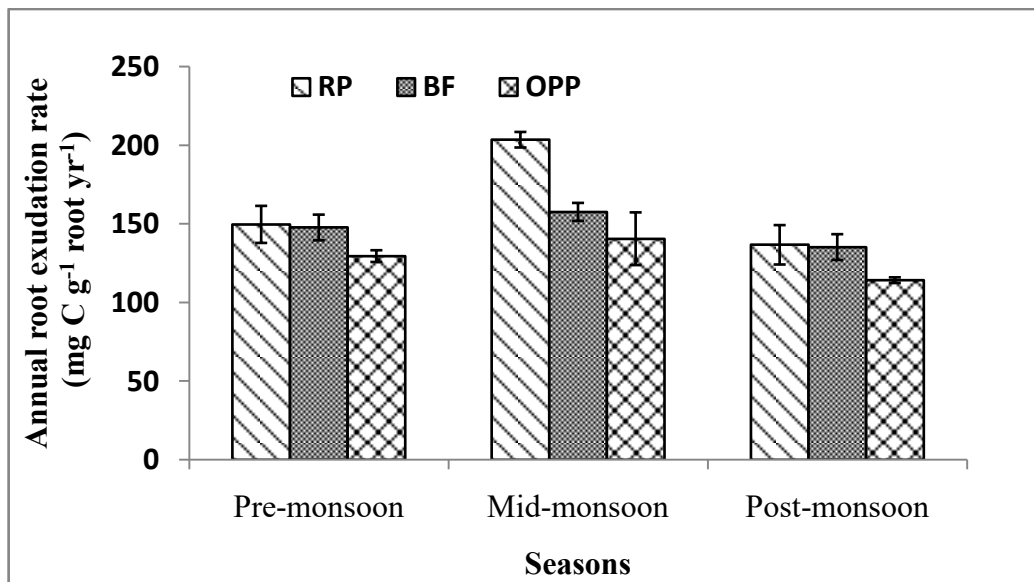


Figure 4.13 Seasonal variation in annual root exudation rates in different land use systems. Values are mean \pm 1SE.

CHAPTER 5

DISCUSSION

5.1 Effect of land use change on soil physico-chemical and biological properties

Land use significantly affected various soil properties. For example, bulk density varied significantly between different land uses. Increase in the bulk density may be related to decreased soil organic matter (SOM) content and altered process of soil aggregation due to conversion of the natural forest to plantations. Similar reports have been made by Lalfakzuala *et al.* (2008) that a heavily cultivated land disturbs the soil productivity because of the loss in organic matter. According to Alexander, (1989) higher SOM content decreases the value of the bulk density of the soils. The FL (secondary forest), BF and NF showed less bulk density than plantation soils due to higher root biomass, accumulation of organic matter and microbial communities. Higher bulk density in rubber plantation soils was earlier reported in Indonesia (Allen *et al.*, 2015). This is probably due to high rate of soil erosion caused by reduced ground vegetation (Guillaume *et al.*, 2015) and increase in soil compaction due to high rubber tree density in plantations.

The sand, silt and clay ratio indicated that land use change affected the native soil properties. RP and OPP showed high clay percent and less amount of sand when compared to BF, FL and NF. This could be attributed to increased removal of top soil through the clearing of ground vegetation by land owner for the management of plantations. Igue, (2004) argued that continuous cropping and intensive land use pattern also affects the particle size distribution.

Soil moisture is abruptly affected by land use change due to the variations in infiltration rate, surface runoff and evaporation (Demir *et al.*, 2007; Zhai *et al.*, 1990). Maximum soil moisture content in NF during mid-monsoon can be attributed to dense vegetation whereas regular surface weeding and poor surface vegetation led to less organic matter in RP and OPP. The seasonal variation in soil moisture content is probably due to annual precipitation pattern.

Variations in soil pH among land use systems reflect differences in uptake of exchangeable bases, N fixation and production of litter (Githae *et al.*, 2011). In the present study, the pH was significantly higher in plantation soils in comparison to the FL and NF. This could be due to high leaching of cations under FL and NF and thus leaving more stable cations like Fe and Al (Table 4). Zaidey *et al.* (2010) reported that the Al concentrations and soil organic matter influences the soil acidity. The strongly acidic nature of the soil may be related to the leaching of exchangeable cations because of high rainfall (>2000 mm) during the monsoon period. The pH range of 3.8 to 5.6 in the present study indicated high soil microbial biomass in all the land use systems. Bier & Singh, (2018) also reported a pH range from strongly acidic to moderately acidic in soils under *jhum* and forest land use system. Arunachalam & Pandey, (2003) have also reported that acid soils supports optimum bacterial and fungal growth.

The nutrients in soil largely depend on nature of vegetation (Mishra, 2010) as the litter layer plays an important role in enhancing soil productivity (Singh & Mudgal, 2000). The organic carbon content in soil is important in regulating nutrient availability and microbial activity in any ecosystem. The higher SOC and TN content in FL and NF might be due to higher organic matter inputs from above- and below-

ground litter (Materechera, 2010). The accumulation of organic matter in soils can be expected in FL and NF due to its vegetation and abundance of forest litter. Higher soil moisture including SOC and TN content in FL and NF soils indicated higher microbial biomass (Table 2 and 3). Conversion of natural forest to plantations modifies the soil physico-chemical properties and the soil bacterial community (Murty *et al.*, 2002). Studies have confirmed that RP resulted in significant decrease in SOC and microbial biomass (Zhang *et al.*, 2007). Study of van Straaten *et al.* (2015) showed that conversion of natural forest to RP and OPP decreases SOC stocks by 50%. Nevertheless, FL was 20 yr old in the present study, the SOC concentration was found lower than NF. Similar result has been reported by Fagotti *et al.* (2012) and Nogueira *et al.* (2006).

Less concentration of SOC and TN in OPP soils contrasting to RP indicated a higher carbon input through leaf litter fall under RP. Similarly, Guillaumea *et al.* (2016) found decreased carbon content in soil under OPP. Further, this shows a slow rate of organic matter decomposition in OPP. The addition of fertilizer in OPP probably decreases the microbial population in soil. Low content of SOC in OPP is also due to poor drainage within the rows. The adventitious root system, use of machinery during the harvesting period and cultural operations involved in OPP could further deteriorate the soil physical conditions restricting the root growth.

Higher P_{avail} content during pre-monsoon and mid-monsoon in RP and OPP is probably due to the presence of higher number of *Pseudomonas* (phosphorous solubilizing bacteria). Some other phosphorous solubilizing bacteria found in different land use were: *Burkholderia*, *Bacillus*, *Rhodococcus*, *Arthrobacter* and *Serratia*. However, Ekukinam *et al.* (2014) found depletion in P availability under

rubber plantation with increasing age of plantation. On the other hand, comparatively low P_{avail} in FL is possibly due to the degradation of phosphorous mobilizing and solubilizing bacteria (PSB) as PSB and fungi are well known in regulating phosphorous availability.

Changes in soil properties due to management can significantly affect mineralization rate in soils. It is well understood that soil and vegetation characteristics (Pajares & Bohannan, 2016) have a significant role in forest N cycling. NH_4 concentration in rhizosphere soil of RP was found greater compared to OPP, BF, FL and NF whereas NF showed the maximum concentration of NO_3 . High NH_4 concentration in RP can be attributed to deciduous nature of RP as a large amount of nutrients are returned as litter fall to the soil (Kush *et al.*, 1990) which acts as the principal organic matter source in RP. Krishnakumar & Potty, (1992) estimated the turnover rate to be about $6 \text{ t ha}^{-1}\text{yr}^{-1}$. RP can also be characterized by faster decomposition of leaf litter that is accumulated on the surface of the soil. Subsequently, low soil pH in FL and NF possibly inhibited the rate of ammonification. On the other hand, low soil pH is also known to accelerate the nitrification rate (Lu *et al.*, 2014). N uptake by soil microorganisms regulates the N balance of forest ecosystems (Zogg *et al.*, 2000). High nitrification rates in NF may also be attributed to higher heterotrophic nitrification. Similar results have been reported in tropical and subtropical soils elsewhere (Pett-Ridge *et al.*, 2013; Zhang *et al.*, 2013). Zhu *et al.* (2015) suggested that fungi are the important driver for heterotrophic nitrification in acidic forest soils.

High $\text{NH}_4\text{-N}$ during mid-monsoon in both rhizosphere and bulk soil suggested that the uptake of $\text{NH}_4\text{-N}$ was reduced during mid-monsoon likewise the

uptake of $\text{NO}_3\text{-N}$ was reduced during post-monsoon. Greater availability of $\text{NH}_4\text{-N}$ during mid-monsoons also depicts the higher rate of ammonification and similarly greater availability of $\text{NO}_3\text{-N}$ during post-monsoons suggested higher nitrification rate in the soils. These both processes were enhanced by higher soil moisture content in the study.

Microbial biomass shows variable effects with land use change (Haripal & Sahoo, 2014; Yang *et al.*, 2010). Results showed high MBC during mid-monsoon season in both rhizosphere and bulk soils across all the land use systems. It is evident from the significant positive correlation observed between soil moisture and microbial biomass carbon that during mid-monsoon the soil moisture stimulated the microbial activity. A similar result has been reported by Silva *et al.* (2012) in tropical soils. Zhong & Makeschin, (2006) found the maximum values of microbial biomass during summer season. The higher microbial biomass during mid-monsoon in the present study may be due to high immobilization rate during the soil organic matter decomposition. Several workers reported a close relation of soil moisture with microbial biomass, which often favours the growth of microbes and fungi during this season (Yang *et al.*, 2010). Increase in soil microbial biomass accumulation in the tropics with increasing rainfall has also been reported (Szott *et al.*, 1999). Seasonal change in soil microbial biomass in tropical dry forest has also been reported by Roy & Singh, (1994).

Soil microbial biomass carbon was also influenced by SOC in the present study. The comparatively high MBC values in FL and NF may be because of the abundance of diverse vegetation and vegetation cover which contributed to high

amount of substrate for the activities of microorganisms. Several workers have reported a close linkage between plant richness and microbial biomass (Broughton *et al.*, 2000; Spechn *et al.*, 2000; Bardget *et al.*, 1999). Reports have shown that higher soil carbon enhance the growth of soil microbes and accumulation of microbial biomass in soil (Tripathi *et al.*, 2008; Chen *et al.*, 2005). The minimum MBC recorded in plantation soils may be attributed to lack of surface vegetation cover. Reduced quality and quantity of organic matter in the soil have resulted in loss of microbial activity (Degens *et al.*, 2000). Influence of quantity and quality of organic matter inputs on soil microbial biomass and activities by different vegetation has been well documented (Jin *et al.*, 2010; Xu *et al.*, 2008).

Soil respiration in terrestrial ecosystems has a major role in carbon cycling and climate change (Jackson *et al.*, 2009; Valentini *et al.*, 2000). Soil respiration rates increased sharply from pre-monsoon to mid-monsoon (Table 3). The higher soil respiration rate is possibly due to higher physiological activity in roots due to plant growth (Hogberg *et al.*, 2001) and heterotrophic respiration because of increased microbial decay of organic matter decomposition. The result of the present study revealed that C released due to soil respiration was high in mid-monsoon than in pre-monsoon or post-monsoon seasons across all the land uses in both rhizosphere and bulk soils. This can be attributed to higher moisture content in soil during mid-monsoon. Similarly, Chen *et al.* (2008) reported higher soil respiration rate was due to higher soil moisture content. Upadhyay *et al.* (2004) also observed seasonal variation in the rates of soil respiration in bamboo forest.

Higher soil respiration rate in RP than in other land use is possibly due to the high quantity of above ground litters thereby conserving soil moisture and making

temperature suitable for the activity of microbial community. The rates of soil respiration in RP are often affected by the age of rubber tree. Puttaso *et al.* (2015) reported highest soil respiration in 27 years plantation followed by 11 years, 17 years and 3 years in Northeast Thailand. In the present study, the lowest soil respiration activity in BF is primarily due to low productivity of the herbaceous vegetation.

The exchangeable cations (Ca, Mg, K, Al and Na) were generally low in the study area and the proportion varied substantially across land uses. High Ca and Mg concentration in RP is possibly due to the presence of herbaceous vegetation and the growth of dense canopy which further protected the soil from the direct impact of rainfall. However, the concentration of exchangeable calcium (Ca) and magnesium (Mg) are reported to reduce drastically with the increasing age of rubber plantation whereas K concentration increases with age (Ekukinam *et al.*, 2014). On the other hand, least concentration of Ca and Mg and higher concentration of Al in FL and NF are possibly due to higher leaching which further led to low soil pH. Furthermore, addition of NH_4 as a replacement of NO_3 in substrate systems may also reduce the uptake of Ca and Mg in FL and NF soils.

The variation in CEC among the land use is probably due to strong association of CEC with SOM and soil texture, indicating that the high sand content reduces the CEC of the soils. Continuous leaching of Ca and Mg could partly lead to low cation exchange capacity in FL and NF soils. The relative amount and type of colloidal substances (OM and clay) in different land use also play important role in the CEC of soil.

5.2 Land use change effects on bacterial communities

5.2.1 Bacterial diversity in different land use systems

The study has major implications for the soil microbial restoration and the management of forest in global change scenario. Profound changes were noticed in bacterial community composition in the rhizosphere soils of various land uses (e.g. OPP, BF, RP, FL and NF). Results showed that soil bacterial community was responsive to nutrient distribution pattern, but such responses differed among phyla. Different species diversity estimator viz., Chao 1, Phylogenetic diversity, Simpson, Shannon-Wiener and observed species matrices showed a total number of bacterial species present in the community. High bacterial diversity in BF is possible due to the large organic inputs such as litter and root exudates that provide a source of organic carbon thereby favouring microbial growth (Phillips, 2007). Changes in microbial groups may be related to variations in environmental factors (i.e. temperature, moisture, O₂ availability and pH) as they provide better adaptability to promote specific microbial groups to the new environment (Eilers *et al.*, 2012). It has been reported that bacterial diversity changes as a function of soil pH (Fierer & Jackson, 2006) and lower pH favours less bacterial diversity. Soils of FL were more acidic than other land uses, and thus in the present study, microbial diversity in different land uses changes as a function of soil pH.

The beta diversity analysis showed that NF and RP had unique bacterial community which is totally different from other land uses. The similarity in % of OTUs of FL and NF depicts that FL was in the recovery stage to NF. This depicts that the bacterial community in other plantations has deviated compared to NF.

5.2.2 Bacterial community composition at the phylum level

The overall bacterial compositions of five different land use system were similar but the relative abundances of each phylum varied among land use types. Land use system possibly affected the distribution and abundances of the bacterial communities. The most abundant phyla were *Acidobacteria*, *Proteobacteria* and *Verrucomicrobia*, and these phyla have been reported to be dominant in tropical forests (Jaitz *et al.*, 2011; Miyashita *et al.*, 2013). Members under the phyla *Acidobacteria* varied with soil properties, soil type and land-use change (Mendes *et al.*, 2015), whereas *Proteobacteria* is regarded as free-living bacteria present in different habitats (Yang *et al.*, 2015). *Acidobacteria* is mostly abundant in forest soils, while *Proteobacteria* occurs in disturbed soils (Chakraborty *et al.*, 2015). It is possibly because *Acidobacteria* are slow growing bacteria species that can be adapted to nutrient limited ecosystems and are replaced by fast-growing bacteria when the soil nutrient content is altered due to anthropogenic influences. Further, the loss of *Acidobacteria* and *Proteobacteria* phyla in the present study could be considered as bio-indicator towards land degradation. The phylogenetic diversity of *Acidobacteria* depends on soil pH (Fierer & Jackson, 2006) which has also corroborated with the present findings. Changes in relative abundance of the dominant phyla in different land use indicate that bacterial communities were modified during land use change. Since, *Acidobacteria* and *Proteobacteria* are the dominant groups, these phyla together with physico-chemical characteristics of the soils shapes the microbial distribution and nutritional status of the studied land use systems.

In terrestrial ecosystem, broad distribution of *Verrucomicrobia* in soils has been reported confirming that they are important members of soil microbiota (Lupatini *et al.*, 2013). Results showed that *Verrucomicrobia* and *Acidobacteria* decreased with the increase in TOC, TN and P_{avail} concentration. The abundance of *Verrucomicrobia* in FL was probably due to the lower concentration of primary soil nutrients which was affected due to Jhum cultivation. Recently, a decrease in abundance of *Verrucomicrobia* has been reported (Huang *et al.*, 2012) after the increase in available nitrogen, phosphorus and potassium, and soil organic matter. In Brazilian Amazonia, lower abundance of *Verrucomicrobia* was reported in deforested soils (Mendes *et al.*, 2015). The presence of *Verrucomicrobia* under diverse soil conditions employs to investigate Verrucomicrobial community as a bioindicator of tropical soils (Navarrete *et al.*, 2015). The distribution of Verrucomicrobial 16S rRNA in soil is also affected by temporal variation of environmental factors like soil history, soil depth, and soil moisture content (Buckley & Schmidt, 2001). The soil nitrogen availability exerts a great impact on the soil Verrucomicrobial communities in certain ecosystems, including oligotrophic environments (Wertz *et al.*, 2012). The present findings of highest abundance of *Verrucomicrobia* in FL corroborated well with the previous observations and we believe that nitrogen and phosphorous played important roles in shaping distribution and abundance of *Verrucomicrobia* in soils.

Besides abundant phylum, the distribution of phyla *Lentisphaerae*, *Chloroflexi*, *Planctomycetes*, *Nitrospirae* and *Actinobacteria* varied due to soil nutritional status in different land use (Pan *et al.*, 2014). *Lentisphaerae* have been reported in soil samples collected from the diverse environmental niche

(Castaneda & Barbosa, 2017). *Chloroflexi* have been reported to be abundant in nutrient poor soils (Will *et al.*, 2010) for example, oligotrophic environments such as soils from high-elevation regions with patchy vegetation (Freeman *et al.*, 2009), alpine tundra soil (Costello & Schmidt, 2006) and hyper-arid polar desert soil (Pointing, 2009). Soil history had a significant impact on the richness of the *Planctomycetes* community. Previously, the heterogeneity in soil NO₃-N levels (nitrate as nitrogen), soil nutritional history, compost amendments, and soil oxygen distribution have been shown to correlate with *Planctomycetes* abundance in soil (Derakshani, 2001). Abundance of *Nitrospira* in soil has been shown to be dependent on soil nitrite oxidation activity (Xia *et al.*, 2011). Previous studies have shown that reduction of soil nitrite oxidation activity favoured increased abundance of *Nitrospira* like nitrite oxidizing bacteria in soil (Attard *et al.*, 2010).

However, highest numbers of Actinobacterial sequences in OPP were identified due to higher proportion of bulk soil following the management practices in OPP compared to other land uses. In general, *Actinobacteria* have been recognized as typical bulk-soil inhabitants (Smalla *et al.*, 2001). Members under this phylum are mostly slow-growing and possess traits like plant growth promotion and biosynthesis of biologically active secondary metabolites (Basilio *et al.*, 2003). The minimal population of *Actinobacteria* shown in FL can be attributed to the effect of *Jhum* agricultural practices which gradually promoted the dominance of other phyla. Together, detection of these bacterial phyla within the dataset confirms the fact that soil nutritional status, available phosphorous, total nitrogen and ongoing shifting cultivation have immense impact in shaping the microbial diversity and distribution in hilly mountainous region of Northeast India.

Previous studies have reported that land use change lead to significant shift in microbial community composition which could be linked to changes in soil abiotic properties such as soil pH (Macdonald *et al.*, 2009; Costa *et al.*, 2006). The significant positive correlation of microbial diversity indices with that of soil pH indicates that the soil pH is a major factor responsible for regulating the microbial composition and diversity in these ecosystems. Decreased microbial diversity with greater acidity suggested that extremely low pH creates stressful condition for unicellular bacteria. Reports showed that the bacterial diversity in tropical regions increases with high soil pH (Ito *et al.*, 2017; Navarrete *et al.*, 2015). In addition, stepwise multiple regression analysis suggested that the soil pH, TN and P_{-avail} are strongest predictor variables explaining 99% variability in the microbial diversity in these ecosystems.

5.3 Effect of land use change on soil aggregate size fractions and aggregate associated OC stock

Land use change significantly affected aggregate size fractions and stability due to variations in land management practices (Somasundaram *et al.*, 2017; Tisdall & Oades, 2012; Dalal *et al.*, 2011), land use type (Kalhor *et al.*, 2017) and nutrient additions (Sarker *et al.*, 2018; Tripathi *et al.*, 2008) in different ecosystems of the world. Among the studied land use practices, plantations of rubber and oil palm significantly altered soil aggregate size distribution with considerably higher proportion of macroaggregates. In NF, BF and FL, the proportional decrease in macroaggregates and increase in meso- and microaggregates was noted (Fig. 4.8). Considerable changes in the proportional changes in aggregate sizes in two sets of

ecosystems appeared to be strongly affected by the root architecture, litter intrinsic quality and soil organic matters (Rabbi *et al.*, 2014). In RP, gum secretion in addition to higher C exudation rates from root systems appeared to support higher macroaggregate formation (Table 4.18). However, in OPP, dense root systems with different intrinsic quality may alleviate the proportion of macroaggregates. Further, in RP, greater earthworm population may contribute to soil aggregation through the formation of organo-mineral complex and occlusion of plant residues by clay minerals (Saidy *et al.*, 2013).

The fractions of microaggregate in NF could be important for long term storage of carbon in the soil by enhancing the carbon sequestration potential of the natural forests. There are reports that the formation and stabilization of soil aggregates is due to quality and quantity of organic and inorganic stabilizing agents (Fink *et al.*, 2016; Cowie *et al.*, 2013). The fine root distribution pattern and penetration into the soil also affects the formation and stability of soil aggregate (Erktan *et al.*, 2016). In this study, stability of aggregate sizes varied with soil moisture content (Table 4.15) as a result of increase clay dispersion (Watts *et al.*, 1996) and decrease macroaggregate stability (Shu *et al.*, 2015). In NF, considering increased clay dispersion and decreased aggregate stability can be suggested as a possible cause of high organic matter and decreased aggregate stability.

Maximum aggregate stability as evident by higher MWD in RP and OPP reflected higher clay content and bulk density, and lower soil moisture content (Table 1). Rubber-based agroforestry systems in tropics have been reported to increase MWD (Chen *et al.*, 2017). Clay content in soil act as cementing agent (Fink *et al.*, 2016; Feng *et al.*, 2013) and therefore, the aggregate stability depends on the

physico-chemical properties of the clay and associated minerals (Denef & Six, 2005). High content of iron and aluminum oxides have been found to enhance aggregate stability (Bissonnais *et al.*, 2018). Soils of the northeastern India are generally rich in iron and aluminum because of high rainfall (Roy *et al.*, 2015), and therefore higher aggregate stability may be related to the climatic conditions of the region.

Low range of the soil C/N ratio in the studied land use systems is due to humid tropical climate of the area which led to a rapid decomposition of organic matter (Chen *et al.*, 2017). The C/N ratio of aggregate fractions in all land use systems reflected faster degradation and mineralization rates in microaggregates (Guo *et al.*, 2012). Vodnik *et al.* (2008) argued that greater C/N ratio in macroaggregates was due to incomplete humified organic plant residues which indicated the role of active binding agents.

In the present study, macroaggregates acquired the maximum amount of soil OC and total N and minimum in microaggregates. Our result is in agreement with Wang *et al.* (2014) & Ayoubi *et al.* (2012). Kushwaha *et al.* (2001) reported that the concentrations of SOC and TN were distinctly greater in the macroaggregate fractions under different tillage practices. Similarly, Li *et al.* (2016) also reported higher SOC and TN concentrations in macroaggregates than either in microaggregates or silt-plus-clay fractions in soils under diverse tropical forest lands in China. High concentration of aggregate-associated soil OC and total N in NF can be attributed to high rate of organic matter decomposition. Further, this may lead to higher C concentration in the soil thereby enhancing belowground C sequestration potential. Forest soils accumulate considerably higher SOC than savannas and agro-

ecosystems (Hagedorn *et al.*, 2001). Less organic carbon due to conversion of tropical rain forest to rubber plantation has been well documented (Li *et al.*, 2012).

The response of aggregate-associated OC stocks to the land use change varied with aggregate size fractions. Higher OC stocks in the macroaggregate fractions than in mesoaggregate and microaggregate have been reported by several workers (Gelaw *et al.*, 2013; Wei *et al.*, 2013). Don *et al.* (2011) reported 25-42 % reduction in soil OC stocks due to conversion of forest to cropland.

5.4 Root exudation rates in different land use species

Root derived C flux is an important source of C inputs into soils affecting the soil microbial communities (Yin *et al.*, 2014; Phillips *et al.*, 2013). It act as C source for the soil microorganisms and the magnitude and composition of root exudates regulates the soil microbial activities (Dennis *et al.*, 2010). Root exudates are also reported to drive the terrestrial C which may affect the nutrient cycling (Finzi *et al.*, 2015; Bardgett *et al.*, 2014). In the present study, root exudation rates were found maximum during the summer season. The roots of *Hevea brasiliensis*, *Melocanna baccifera* and *Elaeis guineensis* exuded more C in the month of July and were followed in decreasing order by October, April and May. Exudation rates in *Hevea brasiliensis* were greater and followed by *Melocanna baccifera* and *Elaeis guineensis*. The difference in root exudation rates among different species are assumed to be determined by nutrient availability, root architecture and densities, and mycorrhizal associations. Yin *et al.* (2014) reported that mass-specific exudation rates in beech, oak, tulip and maple dominated ~80 years old forest ranges from 0.1 to 0.5 mg C g⁻¹root day⁻¹.

The present study revealed that the microbial communities in RP used the carbon exudes from the roots as a source of their assimilation which further led to greater soil respiration in RP soil. The C released by the roots is mostly assimilated by microbial community (Shahzad *et al.*, 2012) due to which the substantial fraction of the labile C present in the rhizosphere of RP is readily depleted. The lowest SOC and highest C exudes in RP suggest that the microbes present within the rhizosphere of RP utilize the root exudates as an important source of carbon (Dennis *et al.*, 2010). Moreover, the microbial population may vary based on the magnitude of C released as root exudates which eventually may lead to change in microbial community composition.

Root-derived C fluxes are responsible for inducing microbial activities in the rhizosphere as reported in several tree species (Cheng *et al.*, 2014; Bengtson *et al.*, 2012). However, the contribution of exudation in land uses is poorly quantified as exudation rate and nutrient distribution pattern may vary between co-occurring tree species. The present study indicated that RP had higher C exudation rate compared to BF and OPP which profoundly contributed to higher soil respiration. It was found that the exudation rates by all the species decreased from the year 2015 to 2016. The results in the present study showed key linkages between the magnitude of root-derived C flux and soil properties in different land use systems.

Furthermore, results suggested that the variability in root-derived C flux estimates in different land uses may be due to seasonal variation and the timing of exudation measurements. However, more intensive further studies would be required to clearly demonstrate the effect of different land uses and seasons on the magnitude of root exudation in this region.

CHAPTER 6

SUMMARY AND CONCLUSION

Land use types and their management activities profoundly affected the soil properties thereby affecting the long term ecological sustainability. Conversion of natural forest to plantations strongly affected soil physico-chemical and bacterial communities. Bamboo forest, in the absence of management practices, act as efficient land use system in sequestering soil carbon. However, Oil Palm and Rubber plantations released more carbon to the atmosphere as a result of varied management practices.

The nutrient distribution in soil largely depends on nature of vegetation as litter layer plays an important role in enhancing soil fertility and nutrient availability. Higher availability of SOC and TN content in fallow land and natural forest reflected high microbial biomass across all the land uses. Soil moisture significantly ($p < 0.05$) positively correlated with SOC, TN, P_{avail} , NH_4-N , NO_3-N , MBC and SR across all the land uses. Soil pH was negatively correlated with SOC whereas TN, P_{avail} , NH_4-N , NO_3-N , MBC and SR showed a significant positive correlation ($p < 0.05$) with SOC in all the land uses. CO_2 efflux was significantly predicted by SOC and MBC in these ecosystems.

The results also indicated that the conversion of natural forest to various land uses have significantly altered proportions of soil aggregates sizes and consequently soil C sequestration potential. Rubber and Oil palm plantations had greater ability to improve the macroaggregate size fractions on the steeply sloped mountainous region,

and thereby they showed greater potential to increase transient C in the system. Increased proportion of microaggregate size fractions in natural forest will significantly improve soil C sequestration potential of the site for a longer period of time. MWD significantly changed as a function of silt and clay contents in different ecosystems of the region. Soil aggregate size fractions (macro-, meso and micro-aggregates) in the present study were significantly regulated by MWD, total nitrogen, bulk density, soil moisture and silt and clay contents depending on the aggregate size fractions. Further, a long term study on the impact of land use on soil aggregation is recommended for sustainability of different land use systems as well as for preserving environmental quality.

The greater microbial diversity in bamboo forest compared to other ecosystems indicated a strong intertwining effect of rhizomicrobiome on soil productivity. Microbial diversity in the present study was strongly governed by soil pH, nitrogen and phosphorous concentrations. Highly diverse bacterial communities promote ecosystem stability and enhance nutrient availability, and thus proper management of microbial community in the ecosystem may greatly enhance ecosystem sustainability. The study has major implications for the soil microbial restoration and the management of forest in global change scenario. The overall bacterial compositions of five different land use system were similar but the relative abundances of each phylum varied among the land use types. Land use system possibly affected the distribution and abundances of the bacterial communities. Long term effect of such changes might have adverse effect on functional activities of soil as well as biogeochemical processes. Hence, further study involving functional

profiling of soil microorganisms is required to understand and assess the sustainability of land use systems.

The changes in the magnitude of root exudates in different land use affected the structural composition of soil microbial communities and can be regarded as important drivers of terrestrial nutrient cycling.

As the present study focused on assessing the impact of conversion of natural forest and bamboo forest to rubber plantation, oil palm plantation and fallow land, the results obtained in this study may be useful in directing farming practices and policy making process for sustainable land use. The Rubber based agroforestry and Oil Palm agroforestry may be given emphasized to make the system more sustainable in contrast to monoculture. Conclusively, this research will help to understand the impact of land use change on soil properties and microbial dynamics.

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PHOTOPLATES



A) Rubber Plantation



B) Bamboo Forest



C) Oil Palm Plantation



D) Fallow Land



E) Natural Forest



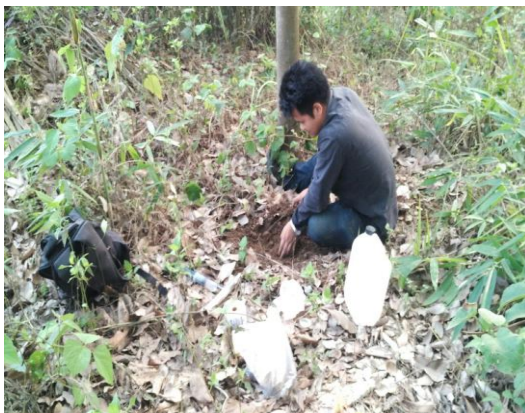
F) Soil sampling



G) Soil respiration experimental setup



H) Commonwealth Forestry Conference



I) Rhizosphere soil collection



J) Tracing fine roots (Rubber)



K) Soil collection in Oil Palm Plantation



L) Soil collection in Fallow land

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Soil properties under different land use systems of Mizoram, North East India

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Abstract

Changes in land use and improper soil management have led to severe land degradation around the globe through the modification in soil physicochemical and biological processes. This study aimed to assess the soil properties of different land use system types. Soil samples (0-15 cm depth) were collected from five land uses; Rubber Plantation (RP), Oil Palm Plantation (OPP), Bamboo Forest (BF), Fallow Land (FL) and Natural Forest (NF) and analyzed for bulk density, soil texture, soil pH, soil moisture, soil carbon, total nitrogen, ammonium, nitrate, soil microbial biomass carbon, soil respiration. Soil pH was lower than 4.9 in all the sites indicating that the surface soil was highly acidic. Soil organic carbon (SOC) and total nitrogen (TN) values ranged from 2.02% to 2.81% and 0.22% to 0.3% respectively. Soil organic carbon (SOC), total nitrogen (TN) and soil microbial biomass (SMBC) were highly affected by soil moisture. $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ ranged from 5.6 mg kg^{-1} to 10.2 mg kg^{-1} and 1.15 mg kg^{-1} to 2.81 mg kg^{-1} respectively. NF soils showed the maximum soil microbial biomass carbon (SMBC) whereas the minimum was observed in BF with values ranging from 340 mg kg^{-1} to 345 mg kg^{-1} . Basal respiration was highest in RP (375 $\text{mg CO}_2 \text{ m}^{-2} \text{ hr}^{-1}$) and lowest in BF (224 $\text{mg CO}_2 \text{ m}^{-2} \text{ hr}^{-1}$). The findings demonstrated significant effect ($p < 0.05$) of land use change on soil nutrient status and organic matter. Findings also indicated that land use change deteriorated native soil physicochemical and biological properties, but that land restoration practices through longer fallow period (>10 years) likely are successful in promoting the recovery of some soil characteristics.

Keywords: Land Use, Oil palm plantation, Organic matter, Rubber Plantation, Soil fertility

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INTRODUCTION

Soil modification due to changes in land use types and patterns is a major threat to sustainable productivity of the soil (Ayoubi *et al.*, 2011) and is considered one of the major factors that affect the distribution patterns of nutrients (Islam and Weil, 2000) in the soil. Northeastern India is drastically affected by land use change (Grogan *et al.*, 2012; Tao *et al.*, 2018), particularly, shifting cultivation, closely linked to ecological, socio-economic, cultural and land tenure systems of tribal communities (Tripathi *et al.*, 2017) profoundly affects the soil fertility and crop productivity (Wapongnungsang *et al.*, 2018).

Mizoram, a region with steep slopes hills in North-east India have undergone different land use change (Lallianthanga and Hmingthanpuii, 2013; Lallianthanga *et al.*, 2014) with more than 60% of the total population depending on small scale agricultural practices as it is the main source of liveli-

hood for rural areas. The significant reduction in Jhum area is mainly due to the implementation of Oil Palm and Rubber plantation. Out of the total geographical area i.e., 2108700 hectare, the total potential area for Oil palm plantation was recorded as 101000 hectare. Rubber Plantation of about 1000 hectare is also established at different districts of the state (Economic survey Mizoram, 2016-2017). Bamboo forest covering 57 % of the total geographical area has shown a positive impact both in terms of forest cover and livelihood (MIRSAC, 2007).

SOC is an important component of the global carbon cycle indicating soil fertility and productivity (Van der Werf *et al.*, 2009) and studies have shown a significant variation with relation to land uses (Ali *et al.*, 2017; Iqbal *et al.*, 2014; Maurya *et al.*, 2014). The land use practices often influences the fluxes of soil carbon stocks and have been reported to vary with the change in land use systems. Various studies have reported a strong de-

crease of SOC after forest conversion to plantations (Guillaume *et al.*, 2015; van Straaten *et al.*, 2015). This has led to raise major concerns about the sustainability of such land use types in the tropics (Lal, 2010).

Although several studies have already been reported related to shifting agriculture (Hauchhum and Tripathi, 2017; Wapongnungsang, 2017; Wapongnungsang *et al.*, 2018), however, the impact of land use change and plantations on soil physicochemical and biological properties is poorly understood. Therefore, the present study aims to investigate the soil properties in five different land use systems, namely, Rubber Plantation, Oil Palm Plantation, Bamboo Forest, Fallow land (~20 yrs) and Natural Forest.

MATERIALS AND METHODS

Site description: Five different land use systems were selected from Mizoram, namely, Rubber Plantation (RP) (23°47.123' N lat and 92°36.831' E long), Oil Palm Plantation (OPP) (23°47.559' N lat and 92°36.492' E long), Bamboo Forest (BF) (23°47.771' N lat and 92°36.080' E long), Fallow land (~20 yrs) (FL) (23°35.392' N lat and 92°42.952' E long) and Natural Forest (NF) (23°35.207' N lat and 92°43.016' E long). Three replicated plots (20m×25m) were established for each land use type to consider true site replicates by maintaining the minimum distance between plots with more than 15m (Mariotte *et al.*, 1997). All the studied land uses experiences moderate humid tropical climate (MIRSAC, 2007).

Soil sampling: Soils were collected from three replicated plots within each land use type following the simple random sampling technique. Soil auger was used to collect the soil samples from 10 different subplots within each replicated plots and further pooled as composite sample. Roots, stones, and debris if any, were removed and hand sieved through a 2 mm mesh sieve and separated into two parts. One part was air dried and the other part was kept in the deep freezer for further analysis.

Analysis of soil properties: Soil Bulk density (BD) was measured by collecting a known volume of soil using a metal ring pressed into the soil (intact core), and determining the weight after drying (McKenzie *et al.*, 2002). Soil texture was determined following the hydrometer method (Bouyoucos, 1962). The textural classification according to the United States Department of Agriculture (USDA) was followed to give the textural class of soil. Soil pH was measured in a soil-water suspension (1:2.5 soil-water ratios) with pH analyzer. The gravimetric method was followed to determine soil moisture content (SMC). Soil organic carbon (SOC) and total nitrogen (TN) was determined by dry combustion in a CHNS/O Elemental Analyzer with autosampler and TCD de-

tector –Euro Vector, Model: EuroEA3000. Ammonium nitrogen ($\text{NH}_4^+\text{-N}$) was estimated by Indophenol-blue method (Rowland, 1983) and Nitrate-nitrogen ($\text{NO}_3^-\text{-N}$) by Phenol disulphonic acid method (Harper, 1924). Soil Microbial Biomass Carbon (SMBC) was determined by following the fumigation extraction method (Vance *et al.*, 1987). Oven-dry equivalent field-moist soil (25 g) was fumigated for 24 h at 25°C with ethanol-free CHCl_3 . Following fumigant removal, the soil was treated with 100 ml of 0.5M K_2SO_4 by horizontal shaking for 1 h and then filtered. The other non fumigated 25 g soil was extracted simultaneously. MBC was calculated using k_{EC} factor of 0.38 (Vance *et al.*, 1987). Soil basal respiration was estimated following the Alkali absorption method (AA-method) (Kirita, 1971).

RESULTS AND DISCUSSION

The studied soil physical properties were found significantly different across the land use systems ($p < 0.05$) and the values are shown in Table 1. Bulk density (BD) values ranged from 1.06 g/cm^3 – 1.27 g/cm^3 with maximum density in RP which was followed by $\text{OPP} > \text{NF} > \text{FL} > \text{BF}$. The minimum bulk density in BF soils could be due to profuse root growth and dense root distribution in BF compared to other land uses. The conversion of the natural forest to plantations probably lead to loss of soil organic matter (SOM) that caused higher bulk density in the plantation soils. Higher bulk densities under intensive rubber plantation were previously reported in Indonesia (Allen *et al.*, 2015; Guillaume *et al.*, 2015). The soil texture of all the land use was determined to be sandy loam with sand, silt and clay values ranging from 62.5% - 71.2%, 17.0% - 20.6% and 11.8% - 16.9% respectively. High sand percent was estimated from BF soils and clay percent was high in plantation soils compared to other land uses.

The soils were found to be acidic in nature with a narrow pH range among the land use systems. Soil pH and moisture content values ranged from 3.9 to 4.9 and 17.4% to 23.4% respectively (Table 1). The NF soil was highly acidic compared to RP, BF, OPP and FL. Similarly, the moisture content was also higher in NF soils. High organic matter content and dense vegetation in NF probably conserve the soil moisture. It has been reported that forest conversion to plantations in Indonesia, Peru and Southern Cameroon led to low moisture availability due to losses in the top soil and vegetation (van Straaten *et al.*, 2015; Guillaume *et al.*, 2016). The values of SOC and TN were found higher in NF followed by FL, BF, OPP and RP (Table 1) and the values ranged from 2.02% to 2.81% and 0.22% to 0.3% respectively. High SOC in NF can be accounted by a considerable amount of litter decomposition and availability of soil nutrients. The higher inputs of organic matter and nutrients

Table 1. Soil properties of different land use systems. Values are mean±1SE (n=5). LSD is shown at $p<0.05$.

SOIL VARIABLES	LAND USE SYSTEMS					LSD
	Rubber Plan- tation	Bamboo Forest	Oil Palm Plantation	Fallow Land	Natural For- est	
Bulk Density	1.27 ±0.04	1.06 ±0.01	1.16 ±0.02	1.09 ±0.01	1.10 ±0.004	0.18
Sand (%)	62.5 ±0.10	71.2 ±0.99	63.7 ±2.08	68.6 ± 0.05	69.2 ±1.91	4.23
Silt (%)	20.6 ±0.04	17.0 ±0.94	19.6 ±1.63	17.8 ±0.55	18.8 ±0.78	2.97
Clay (%)	16.9 ±0.05	11.8 ± 1.94	16.7 ±0.45	13.6 ±0.6	12.0 ±1.13	3.34
Soil pH	4.68 ±0.05	4.91 ±0.08	4.65 ±0.14	4.84 ±0.07	3.93 ±0.12	0.31
Soil Moisture (%)	20.3 ±0.7	21.3 ±1.0	17.4 ±1.14	22.3 ±0.6	23.4 ±0.11	2.88
Soil Carbon (%)	2.02 ±0.15	2.32 ±0.05	2.22 ±0.14	2.36 ±0.15	2.81 ±0.1	0.38
Total Nitrogen (%)	0.22 ±0.01	0.23 ±0.01	0.23 ±0.007	0.26 ±0.008	0.3 ±0.11	0.06
Ammonium (mg/kg)	7.34 ±0.88	10.2 ±1.5	7.58 ±1.3	5.60 ±0.46	8.96 ±0.8	3.14
Nitrate (mg/kg)	1.25 ±0.36	1.94 ±0.5	2.81 ±0.14	1.15 ±0.13	2.81 ±0.3	1.03
Soil Microbial Biomass Carbon (mg/kg)	341.9 ±10.31	340 ±12.3	341.2 ±12.3	343.4 ±3.6	345.2 ±15.0	31.8
Soil Respiration (mgCO ₂ /m ² /hr)	375 ±39.5	224.1 ±42.8	372.9 ±30.2	345.2 ±31.3	345.4 ± 35.7	106

through litter fall have positively shown to affect soil organic matter (Hattori *et al.*, 2005; Ouyang *et al.*, 2007). SOC availability is a good indicator of soil nutrient supply in tropical ecosystems (Chen *et al.*, 2010). It has been reported that soil erosion is the factor affecting the SOC loss in oil palm plantations of Peninsular Malaysia (Gharibreza *et al.*, 2013). In contrast to other land use, less SOC in OPP may be due to the effect of land management activities under plantation. The regular clearance of the surface through weeding and removal of the fallen leaves leads to low microbial activity. Available forms of nitrogen play an important role in N transformation. The values of NH₄⁺-N and NO₃⁻-N ranged from 5.6 mg kg⁻¹ to 10.2 mg kg⁻¹ and 1.15 mg kg⁻¹ to 2.81 mg kg⁻¹ respectively. Higher range of NH₄⁺-N and NO₃⁻-N in NF soils depicted higher rate of ammonification and nitrification which were favoured by high soil moisture content in NF. Results suggested that soil moisture stimulated the soil microbial activity, as also observed in natural and regenerated tropical forests soils of Brazil (Silva *et al.*, 2012).

The values of soil microbial biomass carbon (SMBC) in the land use types ranged from 340 mg kg⁻¹ to 345 mg kg⁻¹. NF soils exhibited the maximum SMBC whereas the minimum was observed in BF. Several workers have found a close relationship between soil moisture and microbial biomass (Devi and Yadava, 2006; Singh *et al.*, 2010). Results showed that SMBC were greatly influenced by SOC. Several reports (Chen *et al.*, 2005; Chen *et al.*, 2017) have shown that higher SOC enhances the growth of microbes and causes accumulation of microbial biomass in soil.

The change in soil respiration rates due to soil temperature and moisture under climate change often contributes to the responses of soil respiration in different ecosystems. Basal respiration in BF was significantly different ($p<0.05$) compared to other land uses with highest in RP followed by

OPP>NF>FL and lowest in BF. The values ranged from 224 to 375 mg CO₂ m⁻² hr⁻¹ (Table 1). The greater soil microbial activity in RP, OPP and NF could release more nutrients from soil organic matter for fine root uptake which further lead to increase in soil respiration compared to FL and BF. Guntinas *et al.* (2013) reported that higher moisture content in the soils of forest, grassland and cropland is responsible for higher soil respiration rate. Low SMBC and basal respiration in BF indicates the proliferation of profuse root systems in BF as to exploit soil nutrients from the greater volume of soil to compensate high productivity of BF.

Conclusion

This study highlights the soil properties of different land use types of Mizoram. Results showed a distinct change in soil properties with the land use change ultimately leading to negative feedbacks between soil property and land use types. It further indicated that land use change to RP and OPP deteriorated native soil physicochemical and biological properties, but land restoration practices through longer fallow period (>10 years) are likely to promote the recovery of inherent soil characteristics. However, a long term observation of the land use change is recommended for further study to follow in order to understand the chrono sequence effect of land use change.

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ABSTRACT

**INFLUENCE OF RHIZOSPHERE ON SOIL FERTILITY IN
DIFFERENT LAND USE SYSTEMS OF MIZORAM**

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Mizoram is a hilly state with a geographical area of 21,087 km² and 85 % forest cover having the second largest forest cover with respect to its geographical area in the country. Tropical and subtropical forests of Mizoram are significantly affected by land use change such as shifting cultivation and plantations. The monoculture practice of rubber and oil palm plantations has occurred widely across the region.

Land use is a physical entity of topography and spatial arrangement of natural resources including soil, minerals, water and biota. Landscape transformations from natural forest ecosystems to multitude of land use types (e.g. secondary forests and plantations) are linked with noticeable changes in structure and functioning of ecosystem at various spatio-temporal scales. Landscape changes are occurring very rapidly in to the ecosystems which lead to land degradation and decline in level of soil fertility. These changes are most profound in North-East India as result of increasing population densities which adversely affect the soil productivity of natural and modified ecosystems. The soils in this hill region are prone to heavy erosion and degradation mostly due to shifting cultivation practices and heavy rainfall during the rainy seasons. The cultivation practices along the slope have caused a rapid loss in tree cover that eventually led to change in soil physico-chemical and biological properties, which further affects process of soil aggregation and C sequestration in the soil. Shifting cultivation locally known as *Jhum* is a traditional means of agriculture practice which include clearing and burning of forests after which the cultivation of multiple crops takes place. Recently, due to increase in population needs and suitability of the soil and climatic conditions, vast area has been developed into Oil Palm and Rubber plantation.

Land use change is ecologically sensitive component of the tropical forest ecosystems affecting all components of the ecosystems. Changes in the ecosystem components significantly affect the fertility of soil by altering rhizosphere structure, function and interactions. Therefore, it is important to understand the effect of land use change on rhizosphere properties and to effectively manage the soil properties as a result of changing land use patterns in the region.

Assessing soil properties of various land use systems is an important component for the sustainable productivity. Aizawl district located in the northern part of Mizoram can be considered as one of the important sites to study the different land use systems. Its natural vegetation is mostly being altered by the various land use types. The natural bamboo ecosystems are widely being altered by slash and burn technique following the cultivation of agricultural crops throughout the year. Therefore, assessment of soil properties and the rhizosphere effects upon different land use systems are important to detect early changes in soil quality. Hence, the present study focuses on the following issues: soil fertility; bacterial communities; soil aggregate stability and magnitude of root exudates in different land use system of Mizoram.

The study was carried out in five different land uses; RP (Rubber Plantation), BF (Bamboo Forest), OPP (Oil Palm Plantation), FL (Fallow Land) and NF (Natural forest). The monoculture of RP (*Hevea brasiliensis*) and OPP (*Elaies guineensis*) were 10 years old with plantation area of ~3.5 ha and ~5 ha respectively. BF (*Melocanna baccifera*), FL, and NF were 12, 20 and >100 years old, respectively. The vegetation of FL was secondary successional type with the dominant species of Broom grass (*Thysanolaena maxima*) and small patches of bamboo along with other

woody species. The NF was a mixed forest dominated by *Schima wallichii* tree species along with other woody associates like *Albizia chinensis*, *Callicarpa arborea*, *Castanopsis tribuloides*, *Duabanga grandiflora*, *Macaranga peltata*, *Sterculia villosa*, *Toona ciliata* etc.

A sample area representing about 1 hectare land was selected in each land use system and within each 1 hectare five replicated plots (100 m²) were established to consider true site replicates. A total of 30 soil cores were collected following random sampling technique from each land use which was further composited into 5 samples (1 sample represent each replicated plot) representing each land use system for soil analysis (bulk density, soil moisture content, total porosity, soil texture, soil organic carbon, total nitrogen, available phosphorous, nitrate nitrogen, ammonium nitrogen, exchangeable cations, microbial biomass carbon and soil respiration).

The rhizosphere soil samples for DNA extraction was collected from 5-6 random locations (ca. 3-5 m away from each other) from each permanent plots and were pooled into sterile tubes, kept to frozen in dry ice and transported to the laboratory for further analysis. Bacterial DNA was extracted from the composites of rhizosphere soil samples of different land use systems using the Fast DNA spin kit (MP Biomedical, Solon, OH, USA). The V4 hyper variable region of the 16S rRNA gene was amplified using 10 pmol/μl of each forward and reverse primer. Paired-end Mi-seq (Illumina) sequencing (2 X 250 bp) was carried out at Scigenome Lab, Cochin, India and the raw data was submitted to NCBI- Sequence Read Archive (SRA): SUB4657919 with accession number PRJNA514616. The bacterial diversity

changes were measured using the alpha diversity metrics: Phylogenetic Diversity, Chao 1 and observed species.

Aggregate size fractions was determined by mechanical sieving into different size fractions (4.75–8 mm; 2–4.75 mm; 1–2 mm; 0.5–1 mm; 0.25–0.5 mm and <0.25 mm).

The collection and analysis of root exudates was done following the modified culture-based cuvette system developed especially for field-based exudate collections. All the samples were analyzed for total organic carbon using TOC- V_{CPH} Total Organic Carbon Analyser, Shimadzu.

The studied soil physical properties varied significantly ($p < 0.05$) across the land use systems. Bulk density (BD) values were maximum in RP and minimum in BF. The clay and silt percent was greater in plantations soils compared to other land uses whereas the sand percent was high in BF soils. The soil moisture (SM) content in rhizosphere soil was highest in NF during mid-monsoon and was followed in decreasing order by BF>FL>RP>OPP. The soil pH values were found to be acidic in nature. FL was more acidic during pre-monsoon compared to other land uses whereas NF was more acidic during mid-monsoon and post-monsoon.

Soil organic carbon (SOC) concentration was highest during mid-monsoon season and were significantly different ($p < 0.05$) across the land uses. Seasonal variation in SOC concentrations in rhizosphere soils was significant at $p < 0.05$. The maximum concentration was found in rhizosphere soil of NF and lowest in RP. Similar trend to SOC was observed in TN concentrations during seasons with higher concentration in NF and less concentration in plantation soils.

The rhizosphere soil of RP showed the higher $\text{NH}_4\text{-N}$ concentration than other land uses during pre-monsoon whereas BF showed the maximum during mid-monsoon and post-monsoon. On the other hand, bulk soil of BF showed high $\text{NH}_4\text{-N}$ concentration during pre-monsoon and OPP showed high $\text{NH}_4\text{-N}$ concentration during both mid-monsoon and post-monsoon. Comparatively bulk soil of NF and FL showed the lowest $\text{NH}_4\text{-N}$ concentration during pre-monsoon. Subsequently higher $\text{NO}_3\text{-N}$ was observed in rhizosphere soil of NF during all the seasons whereas lowest $\text{NO}_3\text{-N}$ was obtained in BF.

The MBC values varied significantly ($p < 0.05$) between the seasons and the highest MBC was shown during mid-monsoon season in both rhizosphere and bulk soils across all the land uses. FL and NF showed the maximum values compared to other land uses during all the seasons in both rhizosphere soil and bulk soil. OPP showed the lowest MBC during all the seasons. Soil MBC was also influenced by SOC in the present study.

The soil respiration rate was significantly greater in rhizosphere soils than in bulk soils with greater values during mid monsoon across all the land uses in both the soils. RP showed the maximum soil respiration rate and lowest in BF. The soil respiration rates increased sharply from pre-monsoon to mid-monsoon which further decreased during post-monsoon.

SM was positively correlated ($p < 0.05$) with SOC, TN, P_{avail} , $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, MBC and SR across all the land use system. Soil pH was negatively correlated with SOC whereas TN, P_{avail} , $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, MBC and SR showed a significant positive correlation ($p < 0.05$) with SOC in all the land use system.

A total of 2076313 sequencing reads were obtained in the present study. The results of alpha diversity indices showed that bacterial diversity was highest in BF compared to other land uses. Operational Taxonomic Units (OTUs) was highest in BF across all the land use system. NF had unique bacterial community which was totally different from other land uses.

The taxonomic distributions of Bacterial phyla exhibited that 61.71% and 0.97% of the OTUs were classified under Bacteria and Archaea, respectively, whereas 37.31% of the OTUs was not taxonomically classified. OTU percentage varied with the different land use systems. Higher OTUs of *Acidobacteria*, *Bacteroidetes* were observed in NF whereas lowest was found in RP. Results also depicted that the % of OTUs of FL and NF were nearly similar i.e. the % of OTUs in FL was in the recovery stage to NF

Acidobacteria, *Proteobacteria* and *Verrucomicrobia* were the three most predominant phyla, totally accounting for more than 50% of all the sequences. NF showed the maximum abundance of *Acidobacteria* and *Proteobacteria* which decreased with the change in land use whereas OPP showed the least distribution of both *Acidobacteria* and *Proteobacteria*. The present dataset also contains a considerable number of sequences affiliated to phylum *Lentisphaerae*, *Chloroflexi*, *Planctomycetes*, *Nitrospirae* and *Actinobacteria*.

The variations in macroaggregate and microaggregate size distributions was significant ($p < 0.05$), whereas, the variations in mesoaggregates were narrow and not significant across land uses. The percent contribution of macroaggregates and mesoaggregates were considerably higher than microaggregates. The proportion of macroaggregates was maximum in plantation soils and minimum in BF. Proportions

of microaggregate size fractions were maximum in NF with a proportionate decrease of 44%, 52%, 16% and 24% in RP, OPP, BF and FL, respectively. The maximum MWD was obtained in plantation soils (RP and OPP) and minimum in BF. Variation in aggregate-associated SOC and TN content was significant ($p < 0.05$) across the land uses. The concentration of both SCO and TN associated with the macroaggregates was noticeably higher than mesoaggregates and microaggregates. The NF had significantly high SOC and TN associated with various aggregate sizes. Soil OC stocks were significantly higher in the macroaggregate fractions than in mesoaggregate and microaggregate.

Root exudation rate was high during the summer season in all the land use systems. RP showed the maximum annual root exudation rate and minimum in OPP. The roots of *Hevea brasiliensis*, *Melocanna baccifera* and *Elaeis guineensis* exuded more C in the month of July and were followed in decreasing order by October, April and May. Exudation rates in *Hevea brasiliensis* was greater and were followed by *Melocanna baccifera* and *Elaeis guineensis*. Our results suggest that there are key linkages between the magnitude of root-derived C fluxes and the proportion of nutrients held in soil.

Land use types and their management activities profoundly affected the soil properties thereby affecting the long term ecological sustainability. Conversion of natural forest to plantations strongly affected physical and chemical soil attributes, as well as the soil bacterial community. Bamboo forest, in the absence of management practices, act as efficient land use system in sequestering soil carbon. The greater microbial diversity in bamboo forest compared to other ecosystems indicated a strong intertwining effect of rhizomicrobiome on soil productivity. Highly diverse

bacterial communities promote ecosystem stability and enhance nutrient availability, and thus proper management of microbial community in the ecosystem may greatly enhance ecosystem sustainability. As the present study focuses on assessing the impact of conversion of natural forest and bamboo forest to rubber plantation, oil palm plantation and fallow land, the results obtained in this study may be useful in directing farming practices and policy making process for sustainable land use in this region.