Study on carbon dynamics of forest, agro-forestry and cropland ecosystems of Sikkim Himalayas

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By

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This is to certify that the Ph.D. thesis entitled "**Study on carbon dynamics of forest, agro-forestry and cropland ecosystems of Sikkim Himalayas**" submitted to Sikkim University in partial fulfillment for the requirements of the degree of Doctor of Philosophy in Botany embodies the research work carried out by **Mr. Nima Tshering Lepcha** at the Department of Botany, School of Life Sciences, Sikkim University. It is a record of *bonafide* investigation carried out and completed by him under my supervision. He has followed the rules and regulations prescribed by the University. The results are original and have not been submitted anywhere else for any other degree or diploma.

It is recommended that this Ph.D. thesis can be placed before the examiners for the evaluation.

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DECLARATION

I declare that the present Ph.D. thesis entitled "Study on carbon dynamics of forest, agro-forestry and cropland ecosystems of Sikkim Himalayas" submitted by me for the award of the degree of **Doctor of Philosophy in Botany** is a bonafide research work carried out by me at the Department of Botany, Sikkim University under the supervision of **Dr N. Bijayalaxmi Devi.**

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Nima Tshering Lepcha

DEDICATED

This thesis is dedicated to my mother's memory

Late **Chumsang Lepcha**

May her memory forever be a comfort and a blessing.

She was the best mother a child could ever have!

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ABBREVIATIONS

AGB:	Aboveground Biomass
AGBC:	Aboveground Biomass Carbon
ANOVA:	Analysis of Variance
ASML:	Above Mean Sea Level
AP:	Available Phosphorous
AT:	Air Temperature
BD:	Bulk Density
BEF:	Biomass Expansion Factor
BGB:	Belowground Biomass
BGBC:	Belowground Biomass Carbon
C:	Carbon
CAF:	Cardamom Agroforestry
CH_4:	Methane
CO_2:	Carbon dioxide
$CuSO_4$	Copper Sulphate
DBH:	Diameter at Breast Height
EC:	Total Ecosystem Carbon
ENVIS:	Environment Information System
FAO:	Food and Agriculture Organization
FSI:	Forest Survey of India
H_2SO_4:	Sulphuric Acid
H_2O:	Water
HCL:	Hydrochloric Acid
IPCC:	Intergovernmental Panel on Climate Change
IVI:	Importance Value Index
K_2SO_4:	Potassium Sulphate

LULC:	Land Use Land Cover
MBC:	Microbial Biomass Carbon
N_2O:	Nitrous Oxide
NaOH:	Sodium Hydroxide
NOAA:	National Oceanic and Atmospheric Administration
NH_4OAc:	Ammonium Acetate
PCA:	Principal Component Analysis
PPM:	Parts Per Million
RF:	Rainfall
RH:	Relative Humidity
SD:	Standard Deviation
SE:	Standard Error
SM:	Soil Moisture
SOC:	Soil Organic Carbon
SOCS:	Soil Organic Carbon Sequestration
ST:	Soil Temperature
STF:	Subtropical Forest
TBCS:	Total Biomass Carbon Sequestration
TECS:	Total Ecosystem Biomass Sequestration
TN:	Total Nitrogen
VBC:	Vegetation Biomass Carbon
WMO:	World Meteorological Organization
WPC:	Wet Paddy Cropland

SYMBOLS AND UNITS

cm	Centimeter
°C	Degree Celsius
g	Gram
Gt	Gigatonnes
ha	Hectare
h	Hour
kg	Kilogram
km	Kilometer
m	Meter
Mt	Metric ton
µg	Microgram
mm	Millimeter
ml	Milliliter
Mg	Megagram
mg	Milligram
%	Percentage
Pg	Picogram
sq km	Square kilometer
Tg	Teragram
t	Tons
yr	Year
ppmv	Parts per million by volume

CHAPTER-1

INTRODUCTION

INTRODUCTION

Carbon is the primary component of the earth's biogeochemical cycle as it enables living organisms to live and flourish. Climate change in recent years is a phenomenon that emerged as one of the leading environmental issue. The increase of greenhouse gas concentration in the atmosphere due to anthropogenic activity is the main reason for developing climate change. Carbon dioxide is the key component among greenhouse gases accounting for 60% of global warming and forcing climate change. Because of an increase in the burning of fossil fuels, deforestation, and land-use conversion, the atmospheric CO_2 concentration increased from the pre-industrial of about 280.00 ppm (parts per million) to 391.19 ppm in 2011 (NOAA 2011). An increase of 2.11 ppm of atmospheric CO_2 was recorded in the previous 10 decades (2005-2014) which expanded more than two-fold to the 1960s (Tans and Keeling 2015). The total amount of CO_2 present in the earth's atmosphere plays an important role to maintain the earth's surface temperature (IPCC 2001). The increasing CO_2 concentration in the atmosphere results in a rise of surface temperature by 0.5^0C in the past 100 years and is projected to rise by 0.6 to 5.0^0C in the next 100 years (IPCC 2014). Fast industrialization, urbanization, deforestation, forest fire, agricultural expansion and land-use change in the past years have all prompt to release of a high level of greenhouse gases, mainly carbon dioxide gas in the atmosphere. Increasing atmospheric CO_2 and its potential consequences on climate are the most significant environmental issues globally (Brown et al. 1989). The major natural carbon sinks on the earth's surface are plants, ocean and soil. The Kyoto Convention of 1997 concludes that capturing CO_2 from the atmosphere through biomass is the only way of mitigation of greenhouse gases especially CO_2 from the air (Bhadwal and Singh

2002). And carbon sequestration in terrestrial sinks can be utilized to balance the emission of greenhouse gases in the atmosphere (Jandl et al. 2007).

Carbon sequestration is the net expulsion of carbon dioxide from the atmosphere and putting away into a long live pool of carbon. These carbon pools can be living over the above-ground biomass such as a tree, bush and herbs, and living biomass in soil i.e. root and microorganism. Absorbing carbon dioxide from the atmosphere and converting it into the biomass of the plants and absorption in the soil is only a practical way of removing greenhouse gases from the atmosphere into the biological system (Ramchandran et al. 2007). For the overall carbon management strategy, carbon sequestration plays a very important role in the reduction and mitigation of global CO_2 emissions. Hence, it is recognized as an efficient and low-cost method for global carbon emission mitigation. The potential of vegetation C sequestration relies on nature, which in turn relies on the association between species, geography, climate, and land-use management practices. So, the carbon sequestration potential changes with the place, region and species composition of an area. Understanding of carbon dynamics of various land-use types and the relationship between carbon stock and the land-use system is essential, as each land-use system has either a positive or negative effect on carbon balance (Toru and Kibret 2019). The amount of carbon stored in any land-use system depends on the function and structures of the different components within the system (Albrecht and Kandji 2003). Land-use change has significantly affected the total ecosystem carbon stock as well as the emission of greenhouse gases (Singh et al. 2018). Increasing demand for food due to population pressure results to change in land-use, which is one of the significant aspects of increasing atmospheric carbon dioxide. Change in land-use systems is frequent in the mountains especially due to less cultivable land, low fertility and landscape. The transformation of natural forests into other forms of managed land-use systems has been the general pattern in the mountainous region (Rai et al. 1994).

In the terrestrial ecosystem, the significant role played by forests in capturing CO_2 from the atmosphere and transforming it into forest biomass through the process of photosynthesis is well known. Five carbon pools have been identified by the Intergovernmental Panel on Climate Change (IPCC) comprising the aboveground biomass, belowground biomass, litter, woody debris and soil organic matter (Eggleston et al. 2006). Because of the presence of woody biomass in the forest, more than 20-50 % of carbon is stored in the forest ecosystem as compare to other land-use systems (Sharma et al. 2010). Also, due to a huge amount of carbon in vegetation as well as in soil (Dixon et al. 1994), approximately 60% of total carbon in the terrestrial ecosystems is store in the forest ecosystems (FAO 2001). Undoubtedly, forests also sequester more carbon than other land-use in the terrestrial ecosystem. Vegetation cover in the forest ecosystems contains about 350,000 Tg (Teragram) of carbon (Dixon et al. 1994) and around 42% of carbon is accumulated by living biomass vegetation, while soil and litter have 49% and deadwood has 9% in the terrestrial ecosystems (Pan et al. 2011). Trees constitute one of the most important components of the forest as they are capable of absorbing a large amount of CO_2 from the atmosphere. The estimation of biomass in the forest is, therefore, necessary to understand the carbon storage and sequestration potential of the forest ecosystem (Wang et al. 2004). Above ground vegetation act as an important factor in soil formation as it gives litter for the formation of organic matter in the soil (Chapman and Reiss 1992). Around 4.1 billion hectares of various forest ecosystems worldwide act as a reservoir of terrestrial carbon stock (Dixon and Wisniewski 1995) which sequestered about 1240 Pg (Picogram) of carbon globally (51% of total ecosystem carbon) in vegetation and soils (Prentice 2001). Carbon sequestration by the forest as a mitigation approach has fascinated and gains much importance, as it has been considered a comparatively low-cost means of addressing climate change instantly (FSI 2017). Estimation of forest biomass including the litter biomass is therefore necessary for estimating and monitoring the amount of carbon lost during deforestation and or any land-use change. Also, this gives the value of carbon storage or sequestration potential by the forest ecosystem. The decomposition of plant litter in terrestrial ecosystems is a predominant process for the flow of carbon and nutrients (Hattenschwiler et al. 2005). According to FAO (2005), around 13 million hectares of land are being deforested every year mainly due to the transformation of forest to the agricultural system or other practices. Transformation of natural forest into cropland or other farming practices lessen soil productivity because of increased erosion. Soil erosion resulted in a decrease in soil fertility and microbial properties which plays a vital role in sustaining soil and environment quality (Kara and Bolat 2008). Different cropping system is characterized by different crop yield which resulted to a difference in carbon inputs through crop residues (Yu et al. 2009). The land-cover pattern in the terrestrial ecosystems will strongly be affected by future agricultural demand which in turn affects the flux of CO_2 and other greenhouse gases (Bhadwal and Singh 2002). Hence, the proper management of agricultural systems is one of the essential activities that can control carbon loss and emissions.

There is a rapid increase in interest in the agroforestry systems as a land-use practice across the globe due to the recognition of the potential of agroforestry systems in carbon sequestration by the International Panel on Climate Change (IPCC 2006). Tropical agroforestry systems in developing countries can store 12.00-228.00 Mg C ha^{-1} carbon (Albrecht and Kandji 2003; Watson et al. 2000) and act as carbon sink both in soil and vegetation to mitigate climate change (Goswami et al. 2014). Watson et al. (2000) assume that by 2040 the carbon sequestration potential of agroforestry would be 586 Mt C per year from the available 630 million hectares areas. Additionally, the agroforestry system provides various ecological advantages to the surrounding inhabitants directly or indirectly including fuel, nourishment, feed, timber, soil and water protection, biodiversity preservation and other usable items (Kumar et al. 2012).

Besides, vegetation carbon stock, carbon store in soil comprises an important component in understanding the carbon cycle of a system. Soil organic carbon in the terrestrial ecosystem is a storehouse of carbon pool and has been identified as an important factor for determining soil fertility as it works as a sink of carbon depending on the land management activities (Lal 2005). Forest ecosystems have around 40% of the total SOC stock in the globe (Eswaran et al. 1999). Soil contains about 1.5 to 3 times more carbon than the vegetation and twice much carbon as in the atmosphere (Batjes 1996; Jobbagy and Jackson 2000). Hence understanding variations in SOC content and sequestration across the different depth of soils in different land-use systems are important. Soil carbon storage transforms rapidly in response to land-use change and relies upon the ecological, biogeochemical and land management factors (Longbottom et al. 2014). Many studies have proposed that land-use change is the fundamental factor determining SOC content because of its effects on soil aggregates (Yang et al. 2009; Fang et al. 2014), microbial activity and biogeochemical cycles (Nsabimana et al. 2004; Yang et al. 2009). Land-use change has been frequently observed to have huge impacts on soil carbon stocks and the transformation of forest to farming lands decreased carbon stock in soil (Chapman et al. 2013). The study of the soil processes in different land-use systems and their effect on soil ecosystem functioning due to human activities is necessary to protect and regenerate the ability of soil to deliver ecosystem services (Van Leeuwen et al. 2017). SOC plays a vital role in agricultural productivity, climate stabilization and other vital ecosystem services for social, ecological and economic sustainability. Subsequently, assessment of the function of soil as a sink for carbon in various land-use systems becomes important as an increase in SOC content could prompt carbon sequestration that can balance the annual atmospheric CO_2 concentration. Several studies highlighted the role of carbon sequestration in maintaining a balance in greenhouse gas emissions and its connection to site composition, i.e. soil structure, soil carbon content and climatic condition (Montagnini and Nair 2004; Nair et al. 2009).

Soil respiration is another key source of releasing carbon from the soil to the atmosphere in the form of CO_2 and plays a vital role in understanding carbon exchange in soil and the atmosphere (Kutsch et al. 2009). For climate change mitigation strategies, the study of soil carbon flux is important because higher carbon is store in soil than in vegetation (Song et al. 2013). Carbon dioxide released by root and microbial respiration constitutes soil respiration (Luo and Zhou 2006). Soil respiration influences net carbon uptake from the atmosphere (Ryan and Law 2005) and is an important factor in soil carbon storage, soil quality, and soil biological activity. The emission of CO_2 from the soil is considered as one of the major carbon fluxes in the carbon cycle globally. Different land-use systems have diverse soil respiration patterns due to the influence of different biotic and environmental factors.

The biological cycle of nutrients in an ecosystem is one of the principal processes that support organic matter production (Sharma et al. 2000). Among geochemical, biogeochemical and biochemical mineral-flow pathways in terrestrial ecosystems, biochemical mineral-flow pathways assume importance in redistribution and conservation of nutrients within the standing crop and in determining the amount of nutrients in litterfall (Sharma and Pande 1989).

Soil microorganisms perform a major function in nutrient cycling and help in understanding and regulating the soil carbon cycle of different ecosystems. For the formation of the organic carbon pool, soil microbial biomass carbon act as a key indicator of soil organic carbon by decomposing organic matter. Microbial biomass carbon in the soil contributed around 1-3% carbon to the total soil organic carbon (Dilly et al. 2003). It also controls nutrient dynamics by affecting the primary productivity of most biogeochemical processes in terrestrial ecosystems (Gregorich et al. 2000; Kara and Bolat 2008). The functioning of an ecosystem depends on the flux of carbon and other chemical nutrients, mediated by the microbial interaction in the soil, plant and animal food web (Seneviratne 2015). The fertility of soil also depends upon soil microbial mechanisms and their mediated processes (Lynch 1984). According to Singh and Gupta (2018), soil microbial biomass acts as a keystone biological driver to the ecosystem functioning. Geographical area, climate variability, soil properties, and the dominant vegetation are the key drivers in controlling microbial biomass carbon dynamics in different land-use types (Singh and Gupta 2018; Wardle 1992). Therefore, estimating soil microbial biomass carbon in the different ecosystems is an important tool for understanding and predicting long-term effects on change in land-use (Sharma et al. 2004).

The physicochemical characteristics of soil depend on the climate, topography, vegetation type of different land-use systems. Nitrogen, phosphorus, and potassium present in soil are major constituent nutrients of vegetation in the terrestrial ecosystem. The soil physicochemical characteristics differ due to differences in the type of vegetation growing upon it and changes in land-use systems (Yadava and Devi 2004). Sequestration of carbon by the green plants has been given much attention as a promising means of natural forest to reduce CO_2 emissions as well as enhancing carbon sinks. Also, it is clearly seen that different vegetation or land-use type has the different potential to influence carbon pools and their dynamics.

Present study postulated that the different land-use practices adopted in Sikkim Himalaya such as forestry, agroforestry and cropland systems have a different impact in terms of carbon stock and sequestration, productivity and soil nutrient distributions. In addition, the distribution pattern of soil nutrient, soil microbial biomass carbon and rate of CO_2 emission from three different ecosystems were discussed in the present study. Therefore, the present work "Study on carbon dynamics of three different ecosystems i.e. forest, agroforestry and cropland ecosystems of Sikkim Himalayas" was carried out to understand the carbon cycle scenario, its impact and its efficiency on sequestration options in different land-use systems of Sikkim Himalayas.

Thus the main objectives of the present research work are:

1. To study the carbon stock in soil and vegetation (including the litter and microbial pools) in the three ecosystems of Sikkim Himalayas.
2. To estimate the rate of soil CO_2 emission in the three major land-use systems.
3. Comparison of the rate of carbon sequestration and to establish a relationship between the rate of carbon sequestration with abiotic and biotic variables in the three ecosystems.

The present thesis has been presented in seven chapters:

Chapter I: Introduction.

Chapter II: Study site and climate.

Chapter III: Soil physicochemical characteristics.

Chapter IV: Carbon stock and sequestration of three different land-use systems.

Chapter V: Soil microbial biomass carbon.

Chapter VI: Soil CO_2 emission.

Chapter VII: General discussion and summary.

CHAPTER-II

DESCRIPTION OF STUDY SITES AND CLIMATE

DESCRIPTION OF STUDY SITES AND CLIMATE

Study Sites

Study sites i.e. subtropical forest, cardamom agroforestry, and rice cropland selected for the present research were situated at Dzongu, North Sikkim (reserved lands for the indigenous Lepcha tribe) which is approximately about 78 km^2 of the state (Bhasin 2011). The altitudinal range of the study sites was 700-6000 m asml. The subtropical forest is dominated by *Alnus nepalensis* with other broad-leaved species while the introduction of large cardamom (*Amomum subulatum*) in the subtropical forest converts it to the cardamom agroforestry system. Paddy cropland is another important cropland of the state as rice forms the staple food of the people of the state and other northeastern states of India. Landuse and land cover map (LULC) and map of the study sites are placed below as (Fig 1 and 2).

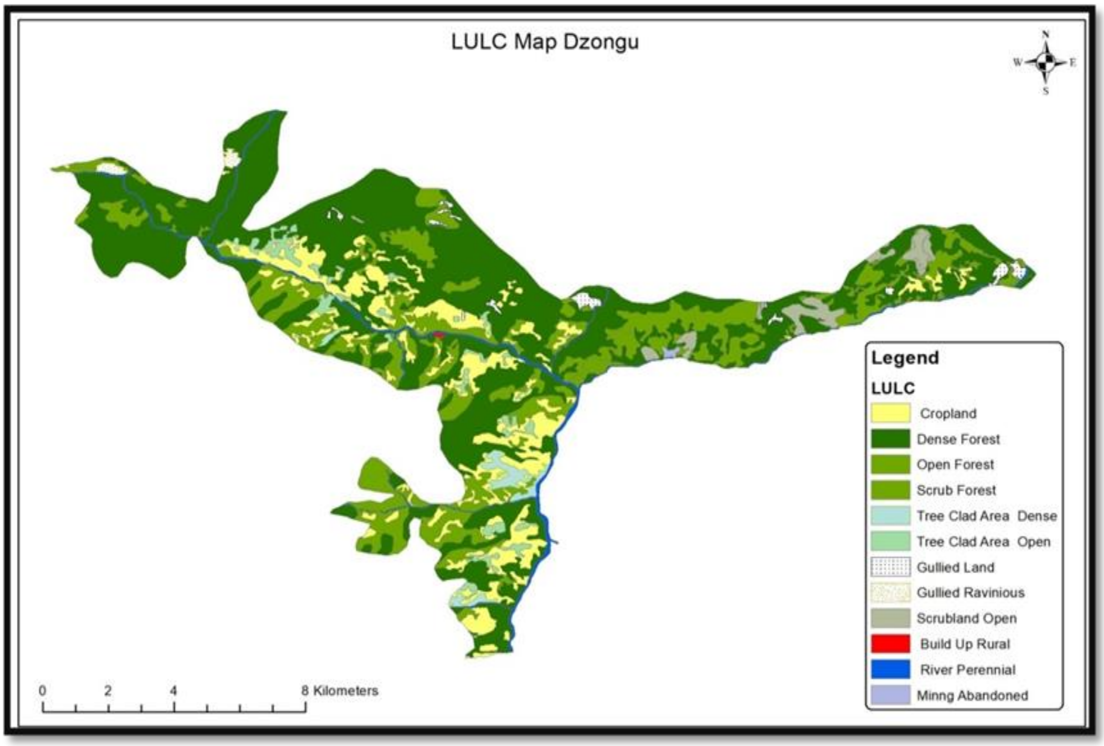


Fig 1. Landuse land cover (LULC) map of Dzongu, N.Sikkim

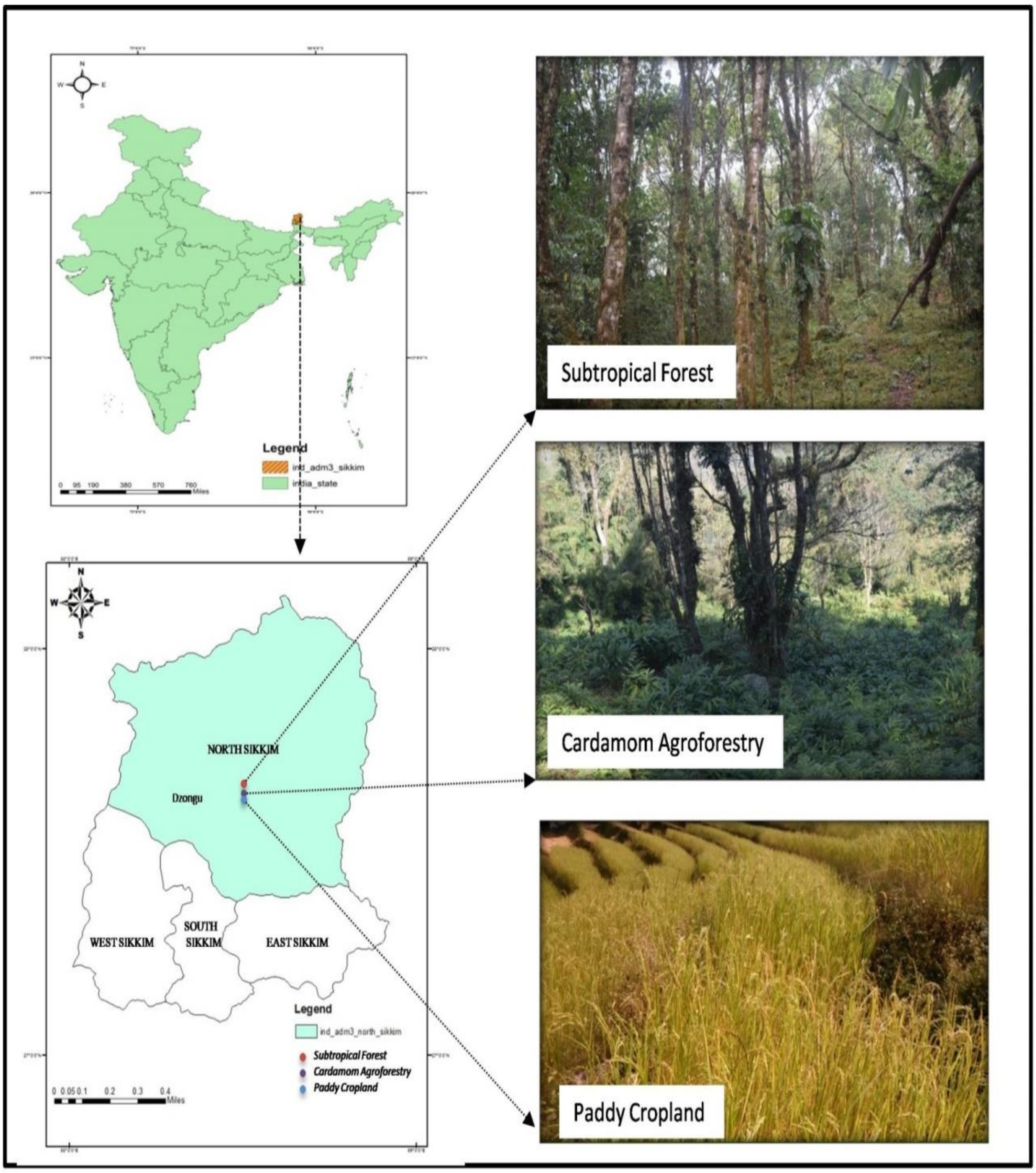


Fig 2. Map of study sites

Subtropical Forest

The subtropical forest was located between $27^0$31.550'N & $88^0$29.722'E and at an altitude ranging from 1400-1700 m asml. Dominated tree species is *Alnus nepalensis* with other species such as *Schima wallichii, Lyonia ovalifolia* and few other tree species. Besides the trees, this forest also housed luxuriant vegetation like bamboos, edible wild plants, firewood, fodder, medicinal plants and other non-timber forest produces. Herbaceous vegetation like *Arisaema sp., Asplenium sp., Begonia sp., Impatiens sp* were also present.

Plate 1: Subtropical Forest

Cardamom Agroforestry

The cardamom agroforestry system is an indigenous one and lies between $27^{0}31.311'$N & $88^{0}24.490'$E at an elevation ranging from 1350-1619 m asml and spread over an area of 100 hectares. *Alnus* species is used as the main shading tree in this system. Cardamom agroforestry system is getting prominence in the state as it provides livelihood benefits to the locals. Some of the variety of large cardamom cultivated were Ramsey, Ramla, Dzongu Golse, Seremna, Sawney and Varlangey (Gudade et al. 2013).

Plate 2: Cardamom Agroforestry

Paddy Cropland

Paddy cropland of the present study lies between $27^{0}31.445'$N & $88^{0}30.380'$E at an elevation of 1200-1400 m amsl. Paddy is grown in almost all places of the state and the total area under paddy cultivation in Sikkim is 106.70 sq km with a production of 19690 tons (2015-2016) of rice (ENVIS Centre, Sikkim). Wet rice (zomal) are grown on waterlogged terraced farms. Some of the local rice varieties cultivated in the state are thulo attey, sanu attey, tukmor jho, chini jho, darmali jho, marbun jho, mumpop jho etc.

Plate 3: Wet Paddy Cropland

Climate

The climate of the study area is monsoonal with three seasons, i.e summer, rainy and winter. About 60-70% of monthly rainfall takes place during the rainy season. Maximum rainfall occurs during the month of July (495.26 mm) and minimum during the month of December (12.16 mm). The mean average temperature varied from 6.40^{0}C (January) to 18.40^{0}C (June) and relative humidity varied from 48.00% (March) to 78.88% (July). An ombrothermic diagram for ten years is placed on (Fig 3). During the study period i.e. (2016-2018) the mean annual rainfall was 3216 mm, average relative humidity ranged from 42.5-72.5% and mean temperature varied from 7.00^{0}C-20.00^{0}C across the months (Meteorological Station Gangtok, Sikkim). The climate data of the study sites during the study period are presented in Fig (4).

Summer Season

The summer season comprises March, April and May and rainfall during this season varied from 34.60 mm (March) to 264.73 mm (April). The mean temperature and relative humidity varied from March (12.67^{0}C and 48.00%) to May (16.67^{0}C and 64.50%) respectively during this season.

Rainy season

The rainy season starts from the month of June till October and the mean monthly rainfall varies from 421.00 mm (October) - 631.46 mm (July) during this season. The mean temperature ranged between 15.67^{0}C - 20.00^{0}C and relative humidity 60.50% - 72.50% in this season. The highest monthly temperature and relative humidity were also recorded during this season in the month of August (20.00^{0}C and 72.50% respectively).

Winter season

The Winter season corresponds to the dry season and starts in November and ends in February. Minimum rainfall is recorded during this season from 12.20 mm (December) to 61.83 mm (January). The lowest temperature and relative humidity were also recorded during this season 6.40^{0}C(January) - 8.20^{0}C(December) and 42.5% - 60.5% respectively across the months.

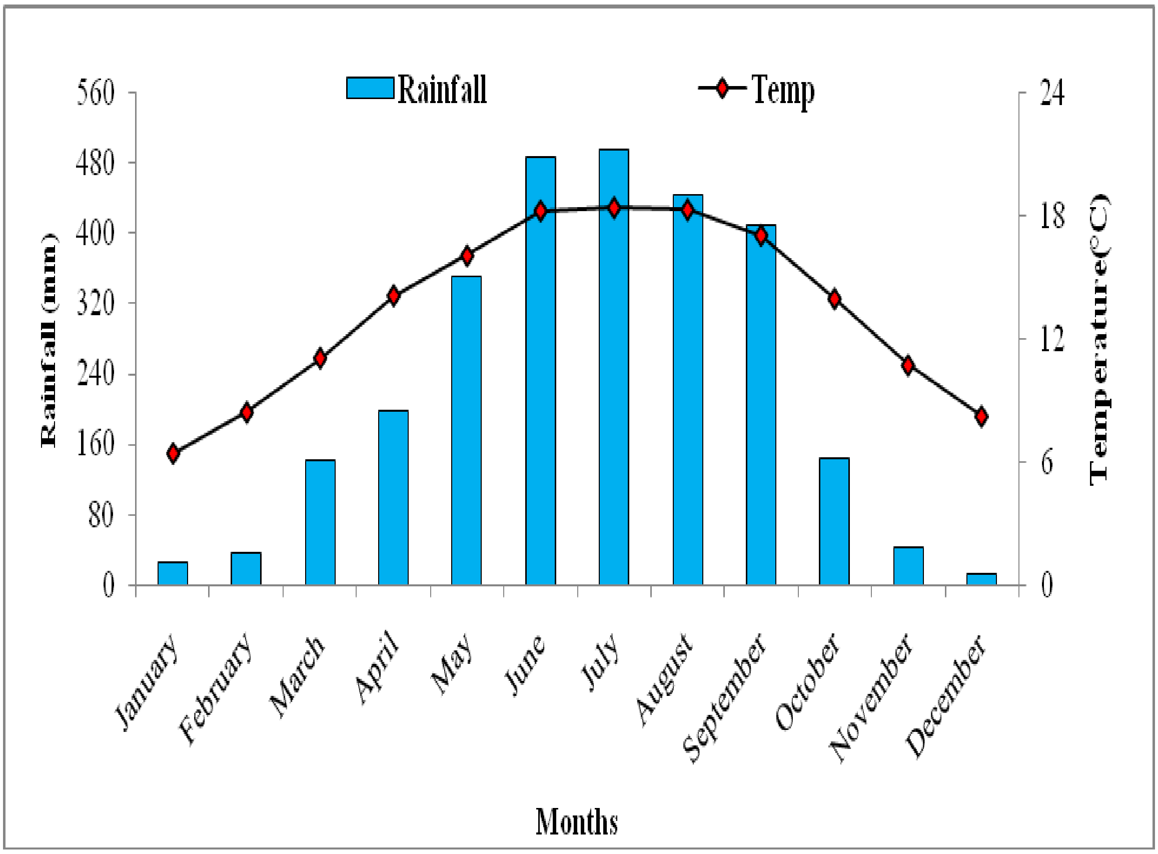


Fig 3. Ten years Ombrothermic diagram of North Sikkim (2009-18)

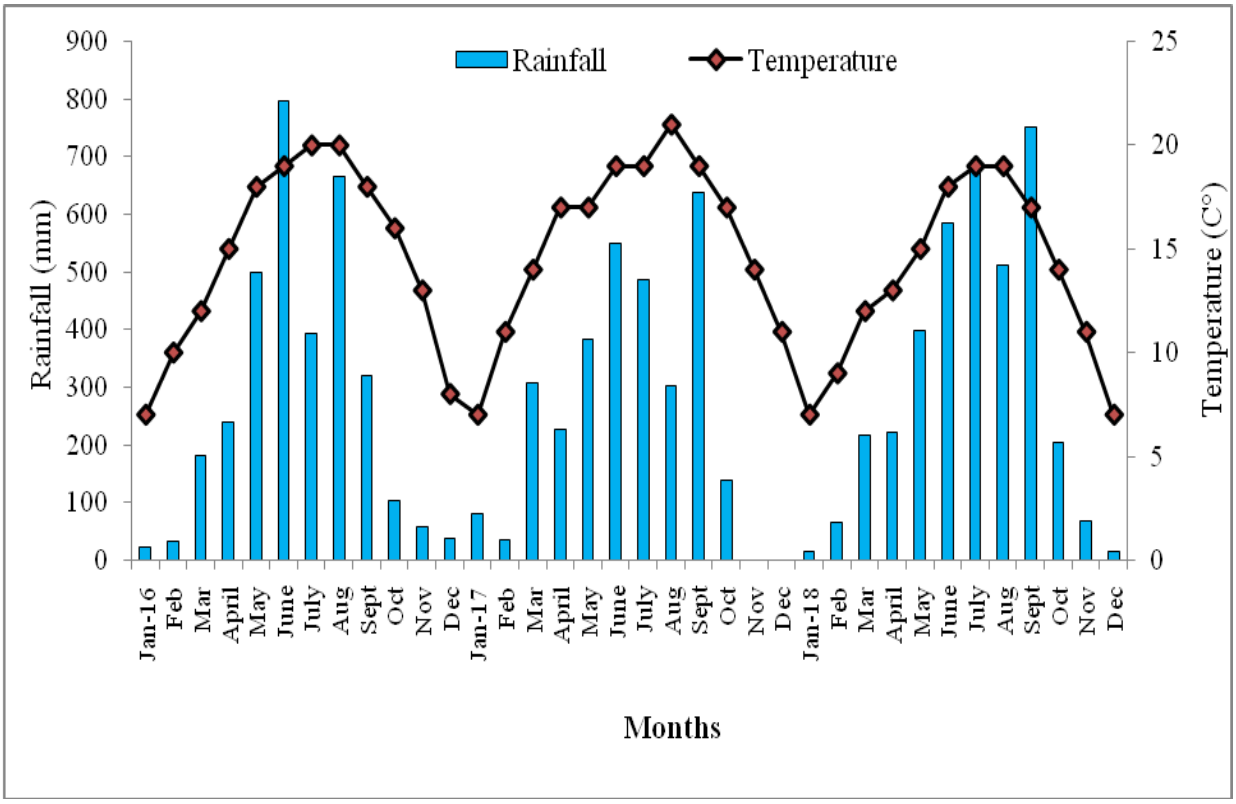


Fig 4. Climate data of all the study sites during the study period (2016-18)

Soil

The soil of Sikkim Himalayas has gneissic rocks as its parent material (Saha 2013). The two tree-based systems (subtropical forest and cardamom agroforestry) have loamy soils while paddy cropland has clayey loam soil. The pH of soil varied between 4.80-5.80 across soil depth and sites. The mean soil moisture across different landuse ranged between 18.22-38.33% while the mean soil temperature varied from 7.00^{0}C-18.03^{0}C. Bulk density (0.56-0.91g cm^{-3}), soil nutrients including organic carbon, nitrogen, phosphorus and potassium ranged from 1.88-4.96%, 0.15-0.37%, 0.010-0.054%, and 0.14-0.26% respectively across the sites.

Vegetation

Subtropical Forest

Alnus nepalensis is the dominant tree species in this forest contributing more than 50% of the total tree density while other tree species including *Lyonia ovalifolia*, *Machilus edulis*, *Symplocos theifolia* and *Schima wallichii* were also found. *S*hrubs are absent as this forest is converted from the cardamom agroforestry system about 35 years ago. However, various herbaceous vegetation like *Eupatorium cannabinum, Ageratum conyzoides, Pouzolzia hirta, Drymaria cordata, Artemisia vulgaris, Impatiens sp., Arisaema sp., Digitaria ciliaris, Oplismenus compositus, Equisetum diffusum and Urtica dioica* are also present. The list of tree species and herbaceous species in this site is placed in Table-1.

Table 1. List of trees and herbaceous species in the subtropical forest site.

Scientific Name	Family
<u>Trees</u>	
Alnus nepalensis D. Don	Betulaceae
Castanopsis indica (Roxb. ex Lindl.) A.DC.	Fagaceae
Ficus racemosa Willd.	Moraceae
Juglans regia L.	Juglandaceae
*Lyonia ovalifolia (*Wall.*)* Drude	Ericaceae
Macaranga pustulata King ex Hook.f.	Euphorbiaceae
Machilus edulis King ex Hook.f.	Lauraceae
Schima wallichi Choisy	Theaceae
Spondias axillaris Roxb.	Anacardiaceae
Symplocos theifolia D. Don	Symplocaceae

Herbs

Arisaema sp	Araceae
Artesimia vulgaris	Asteraceae
Asplenium sp	Aspleniaceae
Ageratum conyzoides	Asteraceae
Begonia sp	Begoniaceae
Centella asiatica	Apiaceae
Deparia boryana	Athyriaceae
Diplazium forrestii	Athyriaceae
Digitaria ciliaris	Poaceae
Drymaria cordata	Caryophyllaceae
Elatostema sp	Urticaceae
Equisetum diffusum	Equisetaceae
Eupatorium cannabinum	Asteraceae
Hydrocotyle javanica	Araliaceae
Impatiens sp	Balsaminaceae
Lecanthus peduncularis	Urticaceae
Nephrolepis exaltata	Nephrolepidaceae
Oxalis corniculata	Oxalidaceae
Oplismenus compositus	Poaceae
Persicaria runcinata	Polygonaceae
Pilea pumila	Urticaceae
Piper sp	Piperaceae
Pouzolzia hirta	Urticaceae
Polygonum molle	Polygonaceae
Pogonatherum crinitum	Poaceae
Selaginella sp	Selaginellaceae
Thelypteris cana	Thelypteridaceae
Urtica dioica	Urticaceae

Cardamom agroforestry

Cardamom (*Ammomum subulatum*) along with other shade provisioning trees and herbs are grown here in this agroforestry system. *Alnus nepalesis* is used as the main shading tree however, other tree species for fodder, timber and firewoods like *Ficus racemosa Juglans regia*, and *Macaranga pustulata* are also planted within the agroforestry system. Shrubs are manually removed but herbaceous species like *Ageratum conyzoides, Drymaria cordata, Oxalis corniculata, Diplazium esculentum, Spilanthes acmella, Galinsoga parviflora, Pouzolzia hirta, Pteridium sp. Urtica dioica* and *Polygonum molle* are present. A list of tree species and herbaceous species in the cardamom agroforestry site is placed in Table- 2.

Table 2. List of trees and herbaceous species in the cardamom agroforestry site.

Scientific Name	Family
Trees	
Alnus nepalensis D. Don	Betulaceae
Ficus racemosa Willd.	Moraceae
Juglans regia L.	Juglandaceae
Spondias axillaris Roxb.	Anacardiaceae
Toona ciliata M.Roem.	Meliaceae
Macaranga pustulata King ex Hook.f.	Euphorbiaceae
Viburnum cordifolium Wall. Ex DC.	Adoxaceae
Herbs	
Amomum subulatum	Zingiberaceae
Arisaema sp	Araceae
Begonia sp	Begoniaceae
Centella asiatica	Apiaceae
Deparia boryana	Athyriaceae

Diplazium forrestii	Athyriaceae
Drymaria cordata	Caryophyllaceae
Elatostema sp	Urticaceae
Equisetum diffusum	Equisetaceae
Eupatorium sp	Asteraceae
Galinsoga parviflora	Asteraceae
Impatiens sp	Balsaminaceae
Lecanthus peduncularis	Urticaceae
Oxalis corniculata	Oxalidaceae
Oplismenus compositus	Poaceae
Persicaria runcinata	Polygonaceae
Pilea pumila	Urticaceae
Polygonum molle	Polygonaceae
Pouzolzia hirta	Urticaceae
Spilanthes acmella	Asteraceae
Thelypteris cana	Thelypteridaceae
Urtica dioica	Urticaceae

Paddy Cropland

In the paddy cropland, trees and shrubs are absent. Besides *Oryza sativa* (rice), other weed species such as *Cyperus rotundus, Cyperus difformis, Paspalum distichum, Fimbristylis maliacea* along with certain grasses were found (Table-3).

Table 3. List of herbaceous species in wet paddy cropland site.

Scientific Name	Family
Oryza sativa	Poaceae
Ageratum conyzoides	Asteraceae
Cyperus difformis	Cyperaceae
Cyperus rotundus	Cyperaceae
Digitaria ciliaris	Poaceae

Echinochloa colona	Poaceae
Echinochloa crus-galli	Poaceae
Fimbristylis miliacea	Cyperaceae
Imperata cylindrica	Poaceae
Paspalum distichum	Poaceae
Pogonatherum crinitum	Poaceae

CHAPTER-III

SOIL PHYSICOCHEMICAL PROPERTIES

SOIL PHYSICOCHEMICAL PROPERTIES

INTRODUCTION

Soil is characterized as a complex mixture of minerals, water, air and soil organic matter and plays a vital role in the global carbon balance. Soil plays a significant role in nutrient cycling in all terrestrial land-use systems as it acts as a source and sinks for nutrients and additionally gives habitat for diverse populations of soil organisms. Soil contains more inorganic and natural carbon than the earth's biosphere (Post et al. 1990) and has long been recognized as the largest organic carbon reservoir of terrestrial systems (Post et al. 1982). Soil helps in maintaining long term site productivity and water quality in forest, and crop productivity in agricultural fields as yield is directly impact by soil nutrient and physical properties (Schoonover and Crim 2015). Various basic components required for plant development, classified as macronutrients or micronutrients are present in soil which can affect the composition and structure of terrestrial flora.

Soil quality and fertility can be estimated through the physical, chemical and biological properties of soil (Shukla et al. 2006). Soils differ in their physicochemical properties depending upon their parent material, topography, climate and vegetation under which soil was formed (Shrikant and Bapat 1993). In the terrestrial ecosystems, the nutrient is slowly accumulated in various forms, such as atmospheric nutrients input, weathering of minerals, release from litterfall and fixation by microorganisms of certain nutrients (Glumphabutr et al. 2007). Physicochemical properties i.e. soil moisture, soil temperature, soil texture, bulk density and other important nutrients like carbon, nitrogen, phosphorus and potassium were studied owing to the importance of these nutrients to plants.

Soil texture is an important physical indicator of the terrestrial ecosystem affecting the balance between water and air (Qin et al. 2010). It is characterized by the amount of sand, silt and clay particle present in the soil. The measurement of the particle size of clay is (<0.002 mm), silt (0.002-0.05 mm), and sand (0.05-2.0 mm) that can help in assigning textural classes of soil. Sandy soils have limited nutrient capacity due to low water holding capacity and limited soil organic matter content (Walpola and Arunakumara 2010). High silt content in the soil increases water holding capacity (Burke et al. 1989) and clay-rich soil has a high water-holding capacity and nutrient content (Hamarashid et al. 2010).

Soil moisture is another key parameter of plant productivity and plays a crucial role in controlling the soil processes including the biological, physical and chemical processes of the soil system (Brevik et al. 2015). Water availability in soil has been considered as one of the most significant environmental parameters regulating plant richness (Lavers and Field 2006). Soil moisture acts as a vital role in the supply of water and nutrient to the plant that helps to increased plant growth and productivity. In the global terrestrial system, the greater part of primary productivity is influenced by water content in the soil (Heimann and Reichstein 2008).

Bulk density is the mass of the soil corresponding to a known volume of soil and is frequently utilized as an indicator of soil compaction. The bulk density of the terrestrial ecosystem is identified with soil textural class and soil porosity.

Soil pH is a proportion of hydronium particles in the soil, which controls the acidity and alkalinity of the soil. Soil pH is one of the most significant elements for the growth and development of plants. Generally, the pH values of natural soils ranged from 3.0-8.4. It varies by region and generally, the soil is acidic in wet climate and alkaline in the dry region where rainfall is limited. A slightly acidic pH ranged between 6.0-7.0 appears to provide optimum nutrient availability to plants (Kimmins 1997) as most macronutrients are available within this range.

Generally, carbon, nitrogen, phosphorous and potassium are primary supplements for plant growth. These elements and their interaction play a crucial role in shaping the earth's landscape and climate system (Post et al. 1982).

Soil organic matter, the key indicator of soil quality and productivity, is formed by the decomposition of living plant parts and animal bodies by microorganisms living in the soil. Soil organic carbon (SOC) is the key component of the soil-plant ecosystem and has an important influence on the physicochemical characteristics of soil as it releases nutrients to the plants through mineralization (Lal 2004). SOC is directly related to the productivity of the ecosystem and acts as an indicator of environmental change (Chapin 2003). In general, soil organic matter improves the health of the soil by bringing changes in soil parameters like water holding capacity, bulk density, soil structure, nutrient availability and microbial population (Kononova 1966).

Nitrogen (N) and Phosphorous (P) are the major components of soil fertilizer for optimal plant growth and are essential for photosynthesis and other processes related to primary productivity (Quilchano et al. 2008; Liu et al. 2013a). Soil nitrogen and phosphorus are closely associated with SOC cycles (Gao et al. 2014) and helps to mitigate the effects of climate change globally (Lal 2004; IPCC 2007; Sardans and Penuelas 2012). The concentrations and distribution of soil carbon, nitrogen and phosphorus varied in diverse plant communities due to different chemical traits and litter content in the soil (Zhao et al. 2010; Deng et al. 2013).

The physicochemical properties of soil i.e texture, moisture, bulk density, pH, carbon, nitrogen, phosphorous and potassium content regulates different ecosystem processes and communities in an ecosystem. Hence, studying the physical and chemical properties of soil in different land-use systems can help in understanding the various processes of these ecosystems. Therefore, chapter III deals with the dynamics of the physical and chemical properties of the soil in three different land-use systems i.e. subtropical forest (STF), cardamom agroforestry (CAF) and wet paddy cropland (WPC) in different seasons of the study period (2016 - 2018).

MATERIAL AND METHODS

Soil physicochemical properties in the three different land-use systems were determined on a seasonal basis during 2016-2018. A total of 405 soil samples from 135 different soil pits (5 each from each land-use system) were collected seasonally across three soil depths, i.e. 0-15 cm, 15-30 cm, and 30-45 cm by using a soil corer (5.2 cm diameter). Soil samples were placed in sterilized polythene bags and brought into the laboratory. Each soil sample is separated into two parts. The first half of fresh soil samples were used for the analysis of soil moisture and bulk density. The remaining soil sample was air-dried, crushed and passed through a sieve (2 mm) to separate coarse material, stones and gravel, and live roots were sorted out manually. A composite soil sample of each soil depth from every land-use was prepared by using different soil samples collected from different soil pits. Then the sieved soil samples were used to analyze for nutrient concentrations in the laboratory following standard procedures and methods. All the analyses of soil were done in 5 replicates of the composite soil samples from all three land-use types.

Soil Texture

Soil texture fraction (silt, clay and sand) was analyzed by the soil hydrometer method (Motsara and Roy 2008) using sodium hexametaphosphate as a dissolving agent. Soil texture was calculated using the following formula.

$$(\%)\text{Clay} = \frac{\text{Corrected 2 hour hydrometer reading x 100}}{\text{Oven dried weight of Soil}}$$

$$(\%)\text{Silt} + \text{clay} = \frac{\text{Corrected } 40 - \text{second hydrometer reading x 100}}{\text{oven dried weight of soil}}$$

(%) Sand= 100-silt(%) + clay

(%) Silt= % Silt + clay- clay(%)

Soil Moisture

Soil moisture content was determined by the gravimetric method. Ten (10 g) of fresh soil is kept in an oven at 105^{0}C until the weight becomes constant and reweighed (Allen et al. 1974). Moisture content in soil was calculated by using the formula.

$$(\%) Soil\ moisture = \frac{(\mathrm{W}^1 - \mathrm{W}^2)\ \mathrm{x}\ 100}{\mathrm{W}^2}$$

W_1=Weight of fresh soil and W_2= Weight of oven-dried soil.

Soil Bulk Density

Soil bulk density was determined by a soil corer of diameter 5.2 cm without disturbing the soil and the cored soil was dried in an oven at 105^{0}C for 48 hours or till constant weight was achieved. Bulk density was determined by the formula of Ravindranath and Ostwald (2008):

$$\text{Bulk density (g cm}^3\text{)} = \frac{\text{Weight of oven dried soil}}{\text{Volume of soil core}}$$

Soil Temperature

Soil temperature was determined using a soil thermometer.

Soil pH

Soil pH was measured by using an auto digital pH meter (Coslab). Ten (10) g of fresh soil sample was dissolved in 50ml of distilled water and subjected to a rotary shaker for 30 minutes and an aliquot was used for determination of pH.

Soil Organic Carbon (SOC)

Organic carbon in soil was determined by the colorimetric method (Anderson and Ingram 1993). One (1g) of the finely grounded air-dried soil sample was added to 10 ml of 5% potassium dichromate. Then 20ml of H_2SO_4 is added and allowed to cool at room temperature and 50 ml of barium chloride was added and left overnight. The supernatant solution was estimated for organic carbon by using a spectrophotometer (Systronics) at 600 nm.

$$\text{Organic Carbon (\%)} = \frac{(\mathrm{K} \times 0.1)}{(\mathrm{W} \times 0.74)}$$

Where K is the carbon concentration of the sample and W is the weight of soil.

Total Nitrogen

Total nitrogen was estimated by using Kjeltec 8500 (FOSS) using 1g of finely sieved air-dried soil sample and 7g of K_2SO_4, 0.8 g $CuSO_4$. $5H_2O$ and 12ml H_2SO_4 followed by distillation with boric acid and NaOH solution and thereafter titrated with 0.1N HCL solution.

$$(\%)\mathrm{N} = \frac{(\mathrm{T} - \mathrm{B})\ \mathrm{x}\ \mathrm{N}\ \mathrm{x}\ 14.007\ \mathrm{x}\ 100}{\text{Weight of soil sample}}$$

T=Titration volume for sample (ml), B=Titration volume for blank (ml), N=Normality of acid

Available Phosphorus

Available phosphorus was determined by the ammonium molybdate stannous chloride method (Allen et al. 1974). 1 gram of air-dried soil sample was extracted with 2.5% acetic acid extract and filtered through Whatman no.42 and 20 ml of distilled water was added to twenty (20) ml of the aliquot. Thereafter, 2ml of acidified ammonium molybdate reagent and stannous chloride were added and volume was adjusted to 50ml. The phosphorus content was determined by using a Spectrophotometer (Systronics) at 700 nm and phosphorus concentration in the soil sample was calculated by:

$$(\%)\ \mathrm{P} = \frac{\mathrm{C\ (mg)\ x\ solution\ volume\ (ml)}}{\mathrm{10\ x\ aliquot\ volume\ (ml)x\ sample\ wt\ (g)}}$$

Where C = mg P obtained from the standard graph.

Exchangeable Potassium

Exchangeable potassium was determined by flame photometer method (Allen et al. 1974). 5 gram of soil samples were extracted with Normal ammonium acetate solution (NH_4OAc) solution and filtered through Wattman paper 1 and the extract was analyzed in flame photometer after calibration.

$$(\%) = \frac{\mathrm{C(ppm)x\ Solution\ volume(ml)}}{\mathrm{10000\ \ x\ \ sample\ wt\ (gm)}}$$

Where C = concentration of K in ppm.

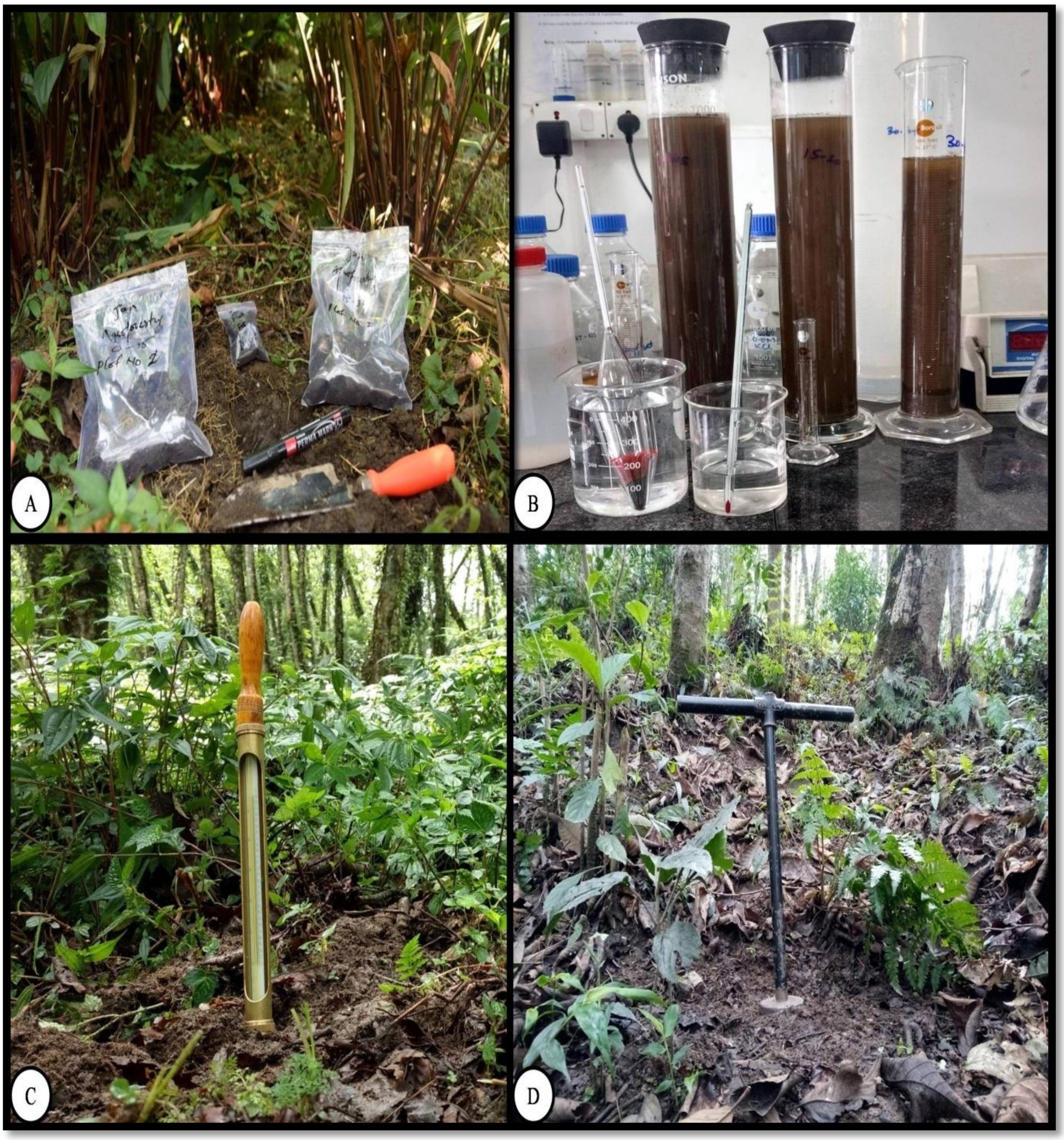


Plate 4: A) Soil sampling from three different soil depth (0-45); B) Soil texture estimation; C) Measurement of soil temperature; D) Soil sampling with a soil corer

Plate 5: E) Soil samples; F) Soil organic carbon estimation; G) Digested soil samples; D) Nitrogen estimation

RESULT

Soil Temperature

A physicochemical property of soil in three different land-use types has been presented in (Table-4). The soil temperature ranged from 6.20^{0}C-16.30^{0}C in the subtropical forest (STF), 6.20^{0}C-17.50^{0}C in cardamom agroforestry (CAF) and 6.10^{0}C-17.50^{0}C in the wet paddy cropland (WPC). Seasonally, the soil temperature was maximum during the rainy season followed by the summer season and minimum in the winter season in all the study sites (Fig 5). Soil temperature exhibited significant variation with land-use, seasons, and interaction between land-use ($p<0.01$) and soil depths ($p<0.001$) (Table-5).

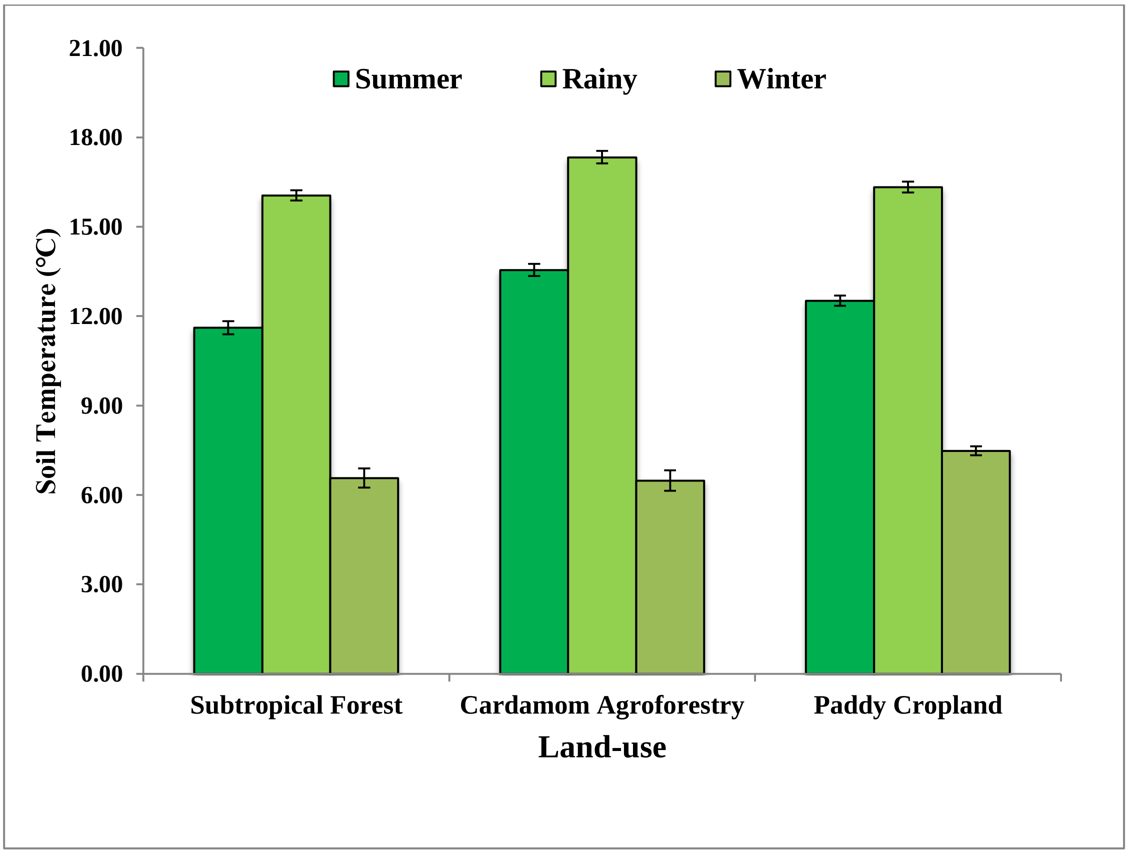


Fig 5. Variation of soil temperature across the seasons of three different study sites(mean±SE)

Table 4. Soil physical and chemical properties in the three land-use systems.

Soil Properties	Land-use Type		
	Subtropical Forest	Cardamom Agroforestry	Paddy Cropland
Sand (%)	42.52	46.72	38.55
Silt (%)	30.82	30.10	31.95
Clay (%)	26.66	23.08	29.50
Soil Type	Loam	Loam	Clayey Loam
Soil Temperature (^{0}C)	6.20-16.30	6.10-17.50	6.20-17.50
Soil pH	4.80-5.70	4.80-5.70	5.20-5.80
BD (g cm^{-3})	0.68-0.91	0.61-0.80	0.56-0.78
Soil Moisture (%)	18.22-36.00	19.00-37.00	20.22-38.33
C (%)	2.30-4.96	2.03-4.53	1.88-3.99
N (%)	0.19-0.37	0.18-0.34	0.15-0.27
P (%)	0.022-0.054	0.016-0.040	0.010-0.033
K (%)	0.26	0.17	0.14
C:N	11.60-15.22	10.10-15.01	9.62-14.82

BD-Bulk density; C-Carbon concentration; N-Total Nitrogen; P-Available Phosphorous; K-Exchange potassium; C:N- Carbon Nitrogen Ratio

Table 5. ANOVA (Two-way) of soil parameters in different land-use system (2016-18).

	pH			**Soil Moisture**		
	F	***P-value***	***F crit***	***F***	***P-value***	***F crit***
Land-use	51.86594	2.08E-12**	3.204317	5.780461	0.00483*	3.204317
Seasons	107.8442	6.83E-18**	3.204317	64.24477	6.51E-14**	3.204317
Interaction	1.594203	0.192294^{ns}	2.578739	0.748919	0.563939^{ns}	2.578739
	Bulk Density			**Soil Temperature**		
	F	***P-value***	***F crit***	***F***	***P-value***	***F crit***
Land-use	95.27539	6.69E-17**	3.204317	119.2769	1.03E-18**	3.204317
Seasons	54.08268	1.07E-12**	3.204317	9110.038	2.05E-59**	3.204317
Interaction	2.506719	0.055196^{ns}	2.578739	38.49591	5.48E-14**	2.578739
	Total Nitrogen			**Available Phosphorous**		
	F	***P-value***	***F crit***	***F***	***P-value***	***F crit***
Land-use	11.98715	6.71E-05**	3.204317	11.45492	9.52E-05**	3.204317
Seasons	6.94743	0.002347*	3.204317	26.08607	3E-08**	3.204317
Interaction	0.435748	0.782063^{ns}	2.578739	1.311475	0.280185^{ns}	2.578739

*Significance at *P-value*<0.01, **Significant at *P-value*<0.001, ^{ns}Not Significant

Soil Texture

The soil texture was loam in STF and AGF study site while in the WPC it is clayey loam in nature (Table-4). Sand percentage content is highest in CAF (46.72%) followed by STF (42.52%) and lowest in the WPC (38.55%). Silt and clay content is higher in WPC (31.95%, 29.50%) followed by STF (30.82%, 26.67%) and CAF (30.10%, 23.09%) (Fig 6).

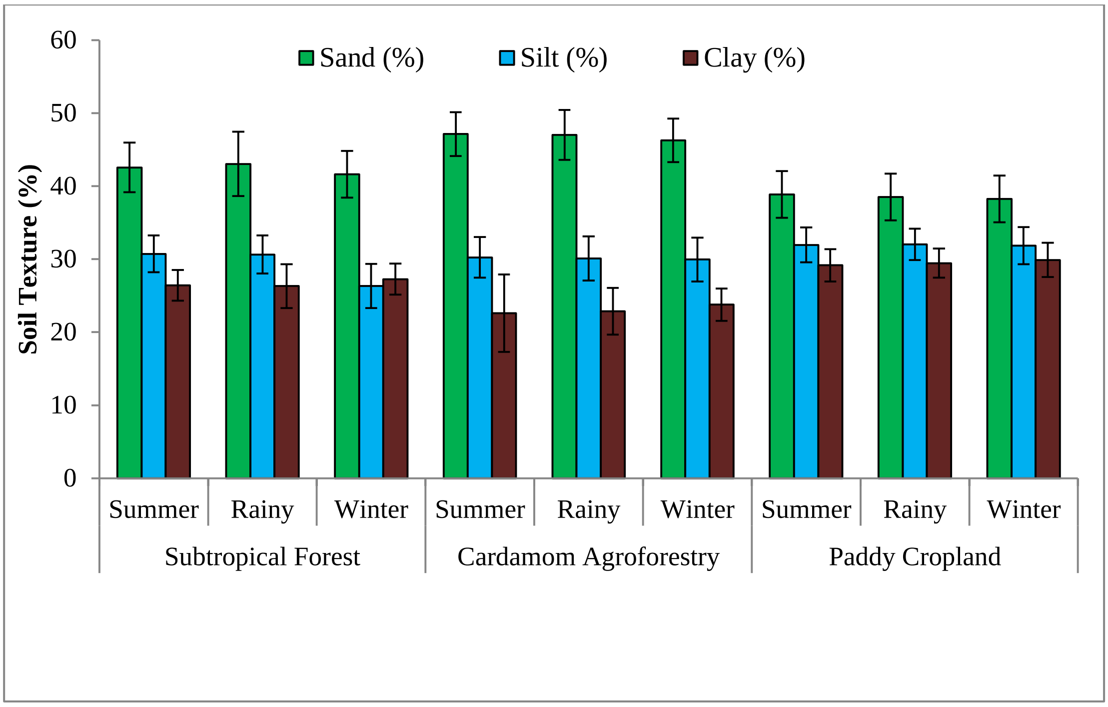


Fig 6. Variation of soil texture across the seasons of three different study sites (mean±SE)

Soil Moisture

Moisture content in soil ranged from 18.22% to 38.33% across different soil depths and seasons in all the study sites. Paddy cropland exhibited maximum soil moisture content (20.22%-38.33%) followed by cardamom agroforestry (19.00%-37.00%) and minimum in the subtropical forest (18.22%-36.00%) (Fig 7). All the study sites show peak soil moisture during the rainy season and the lowest in the winter season. Moisture content decreases along with the soil depth with a maximum in the upper soil layer (0-15 cm) and a minimum in the inner soil layer (30-45 cm). Soil moisture in the present study showed a significant variation with land-use type ($p<0.01$) and seasons ($p<0.001$) (Table-5).

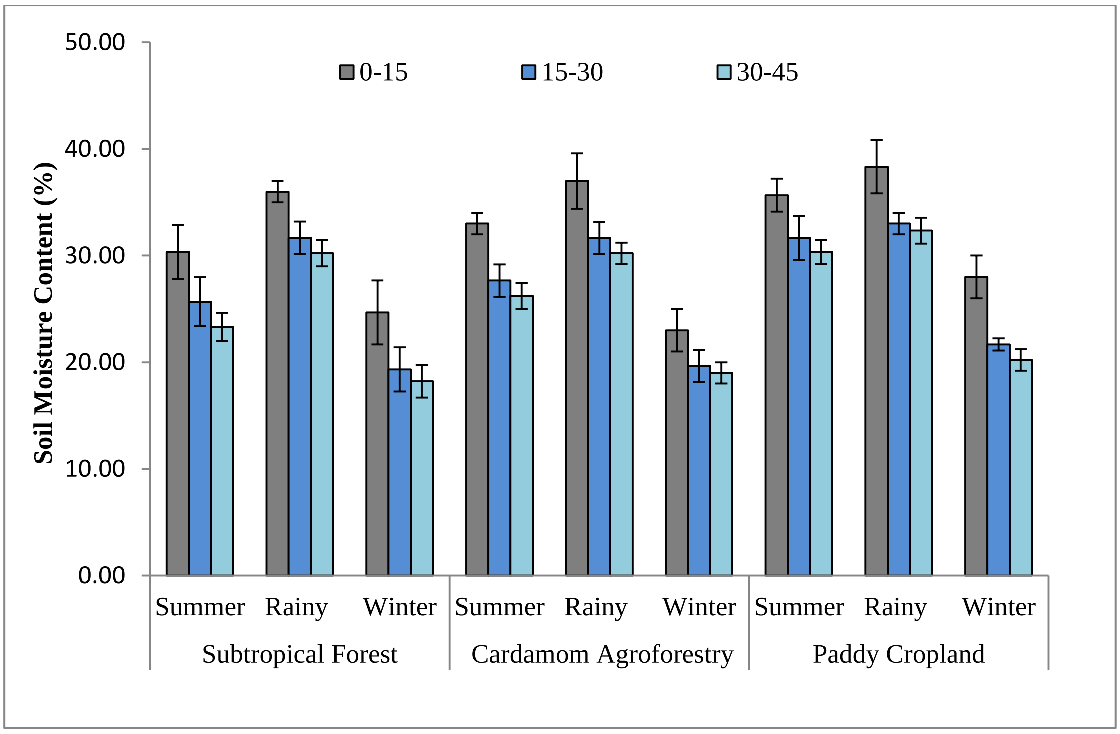


Fig 7. Variation of soil moisture across the seasons and soil depth of three different study sites (mean±SE)

Bulk density

Bulk density varied from 0.56-0.91 g cm^{-3} and increased with soil depth across the land-use systems. Maximum bulk density was recorded in the STF (0.68-0.91g cm^{-3}) followed by CAF (0.61-0.80 g cm^{-3}) and lowest in the WPC (0.56-0.78 g cm^{-3}). Seasonally, bulk density is observed highest during winter and lowest in the rainy season in all the study sites (Fig 8). Soil BD showed a significant variation with land-use type ($p<0.01$) and seasons ($p<0.001$) (Table-5).

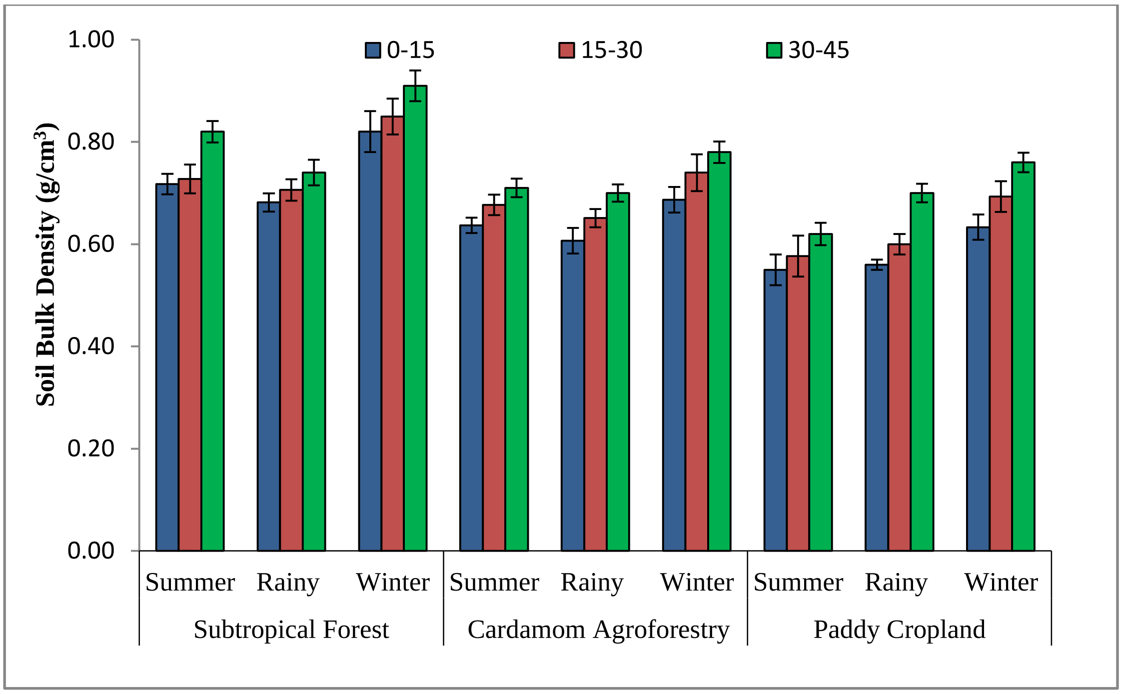


Fig 8. Variation of bulk density across the seasons and soil depth of three different study sites (mean±SE)

Soil pH

The pH in soil was higher in the WPC (5.2-5.8) than STF (4.8-5.7) and CAF (4.8-5.7) and it increases with soil depth across the seasons and land-use types (Table 4). Winter season experienced a slightly higher pH of the soil than the rest of the season while the rainy season showed the minimum in all the land-use types (Fig 9). There is a significant variation in soil pH value with land-use type ($p<0.01$) and seasons ($p<0.001$) (Table-5).

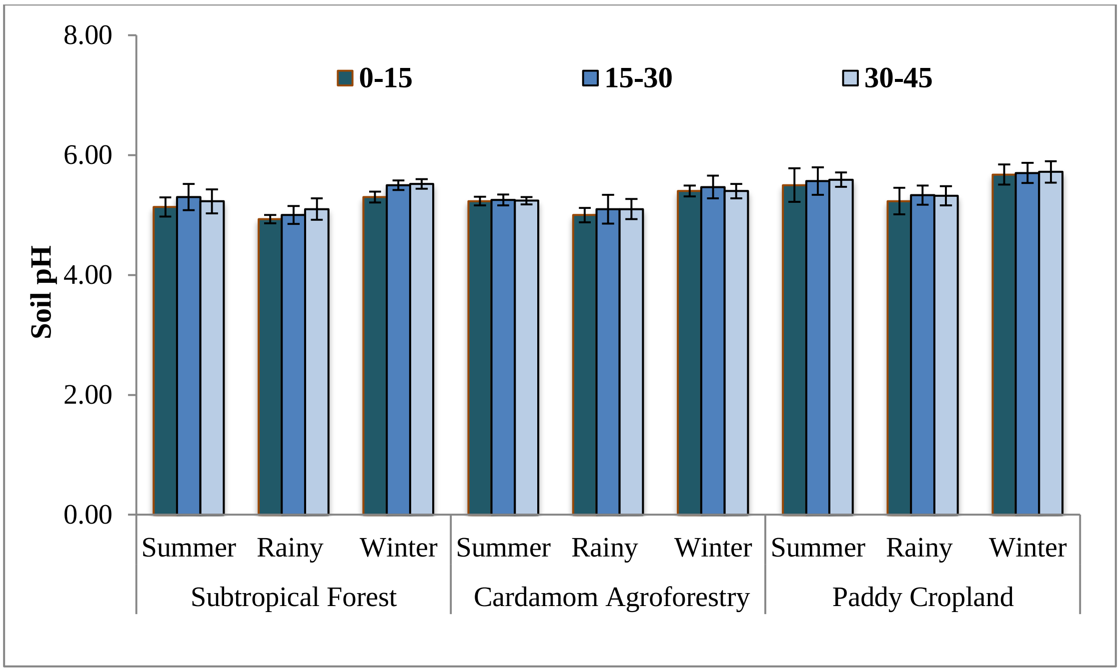


Fig.9. Variation of soil pH across the seasons and soil depth of three different study sites (mean±SE)

Soil Organic Carbon (SOC)

Soil organic carbon (SOC) varied from 1.88% to 4.96% across the soil depths and study sites. In the subtropical forest, SOC ranged between 2.30%-4.96% while in cardamom agroforestry and paddy cropland it varied from 2.03%-4.53% and 1.88%-3.98% respectively. SOC decreases along with soil depth with the highest in the upper soil layer i.e. 0-15cm (3.40%-4.96%), followed by 15-30 cm (2.96%-4.15%) and minimum in the 30-45 cm (1.88%-2.90%). In all the study sites, the maximum value of soil organic C was observed during the rainy season, followed by summer and minimum in the winter season (Fig 10). SOC showed a significant variation and with land-use type ($p<0.01$) and seasons ($p<0.001$) (Table-5).

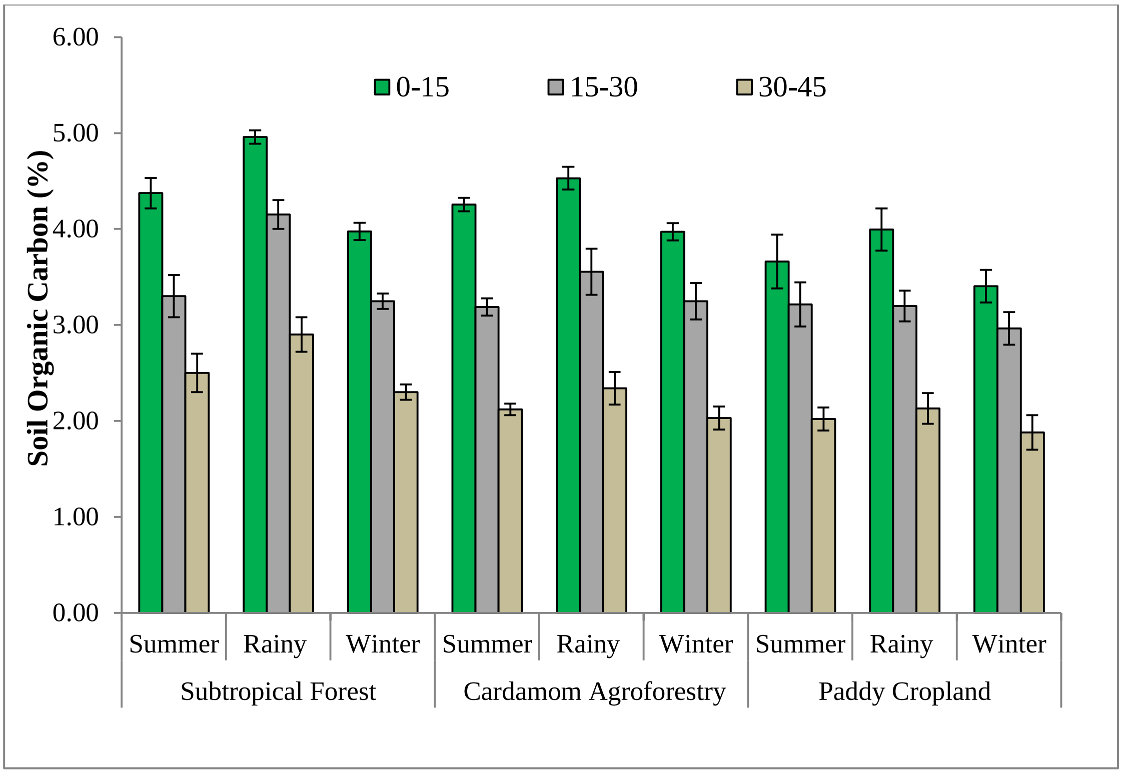


Fig 10. Variation of soil organic carbon across the seasons and soil depth of three different study sites(mean±SE)

Total Nitrogen (N)

Total nitrogen was maximum in the STF (0.19%-0.37%) followed by CAF (0.18%-0.34%) and minimum in the WPC (0.15%-0.27%). Across the seasons and soil depths total nitrogen in soil varied from 0.15% to 0.37%. The seasonal trend was highest in the rainy season and lowest in the winter season in all three study sites (Fig 11). Similarly, total nitrogen also showed a significant variation with land-use type ($p<0.01$) and seasons ($p<0.001$) (Table-5).

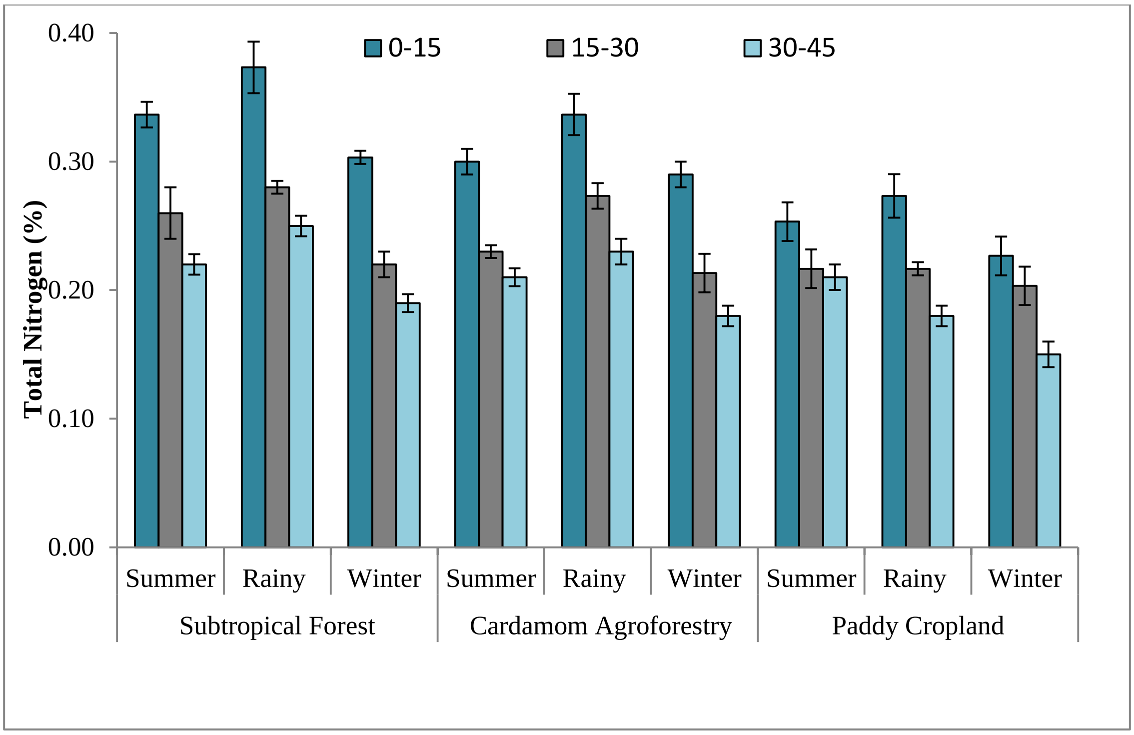


Fig 11. Variation of total nitrogen across the seasons and soil depth of three different study sites(mean±SE)

Available Phosphorous (P)

Across the study sites, available phosphorous ranged from 0.010%-0.054% in different seasons and soil depth. Subtropical forest recorded highest P (0.022%-0.054%), followed by cardamom agroforestry (0.016%-0.040%) and paddy cropland, (0.010%-0.033%). The concentration of available phosphorous decreases along with soil depth and showed a maximum in the rainy season and a minimum in the winter season in all the study sites (Fig 12). Available phosphorous showed a significant variation with land-use type ($p<0.01$) and seasons ($p<0.001$) (Table-5).

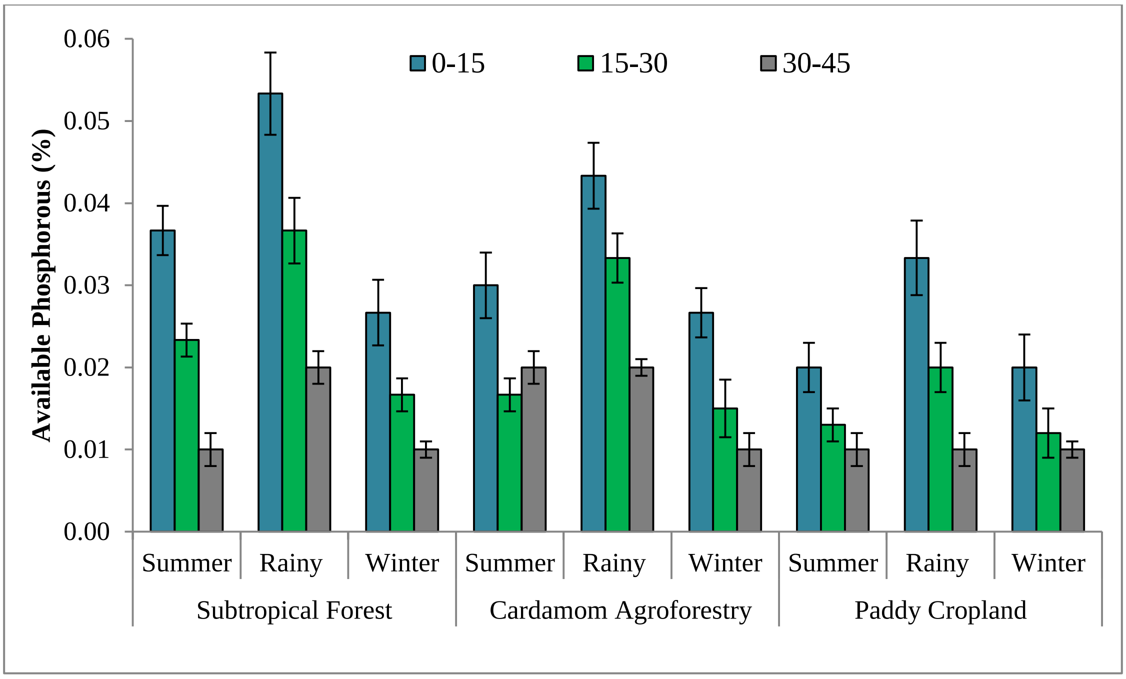


Fig 12. Variation of available phosphorous across the seasons and soil depth in different study sites (mean±SE)

Exchangeable potassium (K)

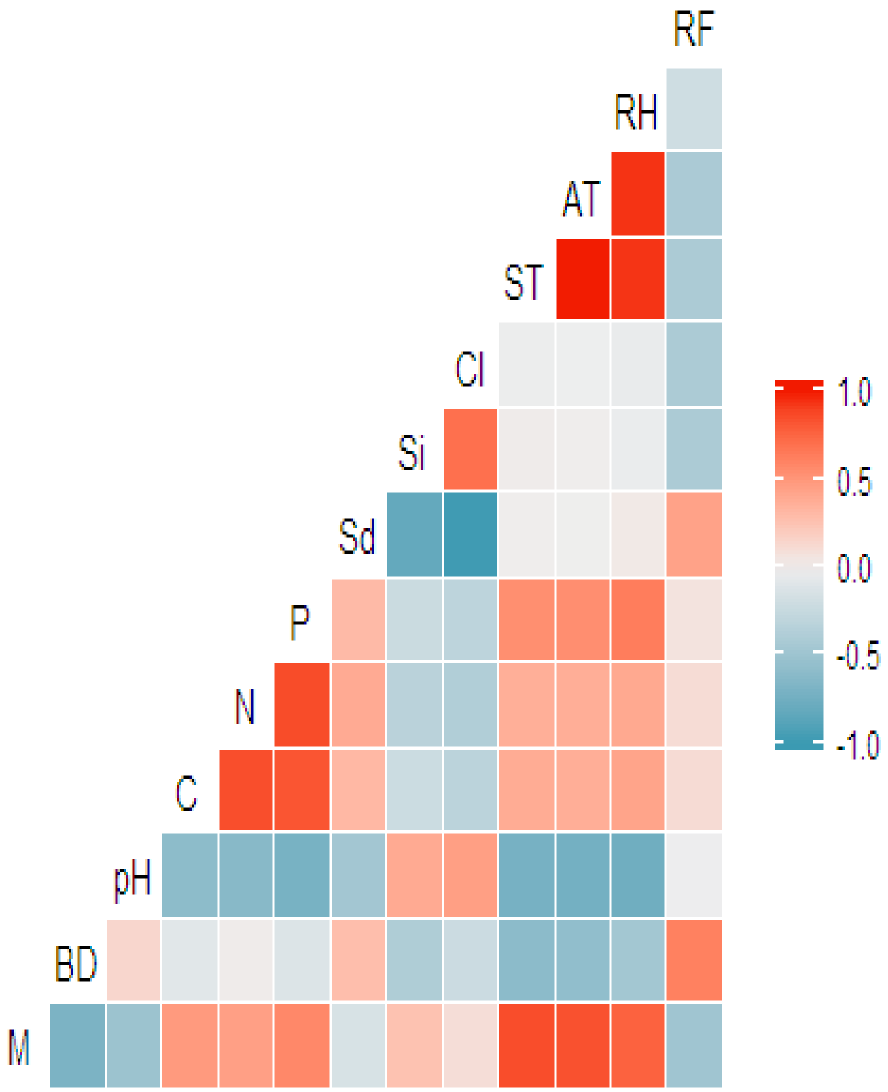
Exchangeable potassium in all study sites varied from (0.14%-0.26%). The concentration of potassium was highest in the STF (0.26%) followed by AGF (0.17%) and lowest in the WPC (0.14%).

Soil Carbon :Nitrogen(C:N) ratio

The C:N ratio of the soil ranged from 11.60-15.22 in the STF, 10.10-15.01 in CAF and 09.62-14.82 in WPC. Maximum C:N ratio was observed during the winter season and minimum during the summer season in all study sites (Table-4).

Relationship between abiotic variables and soil properties

The Pearson correlation matrix between the biotic and abiotic variables of three different land-use systems was shown in Fig 13. The concentration of sand in soil showed a positive significant correlation ($p<0.05$) with bulk density, C, N, P and rainfall but show a negative significant correlation ($p<0.05$) with pH, silt and clay. However, silt exhibited a positive significant correlation ($p<0.05$) with pH and rainfall but show a negative significant correlation ($p<0.05$) with bulk density, total nitrogen, sand and clay. Similarly, clay fraction showed a positive significant correlation ($p<0.05$) with pH, silt and rainfall but show a negative significant correlation ($p<0.05$) with C, N, P and sand fraction. Soil temperature and soil moisture showed a positive significant correlation ($p<0.01$) with C, N, P, and air temperature but a negative significant correlation ($p<0.01$) with bulk density, pH and rainfall. Bulk density exhibited a significant negative correlation ($p<0.05$) with soil moisture, sand, silt, air and soil temperature, and relative humidity. Soil pH showed a significant positive relation ($p<0.01$) with silt and clay only, whereas it is negatively correlated ($p<0.05$) with moisture, C, N, P, sand, soil and air temperature and relative humidity. C, N, P showed a significant positive relation ($p<0.01$) with soil moisture, sand, soil and air temperature and relative humidity however correlation with soil pH, silt and clay was negatively significant ($p<0.05$).



M = Moisture, BD = Bulk Density, C = Soil organic carbon, N =Total nitrogen, P = Available phosphorus, Sd= Sand, Si= Silt, Cl= Clay, ST = Soil temperature, AT= Air temperature, RH = Relative Humidity, RF = Rainfall

Fig 13. Pearson correlation matrix between biotic and abiotic variables in different study sites

DISCUSSION

The results of the present study revealed that all soil physicochemical properties (soil texture, moisture, temperature, bulk density, pH, C, N, P, C:N ratio) vary in different land-use types. This is due to the difference in vegetation composition and other management practices used in each of the land-use types. Several studies also showed a change in soil properties due to land-use change (Pabst et al. 2013; Ravindran and Yang 2015; Reza et al. 2018).

Present study indicated a difference in the sand, silt and clay content with land-use type and soil depth. Similar results were reported by another previous study Yuksek and Yuksek (2011). However, contrasting results were also reported wherein no significant differences in soil texture were observed with the change in land-use types and soil depth by other studies (Evrendilek et al. 2004; Korkonc 2014). In the CAF, sand particles were maximum, followed by STF and minimum in WPC, while clay and silt particles showed a reverse pattern with a highest in WPC and a lowest in CAF study site. The present results agree with the findings of Tellen and Yerima (2018). The reason for this reverse trend may be due to the variation in the management practices of these two agriculture-based systems. In cardamom agroforestry, slightly higher sand content than that of the subtropical forest may be due to the removal of herbaceous layers that makes the soil susceptible to erosion while adopting the terraced farming practice in paddy cropland reduces soil erosion leading to an increase in clay and silt content. Distribution of particle size plays an important role in vegetation as they influence the consistency of soil texture quality and erosion (Aderonke and Gbadegesin 2013). Also, bioclimatic conditions change rapidly in dissected landscapes of Himalaya within short distances resulting in heterogeneous soil types and physical and chemical properties (Baumler 2015).

A maximum mean soil temperature in the subtropical forest than the other two agriculture-based systems may be due to dry soil in the forest as water is used for irrigation in the other two agricultural systems. Higher soil moisture content in the cropland than that of forest and agroforestry soils could be attributed to a higher clay content in the former. Such a trend was observed by several other studies (Sala et al. 1998; English et al. 2005; Kara and Baykara. 2014).

Bulk density (BD) range of 0.6-1.8 g cm^{-3} in the present study is within the range reported by Baumler and Zech (1994) and increased with a decrease in soil depth in all types of land-use. Such a trend was reported by several other studies (Barbhuiya et al. 2004; Li et al. 2013a; Zhang et al. 2014; Francaviglia et al. 2017). Less organic matter and the weight of the overlying horizons are attributed to higher soil bulk density in the inner soil layers (Gruneberg et al. 2014). The variation in the BD of the various systems may be due to the difference in the distribution of particle size. The dependency of bulk density on soil texture is shown by several studies (Smith et al. 1997; Dumig et al. 2006).

The slightly or moderately acidic pH range of the soil in all three land-use types is comparable with the values reported from different land-use types of Arunachal Pradesh (Arunachalam and Arunachalam 2006), and different agroecological zones of Sikkim (Deb et al. 2018). Soil pH concentrations of the present study sites increased with soil depth because of the rich organic matter in the upper soil layer that decomposed to produce more organic acids, leading to a low pH concentration in the top soil layer (Hong et al. 2019).

Soil organic carbon (SOC) decreased with soil depth in all land-use types with a maximum concentration in the forest due to the presence of litter from trees that continuously increases root turnover (Kimmins 2004). The lower amount of organic carbon in the agricultural field may be because of the harvest of all above-ground plant parts for fodder (Shrestha et al. 2004), that lowers the rate of organic matter turnover. Additionally, continuous cultivation for years with minimum inputs of soil organic matter lowers SOC content in the paddy cropland (Poudel and Thapa 2001). A higher SOC in the forest as compared to other land-use types was reported by Soleimani et al. (2019). Similarly, lower annual carbon input in agricultural lands than that of the natural forest lead to lower SOC has been reported by (Huang and Song 2010). The total amount of SOC in the paddy cropland is lowest as compared to cardamom agroforestry and natural forest which confirmed that the transformation of the forest into cultivation land can decrease SOC stock.

The maximum total nitrogen (N) of soil in the subtropical forest is due to the presence of *Alnus nepalensis* nitrogen-fixing tree species, as a dominant tree in both the systems. Rothe et al. (2002) reported that the presence of N-fixing species increases soil total nitrogen content. Further, the high accumulation of litter from trees on the soil surface and the decomposition of organic matter by microorganisms in tree-based systems enhanced nutrient content in the soil. The present trend is consistent with that (Ufot et al. 2016; Chemada et al. 2017), where higher N content was reported in the forest than in the nearby agricultural areas. The available phosphorus and potassium decreased with soil depth in all the systems which are due to a higher organic matter in the upper soil layer, and low soil pH which helps in soil P immobilization (Chase and Singh 2014). Soils of the present study indicate phosphorus limitation which could be due to the acidic nature of these soils. Available P is higher in the forest than agricultural lands which may be due to the high SOC content resulting in the release of organic phosphorus thereby enhancing available P under forest land. A similar finding was reported by (Takele et al. 2014; Tufa et al. 2019).

The maximum exchangeable K recorded in STF can be attributed to a higher input of soil organic matter that increases the ability of soil cationic exchange, thereby reducing leaching rate in the soil (Mbah 2008). Minimum K concentration in WPC may be due to the leaching of soil K through irrigation water, limited crop residue recycling, intensive management practices, and soil erosion which led to the depletion of basic cations on agricultural lands (Lechisa et al. 2014; Akbas et al. 2017).

The C:N ratio was lower in soils of WPC as compared to STF and AGF soils because of intensive management practices and frequent tillage which encourage oxidation of organic matter that leads to loss of carbon and nitrogen from the soil. The present trend of C:N ratio is in agreement with the finding of various researchers that reported higher C:N ratio in the forest soils than in other agricultural land-use systems (John et al 2005; Puget and Lal 2005; Abera and Belachaw 2011).

CHAPTER-IV

CARBON STOCK AND SEQUESTRATION

CARBON STOCK AND SEQUESTRATION

INTRODUCTION

Climate change is now a reality and it's not about trying to find definitive facts, but about taking action. Atmospheric temperature increase known as global warming is one of the main consequences of increase in atmospheric carbon dioxide. An unpredictable rise in the concentration of carbon dioxide in the atmosphere involves the identification of methods for mitigating the global warming (Sheikh et al. 2014). This warming effect will lead to numerous adverse environmental changes, such as the depletion of mountain glaciers, the degradation of coral reefs (IPCC 2007), habitat loss and deforestation (Buizer et al. 2014). Carbon storage by green plants and soils in terrestrial ecosystems can effectively overcome the problems of climate change and global warming for longer periods (Sheikh et al. 2014). The sequestration of terrestrial carbon is recognized as the most relevant and economic way of storing carbon in living plants and thus helps to mitigate the increasing CO_2 emission in the atmosphere.

Biomass of plants and animals origin is considered as a renewable natural resource and can be used to evaluate the amount of carbon stored in the ecosystems. The amount of plant biomass is determined by the net primary productivity which has been produced through the process of photosynthesis (Clark et al. 2001). It is often used to understand the carbon cycling process between the atmosphere and the terrestrial ecosystem concerning global climate change (Cairns et al. 2000). Therefore, the estimation of biomass is important for national development planning and scientific studies of ecosystem productivity (Pandey et al. 2010). Certain methods employed for the estimation of biomass or carbon stock (aboveground and belowground) in vegetation includes destructive, non-destructive, and remote sensing methods (Lu 2006). Carbon can be stored in the aboveground and belowground part of the plants however, carbon can also be stored in soil, detritus or litter on the forest floor. Estimating biomass and carbon in terrestrial systems is gaining importance across the world (Sharma et al. 2010; Ekoungoulou et al. 2014; Salunkhe et al. 2016), and several countries are working in compliance with the greenhouse gas reduction agreements under the United National Framework Conversion on Climate Change (Brown 2002).

Aboveground biomass (AGB) includes all living biomass above the soil i.e stem, branches, leaves, fruit and bark, and below-ground biomass (BGB) includes all live roots (Penman et al. 2003). Both AGB and BGB have great importance for the characterization of the structure and function of the ecosystems. The total amount of carbon stored in the global vegetative biomass exceeds that of atmospheric carbon dioxide, and about 90% of the plant biomass carbon is stored in the form of tree biomass (Komer 2006).

Soils are potentially viable sinks for atmospheric carbon as it stores the largest carbon pool in terrestrial ecosystems and may significantly contribute to the mitigation of global climate change (Lai et al. 1998). Soil organic carbon (SOC) is a key component of the soil-plant ecosystem and is closely associated with soil properties and processes, nutrient buffering and supply, as well as emission and storage of greenhouse gases (Kasel and Bennett 2007; Yang et al. 2009). The assessment of potential C sequestration in soil requires the estimation of carbon pools under existing land-uses and the distribution of soil profiles. Removal of trees from the forest displaces a large amount of sequestered carbon (IPCC 2000) and consequently reduces the SOC held in soil profiles (Glaser et al. 2000). Gradual conversion of forest and grassland to cropland has resulted in significant losses of soil carbon worldwide (Lai 2002). The type of land-use, degree of land-use, change and post-conversion land management harm the degree of variation in SOC content (West et al. 2010). Carbon stored in the soil varies in different ecosystems depending on the species composition, climate, soil types, and other characteristics of the site (Trumper et al. 2009).

Litter or detritus constitute an important source for transferring energy and nutrient cycling from vegetation to soil particularly in low fertile soil where trees greatly rely on the recycling of their nutrients (Okeke and Omaliko 1992; Negash and Starr 2013). It also represents a connecting link between carbon captured through photosynthesis and release through decomposition (Meentemeyer et.al. 1982) and helps us in understanding the global carbon cycle.

All these carbon pools play a vital role in identifying the total amount of carbon captured in the terrestrial vegetation (Chave et al. 2003). Hence, estimation of biomass and carbon stock in different compartments of an ecosystem is necessary for the characterization of structure and function of different ecosystems and in identifying the amount of carbon stored in terrestrial vegetation (Chava et al. 2003). Different regions and land-use types have different biomass and carbon stock or storage potential depending on the type of vegetation or species and management practices adopted in each of the systems. Carbon sequestration by plants is the absorption of CO_2 from the atmosphere by photosynthesis and storing it in different parts of the plants for a very long period in the terrestrial ecosystems. Carbon sequestration depends upon the biomass production capacity of the vegetation, which in turn depends upon the interaction between edaphic, climatic, topographic factors of an area and type of land-use system. Biomass carbon moving from non-tree-based land-use types such as agricultural land, grassland to tree-based land-use types such as agroforestry, plantation and natural forest helps in increasing carbon sequestration (Quinkenstein et al. 2011).

Among the known terrestrial land-use types, forests play a most significant role in the global carbon cycle as these ecosystems act as the most important carbon sink (Houghton 2007). Forests store more carbon than any other terrestrial ecosystem because of greater biomass accumulation in trees. Forest habitats play a crucial role in atmospheric carbon sequestration, which helps to minimize the carbon footprint and helps to moderate global warming and consequent climate change (Ramachandra and Bharath 2019). It has been estimated that the quantification of carbon sequestered by forests worldwide is 861±66 Pg C (Pan et al. 2011). Indian forests can store about 74% of the total carbon stored in vegetation and can sequester 7.35% of total carbon emissions annually (Ramachandra and Shwetamala 2012).

Besides the forests, man-managed ecosystems such as agroforestry ecosystems have huge potential to mitigate climate change and global warming by storing and sequestering carbon (Pala et al. 2015). Tropical agroforestry systems can accumulate 12.00-228.00 Mg C ha^{-1} (Soto-Pinto et al. 2010) and can also simultaneously reduce the problem of land-use and land cover change due to agriculture. Several studies have shown the negative impact of agricultural systems on carbon stock (Girmay et al. 2008; Toru and Kibret 2019). Nevertheless, agriculture systems are one of the important land-use practices that emit as well as sequester carbon dioxide. Indigenous agroforestry systems play a crucial role in maintaining rural communities livelihoods and can help to offset the rising potential impacts of climate change (Sharma et al. 2016).

The increasing human population has led to fast land-use and land cover change (LULC) in the globe affecting the global carbon cycle. Every land-use system has either a positive or negative impact on the carbon balance (Toru and Kibret 2019). The net flux of carbon due to land-use change during 1850-2000 is 148 Pg C (Kaul et al. 2009). The rapid global change in land-use through forest-to-cropland transition has increased fivefold over the last few decades from about 3 to about 15 million km^2 during the year 1700-2007 (Ramankutty et al. 2018). The area of South East Asia also experienced 11.30 percent of the total forest cover loss during the period 2000 to 2014, i.e. 29.3×10^{10} m^2 (Zeng et al. 2018). In the Sikkim Himalaya, Kanade and John (2018) have recorded a decrease in primary forest cover and an increase in secondary forest and agriculture by 30 percent and 16 percent of primary forest at an altitude range of 800-2200 m and 2200-2800 m.

Sikkim, one of the tiny mountainous states of India located at the Eastern Himalayas hosts several types of land-use systems including forests, grassland, agricultural land, etc. However, increasing demand for resources due to several pressures lead to a change in land-use type in this region too. Approximately 8.30 km^2 and 5.26 km^2 have been transformed from open forest to agricultural land and agricultural land to agroforestry systems between 2007 and 2018 in Sikkim Himalaya (Mishra et al. 2019).

This chapter discusses:

I. Biomass carbon of vegetation and soil in three different land-use systems i.e. subtropical forest, cardamom agroforestry and wet paddy cropland.

II. Total ecosystem carbon and sequestration potential of different carbon pools in the three land-uses.

III. Changes in carbon stocks and sequestration in three different land-use systems.

METHODS

Plant Sampling and Analysis

Vegetation

For the sampling of vegetation, both destructive methods and non-destructive methods were used depending on the type of vegetation to be sampled. In the present study sites, the plant of different categories such as trees and herbs were sampled by using appropriate quadrats of different sizes.

Trees

For the sampling of, tree species, 10 permanent quadrats, five in each land-use of 31.6 x 31.6 m (0.1 ha) size were earmarked in subtropical forest and agroforestry systems, (Fig.14). All individual trees present in each quadrat were enumerated, marked, and identified, and geo locations of each permanent plot were also recorded. Diameter at breast height (dbh) of each tree was measured at 1.3 m height of the tree trunk and the height of each tree was measured by using a hypsometer (Forestry Pro, Nikon).

Shrubs

To estimate the shrubs present in the study sites, permanent quadrats of 5m x 5m were established within the opposite corner of the 31.6m x 31.6m permanent plots. All the shrubs within the plots were marked, enumerated, and dbh was measured.

Herbs

All the herbaceous vegetation present within a 1m x 1m quadrat located within the bigger quadrat of shrubs were harvested and transferred to the laboratory in an airtight polythene bag to estimate the fresh weight. Soil particles and other debris from the roots were removed and washed under running tap water whenever necessary and allow to drip water for some time. Thereafter, fresh weight was measured and the plants are oven-dried at 80^0C until the constant weight was achieved.

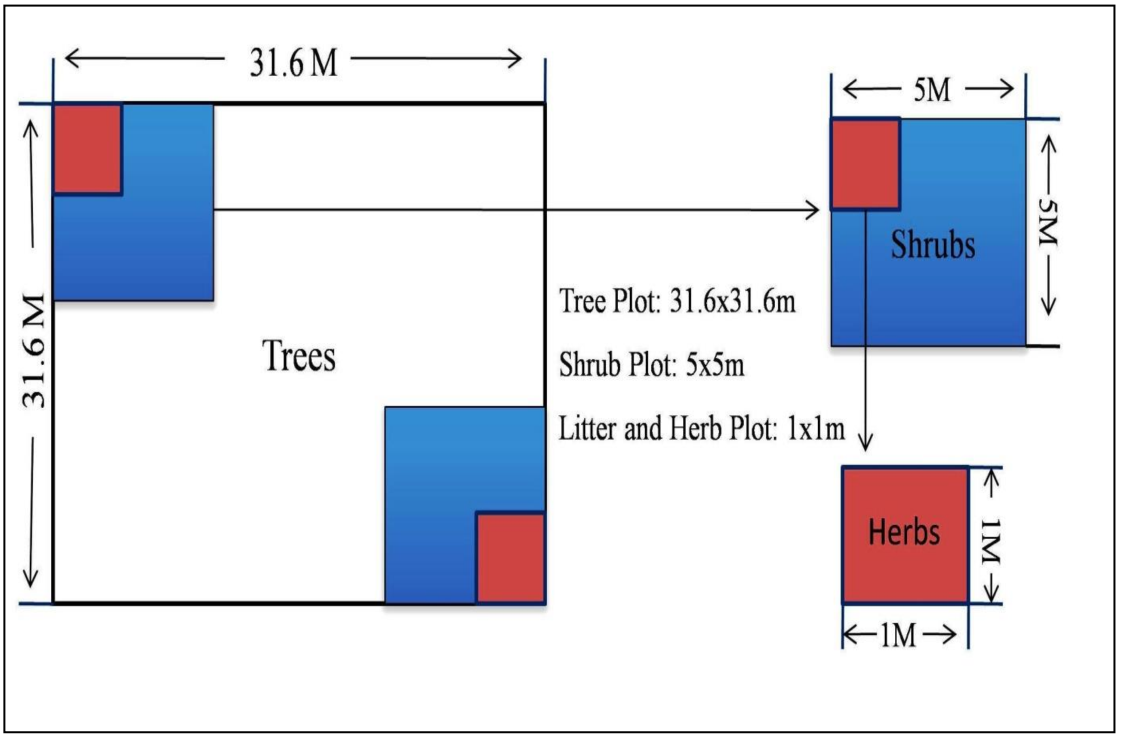


Fig 14. Sampling design layout

Importance value index (IVI)

Density

Tree densities of all tree species within ten (10) quadrats of 0.1 ha (31.6 x 31.6 m) each in the subtropical forest and cardamom agroforestry systems were estimated. Species of trees were identified from Flora of Bhutan (Gierson and Long 1983) and the density of each tree species was calculated by the formula.

$$\text{Tree density (individuals ha}^{-1}) = \frac{\text{No of individuals per species}}{\text{Sampled area}}$$

Frequency

The frequency of each tree species within the quadrat was estimated by the given formula,

$$\text{Tree frequency (\%)} = \frac{\text{Total number of individuals per species}}{\text{Total no of quadrat studied}}$$

Basal area

Basal area of the tree species was determined by using the girth size (m) of the tree from the sample plots of 31.6 x 31.6 m (0.1ha),

$$\text{Basal area (m}^2) = 3.14\frac{(\text{ D})^2}{4}$$

From the density, frequency and basal area of each tree species relative frequency, relative density and relative basal area of each tree species were calculated and added up to calculate the IVI of each tree species across different land-use systems (Misra 1968).

$$\text{Relative density} = \frac{\text{Density of individual species}}{\text{Total density of all species}} \text{ x } 100$$

$$\text{Relative frequency} = \frac{\text{Frequency of the individual species}}{\text{Total frequency of all the species}} \text{ x } 100$$

$$\text{Relative basal area} = \frac{\text{Basal area of the individual species}}{\text{Total basal area of all the species}} \text{ x } 100$$

IVI (%) = Relative density+ Relative frequency +Relative basal area

Diversity and other indices

Different diversity indices like Simpson and Shannon Weiner indices along with dominance, evenness and species richness indices were calculated from two different tree-based land-use types using the following formulas:

Simpson's dominance index (Simpson 1949)

$$D=1-¡\sum(n(n-1)/N(N-1)$$

Where n=no of individual of each species and N=total number of individuals of all the species

Shannon Weiner- diversity index (Shannon and Weaver 1963)

$$H= SUM [(Pi)*\ln(Pi)]$$

Where pi is the proportion of individuals in the i^{th} species i.e. (ni/N)

Pielous Evenness (Pielou 1966)

$$Evenness=H/\ln N$$

Where H= Shannon index and N= Number of species

Margalef species Richness index (Margalef 1958)

$$d= (S-1)/\log (N)$$

Where S= Number of species and N= Number of individual species.

Biomass, carbon stock and sequestration

Aboveground biomass carbon

Biomass carbon in the vegetation of three different land-use types was estimated by demarcating five plots in each land-use and inside each plot, two random quadrats of 31.6m X 31.6m were earmarked. All individual trees ≥ 10cm diameter at breast height (DBH) 1.3 m within the quadrats were enumerated and numbered for the first and third year (2016 and 2018). Wood specific gravity for each tree species was also determined by oven drying the wood samples of trees collected by using an increment borer at a height of 1.3 m above the ground. FSI species-specific volume equations (FSI 1996) were used to calculate the tree volume of each tree species and aboveground biomass was computed by using the procedure of (Ravindranath and Ostwald 2008).

Aboveground Biomass = Volume of tree × Specific gravity × BEF (IPCC 2006)

Where BEF is the Biomass Expansion Factor

Belowground biomass carbon

Quantification of belowground biomass (BGB) of trees was done using standard root to shoot ratio default value of 0.26 (Ravindranath and Ostwald 2008).

Total biomass was calculated by adding aboveground biomass and belowground biomass and carbon stock in trees were computed using the following formula:

C= Total AGB x 0.47 (Ravindranath and Oswald, 2008)

Where AGB is aboveground biomass,

C is a carbon (Mg ha^{-1}), 0.47 is the default carbon fraction.

Plate 6 (A,B,C,D): Above-ground biomass estimation

Wood specific gravity

The specific gravity of each tree species was estimated following the maximum moisture content method (Smith 1954), where the wood core of tree species was extracted by increment borer (Hagloff, Sweden) and the wood core was weighed and oven-dried at 105°C until the weight becomes constant. The specific gravity of each tree species was computed using:

$$\text{Specific gravity } (gm/cm^3) = 1/(Mm\text{-}Mo/Mo+1/Gso)$$

Where, Mm= Initial fresh weight of the wood core, Mo= Oven-dried weight of the core and Gso = 1.53(Moisture correction factor).

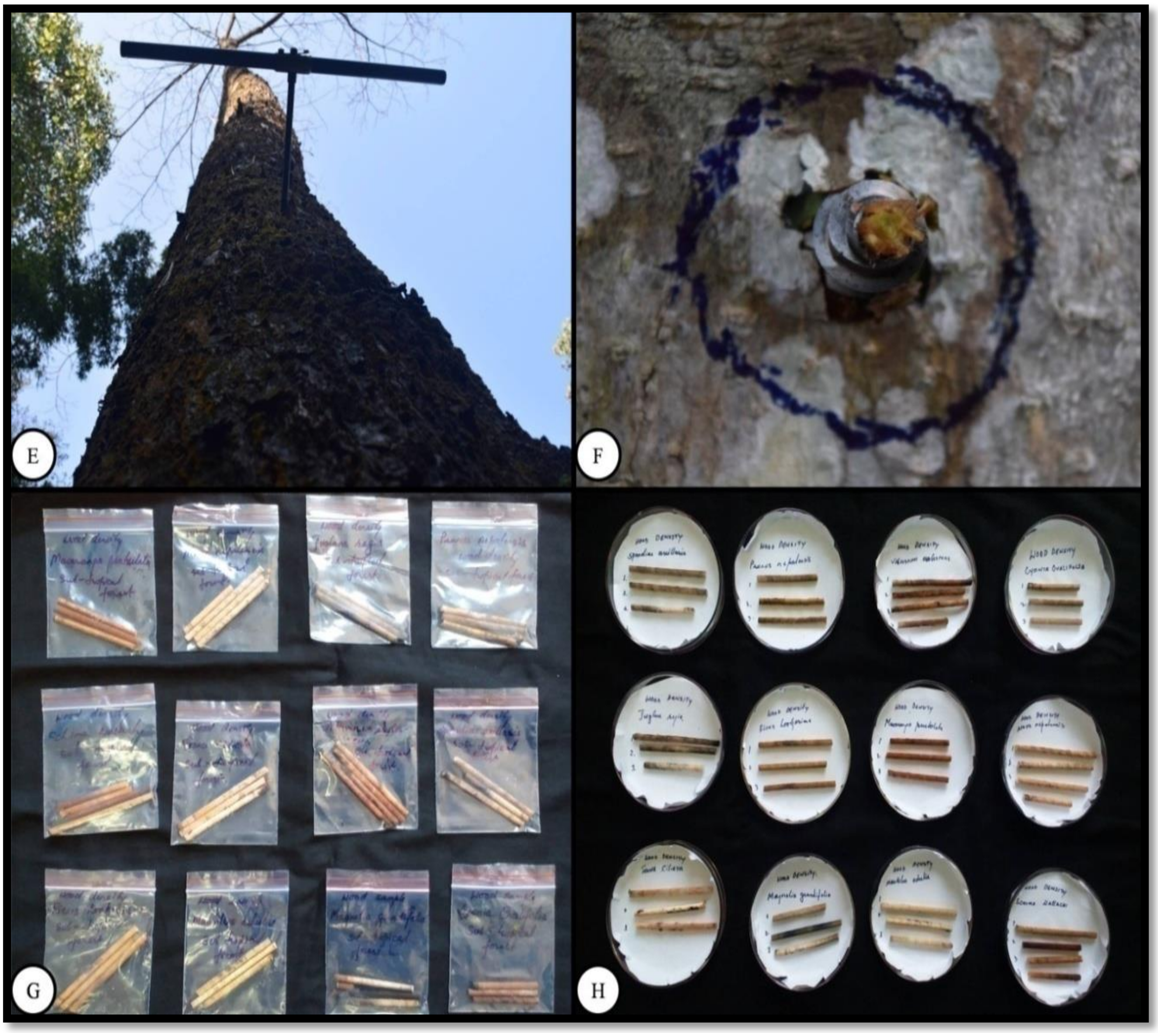


Plate 7 (E,F,G,H): Wood density estimation

Herbaceous biomass carbon

All herbaceous vegetation from the three study sites including the rice cropland were harvested completely from ten quadrats of 1m x 1m size during the rainy season and growing season of rice (2016 and 2018). All herbaceous plants, including cardamom, were brought to the laboratory, washed, weighed and oven-dried (80^{0}C) to achieve a constant weight and reweighed to estimate biomass. Carbon in the herbs was computed by using the default carbon fraction (0.50) of IPCC (2006).

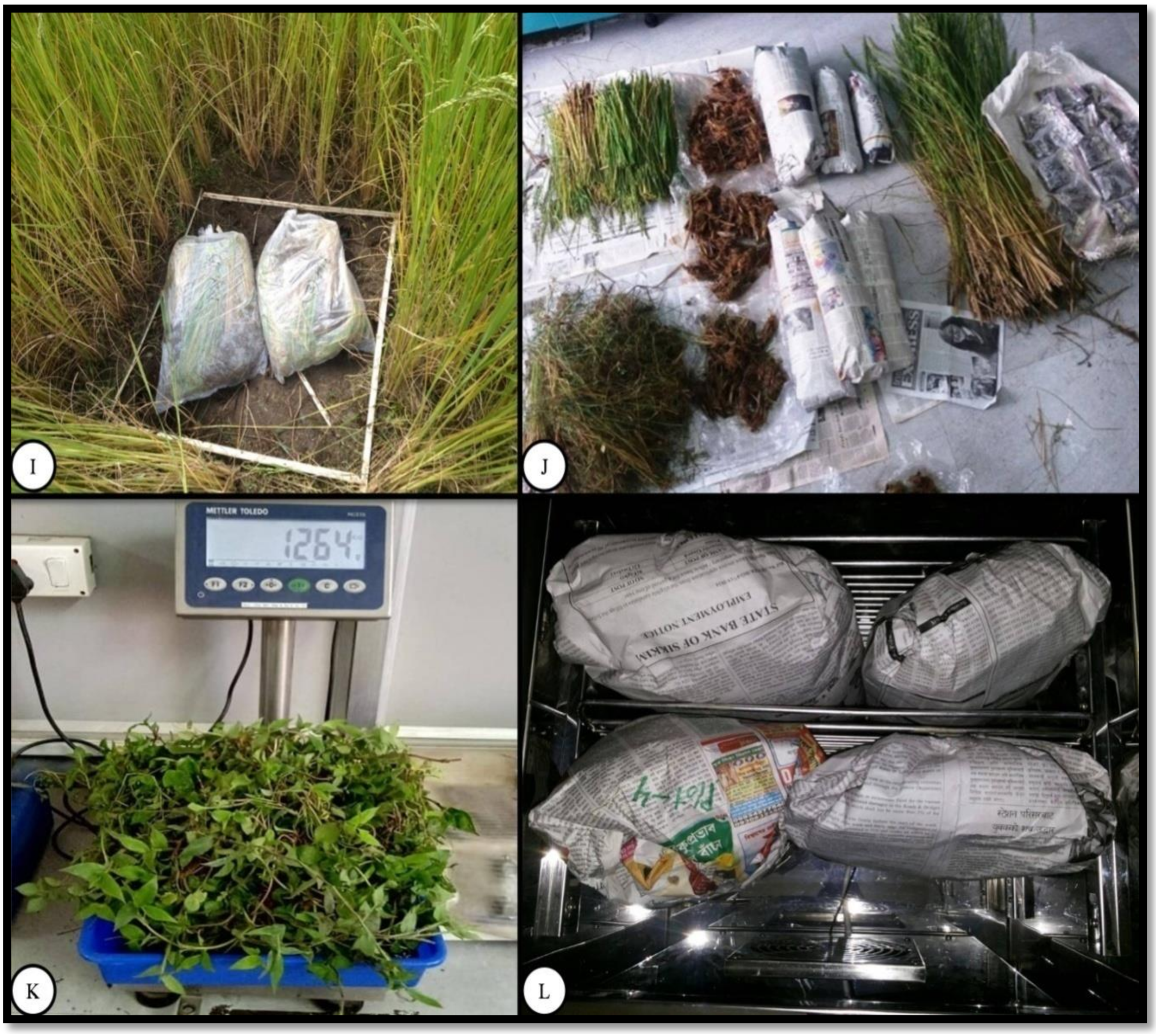


Plate 8(I,J,K,L): Herbaceous biomass estimation

Litter biomass carbon

Monthly litter from ten (10) permanent litter traps of 1m x 1m size was collected during the year 2016 and 2018 in the subtropical forest and cardamom agroforestry systems to quantify the carbon stock in litters. Litters from the traps were transported to the laboratory, washed and dried in an oven at 80^{0}C until weight became constant and weighed to determine the total value of biomass. The annual litter biomass of the study site was then estimated by summing up all the litter collected in different months of the year. The carbon in the litter was estimated using a carbon default value of 0.50 (IPCC 2006) from the litter biomass. In the cropland, no litter was estimated however the belowground parts of the rice left after harvesting of the crops were used as litter in the present study.

Plate 9 (M,N,O,P): Litter biomass estimation

Soil carbon stock and sequestration

A total of 540 soil samples from thirty different soil pits (10 each from each land-use system) were collected for the estimation of SOC stock and concentration using a soil corer (5.2 cm diameter) across three soil depths, i.e. 0-15 cm, 15-30 cm, and 30-45 cm. Samples were air dried, crushed and passed through a sieve (2mm) to separate coarse material and gravel and live roots were sorted out manually. Then the sieved soil samples were colorimetrically analyzed for organic carbon content (Anderson and Ingram 1993). Bulk density and SOC for each soil depth was estimated using the formula provided by Ravindranath and Ostwald (2008).

BD = Weight of known volume of soil / Volume of soil

SOC (Mg ha^{-1}) = bulk density (g cm^{-3}) x soil depth interval (cm) x SOC (%)

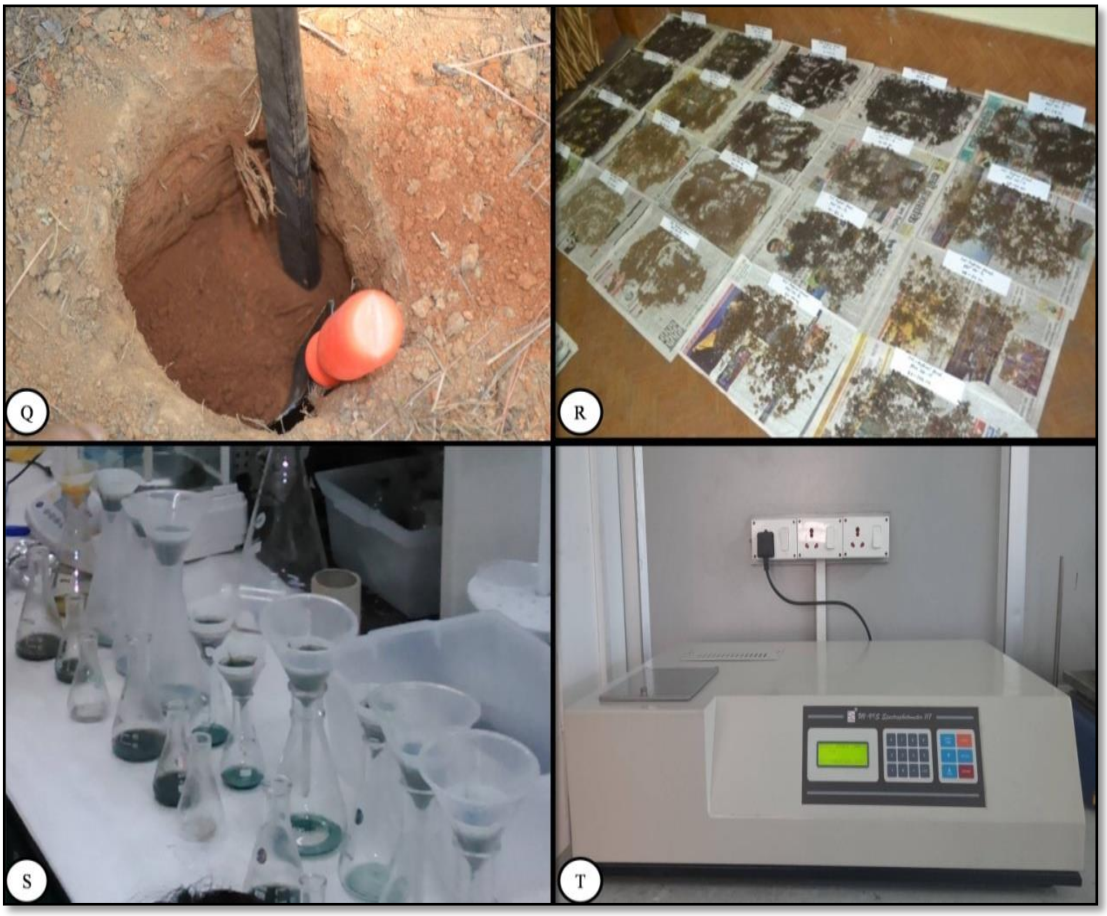


Plate 10(Q,R,S,T): Soil organic carbon estimation

RESULT

Tree Density, basal area and Important Value Index (IVI)

The total number of tree species in the subtropical forest was 10. *Alnus nepalensis* was the dominant species in the subtropical forest (IVI 128.55) and *Machilus edulis, Lyonia ovalifolia, Juglans regia, Schima wallichi* (IVI 51.78, 37.41, 20.32 and 15.15 respectively) were co-dominant tree species (Table-6). The total tree density in the subtropical forest was (188.28 individual/ha) (Table-6). *Alnus nepalensis* has the highest tree density (98.14 individual/ha) contributing more than 50% of the total tree density of the forest. The basal area of tree species in the present study site was 33.52 m^2 ha^{-1} with the highest contribution by Alnus *nepalnesis* (10.21 m^2 ha^{-1}) (Table-7).

Table 6. List of dominant tree species in the subtropical forest site.

Name of Species	**Density**	**Frequency**	**IVI**
Alnus nepalensis D. Don	98.14	100	128.55
Machilus edulis King ex Hook.f.	17.02	30	51.78
Schima wallichi Choisy	9.01	10	15.15
*Lyonia ovalifolia (*Wall.*)* Drude	25.03	50	37.41
Juglans regia L.	8.01	30	20.32
Symplocos theifolia D. Don	12.02	20	13.19
Spondias axillaris Roxb.	2.00	10	5.4
Castanopsis indica (Roxb. ex Lindl.) A.DC.	3.00	10	5.68
Macaranga pustulata King ex Hook.f.	9.01	20	10.96
Ficus racemosa Willd.	5.01	30	11.6
Total	**188.25**	**310**	**300.04**

Table 7. Density (individual ha^{-1}) and basal area (m^2 ha^{-1}) of trees in forest and agroforestry sites.

Major Land-use	Mean age (years)	Trees	
		Density (Individual/ha)	**Basal Area** (m^2 ha^{-1})
Subtropical Forest	40	188.28	33.52
Cardamom Agroforestry	25	124.90	18.28

In the cardamom agroforestry system, the total number of tree species was seven (7) and *Alnus nepalensis* (IVI-175.74) is the main shading tree. *Ficus racemosa* (IVI-46.60) and *Macaranga pustulata* (IVI-22.16) were other co-dominant shading trees (Table-8). Tree density in the present site was 124.90 individual/ha and *Alnus nepalensis* (80.13 individual/ha) had the maximum tree density (Table-8). The total basal area of the cardamom agroforestry site was 18.28 m^{2}ha^{-1} with the highest basal area contributed by *Alnus sp.* (11.54 m^{2}ha^{-1}) (Table-7).

Table 8. List of dominant tree species in the cardamom agroforestry site.

Name of Species	Density	Frequency	IVI
Alnus nepalensis D. Don	80.13	100	175.74
Ficus racemosa Willd.	26.04	60	46.60
Juglans regia L.	4.01	20	16.27
Toona ciliata M.Roem.	4.01	40	19.27
Macaranga pustulata King ex Hook.f.	6.01	40	22.61
Spondias axillaris Roxb.	2.00	20	10.50
Viburnum cordifolium Wall. Ex DC.	2.00	20	8.95
Total	**124.20**	**300**	**300.04**

Simpson's dominance, Shannon-Weiner diversity

Different indices i.e. Simpson's dominance, Shannon-Weiner diversity, Pielou's evenness index and Margalef's species indices of the tree species in the subtropical forest and cardamom agroforestry were presented in (Table-9). The subtropical forest has a higher value of all the indices as compared to the cardamom agroforestry system.

Table 9. Dominance, Diversity and Evenness of trees across two study sites.

Land-uses	Tree			
	Simpson's dominance index	Shannon - Weiner diversity index	Pielou's evenness index	Margalef's species richness index
Subtropical Forest	0.677	1.653	0.689	1.909
Cardamom Agroforestry	0.562	1.241	0.596	1.696

Tree above-ground and below-ground biomass

Subtropical forest

Above-ground biomass of trees in the present subtropical forest was 123.41 Mg ha^{-1}in the first year and 128.42 Mg ha^{-1} in the third year respectively (Table-10). *Alnus nepalensis* recorded the highest aboveground biomass in the first year (54.45 Mg ha^{-1}) and 55.87 Mg ha^{-1} in 3rd year contributing 44.12% and 43.51% respectively to the total aboveground biomass. The lowest aboveground biomass value was contributed by *Ficus racemosa* (0.32 Mg ha^{-1} and 0.44 Mg ha^{-1}) in the 1st and 3rd year respectively.

Belowground biomass (BGB) value in the present study was 32.09 Mg ha^{-1} and 33.39 Mg ha^{-1} in the first and third years respectively. The highest BGB was recorded for *Alnus nepalensis* (14.16 Mg ha^{-1}in 1st year and 14.53 Mg ha^{-1}in 3rd year) and the lowest for *Ficus racemosa* (0.08 Mg ha^{-1}, 0.11 Mg ha^{-1}) in both the years. Total biomass (tree aboveground + belowground) in the subtropical forest was 155.50 Mg ha^{-1}in the first year and 161.81 Mg ha^{-1} in the third year respectively (Table 10).

Table 10. Above-ground, Below-ground biomass (Mg ha^{-1}) and Total Biomass (Mg ha^{-1}) in different species of subtropical forest (2016 and 2018).

Sl No	Species	2016			2018		
		AGB (Mg ha^{-1})	**BGB** (Mg ha^{-1})	**Total** (Mg ha^{-1})	**AGB** (Mg ha^{-1})	**BGB** (Mg ha^{-1})	**Total** (Mg ha^{-1})
1	*Alnus nepalensis* D. Don	54.45	14.16	68.61	55.87	14.53	70.40
2	*Machilus edulis* King ex Hook.f.	37.89	9.85	47.74	38.65	10.05	48.70
3	*Schima wallichi* Choisy	11.85	3.08	14.93	12.42	3.23	15.65
4	*Lyonia ovalifolia* (Wall.) Drude	8.18	2.13	10.31	8.87	2.31	11.18
5	*Juglans regia* L.	6.41	1.67	8.08	6.86	1.78	8.64
6	*Symplocos theifolia* D. Don	1.08	0.28	1.36	1.38	0.36	1.74
7	*Spondias axillaris* Roxb.	1.36	0.35	1.71	1.66	0.43	2.09
8	*Castanopsis indica* (Roxb. ex Lindl.)A.DC.	1.00	0.26	1.26	1.26	0.33	1.59
9	*Macaranga pustulata* King ex Hook.f.	0.87	0.23	1.10	1.01	0.26	1.27
10	*Ficus racemosa* Willd.	0.32	0.08	0.40	0.44	0.11	0.55
	Total	**123.41**	**32.09**	**155.50**	**128.42**	**33.39**	**161.81**

Cardamom agroforestry

The total aboveground biomass for trees was 65.01 Mg ha^{-1} and 69.04 Mg ha^{-1} in the first and third year respectively (Table-11). Among the tree species, the highest AGB was observed for *Alnus nepalensis* with a value of 45.61 Mg ha^{-1} in 1st year and 47.84 Mg ha^{-1} in 3rd year contributing 70.67% and 69.86% respectively to the total aboveground biomass. The lowest AGB was recorded for *Viburnum cordifolium* contributing 0.45% (0.28 Mg ha^{-1}) in 1st year and 0.60% (0.41 Mg ha^{-1}) in the 3rd year to the total aboveground biomass.

Belowground biomass of trees was 16.90 Mg ha^{-1} and 17.96 Mg ha^{-1} in the 1st and 3rd year respectively. The highest BGB was recorded for *Alnus nepalensis* with a value of 11.86 Mg ha^{-1} in 1st year and 12.44 Mg ha^{-1} in 3rd year. *Viburnum cordifolium* has the lowest BGB with a value of 0.07 Mg ha^{-1} in 1st year and 0.11 Mg ha^{-1} in 3rd year. Therefore the total tree biomass (above and belowground) of the cardamom agroforestry system was 81.91 Mg ha^{-1} in 1st year and 86.99 Mg ha^{-1} in 3rd year respectively (Table-11).

Table 11. Above-ground, Below-ground biomass (Mg ha^{-1}) and Total Biomass (Mg ha^{-1}) in different species of Cardamom Agroforestry(2016 and 2018).

Sl No	**Species**	**2016**			**2018**		
		AGB (Mg ha^{-1})	**BGB** (Mg ha^{-1})	**Total** (Mg ha^{-1})	**AGB** (Mg ha^{-1})	**BGB** (Mg ha^{-1})	**Total** (Mg ha^{-1})
1	*Alnus nepalensis* D. Don	45.61	11.86	57.47	47.84	12.44	60.28
2	*Ficus racemosa* Willd.	8.15	2.12	10.27	8.66	2.25	10.91
3	*Juglans regia* L.	4.01	1.04	5.05	4.42	1.15	5.57
4	*Toona ciliata* M.Roem.	3.46	0.90	4.36	3.77	0.98	4.75
5	*Macaranga pustulata* King ex Hook.f.	3.19	0.83	4.02	3.44	0.89	4.33
6	*Spondias axillaris* Roxb.	0.31	0.08	0.39	0.50	0.13	0.63
7	*Viburnum cordifolium* Wall. Ex DC.	0.28	0.07	0.35	0.41	0.11	0.52
	Total	**65.01**	**16.90**	**81.91**	**69.04**	**17.96**	**86.99**

Tree carbon stock

Subtropical forest

The total tree carbon stock in the subtropical forest site was 73.08 Mg C ha^{-1} and 76.05 Mg C ha^{-1} in the 1st and 3rd year respectively (Table-12). The above-ground biomass carbon stock was 58.00 Mg C ha^{-1} in 1st year and 60.36 Mg C ha^{-1} in 3rd year in this forest while the carbon stock in the belowground biomass was recorded to be 15.08 Mg C ha^{-1} and 15.69 Mg C ha^{-1} in the 1st and 3rd year respectively. Carbon density showed a positive and significant relation (Fig 15) with tree density ($p<0.10$), basal area ($p<0.05$), and aboveground biomass ($p<0.001$).

Table 12: Above-ground, Below-ground biomass carbon (Mg C ha^{-1}) and Total biomass carbon (Mg C ha^{-1}) in different species of subtropical forest (2016 and 2018).

Sl No	Species	**2016**			**2018**		
		ABGC (Mg C ha^{-1})	**BGBC** (Mg C ha^{-1})	**Total** (Mg C ha^{-1})	**ABGC** (Mg C ha^{-1})	**BGB** (Mg C ha^{-1})	**Total** (Mg C ha^{-1})
1	*Alnus nepalensis* D. Don	25.59	6.65	32.25	26.26	6.83	33.09
2	*Machilus edulis* King ex Hook.f.	17.81	4.63	22.44	18.17	4.72	22.89
3	*Schima wallichi* Choisy	5.57	1.45	7.02	5.84	1.52	7.36
4	*Lyonia ovalifolia* (Wall.) Drude	3.84	1.00	4.84	4.17	1.08	5.25
5	*Juglans regia* L.	3.01	0.78	3.80	3.22	0.84	4.06
6	*Symplocos theifolia D.* Don	0.51	0.13	0.64	0.65	0.17	0.82
7	*Spondias axillaris* Roxb.	0.64	0.17	0.81	0.78	0.20	0.98
8	*Castanopsis indica* (Roxb. ex Lindl.)A.DC.	0.47	0.12	0.59	0.59	0.15	0.75
9	*Macaranga pustulata* King ex Hook.f.	0.41	0.11	0.52	0.47	0.12	0.60
10	*Ficus racemosa* Willd.	0.15	0.04	0.19	0.21	0.05	0.26
Total		**58.00**	**15.08**	**73.08**	**60.36**	**15.69**	**76.05**

Cardamom agroforestry

In the agroforestry study site, the total carbon stock was recorded to be 38.50 Mg C ha^{-1} and 40.89 Mg C ha^{-1} in the 1st and 3rd year respectively (Table-13). The aboveground biomass carbon stock was 30.55 Mg C ha^{-1} in 1st year and 32.45 Mg C ha^{-1} in 3rd year. Below-ground biomass carbon stock was 7.94 Mg C ha^{-1} and 8.44 Mg C ha^{-1} in the first and third year respectively. Carbon density exhibited a positive and significant relation (Fig 15) with tree density ($p<0.10$), basal area ($p<0.05$), and aboveground biomass ($p<0.001$) in this system too.

Table 13. Above-ground, Below-ground biomass (Mg ha^{-1}) and Total Biomass (Mg ha^{-1}) in different species of cardamom agroforestry (2016 and 2018).

Sl No	Species	2016			2018		
		AGBC (Mg C ha^{-1})	**BGBC** (Mg C ha^{-1})	**Total** (Mg C ha^{-1})	**AGBC** (Mg C ha^{-1})	**BGBC** (Mg C ha^{-1})	**Total** (Mg C ha^{-1})
1	*Alnus nepalensis* D. Don	21.44	5.57	27.01	22.48	5.85	28.33
2	*Ficus racemosa* Willd.	3.83	1.00	4.83	4.07	1.06	5.13
3	*Juglans regia* L.	1.88	0.49	2.37	2.08	0.54	2.62
4	*Toona ciliata* M.Roem.	1.63	0.42	2.05	1.77	0.46	2.23
5	*Macaranga pustulata* King ex Hook.f.	1.50	0.39	1.89	1.62	0.42	2.04
6	*Spondias axillaris* Roxb.	0.15	0.04	0.18	0.24	0.06	0.30
7	*Viburnum cordifolium* Wall. Ex DC.	0.13	0.03	0.16	0.19	0.05	0.24
Total		**30.55**	**7.94**	**38.50**	**32.45**	**8.44**	**40.89**

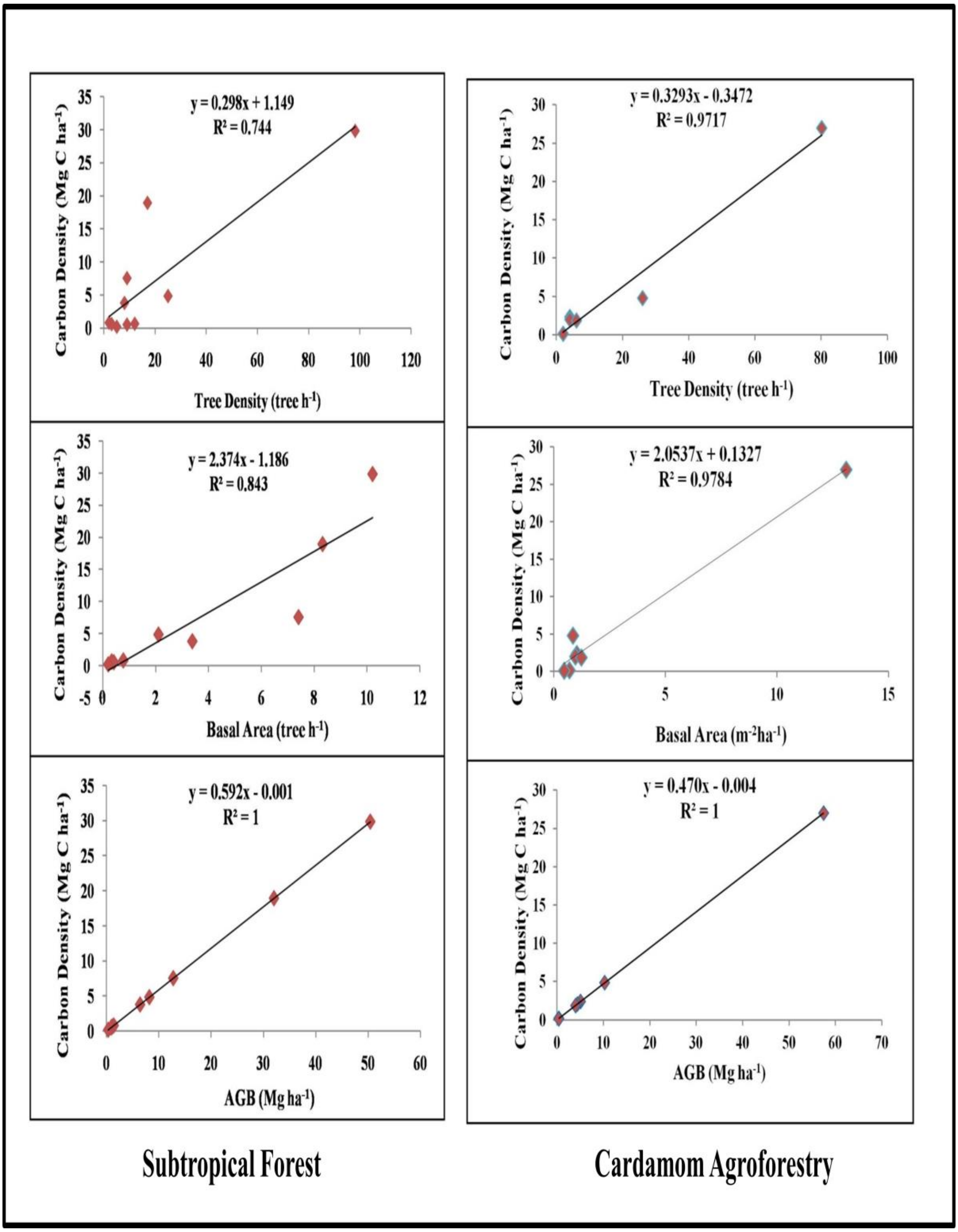


Fig 15. Regression models between carbon density and tree density, basal area, and biomass stock in STF and AGF system

Herbaceous biomass and carbon stock

The herbaceous biomass in the 1st and 3rd year of the present study sites was 8.44±0.76 Mg ha^{-1}; 8.74±0.49 Mg ha^{-1} (forest), 4.69±0.50 Mg ha^{-1}; 5.49±0.48 Mg ha^{-1} (agroforestry) and 10.66±0.63Mg ha^{-1}; 10.86±0.72 (cropland) respectively. The herbaceous carbon stock in the two years was observed to be highest in cropland (5.33±0.31 Mg C ha^{-1}; 5.43±0.36 Mg C ha^{-1}) followed by forest (4.22±0.38 Mg C ha^{-1}; 4.37±0.24 Mg C ha^{-1}) and lowest in the subtropical forest (2.34±0.25 Mg C ha^{-1}; 2.70±0.27 Mg C ha^{-1}) (Table-14). Herbaceous carbon stock value of forest system contributes 5.46% of carbon in the 1st year and 5.88% in the 3rd year to the total vegetation carbon stock. Similarly in cardamom agroforestry, herbaceous carbon stock contributes 5.24% and 5.62% of carbon to the total vegetation carbon of the site in the 1st and 3rd year respectively. Herbaceous biomass and carbon stock were highest in the paddy cropland followed by subtropical forest and lowest in the cardamom agroforestry.

Table 14. Herbaceous biomass (Mg ha^{-1}) and carbon (Mg C ha^{-1}) in three different land-use types (2016 and 2018).

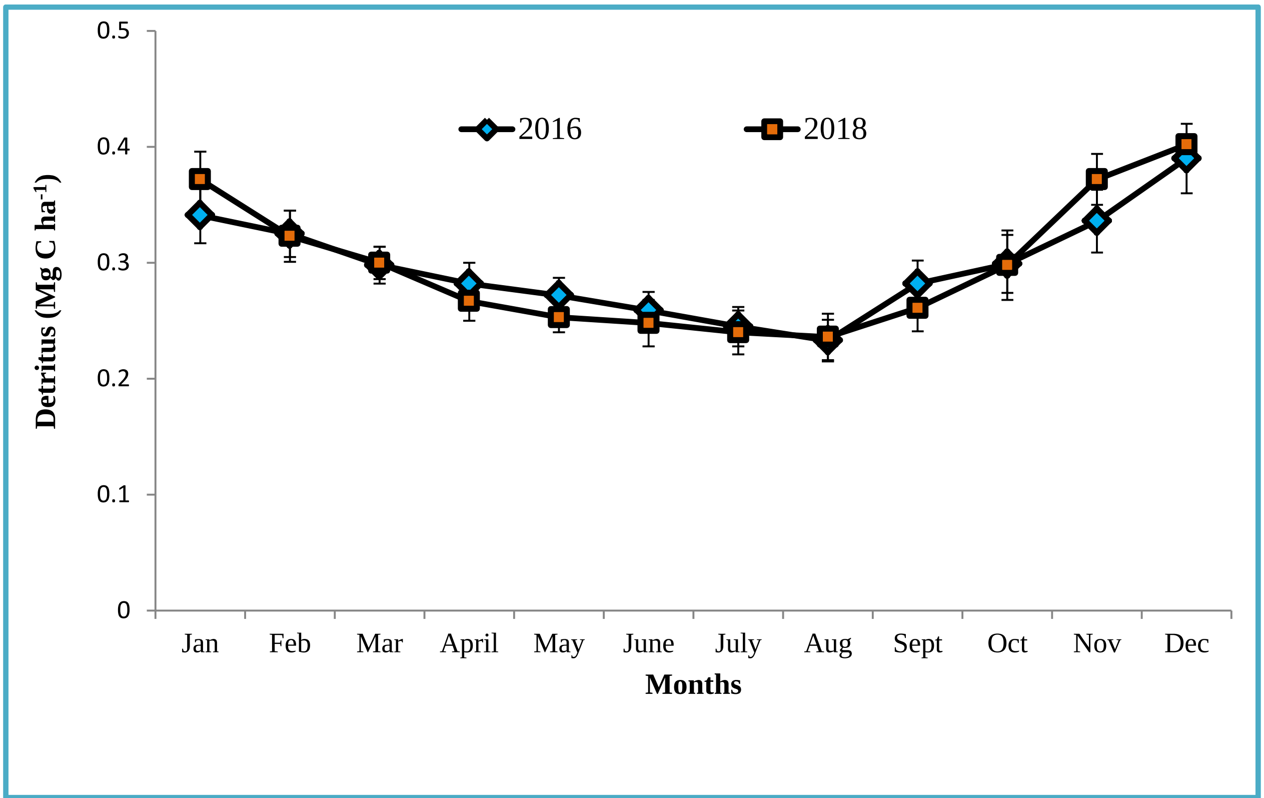
Land-use	2016		2018	
	Herbaceous Biomass (Mg ha^{-1})	**Herbaceous Carbon** (Mg C ha^{-1})	**Herbaceous Biomass** (Mg ha^{-1})	**Herbaceous Carbon** (Mg C ha^{-1})
Subtropical Forest	8.44±0.76	4.22±0.38	8.74±0.49	4.37±0.24
Agroforestry Cardamom	4.69±0.50	2.34±0.25	5.49±0.48	2.70±0.27
Wet Paddy Cropland	10.66±0.63	5.33±0.31	10.86±0.72	5.43±0.36

Litter biomass and carbon stock

Annual litter biomass and carbon of subtropical forest were recorded to be 7.12±0.53 Mg ha^{-1}and 3.56±0.27 Mg C ha^{-1} respectively in 1st year, and 7.16±0.38 Mg ha^{-1} and 3.58±0.20 Mg ha^{-1} in 3rd year (Table-15). Similarly, the total annual detritus biomass and carbon of cardamom agroforestry were 6.02±0.36 Mg ha^{-1}and 3.56±0.27 Mg C ha^{-1} in 1st year and 6.18±0.43 Mg ha^{-1} and 3.09±0.21 Mg C ha^{-1} in the 3rd year. Litterfall contributed 1.84 % and 1.95% to the total ecosystem carbon in subtropical forest and agroforestry study sites respectively. Litter carbon in the subtropical forest was slightly higher than agroforestry with a peak litterfall during the winter season (Nov-Feb) and least during the rainy season (June-Oct) in all the study sites. The monthly litterfall ranged from 0.23-0.40 Mg C ha^{-1} in the subtropical forest and was highest in December (0.40 Mg C ha^{-1}) and lowest in August (0.23 Mg C ha^{-1}) (Fig 16). In the agroforestry system, the litterfall ranged from 0.20-0.34Mg C ha^{-1} with the maximum in December (0.34 Mg C ha^{-1}) and minimum in June (0.20 Mg C ha^{-1}) (Fig 16).

Table 15: Litter biomass (Mg ha^{-1}) and litter biomass carbon (Mg C ha^{-1}) in three different land-use types (2016 and 2018).

Land-use	2016		2018	
	Litterfall Biomass (Mg ha^{-1})	**Litterfall Carbon** (Mg C ha^{-1})	**Litterfall Biomass** (Mg ha^{-1})	**Litterfall Carbon** (Mg C ha^{-1})
Subtropical Forest	7.12±0.53	3.56±0.27	7.16±0.38	3.58±0.20
Agroforestry Cardamom	6.02±0.36	3.01±0.18	6.18±0.43	3.09±0.21



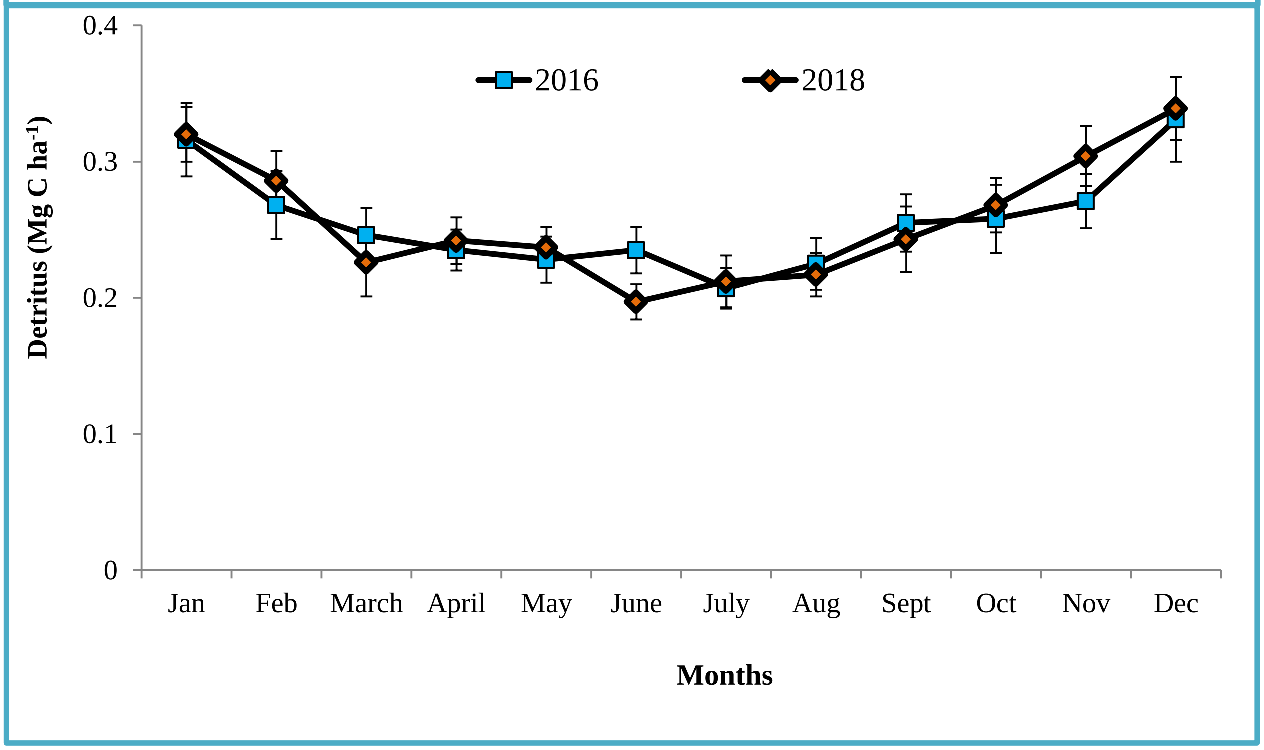


Fig 16. Monthly litterfall carbon in the subtropical forest and cardamom agroforestry systems during the study period (mean±SE)

Soil organic carbon stock (SOC)

The soil organic carbon stock in 0-45cm in the three different land-use systems varied from 87.41±3.22 to120.93±4.15 Mg C ha^{-1} in 1^{st} year and 87.52±4.27 to 122.40±5.37 Mg C ha^{-1} in the 3^{rd} year (Table-16). SOC stock and concentrations in different soil layers were highest in the subtropical forest in both the sampling years (110.15±10.72 and 112.76±10.06 Mg C ha^{-1}) followed by cardamom agroforestry (105.24±9.92 and 107.43±9.21 Mg C ha^{-1}) and paddy cropland (92.01±10.23 and 92.74±11.53 Mg C ha^{-1}). SOC decreases with an increase in soil depth, and maximum soil carbon was recorded in the rainy season and minimum in the winter season in all the study sites (Fig 17).

The Analysis of variance (ANOVA) in subtropical forest indicated a significant difference in SOC stock between different sampling months of summer ($P<0.01$), rainy ($P<0.001$), winter ($P<0.05$) and annually ($P<0.05$) (Table-17). Similarly, in cardamom agroforestry study sites, the analysis of variance (ANOVA) shows a significant difference between sampling months of summer ($P<0.05$), Rainy ($P<0.05$), winter ($P<0.05$) and annually ($P<0.001$) (Table-18). The Analysis of variance (ANOVA) in paddy cropland indicated a significant difference in SOC stock between different sampling months of summer ($P<0.05$), Rainy ($P<0.05$), winter ($P<0.001$) and annually ($P<0.001$) respectively (Table-19). The Analysis of variance (ANOVA) in different land-use shows a significant difference in SOC stock between and within different land-use systems ($P<0.05$) (Table-20).

Table 16.Soil organic carbon (SOC) concentration and soil organic carbon stock at various soil depths in different land-use systems (2016 and 2018).

Land-use types	SOC (%) (2016)				SOC stock (Mg C ha^{-1}) (2016)			
	0-15	*15-30*	*30-45*	*Total*	*0-15*	*15-30*	*30-45*	*Total*
Subtropical Forest	4.04±0.56	3.45±0.45	2.21±0.35	9.70±1.35	40.99±4.01	38.17±3.78	31.00±2.93	110.15±10.72
Cardamom Agroforestry	3.85±0.42	3.32±0.35	2.04±0.40	9.21±1.17	40.14±3.41	35.89±3.63	29.21±2.88	105.24±9.92
Paddy Cropland	3.23±0.59	3.20±0.32	1.88±0.47	8.31±1.38	34.16±4.02	31.64±3.22	26.21±2.98	92.01±10.23
	SOC (%) (2018)				SOC stock (Mg C ha^{-1}) (2018)			
Subtropical Forest	3.96±0.43	3.42±0.56	2.51±0.41	9.89±1.40	42.03±3.51	36.76±3.32	33.97±3.23	112.76±10.06
Cardamom Agroforestry	3.63±0.46	3.45±0.38	2.31±0.31	9.42±1.13	38.72±3.42	36.21±3.01	32.50±2.78	107.43±9.21
Paddy Cropland	3.36±0.55	3.11±0.51	1.96±0.24	8.43±1.30	35.22±4.22	30.41±4.03	27.11±3.28	92.74±11.53

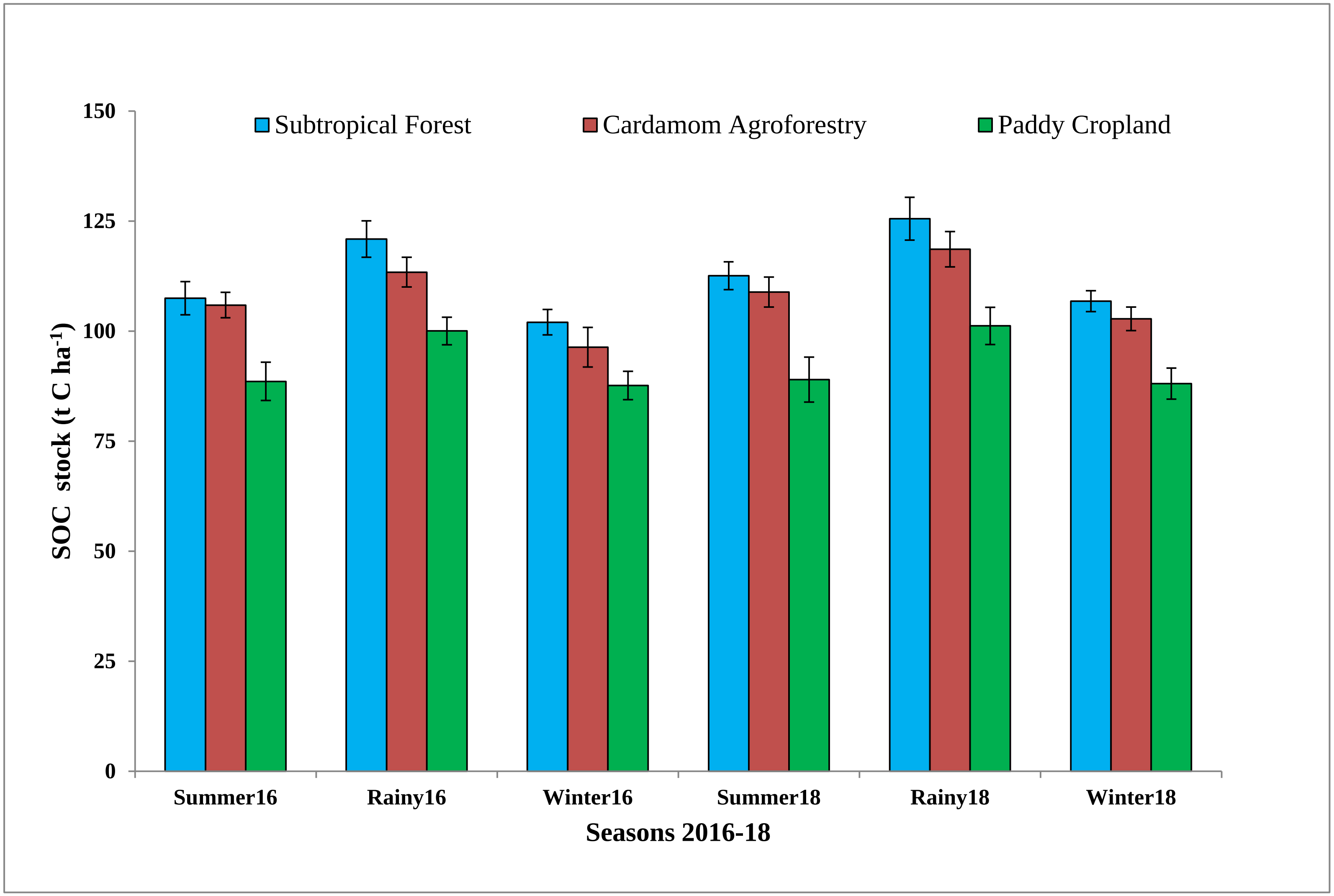


Fig 17. Seasonal SOC stock in the three land-use systems during the study period (mean±SE)

Table 17. ANOVA (One-way) of SOC in a subtropical forest.

Seasons	**Source of Variation**	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Summer	Between Months	157.823	2	78.91151	10.9123	0.003927**	4.25649
	Within Month	65.0832	9	7.231466			
Rainy	Between Months	885.046	4	221.2614	9.05804	0.000629***	3.0555
	Within Month	366.406	15	24.42708			
Winter	Between Months	127.867	3	42.62239	5.91158	0.010239*	3.49029
	Within Month	86.5197	12	7.209978			
Annual	Between Months	127.867	3	42.62239	5.91158	0.010239*	3.49023
	Within Month	86.5197	12	7.209978			

**Significant at P-value< 0.05, **Significant at P-value< 0.01, ***Significant at P-value< 0.001*

Table 18 ANOVA (One-way) of SOC stock in a cardamom agroforestry.

Seasons	Source of Variation	SS	df	MS	F	P-value	F crit
Summer	**Between Months**	211.2058	2	105.6029	5.753374	**0.024579***	4.256495
	Within Month	165.1945	9	18.35495			
Rainy	**Between Months**	1487.276	4	371.8189	3.305527	**0.039428***	3.055568
	Within Month	1687.26	15	112.484			
Winter	**Between Months**	270.2677	3	90.08922	4.306124	**0.027996***	3.490295
	Within Month	251.0542	12	20.92119			
Annual	**Between Months**	6407.155	11	582.4686	3.869635	**0.000992*****	2.066608
	Within Month	*5418.824*	*36*	*150.5229*			

**Significant at P-value< 0.05, **Significant at P-value< 0.01, ***Significant at P-value< 0.001*

Table 19 ANOVA (One-way) of SOC stock in a wet paddy cropland.

Seasons	Source of Variation	SS	df	MS	F	P-value	F crit
Summer	**Between Months**	108.2113	2	54.10563	4.649852	**0.041029***	4.256495
	Within Month	104.7239	9	11.63599			
Rainy	**Between Months**	357.7441	4	89.43601	3.456239	**0.034269***	3.055568
	Within Month	388.1503	15	25.87669			
Winter	**Between Months**	562.7523	3	187.5841	11.81305	**0.00068*****	3.490295
	Within Month	190.5527	12	15.87939			
Annual	**Between Months**	3097.608	11	281.6007	9.557653	**9.65E-08*****	2.066608
	Within Month	1060.681	36	29.46337			

**Significant at P-value< 0.05, ***Significant at P-value< 0.001*

Table 20. Analysis of variance (ANOVA) between soil carbon stock of different land-use types.

ANOVA Source of Variation	Sum of Squares	df	Mean Square	F	Sig (P)
Within Land-use	1145.431	2	572.7156	115.9641	P<0.05
Between Land-use	743.7419	5	148.7484	30.11875	
Total	1938.56	17			

Total ecosystems carbon pool

Total ecosystem biomass carbon in the present subtropical forest was 191.01 Mg C ha^{-1} in 1st year and 196.76 Mg C ha^{-1} in the 3rd year (Table-21). Soil carbon has the highest carbon stock and contributed 57.67% (1st year) and 57.31% (3rd year) of the total ecosystem carbon while the contribution of litter is least with 1.86% and 1.82% in the 1st and 3rd year respectively (Fig 18).

In the agroforestry system, the total ecosystem carbon value was 153.56 Mg C ha^{-1} and 158.62 Mg C ha^{-1} in the 1st and 3rd year respectively (Table-21). Among the different carbon pools, soil carbon contributed maximum carbon 68.53%, and 67.73% to the total ecosystem carbon in the first and third year respectively.

The total ecosystem carbon in the paddy cropland during the first and third year i.e. 2016 and 2018 was 97.34 Mg C ha^{-1} and 98.17Mg C ha^{-1} respectively (Table-21). As in the other two ecosystems, the highest contribution to total ecosystem carbon was by soil 94.52% in 1st year and 94.47% in 3rd year, while the contribution of the vegetation to the total ecosystem carbon is least in this system 5.48% and 5.53% in first and third year respectively (Fig 18).

Table-21. Vegetation Biomass Carbon (VBC), Soil Organic Carbon (SOC) and Total Ecosystem Carbon (EC) stock expressed in Mg C ha^{-1} of study sites during study periods (2016 and 2018).

Major land-uses	**VBC**				**SOC Stock** Mg C ha^{-1}	**EC stock** Mg C ha^{-1}
	AGBC Mg C ha^{-1}	BGBC Mg C ha^{-1}	HERBS Mg C ha^{-1}	LITTER Mg C ha^{-1}		
2016						
Subtropical Forest	58.00±3.38	15.08±0.87	4.22±0.38	3.56±0.27	110.15±10.72	191.01±15.62
Cardamom Agroforestry	30.55±2.12	7.94±0.55	2.34±0.25	3.01±0.18	105.24±9.92	149.08±13.02
Paddy Cropland	0	0	5.33±0.31	0	92.01±10.23	97.34±10.54
2018						
Subtropical Forest	60.36±3.63	15.69±0.94	4.37±0.24	3.58±0.20	112.76±10.06	196.76±15.07
Cardamom Agroforestry	32.45±3.02	8.44±0.78	2.70±0.27	3.09±0.21	107.43±9.21	154.11±13.49
Paddy Cropland	0	0	5.43±0.36	0	92.74±11.53	98.17±11.89

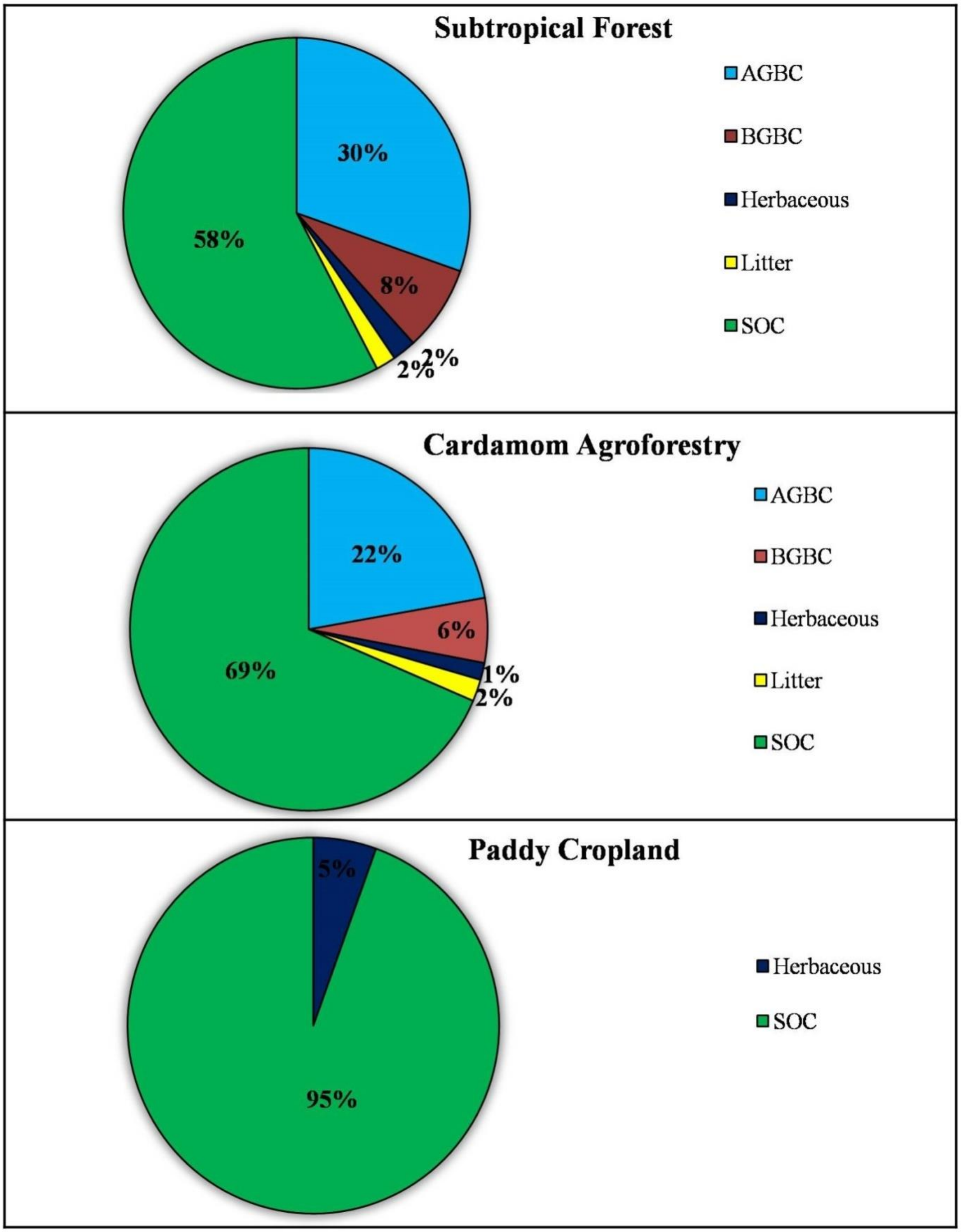


Fig 18. Different carbon pools in three different land-use systems

Carbon Sequestration

Carbon sequestration of the subtropical forest was 5.75 Mg C ha^{-1} yr^{-1} with the highest sequestration by SOC (2.61 Mg C ha^{-1} yr^{-1}) followed by AGB (2.36 Mg C ha^{-1} yr^{-1}), BGB (0.61 Mg C ha^{-1} yr^{-1}), herbaceous plants (0.15 Mg C ha^{-1} yr^{-1}) and lowest by the detritus (0.02 Mg C ha^{-1} yr^{-1}) (Table-22). Percent contribution of different carbon pools to the total ecosystem carbon sequestration in the forest was SOC- 57.67%, AGB- 30.36%, BGB- 7.89%, herbs - 2.21% and litterfall -1.86%.

Similarly in the cardamom agroforestry, the total carbon sequestration was 5.03 Mg C ha^{-1} yr^{-1} of which SOC sequester 2.19 Mg C ha^{-1} yr^{-1} followed by AGB 1.90 Mg C ha^{-1} yr^{-1}, BGB 0.50 Mg C ha^{-1} yr^{-1}, herbs 0.36 Mg C ha^{-1} yr^{-1} and least by the litterfall 0.08 Mg C ha^{-1} yr^{-1} (Table-22). SOC contributed 68.53%, AGB-22.21%, BGB-5.78%, detritus-1.96% and herbs-1.52% respectively to the total ecosystem carbon sequestration of the site.

Paddy cropland, sequester 0.83 Mg C ha^{-1} yr^{-1} with the highest contribution by SOC (0.73 Mg C ha^{-1} yr^{-1}) contributing 94.52% to the total ecosystem carbon and lowest by the herbs or vegetation (0.10 Mg C ha^{-1} yr^{-1}) (Table-22) contributing only 5.48% to the total ecosystem carbon sequestration.

Table 22. Carbon sequestration in different components of three land-use systems.

Different components	Carbon Sequestration		
	Subtropical Forest	Cardamom Agroforestry	Paddy Cropland
Aboveground Biomass	2.36	1.90	0.00
Belowground Biomass	0.61	0.50	0.00
Herbs	0.15	0.36	0.10
Litter	0.02	0.08	0.00
Total vegetation Carbon	**3.14**	**2.84**	**0.10**
Soil Organic Carbon	**2.61**	**2.19**	**0.73**
Total ecosystem carbon	**5.75**	**5.03**	**0.83**

Biomass and Carbon Density Map

Biomass and carbon density maps of the year 2016 and 2018 (Fig 19 and 20) revealed that there is a net increase in carbon stock and sequestration in forest and agroforestry systems except for the agricultural systems where there is a decrease in carbon stock and sequestration.

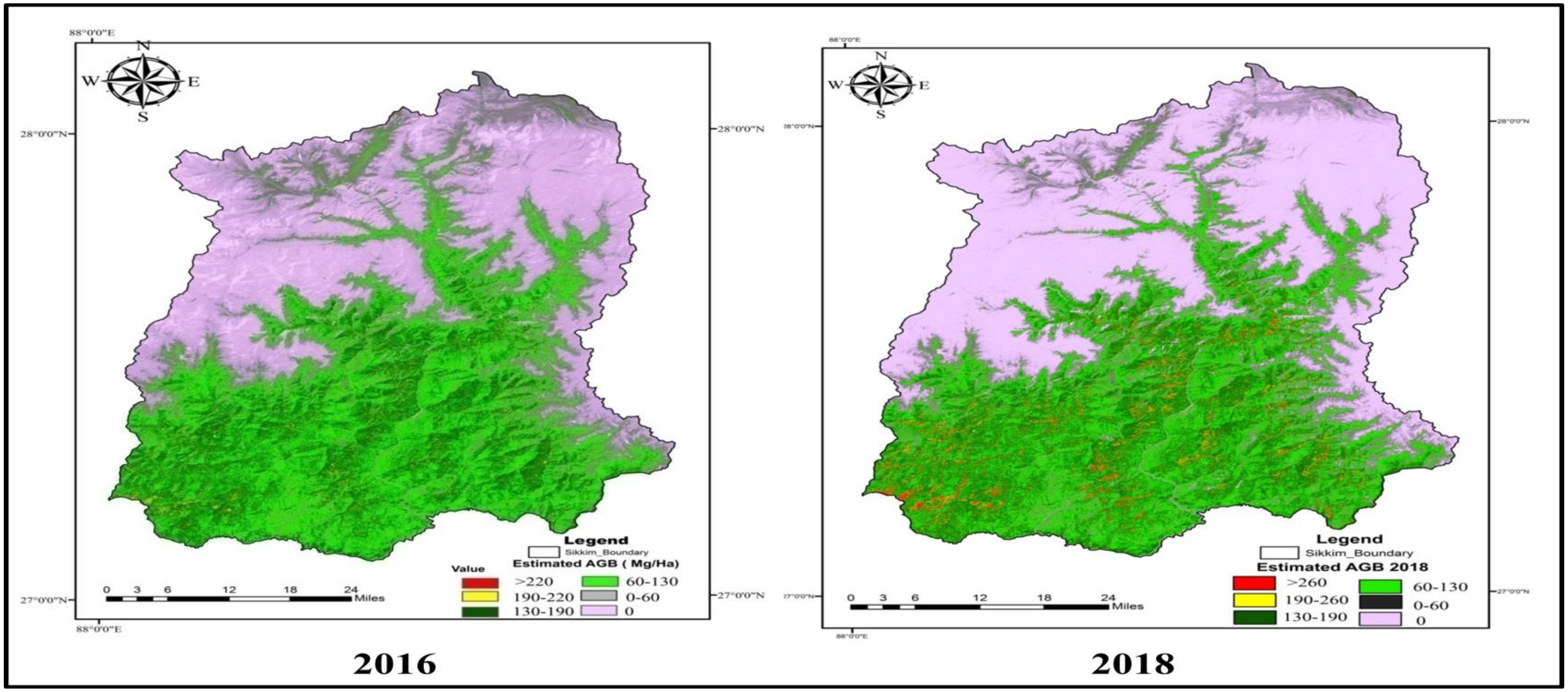


Fig 19. Biomass Map of Sikkim in 2016 and 2018

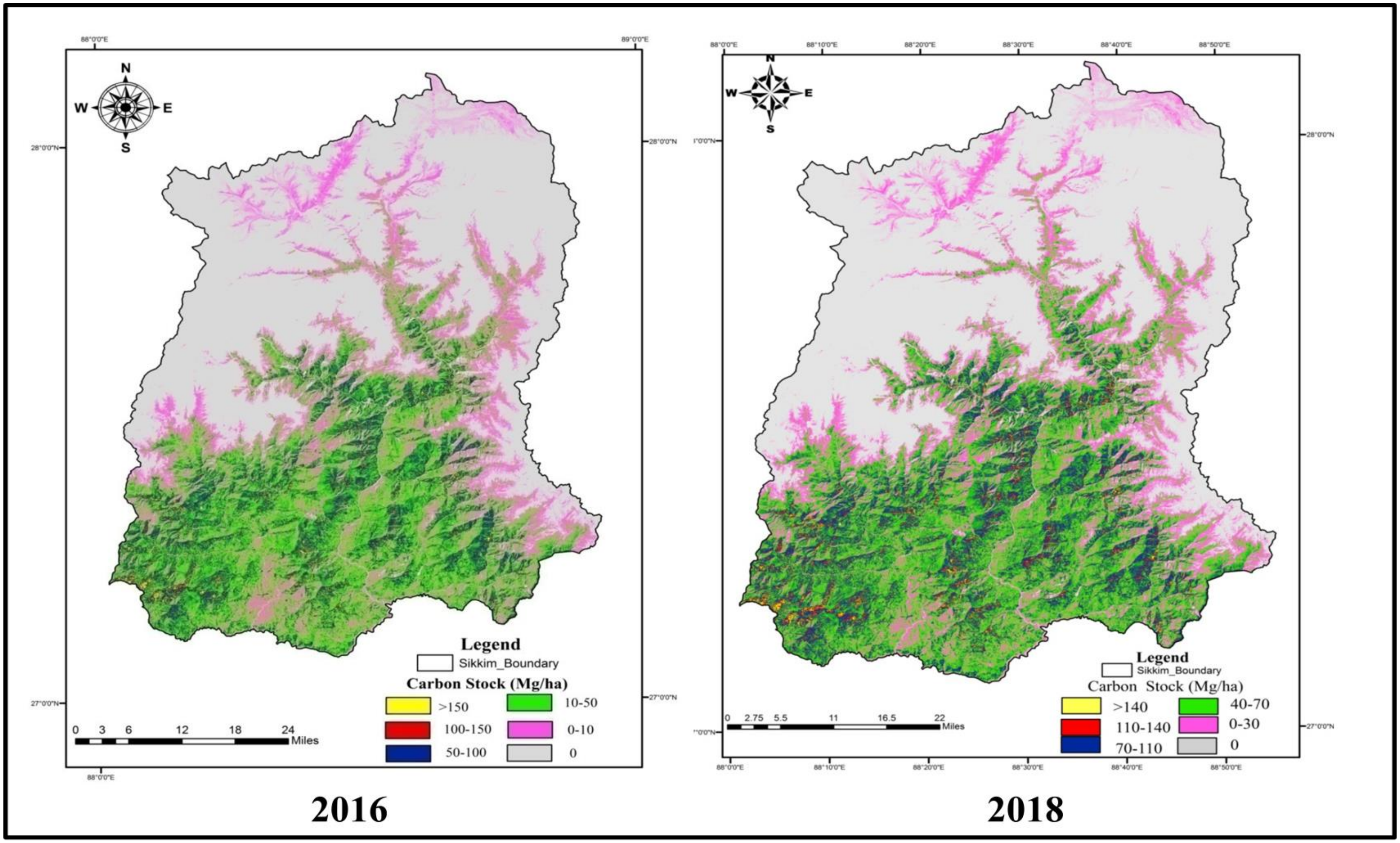
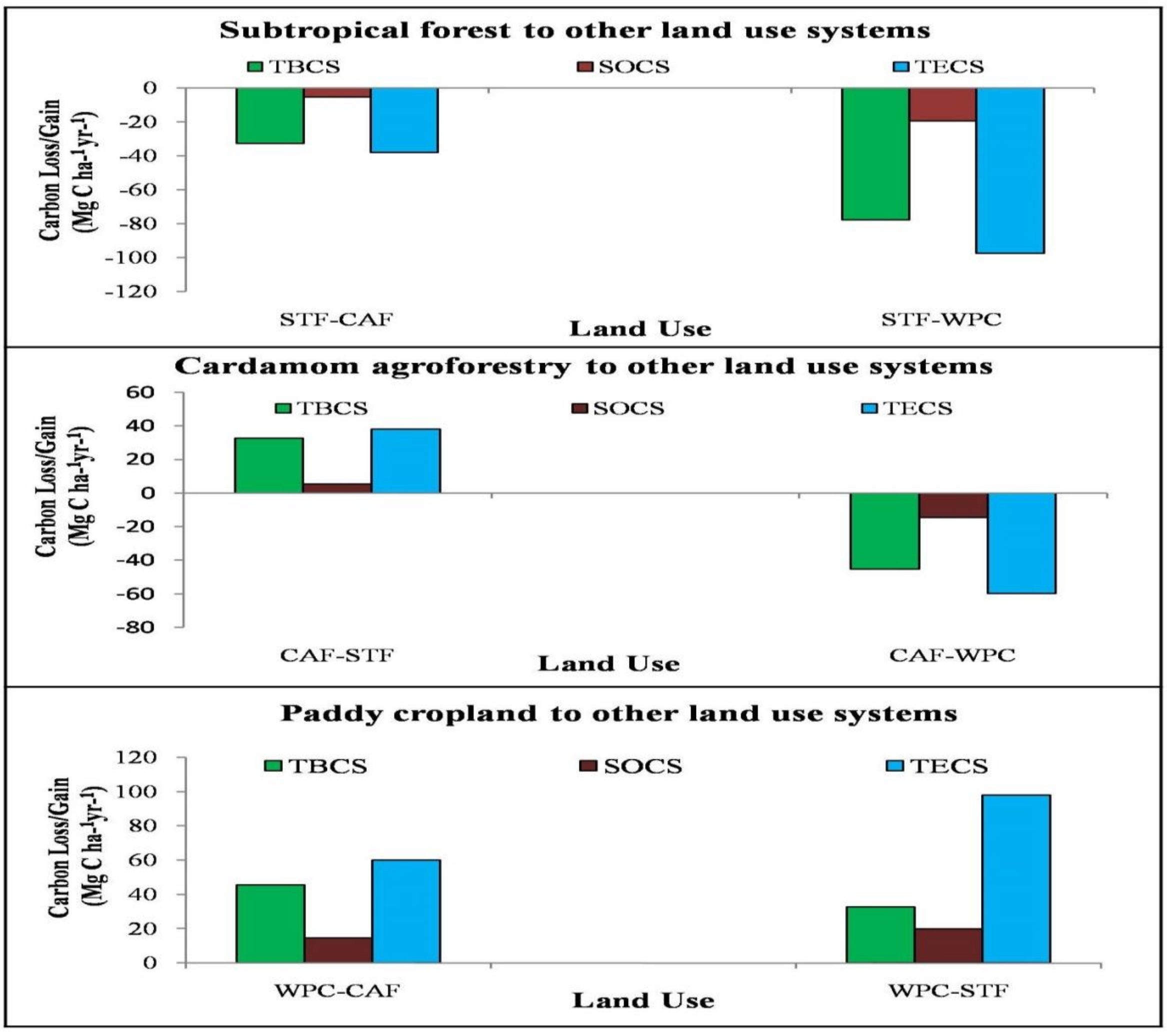


Fig 20. Carbon density Map of Sikkim in 2016 and 2018

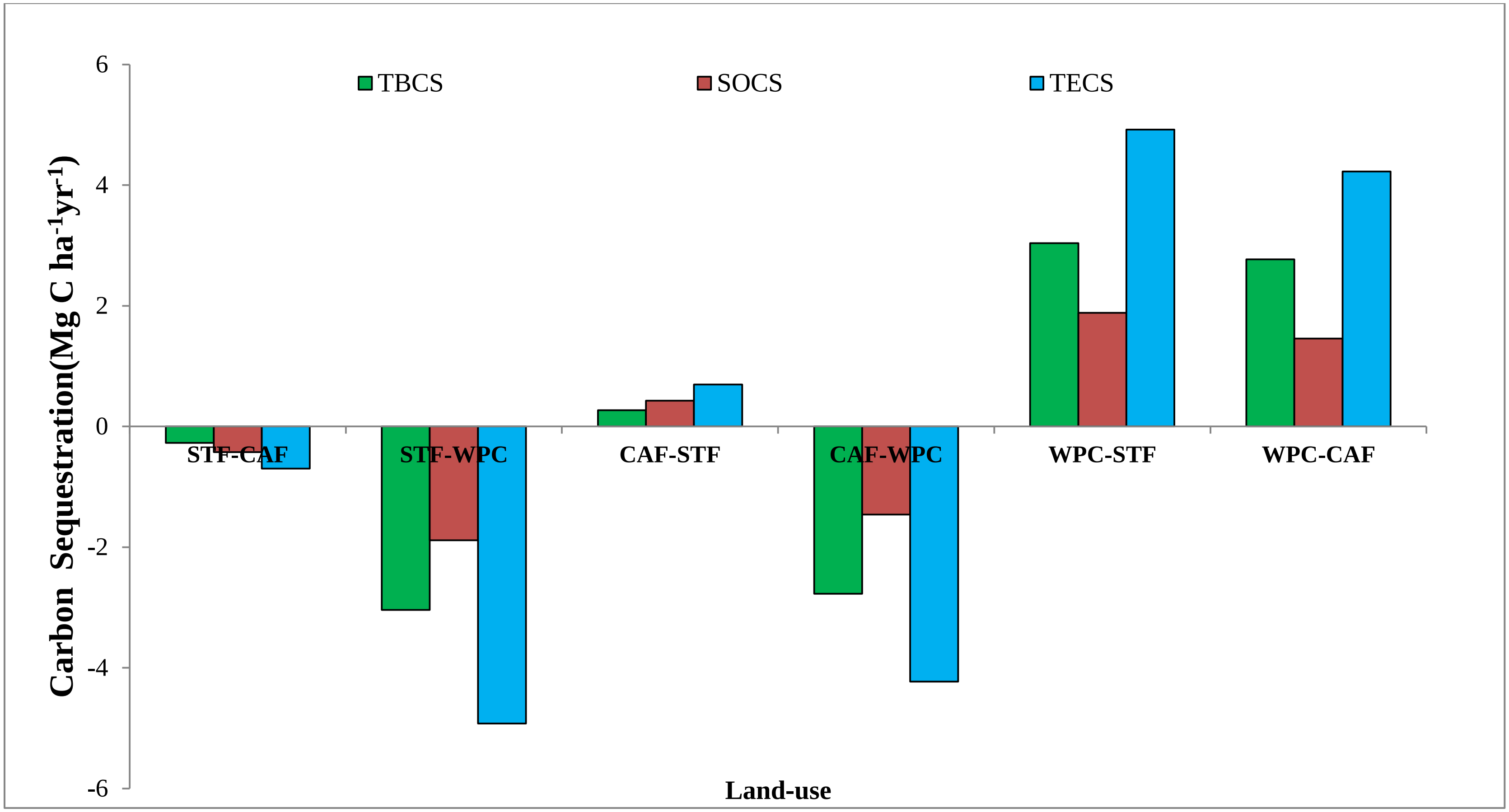
Changes in carbon stocks in the different land-use system

Carbon is lost when a forest is converted to agroforestry and cropland ecosystems. However, the reverse process leads to an increase in carbon stock (Fig 21). The same trend was also observed for carbon sequestration (Fig 22).



STF- Subtropical Forest, CAF- Cardamom agroforestry, WPC- Wet paddy cropland, TBCS- Total biomass carbon sequestration, SOCS- Soil organic carbon sequestration, TECS- Total ecosystem biomass sequestration.

Fig.21. The magnitude of changes in carbon stock from the conversion of STF to other land-uses, CAF to other land-use and WPC to other land-use sys



STF- Subtropical Forest, CAF- Cardamom agroforestry, WPC- Wet paddy cropland, TBCS- Total biomass carbon sequestration, SOCS- Soil organic carbon sequestration, TECS- Total ecosystem biomass sequestration.

Fig 22. Carbon sequestration rate due to a change in land-use systems of present study

DISCUSSION

Tree vegetation characteristics of the different land-use systems

In the present land-uses, species richness was higher in the subtropical forest with ten (10) species than that of cardamom agroforestry with six (6) species only. Low species richness in the present cardamom agroforestry system is because of the preference of nitrogen-fixing tree species *Alnus nepalensis* over other tree species by the local's agroforestry managers.

The total tree density was higher in the subtropical forest (188.28 individual ha^{-1}) than in the agroforestry system (124.90 individual ha^{-1}). Higher tree density in the subtropical forest is due to the removal of the tree from the agroforestry systems for various purposes. Anthropogenic interventions and management practices significantly affect the tree density in different types of land-uses (Schall and Ammer 2013), leading to different tree densities. The tree density of the present subtropical forest is less than the tree density of *Dipterocarpus* forests 155.00-460.00 individual ha^{-1} (Rabha et al. 2014) and different tropical forests of Assam (Borah et al. 2013, 2015). The low tree density of the present forest is because of anthropogenic disturbance and the young age of the forest as this forest was converted from an agroforestry system about twenty-five (25) years ago. However, tree density of the cardamom agroforestry system was similar to that of poplar intercropping systems of Northwestern Jiangsu, China (Fang et al. 2010) but lower than that of poplar agroforestry system of Northwestern India (Rizvi et al. 2011) and coffee agroforestry of Guatemala (Schmitt-Harash et al. 2012).

The tree basal area of the present subtropical forest was higher than the cardamom agroforestry system but comparable to the range reported from different tropical forests of Assam 5.37-37.09 m^2 ha^{-1} (Borah et al. 2013). The basal area of the present forest is slightly higher than that of Sal subtropical submontane zone of Garhwal Himalaya 26.10 m^2 ha^{-1} (Tiwari et al. 2010), tropical dry deciduous forest of Aravally mountains of Rajasthan 26.26 m^2 ha^{-1} (Kumar et al. 2010) and open forest of Mizoram 19.80 m^2 ha^{-1} (Singh et al. 2018). However, the value of the basal area of the subtropical forest is lower than that of the evergreen rainforest of Eastern Himalayas 37.40 m^2 ha^{-1} (Gogoi et al. 2017), subtropical broad-leaved forest of Meghalaya 39.19-59.44 m^2 ha^{-1} (Chaudhury and Upadhaya 2016) and subtropical evergreen broad-leaved forest of China 39.6-48.00 m^2 ha^{-1} (Lin et al. 2012). The total tree basal area of cardamom agroforestry (18.28 m^2 ha^{-1}) was lower than the tea agroforestry of Assam (Kalita et al. 2016) and managed plantation and jhum fallow agroforestry of Tripura (Chaudhary et al. 2016). But, the present value is higher than the plantation system of Aravally mountains, Rajasthan 13.88 m^2 ha^{-1} (Kumar et al. 2010), and different systems in the mid-hills of Indian Himalayas (6.11 m^2 ha^{-1}) (Yadav et al. 2017).

The Shannon diversity, Simpson dominance and Species evenness indices in trees of the subtropical forest were comparatively higher than the cardamom agroforestry systems due to selective tree retention and removal of a closed tree in the agroforestry system. The Shannon diversity index of the present (1.24-1.65) forest is comparable with the different tropical forests of Assam 1.01-1.55 (Borah et al. 2013) but lower than the tropical evergreen forest of Assam 1.94-2.52 (Gogoi et al. 2017). The higher value of Simpson dominance and lower value of evenness index and species richness was recorded in the present study as compared to the finding of (Borah et al. 2013; Gogoi et al. 2017) in the different tropical forests.

Biomass and carbon stock potential in three different land-use systems

Total vegetation carbon in the present study of three different land-use types ranged between 3.05-84.00 Mg C ha^{-1}. The present value of carbon is comparable to other findings reported from different land-use systems all over the world i.e. 5.00-191.00 Mg C ha^{-1} in Himalayan Watershed, Sikkim (Sharma and Rai 2007), 0.04-134.34 Mg C ha^{-1} in Gera, South Western Ethiopia (Mohammod and Bekela 2014), 3.34-52.88 Mg C ha^{-1} in Western Himalayas (Chisanga et al. 2018), 7.44-131.66 Mg C ha^{-1} in Mizoram, Northeast India (Singh et al. 2018) and 20.69-139.75 Mg C ha^{-1} in Eastern Ethiopia (Toru and Kibret 2019). Vegetative biomass carbon stock (VBCS) was highest in the subtropical forest (STF) followed by cardamom agroforestry (CAF) and lowest in the paddy cropland (WPC). Compared to WPC, higher vegetative carbon in STF and CAF is primarily due to the presence of more woody vegetation in the form of trees in two tree-based systems. In addition, lower biomass and carbon densities in agricultural land-use systems could be due to poor productivity and intensive management practices (Chisanga et al. 2018). The difference in the biomass and carbon density of each land-use system is due to the difference in the production of biomass. A high tree density and basal area in the forest systems enhances biomass storage (Pibumrung et al. 2008). Several studies also show a high carbon in the forests than other land-use systems of the world (Chen et al. 2005; Pibumrung et al. 2008; Kumar et al. 2010; Ahmad and Nizami 2015).

The average tree biomass (Aboveground+ Belowground) value in the present subtropical forest was 156.66 Mg ha^{-1} which contributes 38% to the total ecosystem carbon. Present data is consistent with the biomass value of tropical rain forests of Thailand, 96.00-276.00 Mg ha^{-1} (Terakunpisut et al. 2007), 32.47-261.80 Mg ha^{-1} in tropical forests of

Cachar district of Assam (Borah et al. 2013) and 49.00-178.40 Mg ha^{-1} in different forest type of Manipur (Sharma et al. 2020). However, the value is lower than other subtropical forests (180.00-261.00 Mg ha^{-1}) of the world (Nizami et al. 2009; Thokchom and Yadava 2013; Ali et al. 2014; Cao et al. 2014; Chaudhury and Upadhyaya 2016). The average aboveground biomass carbon stock (AGBC) in the present subtropical forest was 74.56 Mg C ha^{-1} which comes within the range of 15.40-214.90 Mg C ha^{-1} reported from different subtropical forests of China (Sun and Guan 2014; Li et al. 2019) and 60.09-121.43 Mg C ha^{-1}in the different forests of Manipur (Thokchom and Yadava 2017). The present carbon value is slightly higher than the value of tropical forest (67.64-73.21Mg C ha^{-1}) of Assam (Borah et al. 2015). However, it is lower than subtropical broad-leaved forest (88.58-138.87 Mg C ha^{-1}) of Meghalaya (Chaudhury and Upadhaya 2016), subtropical forests (90.53 Mg C ha^{-1}) of Eastern China (Ali et al. 2014) and subtropical broad-leaved forest (133.60-140.40 Mg C ha^{-1}) of Meghalaya (Gogoi et al. 2020). The lower carbon values in the present forest could be attributed to the relatively low biomass yield (Segura and Kanninen 2005).

The average tree biomass carbon (aboveground + belowground) of cardamom agroforestry was 39.69 Mg C ha^{-1} contributing 22% to the total ecosystem carbon. The present value is comparable with the value reported from a traditional agroforestry (0.70-54.00 Mg C ha^{-1}) of Sahel, West African (Takimoto et al. 2008), polar based agroforestry systems (6.28-83.07 Mg C ha^{-1}) of Haryana (Rizvi et al. 2011), farm forestry (1.91-62.05 Mg C ha^{-1}) and agroforestry systems (1.61-57.71Mg C ha^{-1}) of Andhra Pradesh (Prasad et al. 2012). The present carbon value of the agroforestry system is slightly lower than the value of tea agroforestry (44.80-56.70 Mg C ha^{-1}) of Assam (Kalita et al. 2016). However it is higher from the carbon storage range of agroforestry systems (0.29-15.21 Mg C ha^{-1}) reported by Nair et al. (2010), 25.03 Mg C ha^{-1} in cardamom agroforestry in Mamlay watershed (Sharma and Rai 2007), temperate agroforestry systems of China, 24.10-36.50 Mg C ha^{-1} (Xie et al. 2017), and different production systems of Almora, Uttarakhand, 1.17-25.30 Mg C ha^{-1} (Yadav et al. 2017). Maximum carbon stock by *Alnus nepalensis* (27.01±1.04 Mg C ha^{-1}) and minimum by *Viburnum cordifolium* (0.16±0.03 Mg C ha−1) in the present agroforestry corresponds to the highest tree density and basal area of *Alnus* and *Viburnum sp.* (Table-2). The rate of carbon fixation and capture of carbon by vegetation depends primarily on the geographical position, plant types, variety of species, and age of a tree (Liu et al. 2015). In the present study, maximum C storage correlates with high tree densities and basal areas of trees rather than the girth size of trees, which agrees with the report of Kalita et al. (2016) from tea agroforestry systems of Assam, but contrasting to that of a *Dipterocarpus* forest of Manipur (Devi and Yadava 2015), indicating the dependence of carbon densities on tree girth size.

Carbon density in the tree-based systems (subtropical forest and agroforestry systems) of the present study showed a positive and significant relation (Fig.7) with tree density ($p<0.10$), basal area ($p<0.05$), and aboveground biomass ($p<0.001$) which is consistent with the findings of several researchers from different subtropical land-use systems of India (Borah et al. 2015; Chaudhary et al. 2016; Gogoi et al. 2017).

Herbaceous biomass carbon of the present study contributed 5% in WPC, 2% in STF and 1% CAF to the total ecosystem carbon. In wet paddy cropland the herbaceous biomass carbon was 5.38 Mg C ha^{-1} which is comparable with the values reported by

(Gnanavelrajah et al. 2008), 5.40 Mg C ha^{-1} from a rice field of the eastern coast of Thailand but higher than that of 1.10-1.40 Mg C ha^{-1} in wet irrigated rice system of Philippines (Witt et al. 2000); agricultural land-use systems of Himachal Pradesh, 3.34 Mg C ha^{-1} (Chisanga et al. 2018) and 0.81-1.33 Mg C ha^{-1} in rice paddy of Thailand (Bridhikitti 2017). Present herbaceous biomass carbon in the STF (4.29 Mg C ha^{-1}) and AGF (2.52 Mg C ha^{-1}) is higher from *Dipterocarpus* forest of Manipur, 0.56-1.00 Mg C ha^{-1} -Devi and Yadava (2015) and three land-use systems (natural forest, managed plantation and jhum fallow) of Tripura, 0.17-1.22 Mg C ha^{-1} -Chaudhary et al. (2016).

Litter biomass carbon

The total average annual litter biomass carbon was slightly higher in the subtropical forest (3.57 Mg C ha^{-1}) than cardamom agroforestry (3.05 Mg C ha^{-1}) and contributed 2% each carbon value to the total ecosystems carbon. Litter biomass was absent in WPC due to the absence of trees and shrubs in this ecosystem and removal of plant parts for fodder after harvesting. High litter input in the STF than CAF is because of the presence of trees that enhances litter productivity. Litter biomass carbon is influenced by the vegetation, site status and type of land-use management practices (Takahashi et al. 2010). The present litter carbon is comparable with that of montane sub-tropical forests (2.20 - 22.60 Mg C ha^{-1} -Vogt et al. 1986), subtropical forest and monoculture plantation of China, 1.25- 4.35 Mg C ha^{-1} (Chen et al, 2005), subtropical board-leaved forest of China, 0.040 6.50 Mg C ha^{-1} (Zeng et al. 2013) and coffee agroforestry system (3.30-4.80) of Costa Rica (Hager 2012). The present value however is lower than subtropical forest (10.70-19.50 Mg C ha^{-1}) of Northeast India reported by Arunachalam et al. (1998), in Oak forest of Garhwal Himalaya, 7.30 Mg C ha^{-1} (Pant and Tiwari 1992), subtropical forest of Manipur, 10.90 Mg C ha^{-1} (Devi and Yadava 2010) and in the subtropical broad-leaved forest of Meghalaya, 6.66-10.44 Mg C ha^{-1} (Chaudhury and Upadhaya 2016). But, it is higher than the value of a subtropical forest (2.12-2.64 Mg C ha^{-1}) of China (Sun and Guan 2014). Peak detritus during the winter season and least during the summer season was observed in the present study which may be due to the plant's physiological response to the dry winter season. Similar observations were reported from earlier studies of in different ecosystems (Arunachalam et al. 1998; Yang et al. 2004; Kamei 2007; Devi and Yadava 2010).

Soil Organic Carbon

The total soil organic carbon stock (SOC) in the different soil layers of the present study ranged from 92.01 Mg C ha^{-1} in paddy cropland to 112.76 Mg C ha^{-1} in a subtropical forest. Comparing the three land-uses of the present study, the average SOC stock was highest in the STF (111.45 Mg C ha^{-1}) followed by CAF (106.33 Mg C ha^{-1}) and lowest in the WPC (92.37 Mg C ha^{-1}). Higher SOC stock in the subtropical forest may be due to the higher litter input in the soil from the trees, thereby increasing soil organic carbon. Also, roots of the trees in forest and agroforestry are major determinants of soil organic matters in tree-based systems (Jha et al. 2012; Toru and Kibret 2019). A lower SOC value in the cropland systems may be due to intensive management practices (Melero et al. 2011), and tilling of soil and removal of aboveground parts of the crops and grains for domestic use and fodder in the WPC results to decrease of carbon from soil (Amanuel et al. 2018). Further, the waterlogged condition of rice farming reduces the microbial activity and decomposition of soil organic matter thereby retarding the input of soil organic carbon. The present result is inconsistent with the findings of several studies (Schmitt-Harsh et al. 2002; Fantaw et al. 2007; Kumar et al. 2010; Jha et al. 2012; Amanuel et al. 2018; Sahoo et al. 2019; Toru and Kibret 2019) that recorded higher SOC stocks of the forest than other land-use systems. However, Singh et al. (2018) reported higher SOC in agroforestry than forested land which is contradictory to present study. The value of SOC stock in the present three land-use systems was within the reported range of soil carbon stock of Amazonian rain forest (72.00-149.00 Mg C ha^{-1} - Glaser et al. 2003), different agroforestry systems (30.00-300.00 Mg C ha^{-1} - Nair et al. 2010), and land-use systems namely natural forest, managed plantation and jhum fallows of Tripura, North-East India (85.34-121.87 Mg C ha^{-1} -Chaudhary et al. 2016). However, the present values were lower than that of different land-use types of Ethiopia (138.00-339.19-Toru and Kibret 2019) but higher from the values reported by (16.00-62.58 Mg C ha^{-1}- Singh et al. 2018) (22.92-52.74 Mg C ha^{-1} - Sahoo et al. 2019) from different land-use systems and soils of Mizoram. Soil organic carbon and bulk density show an inverse trend across the soil depth which is because of high organic matter content in the upper soil layers. Many studies reported a similar trend where SOC values were higher in the upper layer than that of subsurface soil layers (Shrestha et al. 2004; Gruneberg et al. 2010; Sharma et al. 2014; Amanuel et al 2018; Toru and Kibret 2019).

Total ecosystems carbon pools and carbon sequestration

Total average ecosystem biomass carbon value in the three different sites was 193.88 Mg C ha^{-1} (STF), 151.59 Mg C ha^{-1} (CAF), 97.75 Mg C ha^{-1} (WPC), respectively (Table - 21). Soil contributed the highest carbon, followed by trees, herbs and least by detritus to the total ecosystem carbon pool (Fig 18). The present value of total ecosystem carbon pools is comparable with the findings reported from different land-use systems of Tripura, 1.76-213.16 Mg C ha^{-1} (Chaudhary et al. 2016), Himachal Pradesh, 88.25-166.36 Mg C ha^{-1} (Chisanga et al. 2018) and Mizoram, 41.43-168.59 Mg C ha^{-1} (Singh et al. 2018).

The carbon sequestration of the present study was estimated to be 0.83-5.76 Mg C $ha^{-1}yr^{-1}$ across the different study sites. The rate of carbon sequestration in the vegetation components varied between 0.10-3.14 Mg C ha^{-1} yr^{-1} across the study sites. STF has the highest sequestration rate followed by CAF and lowest by the WPC. The higher rate of sequestration in STF may be due to the presence of higher tree density in the forest ecosystem. WPC has the least carbon sequestration rate which is due to the annual removal of the crops and plant biomass for domestic use resulting in less C return to the soil. Previous other studies on carbon sequestration also reported less carbon store and sequestration in agricultural systems (Murty et al. 2002; Deng et al. 2016). In the tree-based systems, vegetation sequestered a higher amount of carbon than soil whereas in the waterlogged cropland sequestration is higher in soil than vegetation. The present value of carbon sequestration in the three study sites (0.10-3.14 Mg C $ha^{-1}yr^{-1}$) is lower than the sequestration rate in the Chyandanda community forest, 5.02 Mg C $ha^{-1}yr^{-1}$ -Mandal et al. (2016) and Sal forest of Central Himalaya, 3.45 Mg C $ha^{-1}yr^{-1}$- Singh et al. (2019). Soil carbon sequestration rate in the present study sites varies from 0.73-2.61 Mg C $ha^{-1}yr^{-1}$ with a maximum in the STF and a minimum in the WPC. The present value is higher than the sequestration rate of 0.24-0.69 Mg C $ha^{-1}yr^{-1}$ for a re-vegetated hilly Plateau of Loess, China (Wang et. al. 2012).

Changes in carbon stocks and sequestration in three different land-use systems

Land-use change affected the biomass carbon stock in the present study (Fig 21). Carbon is lost when a forest is converted to any other agricultural land-use systems and the reverse process leads to an increase in carbon stock. A similar trend was also observed for carbon sequestration. Transformation of wet paddy cropland to a forest leads to the highest increase in carbon stock while the reverse will result in the loss in carbon. The present findings agree with that of Milne and Brown (1997) and Mohammod and Bekele (2014) where the transformation of the forest into agroforestry results in carbon loss. Several studies also reported an increase in biomass and carbon due to the introduction of trees in agricultural systems which conform to present study (Fang et al. 2010; Murthy et al. 2013; Goswami et al. 2014; Chisanga et al. 2018).

CONCLUSION

Tree-based land-use systems have a better potential for carbon stock and sequestration in vegetation than that of the other agricultural systems. The present study suggests that biomass carbon loss can be minimized by reducing anthropogenic activities such as the conversion of forests into agroforestry systems or other agricultural systems. The conversion of land-use systems has an impact on carbon stored in soil and vegetation due to changes in various environmental factors in the site. Progressive land-use change leads to loss of carbon while a retrogressive land-use change results in to increase in carbon stock and sequestration.

CHAPTER-V

SOIL MICROBIAL BIOMASS CARBON

SOIL MICROBIAL BIOMASS CARBON

INTRODUCTION

Soil microorganisms perform a major function in understanding and regulating the carbon cycling of different ecosystems. Soil hosts an enormous number of microbes such as bacteria, fungi and other microfauna which act as an important biota to maintain soil health and quality. The soil microbes constitute a very small component in soil but play an important role in most terrestrial ecosystems since microbes are the driving force for nutrient transformations and thus have a major role in maintaining soil fertility and in functioning of an ecosystem (Inagaki and Miura 2002; Giesler et al. 2004). The microbial biomass carbon in soil (MBC) forms an active pool of soil nutrients and comprises 1-3% carbon to the total soil organic carbon (Dilly et al. 2003). During the process of nutrient cycling, all organic materials are transformed by microorganisms to produce and deliver energy and new cell metabolites to support growth and development (Tracy and Frank 1998). For the formation of the organic pool, soil microbial biomass carbon acts as a key indicator of soil organic carbon by decomposing organic matter and controlling nutrient dynamics which affect the primary productivity in most biogeochemical processes in the terrestrial ecosystem (Gregorich et al. 2000; Kara and Bolat 2008). Not only this, the microbial biomass carbon is a labile fraction of soil organic matter and plays a significant role in maintaining soil fertility and the availability of plant nutrients (Amatya et al. 2002). Generally, plants serve as a carbon source for the microbial community while the microbes in return give nutrients to the plants for growth through nutrient mineralization, by decomposing soil organic matter and animal residues (Srivastava and Singh 1991).

The top upper layer of the soil comprises of abundant microorganism which actively takes part in nutrient conversion and decomposition of litter and production of humus. A decrease and increase in soil microbial biomass could result in mineralization and immobilization of nutrients (Me Grill et al. 1986). The MBC has been recognized as a very sensitive indicator of soil organic matter as microbial fraction changes quickly and these differences are noticeable before they occur in total soil organic matter (Gama-Rodrigues et al. 2008). The productivity and availability of nutrients in the ecosystem depend on the size and activity of the microbial biomass carbon (Chattopadhyay et al. 2012). The cycling of soil organic matter may be affected by the changes in soil microbial biomass and therefore, the microbial activity in the soil has a direct influence on the fertility and stability of ecosystems. To explain the interrelationships and controlling mechanisms of the input/output of carbon fluxes of nutrients and energy in the soil a solid measurement of the microbial biomass is therefore essential.

The microbial population and biomass are influence by the environmental factors in an ecosystem such as soil type, climate and land-use management practices (Devi and Yadava 2006). Besides these, soil physicochemical properties such as soil pH, clay content, and soil organic carbon also influenced microbial biomass carbon in various land-use systems (Srivastava 1992; Kara and Bolat 2008). According to Singh and Gupta (2018), soil microbial biomass acts as a keystone biological driver to the ecosystem functioning as it depends on the flux of carbon and other chemical nutrients, mediated by the microbial interaction in the soil, plant and animal food web (Seneviratne 2015). Effect on soil ecosystem functioning due to human activities such as land-use change is necessary to protect and regenerate the ability of soil to deliver ecosystem services (Van Leeuwen et al. 2017). The change in climate and other anthropogenic disturbances are critical drivers to regulate the microbial diversity in the ecosystems. Land-use types, geographical area, climate variability, soil properties and the dominant vegetation composition are the key drivers for controlling microbial biomass carbon dynamics in different land-use types (Wardle 1992; Singh and Gupta 2018). Also, a change in plant species composition influences the microbial community due to a change in soil organic nutrient (Zhang et al. 2016), due to the sensitivity of microbes to differences in the litter composition and root turnover rates (Hooper and Vitousek 1998). Availability of different substrates induced better growth and function of soil microbes (Jagadamma et al. 2014), hence microbial biomass can be used as a means for evaluation of the quality of soil in different vegetation types (Groffman et al. 2001a) Nutrient regulations to climate change through the carbon cycle by soil microbes are crucially important in carbon-climate reaction (Bardgett et al. 2008). Therefore, the estimation of microbial biomass carbon and understanding the land-use influence to soil microbial diversity is very important for different land-use systems to predict long-term effects on change in land-use (Sharma et al. 2004).

For the estimation of microbial biomass in the soil, various approaches are used. Some of the techniques used for the determination of microbial biomass are the fumigation-extraction method (Vance et al. 1987), fumigation-incubation method (Jenkinson and Powlson 1976), substrate-induced respiration method (Anderson and Domsch 1978), ATP extraction method (Tate and Jenkinson 1982), and Microcolorimetic method (Critter et al. 2002). For the estimation of microbial biomass carbon in the present study, the fumigation-extraction method was used since this method is reliable for soils of various land-uses with pH values above 3.7 (Wu et al. 1994) and waterlogged soil (Inubushi et al. 1991).

Sikkim Himalaya, located in the mountainous region of Eastern Himalaya experienced frequent land-use change. Natural forests of this region are often transformed into croplands and agroforestry systems to meet the livelihood requirement of the locals. This transformation results to change in various soil conditions leading to soil degradation and fertility loss. Hence, understanding the change in soil fertility and quality becomes crucial for proper management of soil and identification of suitable farming systems in this region.

Therefore, this chapter (V) discusses the variance in microbial biomass C across land-use types and season, contribution of microbial C to soil organic carbon, a relation of microbial biomass C with abiotic variables and comparison of the microbial C values with the values of other different land-use types.

MATERIAL AND METHODS

To estimate the soil microbial biomass carbon, random soil samples were collected from five plots of each of the land-use types in different seasons for two consecutive years. A total of 180 random soil samples from 90 soil pits from two different soil depths (0-15 and 15-30cm) were collected with a stainless steel soil corer (5 cm diameter) and mixed to form a composite sample for each depth. The distance between two random sampling points was at least 50m apart. Soils were transported to the laboratory in sealed polythene bags and roots, stones and organic residues were removed from the samples and each soil sample and stored at 4^0C to determine soil microbial biomass (MBC).

Microbial biomass carbon was estimated by the chloroform fumigation and extraction method (Vance et al. 1987). Each soil subsample for the determination of MBC was separated into two sections. The first section of the samples from each soil depth was fumigated with 30ml of alcohol-free chloroform by using a vacuum pump and incubated for 5 days and extracted with 0.5M potassium sulphate. The other halves of the soil samples were extracted with 0.5M potassium sulphate and used for the estimation of microbial biomass carbon. The microbial carbon content in the filtered extract of both fumigated and the unfumigated sample was analyzed for organic carbon using the titration method (Anderson and Ingram 1993). Soil microbial biomass carbon was calculated using the following expression:

$$MBC = E_C \times 2.64$$

E_C is C fumigated – C unfumigated soil samples

Plate 11: Estimation of soil microbial biomass carbon.

Data and statistical analyses

A three-way ANOVA was used to study the effects of land-use, season and soil depth on soil microbial biomass carbon and soil properties. Pearson's multiple correlation analysis was performed to determine the correlation between soil parameters and microbial biomass in different land-use systems. Data of soil parameters from all the land-use types were also subjected to Principal component analysis (PCA) by using R commander. All data are an average of five replicates ±SE of the composite soil samples.

RESULT

Soil microbial biomass carbon (MBC)

Soil microbial biomass carbon was highest in the subtropical forest (234.70±6.34 µg g^{-1} to 746.25±17.22 µg g^{-1}), followed by cardamom agroforestry (222.67±5.99 µg g^{-1} to 604.75±13.45 µg g^{-1}) and wet paddy cropland (186.54±7.88 µg g^{-1} to 458.23±17.33 µg g^{-1}) across soil depth and years (Table-23). However, the value of MBC increases slightly in the second year as compared to the first year (Table-23). MBC decreased with an increase in soil depth in all the three land-use systems showing the highest concentration in the rainy season (962.55±24.91 µg g^{-1}) and lowest in winter (625.97±16.83 µg g^{-1}) (Fig 23 and 24). Three-way Analysis of variance (ANOVA) of soil microbial biomass carbon showed a significant difference between land-use types season and soil depth in all the study sites ($p<0.001$). Also, the interaction between land-use type and season significantly affected the MBC in all the study sites (Table-24).

Table 23. Seasonal and annual microbial biomass carbon (MBC) across soil depths (0-30cm) in the study site (Means ±SE).

Land-use type	Soil Depth	2016-17 MBC (µg g^{-1})				2017-18 MBC (µg g^{-1})			
	(cm)	Summer	Rainy	Winter	Mean	Summer	Rainy	Winter	Mean
Subtropical Forest	0-15	539.30±12.32	758.29±17.22	442.23±13.34	579.94±14.29	546.10±17.23	764.30±21.42	436.20±12.34	582.20±16.99
	15-30	318.96±09.21	403.22±08.78	234.70±06.34	318.96±08.11	324.98±07.87	421.27±06.67	270.81±05.32	339.02±06.62
	Total	**858.26±21.53**	**1161.51±26.00**	**676.93±19.68**	**898.90±22.40**	**871.08±25.10**	**1185.57±28.09**	**707.01±17.66**	**921.22±23.61**
Cardamom Agroforestry	0-15	461.08±11.65	604.75±13.45	417.90±09.89	494.57±11.66	471.20±10.43	592.41±08.21	427.70±07.87	497.10±08.83
	15-30	252.76±08.11	394.94±08.32	222.67±05.99	290.12±07.47	257.59±07.54	388.71±08.32	222.70±07.66	289.66±07.89
	Total	**713.84±19.76**	**999.69±21.77**	**640.57±15.88**	**784.69±19.13**	**728.79±17.97**	**981.12±16.53**	**650.40±15.53**	**786.76±16.72**
Paddy Cropland	0-15	399.80±13.32	458.23±17.33	356.91±8.76	404.98±13.13	400.10±12.32	435.55±15.34	344.87±6.78	393.51±11.48
	15-30	252.76±10.23	258.78±11.56	192.58±8.78	234.71±10.19	228.64±9.79	294.88±12.83	186.54±7.88	236.69±10.16
	Total	**652.56±23.55**	**717.01±28.89**	**549.49±17.54**	**639.69±23.32**	**628.74±22.11**	**730.43±28.17**	**531.41±14.66**	**630.20±21.64**

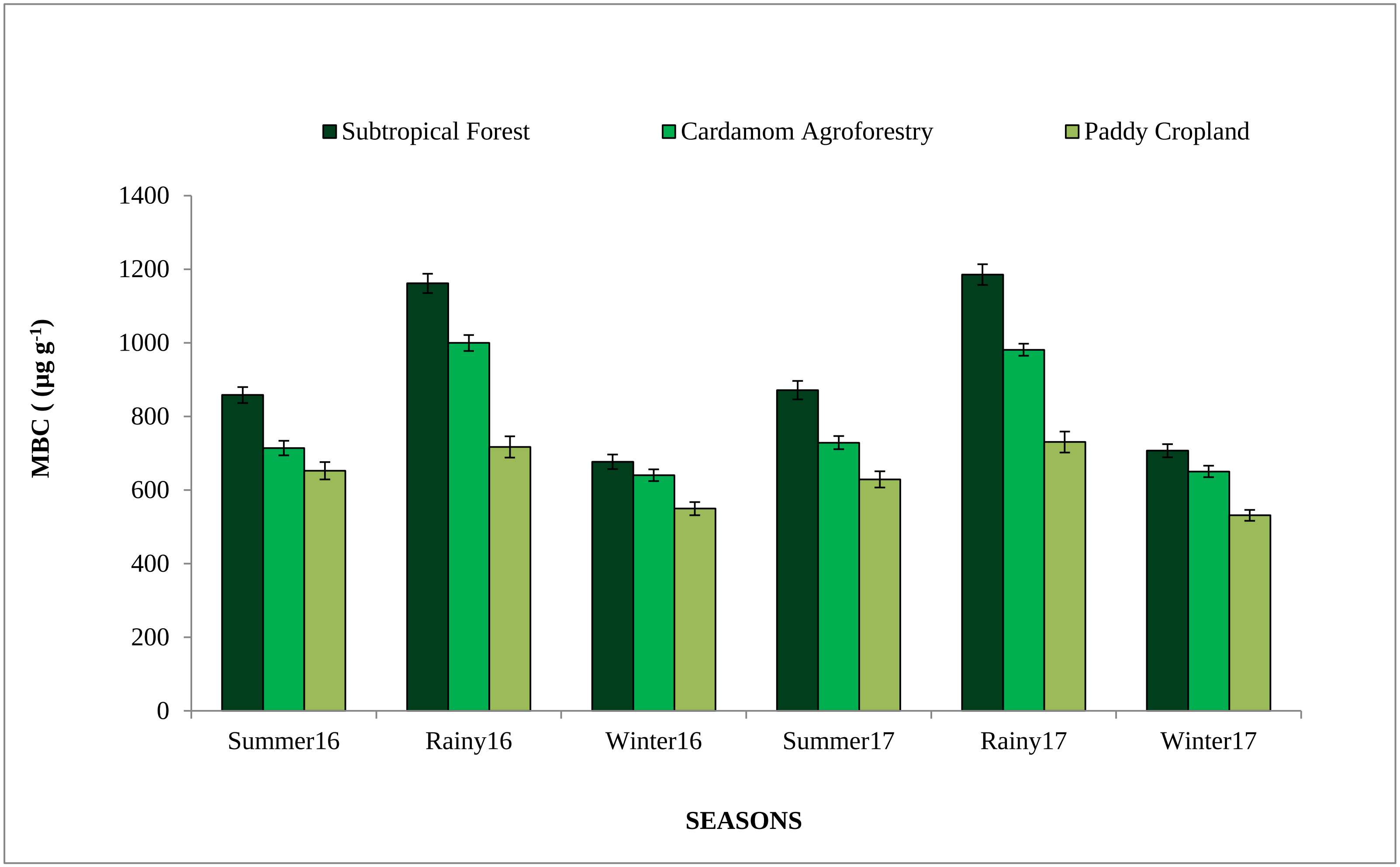


Fig 23. Seasonal Microbial Biomass Carbon (MBC) across soil depth 0-30 cm in the three different land-use types (mean±SE).

Table 24. Three-way ANOVA of soil microbial biomass carbon (MBC) in the three different land-uses showing variance due to season, soil depth and land-use.

Source	Type III Sum of Squares	df	Mean Square	F	P-value
SEASON	198343.952	2	99171.976	19.463	**.000*** **
LAND-USE	56975.493	2	28487.746	5.591	**.008** **
DEPTH	673568.660	1	673568.660	132.192	**.000*** **
SEASON * LAND-USE	94126.429	4	23531.607	4.618	**.004** **
SEASON * DEPTH	25557.996	2	12778.998	2.508	**.096^{NS}**
LAND-USE * DEPTH	13099.670	2	6549.835	1.285	**.289^{NS}**
SEASON * LAND-USE * DEPTH	34283.282	4	8570.821	1.682	**.175^{NS}**

*** Significant at $p < 0.001$; ** significant at $p < 0.01$; ^{NS} Not significant.

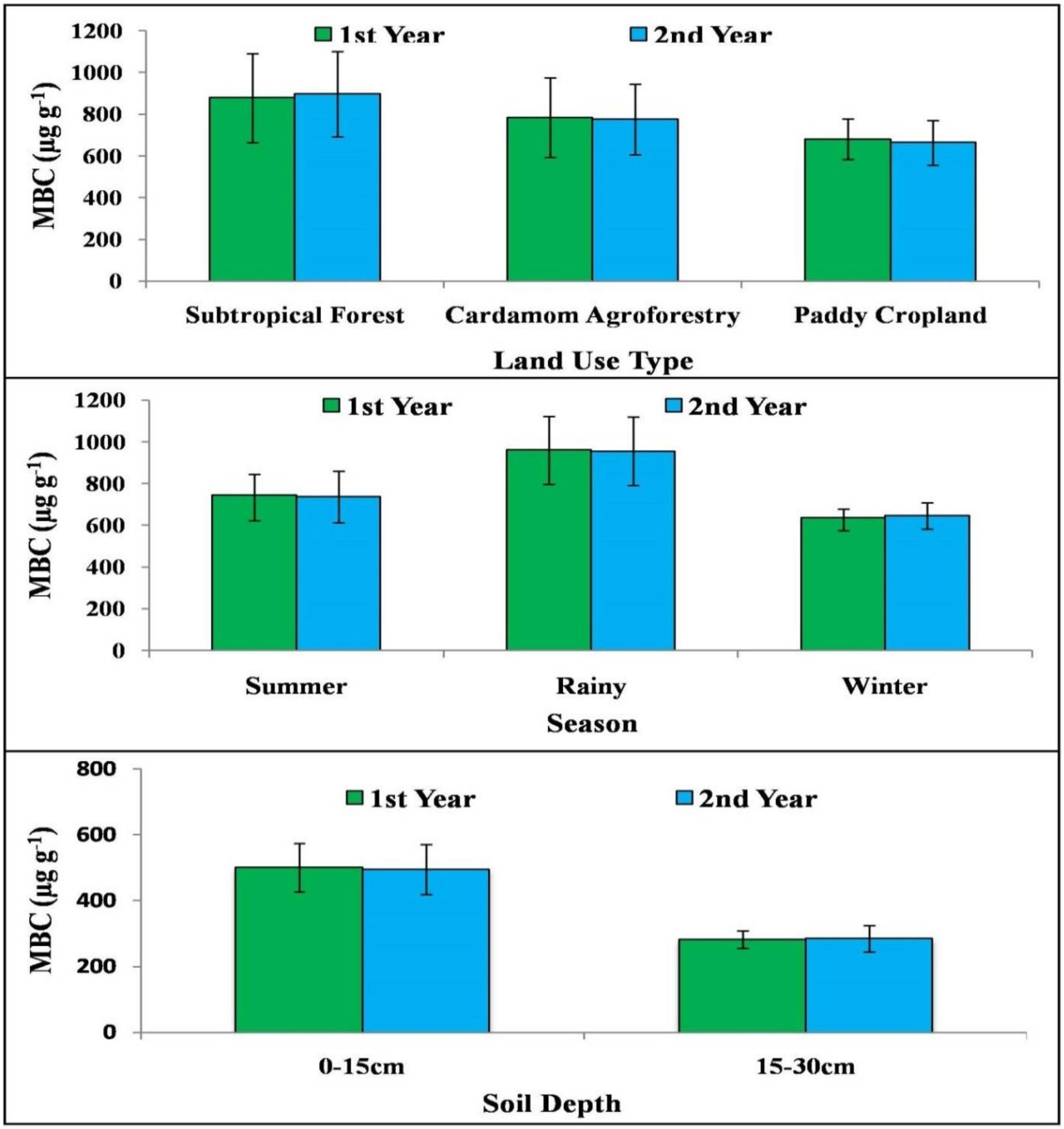


Fig 24. Variance of microbial biomass carbon (µg g^{-1}) due to land-use type, season and soil depth. (mean±SE).

Correlation matrix and principal component analysis

Pearson correlation analysis exhibited a strong positive significant relationship between microbial biomass carbon and edaphic and climatic variables ($p<0.01$) except for bulk density and soil pH (Table-25) where it is negatively significant ($p<0.05$). Principal component analysis (PCA) of microbial biomass C with different soil parameters in the land-use types explained 49.0% variability in the first component and 26.5% in the second component (Fig 25).

***Microbial biomass quotient* (MBC/SOC) %**

Microbial biomass quotient ranged from 1.88-2.16 % across the soil depth and land-use types (Table-26). The highest value of microbial biomass quotient was in the cardamom agroforestry system followed by the wet paddy cropland while the least was in the subtropical forest. In the present study, the trend of microbial biomass quotient was winter > summer > rainy and these values decreased across soil depths, and in the second year of the sampling period (Table-26).

Table 25. Pearson correlation coefficient between soil microbial biomass and soil and climatic variables in the study sites.

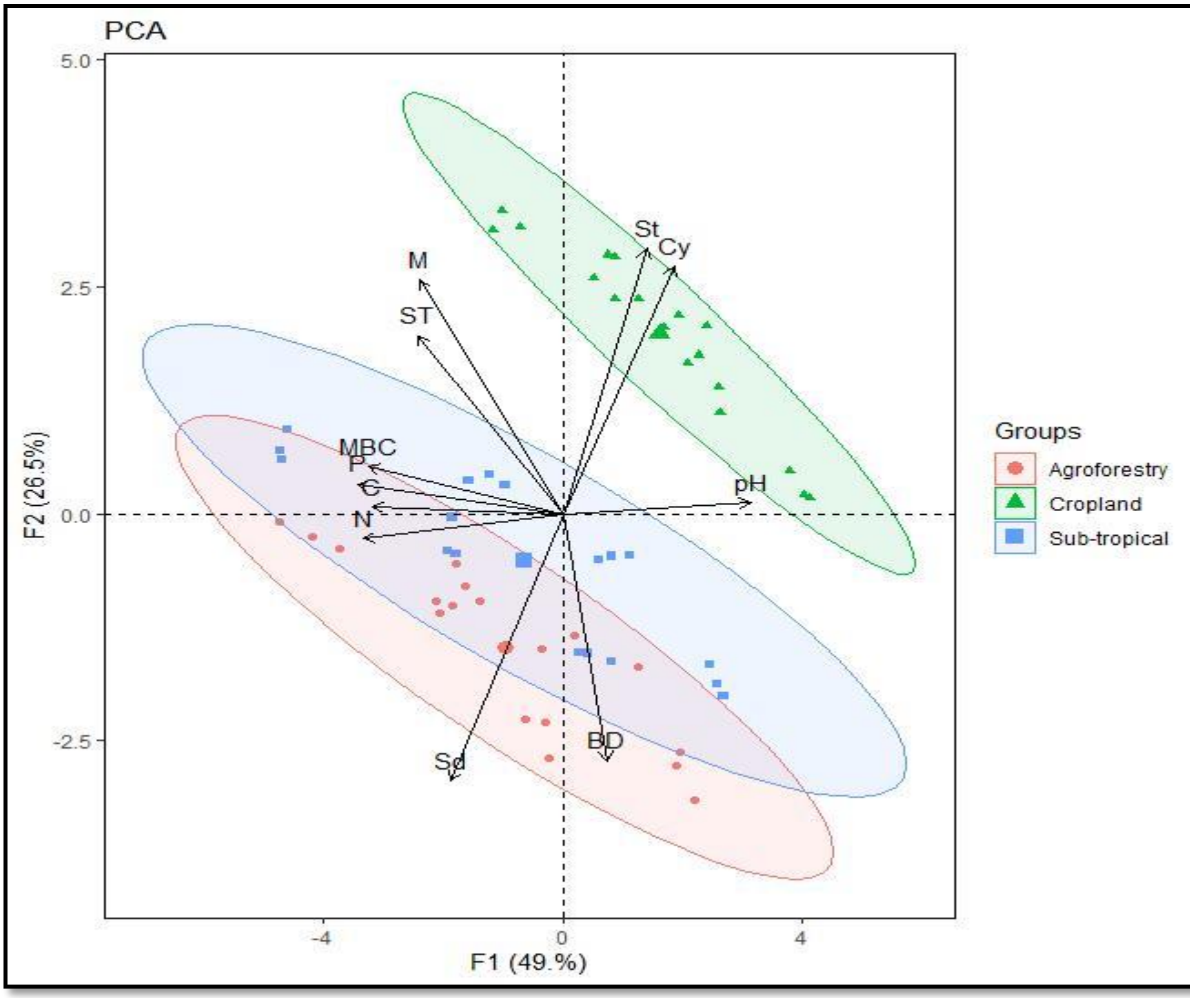
	MBC	M	BD	pH	SOC	N	P	ST	RH	R
MBC	1									
M	0.495*	1								
BD	-0.483*	-0.693**	1							
pH	-0.566*	-0.508*	0.137 NS	1						
SOC	0.875**	0.157NS	-0.530*	-0.508*	1					
N	0.851**	0.193 NS	0.422 NS	-0.625**	0.962**	1				
P	0.843**	0.244 NS	0.404 NS	-0.712**	0.892**	0.924**	1			
ST	0.754**	0.825**	-0.483*	0.736**	0.479*	0.448	0.492*	1		
RH	0.804**	0.774**	-0.369 NS	0.756**	0.499*	0.472*	0.533*	0.950**	1	
R	0.698**	0.451 NS	-0.058 NS	-0.007NS	0.430 NS	0.358 NS	0.474*	0.660**	0.840**	1

**Significant at $p<0.01$; *Significant at $p<0.05$; NS Non-Significant

MBC: Microbial Biomass Carbon, M: Soil Moisture, BD: Bulk Density, SOC: Soil Organic Carbon Stock, N: Total Nitrogen, P: Available Phosphorous, ST: Soil Temperature, RH: Relative Humidity, R: Rainfall.

Table 26. Variation of soil microbial quotient (MBC/SOC)% across soil depth and seasons in the study sites.

Land-use type	Soil depth	1st Year				2nd Year			
	(cm)	Summer	Rainy	Winter	Mean	Summer	Rainy	Winter	Mean
Subtropical Forest	0-15	0.76±0.11	0.60±0.16	0.90±0.07	0.75±0.11	0.80±0.12	0.58±0.12	0.95±0.09	0.78±0.11
	15-30	1.07±0.07	1.01±0.12	1.49±0.08	1.19±0.09	1.10±0.05	0.94±0.10	1.26±0.05	1.10±0.07
	Total	**1.83±0.18**	**1.61±0.28**	**2.38±0.15**	**1.94±0.20**	**1.90±0.17**	**1.52±0.22**	**2.21±0.14**	**1.88±0.18**
Cardamom Agroforestry	0-15	0.89±0.09	0.73±0.07	0.95±0.06	0.86±0.07	0.87±0.08	0.71±0.15	0.91±0.05	0.83±0.09
	15-30	1.42 ±0.03	1.00±0.04	1.48±0.06	1.30±0.04	1.41±0.06	1.04±0.06	1.50±0.06	1.31±0.06
	Total	**2.31±0.12**	**1.73±0.11**	**2.44±0.12**	**2.16±0.11**	**2.28±0.14**	**1.75±0.21**	**2.41±0.11**	**2.15±0.15**
Paddy Cropland	0-15	0.76±0.06	0.77±0.11	0.94±0.07	0.84±0.08	0.79±0.08	0.79±0.09	0.88±0.07	0.82±0.08
	15-30	1.16±0.05	1.21±0.06	1.44±0.03	1.27±0.05	1.21±0.04	1.07±0.05	1.44±0.04	1.24±0.04
	Total	**1.92±0.11**	**1.97±0.17**	**2.37±0.10**	**2.10±0.13**	**2.01±0.12**	**1.86±0.14**	**2.32±0.11**	**2.06±0.12**



MBC: Microbial biomass carbon, C: Carbon stock, N: Nitrogen, P: Phosphorous, ST: Soil temperature, M: Soil Moisture, BD: Bulk Density, St: Silt, Cy: Clay, Sd: Sand

Fig 25. Principal component analysis (PCA) of different soil parameters in the three land-use types.

DISCUSSION

Annual mean soil microbial biomass carbon (0-30 cm) of the present study was found to be highest in the subtropical forest (910.06 µg g^{-1}) followed by cardamom agroforestry (785.57 µg g^{-1}) and wet paddy cropland (634.94 µg g^{-1}). The highest MBC in the subtropical forest (STF) coincides with diverse tree species leading to an increase in organic matter, fine roots and litter diversity that enhances microbial activities in the soil thereby promoting better soil health. Also, a higher soil organic carbon content in the forest soils results to a greater quantity of microbial biomass carbon. Previous other studies also reported higher MBC in forest soils than that of other ecosystems which agree with the present findings (Arunachalam et al. 1999; Wu et al. (2016a). A significant positive correlation between soil microbial biomass carbon and soil organic matter (Table-25) in the present study shows that soil MBC is highly influenced by soil organic matter content. Consistent relation of MBC and soil organic matter were reported by many researchers from different ecosystems of the world (Chen et al. 2006; Wang and Wang 2011; Chen et al. 2017; Bargali et al. 2018; Padalia et al. 2018; Lepcha and Devi 2020). Absence of tree species resulted in less soil organic matter input in soil and intensive plowing and tillage of the soil disturbed the soil micro-fauna in wet paddy cropland which therefore recorded the least soil microbial biomass. Furthermore, the excessively high soil moisture content in the wet paddy cropland (WPC) due to waterlogged conditions limits the microbial activity and biomass in the soil (Lepcha and Devi 2020). In the tree-based systems, STF has higher soil microbial biomass than CAF due to higher clay content in the soil of the forest that increases the moisture retention capacity of soil and microbial biomass. Jenkinson and Powlson (1976) reported that soil with more clay content has high microbial biomass carbon. A slightly higher microbial biomass carbon in the cardamom agroforestry than that in wet paddy cropland is because of the presence of a litter layer in the former retaining soil moisture that promotes microbial activity. Afforested soils with higher litter inputs reported a higher MBC (Wu et al. 2016a) which is consistent with the result of the present study.

Soil microbial biomass carbon exhibited strong seasonal variations in all the land-use systems showing a peak value during the wet rainy and lowest in the dry winter season (Fig 24). Highest MBC during the rainy season is due to the warm and wet climate that accelerates litter decomposition due to the peak microbial activities and decomposition during this season which enhances the immobilization of nutrients by the microbes (Upadhyay et al. 1989; Usman et al. 2000; Lepcha and Devi 2020). In addition, high relative humidity during the wet period accelerates fungal growth leading to an increase in microbial biomass carbon (Acea and Carballas 1990). Least MBC during the dry and cold winter seasons correlates with low temperatures and less soil moisture, leading to the death of microorganisms that release organic carbon (Lepcha and Devi 2020), and freeze-thaw action can facilitate the decomposition of organic detritus and mineralization of carbon (Groffman et al. 2001b). Similar seasonal trends of MBC were observed in different ecosystems by several researchers (Devi and Yadava 2006; Iqbal et al. 2010; Otieno et al. 2010; Patel et al. 2010; Tan et al. 2013; Huang et al. 2016). However, present result is contrasting with finding reported by Singh et al. (1989) and Arunachalam and Arunachalam (2000) which show highest microbial biomass C in summer and winter respectively indicating that the microbial biomass C is strongly influenced by the species composition, location, elevation and pattern of rainfall of the site.

The depth of soil is another significant factor influencing MBC and in all land-use types, the upper soil layer has more MBC than the subsoil (Fig 24). The decrease of MBC with soil depth may be because of lower carbon and nitrogen content in the inner soil layer and more organic matter in the top humus soil that promotes microbial activity (Fall et al. 2012; Lepcha and Devi 2020). Previous studies on MBC across soil depth in various land-use types also reported similar such findings (Blume et al. 2002; Wichern et al. 2003; Fall et al. 2012).

The present values of MBC in the three study sites (186.54 µg g^{-1} to 764.30 µg g^{-1}) falls within the reported range of tropical soils of Costa Rica (Henrot and Robertson 1994), Arunachal Pradesh (Barbhuiya et al. 2004), subtropical soils of Manipur (Devi and Yadava 2006), different land-use systems of Turkey (Kara and Bolat 2008) and subalpine temperate forest of Taiwan (Ravindran and Yang 2015). However, these values are lower than that of the subtropical forest of Meghalaya (Arunachalam and Arunachalam 2000) but slightly higher than that of different agroforestry systems (Kaur et al. 2000), subtropical grassland and agroecosystems of Manipur (Singh and Yadava 2006), and different land-use system of Uttarakhand (Bargali et al. 2019). Comparison of soil microbial biomass carbon with previous work in different land-use systems is presented in (Table-27).

Table -27. Comparison of soil microbial biomass carbon values in different land-use systems.

Land-use type	Place	Soil Depth (cm)	MBC ($\mu g\ g^{-1}$)	References
Humid tropical forest	Costa Rica	0-15	106.00–2073.00	Henrot and Robertson 1994
Subtropical humid forest	Meghalaya, India	0-20	203.62- 1087.70	Maithani et al.1996
Different agroforestry systems	Haryana, India	0-30	76.10- 153.40	Kaur et al. 2000
Major land-use systems	Sikkim, India	0-15	219.00-864.00	Sharma et al. 2004
Tropical wet forest	Arunachal Pradesh, India	0-30	121.00-3232.00	Barbhuiya et al. 2004
Tropical dry forests	Uttar Pradesh, India	0-15	289.16-749.83	Singh et al. 2010
Traditional agroforestry	Arunachal Pradesh, India	0-30	47.50-1167.00	Tangjang et al. 2010
Tropical rice agroecosystems.	Odisha, India	0-30	59.00-514.00	Haripal and Sahoo 2014
Different forest type	Uttarkhand, India	0-15	416.00-763.00	Bargali et al. 2018
Different land-use systems	Uttarakhand, India	0-15	16.00–397.00	Bargali et al. 2019
Subtropical Forest	**Sikkim, India**	**0-30**	**234.70-764.30**	**Present study**
Cardamom Agroforestry	**Sikkim, India**	**0-30**	**222.69-604.75**	**Present study**
Paddy Cropland	**Sikkim, India**	**0-30**	**186.54-458.23**	**Present study**

Principal Component Analysis (PCA) of different soil parameters and microbial biomass carbon in the three different land-uses of the present study (Fig 25) indicates 75.50 % of the overall variance. The PCA component F1 explained 49.00%, while the second component F2 explained 26.50% of the variation. PC1 revealed that the microbial activity in subtropical forest is positively influenced by macro-elements, soil moisture, and temperature while soil pH exhibited an inverse relation with it. PC2 indicates that soil properties such as texture (silt, clay, and sand) and bulk density show a strong influence on land-use. In wet paddy cropland, the most important factors are silt and clay content however sand and bulk density of soil play an important role in the cardamom agroforestry system.

In the present study, the cardamom agroforestry system reported a higher microbial quotient which indicates more carbon immobilization by the microbes from the organic substrates. However, the least microbial quotient in the forest may be a result of carbon mineralization from the microbes to support vegetation. The microbial quotients were more in agricultural-based systems (cropland and agroforestry) than that of the forest. Further, the highest microbial quotient ratio in the agroforestry system among the agriculture-based systems probably suggests that the carbon immobilization capabilities of microbes increased in the agri-silviculture system than in monoculture. A previous study on the microbial quotient in different land-use also reported a higher value in agricultural soils than that of forest soils (Kara and Baykara 2014). The microbial quotient showed a strong significant variance with the season and soil depth. Winter season reported more immobilization of carbon and rainy season the least in all land-use type which may be due to the availability of more substrate in winter season. An increase in the microbial quotient with soil depth denotes the presence of more active carbon pools in the subsurface soil. Some studies reported that subsurface soil layers act as a store of microbial inoculation (Yi et al. 2006; Wei et al. 2009). The microbial biomass quotient slightly decreases in the second year as compared to the first year indicating a decrease in microbial carbon immobilization and organic carbon in the soil which may be because of a change in rainfall pattern in the second year. The soil microbial quotient (MBC/SOC) of the present study 1.88-2.16% falls within the range of tropical forests, 1.50-5.30F% (Luizao et al. 1992) and temperate forest soils, 1.80-2.90% (Vance et al. 1987) and those of agricultural soils (2-6%) reported by Brookes et al. (1985).

CONCLUSION

The present study concluded that microbial activity is more in tree-based systems i.e. forest and agroforestry systems than single-crop agricultural systems. Microbial biomass carbon shows strong seasonality and is influenced by seasonal changes in soil parameters as well. However, vegetation type, quantity and quality of litter input on the forest floor and soil depth also exhibit significant influence on microbial biomass and activity too. Human disturbance such as intensive cultivation, plowing and tillage of the soil decreases microbial activity and soil fertility. Also, microbial C immobilization is more in the agricultural-based systems than that of the forest, and the agri-silvicultural farming method improves soil fertility and quality by increasing microbial soil C immobilization. Therefore, the adoption of the tree-based agricultural system is recommended for the mountainous regions especially to prevent soil erosion and maintain soil quality and fertility for the sustainability of the region.

CHAPTER-VI

SOIL CO_2 EMISSION

SOIL CO_2 EMISSION

INTRODUCTION

Anthropogenic activities such as land-use change, fire, burning of fossil fuels and industrialization have led to the rapid rise in atmospheric CO_2 gas resulting to the global warming and climate change (Pachauri et al. 2014). There has been a rapid increase in the rate of greenhouse gases emissions such as carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4) which have led to increase in the earth's temperature in the last few decades (Rahman 2013). Carbon dioxide gas one of the important greenhouse gases contributes about 60% to global warming and significantly affects the regional and global climate (Rastogi et al. 2002). The rate of atmospheric CO_2 concentration has increased rapidly from 280 ppmv to 391 ppmv from early industrial revolution time to the present day (WMO 2012). CO_2 emissions from fuel combustion in India have begun to increase since the industrial revolution and have reached their peak in the last two decades. It grew from 0.66 billion tonnes in 1970 to 1.84 billion tonnes in 2010, which is 2.8 times more than in the past year (IEA 2011). There are two major natural carbon sinks in the study of climate change and carbon cycling interaction, namely the ocean and terrestrial biosphere, which have consumed almost half of all human emissions of carbon dioxide (Tan et al. 2013). Vegetation and soils of the terrestrial ecosystems are the main storage sinks of atmospheric CO_2 (Franzluebbers and Doraiswamy 2007).

Soil acts as an important source of atmospheric carbon dioxide (Rastogi et al. 2002) and sinks as well by storing about 80% of terrestrial carbon worldwide (Nielsen et al. 2011). Soil also plays an important role in carbon and nutrient cycling and with the increased in global atmospheric greenhouse gases and climate change, it can also be a net source or sink of CO_2 in the nearby future (Thokchom and Yadava 2014). According to Lal (2008), soil contains 2700 Gt of carbon, which is more than the total amount of atmospheric carbon (780Gt) and biomass carbon (575Gt). Besides, its contribution to global warming, soil CO_2 emission influences soil organic matter loss, fertility and productivity (Rastogi et al. 2002).

Soil respiration is the key ecosystem process releasing carbon from soil as carbon dioxide by soil microorganisms, soil fauna and plant roots and through the decomposition of SOC matter (Rastogi et al. 2002; Millard et al. 2010). Soil microorganism contributes 99% while plant roots contribute 50% of the total soil respiration and the contribution of soil fauna is comparatively very less (Hanson et al. 2000; Rastogi et al. 2002). In the terrestrial C cycle, soil respiration is regarded as the main mechanism for the release of carbon with an annual release of 98 Pg C per year into the atmosphere (Bilandžija et al. 2016; Zhao et al. 2017). Globally, soil CO_2 emission is the second highest carbon flux between the atmosphere and the terrestrial biosphere (Schlesinger and Andrews 2000). The emission of CO_2 from the soil is the main factor used for evaluation of biological activity in the soil of terrestrial ecosystem (Santruckova 1992) and the addition of organic matter influences the rate of decomposition and soil respiration.

CO_2 emission from the soil is very sensitive to biotic and abiotic variables of soil (Bain et al. 2005) and mainly depends upon soil temperature and moisture content (Carlyle and Than 1988; Raich and Tufekcioglu 2000; Qi and Xu 2001; Devi and Yadava 2008; Li et al. 2008; Yohannes et al. 2011; Zhou et al. 2013). Previous studies have shown that the rate of soil CO_2 emission is strongly influenced by the amount of organic materials present on the soil, interaction among soil physicochemical & biological processes and environmental conditions like temperature, humidity, rainfall, etc. (Agehara and Warncke 2005; Lee et al. 2006; Evans and Burke 2013). Seasons and vegetation types also influence the soil respiration rate in ecosystems (Raich and Tufekcioglu 2000). Further, different management practices, including land-use transformation (Zhao et al. 2006; Iqbal et al. 2010) influenced CO_2 emission from the soil and deforestation contributes 12.5% of global CO_2 emissions (IPCC 2013).

CO_2 emission from the soil can be estimated by various techniques in different ecosystems. Some of these techniques are open flow infrared gas analyzer technique where ambient air flows through a chamber and the CO_2 flux is determined from the difference in concentration between inlet and outlet air (Nakadai et al. 1993). The closed chamber technique where CO_2 in a closed chamber is regularly sampled and the efflux is determined through the increase rate in the chamber CO_2 concentration (Bekku et al. 1995). The dynamic closed chamber technique in which air circulates and returns to the chamber from the gas analyzer (Rochette et al. 1992) and alkali absorption methods in which carbon dioxide from the soil is absorbed in a closed chamber (Buyanovsky et al. 1986). In the present study alkali absorption method was used in the field for its convenience and capability in different ecosystems by several studies (Kucera and Kirkham 1971; Buyanovsky et al. 1986; Singh et al. 1988; Anderson and Ingram 1993; Devi and Yadava 2008).

It has been observed that a high quantity of CO_2 goes through the soil consistently, which is over ten times more of CO_2 released from fossil-fuel combustion (Raich and Potter 1995). Hence, small changes in CO_2 emission concentration may strongly influence the global carbon dynamics (Wei et al. 2014). Soil CO_2 emission therefore can be used as an important parameter for the study of biological activity in the soil, carbon cycling, and the energy flow in an ecosystem (Singh and Gupta 1977). Different land-use types have variances in climate, vegetation, geographical characteristics, slope, management practices, etc which therefore led to a different rate of emission. Therefore, measurement of soil respiration in different land-use types is important to understand the characteristics of soil respiration in the entire terrestrial ecosystem as well as to recognize the diverse biotic and abiotic factors that regulate soil CO_2 outflows.

Therefore, the present chapter (VI) on soil CO_2 emission from different land-use systems of Sikkim Himalaya provides information on:

i) Monthly and seasonal changes in the rate of soil CO_2 emission from three different land-use systems

ii) The relationship between soil CO_2 emission with biotic and abiotic variables of the three different land-use types in the years 2016 and 2017. This information will help to understand the different CO_2 emission capacity of different ecosystems in this region and help in identification of the system with minimum emission.

METHODS

Monthly soil CO_2 emission of a subtropical forest, cardamom agroforestry and paddy cropland were measured by using the alkali absorption method during the 2016 - 2017 (Anderson and Ingram 1993). Thirty-six (36) open-ended cylinders with a diameter of 13cm and a height of 25cm were inserted up to 15cm in the soil of each of the study sites as shown in (Photo-12). Three cylinders out of the thirty-six (one in each site) were used as a blank sample, and the entire vegetation and soil surface within these cylinders were sealed. All the herbaceous plants and rocks inside the other cylinders were removed manually. Fifty (50 ml) of 0.25N NaOH solution was kept within each cylinder in small vials including the blank cylinder and sealed with anchor grip to make the whole setup airtight and left for 24 hours. After 24 hours, NaOH was titrated with a 0.25N HCL solution using phenolphthalein indicator until the pink colour disappeared. CO_2 absorbed from the soil was calculated using the formula:

$$CO_2 \text{ mg} = V x N x 22 \text{ (Anderson and Ingram 1993)}$$

Where V is the volume of HCL, N is the normality of HCL

Photo 12: Determination of soil CO_2 emission by alkali absorption method.

RESULT

Subtropical forest

The monthly rate of soil CO_2 emission in the subtropical forest varied from 114.58-259.13 mgCO_2m^{-2}hr^{-1}in different months during 2016-17(Fig 26). Minimum soil respiration rate was during the month of February in both the years and thereafter it consistently increased upto August in 1st year and July in 2nd year and decreases again. Seasonally, the rate of CO_2 emission was highest in the rainy season followed by summer and winter season (Table-28), and contributed 43.97%, 29.51%, and 26.52% respectively to the total soil CO_2 emission of this system. The analysis of variance in subtropical forest indicated a significant variation in different sampling months of summer ($P<0.01$), rainy ($P<0.001$), winter ($P<0.01$) and annually ($P<0.001$) (Table-29).

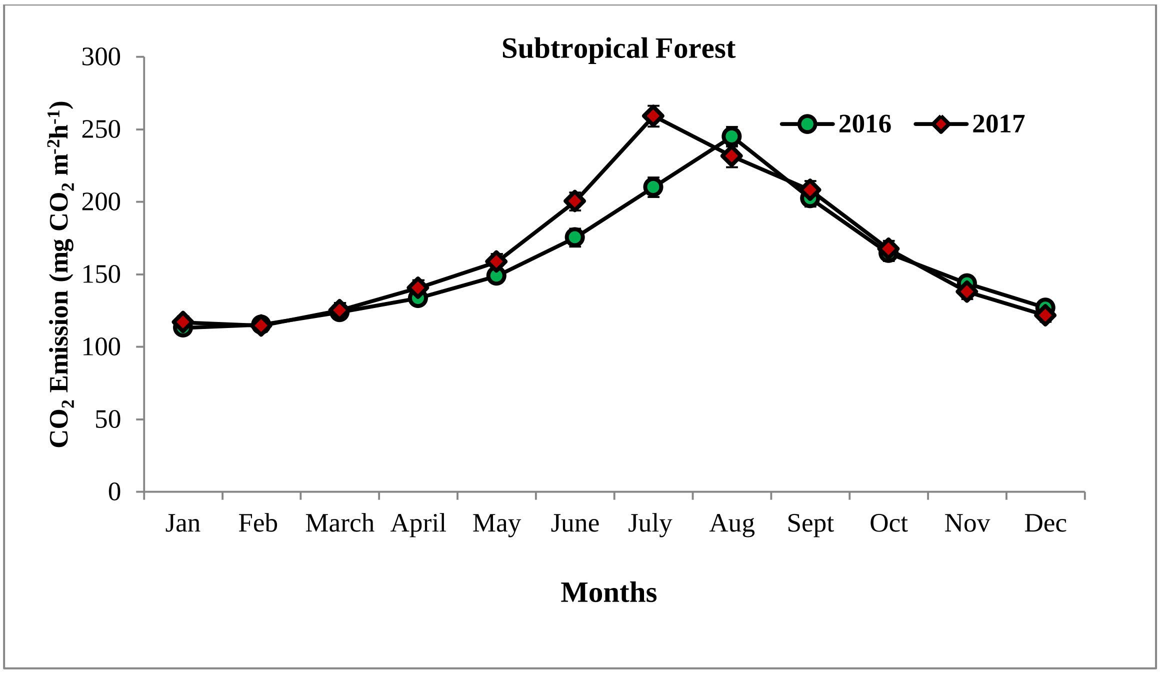


Fig 26. Monthly soil CO_2 emission in the subtropical forest (Mean ± SE)

Table 28: Seasonal variation in the rate of soil respiration (mg CO_2 $m^{-2}h^{-1}$) in different land-use types.

	2016			2017		
Seasons	**Subtropical Forest**	**Cardamom Agroforestry**	**Paddy Cropland**	**Subtropical Forest**	**Cardamom Agroforestry**	**Paddy Cropland**
Summer	135.46±6.07	134.82±6.13	111.56±5.83	141.49±5.93	133.18±6.20	111.72±5.80
Rainy	199.47±7.72	170.99±6.94	151.72±5.56	213.30±6.44	172.71±6.36	151.64±5.86
Winter	125.92±6.08	122.88±5.88	106.72±4.50	122.77±5.03	117.16±5.23	104.71±4.55

Table 29: ANOVA (One-way) of CO_2 emission in different months of seasons in subtropical forest.

Seasons	*Source of Variation*	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Summer	**Between Months**	1297.569	2	648.78	19.1734	**0.002477****	5.14325
	Within Months	203.025	6	33.837			
Rainy	**Between Months**	12941.85	4	3235.463	171.620	**3.62E-09*****	3.47805
	Within Months	188.523	10	18.852			
Winter	**Between Months**	877.102	3	292.367	3.3501	**0.00617****	4.06618
	Within Months	698.159	8	87.269			
Annual	**Between Months**	57402.06	11	5218.369	114.930	**5.52E-18*****	2.21630
	Within Months	1089.709	24	45.4045			

**Significant at *P-value*< 0.01, ***Significant at *P-value*< 0.001

Cardamom Agroforestry

In the cardamom agroforestry, the soil CO_2 emission rate ranged from 118.54 $mgCO_2m^{-2}hr^{-1}$ to 219.29 mg $CO_2m^{-2}hr^{-1}$in different months of the sampling period (Fig 27). The highest soil CO_2 emission rate was recorded in August in both the years and the lowest was in January in the first year and December in the second year respectively. The rate of CO_2 emission trend was observed as: rainy>summer>winter season (Table-28). Seasonally, summer rainy and winter contributed (31.46%), (40.36%) and (28.18%) respectively to the total soil CO_2 emission in the agroforestry ecosystem. One-way analysis of variance (ANOVA) of the different values of soil CO_2 emission rate in cardamom agroforestry indicated a significant variation in different sampling months of summer (P<0.01), rainy (P<0.001), winter (P<0.01) and annually (P<0.001) (Table-30).

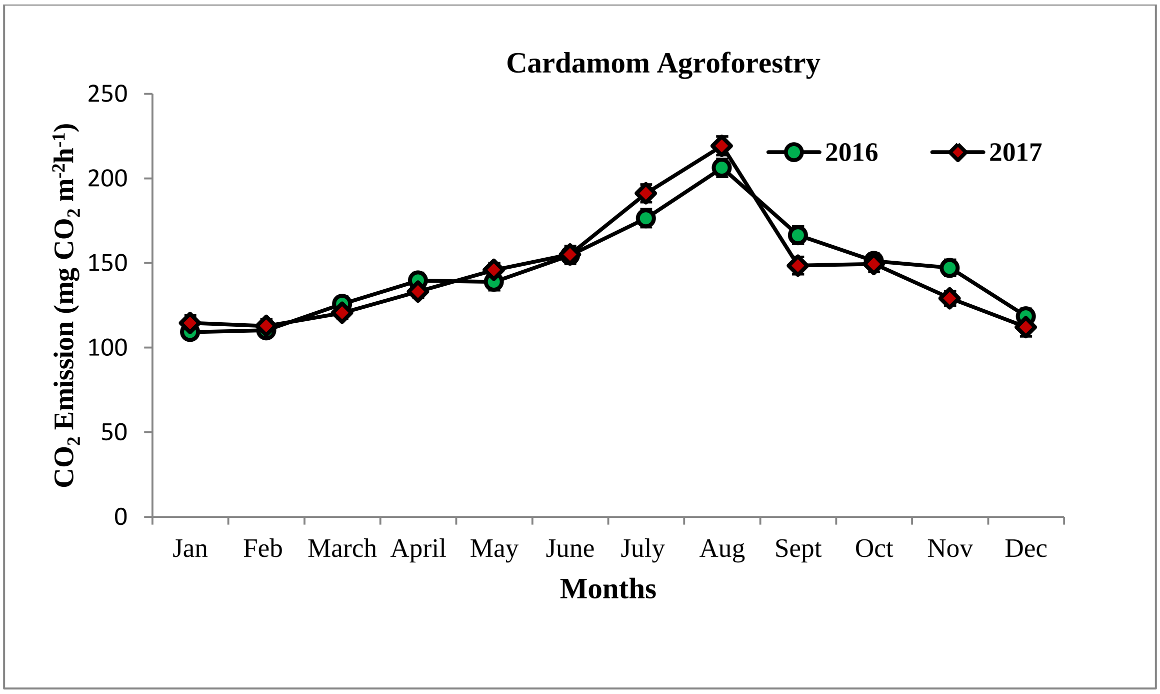


Fig 27. Monthly soil CO_2 emission in the cardamom agroforestry (Mean ± SE)

Table 30: ANOVA (One-way) of soil CO_2 emission in different sampling months of the seasons in cardamom agroforestry.

Seasons	*Source of Variation*	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Summer	**Between Months**	1048.966	2	524.483	16.6923	**0.003536****	5.14325
	Within Months	188.523	6	31.420			
Rainy	**Between Months**	6498.065	4	1624.516	24.7365	**3.61E-05*****	3.4780
	Within Months	656.726	10	65.6726			
Winter	**Between Months**	1631.198	3	543.7325	14.9976	**0.001198****	4.06618
	Within Months	290.0367	8	36.25459			
Annual	**Between Months**	25457.89	11	2314.353	48.9255	**1.04E-13*****	2.21630
	Within Months	1135.287	24	47.30361			

**Significant at *P-value*< 0.01, ***Significant at *P-value*< 0.001

Paddy Cropland

Monthly soil CO_2 emission rate in paddy cropland varied from 89.31 $mgCO_2m^{-2}hr^{-1}$ to178.04 $mgCO_2m^{-2}hr^{-1}$ in different months throughout the sampling period (Fig 28) with maximum emission in the August and September in the first and second year respectively. The minimum rate of CO_2 emission was observed in the month of January in both the years. Rainy season exhibited the highest rate of CO_2 emission followed by summer season and winter season (Table-28) and contributing 41.10%, 30.28%, and 28.65% respectively to the total soil CO_2 emission in the paddy cropland. The analysis of variance in cropland indicates significant seasonal, (summer rainy and winter (P<0.01), and annual variations (P<0.001) (Table-31).

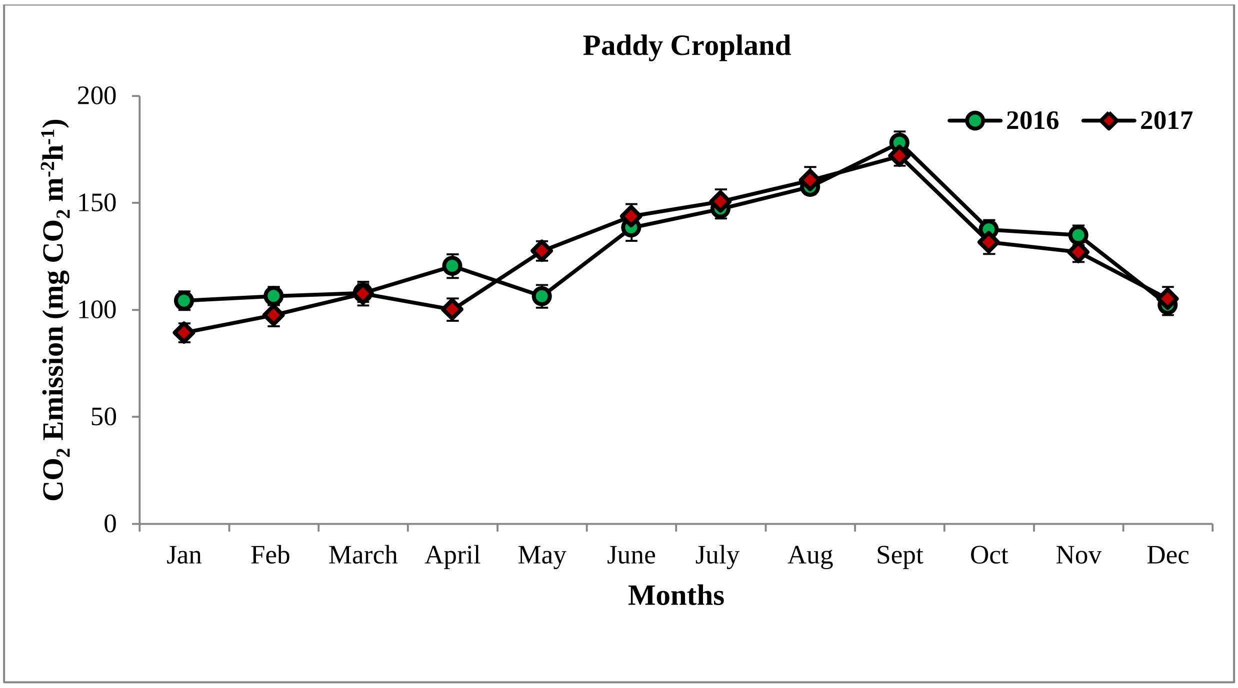


Fig 28. Monthly soil CO_2 emission in the wet paddy cropland (Mean ± SE)

Table 31: One-way ANOVA for soil CO_2 emission in different sampling months of seasons in wet paddy cropland.

Seasons	*Source of Variation*	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Summer	**Between Months**	520.6535	2	260.3268	15.19034	0.004486**	5.14325
	Within Months	102.8259	6	17.1376			
Rainy	**Between Months**	2625.661	4	656.4152	4.241633	0.029073*	3.47805
	Within Months	1547.553	10	154.7553			
Winter	**Between Months**	4315.332	3	1438.444	19.9092	0.000456***	4.06618
	Within Months	578.0017	8	72.25022			
Annual	**Between Months**	23122.400	11	2102.036	17.34441	7.93E-09***	2.21630
	Within Months	2908.654	24	121.193			

**Significant at *P-value*< 0.01, ***Significant at *P-value*< 0.001

Table-32: ANOVA (Two-way) for Soil CO_2 emission exhibiting the influence of land-use types and seasons.

Source	*F*	*P-value*	*F crit*
Land-use	27.36497	3.21E-07***	3.354131
Seasons	7.349842	0.002829**	3.354131
Land-use x Seasons	0.874378	0.492125^{NS}	2.727765

**Significant at *P-value*< 0.05, ***Significant at *P-value*< 0.001

Relationship between soil CO_2 emission and abiotic variables

CO_2 emission in soil was significantly affected by land-use types and season in all the study sites (p<0.05) (Table-32). The rate of soil CO_2 emission was positively significant with all the abiotic variables (soil and air temperature, soil moisture, soil organic carbon, total nitrogen, available phosphorous, soil microbial carbon, relative humidity and annual rainfall) except for soil PH and bulk density. Linear regression equations between soil carbon dioxide emission with biotic and abiotic variables are presented below:

$$Y=0.143X_1+7.99 \qquad (R^2=0.36) \qquad (1)$$

$$Y=0.004X_2+5.91 \qquad (R^2=0.24) \qquad (2)$$

$$Y=-1E-05X_3+0.67 \qquad (R^2=3E-05) \qquad (3)$$

$$Y=0.010X_4+2.20 \qquad (R^2=0.69) \qquad (4)$$

$$Y=0.000X_5+0.14 \qquad (R^2=0.60) \qquad (5)$$

$$Y=0.000X_6-0.01 \quad (R^2=0.59) \quad (6)$$

$$Y=0.099X_7-1.60 \quad (R^2=0.63) \quad (7)$$

$$Y=0.099X_8-1.60 \quad (R^2=0.61) \quad (8)$$

$$Y=0.022X_9+26.28 \quad (R^2=0.57) \quad (9)$$

$$Y=4.976X_{10}+463.40 \quad (R^2=0.58) \quad (10)$$

$$Y=5.337X_{11}+44.34 \quad (R^2=0.68) \quad (11)$$

Where Y=Soil CO_2 emission, X_1=Soil moisture, X_2=Soil pH, X_3=Bulk density, X_4= Soil organic carbon, X_5= Total nitrogen, X_6= Available phosphorous, X_7= Soil temperature, X_8= Air temperature, X_9= Relative humidity, X_{10}= Rainfall, X_{11}= Soil microbial carbon.

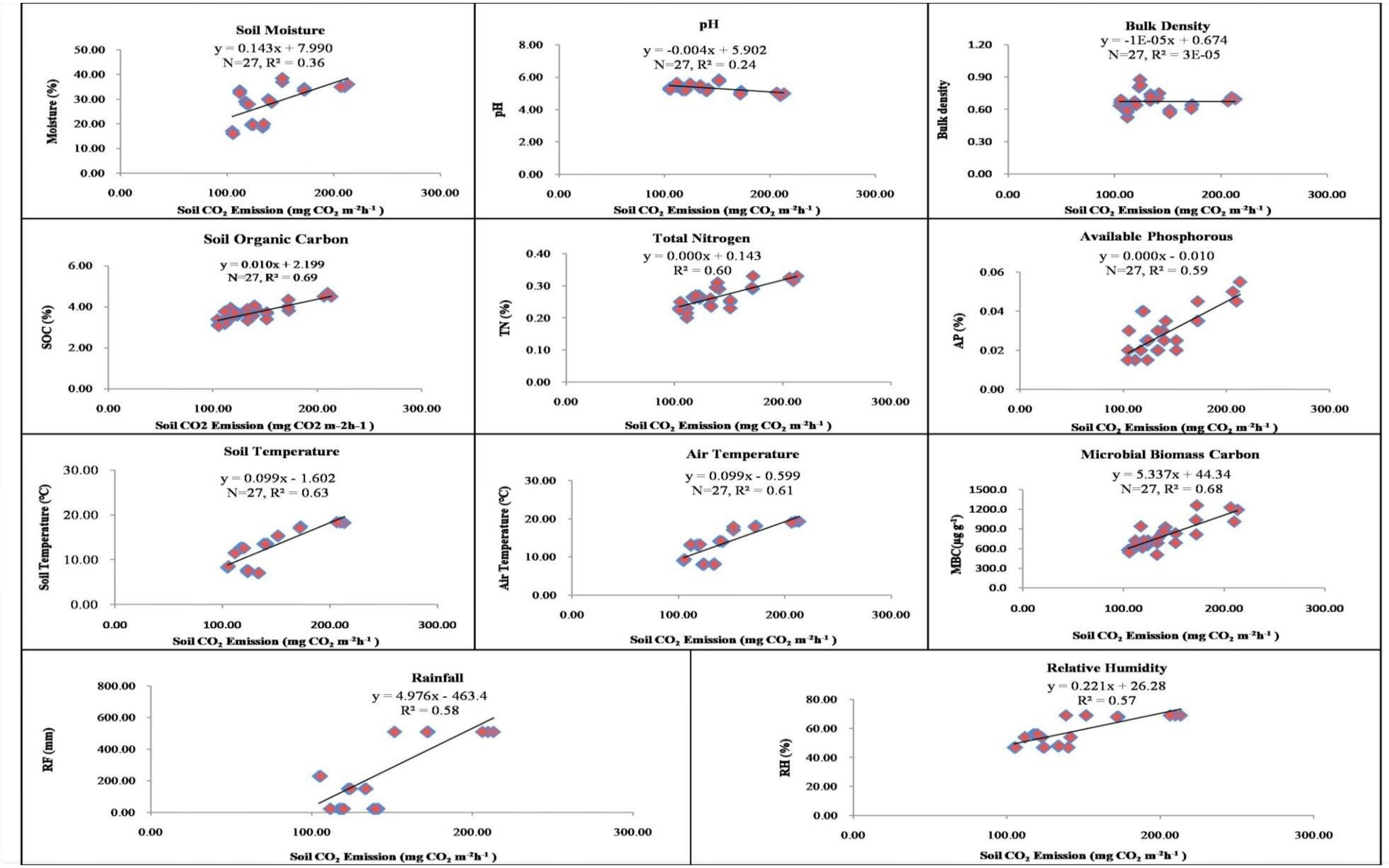


Fig 29. Relationship between soil CO_2 emission and soil properties and other climatic factors of three land-use systems

DISCUSSION

The rate of CO_2 emission significantly varies in the different land-use types and is comparatively higher in the subtropical forest (STF) (114.58-259.13 $mgCO_2m^{-2}hr^{-1}$) than that of cardamom agroforestry (AGF) (118.54-219.29 mgCO2$m^{-2}hr^{-1}$), and wet paddy cropland (WPC) (89.31-178.04 $mgCO_2m^{-2}hr^{-1}$). The higher rate of CO_2 emission in the subtropical forest site is due to high litter input in soil from the different plant species favoring the growth of microbes, which ultimately increased the release of CO_2 from the soil. Furthermore, forest has high amount of vegetation than the other ecosystems and it has been reported that land-use with vegetation cover emitted more CO_2 than bare land with higher soil organic carbon and total nitrogen content (Shi et al. 2014). Also, presence of belowground roots contributed to heterotrophic soil respiration by the microorganism (Kuzyakov and Chen 2001). Moreover, vegetation cover can change soil temperature and humidity conditions, and thus can influence on the rate of soil respiration (Raich and Tufekcioglu 2000). The lowest soil respiration rate in the paddy cropland of the present study coincides with the waterlogged soil condition during the growing period of rice in this system which inhibits the growth of microorganisms due to oxygen depletion thereby lowering the microbial CO_2 emission from soil (Liu et al. 2013b; MacCarthy et al. 2018).

Low rate of CO_2 emission in AGF than that of STF could be due to the removal of herbaceous plants and less tree density, resulting in low organic matter and microbial activity. Many studies reported enhancement of CO_2 release from the soil due to higher substrate and microbial activity (Wang et al. 2003; Zhou et al. 2013; Jiang et al. 2017), vegetation types (Raich and Tufekcioglu 2000; Law et al. 2001; Grand et al. 2016), litterfall (Bond-Lamberty et al. 2004; Yan et al. 2006), and root biomass (Han et al. 2017; Zhu et al. 2019). The present values of CO_2 emission are comparable to the values reported by several studies in different land-use systems of world (Tewary et al. 1982; Devi and Yadava 2008; Devi and Singh 2016; Jeong et al. 2018; MacCarthy et al. 2018) but less than that reported by Laishram et al. (2002) in mixed oak forest of Manipur and tropical rainforest soils (Schwedenmann et al. 2003) (Table-33).

Table 33. Comparison of soil CO_2 emission rate in different land-use systems of the world.

Land-use type	Place	CO_2 emission rate ($mgCO_2m^{-2}hr^{-1}$)	References
Tropical grassland	Haryana, India	44.00–448.00	Gupta and Singh 1981
Tropical forest	Haryana, India	90.00-1120.00	Rajvanshi and Gupta 1986
Mixed oak-conifer forest	Uttarakhand, India	101.30-270.00	Tewary et al.1982
Tropical forest	Brazil	216.00–510.00	Fernandes et al. 2002
Mixed oak forest	Manipur, India	410.80-604.00	Laishram et al. 2002
Pecan-cotton alley cropping system	USA	177.00 -776.00	Lee and Jose 2003
Tropical rainforest	Costa Rica	430.00–675.00	Schwedermann et al. 2003
Forest stand	Irish midlands, Ireland	24.00-220-00	Siaz et al. 2006
Subtropical paddy ecosystem	China	178.50-259.90	Ren et al. 2007
Subtropical mixed oak forest	Manipur, India	138.49-250.94	Devi and Yadava 2009
Dry dipteroocarp forest	Thailand	200.00–700.00	Hanpattanakit et al. 2009
Natural forest	China	93.30- 514.60	Zhou et al. 2010
Different land-use types	Arunachal Pradesh, India	135.54-296.54	Bhuyan et al. 2014
Different Ecosystems	Manipur, 2014	124.33- 586.03	Thokchom and Yadava 2014
Subtropical mixed oak forest	Manipur, India	169.24-373.20	Devi and Singh 2016

Temperate deciduous forest	Korea	173.80- 383.80	Eom et al. 2018
Temperate deciduous forest and alpine pasture	Korea	21.10-693.70	Jeong et al. 2018
Different Land-use system	Ghana	33.70-256.70	MacCarthy et al. 2018
Different Land-use system	Mizoram, India	224.00-375.00	Manpoong and Tripathi 2019
Subtropical Forest	**Sikkim, India**	**114.58-259.13**	**Present study**
Cardamom Agroforestry	**Sikkim, India**	**118.54-219.29**	**Present study**
Paddy Cropland	**Sikkim, India**	**89.31-178.04**	**Present study**

The trend of CO_2 emission rate in the present study sites exhibited a clear significant seasonal pattern in all three different land-uses with a maximum during the rainy season and minimum during the winter season. Higher CO_2 emission during the wet rainy season and least in the dry winter seasons in all the study sites coincides with favorable environmental conditions such as high soil moisture, relative humidity, soil and air temperature that accelerates the microbial activity in soil and decomposition of organic matter leading to more CO_2 emission from the soil. Other studies also reported high soil respiration rates during the wet season when plant growth and microbial activity is maximum (Laishram et al. 2002; Dechaine et al. 2005; Li et al. 2006). Also, transition from dry to the wet season, along with rapid increase in temperature and soil moisture enhanced soil CO_2 emission rates (Wu et al. 2016b). Contrastingly, dry winter season retards the microbial activity and decomposition due to unfavorable environmental factors leading to low soil respiration in soil (Devi and Yadava 2008). Thus, a low microbial respiration and root respiration during the dry winter season decrease the rate of soil CO_2 emission (Verchot et al. 2000). Similar seasonal trend of soil respiration are reported from different ecosystems which conforms with present study (Laishram et al. 2002; Saraswathi et al. 2008; Devi and Yadava 2009; Chen et al. 2013; Thokchom and Yadava 2014; Jeong et al. 2018).

Biotic and abiotic factors of the study sites also influenced the rate of CO_2 emission from soil (Fig 29). The correlation equations between the rate of soil CO_2 emission with the environmental factors in the present study (Fig 29) indicates strong influence of soil respiration by the climate, substrate availability and microbial activity of the study sites. Several other studies from different ecosystems also reported positive significant relation of CO_2 emission with these parameters (Qi et al 2002; Lee and Jose 2003; Thokchom and Yadava 2014, Zhang et al. 2015) which is in conformity with our findings. Soil temperature (R^2=0.63, $p<0.001$) and soil organic carbon (R^2=0.69, $p<0.001$) appear to be the main driving factors for the rate of soil CO_2 emission in the present study (Fig 29). Present finding is supported by several other studies from different ecosystems where soil temperature (Lee and Jose 2003; Mo et al. 2005; Devi and Yadava 2008; Shi et al. 2012; Chen et al. 2013, Song et al. 2013; Wu et al. 2016b; Jiang et al. 2017) and soil organic carbon (Liu et al. 2011; Yang et al, 2011; Li et al. 2013b; Zhou et al. 2013, Fan et al. 2015) are reported to be influential factors. However, several other studies also reported soil moisture as more influential factors (Lee et al. 2006; Devi and Yadava 2008; Deng et al. 2010; Wang et al. 2010; Arora and Chaudhry 2017; Meena et al. 2020) which is contrasting to the present study.

Microbial biomass C in soil shows a strong correlation with CO_2 emission (R^2= 0.68, $p<0.001$) in the present study which confirms that microbes have a significant contribution in CO_2 flux from the soil as microbes release more nutrients from soil organic matter, which further increases soil respiration (Lee and Jose 2003; Devi and

Yadava 2008; Iqbal et al. 2010; Arora and Chaudhary 2017; Manpoong and Tripathi 2019). Besides the above mentioned factors, other studies have shown positive influenced of soil CO_2 emission due to the amount of total nitrogen (R^2=0.60, $p<0.001$) (Fan et al. 2015; Zhu et al. 2019), available phosphorous (R^2=0.59, $p<0.001$) (Fan et al. 2015), relative humidity (R^2=0.57, $p<0.001$) and rainfall (R^2=0.58, $p<0.001$) but (Devi and Yadava 2009; Devi and Singh 2016) which is consistent with the present study. Least effect of soil pH and bulk density among the abiotic variables to soil CO2 emission in the present study is supported by the findings of Arora and Chaudhary (2017).

Conclusion

The STF has the highest rate of soil CO_2 emission followed by AGF and lowest in the WPC. Waterlogged soil condition in rice cultivation leads to least carbon emission from paddy cropland. However, croplands other than paddy may have higher soil CO_2 emission. The concentration of soil CO_2 emission is significantly affected by various abiotic parameters such as soil and air temperature, rainfall, relative humidity, soil moisture, SOC, TN, AP and soil microbial biomass. Overall, soil respiration variations in the present study were primarily controlled by soil temperature and soil organic carbon. The findings of the present investigation have shown that forests have higher emission than agroforestry and adoption of agroforestry systems will reduce carbon emission from soil.

CHAPTER-VII

GENERAL DISCUSSION AND CONCLUSIONS

GENERAL DISCUSSION AND CONCLUSIONS

The present research work entitled "Study on carbon dynamics of three different ecosystems i.e forest, agroforestry and cropland ecosystems of Sikkim Himalayas was taken up with the following objectives: i) to study the carbon stock in soil and vegetation (including the litter and microbial pools in the three ecosystems of Sikkim Himalayas ii) to estimate the rate of soil CO_2 emission in the three major land-use systems iii) comparison of the rate of carbon sequestration and to establish a relationship between the rate of carbon sequestration with abiotic and biotic variables in the three ecosystems.

The present study was carried out in three different subtropical landuse types, namely subtropical forest (STF) ($27^0$31.550'N & $88^0$29.722'E), cardamom agroforestry (AGF) ($27^0$31.311′N & $88^0$24.490′E) and wet paddy cropland (WPC) ($270^0$31.445'N & $88^0$30.380'E) located at Dzongu, North Sikkim, India with elevation ranging from 1200-1700 m asl. All the study sites are situated in sloped area, but the paddy cropland has terraced beds on the sloped surface. The study sites experienced monsoonal climate with three distinct seasons, namely summer (March-May), rainy (June-Oct) and winter seasons (Nov-Feb) and have a mean air temperature ranging from 7-22^{0}C, relative humidity 31-95% and an average annual rainfall of 2663 mm.

A total of 332 trees (≥ 30.00 cm cbh) and herbs from the STF and AGF during the study period belonging to 12 families were registered in this current study. A total of 10 species from forests and 7 species from agroforestry systems were recorded. In the STF, the tree densities and basal area were higher (188.28 individual ha^{-1}; 33.52 m^2 ha^{-1}) than that of the AGF (124.90 individual ha^{-1}; 18.28m^2ha^{-1}).

Alnus nepalensis forms the dominant tree species in tree-based landuse systems of STF (IVI 128.55) and AGF (IVI 175.74), as this tree species is favor by farmers as a shade-providing tree due to its multipurpose uses and nitrogen fixing potential. However, few other trees such as *Ficus hookeri, Schima wallichii, Machilus edulis, Lyonia ovalifolia, Macaranga pustulata, Juglans regia, Spondias axillaris* were alos reported from the study sites.

Soils of study sites has gneissic rocks as its parent material and has sandy loam texture in subtropical forest and cardamom agroforestry while in the wet paddy cropland it is clayey loam. The physical and chemical properties of the soil showed variations between land- use because of the differences in plant species composition, microclimatic conditions of the study sites and management practices adopted by each of the land use. Such a result was also reported by several studies from different land use types of the world where a change in soil properties occurred due to land-use change (Pabst et al. 2013; Ravindran and Yang 2015; Reza et al. 2018). The soil parameters also vary across the various seasons, but didn't show consistent pattern of seasonal change over land use but it vary significantly with the soil depth and show a decreasing trend across soil depth except for the clay content and bulk density in all the study sites. This is due to the shift in climatic variables and plant growth pattern due to alteration of plants physiological events such as phenology as season changes. Soil nutrients in the forest and agroforestry systems were more in the tree based sural systems than that of purely conventional agricultural systems which is due to the return of nutrients in soil by the litter from tree species and presence of nitrogen fixing tree species in the former two sites. Such a finding was reported by (Rothe et al 2002; Huang and Song 2010; Soleimani et al 2019). Changes in bioclimatic variables rapidly changes the physical and chemical properties of soil in Himalayas has been reported by (Baumler 2015).

Biomass and carbon stock from present study show that carbon stock both in vegetation, soil and litter were more in the forests and cardamom agroforestry than agricultural systems which clearly shows that trees are better carbon stores than other plants and presence of trees increases the soil carbon too. Also rate of carbon sequestration is more in those systems where trees are more than non tree based ecosystems or agricultural lands. Previous other studies on carbon sequestration also reported less carbon store and sequestration in agricultural systems (Murty et al. 2002; Deng et al. 2016). Transformation of forests to agriculture based systems lead to loss of carbon but the reverse process lead to increase in carbon sequestration. Other studies also reported carbon loss due to transformation of the forest into agroforestry (Milne and Brown 1997; Mohammod and Bekele 2014). However, adoption of tree based agricultural systems such as agroforestry can minimize the loss in carbon to some extent and bring sustainability. Increase in biomass and carbon due to the introduction of trees in agricultural systems has been reported by several studies which conform with our study (Fang et al. 2010; Murthy et al. 2013; Goswami et al. 2014; Chisanga et al. 2018). The same trend was also observed for carbon sequestration.

Soil microbial biomass carbon was significantly affected by land-use types ($p<0.001$) and season($p<0.001$) in all the study sites. Forest reported highest soil microbial biomass than cardamom agroforestry and wet paddy cropland. This is due to the less disturbance and more organic matter on the forest floor which increases microbial activity and biomass. Intensive agriculture in paddy cropland along with less organic matter and water logged soil conditions decreases soil MBC. Afforested soils with higher litter inputs reported a higher MBC (Wu et al. 2016a). Also presence of more nitrogen in the forest and agroforestry due to *Alnus sp*. increases microbial C in these two sites (Sharma et al. 2019). Soil depth also influenced MBC in all the three land-use systems due to differences in soil and biotic parameters such as bulk density and litter input in the different soil layers. Previous studies on MBC across soil depth in various land-use types also reported similar such findings(Blume et al. 2002; Wichern et al. 2003; Fall et al. 2012). Furthermore, all the land use exhibit highest concentration in the rainy season and lowest in winter and an increase in MBC was recorded in the second year as compared to the first year. The warm and wet climate during the rainy season accelerates litter decomposition due to the peak microbial activities and decomposition during this season which enhances the immobilization of nutrients by the microbes (Upadhyay et al. 1989; Usman et al. 2000; Lepcha and Devi 2020). In addition, high relative humidity during the wet period accelerates fungal growth leading to an increase in microbial biomass carbon (Acea and Carballas 1990). Least MBC during the dry and cold winter seasons correlates with low temperatures and less soil moisture, leading to the death of microorganisms that release organic carbon (Lepcha and Devi 2020), and freeze-thaw action can facilitate the decomposition of organic detritus and mineralization of carbon (Groffman et al. 2001).Similar seasonal trends of MBC were observed in different ecosystems by several researchers (Devi and Yadava 2006; Iqbal et al. 2010; Otieno et al. 2010; Patel et al. 2010;Tan et al. 2013;Huang et al. 2016).

In the present study, the cardamom agroforestry system reported a higher microbial quotient which indicates more carbon immobilization by the microbes from the organic substrates. However, the least microbial quotient in the forest may be a result of carbon mineralization from the microbes to support vegetation. The microbial quotients were more in agricultural-based systems (cropland and agroforestry) than that of the forest. Further, the highest microbial quotient ratio in the agroforestry system among the agriculture-based systems probably suggests that the carbon immobilization capabilities of microbes increased in the agri-silviculture system than in monoculture. Therefore, the adoption of the tree-based agricultural system is recommended for the mountainous regions especially to prevent soil erosion and maintain soil quality and fertility for the sustainability of the regionPrevious other study on the microbial quotient in different land-use also reported a higher value in agricultural soils than that of forest soils (Kara and Baykara 2014). The microbial quotient showed a strong significant variance with the season and soil depth. Winter season reported more immobilization of carbon and rainy season the least in all land-use type which may be due to the availability of more substrate in winter season. An increase in the microbial quotient with soil depth denotes the presence of more active carbon pools in the subsurface soil. Some studies reported that subsurface soil layers act as a store of microbial inoculation (Yi et al. 2006; Wei et al. 2009).

Soil respiration was highest for the subtropical forest due to high litter input in the forest than that of other systems which increases microbial activity and respiration. The lowest soil respiration rate in the paddy cropland of the present study coincides with the waterlogged soil condition during the growing period of rice in this system which inhibits the growth of microorganisms due to oxygen depletion thereby lowering the microbial CO_2 emission from soil (Liu et al. 2013b; MacCarthy et al. 2018). Removal of herbaceous plants and less tree density, results in low organic matter and microbial activity and CO_2 emission in AGF than that of STF. Many studies reported enhancement of CO_2 release from the soil due to higher substrate and microbial activity (Wang et al. 2003; Zhou et al. 2013; Jiang et al. 2017), vegetation types (Raich and Tufekcioglu 2000; Law et al. 2001; Grand et al. 2016), litterfall(Bond-Lamberty et al. 2004; Yan et al. 2006), and root biomass (Han et al. 2017; Zhu et al. 2019). CO_2 emission in soil was significantly affected by landuse types ($p<0.05$) and season ($p<0.05$) in all the study sites. Soil CO_2 emission showed a strong positive significant relationship with soil properties and climate data of three different land-use systems ($p<0.05$) except for soil pH where it is negatively significant ($p<0.05$). However, soil respiration in the present study were more influenced by soil temperature and soil organic carbon.

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APPENDIX

APPENDIX I: Rainfall in millimeters (R/F) in study sites (2009-2018).

RAIN FALL (mm)	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
January	0.6	0.0	31.8	96.4	9.6	0.0	22.0	81.8	7.7	15.2
February	1.6	7.1	72.4	39.5	62.8	29.8	34.0	34.9	9.0	66.4
March	16.8	26.2	79.7	208.3	120.8	163.0	180.6	306.8	112.3	216.2
April	31.2	77.4	150.3	365.3	200.5	194.4	239.4	226.7	266.1	221.0
May	131.5	424.9	190.8	139.7	615.5	417.0	498.4	383.7	315.7	397.3
June	76.5	582.8	393.8	599.2	313.6	651.6	796.4	548.0	318.3	584.2
July	192.2	531.6	442.8	595.9	530.2	605.1	393.6	487.3	485.7	688.2
August	313.0	599.1	397.2	316.2	301.8	629.8	664.7	301.6	400.6	510.8
September	125.9	329.1	261.5	785.4	310.3	297.5	320.2	638.1	274.5	751.9
October	111.3	106.7	126.8	302.4	226.7	36.7	102.8	139.8	83.3	203.5
November	6.1	111.8	68.8	0.0	82.4	25.2	59.0	0.0	6.5	68.9
December	3.9	11.2	12.4	17.5	10.4	13.6	37.5	0.0	0.0	15.1
AVERAGE	**1010.6**	**2807.9**	**2228.3**	**3465.8**	**2784.6**	**3063.7**	**3348.6**	**3148.7**	**2279.7**	**3738.7**

APPENDIX II: Air temperature (°C)in study sites (2009-2018).

TEMPERATURE (°C)	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
January	6.5	6.5	5.0	4.0	5.0	6.0	7.0	7.0	8.0	9.0
February	8.0	7.5	6.5	6.5	7.5	7.0	9.5	10.5	11.0	10.0
March	10.0	11.0	10.0	9.5	11.0	9.5	12.0	14.0	11.5	12.0
April	13.5	14.0	12.5	12.5	13.0	14.0	15.0	16.5	16.0	14.0
May	14.0	15.0	15.5	15.5	15.5	16.0	18.0	17.0	18.0	16.0
June	16.5	16.5	17.5	17.5	18.5	18.5	19.5	19.5	19.5	19.0
July	17.0	16.5	17.5	18.5	18.5	18.5	20.0	19.0	19.5	19.0
August	16.5	18.0	17.0	18.5	17.0	18.0	18.5	20.5	19.5	19.5
September	16.5	16.5	15.0	17.0	16.5	16.5	17.0	19.0	18.5	18.0
October	13.0	13.5	13.0	12.5	14.5	13.0	14.5	17.0	15.5	13.0
November	10.0	9.5	9.0	9.0	11.0	10.0	12.0	13.5	12.5	10.5
December	6.5	7.0	6.5	7.0	8.0	7.5	9.0	11.0	12.0	7.5
AVERAGE	12.3	12.6	12.1	12.3	13.0	12.9	14.3	15.4	15.1	14.0

APPENDIX III: Rel. Frequency, Rel. Density, Rel. Basal Area and IVI of tree species of subtropical forest.

SL NO.	SPECIES NAME	REL. FREQUENCY	REL. DENSITY	REL. BASAL AREA	IVI
1	*Alnus nepalensis*	32.26	52.13	44.16	128.55
2	*Macaranga pustulata*	6.45	4.79	0.66	11.90
3	*Juglans regia*	9.69	4.25	6.40	20.34
4	*Lyonia ovalifolia*	16.13	13.30	5.28	34.71
5	*Schima wallichi*	3.23	4.79	7.14	15.15
6	*Machilus edulis*	9.68	9.04	33.06	51.78
7	*Symplocos theifolia*	6.45	6.38	1.07	13.90
8	*Spondias axillaris*	3.23	1.06	1.11	5.40
9	*Ficus racemosa*	9.68	2.66	0.27	12.60
10	*Castanopsis indica*	3.23	1.60	0.86	5.68
	Total	**100**	**100**	**100**	**300.00**

APPENDIX IV: Frequency, Rel. Density, Rel. Basal Area and IVI of tree species of cardamom agroforestry.

SL NO.	SPECIES NAME	REL. FREQUENCY	REL. DENSITY	REL. BASAL AREA	IVI
1	*Alnus nepalensis*	33.33	64.52	77.89	175.74
2	*Macaranga pustulata*	13.33	4.84	4.44	22.61
3	*Juglans regia*	6.67	3.23	6.38	16.27
4	*Toona ciliate*	13.33	3.23	3.17	19.72
5	*Spondias axillaris*	6.67	1.61	2.12	10.40
6	*Viburnum cordifolium*	6.67	1.61	0.37	8.65
7	*Ficus hookeriana*	13.33	14.52	4.36	32.21
8	*Ficus racemosa*	6.67	6.45	1.27	14.39
	Total	**100**	**100**	**100**	**300.00**

APPENDIX V: Change in magnitude of soil carbon stock, vegetation biomass carbon stock and total ecosystem carbon stock through land use changes (2016-18).

Land use changes types	**SOCS Changes**				**VBCS Changes**			**TECS Changes**
	0-15 cm	15-30 cm	30-45 cm	0-45 cm	AGBC	BGBC	Total	
2016								
STF to CAF	-0.85	-2.28	-1.79	-4.91	-27.45	-7.14	-34.59	**-41.93**
STF to WPC	-6.83	-6.53	-4.79	-18.15	-54.10	-13.65	-67.75	**-93.67**
CAF to STF	0.85	2.28	1.79	4.91	27.45	7.14	34.59	**41.93**
CAF to WPC	-5.98	-4.25	-3	-13.24	-26.65	-6.51	-33.14	**-51.74**
WPC to STF	6.83	6.53	4.79	18.15	54.10	13.65	67.75	**93.67**
WPC to CAF	5.98	4.25	3	13.24	26.65	6.51	33.16	**51.74**
2018								
STF to CAF	-3.31	-0.55	-1.47	-5.33	.27.91	-7.25	-35.16	**-42.65**
STF to WPC	-6.81	-6.35	-6.86	-20.02	-56.34	-14.29	-70.63	**-98.59**
CAF to STF	3.31	0.55	1.47	5.33	27.91	7.25	35.16	**42.65**
CAF to WPC	-3.5	-5.8	-5.39	-14.69	-28.43	-7.04	-35.47	**-55.94**
WPC to STF	6.81	6.35	6.86	20.02	56.34	14.29	70.63	**98.59**
WPC to CAF	3.5	5.8	5.39	14.69	28.43	7.04	35.47	**55.94**

APPENDIX VI: Change in magnitude of soil carbon sequestration, vegetation biomass carbon sequestration and total ecosystem carbon stock through land use changes (2016-2018).

STOCK SEQUESTRATION 2016-2018 (TECS)			
Land use changes types	**SOCS Sequestration (0-45CM)**	**VBCS Sequestration**	**TECS Sequestration**
STF to CAF	-0.3	-0.42	**-0.72**
STF to WPC	-3.04	-1.88	**-4.92**
CAF to STF	0.3	0.42	**0.72**
CAF to WPC	-2.74	-1.46	**-4.2**
WPC to STF	3.04	1.88	**4.92**
WPC to CAF	2.74	1.46	**4.2**

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Carbon storage potential of a traditional cardamom agroforestry system of Sikkim Himalayas

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ABSTRACT

The carbon storage potential of a 20-year-old traditional cardamom agroforestry system of Sikkim Himalayas was studied by using volume equations, wood specific gravity, biomass expansion factor and default carbon fraction for trees species and complete harvest of herbaceous vegetation. The aboveground biomass (AGB) and aboveground biomass carbon (AGBC) of trees were 81.91± 9.04 Mg ha^{-1} and 38.47 ± 4.25 Mg C ha^{-1} while herbaceous carbon was 2.20±0.22 Mg C ha^{-1}, of which cardamom crop contributed 1.02±0.10 Mg C ha^{-1}. Carbon density showed a positive and significant relation with tree girth size, tree density, basal area, and above ground biomass ($p<0.01$). Annual detritus carbon was 3.64±0.20 Mg C ha^{-1}while total soil organic carbon varied from 89.90±2.16-117.91±3.12 Mg C ha^{-1}. SOC contributed highest carbon (69.5%) followed by AGBC (21%), BGBC (5.5%), detritus (2.5%) and herbaceous (1.5%) to the total ecosystem carbon. Traditional cardamom agroforestry practices of the Himalayan region besides providing livelihood benefits to the locals also act as a potential carbon store in the Himalayas.

Keywords: *Aboveground biomass, Soil organic carbon, Carbon stock, Livelihood benefit, Agroforestry.*

Introduction

Increasing human population and demand for food leads to change in land-use system which is one of the important aspects of increasing atmospheric carbon dioxide. Various practices of different land use, such as reforestration, afforestration, and agroforestry reduce the CO_2 concentration in the atmosphere (Canadell and Raupach, 2008).This conversion of land use system result in loss of the carbon value in both vegetation and soil (Post and Know 2000). Agroforestry system work as carbon sinks in both soil and vegetation to mitigate climate change (Goswami *et al.*, 2014). Fourth assessment report on climate change by International Panel on Climate Change(IPCC 2006) has recognized that agroforestry own high potential for sequestering carbon of all land uses in developing countries (Watson *et al.*, 2000; Smith *et al.*, 2007). The tropical agroforestry system has high potential to store carbon at the range of 12-228 Mg ha^{-1} (Albercht and Kandji 2003). According to (Watson et al. 2000), by 2040 the potential of carbon sequestration of agroforestry would be 586 Mt C per year from the available 630 million hectares areas. In any agroforestry systems, the carbon value depends on the structure and function of the different components present in different systems (Albercht and Kandji 2003). Besides, carbon capture, these land use system also provide other environmental benefits to the surrounding inhabitants directly or indirectly, such as fuel, food, fodder, timber, soil and water conservation, biodiversity conservation etc. (Kumar et al. 2012).

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RESEARCH ARTICLE

Carbon cycling and balance in a traditional cardamom based agroforestry system of Sikkim Himalayas

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Abstract
The pattern of carbon cycling dynamics in a 20 year old traditional cardamom agroforestry system located at Sikkim Himalaya was studied for two years to understand the dynamics of different carbon pools and carbon emission patterns in the system. Total biomass (aboveground + belowground) and carbon in trees were 81.91 ± 9.04 Mg ha^{-1} and 38.47 ± 4.25 Mg C ha^{-1}, respectively and contributed 27% of the total ecosystem carbon. Herbs and detritus carbon were 2.34 ± 0.24 Mg C ha^{-1} and 3.64 ± 0.20 Mg C ha^{-1} respectively while cumulative soil organic carbon (SOC) stock ranged from 89.90 ± 2.16 (January) to 117.91 ± 3.12 Mg C ha^{-1}(August) in the 0–45 cm soil layer. Seasonal microbial biomass carbon (MBC) stock varied from 501.34 to 857.77 μg g^{-1} while annual CO_2 emission (SR) ranged from 112.11–219.29 mg CO_2 m^{-2} h^{-1}. SOC, MBC and SR exhibited strong seasonality with a peak value in rainy and least in the winter season. Carbon density of trees exhibited a positive and significant relation with tree density ($P < 0.05$), basal area ($P < 0.01$) and aboveground biomass ($P < 0.01$). All the abiotic variables with the exception of bulk density and soil pH showed strong positive and significant relationship with SOC, MBC and SR. Total carbon sequestration of 11.91 Mg C ha^{-1} year^{-1} and release of 3.46 Mg C ha^{-1}year^{-1} resulting to a net ecosystem carbon balance of 8.45 Mg C ha^{-1} year^{-1} by a traditional agroforestry system suggests that besides provision of livelihood opportunities it can be used as an adaptation strategy in agricultural systems for mitigation of climate change.

Keywords Aboveground biomass · Carbon stock · CO_2 emission · Climate change · Microbial biomass · Carbon balance · Soil organic carbon

Introduction

Agroforestry are land use systems where woody perennials like trees, shrubs, palms and bamboos are incorporated along with agricultural crops and livestock (Nair 1993; Negash and Kanninen 2015). It is an old practice of farming system adopted by farmers all throughout the world but recently came to limelight due to policies adopted by World Bank and Food and agriculture organization (FAO) and its multiple benefits including provision of food security, reduction in poverty, ecological benefits and many more (Nair 1993). The developing world especially of Asia and Africa is facing certain challenges such as food and water scarcity, population pressure, poverty, deforestation, fertility loss, land degradation and climate change (Kay et al. 2019). Among the challenges, the risk of changing climate to yield, adaptability and suitability of crop and effort for sustainable crop production is a major concern for the farmers. In spite of adoption of different strategies for sustainable crop production and other greening techniques to reduce ecological damage, the agricultural sector is one of the major causes of pressure on natural resources and environment (EEA 2017). To address such issues certain policies are adopted by different countries including the Nitrate Directive, 1991 and Water Framework Directive, 2000 adopted by European commission and National Agroforestry policy, 2014 adopted by India. Also, recently the COP21 Paris Agreement (UNFCCC 2015) the Effort Sharing 2021–2030 (REGULATION (EU) 2018/842) includes agricultural practices, aiming to reduce greenhouse gas (GHG) emissions or balance with an equal amount of GHG sequestration (Kay et al. 2019). Appropriate agroforestry system helps in the maintenance of organic matter and physical characteristics reduce soil

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erosion, augment nitrogen build up through nitrogen fixing trees elevating efficient nutrient cycling (Patiram et al. 2003). Several works highlighted agroforestry practice as one of the agricultural practices that can adapt and mitigate climate change (Aertsens et al. 2013; Hart et al. 2017); sequester carbon in biomass and soil (Kim et al. 2016); increase soil organic matter and water availability (Murphy 2015); protect crops, pastures and livestock against harsh environmental events (Sànchez and McCollin 2015). Keeping in view this background we intend to study the carbon sink and source function of one of the largest agroforestry systems of Eastern Himalayas i.e. a cardamom agroforestry system. Cardamom agroforestry system of Sikkim, a mountainous state of India located in the Eastern Himalayas, a biodiversity hotspot of world and other Himalayan regions are transformed from natural forests by the locals in order to sustain their livelihood (Rai et al. 1994) are very close to a natural forest and provide ecosystem services similar to a natural forest (Sharma et al. 2007). Previous studies on cardamom agroforestry reported from the Eastern Himalayan region mainly focused on the ecological services (Sharma et al. 2007), carbon sequestration and soil and water conservation (Sharma et al. 2008), management diversity (Sharma et al. 2009). However, the carbon cycling pattern of this important agroforestry of the Eastern Himalayas remained poorly understood. Therefore, this study estimates (i) carbon storage in vegetation, litter, soil and microbes, (ii) CO_2 release from soil to the atmosphere, and (iii) carbon sequestration and balance in a traditional cardamom agroforestry system of Sikkim Himalayas.

Material and methods

Study area

The study site i.e. traditional cardamom agroforestry system was located at Dzongu, North Sikkim, India situated at 27°31.311′ E and 88°24.490′ N at an altitude ranging from 1350 to 1619 m above mean sea level (Fig. 1). Approximate area of the study site is 15.5 hectares and this agroforestry system was transformed from a rice field about 20 years ago. The climate of the area is monsoonal with three distinct seasons namely summer, rainy and winter seasons. Main shading tree in the study area is *Alnus nepalensis,* however, few other tree species such as *Schima wallichii, Macaranga pustulata,* and herbaceous species such as *Ageratum conyzoides, Drymaria cordata, Oxalis corniculata, Diplazium esculentum, Spilanthes calva, Galinsoga parviflora, Persicaria runcinata, Pilea macrophyla, Pouzolzia hirta, Cynodon* sp., *Eupatorium* sp., *Setaria* sp., *Carex* sp., *Sonchus* sp., *Achyanthes* sp., *Persicaria* sp., *Cypesus* sp., *Athyrium* sp. were also present. During the study period, average temperature ranged from 10 to 24 °C; relative humidity varied from 31 to 95% and annual rainfall was 2663 mm (Meteorological Station, Gangtok). Soil was loamy with a pH ranging from 4.2 to 5.8 in the study site.

Soil sampling for physicochemical characteristics

For the analyses of physicochemical characteristics of soil, seasonal soil samples collected from ten different locations across three soil depths, i.e. 0–15 cm, 15–30 cm, and 30–45 cm by using a soil corer of 5.2 cm diameter for two consecutive years (2016 and 2017). Five replicates of each soil sample from each soil depth were used for analysis. Soil samples were brought into the laboratory in sealed polythene bags, coarse material, gravel and live roots were sorted out manually and each soil sample was separated into two parts. The first half of fresh soil samples were used for the analysis of soil moisture by the gravimetric method (80 °C for 48 h). Soil temperature was determined by using a soil thermometer and bulk density was determined by the oven-drying of known volume of fresh soil (80 °C) using the formula of Ravindranath and Ostwald (2008). The remaining part of soil sample was air-dried, crushed and passed through a sieve (2 mm) and the sieved soil samples are used for analyses of soil pH using an auto digital pH meter (1:5 soil distilled water suspension), texture by hydrometer method (Allen et al. 1974) and total nitrogen and available phosphorous in soil were determined by using Kjeltec 8500 (FOSS) and ammonium molybdate stannous chloride method (Devi and Yadava 2006) respectively (Table 1).

Aboveground biomass carbon estimation and sequestration

The study site was demarcated into five plots and inside each plot two random quadrats of 31.6 m × 31.6 m were earmarked. Aboveground biomass of trees for two consecutive years (2016 and 2017) was estimated allometrically to estimate biomass carbon and sequestration of carbon in the study site. All individual trees ≥ 10 cm diameter at breast height (DBH) 1.3 m within the plots were enumerated and numbered. Specific gravity of wood for each tree species was computed by oven drying the wood samples collected by using an increment borer at a height of 1.3 m above the ground. Volume equations of FSI (FSI 1996) were used to calculate the tree volume of each tree species which is used to compute the aboveground biomass (IPCC 2006; Ravindranath and Ostwald 2008).

$$\text{Aboveground Biomass} = \text{Volume of tree} \times \text{Specific gravity} \times \text{BEF},$$

where BEF is the biomass expansion factor.

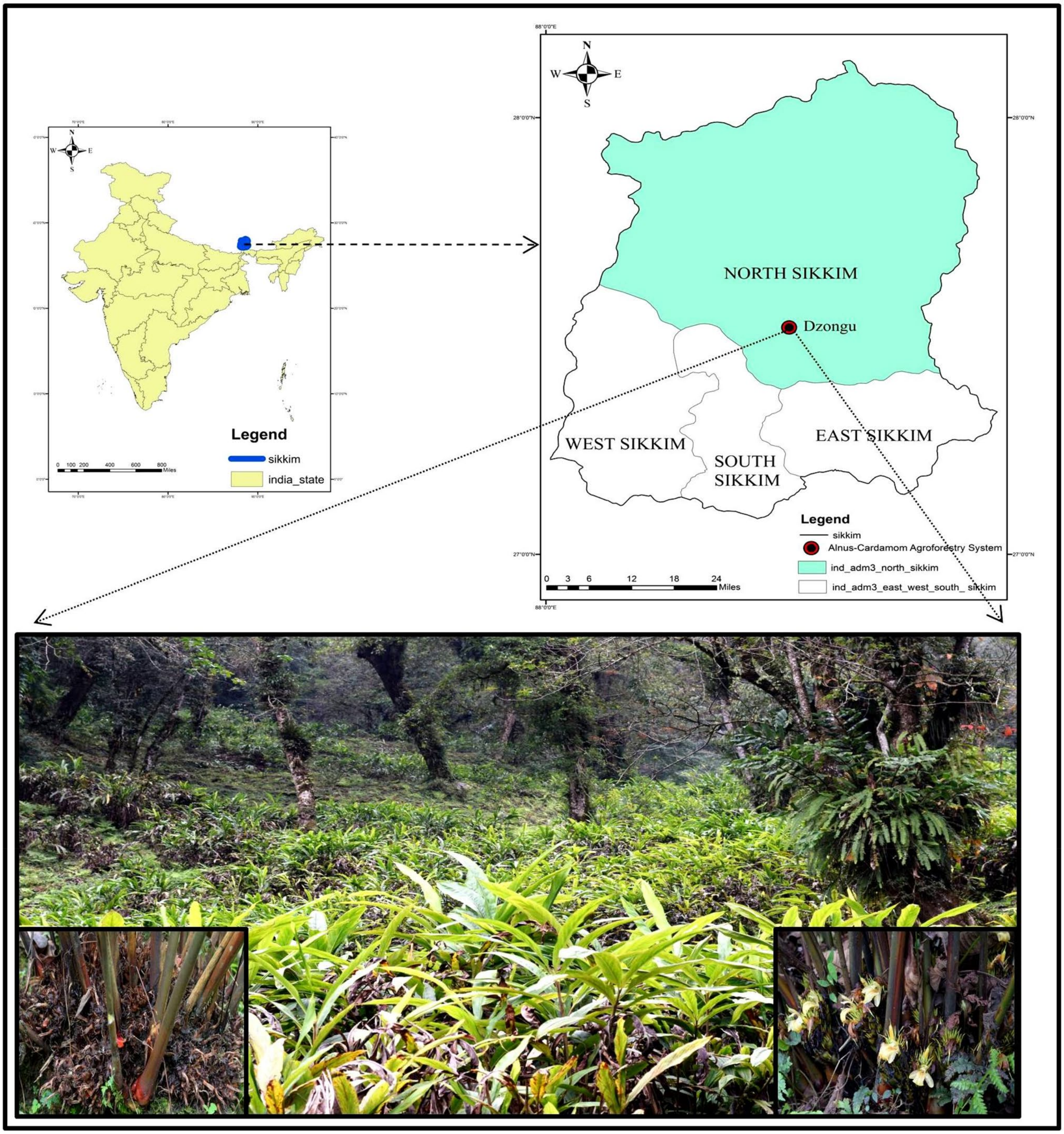

Fig. 1 Map showing study site of cardamom agroforestry of Sikkim Himalayas

Quantification of belowground biomass (BGB) of trees was done using standard root to shoot ratio of 0.26 (Ravindranath and Ostwald 2008).

Total biomass was calculated by adding aboveground biomass and belowground biomass and carbon stock in trees was computed using the following formula (Ravidranath and Ostwald 2008):

$$C = \text{ Total AGB } \times 0.47,$$

where AGB is aboveground biomass, C is a carbon (Mg ha^{-1}), 0.47 is the IPCC default carbon fraction.

The annual carbon sequestration of trees was estimated from the difference of annual carbon stock.

Table 1 Soil properties of cardamom agroforestry system

Soil parameter	Cardamom agroforestry system
Soil properties	
Soil pH	5.00 ± 0.43
Moisture content (%)	37.00 ± 5.62
Soil temperature (°C)	15.60 ± 3.41
Bulk density (g cm^{-3})	0.79 ± 0.31
Soil texture	
Clay (%)	23.00 ± 5.32
Sand (%)	30.00 ± 2.78
Silt (%)	47.00 ± 3.01
Organic carbon (%)	3.35 ± 0.21
Total nitrogen (%)	0.37 ± 0.01
Available phosphorous (%)	0.01 ± 0.01
C:N Ratio	9.05

$$\text{C Sequestration} = \text{BC}_2 - \text{BC}_1,$$

where BC_1 = Biomass carbon of the first year and BC_2 = Biomass carbon of the second year.

To estimate herbaceous biomass, all the herbs from 10 quadrats of 1 m × 1 m size located inside each of the above-described plots were harvested during the rainy seasons of 2016 and 2017. All herbaceous plants, including cardamom, were brought to the laboratory, washed and weighed and oven-dried (80 °C) to achieve a constant weight and weighed to estimate biomass. Carbon in the herbs was computed using the default carbon fractions of IPCC (2006).

Detritus biomass

Monthly litter collected from ten quadrats (1 m × 1 m size) during 2016 and 2017 were brought to laboratory, washed and dried in an oven at 80 °C till weight becomes constant and weighed. Annual detritus carbon was computed from the average annual litterfall using carbon default value 0.47 (IPCC 2006).

Soil organic carbon stock and sequestration

Thirty soil samples were collected from ten different soil profiles to estimate soil carbon stock and sequestration in soil (SOC) from three soil depths, i.e. 0–15 cm, 15–30 cm, and 30–45 cm on monthly interval of 2016 and 2017 by using a soil corer (5.2 cm diameter). Samples were crushed and passed through a sieve (2 mm) to separate out coarse material and gravel and live roots were sorted out manually. The sieved soil samples were colorimetrically analyzed for organic carbon content (Anderson and Ingram 1993). SOC for each soil depth was estimated using the formula provided by Ravindranath and Ostwald (2008).

$$\text{SOC}\left(\text{Mg ha}^{-1}\right) = \text{bulk density}\left(\text{g cm}^{-3}\right) \times \text{soil depth interval (cm)} \times \text{SOC (\%)}.$$

Microbial biomass carbon (MBC)

Microbial biomass carbon in soil was analyzed seasonally from sixty fresh soil samples collected from the study site from ten different pits across two different soil depths (0–15 cm, 15–30 cm). Samples were immediately brought to the laboratory in sealed polythene bags, removed rocks and other debris including roots and stones. Half of the soil samples from each soil depth (0–15 cm and 15–30 cm) were fumigated with 30 ml of alcohol-free chloroform by using a vacuum pump and incubated for 5 days and extracted with 0.5 M potassium sulphate. The other half of the soil samples were extracted with 0.5 M potassium sulphate, incubated for 5 days and used for the estimation of microbial carbon following the procedure of Anderson and Ingram (1993). The microbial biomass carbon was calculated using the formula (Haripal and Sahoo 2014):

$$\text{MBC} = \text{E}_\text{C} \times 2.64,$$

E_C is C fumigated–C unfumigated soil samples.

Soil CO_2 emission

Emission of CO_2 from soil was measured by the alkali absorption method (Anderson and Ingram 1993). Six open-ended cylinders with 13 cm diameter 25 cm height were inserted up to 15 cm in the soil. All the green plants and stones inside the cylinder were removed. One cylinder was used as a blank sample out of the six cylinders and the entire vegetation and soil surface were sealed. Fifty (50 ml) of 0.25 N NaOH solution was kept in each cylinder including the blank cylinder and sealed to make the whole setup airtight by using anchor grip, kept for 24 h and thereafter titrated with 0.25 N HCL solution using phenolphthalein indicator. CO_2 absorbed from the soil was calculated using the formula (Anderson and Ingram 1993):

$$\text{CO}_2\text{mg} = \text{V} \times \text{N} \times 22,$$

where V is the volume of HCL, N is the normality of HCL.

Data analysis

Regression analysis between carbon density of trees and other biotic variables like tree density, basal area, and biomass stock was performed. The relation between biotic (soil organic carbon, microbial biomass carbon and soil respiration) and abiotic variables in the study site was studied using Pearson's multiple correlation analysis using SPSS software (version 21.0).

Result and discussion

Biomass carbon and sequestration

Highest tree density for *Alnus nepalensis* (80.13 tree ha^{-1}) and lowest for *Rhus semmilata* and *Viburnum* sp. (2.00 trees ha^{-1} each) is because of the preference and retention of nitrogen fixing *Alnus* tree to maintain soil fertility and productivity (Sharma et al 1994) by the farmers over other tree species (Table 2). Total tree density (124.19 trees ha^{-1}) of the present cardamom agroforestry system was comparable to poplar agroforestry systems of China (Fang et al. 2010). However, lower tree densities in poplar agroforestry of Northwestern India (Rizvi et al. 2011) and coffee agroforestry of Guatemala (Schmitt-Harsh et al. 2012) indicates that cardamom agroforestry systems of Eastern Himalayas promote and encourage higher tree growth and is very closely similar to a natural forest (Sharma et al. 2007). Lower Basal area of trees in the present study (18.28 m^2 ha^{-1}) as compare to tea agroforestry of Assam (Kalita et al. 2016) and managed plantation and jhum fallow agroforestry of Tripura (Chaudhary et al. 2016) may be due to young age of the trees in the agroforestry system.

Total biomass (aboveground + belowground) and carbon in trees were 81.91 ± 9.04 Mg ha^{-1} and 38.47 ± 4.25 Mg C ha^{-1}, respectively (Table 2) which contributed 27% of the total ecosystem carbon (Fig. 2). Present value of biomass and carbon is comparable to those of other agroforestry systems in the world (Takimoto et al. 2008; Rizvi et al. 2011; Prasad et al. 2012; Kalita et al. 2016), however, it is higher from the values reported from other agroforestry systems in India and China (Nair et al. 2010; Xie et al. 2017; Yadav et al. 2017). Among the tree species maximum carbon stock (27.01 ± 1.04 Mg C ha^{-1}) was contributed by *Alnus nepalensis* and minimum by *Viburnum cordifolium* (0.16 ± 0.03 Mg C ha^{-1}) corresponds to highest tree density and basal area of *Alnus* and lowest of *Viburnum sp*. (Table 2). Carbon storage in the trees of different girth sizes did not have a specific pattern but high C storage coincides with high tree densities and basal areas rather than the girth size of trees (Table 2). Our finding agrees with the report of Kalita et al. (2016) from a tea agroforestry system of Assam but contrasting to that of a *Dipterocarpus* forest of Manipur (Devi and Yadava 2016) where tree carbon densities increased with an increase in girth size that may be related to the difference in the carbon

Table 2 Tree density, aboveground biomass, belowground biomass, total biomass stock and carbon stock in trees of cardamom agroforestry system

Species	Tree density (tree ha^{-1})	Basal Area (m^2 ha^{-1})	Biomass (Mg ha^{-1})	Carbon (Mg C ha^{-1})
Alnus nepalensis D. Don	80.13	13.1	57.47 ± 2.21	27.01 ± 1.04
Ficus racemosa Willd	26.04	0.85	10.27 ± 1.22	4.83 ± 0.57
Juglans regia L	4.01	1.02	5.05 ± 0.89	2.37 ± 0.42
Toona ciliata M.Roem	4.01	0.95	4.36 ± 0.76	2.04 ± 0.34
Macaranga pustulata King ex Hook.f	6.01	1.22	4.01 ± 0.66	1.88 ± 0.31
Spondias axillaris Roxb	2	0.69	0.39 ± 0.11	0.18 ± 0.05
Viburnum cordifolium Wall. Ex DC	2	0.45	0.35 ± 0.08	0.16 ± 0.03
Total	124.2	18.28	81.91 ± 5.89	38.47 ± 2.77
Herbs	–	–	4.69 ± 0.48	2.34 ± 0.22
Detritus	–	–	7.28 ± 0.49	3.64 ± 0.20

Diameter class(cm)	Biomass (Mg ha^{-1})			Tree DBH (m)	Carbon (Mg C ha^{-1})	
	AGB	BGB	Total			
30–60	9.78 ± 0.20	2.54 ± 0.05	12.32 ± 0.25	5.76 ± 0.08	5.80 ± 0.12	$P < 0.01$ $r^2 = 0.90$
60–90	2.67 ± 0.29	0.68 ± 0.08	3.35 ± 0.37	2.78 ± 0.11	1.58 ± 0.17	
90–120	8.77 ± 0.34	2.28 ± 0.09	11.05 ± 0.44	9.59 ± 0.08	5.19 ± 0.20	
120–150	19.68 ± 0.47	5.12 ± 0.12	24.80 ± 0.59	26.51 ± 0.10	11.66 ± 0.28	
150–180	19.27 ± 0.85	5.01 ± 0.22	24.28 ± 1.07	20.9 ± 0.07	11.41 ± 0.51	
< 180	4.85 ± 0.26	1.26 ± 0.07	6.11 ± 0.33	6.45 ± 0.30	2.87 ± 0.16	
Total	65.02 ± 2.41	16.89 ± 0.63	81.91 ± 3.04	71.99 ± 0.74	38.51 ± 1.44	

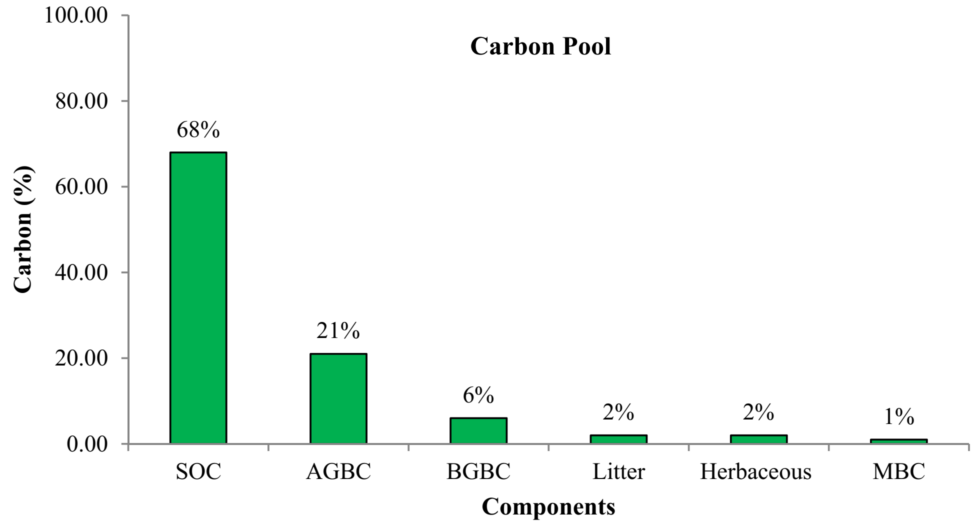

Fig. 2 Contribution of different carbon pools to the total ecosystem carbon

capture pattern of different species. Also, carbon capture rate in vegetation is influenced by geographical area, plant species, species diversity, and age of a tree (Liu et al. 2015). Linear regression models between carbon density and tree density ($r^2 = 0.63$, $P < 0.05$), basal area ($r^2 = 0.79$, $P < 0.01$) and aboveground biomass ($r^2 = 1.00$, $P < 0.01$) indicates strong influence of these parameters to carbon density (Fig. 3). Our findings are in agreement with those of different subtropical ecosystems of Northeast India (Borah et al. 2015; Chaudhary et al. 2016).

Herbaceous and detritus biomass

Herbaceous biomass and carbon including the contribution of cardamom crops was 4.69 ± 0.48 Mg ha^{-1} and 2.34 ± 0.24 Mg C ha^{-1} respectively in the study site (Table 2) and contributed 2.00% to the total ecosystem carbon (Fig. 2), which is higher from different ecosystems (natural forests, managed plantation and jhum fallow) of North east India (Chaudhary et al. 2016; Devi and Yadava 2016). Annual detritus carbon (3.64 ± 0.20 Mg C ha^{-1}) contributed 2.0% to the total ecosystem carbon in agroforestry (Fig. 2) with a peak detritus during the winter season and least during the summer season which agrees with other studies from other agroforestry systems (Hager 2012; Ramesh et al. 2015) of the world.

Soil organic carbon stock (SOC) and sequestration

The cumulative monthly soil organic carbon stock in 0-45 cm soil depth varied significantly between the months 89.90 ± 2.16 (January) to 117.91 ± 3.12 Mg C ha^{-1}(August) showing highest values in the rainy months and lowest during the winter months (Fig. 4). The highest SOC stock in the month of August (117.91 ± 3.12 Mg C ha^{-1}) during the rainy season coincides with higher soil moisture and temperature that probably accelerated the rate of litter decomposition by the microbes as revealed from the highest microbial biomass carbon during this season (857.77 µg g^{-1}). Limitation of moisture along with low air and soil temperatures in the dry winter months result to less microbial activities and SOC. Several studies in different systems also reported similar trend of SOC (Upadhyay and Singh 1989; Devi and Yadava 2006; Bargali et al. 2018). The present range of SOC stock is within the reported range of Nair et al. (2010) (30–300 Mg C ha^{-1}) for agroforestry systems. Annual sequestration of 6.04 Mg C ha^{-1} calculated from the difference of soil carbon stocks of two consecutive years (100.61 Mg C ha^{-1} in the first year and 106.65 Mg C ha^{-1} in the second year) agrees with the values reported from different land-use systems namely natural forest, managed plantation and jhum fallows of Tripura, North-East India (Chaudhary et al. 2016), and three agro-climatic zones of Chiapas agroforestry, Mexico (Sata-Pinto et al. 2010). The bulk density increases with soil depth while SOC decreases due to more organic matter content in the upper soil layer than that of sub surface layers. Similar findings were reported by many studies (Jobbágy and Jackson 2000; Dar and Somaiah 2015; Singh et al. 2018; Soleimani et al. 2019). SOC exhibited strong positive relation with all the abiotic and biotic variables with the exception of soil pH and bulk density where the relation is insignificant and negative (Table 3). Such relationships were reported from different land-use system of India (Shrestha et al. 2004; Ramesh et al. 2015; Chaudhary et al. 2016).

Soil microbial biomass carbon (MBC)

Soil microbial biomass carbon in the present study varied from 501.34 to 857.77 µg g^{-1} (0.736 Mg C ha^{-1}) across different seasons (Table 4) and contributed 0.53% carbon to

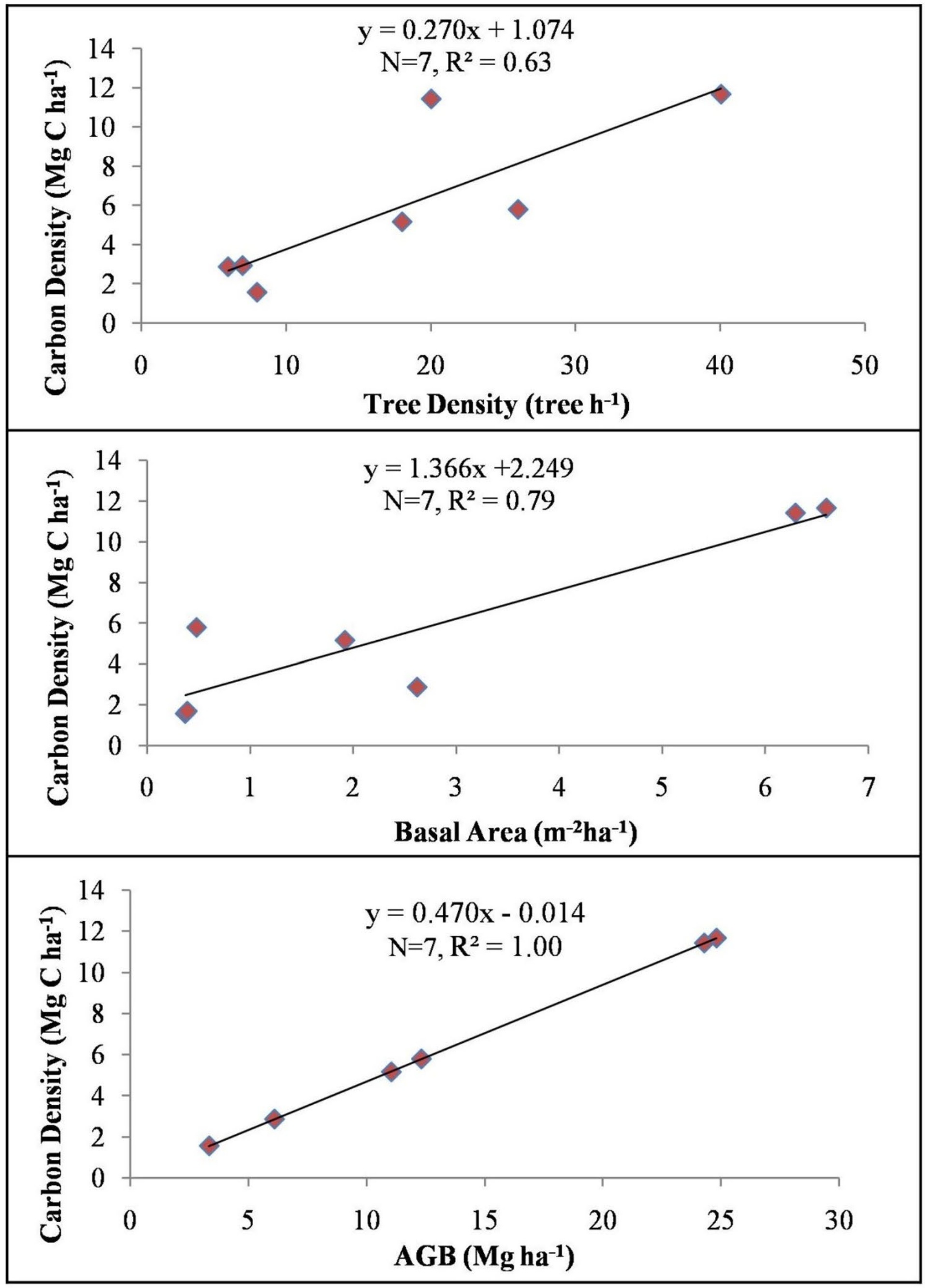

Fig. 3 Regression models between carbon density and tree density, basal area, and biomass stock in study site

total ecosystem carbon. Microbial biomass carbon decreased with soil depth (Table 4) due to the presence of more organic matter in the top humus soil that promotes microbial activity. Maximum MBC during the rainy season and minimum during the winter season may be attributed to availability of substrate carbon from the last fall and favorable congenial environment for the microbes during the warm rainy season while a low temperature during the dry winter season retards the activity of the microbes. MBC increased with moderate warming (Liu et al. 2011) provided water or moisture is not a limitation (Allison and Treseder 2008; Xu et al. 2013). The present value of MBC is comparable to various systems of Northeast India i.e. traditional agroforestry system – 47.50 to 1167.00 μg g^{-1}, (Tangjang et al. 2010), subtropical humid forest of Meghalaya – 203.74 to 1087.70 μg g^{-1} (Maithani et al. 1996), and mixed oak forest of Manipur – 71.10 to 1412.60 μg g^{-1}, (Devi and Yadava 2006) however, the present value is higher than that of various ecosystems of Northern India i.e. rice-berseem cropping, tree plantations and agroforestry systems – 90.56 to 168.00 μg g^{-1}, (Kaur et al. 2000). A positive and significant relationship between microbial biomass carbon and soil organic carbon, soil

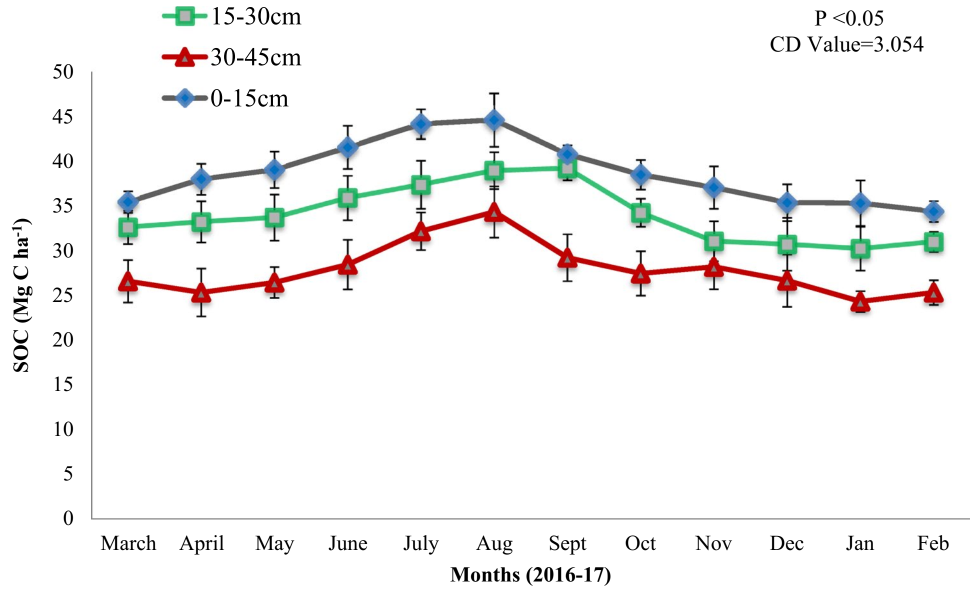

Fig. 4 Monthly soil organic carbon (SOC) concentration in different soil layers (mean ± SE)

Table 3 Pearson correlation matrix between the abiotic variables and MBC, SOC and SR

	SOC	MBC	SR	M	BD	pH	TN	AP	ST	AT	RH	RF
SOC	1											
MBC	0.96**	1										
SR	0.96**	0.99**	1									
M	0.91**	.88**	0.89**	1								
BD	−0.43	−0.47*	−0.42	−0.73**	1							
pH	−0.28	−0.30	−0.27	−0.61**	0.90**	1						
TN	0.84**	0.90**	0.90**	0.86**	−0.41	−0.33	1					
AP	0.66**	0.71**	0.77**	0.69**	−0.44	−0.38	0.073**	1				
ST	0.89**	0.87**	0.88**	0.98**	−0.77**	−0.67**	0.82**	0.75**	1			
AT	.88**	0.87**	0.88**	0.98**	−0.78**	−0.67**	0.83**	0.76**	0.99**	1		
RH	0.97**	0.99**	0.99**	0.93**	−0.51*	−0.36	0.90**	0.78**	0.93**	0.93**	1	
RF	0.83**	0.89**	0.89**	0.62*	0.01	0.18	0.78**	0.60*	0.58*	0.58*	0.84**	1

SOC soil organic carbon, *SR* soil respiration, *MBC* microbial biomass carbon, *M* moisture, *BD* bulk density, *N* nitrogen, *P* phosphorus, *ST* soil temperature, *AT* air temperature, *RH* relative humidity, *RF* rainfall

*Significance at P value < 0.05

**Significant at P value < 0.01

Table 4 Average seasonal and annual microbial biomass carbon (MBC) across soil depths (0–30 cm)

Season	Soil depth (0–15) (μg g^{-1})	Soil depth (15–30 cm) (μg g^{-1})	Soil depth (0–30 cm) Total (μg g^{-1})
Summer	376.96 ± 17.30	226.17 ± 23.50	603.13 ± 35.14
Rainy	533.19 ± 24.45	324.35 ± 14.90	857.77 ± 24.54
Winter	302.75 ± 12.90	198.59 ± 09.90	501.34 ± 17.65
Annual	404.30 ± 117.62	249.68 ± 66.11	653.98 ± 183.47

respiration, soil moisture, total nitrogen, available phosphorous, soil and air temperature, relative humidity and rainfall, indicates that soil microbial activity is a collective function of biotic and abiotic variables of the site (Table 3). Many studies from different ecosystems of the India also reported similar results (Devi and Yadava 2006; Haripal and Sahoo 2014; Bargali et al. 2018) However, the inverse significant relation of microbial biomass carbon with soil pH may be due to the less intensive land management practice adopted in the present acidic soil agroforestry system that helps in increasing the microbial efficiency through channeling of substrates into biomass synthesis. In low pH soils (<6.2) intensive land use practices leads to acid related alleviation of microbial growth and organic matter degradation, leading to large losses of carbon through microbial decomposition (Malik et al. 2018).

Soil CO_2 emission (SR)

Monthly soil CO_2 emission ranged from 112.11 to 219.29 mg CO_2 m^{-2} h^{-1} (values are average of two years) and the annual emission rate was 145.11 mg CO_2 m^{-2} h^{-1} ~ 12.71 Mg CO_2 ha^{-1} $year^{-1}$ (Fig. 5). Highest CO_2 emission rate during rainy season coincides with high soil moisture, relative humidity, soil and air temperature that triggers the microbial activity and decomposition of organic matter leading to more CO_2 evolution from the soil. In contrast winter season due to unfavorable climatic conditions such as low temperature and moisture in the soil retards the microbial respiration and decomposition rate. Several studies from different ecosystems also exhibited a high rate of soil CO_2 emission during the wet period (Saraswathi et al. 2008; Devi and Yadava 2009; Thokchom and Yadava 2014; Jeong et al. 2018) which agrees with our findings. Pearson correlation matrix revealed positive significant relation between soil CO_2 emission rate and soil organic carbon ($P<0.01$), microbial biomass carbon ($P<0.01$), soil moisture ($P<0.01$), total nitrogen ($P<0.01$), available phosphorous ($P<0.01$) and soil and air temperature ($P<0.01$) (Table 3) which agrees with the findings of several other studies (Qi et al. 2002; Lee and Jose 2003; Thokchom and Yadava 2014; Zhang et al. 2015).

Carbon cycling and balance in agroforestry system

Total vegetation carbon including (trees, herbs + cardamom crop) in the present agroforestry system can sequester 5.46 Mg CO_2 ha^{-1} $year^{-1}$ from the atmosphere (calculated from the annual carbon sequestered by vegetation i.e.1.49 Mg C ha^{-1} $year^{-1}$) while 3.64 Mg C ha^{-1} $year^{-1}$ has been input to the soil through litter biomass. Annual soil organic carbon sequestration was 6.04 Mg C ha^{-1} $year^{-1}$ (calculated from the difference between the SOC of the first and second year) of which 0.74 Mg C ha^{-1} $year^{-1}$ was microbial contribution. Annual release of carbon (CO_2) from the soil through microbial and root respiration, decomposition of litters was 12.71 Mg CO_2 ha^{-1} $year^{-1}$ ~ 3.46 Mg C ha^{-1} $year^{-1}$ which is lower from different landuse systems of Northeast India namely grassland (25.50 Mg CO_2 ha^{-1} $year^{-1}$), bamboo (24.63 Mg CO_2 ha^{-1} $year^{-1}$) and forest (37.59 Mg CO_2 ha^{-1} $year^{-1}$) reported by Thokchom and Yadava (2014). A low CO_2 emission in the present agroforestry sytem can be explained due to slightly low pH of soil as acidic soil lowers soil emissions (Oertel et al. 2016) and long term N addition in soil (Bowden et al. 2004) due to use of nitrogen fixing *Alnus* species as main shading tree. The carbon balance in the soil (0.18 Mg C ha^{-1} $year^{-1}$) indicates accumulation of organic carbon through the litter and microbes which is contrasting to that of three land-use systems namely grassland (3.26 Mg C ha^{-1} $year^{-1}$), bamboo (3.30 Mg C ha^{-1} $year^{-1}$) and Dipterocarpus forest (7.19 Mg C ha^{-1} $year^{-1}$) of Manipur, Northeast India where soil carbon loss is reported (Thokchom and

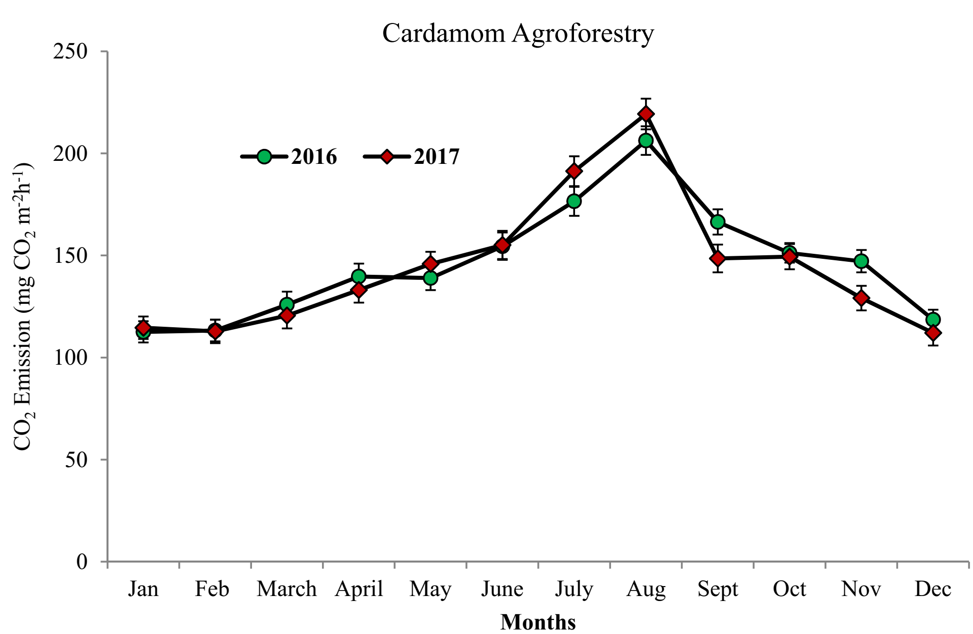

Fig. 5 Monthly rates of soil CO_2 emission from the cardamom agroforestry system during 2016 and 2017. (mean ± SE)

Yadava 2014). Higher N availability decreases total microbial biomass and loss of CO_2 from soil (Rousk et al. 2011). Previous studies on different traditional agroforestry systems of Sikkim Himalayas concluded of highest N in *Alnus*-cardamom agroforestry systems (Sharma et al. 2016). Fontaine et al. (2004) reported that carbon is lost from the soil when microbes are nutrient-limited, therefore nutrient availability in the soil and microbial competition are the main factors controlling SOC decomposition. Also, litters of different quality ultimately create a difference in microbial communities resulting in different SOC decomposition. A traditional cardamom agroforestry system can sequester 11.91 Mg C ha^{-1} $year^{-1}$ in different pools and release 12.71 Mg CO_2 ha^{-1} $year^{-1}$ ~ 3.46 Mg C ha^{-1} $year^{-1}$ through soil respiration or soil CO_2 flux with a net annual ecosystem balance of 8.45 Mg C ha^{-1} $year^{-1}$ and reveals that traditional agroforestry systems of Sikkim Himalayas besides providing livelihood security to the people acts as an effective carbon sink.

Conclusion

The present study revealed that cardamom based traditional agroforestry system of Himalayas act as a potential carbon sink both in vegetation and soil due to its high tree density and non intensive farming practice. Non intensive agroforestry practices in this region can be used as an agricultural adaptation to mitigate climate change in this region because of its less soil CO_2 emission and high ecosystem carbon balance besides the provision of livelihood benefits to the locals.

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Ecological Processes

RESEARCH Open Access

Effect of land use, season, and soil depth on soil microbial biomass carbon of Eastern Himalayas

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Abstract

Background: Soil microbial biomass, an important nutrient pool for ecosystem nutrient cycling is affected by several factors including climate, edaphic, and land-use change. Himalayan soils are young and unstable and prone to erosion and degradation due to its topography, bioclimatic conditions and anthropogenic activities such as frequent land-use change. Through this study, we tried to assess how soil parameters and microbial biomass carbon (MBC) of Eastern Himalayan soils originated from gneissic rock change with land-use type, soil depth and season. Chloroform fumigation extraction method was employed to determine MBC from different land-use types.

Results: Soil physical and chemical properties varied significantly with season, land-use and soil depth ($p < 0.001$). The maximum values of soil properties were observed in the rainy season followed by summer and winter season in all the study sites. Annual mean microbial biomass carbon was highest in the forest (455.03 μg g^{-1}) followed by cardamom agroforestry (392.86 μg g^{-1}) and paddy cropland (317.47 μg g^{-1}). Microbial biomass carbon exhibited strong significant seasonal difference ($p < 0.001$) in all the land-use types with a peak value in the rainy season (forest-592.78 μg g^{-1}; agroforestry- 499.84 μg g^{-1} and cropland- 365.21 μg g^{-1}) and lowest in the winter season (forest – 338.46 μg g^{-1}; agroforestry – 320.28 μg g^{-1} and cropland – 265.70 μg g^{-1}). The value of microbial biomass carbon decreased significantly with soil depth ($p < 0.001$) but showed an insignificant increase in the second year which corresponds to a change in rainfall pattern. Besides, land-use type, season and soil depth, soil properties also strongly influenced microbial biomass carbon ($p < 0.001$). Microbial quotient was highest in the agroforestry system (2.16%) and least in the subtropical forest (1.91%).

Conclusions: Our results indicate that land-use, soil depth and season significantly influenced soil properties and microbial biomass carbon. The physical and chemical properties of soil and MBC exhibit strong seasonality while the type of land-use influenced the microbial activity and biomass of different soil layers in the study sites. Higher soil organic carbon content in cardamom agroforestry and forest in the present study indicates that restoration of the litter layer through retrogressive land-use change accelerates microbial C immobilization which further helps in the maintenance of soil fertility and soil organic carbon sequestration.

Keywords: Soil properties, Inceptisols, Cardamom agroforestry, Paddy cropland, Subtropical forest, Soil organic carbon

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Introduction

Carbon cycle plays a vital role in shaping the earth's atmosphere and climate systems. Soil microorganisms perform a major function in the soil carbon cycle of different ecosystems and regulating the ecosystem cycling. For the formation of the organic pool, soil microbial biomass carbon acts as a key indicator of soil organic carbon by decomposing organic matter and controlling nutrient dynamics which affect the primary productivity of the terrestrial ecosystem (Kara and Bolat 2008). During the last few decades, rapid global land-use change through the transformation of forest to cropland expanded fivefold (~ 3 to ~ 15 million km^2 during 1700–2007) (Ramankutty et al. 2018). The Southeast Asian region also experienced 11.3% of the total forest cover loss, i.e., 29.3 × 10^{10} m^2 during the period 2000 to 2014 (Zeng et al. 2018). Kanade and John (2018) also reported a decline in primary forest cover and increase in secondary forest and agriculture in Sikkim Himalaya by 30% and 16% of primary forest at an altitudinal range of 800–2200 m and 2200–2800 m, respectively.

Research on the effect of land-use change on soil ecosystem functioning due to human activities is necessary to study the soil processes in different land-use systems and to protect and regenerate the ability of soil to deliver ecosystem services (Van Leeuwen et al. 2017). Ecosystem functioning depends on the flux of carbon and other chemical nutrients, mediated by the microbial interaction in the soil, plant, and animal food web (Seneviratne 2015). Soil microbial biomass acts as a keystone biological driver to the ecosystem functioning (Singh and Gupta 2018). The unpredictable rise of climate and anthropogenic disturbances affects the microbial diversity in the ecosystems (Singh and Gupta 2018). Land-use types along with its geographical area, climate variability, soil properties, and the dominant vegetation composition are the key drivers in controlling microbial biomass carbon dynamics in different land-use types (Wardle 1992; Singh and Gupta 2018). Microbes are very sensitive to land-use change due to the differences in the litter composition and root turnover rates (Hooper and Vitousek 1998). The substrate quality of an ecosystem therefore plays a substantial role in the availability of microbes as it regulates the rate of microbial decomposition of freshly added and native soil organic carbon (SOC) (Jagadamma et al. 2014). Microbial biomass carbon in the soil contributed around 1–3% carbon to the total soil organic carbon (Dilly et al. 2003). Nutrient regulations to climate change through the carbon cycle by soil microbes are crucially important in carbon-climate reaction (Bardgett et al. 2008) due to the export of dissolved carbon through hydrological leaching and CO_2 efflux by organic matter decomposition (Jenkinson et al. 1991; Davidson and Janssens 2006).

There is an increased interest for researchers for determining soil microbial biomass in ecosystem functioning (Azam et al. 2003) due to its ability to change soil quality following a land-use change (Bini et al. 2013). Study on soil microbial biomass carbon in the different land-use systems has been carried out globally by several researchers (Fang et al. 2014; Van Leeuwen et al. 2017; Bargali et al. 2018; Padalia et al. 2018; Singh et al. 2018; Soleimani et al. 2019). However, previous studies on microbial biomass carbon from Eastern Himalaya are limited only to forest (Maithani et al. 1996; Arunachalam et al. 1999; Devi and Yadava 2006) or more focussed on the jhum lands (Arunachalam and Pandey 2003; Ralte et al. 2005) and topsoil only (Sharma et al. 2004). But Eastern Himalaya, a biodiversity hotspot of the world, is a fragile region due to frequent land-use transformation/change through deforestation, land degradation, and disruption of the hydrological cycle (Tiwari 2008). Also, because of the high variation in the landscape of the Himalayas, the bioclimatic conditions change rapidly within a very short distance resulting in different soil properties and types (Baumler 2015). Microbial activities are significantly affected by the climate and human management (Rosenzweig et al. 2016), and the microbial carbon use efficiency varies across soil types due to several factors such as substrate quality and quantity, edaphic factors, stoichiometric constraint, and soil biodiversity (Lee and Schmidt 2014; Sinsabaugh et al. 2016). We hypothesize that land-use change alters the soil fertility and microbial biomass carbon that affects the soil organic carbon across soil depth. Hence, this study examined (i) the variance in soil characteristics and microbial biomass carbon in three different land-use types and (ii) the effect of season, land-use type, and soil depth on soil microbial biomass carbon.

Materials and methods

Study area and climate

Three different subtropical land-use types located at Dzongu, North Sikkim, India, namely a subtropical forest (NF) (27° 31.550′ N and 88° 29.722′ E) at an altitude ranging from 1400 to 1700 m asl, a cardamom agroforestry system (AGF) (27° 31.311′ N and 88° 24.490′ E) position at an altitudinal range of 1350–1619 m asl, and paddy cropland (PC) (27° 31.445′ N and 88° 30.380′ E) situated at an elevation of 1200–1400 m asl, were chosen for the study (Fig. 1). The cardamom agroforestry system was converted from paddy cropland about 20 years ago. All the study sites are located in a sloped position (Table 1), but the paddy cropland has terraced beds on the surface. No tilling and chemical fertilizers have been added in any of the sites except for the paddy cropland where tilling has been carried out for the cultivation of paddy. Herbs from AGF were removed manually twice a

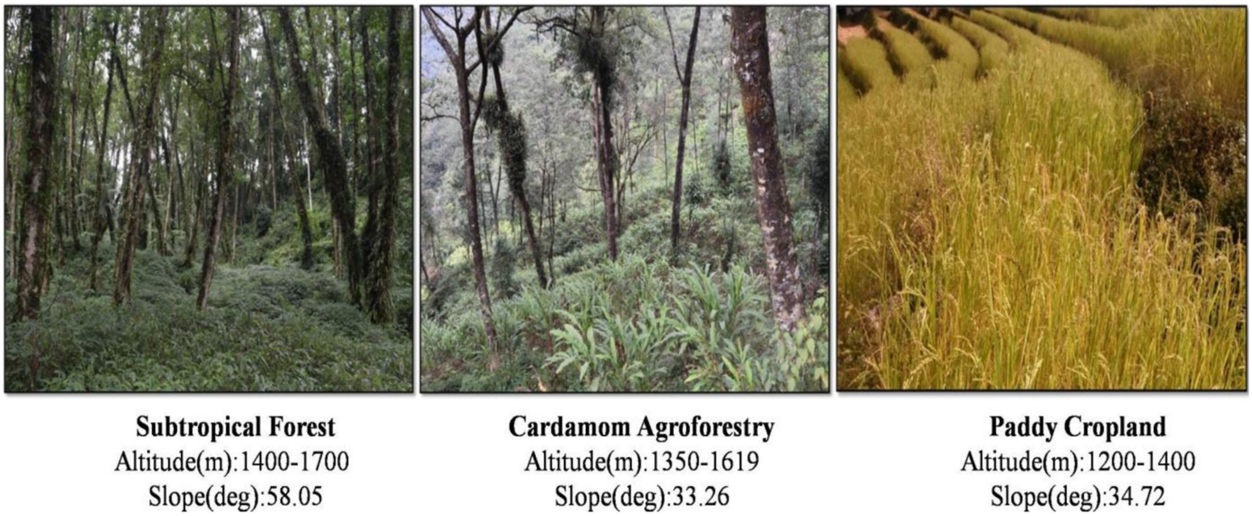

Fig. 1 Picture of the three different study sites along with altitude and slope

year for the cultivation of large cardamom. *Alnus nepalensis* forms the dominant tree species both in the subtropical forest and cardamom agroforestry as it is favored by the farmers as a shade-providing tree due to its multiple uses. However, few other trees such as *Ficus hookeri*, *Schima wallichii*, *Machilus edulis*, *Lyonia ovalifolia*, *Macaranga pustulata*, *Juglans regia*, and *Spondias axillaris* were also present in both the study sites. Tree densities were higher in the forest than in the cardamom agroforestry. In the cardamom agroforestry system, only large cardamom (*Ammomum subulatum*) was planted along with trees on the sloped surface. Herbs are however present in the forest. All the study sites experienced a monsoonal climate with three distinct seasons, namely summer (March to May), rainy (June to October), and winter (November to February) seasons. However, summer is mild while winter is cold, and the rainy season is extremely wet. March and October are transitional months between winter and summer and rainy and winter, respectively. All the study sites have a mean air temperature that varied from 7 to 22 °C, relative humidity 31–95%, and an average annual rainfall of 2663 mm (2007–2016) (Meteorological Station Gangtok, Sikkim). Meteorological data of the study sites during the study period are shown in Fig. 2.

Geology

Geologically, the present study sites were located in the Lesser Himalayan Zone or sub-Himalayan region. This region has gneissic rocks derived from the Daling series (Saha 2013; Singh 2013). The National Bureau of Soil Survey and Land Use Planning (NBSSLUP) classified soils of Sikkim as inceptisols (42.83%), entisols (42.52%), and mollisols (14.64%). All the study sites have gneissic rock origin. Subtropical forest and cardamom agroforestry have loamy soils while paddy cropland has clayey loam soil.

Soil sampling and analyses

Random soil samples were collected from five quadrats of 10 × 10 m established within each of the three land-use types in different seasons (i.e., summer, rainy, and winter) for two consecutive years. However, the distance between two quadrats in each of the study sites was at least 50 m apart. Soil samples were collected every alternate month, i.e., six times a year. A total of 60 soil samples from 15 soil pits from two different soil depths, viz., 0–15 and 15–30 cm, were taken from each of the land-use types by using a stainless steel soil corer (5 cm diameter) and mixed to form a composite sample for each depth. Roots, stones, and organic residues were removed from the samples, and each soil sample was divided into two parts. Half of each of the soil samples was stored at 4 °C to determine soil microbial biomass carbon (MBC), and the remaining half was air-dried for the determination of soil physical and chemical properties.

Soil moisture was analyzed by the gravimetric method and bulk density by oven drying of a known volume of fresh soil (80 °C). Air-dried soil samples were analyzed for texture by the hydrometer method (Allen et al. 1974), and pH was measured using an auto digital pH meter (1:5 soil distilled water suspension). Soil temperature was measured by using a soil thermometer. Soil organic carbon (SOC) was estimated by the colorimetric method (Anderson and Ingram 1993), and SOC stock was calculated following the procedure of Ravindranath and Ostwald (2008). The total nitrogen and available phosphorous in soil were determined by using the Kjeltec 8500 (FOSS) and ammonium molybdate

Table 1 Soil physicochemical properties in different land-use types (means ± SE with a common letter are not statistically different by HSD test at 5% level of significance). Values are the mean of three seasons and 2 years

Soil properties	Subtropical forest			Cardamom agroforestry			Paddy cropland		
	0–15 cm	**15–30 cm**	**Mean**	**0–15 cm**	**15–30 cm**	**Mean**	**0–15 cm**	**15–30 cm**	**Mean**
Sand (%)	42.80 ± 4.40^{a}	42.23 ± 3.20^{a}	42.52 ± 3.80^{a}	47.00 ± 3.01^{a}	46.63 ± 3.41^{a}	46.82 ± 3.21^{a}	39.00 ± 3.40^{a}	38.11 ± 3.21^{a}	38.56 ± 3.31^{a}
Silt (%)	30.62 ± 2.80^{a}	31.02 ± 3.21^{a}	30.82 ± 3.01^{a}	30.00 ± 2.78^{a}	30.21 ± 3.02^{a}	30.11 ± 2.90^{a}	32.70 ± 3.40^{a}	31.21 ± 2.55^{a}	31.96 ± 2.98^{a}
Clay (%)	26.58 ± 3.20^{a}	26.75 ± 2.41^{a}	26.67 ± 2.81^{a}	23.00 ± 5.32^{a}	23.16 ± 3.21^{a}	23.08 ± 4.26^{a}	28.30 ± 4.20^{a}	30.68 ± 2.34^{a}	29.49 ± 3.27^{a}
BD (g cm^{-3})	0.75 ± 0.05^{a}	0.81 ± 0.06^{b}	0.81 ± 0.06^{ab}	0.70 ± 0.05^{a}	0.73 ± 0.05^{b}	0.72 ± 0.05^{ab}	0.62 ± 0.07^{a}	0.77 ± 0.04^{b}	0.70 ± 0.06^{ab}
Moisture (%)	35.40 ± 3.60^{a}	24.21 ± 0.23^{b}	29.81 ± 1.92^{ab}	37.00 ± 5.62^{a}	31.32 ± 5.62^{b}	34.16 ± 5.62^{ab}	41.02 ± 0.89^{a}	34.21 ± 0.45^{b}	37.62 ± 0.67^{ab}
pH	5.61 ± 0.07^{a}	5.57 ± 0.05^{a}	5.59 ± 0.06^{a}	5.40 ± 0.05^{a}	5.34 ± 0.03^{a}	5.37 ± 0.04^{a}	5.05 ± 0.06^{a}	5.00 ± 0.05^{a}	5.03 ± 0.06^{a}
C (%)	4.34 ± 0.30^{a}	3.65 ± 0.45^{b}	4.00 ± 0.38^{c}	4.09 ± 0.35^{a}	3.64 ± 0.36^{b}	3.87 ± 0.35^{c}	3.16 ± 0.19^{a}	2.85 ± 0.03^{b}	3.01 ± 0.11^{c}
TN (%)	0.32 ± 0.07^{a}	0.25 ± 0.03^{b}	0.29 ± 0.05^{c}	0.30 ± 0.05^{a}	0.25 ± 0.04^{b}	0.28 ± 0.05^{c}	0.22 ± 0.03^{a}	0.20 ± 0.04^{b}	0.21 ± 0.04^{ab}
P (%)	0.05 ± 0.01^{a}	0.03 ± 0.01^{b}	0.04 ± 0.01^{ab}	0.04 ± 0.01^{a}	0.02 ± 0.01^{b}	0.03 ± 0.01^{c}	0.02 ± 0.01^{a}	0.01 ± 0.01^{b}	0.02 ± 0.01^{ab}
SOC stock (Mg C ha^{-1})	40.99 ± 4.01^{a}	38.17 ± 3.78^{b}	39.58 ± 3.90^{c}	40.41 ± 2.93^{a}	35.89 ± 4.80^{b}	38.15 ± 3.86^{ab}	34.16 ± 2.69^{a}	31.64 ± 1.42^{b}	32.90 ± 2.06^{ab}
C/N (%)	13.56^{a}	14.4^{b}	13.98^{ab}	13.63^{a}	14.56^{a}	14.10^{a}	14.36^{a}	14.25^{a}	14.31^{a}
HSD (α = 5%) = 4.33									

BD bulk density, *C* carbon concentration, *SOC* soil organic carbon stock, *TN* total nitrogen, *P* available phosphorous, *C/N* carbon/nitrogen ratio, *HSD* Tukey's honestly significant difference at 5% level of significance

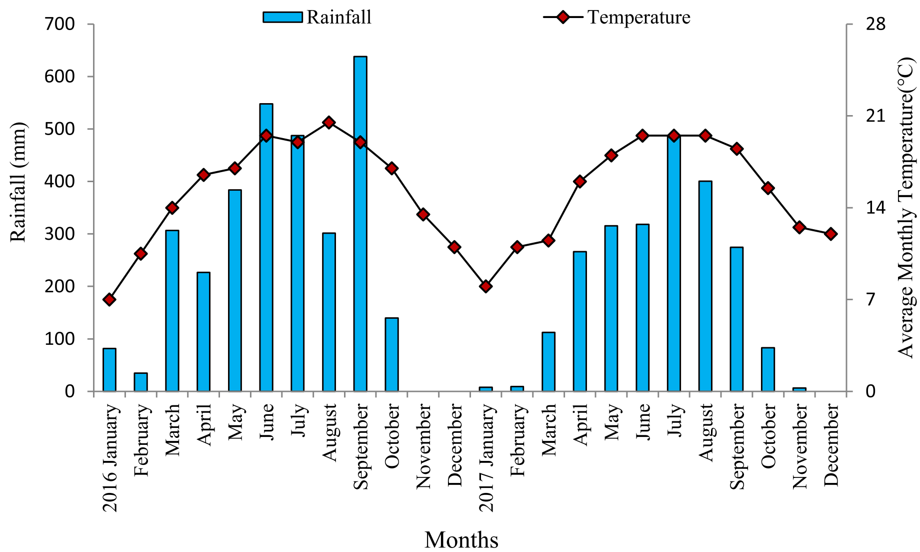

Fig. 2 Meteorological data of the study sites during the study period (2016–2017)

stannous chloride method (Sparling et al. 1985), respectively.

Microbial biomass carbon was estimated by the chloroform fumigation and extraction method (Anderson and Ingram 1993) and calculated using the following formula (Vance et al. 1987):

MBC = $E_C \times 2.64$

where E_C is the difference between C fumigated and unfumigated soil samples.

Data and statistical analyses

The statistical analyses were carried out using SPSS 18.0. Tukey's honestly significant difference (HSD) test was used to compare the means of soil parameters, microbial biomass, and microbial quotients of different land-use types. The influence of land use and season, soil depth, and land use on the soil properties and microbial biomass carbon was studied by using the two-way ANOVA. Pearson's multiple correlation analysis was carried out to determine the relationship between soil parameters and microbial biomass in different land-use types. Soil parameter data from different land-use types were subjected to principal component analysis (PCA) by using R commander. All data are an average of five replicates ± SE of the composite soil samples.

Results

Soil properties

The physical and chemical properties of soil in three different land-use types are presented in Table 1. The physical and chemical properties of soil indicate the significant differences among land-use types and seasons (Table 2). However, no significant interaction between land use and season was observed for all the soil parameters except for microbial biomass carbon (MBC). The highest sand percentage was recorded in AGF, i.e., cardamom agroforestry (46.82%), followed by NF, i.e., subtropical forest (42.52%), and lowest in the PC, i.e., paddy cropland (38.56%). PC reported more silt and clay content (31.96% and 29.49%) than NF (30.82% and 26.67%) and AGF (30.11% and 23.08%) systems, respectively. Soil moisture content ranged from 22.00 to 33.67% in the NF, 24.33 to 35.00% in AGF, and 25.83 to 39.17% in PC with a maximum value in the rainy season and minimum in winter season in all the sites. Soil parameters vary across the different seasons; however, there is no consistent trend of seasonal variation across the land-use types (Table 2 of supplementary file). The physical and chemical properties of soil in the study sites differ significantly with soil depth (Table 2) and exhibit a decreasing trend across soil depth except for clay content and bulk density (Table 1). The bulk density varied from 0.62 to 0.81 g cm^{-1} in different land-use types and increased with soil depth. The maximum bulk density was recorded in NF (0.78 g cm^{-3}) and lowest in the PC (0.70 g cm^{-3}) while the soil temperature ranged from 7 °C (winter) to 21 °C (summer) across the sites.

Soil pH decreased with soil depth and ranged from 5.0 to 5.6 across the sites and soil depth (0–30 cm) with a maximum in the NF and minimum in PC (Table 1). The highest SOC, TN, AP, and SOC stock were in the NF (4.34%, 0.32%, 0.05%, and 40.99 Mg C ha^{-1}, respectively) followed by AGF (4.09%, 0.30%, 0.04%, and 40.41 Mg C ha^{-1}, respectively) and lowest in the PC (3.16%, 0.22%, 0.02%, and 34.16 Mg C ha^{-1}, respectively) in the upper soil layer.

Table 2 Two-way ANOVAs showing significant differences in soil characteristics

Source	Moisture (%)	BD (g cm^{-3})	pH	TN (%)	P (%)	SOC (Mg C ha^{-1})	MBC (μg g^{-1})
Land use and season							
Land use	5.78**	95.27***	51.87***	11.99***	11.45***	134.44***	29.19***
Season	64.24***	54.08***	107.84***	6.95**	26.09***	219.62***	19.46***
Land use × season	0.75^{NS}	2.51^{NS}	1.59^{NS}	0.44^{NS}	1.31^{NS}	2.42^{NS}	3.02**
Soil depth and land use							
Soil depth	87.36***	47.88***	47.11***	64.29***	74.05***	32.42***	40.52***
Land use	19.50***	61.83***	55.05***	80.32***	63.64***	22.57***	13.18***
Soil depth × land use	0.97 ^{NS}	3.04**	1.20 ^{NS}	3.08**	3.74**	1.68 ^{NS}	1.59 ^{NS}

Values represent *F* values. **$p(F) < 0.01$; ***$p(F) < 0.001$
BD bulk density, *TN* total nitrogen, *P* available phosphorous, *SOC* soil organic carbon stock, *MBC* microbial biomass carbon

However, silt content slightly increases in the subsurface soil layer of NF and AGF (Table 1). In the present study, SOC exhibits a negative significant relationship with bulk density and pH ($p < 0.05$) (Table 3). In the NF and AGF, the C/N ratio of soil increased with soil depth; however, it decreased slightly in the case of PC.

Soil microbial biomass carbon

Land-use types, season, and their interaction significantly influenced soil microbial biomass carbon in all the study sites (Table 2). Mean MBC varied from 592.78 ± 14.04 μg g^{-1} to 265.70 ± 7.33 μg g^{-1} in 0–30-cm soil layer across the seasons and land-use types (Table 4). An increase in soil depth showed a decreasing trend in MBC in all the three land-use systems while the highest concentration of MBC was in the rainy season and lowest in winter (Fig. 3). The microbial biomass carbon exhibited a significant difference ($p < 0.001$) with soil depth in all the land-use types (Table 2) however, it showed an insignificant increase in the second year. Soil of the NF (455.03 μg g^{-1}) had the highest mean value of soil microbial biomass carbon, followed by AGF (392.86 μg g^{-1}) and lowest in PC (317.47 μg g^{-1}).

Microbial biomass quotient (MBC/SOC) %

Microbial biomass quotient ranged from 1.57 to 2.43% across the soil depth and land-use types (Table 5). The highest value of microbial biomass quotient was in the AGF followed by PC while the least was in NF. In the present study, the seasonal trend of microbial quotient was winter > summer > rainy season showing a slight decrease in the second year as well as with soil depth.

Correlation matrix and principal component analysis

The correlation matrix between the soil microbial biomass carbon with climatic and soil variables of three different land-use systems (Table 3) showed a strong significant positive relationship with all the parameters ($p < 0.01$) except for bulk density and soil pH where it is negatively significant ($p < 0.05$). SOC exhibited a positive significant relation with total N, available P, soil temperature, relative humidity, and microbial biomass carbon but showed negative significance with soil pH and bulk density ($p < 0.05$). The principal component analysis (PCA) of soil parameters in the different land-use types explained 49.0% variability in the first component and 26.5% in the second component (Fig. 4).

Table 3 Pearson correlation coefficient between soil microbial biomass and soil characteristics

	MBC	M	BD	pH	SOC	N	P	ST	RH	RF
MBC	1									
M	0.495*	1								
BD	− 0.483*	− 0.693**	1							
pH	− 0.566*	− 0.508*	0.137^{NS}	1						
SOC	0.875**	0.157^{NS}	− 0.530*	− 0.508*	1					
N	0.851**	0.193^{NS}	0.422^{NS}	− 0.625**	0.962**	1				
P	0.843**	0.244^{NS}	0.404^{NS}	− 0.712**	0.892**	0.924**	1			
ST	0.754**	0.825**	− 0.483*	0.736**	0.479*	0.448	0.492*	1		
RH	0.804**	0.774**	− 0.369^{NS}	0.756**	0.499*	0.472*	0.533*	0.950**	1	
RF	0.698**	0.451^{NS}	− 0.058^{NS}	− 0.007^{NS}	0.430^{NS}	0.358^{NS}	0.474*	0.660**	0.840**	1

MBC microbial biomass carbon, *M* moisture, *BD* bulk density, *pH* soil pH, *SOC* soil organic carbon, *N* nitrogen, *P* phosphorus, *ST* soil temperature, *RH* relative humidity, *RF* rainfall
*Significant at 0.05, **significance at $p < 0.01$, ***significant at $p < 0.001$, ^{NS}non-significant

Table 4 Seasonal and mean annual microbial biomass carbon (MBC) across soil depths (0-30 cm) in study site (Means ±SE followed by common letter are not statistically different by Tukey's HSD test at 5% level of significance)

Land-use type	Soil Depth (cm)	MBC (μg g^{-1})				MBC (μg g^{-1})			
		Summer	Rainy	Winter	Annual Mean	Summer	Rainy	Winter	Annual Mean
		1st Year				2nd Year			
Subtropical Forest	0–15	539.30 ± 12.32 ^{a}	758.29 ± 17.22 ^{b}	442.23 ± 13.34 ^{c}	579.94 ± 14.29 ^{d}	546.10 ± 17.23 ^{a}	764.30 ± 21.42 ^{b}	436.20 ± 12.34 ^{c}	582.20 ± 16.99 ^{ad}
	15–30	318.96 ± 09.21 ^{a}	403.22 ± 08.78 ^{b}	234.70 ± 06.34 ^{c}	318.96 ± 08.11 ^{ad}	324.98 ± 07.87 ^{a}	421.27 ± 06.67 ^{b}	270.81 ± 05.32 ^{c}	339.02 ± 06.62 ^{ad}
	Mean	**429.13 ± 10.76** ^{a}	**580.75 ± 13.00** ^{b}	**338.46 ± 09.84** ^{c}	**449.44 ± 11.20** ^{ad}	**435.54 ± 12.55** ^{a}	**592.78 ± 14.04** ^{b}	**353.50 ± 08.83** ^{c}	**460.61 ± 11.80** ^{ad}
Cardamom Agroforestry	0–15	461.08 ± 11.65 ^{a}	604.75 ± 13.45 ^{b}	417.90 ± 09.89^{ac}	494.57 ± 11.66 ^{d}	471.20 ± 10.43 ^{a}	592.41 ± 08.21 ^{b}	427.70 ± 07.87 ^{c}	497.10 ± 08.83 ^{d}
	15–30	252.76 ± 08.11 ^{a}	394.94 ± 08.32 ^{b}	222.67 ± 05.99 ^{c}	290.12 ± 07.47 ^{d}	257.59 ± 07.54 ^{a}	388.71 ± 08.32 ^{b}	222.70 ± 07.66 ^{c}	289.66 ± 07.89 ^{d}
	Mean	**356.92 ± 09.88** ^{a}	**499.84 ± 10.88** ^{b}	**320.28 ± 07.94** ^{c}	**392.34 ± 09.56** ^{d}	**364.39 ± 08.98** ^{a}	**490.56 ± 08.26** ^{b}	**325.20 ± 07.76** ^{c}	**393.38 ± 08.36** $^{\mathbf{d}}$
Paddy Cropland	0–15	399.80 ± 13.32 ^{a}	458.23 ± 17.33 ^{b}	356.91 ± 8.76 ^{c}	404.98 ± 13.13 ^{ad}	400.10 ± 12.32 ^{a}	435.55 ± 15.34 ^{b}	344.87 ± 6.78 ^{c}	393.51 ± 11.48 ^{ad}
	15–30	252.76 ± 10.23 ^{a}	258.78 ± 11.56 ^{a}	192.58 ± 8.78 ^{c}	234.71 ± 10.19 ^{ad}	228.64 ± 9.79 ^{a}	294.88 ± 12.83 ^{b}	186.54 ± 7.88 ^{c}	236.69 ± 10.16 ^{ad}
	Mean	**326.56 ± 11.77** ^{a}	**358.50 ± 14.44** ^{b}	**274.74 ± 8.77** ^{c}	**319.84 ± 11.66** ^{ad}	**314.37 ± 11.05** ^{a}	**365.21 ± 14.08** ^{b}	**265.70 ± 07.33** ^{c}	**315.10 ± 21.64** ^{ad}
					HSD (α = 5%) = 4.53				

HSD = Tukey's honestly significant difference at 5% level of significance

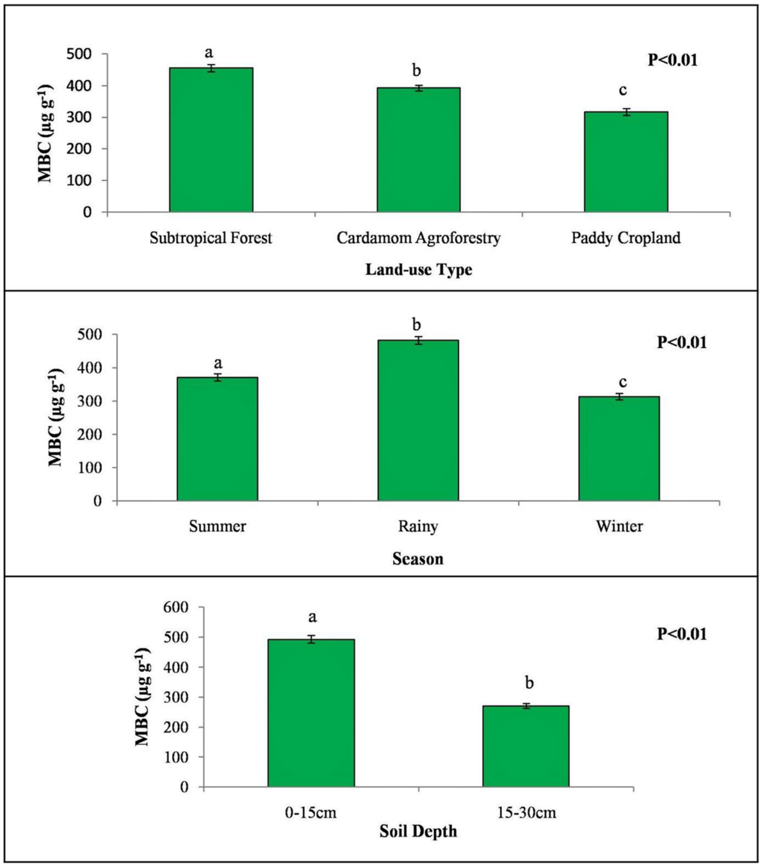

Fig. 3 Variance of microbial biomass carbon (µg g^{-1}) due to land-use type, season, and soil depth (average value of 2 years ± SE)

Discussion

The results of the present study revealed that the soil physical (soil moisture, bulk density, and pH) and chemical (C, N, and P) properties and microbial biomass carbon differ significantly in the three different land-use types with the highest in NF followed by AGF and PC except for soil moisture which exhibits a reverse trend. This is a result of the integrative response of topography and management practices adopted by each of the study sites. Adoption of terraced beds in sloped land of PC to conserve soil moisture, silt, and clay and removal of herbs from the sloped AGF to reduce competition with cardamom crop enhances soil erosion during heavy torrential rains resulting in a difference in the microclimate

Table 5 Soil microbial quotient (MBC/SOC) % in different land-use types of Eastern Himalayas. Means ± SE sharing the same letter are not statistically different by HSD test at 5% level of confidence

Land-use type	Soil depth (cm)	Summer	Rainy	Winter	Mean
Subtropical forest	0–15	0.78 ± 0.12[a]	0.59 ± 0.14[a]	0.93 ± 0.08[ab]	0.77 ± 0.11[a]
	15–30	1.09 ± 0.06[a]	0.98 ± 0.11[a]	1.38 ± 0.07[b]	1.15 ± 0.08[ac]
	Mean	**1.87 ± 0.18**[a]	**1.57 ± 0.25**[a]	**2.30 ± 0.15**[ab]	**1.91 ± 0.19**[a]
Cardamom agroforestry	0–15	0.88 ± 0.09[a]	0.72 ± 0.11[a]	0.93 ± 0.06[a]	0.85 ± 0.08[a]
	15–30	1.42 ± 0.05[a]	1.02 ± 0.05[b]	1.49 ± 0.06[ac]	1.31 ± 0.05[ad]
	Mean	**2.30 ± 0.13**[a]	**1.74 ± 0.16**[b]	**2.43 ± 0.12**[ac]	**2.16 ± 0.13**[ad]
Paddy cropland	0–15	0.78 ± 0.07[a]	0.78 ± 0.10[a]	0.91 ± 0.07[a]	0.83 ± 0.08[a]
	15–30	1.19 ± 0.05[a]	1.14 ± 0.06[a]	1.44 ± 0.04[c]	1.26 ± 0.05[ad]
	Mean	**1.97 ± 0.12**[a]	**1.92 ± 0.16**[a]	**2.35 ± 0.11**[c]	**2.08 ± 0.13**[a]
HSD (α = 5%) = 4.53					

HSD Tukey's HSD test at 5% level of significance

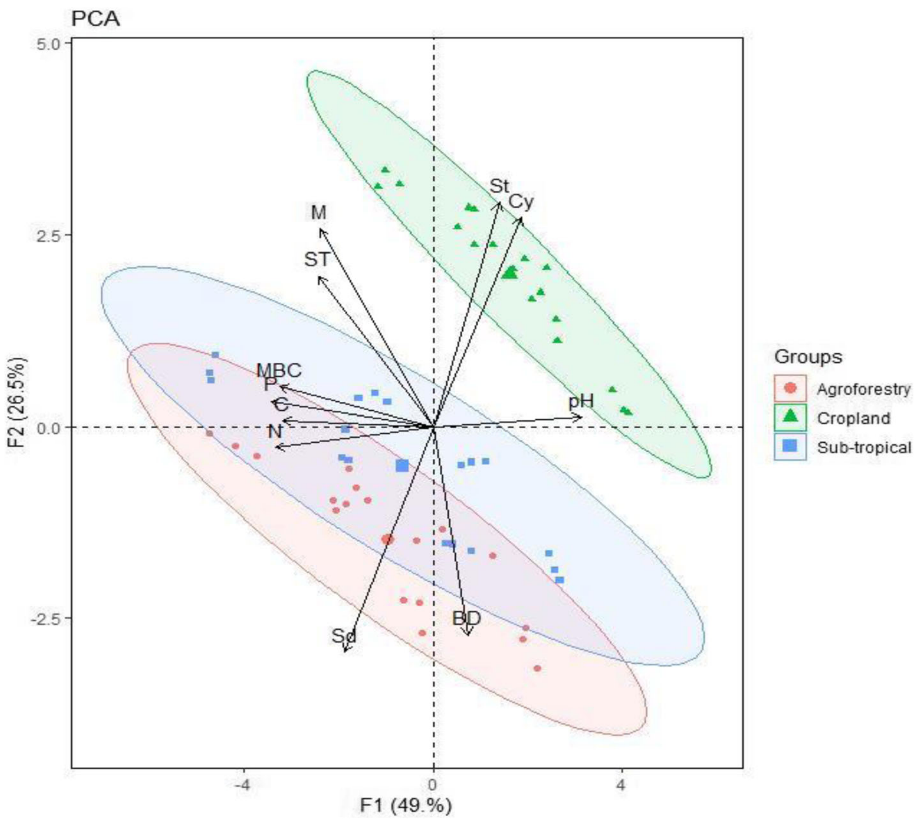

Fig. 4 Principal component analysis (PCA) of soil parameters in the three land-use types

of the two sites which ultimately leads to an alteration of soil properties. Also, vegetation composition and amount of organic matter are different in the study sites which ultimately affect the microbial activity of soil. Several studies reported the influence of land use and change in soil management practices (George et al. 2013; Gonnety et al. 2013; Malik et al. 2018), topography, space and time, vegetation cover, climate, weathering processes, and microbial activities (Paudel and Sah 2003) on physico-chemical properties of soil. Bulk density (BD) of the present study (0.62–0.83 g cm^{-3}) is within the range reported by Baumler and Zech (1994) (0.6–1.8 g cm^{-3}), and it increases with a decrease in soil depth in all the land-use types. Our results agree with the report of several other studies from different land-use types of the world (Zhang et al. 2014; Francaviglia et al. 2017). Higher soil bulk density in the inner soil layers is due to less organic matter and weight of the overlying horizons (Grüneberg et al. 2014). The difference in BD of the different systems could be due to the difference in the particle size distribution of soils in the study sites, and a similar finding was reported by Dumig et al. (2006). A slight decrease in soil pH with soil depth in the present study coincides with abundant rainfall in the study sites which might lead to leaching of calcium and magnesium ions in the lower soil layers thereby leading to a decrease in pH of soil. Zhao et al. (2018) also reported a reduction in pH of subsoil due to leaching of calcium and magnesium ions in high rainfall areas which is in conformity with our report. Waterlogged soil condition due to paddy cultivation leads to more acidic soil in the cropland while the presence of low acidic soil in subtropical forest relates to the slope and topography of the forest which could not retain water or moisture for a long time leading to an increase in pH of soil. Several studies reported that soil pH is affected by slope, topography, terrain features, and topographic wetness index in the agricultural landscape and mountains (Chen et al. 1997) which agrees with our findings.

Our study indicated a difference in the sand, silt and clay content with land-use type, and soil depth (Table 2) which agrees with the report of Yusek and Yuksek (2011). However, contrasting results were reported from different land-use types (forest, grassland, cropland, and bare land) of Turkey (Evrendilek et al. 2004; Korkonc 2014) wherein no significant differences in the soil texture were observed with the change in land-use types and soil depth. Sand particles were highest in the AGF followed by NF and lowest in PC while clay and silt particles show a reverse trend with a maximum in PC and minimum in AGF. The reason for this reverse trend corresponds to the sloped position of the former two sites which enhances soil erosion in contrast to PC which has terraced beds on sloped land. Slightly higher sand in AGF than in NF could be due to the removal of herbaceous layers making the soil susceptible to erosion. The role of soil particle size distribution on vegetation, soil texture quality, and erosion has been reported by Aderonke and Gbadegesin (2013) which complies with our findings.

Soil moisture was higher in the PC (37.61%) than in NF and AGF soils (29.80% and 34.16%, respectively) because of higher clay content in the former. Such a relationship was established by many studies (English et al. 2005; Kara and Baykara 2014), but a reverse trend was reported by Amanuel et al. (2018).

Soil organic carbon (SOC) decreased with soil depth in all land-use types with maximum content in topsoil due to the availability of more organic matter from trees. The presence of trees continuously adds litter in the upper layer and increases root turnover (Kimmins 2004) which further enhanced SOC due to positive priming (Wu et al. 1993). Such a finding was reported by Soleimani et al. (2019) which conforms with our report. Also, annual carbon input in agricultural land through plant residue, a source of labile carbon, is lower than that of the natural forest leading to a low soil organic C (Hooker and Stalk 2008). Low SOC in the agricultural lands of the present study agrees with the reports of several studies (Huang and Song 2010; Reza et al. 2018). Higher total N in soils of the subtropical forest (NF) and AGF is related to the presence of the *Alnus nepalensis* nitrogen-fixing tree species, as a dominant tree in both the systems. Rothe et al. (2002) reported that the presence of N-fixing species increases soil total nitrogen content. A decrease in available phosphorus with soil depth in all the systems coincides with a low soil pH and that enhances P immobilization (Chase and Singh 2014). Soils of the present study indicate phosphorus limitation which could be due to the acidic nature of these soils. Transformation of the forest to cropland results in a reduction in the soil nutrients while the reverse process, i.e., PC → AGF or to a forest (NF), increased the nutrient content and soil organic carbon stock (PC → AGF ~ 5.25 Mg C ha^{-1} and AGF → NF ~ 1.43 Mg C ha^{-1}) and lowers the acidity of the soil.

The microbial biomass carbon in soil of the present study ranged from 186.54 to 764.30 μg g^{-1} across the season, soil depth, and land use (Table 4), and this is within the reported range of tropical soils (106–2073 μg g^{-1}) by Henrot and Robertson (1994). The highest MBC in the forest is due to the production of litter and deep root systems of the tree allowing more microbial activities than other agricultural land-use systems (Arunachalam et al. 1999). Low MBC in the agricultural systems is because of the different agricultural practices, resource availability, and plant composition (Van Leeuwen et al. 2017). A similar trend was reported by several studies in various ecosystems (Bardgett 2005; Soleimani et al. 2019). Furthermore, high moisture in the soil of paddy cropland (PC) due to waterlogging limits the microbial activity in the soil. A slightly higher MBC in the cardamom agroforestry (AGF) than that in PC is because of the presence of a litter layer in the former retaining soil moisture that promotes microbial activity. Wu et al. (2016) also reported a higher MBC in afforested soils with higher litter inputs which agree with our findings. A significant positive correlation between soil organic matter and soil microbial biomass (Table 3) in our study supports the findings of Chen et al. (2006) that soil MBC is highly influenced by soil organic matter present in different ecosystems. Such a result was supported by many researchers (Wang and Wang 2011; Chen et al. 2017; Padalia et al. 2018). Further, high soil N in the natural forest and cardamom agroforestry system is due to the presence of *Alnus nepalensis* which might result in a higher microbial biomass C in these sites. Wardle (1992) concluded that soil N showed more influence than C in organic C microbial immobilization in most of the systems which is consistent with our findings.

Distinct seasonal variations in soil MBC showing a peak value during the rainy (wet) season and a trough in the winter (dry) season (Fig. 3) in all the land-use types of the present study agree with the findings of previous studies from various tropical ecosystems (Devi and Yadava 2006; Iqbal et al. 2010; Patel et al. 2010). Seasonal variation of soil MBC is an indicator of immobilization and mineralization of soil carbon, and an increase in soil microbial biomass indicates immobilization, while a decrease denotes mineralization of nutrients (Yang et al. 2010). Warm and wet weathers during the rainy season accelerate litter decomposition as microbial activities and decomposition are at peak during this season thereby increasing the immobilization of nutrients by the microbes (Usman et al. 2000; Devi and Yadava 2010). Also, high relative humidity during the wet period accelerates the growth of fungi which further increases microbial biomass carbon (Acea and Carballas 1990). Least MBC during the dry and cold winter seasons coincides with a low temperature and less moisture in the soil leading to the death of microorganisms that release organic carbon, and freeze-thaw action can facilitate the decomposition of organic detritus and mineralization of carbon (Groffman et al. 2001). However, dry tropical deciduous forest (Singh et al. 1989) and humid subtropical forest of India (Arunachalam and Arunachalam 2000) show the highest microbial biomass C in summer and winter, respectively, indicating that the microbial biomass C is highly influenced by the species composition, location, elevation, and pattern of rainfall of the site.

Besides land-use type and season, another important factor controlling MBC is soil depth. MBC was more in the upper soil layer and less in the subsoil (Fig. 3) in all the land-use types. This pattern is because of lower carbon and nitrogen content in the lower subsoil and more organic matter in the top humus soil that promotes microbial activity. Previous studies on MBC across soil depth in various land-use types also reported similar findings (Fierer et al. 2003; Fall et al. 2012; Soleimani et al. 2019). In the present study, soil microbial biomass carbon was studied to a depth of 30 cm only, and due to this limitation of soil depth, the presence of the considerable amount of microbial biomass C in the deeper soil layers, i.e., 40–60 cm soil layer as reported by Soleimani et al. (2019), cannot be explored and further study is needed in this context from this region.

Principal component analysis (PCA) on soil physical and chemical properties of the three different land-use types of the present study (Fig. 4) shows 75.5% of the total variation along with the two principal components. PCA component F1 explained 49.0%, while the second component F2 explained 26.5% of variation. Change in the land-use type influenced the characterization capacity of soil parameters significantly. PC1 revealed that the microbial activity in NF is positively influenced by macroelements, soil moisture, and temperature while soil pH exhibited an inverse relation with it. PC2 indicates that soil properties such as texture (silt, clay, and sand) and bulk density show strong influence with land use. However, sand and bulk density of soil play an important role in the cardamom agroforestry system, but silt and clay content show an inverse relation with sand.

Soil microbial quotient (MBC/SOC) of the present study agrees with the value of tropical forests, 1.5–5.3% (Luizao et al. 1992), and temperate forest soils, 1.8–2.9% (Vance et al. 1987), and those of agricultural soils (2–6%) reported by Brookes et al. (1985) (Table 5). The higher microbial quotient of the present subtropical forest than that of a humid subtropical forest of Northeast India, 0.7–1.77% (Maithani et al. 1996), indicates a higher microbial C immobilization. The microbial quotient varied significantly with the season and soil depth, and more immobilization of carbon in the winter season and least during the rainy season in all the land-use types is due to the availability of more substrate in winter. An increase in the microbial quotient with soil depth denotes the presence of more active carbon pools in the subsurface soil. Some studies reported that subsurface soil layers act as a store of microbial inoculation (Yi et al. 2006; Wei et al. 2009). A slight decrease in the microbial biomass quotient in the second year indicates a decrease in microbial immobilization of carbon and ultimately organic carbon in the soil which may be related to the change in environmental factors especially rainfall patterns in the second year (Fig. 2).

A higher microbial quotient in the cardamom agroforestry (AGF) indicates more carbon immobilization by the microbes from the organic substrates while the least microbial quotient in the forest (NF) may be a result of carbon mineralization from the microbes to support vegetation. Further, the exhibition of the highest microbial quotient ratio by the cardamom agroforestry system probably suggests better carbon immobilization capabilities of microbes in the agri-silviculture system than in the forest and cropland. Waid (1999) also reported that microbial diversity is affected by the type of vegetation, quantity, and chemical composition. Kara and Baykara (2014) stated that the MBC/SOC ratio is determined by the amount of labile organic matter and not by the size of the microbial biomass carbon, and their findings agree with our study. A previous study on the microbial quotient in different land-use types also reported a higher value of microbial quotient in agricultural soils than that of forest soils (Kara and Baykara 2014). Sparling et al. (1992) also suggested the percentage of organic C present as microbial biomass C as an indicator of changes in the quality of soil organic matter.

Conclusion

The results of the present study revealed that land use, season, and soil depth significantly influence the physical and chemical properties of soil and microbial biomass carbon. Organic matter or litter layer in tree-based systems increased SOC thereby helping in the restoration of better soil health and fertility. Microbial biomass carbon and soil parameters showed strong seasonality, and land-use type and soil depth strongly influenced the topsoil of all the study sites. Forest had the highest microbial biomass C and least microbial quotient while the reverse trend exhibited by the cardamom agroforestry system suggests better C immobilization in the agroforestry system. Low SOC and MBC in paddy cropland confirmed that the lack of organic matter inputs and intensive land management practices such as plowing and tillage of the soil decreased soil fertility and microbial activity. Hence, a tree-based agricultural system promotes microbial activity and soil fertility through the immobilization of nutrients by microbes.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13717-020-00269-y.

Additional file 1: Supplementary tables.

Abbreviations
MBC: Microbial biomass carbon; SOC: Soil organic carbon; NF: Subtropical forest; AGF: Cardamom agroforestry; PC: Paddy cropland; NBSSLUP: National Bureau of Soil Survey and Planning; PCA: Principal component analysis; TN: Total nitrogen; AP: Available phosphorous; BD: Bulk density

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Authors' contributions
NTL collected and analyzed the data. NBL conceptualized and designed the study. Both authors wrote the manuscript. The authors read and approved the final manuscript.

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Availability of data and materials
All data are included in the manuscript, and additional data are provided in the supplementary file.

Ethics approval and consent to participate
Not applicable

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The authors declare that they do not have any competing interests.

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