HAWASSA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE DEPARTMENT OF BIOLOGY

ASSESSMENT OF THE SOIL MICROBIAL BIOMASS AND PHYSICOCHEMICAL PROPERTIES OF DIFFERENT LAND USE SYSTEM IN WONDO GENET, SIDAMA REGION, ETHIOPIA

BY TESEMA TAYE

MAJO ADVISORY: MR. WELDESEMAYAT GOREMS CO-ADVISOR: BEYENE DOBO (PhD)

MSc. THESIS

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HAWASSA, ETHIOPIA

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MAJOR ADVISORY: MR. WELDESEMAYAT GOREMS (AST. PROFF) CO-ADVISOR:

BEYENE DOBO (PhD)

SUBMITTED TO THE DEPARTMEMT OF BIOLOGY, HAWASSA UNVERSITY COLLEGE OF NATURAL AND COMPUTATIONAL, IN PARTIAL FULFILLMENT OF REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE.

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SCHOOL OF GRADUATE STUDIES HAWASSA UNIVERSITY ADVISOR'S APPROVAL SHEET

This is to certify that the Thesis entitled with "*assessment of the soil microbial biomass and physicochemical properties of different land use type"* submitted in partial fulfillment of the requirements for the Master`s degree with specialization in Applied Microbiology, the graduate program of Department of Biology, and has been carried out by Tessema Taye (GpApMiR/0010/14) under my supervision. Therefore, I recommend that the student fulfill the requirements and hence thereby can submit the Thesis to the department.

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Co-advisor	----------	----------

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We, the undersigned, members of the Board Examiners of the final open defense by **Tessema Taye** have read and evaluated his thesis entitled "*ecological analysis of impact of land use practice on the spatial variability of soil microbial biomass and physicochemical properties* " and examined the candidate. This is therefore to certify that the thesis has been accepted in partial fulfillments of the requirements for degree of Master of Science in Biology with specialization in Applied Microbiology.

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LIST OF ABBREVIATION

ANOVA	*Analysis of variance*
BD	*Bulk density*
CEC	*Cationic exchange capacity*
CFE	*Chloroform fumigation extraction*
CHCl3	*Chloroform/* trichloromethan
Conc.	*Concentration*
DDW	Double-distilled water
EC	*Enset -coffee*
ECA	*Enset-coffee-avocado*
EN	*Enset*
FAS	*Ferrous ammonium sulfate*
GIS	*Geographic Information System*
LSD	*Least significant difference*
LUT	*Land use type*
m.a.s.l	*Meter above sea level*
MA	*Macro-aggregate*
MC	*Moisture content*
ME	*Meso- aggregate*
MI	*Micro -aggregate*
NS	*Non-significant*
SMBC	*Soil microbial biomass carbon*
SMBN	*Soil microbial biomass nitrogen*
SOC	*Soil organic carbon*
SOM	*Soil organic matter*
SPSS	*Statistical Package of Social Science*
STN	*Soil total nitrogen*

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Abstract

Land use change can have both negative and positive effects on soil physico-chemical properties and microbial biomass. The agricultural activity primarily strongly influence the soil physico-chemical and microbial futures, it also regulates annual carbon turn over. Studying the soil microbial biomass and physico-chemical properties under different land use types is great practical significant for land use and soil management regarding soil carbon dynamics and climate change mitigation. The objective of this study was, to investigate the impact of land-use change on physical, chemical and microbial biomass properties in Wondo Genet Woreda, Sidama, Ethiopia. Soil samples were collected from three different agroforestry systems, namely Enset, Enset-Coffee and Enset-coffee-avocado land use type and two soil depths (0–15cm and 15-30 cm) and 20 samples were collected in each land use types. Soil organic carbon (SOC) and total nitrogen (tN), soil microbial biomass were determined by titration method, micro-kjeldahl method, and fumigation extraction method, respectively. The study found that microbial biomass carbon, microbial biomass nitrogen and soil pysico-chemical properties were significantly varied among agroforestry systems across the soil depth. The result show that among all agroforestry systems the highest microbial biomass carbon and nitrogen were observed in the Enset-coffee- avocado, Enset-coffee and Enset which accounts for (1601.3±206.31µg/g), (138.03±17.78µg/g), (288.24±39.06µg/g) on the upper parts of the soil, respectively. The microbial biomass ratio observed as the same fashion in all agroforestry. Enset-coffee-avocado (11.6 µg/g), Enset-coffee (10.64 µg/g) and Enset (9.2 µg/g) in the upper parts of the soil. Soil OC was found to be higher in enset- coffee-avocado (3.43±0.35%) followed by enset-coffee (2.14±0.22%) and Enset (1.81±0.28%) land use types. tN was found to be higher in enset-coffee-avocado (0.29±0.03%), followed by enset-coffee (0.18±0.01%) and enset(0.16±0.02%). The result of the study indicates that the microbial biomass and soil physico-chemical properties highly correlated with the type of vegetation and soil depths. The values of these parameters in enset-coffee- avocado were higher. Thus enset-coffee-avocado based land type has the potential for improving soil quality and properties in the short term in the tropics.

***Keywords**: Land use type; microbial biomass; soil chemical properties; soil physical properties*

1. Introduction

1.1 Background of the study

Soil microorganisms are important components of terrestrial ecosystems (Chandra *et al*., 2016) and drive many soil services such as nutrient cycles (Yin *et al*., 2010; and maintenance of soil function which is directly involved in maintaining soil fertility and its structure (Patel *et al*., 2014). The soil microbial biomass carbon (SMBC) and soil microbial biomass nitrogen (SMBN) are the small part of soil organic carbon pool, but the most important and active parts in soil ecosystem (Jenkinson, 1981). They participate in soil C and N biogeochemical cycle and play a critical role in nutrient retention and soil fertility in terrestrial ecosystems (Jenkinson, 1988).

The soil organic carbon (SOC), soil total nitrogen (STN) and soil microbial C (SMBC) and N (SMBN) are some of the soil properties that are used as basic indicators in assessing soil quality (Reich, *et al*., 1997). As reported by (Adeboye, *et.al*., 2011) the soil organic carbon (SOM) and soil total nitrogen (STN) are the major determinants and indicators of soil quality and fertility and are closely related to soil productivity in an agricultural ecosystem.

The soil microbial biomass (SMB) is a small but key component of the active soil organic matter (SOM) pool and serves as a source and sink of soil nutrients (Vitousek, Howarth, 1991). It has been used to understand soil nutrient dynamics and as an ecological marker (Jenkinson, 1981). The SOM and STN are the major determinants and indicators of soil quality and fertility and are closely related to soil productivity in an agricultural ecosystem (Shen, *et al*.,2001). The reduction of SOC and STN will lead to decrease in soil fertility, soil nutrient supply, porosity and an increase in soil erosion (Titov, 2000). Soils represent an important terrestrial stock of C and approximately two to three times as much as terrestrial vegetation and atmosphere respectively and the C in the SOM of agricultural land is composed of dominant terrestrial C stock (Zhou *et al*., 2005). Thus the dynamics of SOC as affected by agro-ecosystem to a large extent affects the carbon dioxide concentration in the atmosphere as well as even the global climate change (Cheng, Luo, 2004). Soil microorganisms regulate soil ecosystem functions and are indicators of soil quality (Sharma, *et al*., 2017). They promote nutrient cycling and organic matter transformation, improve plant productivity, and control soil borne diseases (Brugman *et al* ., 2018). Different studies revealed that the soil microbial activity, biomass, and composition depend on plant type, parent material, and agricultural practices. Microbial abundance, composition, and activity largely determine

sustainable agricultural productivity (Bender, *et al*., 2014). The soil microbiome may be positively or negatively affected by soil disturbances and management practices that alter soil microbiome classification and function (Philippot, *et al*., 2013). The changes in SOC would result in storage/loss of soil C and N, which are likely to influence the pool size and activity of the SMB (SMBC and SMBN).

The SOC is composed of diverse fractions varying in their degree of decomposition, recalcitrance, and turnover rate (Huang *et al*., 2008). Have also been suggested as indicators of the land use effects on biological activity and soil quality because of their sensitive response to land use change (Zimmerman *et al*., 2011). The SOM components with relatively short residence times consist of microbial biomass and metabolites In this study, the concentrations of microbial biomass carbon (SMBC) and microbial biomass nitrogen (SMBN), soil organic carbon (SOC), total nitrogen (TN) and the relationships between soil organic matter and microbial biomass of surface soil (0 to 15cm) and sub-soil (15 to 30cm) soil layers were compared between the stands of EN, EC and ECA in the Sidama, Regional state, Wondogenet Woreda. The specific objectives of this study were to determine: (i) How land use type affected microbial biomass and soil organic matter and soil quality and (ii) How land use type affected soil physic-chemical properties of the soil.

The present study aimed to ascertain the changes, concentrations and the relationship among SMBC, SMBN, SOC and STN and soil pysicho-chemical properties on land use type. The results are expected to fill up the gap of knowledge about the soil C, N cycle and soil fertility status of Wondogenet Werada and to provide reference for further studies.

1.2 Statement of the Problem

The need to increase agricultural production to meet the growing food demand has resulted, inevitably, in an expansion of the agricultural land consequent removal of natural forest leads to land use change. Land use change affects important ecosystem functions including carbon sequestration, climate regulation or water purification, and all of these are intimately connected to microbial activities. In fact, agricultural intensification is perceived as one of the greatest threats to global biodiversity Convention on Biological Diversity (Zimmerer, 2010). Moreover, habitat fragmentation, degradation and destruction due to land use, change arising from conversion, intensification of production systems,

abandonment of traditional (often biodiversity–friendly) practices, construction and catastrophic events including fires. Other key pressures are excessive exploitation of the environment, pollution and the spread of invasive alien species. Among the most common one are agricultural intensification and expansion, fuel production, timber production, urbanization etc. Thus, how these land use changes affect the microbial biomass and other physicochemical properties of the soil in the study area.

The major land use change in the study area, the conversion of natural forest to other land-use types, leads to not only climate change, loss of biodiversity, change in ecosystem services etc but also affects soil biological and physicochemical properties (Tilman *et al*., 2001; Ashagrie *et al.,* 2007). Several studies have documented that the conversion of natural forest to other land-use types significantly influenced soil health and quality and particularly in temperate regions (Kumar and Ghoshal, 2017). However, restoration of forests poses a major challenge globally, particularly in the tropics, as the forests in these regions are more vulnerable to land-use change (Kumar and Ghoshal, 2017; Jackson *et al*., 2007). Therefore, how land-use change affects the community composition in terms of disturbance and ecosystem restoration in the dry tropics has yet not been studied (Kumar and Ghoshal, 2017).

1.3 Significance of this study

In Ethiopia, agroforestry systems have various socio-economic and ecological benefits (Muleta *et al*., 2007). The major agroforestry systems in the study area are *Enset* based agroforestry system, *Enset-coffee* based agroforestry system and *Coffee-Avocado-tree-enset* based agroforestry system. Despite the immense socio-economic and ecological role that agroforestry plays in the soil, limited studies and poorly understood with regard to microbial property, biomass, and soil physicochemical properties. Moreover, the knowledge and study of the impact of different agroforestry systems (*effect* of agroforestry management) on microbes in agroforestry practices is limited. Therefore, the purpose of the present study is to generate knowledge and develop conservation strategies for efficient storage of C pool in the soils. The study will help to develop future plan about land use and soil management regarding soil carbon dynamics and climate change mitigation. To achieve this objective, study of soil microbial property was conducted in three agroforestry systems in central rift valley, Wendogenet , Sidama , Ethiopia. Present study

help Farmers to Understanding soil microbial biomass and its relationship with soil properties can help farmers optimize their agricultural practices, leading to improved crop yields and sustainable farming methods. The study can provide valuable information for training programs aimed at enhancing soil management practices. And the study laid the base for the further research investigation.

1.4 Scope of the Study

Taking the time limitation into account the scope of the study was delimited in Wondogenet woreda in Sidama regional state. Land use practices and its effect on soil microbial community and physicochemical properties in three different agroforestry systems. The study mainly focused on soil microbial properties and physicochemical properties across different land use practices.

1.5 Limitation of the Study

The present study was assessed the impact of the land use type on microbial biomass and physicochemical properties, in Wondogenet, Sidsma, Ethiopia. Due to time and resource constraint this study did not incorporate all types of soil microbial influencing factors. In terms of site, the study confined to Wondogenet, Sidama, Ethiopia.

1.6 Objective(s)

1.6.1 General objective

The main objective of the study was to assess the impact of land use practices on soil microbial biomass and physicochemical properties in three different agroforestry systems: *Enset* based agroforestry system, *Enset-coffee* based agroforestry system, *Coffee-Avokado* tree *Enset* based agroforestry system.

1.6.2 Specific objectives

- To determine the impact on of land use type difference on SMBC and SMBN across soil depth.
- To determine soil microbial C(SMBC) and N(SMBN) to evaluate spatial variability of microbial and soil physicochemical properties in the three land use types.
- To assess the effect of land use practices on soil microbial properties in the selected land use type.

1.7 Research questions

- How the impact of land use type was determines the spatial variability of soil physicochemical properties: *Enset* based agroforestry system, *Enset-coffee*-based land use type, and *Coffee-Avocado* tree-*Enset* based land use type?
- What are the impacts of land use changes on soil microbial biomass in the Enset-based agroforestry system, *Enset-coffee*-based agroforestry system, and Coffee-Avocado tree-*Enset* based land use type ?
- How soil depth determines the soil microbial and physicochemical properties in the stud sites: *Enset* based *agroforestry system, Enset-coffee based* land use type*, Coffee-Avokado* tree *enset* based land use type ?

2. Review of literature

2.1 Pysco-chemical properties of soil

2.1.1 Soil moisture

Estimating soil moisture can be done directly or indirectly. While indirect methods estimate the amount of water through the properties of water in the soil, direct methods include measuring the moisture content of the soil. The most accurate methods for estimating moisture content are volumetric measurement or oven-drying thermo-gravimetric procedures. Soil water demonstrates remarkable spatial and temporal heterogeneity (Gomez *et al.,* 2000). Thus, in order to restore vegetation and manage water resources, both spatial and temporal fluctuations in soil moisture have always been crucial, particularly in semi-arid and arid ecosystems (Brevik *et al*., 2015). The water content of the soil affect the physiological state of the micro-organisms (Walker *et al*., 2003). Well-moist soils hold more functionally diverse microbial communities. However, excessive soil moisture may lead to a lower biomass of microorganisms (Silva *et al.,* 2008; Unger *et al*., 2009). The diversity of heterotrophic bacteria and fungi was higher in air-dry soils than in the wet ones. However, the diversity of actinomycetes was similar in all soil samples, irrespective of their moisture. Also, (Schjønning *et al*., 2011) showed that dry soils were characterized by a greater diversity of microorganisms than irrigated ones. However, there are also studies (Kim *et al*., 2008). Which demonstrate that the diversity of bacteria is not enriched when soil is watered. more rapidly growing microorganisms there are in soil, the more easily fresh organic matter is degraded (Zaborowska *et al*., 2015). However, such microorganisms are less stable in the environment than those which grow more slowly, And it is the slow-growing microorganisms that are responsible for maintaining the homeostasis of soil (Borowik and Wyszkowska, 2016)

2.1.2 Soil aggregates

Aggregates are defined as structural elements that work cohesively to sustain and influence the physical soil system, collectively referred to as soil aggregation (Blanco & Lal, 2008). Soil aggregation is a “two-tiered process” involving durable micro-aggregates that are encapsulated and held together by less stable macro-aggregates (Tisdall and Oades, 1982; Singh *et al*., 2013). They are formed and arranged in closely packed clusters in accordance with an aggregate hierarchy based off size and mechanism (Snyder & Vázquez, 2005), composed of bacteria, silt, clay,

minerals, fungal hyphae, fine roots and polysaccharides (Singh *et al*., 2013) smaller than 0.2 μm to form micro-aggregates (<250 μm in diameter), which then bind together and form macro-aggregates (>250 μm in diameter) (Cardoso *et al*., 2013). Many function's aggregation is responsible for is the accumulation of carbon, water storage and nutrient cycling, housing microorganisms and playing a large role in the stabilizing of SOM (Singh *et al*., 2013). In turn, the microorganisms and additional soil fauna living within the soil aggregation positively affect the structural integrity of the aggregates (Cardoso *et al*., 2013). Given the right ecosystem, microbial communities can increase aggregation by releasing certain by-products (Degens & Harris, 1997). As these aggregates build, they form the appropriate physical protection carbon needs from mineralization (Loaiza-Puerta *et al*., 2018). It's also known that higher organic carbon (C) increases the size of aggregates (Warrick *et al*., 2002) and functions as a "glue" to bind aggregates together, ultimately facilitating the protection of SOM (Acir *et al*., 2020). Numerous studies focus on the SOM and aggregate relationship as they increase overall water stable aggregates (Lynch and Bragg, 1985), and contribute heavily to the nutrient cycling process (Dapaah and Vyn, 1998).

2.1.3 Bulk density/particle density/

Bulk density is determined by removing a block of soil from site, allowing no compaction or crumbling. This is often will be done by hammering a can or metal ring into the soil and digging the ring or can out when full of soil. The soil is then dried in an oven and weighed. The volume is determined by measuring the volume of the container used to extract the soil. The bulk density is expressed in units grams of oven-dry soil per cubic centimeter (Blake & Hartge, 1986). Soil bulk density will be determined in three replicates by removing a known volume of soil using metal tubes and oven drying it at 105°C for 24hrs.

2.1.4 Soil organic carbon

Soil organic carbon (SOC) accounts for about 60 % of soil organic matter, and it plays an important role in improving soil fertility and sustaining soil productivity (Chen *et al*., 2017). Dissolved organic C is widely known to play a critical role in several soil processes and can affect the mobility and availability of soil nutrients such as nitrogen (N), phosphorus (P) and sulphur (S) (Kalbitz *et al.,* 2000). Specifically, greater accumulation of SOC in agro-ecosystems with greater

crop diversity mainly occurs through increases in the quality, quantity, and chemical diversity of plant-derived carbon inputs to soils, thereby fostering the growth and diversity of soil microbial communities which enhance the formation and storage of SOC (Zhang *et al*., 2021). These patterns align with the positive local-scale relationships observed in natural ecosystems among plant diversity, soil microbial diversity and soil organic carbon (SOC) (Porazinska *et al*., 2018). The link between microbial communities and the dynamic and inherent soil properties in relation to the carbon cycle and its interaction with other biogeochemical cycles (Gärdenäs *et al.*, 2011). The higher plant diversity in ecosystem increasing soil microbial activity and soil carbon storage (Lange *et al.*, 2015).

2.2 Soil microbes

The key driving factor for sustainable agriculture is soil ecosystem, where pivotal services are provided by the soil biota, 'the biological engine of the earth', which can act as early warning signals of ecosystem health, and can be of use in environmental diagnosis (Amrit Kaur *et al*., 2005). Soil ecosystems are significantly impacted by the diverse range of land use activities that utilize soil resources to further human economic or social objective (Yang *et al*., 2017). Poor land-management practices and environmental change are affecting below ground communities globally, and the resulting declines in soil biodiversity reduce and impair these benefits (Wall *et al.*, 2015). Soil bacterial species and diversity are important as they are major decomposers of soil organic matter and can influence carbon and nitrogen turnover rates (Hättenschwiler *et al*., 2005), which provides substrates to microbes and nutrients to plants. Nitrogen mineralization in the soil also depends on the abundance and diversity of bacterial taxa (De Ruiter *et al*., 1993). Higher bacterial diversity is crucial for the breakdown of organic matter that involves complex processes such as chitin degradation (Beier & Bertilsson, 2013). Human activity in soils, including increased use of chemical fertilizers, pesticides, and tillage, has led to a decrease global soil biodiversity (Hirsch, 2010). Conventional farming systems, crop monocultures, use of chemical fertilizers, and intensive use of agrochemicals eliminate certain group of microbes and decrease overall soil microbial diversity (Stagnari *et al*., 2014). The important role that soil microorganisms play in the nutrient and energy-flow relationships of natural as well as man-manipulated environments has given rise to the need for easily measured biological indicators of ecosystem development and disturbance. Soil microorganisms are also agents that promote aggregate stability and good soil

structure. Soil microbial biomass changes more quickly than does soil organic matter as a whole due to changes in soil management (Linzhang *et al* ., 2003).

2.2.1 Soil microbial biomass

The soil microbial biomass (SMB) is a small but key component of the active soil organic matter (SOM) pool and serves as a source and sink of soil nutrients (Smith and Paul, 1990). The microbial biomass has been characterized as a sensitive index for changes in the soil organic carbon that result from management and land use (Brookes, 2006). It has been used to understand soil nutrient dynamics and as an ecological marker (Paul and Voroney, 1980). The SOM and STN are the major determinants and indicators of soil quality and fertility and are closely related to soil productivity in an agricultural ecosystem (Reeves, 1997). Soil microbial biomass acts as a keystone biological driver to the ecosystem functioning (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). One of the most successful, rapid and reliable methods for a direct quantification of the SMB in aerobic soils is the chloroform-fumigation extraction (CFE). Soils are fumigated with chloroform vapor inducing a lysis of microbial cell membranes and thus, the subsequent release of microbial constituents which can be extracted, as microbial biomass C (Vance *et al*., 1987) or microbial biomass N (Brookes *et al*., 1985). While efficient lysis of microbial cells in aerobic soils can easily be achieved with chloroform vapor, (Inubushi *et al*., 1991) proposed the direct addition of chloroform to anaerobic soil in order to overcome limitations in the fumigation efficiency when exposing water-saturated Results suggested that the adapted CFE assay generates reliable and reproducible estimates of microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) (Christian and John, 1999).

2.2.2 Factors affecting microbial biomass

In tropical nations like Ethiopia, the conversion of natural forests into other land uses is a common occurrence that affects the physical, chemical, and biological characteristics of the soil in addition to causing changes in ecosystem services, biodiversity loss, and climate changes (Tripathi, *et al.,* 2007). While unreasonable land use management can result in a sharp decline in soil biodiversity and the degradation of ecological functions, which can affect the sustainable development of soil resources, reasonable land use management can improve soil quality and increase the resistance to

external disturbance (Yao *et al* ., 2000; Lal, 2004). The diversity and abundance of soil microbial communities fluctuate in response to external influences like human activity and global climate change. It is crucial to comprehend how soil ecosystem processes, soil microbial community changes, and soil diversity are affected by both human activity and global climate change in order to comprehend soil quality and nutrient turnover (Simpson *et al.,* 2007). Soil biodiversity is often negatively affected by the interaction between poor land management practices and drivers of climate change, both of which ultimately compromise ecosystem function and services that are essential for human health (control of pests and pathogens, production of nutritious food, cleansing water and reducing air pollution). Responses to reduced human health can in turn affect management decisions that govern land use and climate change (Wall *et al.*, 2015).

2.2.3 Function of soil microbial biomass

Although soil microbes are essential to many soil processes, precisely quantifying the contributions of microbial biomass remains a major analytical challenge because of the structural complexity of soil organic matter (Simpson *et al*., 2007). The amount of microorganisms in the soil influences the soil's transformation and nutritional state (*Norouzi et al.,* 2010). Ecology has long debated the relationship between biomass and biodiversity. Biomass and biodiversity in the soil are vital components that propel ecosystem processes. Nevertheless, in contrast to plant communities, little is understood about the relationships between the diversity and biomass of soil microbial communities across globally distributed biomes, and how changes in these relationships affect the functioning of ecosystems (Bastida *et al.,* 2021). The primary regulators of essential ecosystem processes, including organic matter decomposition, nutrient cycling, and gaseous fluxes, are the diversity and biomass of soil microbial communities (Delgado-Baquerizo *et al*., 2020).

3. Materials and methods

3.1 Description of the study area

The research was carried out in the Wondo Genet watershed, Wondo Genet, Sidama region, South Easter Ethiopia. Wondo Genet has situated 263 km from the capital Addis Ababa, 38 km from the regional capital Hawassa and 13 km from the nearby town of Shashemene, West Arsi zone in Oromia Regional State. It is located between 7° 00' 60.00" N latitude and 38°37' and 38° 42' E longitude. The area falls within an altitudinal range of 1600 and 2500 m.a.s.l. The area comprises a series of hills that are the southwestern spur of the Bale Mountains. The agro-climatic zone of the district is traditionally categorized under Woyna-Dega (mid-highland). The area receives a bimodal rainfall pattern (short rains between February and April, and long rains between June and September) with a total annual rainfall ranging between 700mm and 1400mm.

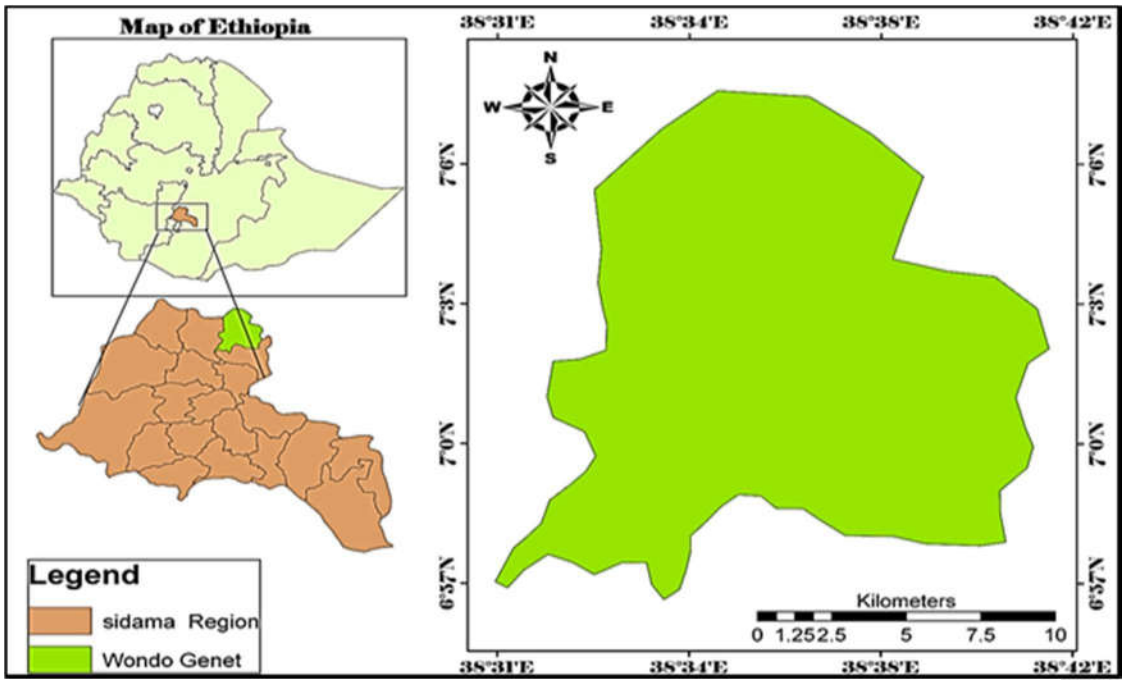

Figure 1: Map of the study area Source : GIS and remote sensing based ecotourism suitability analysis: the case of Wondo genet, Sidama, Ethiopia.

3.2 Study design (Study type, Sample size and Sampling Procedure)

The target population for this particular study was been small holder farming households in wider Wondo Genet Watershed who are directly and indirectly managing agroforestry for its economic, social and climate adaption purposes. Given the time and financial constraint of involving all small-holder farmers in the watershed for the research; a stratified random sampling approach were

used to identify sample households and farm plots soil physicochemical and microbial biomass roperties. The land use type were selected at similar altitudinal locations gradient of the landscape to minimize variation in climatic variables and slope. Within each agroforestry study site, 3 sampling units, 10 farms for each sampling units were randomly selected. The altitude, slope, GPS location, land use type, age of each land use type and site history were also be recorded. For this study three dominant land use type were used: *Enset* based land use type, *Enset-coffee* land use type system, *Coffee-Avokado* tree-*Enset* based land use type (Annex 1).

3.3 Soil sampling techniques

A total of 60 composite soil samples (3 land use types*10 farms site for each* 2 soil depths: 0-15 and 15-30cm) were collected from the land-use types. Then ten (10) experimental plots were selected randomly from each dominant land use type. A total of 30 sampling sites was selected and three sampling unit were determined from each sampling sites, and also the two depth were considered (0-15cm and 15-30cm). The first transect lines were laid out 20m away from the border randomly. From each sampling unit, five soil samples (20m x 20m) were collected by using W-shape sampling technique (i.e four from the corners and one from the center and then the composited single sample were taken for the analysis). The samples was collected from two soil depth that is (0-15cm and 15-30cm) by using core borer. A total of 60 soil samples were collected for the study. The core borer was 15cm length and 7cm diameter. Then the sampled soil was immediately brought to the laboratory by clean, dry and sterile polythene bags.

3.4 Soil analysis

The collected soil samples were air dried, crushed and sieved through a 2-mm sieve and the soil pH, organic carbon (OC), total nitrogen (tN) were analyzed by standard methods as suggested by (Allen et al., 1974; Waksman, 1952).

3.4.1 Soil physico-chemical analysis

Soil pH: Standard laboratory procedures for measuring soil (pH (H2O) were followed as proposed by (Lam, 1983; and Li and Heap, 2011). For measuring soil pH, 1g of fresh soil was dissolved in 5ml of sterilized distilled water and then measured the pH by using pH meter.

Soil moisture content: Standard laboratory procedures for measuring soil moisture were followed as proposed by (Lam 1983; and Li and Heap, 2011). For measuring soil moisture content, 5g of fresh soil was dried at $105^{O}C$ in oven to constant weight. Soil moisture content was calculated as:

$$\text{Soil moisture content (\%)} = \frac{\text{Weight of fresh soil} - \text{Weight of dry soil}}{\text{Weight of dry soil}} * 100$$

Bulk density: Bulk density were determined by removing a block of soil from site, allowing no compaction or crumbling. This was done by metal ring into the soil and digging the ring or can out when full of soil. The soil is then dried in an oven and weighed. The volume was determined by measuring the volume of the container used to extract the soil. The bulk density was expressed in units grams of oven-dry soil per cubic centimeter (Blake & Hartge, 1986). Soil bulk density was determined by removing a known volume of soil using metal tubes and oven drying it at 105°C for 24 h. the bulk density is calculated by the following formula:

$$\text{Bulk density} = \frac{\text{Oven dry soil } (g)}{\text{soil (cm3)}}$$

Soil aggregates: The dry method developed by (Khormali and Nabiallahy, 2009) was used to estimate soil aggregates. Air dried soil samples (100 g) will be placed on top of the nest of sieves and sieved for 3 min on a horizontal shaker (92 rpm), and three dry aggregate size classes separated were,1000 mm (macro-aggregate), 212-500 mm (meso-aggregate) and 53-150 (micro-aggregate).

Soil organic carbon and total nitrogen

Soil organic C was estimated by the dichromate oxidation and titration method (Kalembasa and Jenkinson, 1973). Whereas total N concentration was measured by the micro kjeldahl method (Jackson, 1973) by using a Gerhardt digester and distillation unit. The calculation was done by the following formulas:

$$\text{\% organic carbon} = \frac{}{\text{Sample weigh (g)}}$$

And

$$\%N = \frac{(B-T)*0.15}{(1-B)*0.1*1.4 \text{ x sample}}$$

3.4.2 Soil Microbial biomass analysis

Microbial biomass Carbon

Microbial biomass carbon (MBC) was estimated using the chloroform fumigation extraction method**,** following the procedure described by (Brookes *et al*., 1985) and (Vance *et al.*, 1987). Fresh soil samples (20 g) were incubated for 7 days at room temperature in a closed container. After incubation, two subsamples were prepared for each soil sample: Sample A (without chloroform**)** and Sample B (with chloroform) to measure soil microbial biomass carbon. For fumigation, purified chloroform ($CHCl_3$) was added in proportion to the soil mass. Specifically, 0.25 ml of chloroform was added per 5 g of soil**,** and 0.5 ml per 10 g of soil. In this experiment, 10 g of incubated soil was used for Sample B, to which 0.5 ml of chloroform was added. The flasks were then sealed and maintained for 24 hours to create a chloroform-saturated micro-atmosphere. Excess chloroform was removed by introducing a flux of air CO_2 free for the 30 sec. Following fumigation, 20 ml of 0.5 M K_2SO_4 was added to each sample. The mixture was then mechanically shaken for 1 hour and filtered using conventional filter paper to extract the microbial biomass carbon.

A mixture was prepared by adding 8 ml of extracts, 2 ml of $K_2Cr_2O_7$, 15 ml of di-acid, and 0.07 g of H_2O in a round-bottom flask. The solution was then thoroughly mixed and refluxed for 30 minutes at 70–80°C. After the refluxing process, the mixture was cooled and diluted with 25 ml of double-distilled water (DDW). It was then titrated using 33.5 mM ferrous ammonium sulfate (FAS) with three drops of phenanthroline indicator. The titration process involved detecting color changes from greenish-blue to light green and finally to maroon/bloody. Additionally, a cold blank (non-fumigated sample) was also processed following the same steps to ensure accuracy in measurement. Microbial biomass carbon (µg dry soil) is calculated by the following formula:

$$\text{MBC} = \frac{NF-F}{B} * 3168.$$

Where: NF, non-fumigated; F, fumigated; B, blank

Microbial biomass nitrogen

Microbial biomass nitrogen (MBN) concentration was measured by the micro Kjeldahl method (Jackson, 1973) by using a Gerhardt digester and distillation unit. A mixture was prepared by combining 50 ml of extracts, 10g of K_2SO_4, 0.5 g of $CuSO_4$, and 15 ml of concentrated H_2SO_4 in a boiling tube. The mixture was then set aside for 10 minutes to allow for initial reaction stabilization. After this resting period, the prepared sample was loaded onto the digestion rack for further processing. A blank sample was digested along with seven other samples, making a total of eight tubes per batch during the digestion process. Microbial biomass nitrogen (µg dry soil) is calculated by the following formula:

$$\text{Microbial biomass N} = (Fu - NFu) * 207.407$$

Were ; Fu = fumigated , NFu , non-fumigated

3.5 tatistical analysis

A one way ANOVA (analysis of variance) was performed to identify significant differences in microbial biomass and soil physicochemical priorities. The Pearson correlation method was used to analyze the correlation between MBC, MBN, SOC and TN and other correlation analysis was performed using SPSS version 27. The figures were generated using the origin version 28. $P < 0.05$ was considered to be statistically significant. Mean ±standard error mean was used by software *statistix10* shown in tables.

4. Result and Discussion

4.1 Results

4.1.1 Soil physicho-chemical properties

Soil moisture content, pH and Bulk density

The major physical properties of soils of different sites investigated are presented in Table 1 (pH, moisture content, aggregates, porosity and bulk density). As shown in table (1), moisture contents were lowest in *Enset* (0.59 ± 0.08^{c}); while highest was found in *Enset-coffee-avocado* land use type (0.88 ± 0.08^{a} %), however, no significant difference among the land use changes was observed at $p<0.05$. The content of moisture in *Enset-coffee* agroforestry system was (0.77 ± 0.03^{a}) the surface of the soil.

Table 1: Soil physico-chemical properties: pH, bulk density and moisture content across the land use types

LUT	Soil Depth cm	pH	BD (g/cm^3)	MC (%)
EN	0-15	7.17 ± 0.06^{a}	619.95 ± 21.08^{a}	0.59 ± 0.08^{c}
	15-30	7.04 ± 0.27^{a}	680.01 ± 16.20^{a}	0.62 ± 0.06^{c}
EC	0-15	7.24 ± 0.50^{a}	614.02 ± 18.75^{a}	0.77 ± 0.03^{a}
	15-30	7.26 ± 0.55^{a}	651.56 ± 28.92^{a}	0.73 ± 0.03^{b}
ECA	0-15	7.10 ± 0.22^{a}	509.11 ± 37.76^{a}	0.88 ± 0.08^{a}
	15-30	6.70 ± 0.44^{a}	647.98 ± 22.13^{b}	0.76 ± 8.53^{b}

Values are mean ± SE. In each columns values having different superscript are significantly different from each other ($P < 0.05$). EN(Enset), EC (Enset-coffee), and EC (Enset-coffee-Avocado) , pH , BD (Bulk density) , MC (moisture content).

Bulk density was not significantly affected by land use change; it was higher in *Enset* land use type (619.95 g/cm^3) followed in decreasing order in *Enset-coffee* (614 g/cm^3) and *Enset-Coffee-Avocado* land use type (509.11g/cm^3) in the upper parts of the soil. Bulk density increased when the soil depth increased. Similarity, in the deeper of the soil the bulk density was higher in *Enset* followed by *Enset-coffee* and *Enset-coffee-avocado* land use type.

Soil Aggregates

Soil aggregate is the naturally occurring cluster or group of soil particles and measures formation of organo-mineral complex (union of mineral and organic matter) in the soil. Across different land use types, macro-aggregates constituted (47.60-80.71%) of total soil followed by meso-aggregates (22.18 to 35.94%) and micro-aggregates (3.11–7.16%) (Table 2). Macro-aggregates were significantly higher in *Enset-coffee-avocado* tree land use type (80.71%) followed by *Enset* and *Enset-coffee* land use type (59.71%) and (55.10%), respectively. Meso and micro- aggregates were higher in *Enset-coffee-avocado* and *Enset-coffee* land use type. The highest percentage in Enset soil depth at 0-15cm (59.7%) and the lower at 15-30cm (53.08%); the macro- aggregate *Enset-coffee* soil depth at 0-15cm (55.10%) and lower at 15-30cm (47.60%); the highest percentage in *Enset-coffee-avocado* at the soil depth of 0-15cm (80.71%) and the lower at 015- 30cm (57.932 %). The analysis of variance revealed that no significant differences were observed between the land use types as well as soil depths. The micro-aggregates were also not significantly affected by land use change, but the highest was recorded in *Enset-Coffee-avocado* tree land use type.

Table 2. Percentage of distribution of different dry aggregate soil size classes in different land use types

		Land use type		
Soil aggregates (%)	Depth	EN	EC	ECA
Macro-aggregates	0-15cm	59.71±3.28^{a}	55.10±8.89^{bc}	80.71±3.23^{bc}
	15-30cm	53.08±4.32^{ab}	47.60±2.62^{bc}	57.93±4.94^{c}
Meso aggregates	0-15cm	29.96±5.16^{a}	24.73±1.65^{ab}	33.46±1.36^{a}
	15-30cm	28.10±6.19^{ab}	22.18±3.54^{ab}	35.94±5.34^{b}
Micro-aggregates	0-15cm	4.17±3.37^{a}	3.21±1.07^{a}	3.79±1.90^{a}
	15-30cm	3.11±1.47^{a}	7.16±4.39^{a}	6.22±2.50^{a}

Values are mean ± SE. In each rows, values having different superscript are significantly different from each other ($P < 0.05$). Were EN; *Enset* , EC; *Enset-coffee*, ECA; *Enset-Coffee-Avocdo.*

While mico-aggregate showed the reverse of the macro-aggregate. So micro-aggregate increased along the soil depth except *Enset* land use type. The micro aggregates in *Enset-coffee* and *Enset-coffee-avocado* at surface soil were (4.02%) and (3.76%) whereas at the sub soil micro- aggregate (4.12%) and (4.02%), respectively. Thus at the surface the soil micro-aggregate

is lesser due to higher macro-aggregate and higher organic matter.

Soil texture

The sandy soil is less efficient for the microbial mobilization, and the agricultural productivity, Compared to the other soil types. The sandy soil percentage is higher in the *Enset-coffee-avocado* land use type, while medium by percent in the *Enset-coffee* land use type soil and relatively lower in *Enset* land use type soil, and the sand soil decreased with increased of the depth (Table 3).

Table 3: soil texture means in agro-ecosystem.

Lut	depth	Sand(%)	Clay(%)	Silt(%)
EN	0-15cm	37.50±0.50^{a}	25.00±1.73^{a}	37.50±2.06^{a}
	15-30cm	34.00±2.00^{a}	31.00±1.73^{b}	35.00±2.38^{a}
EC	0-15cm	45.00±3.31^{a}	19.50±2.21^{c}	36.00±1.15^{a}
	15-30cm	45.00±2.51^{a}	23.00±0.57^{c}	31.50±2.87^{a}
CEA	0-15cm	50.50±1.44^{b}	14.75±1.10^{d}	35.00±1.00^{a}
	15-30cm	47.50±2.36^{b}	20.00±2.00^{d}	35.00±2.51^{a}

Values are mean ± SE. In each columns values having different superscript are significantly different from each other (P<0.05). Were EN; Enset, EC;Enset-Coffee, ECA; Enset-Coffee-Avocado

Chemical properties of the soil

The present study was determines the chemical properties of soil, particularity organic carbon and total nitrogen, *Enset-coffee-avocado*, *Enset-coffee* and *Enset* land use type (Table 3). Soil organic carbon and total nitrogen was distinctly varies to the land use types. The highest soil organic carbon was obtained from Enset coffee avocado land use type (3.43%) followed by *Enset- coffee* and *Enset* land use type (2.14%) and (1.81%), respectively in the upper parts of the soil. Soil organic carbon in *Enset-coffee-avocado* land use type was significantly different with *Enset coffee* and *Enset* land use type; whilst there was no significant difference recorded between *Enset- coffee* and *Enset* land use type in the upper parts of the soil.

Table 4: Soil organic carbon (SOC), soil total nitrogen (STN), Microbial biomass carbon (MBC) and Microbial biomass nitrogen (MBN) under different agroforestry systems.

LUT	Depth(cm)	MBC(µg)	MBN(µg)	OC (%)	TN (%)
EN	0-15	288.24±39.06^{a}	31.30±7.79^{a}	1.81±0.28^{a}	0.16±0.02^{a}
	15-30	141.80±35.88^{b}	15.47±2.95^{b}	1.07±0.21^{b}	0.09±0.01^{b}
EC	0-15	812.26±408.43^{bc}	76.29±41.45b^{c}	2.14±0.22b^{c}	0.18±0.01^{b}
	15-30	152.31±43.14^{bc}	14.63±5.19b^{c}	1.40±0.16b^{c}	0.12±0.01^{bc}
ECA	0-15	1601.3±206.31^{c}	138.03±17.78^{c}	3.43±0.35^{c}	0.29±0.03^{bc}
	15-30	395.85±90.91^{c}	65.56±12.08^{c}	1.51±0.19^{c}	0.10±0.01^{c}

Values in Mean ± SE, across the columns different letters (a, b, c) show that there is difference among the mean values at the significance level $P < 0.05$. MBC; microbial biomass carbon,MBN; microbial biomass nitrogen, OC; organic carbon , TN; total nitrogen.

Similarly, variation in soil total nitrogen concentration along the various land use types was found to be highest in *Enset-coffee-avocado* land use type (0.29%) followed in decreasing order *Enset-coffee* (0.18%) and *Enset* land use type (0.16%). The analysis of variance showed that there was significant difference between across the land use type at $P<0.05$ and along the soil depths (Table 4).

Soil Microbial Biomass

As shown in Table 4, results of the microbial biomass carbon and nitrogen varied significantly across the agroforestry systems at ($p <0.05$). The highest microbial biomass carbon and nitrogen was obtained from *Enset-coffee-avocado* land use type, whereas the least was recorded in *Enset* land use type. The mean values for microbial biomass C were 1601.3, 812.26 and 288.24µg/g in the *Enset-coffee-Avocado*, *Enset-coffee* and *Enset* land use type, respectively in the upper parts of the soil. Along the depth the soil microbial biomass carbon decreased in all land use type, but the highest was recorded in *Enset-coffee-avocado* land use type followed by *Enset- coffee* and *Enset* land use type. Likewise, the mean microbial biomass N values under the *Enset* coffee avocado, *Enset-coffee* and *Enset* land use type soils were 138.03, 76.29 and 31.30µg/g, respectively. Microbial biomass nitrogen obtained from *Enset-coffee-avocado* was significantly differs with other land use types. Additionally, *Enset-coffee* was significantly varied with *Enset* land use type in microbial biomass nitrogen. The microbial biomass nitrogen decreased along the soil depth but no significant differences was observed among the three agroforestry land use type.

The ratio of SMBC/SMBN%, SMBC/C%, and SMBN/ N%

The soil MBC to SMBN ratio was significantly different across study land use types and the soil depth. SMBC/SMBN ratio was higher in *Enset-coffee* land use type (11.6%) at 0-15cm, followed by *Enset-coffee* land use type (10.64%) at 0-15cm and then *Enset* land use type (9.2%) at 0-15cm (Table 5). The ratio revealed decreased in all land use type with increase the depth. The soil SMBC/C ratio was also significantly different among the land use types and depths. The highest SMBC/C ratio was found in the *Enset-coffee-avocado* land use type (4.66%) at 0-15cm, followed by *Enset-coffee* (3.7%) at 0-15cm and then *Enset* (1.6%) at (0-15cm). The soil MBN/N ratio show the same trend. The highest MBN /N ratio were found in the *Enset-coffee-avocado*

(0.5%) at 0-15cm, followed by *Enset-coffee* (0.4%) at 0-15cm and *Enset* (0.2%) at (0-15cm) , but it decreased were the soil depth decreased in all land use type in study area.

Table 5: ratio of soil organic carbon, total nitrogen and microbial properties at 0-15 cm and 15- 30cm depth of the agroecosystems.

LUT	DEPTH	SMBC/ SMBN	OC%	TN%	SMBC/C%	SMBN/N%
EN	0-15CM	9.2	1.81	0.16	1.6	0.2
	15-30CM	9.16	1.07	0.09	1.3	0.2
EC	0-15CM	10.64	2.14	0.18	3.7	0.4
	15-30CM	10.41	1.40	0.12	1.08	0.1
ECA	0-15CM	11.6	3.43	0.29	4.66	0.5
	15-30CM	6.03	1.51	0.1	2.3	0.5

Correlation

Pearson's correlation coefficients between MBC, SOC, MBN, STN, moisture content, soil aggregates and bulk density is given in (Table 6).

Table 6: Correlation matrix for physical, chemical, and microbiological characteristics of soils from different land uses.

CORRE	SMBC	SMBN	SOC	STN	pH	BD	MC	MI	ME	MA	SA	CL	SI
SMBC	1												
SMBN	0.94**	1											
SOC	0.73**	0.64**	1										
STN	0.72**	0.63**	0.99**	1									
pH	0.08 ^{NS}	-0.03 ^{NS}	0.17 ^{NS}	0.17 ^{NS}	1								
BD	-0.67**	-0.55**	-0.64**	-0.54**	0.03 ^{NS}	1							
MC	-0.41*	-0.39 ^{NS}	-0.41*	-0.41*	0.14 ^{NS}	0.51*	1						
MI	-0.17^{NS}	-0.03 ^{NS}	-0.14 ^{NS}	-0.15 ^{NS}	-0.46*	0.03 ^{NS}	0.06 ^{NS}	1					
ME	0.15 ^{NS}	0.23 ^{NS}	0.25 ^{NS}	0.25 ^{NS}	-0.26 ^{NS}	-0.12 ^{NS}	0.08 ^{NS}	0.23 ^{NS}	1				
MA	0.53**	0.50*	0.72**	0.72**	0.16 ^{NS}	-0.53**	-0.45*	-0.18 ^{NS}	0.37 ^{NS}	1			
SA	0.57**	0.60**	0.33 ^{NS}	0.32 ^{NS}	-0.11 ^{NS}	-0.40*	-0.48*	0.26 ^{NS}	0.01 ^{NS}	0.12 ^{NS}	1		
CL	-0.65**	-0.71**	-0.55**	-0.54**	0.13 ^{NS}	0.40 ^{NS}	0.47*	0.02 ^{NS}	0.19 ^{NS}	-0.46*	-0.70**	1	
SI	0.02 ^{NS}	0.03 ^{NS}	0.09 ^{NS}	0.10 ^{NS}	-0.08 ^{NS}	0.06 ^{NS}	0.11 ^{NS}	-0.48*	0.30 ^{NS}	0.32 ^{NS}	-0.43*	-0.25 ^{NS}	1

*significant at 0.05, **significant at p<0.01, ***p<0.001, and ^{NS} non-significant

The soil properties such as MBC, SOC, MBN, STN and soil macro aggregates were strongly positively correlated to each other, and negatively correlated with bulk density, meso and micro soil aggregates. Additionally, soil organic carbon and total nitrogen were positively correlated (r=0.99). In contrast, soil organic carbon and nitrogen negatively correlated with bulk density (r=- 0.64, and -0.54, p<0.05, respectively). Soil SMBC strongly correlated with the SMBN (r = 0.94, p<0.01). SMBC to SOC was strongly related (P< 0.01) to STN (r = 0.73**) and SMBC to STN also strongly correlated (r=0.72**, P< 0.01). While SMBN was strongly correlated to SOC (r = 0.65**, P ≤ 0.01). SMBN to STN were also strongly correlated (r=0.63**, P ≤ 0.01).

4.2 Discussion

The study results indicate significant differences in soil physico-chemical properties including soil moisture, bulk density, pH, and microbial biomass across the three land-use types. These variations are influenced by the management practices specific to each site. Previous research has also highlighted the effects of land use and soil management changes on these properties (Malik *et al*.,2013). The soil bulk density increased along the soil depth (Table 1), this study is comparable to (Moges *et al*., 2013). The increasing soil bulk density with increasing soil depth indicates that sub layers the organic matter were getting lower, and the same study reported by (Moges *et al* .,

2013). Bulk density typically increases with soil depth since the lower soil are more compacted and have less organic matter, less aggregation, and less root penetration compared to surface layers, therefore contain less pore space (Singh *et al .,* 2015). 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 bulk density strong negatively correlated with SMBC, SMBN, SOC, and STN (r=-0.67**), (-0.55**), (-0.55**), (-0.54**) and ($p< 0.01$). This indicates that when the bulk density increased the soil microbial biomass become decreased (Table 6).

Soil pH did not show any significant variation across land use types or soil depths ($p<0.05$, Table 4). Similar report showed by (Yohannes, 2017). At the surface of the soil in *Enset* and *Enset-coffee-avoacado* land use type, the pH revealed slightly higher or displayed alkaline property except *Enset-coffee*, where as the depth increased it become decreased shows the neutrality (Table 4). 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 this report. Also the report of (Lepcha, & Devi 2020) the soil pH decreased with the increase of the soil density.

The study found that the soil moisture content was significantly affected by the land use type and the soil depth. The moisture content in the study area were significantly different ($p< 0.05$) in all agro ecosystem at both the surface soil and sub-surface soil. The *Enset* agroforestry land use type soil was less moisture content than *Enset-coffee* and *Enset-coffee-avocado* land use type use type. This indicates that relatively the *Enset* agroforestry may have low macro-aggregate, and organic matter content than study land use types. Optimal moisture affects the solubility and availability of nutrients in the soil. Optimal moisture content can enhance nutrient mobility, making them more accessible to microbes, which can lead to an increase in microbial biomass (Table 4). Both excessively dry and wet soil may lead to a decrease in the biomass of microorganisms (Landes Man and Dighton, 2010).

Soil aggregation is a product of interactions of the soil microbial community and mineral and organic composition and is influenced by many factors such as soil environment, management practices, land use patterns, as reported by (Kabelka *et al* ., 2025). Excessive tillage destroys soil organic matter and weakens the natural stability of soil aggregates making them susceptible

to erosion caused by water and wind (Arshad, *et al*., 1997). The analysis of variance revealed that soil macro aggregate significant difference were observed between the land use types as well as soil depths and it significantly affected by land use type. The micro-aggregates was not significantly affected by land use change, but the highest soil macro-aggregate was recorded in *Enset-Coffee-avocado* tree land use type, this due to leaf falls and the presence of the high organic carbon.

The soil texture class varied based on the land use type and they are significantly different from agroforestry to agroforestry in this study area (Table 2). The present study were comparable to the study reported by (Yimer *et al* ., 2006). The clay soil fraction is relatively lower at the top soil while it revealed higher when the depth increased. The increase in clay fraction with increasing depth and the lowest overall mean proportion of clay fraction compared to the sand and silt fractions with the results of other studies (Yimer *et al .,* 2006). Were observed with the change in land-use types and soil depth. Sand particles were highest in the *Enset-coffee-avocado* land use types (50.50%) followed by *enset-coffee* land use types (45.00%) and lowest in *Enset* land types (37.50%), While clay soil particles show a reverse trend with a maximum in *Enset* land use types(25.00%) and minimum in *Enset-coffee-avocado* land use types (14.75%). The reason for this reverse trend corresponds to the microbial biomass decreased due to the low litters (duff) fall and plant remains, The small particle size of clay can make clay soils susceptible to compaction under pressure, especially when wet (Wagner, 2013). Soil moisture were higher in the *Enset,* followed *Enset-coffee* and *Enset-coffee-avocado* soils (4.27%), (4.12%) and (4.02%) respectively. In this the result reveals that the clay property of the soil increased, the soil moisture content also increased.

Soil organic C and N is considered to be one of the major attributes of soil fertility and agricultural sustainability (Lal, 2002). As per the finding of this study, the highest OC and tN was found to be under *Eset-Coffee-Avokado* land use type (3.43%, 0.29%) and the lowest OC and tN was found to be under *Enset* agroforestry system (1.81%, 0.16%) (Table 4). This is comparable with other similar studies elsewhere (Pereira *et al*., 2013; and Gol, 2009). The study indicates the conversion of diverse agroforestry system into mono-culturing like *Enset* agroforestry practice was significantly decreased soil OC and tN. The regular addition of plant litter (above and below ground plant parts), high plant biodiversity and root exudates (Srivatava and Singh, 1991, and Iqbal and Goni, 2015) may contribute for high soil organic carbon concentration. Additionally,

the higher soil OC and tN in *Enset-coffee* land use type as compared to *Enset* land use type was probably due to addition of nutrient rich leaf litter to soil and also due to recycling of these nutrients (Chaudhary *et al*., 2008; Tripathi and Singh, 2007; Saha *et al*., 2010; Vesterdal and Leifeld, 2010; Tripathi and Singh, 2007) low SOC in the *Enset* was found. Moreover, soil organic carbon (SOC) and total nitrogen decreased across soil depth in all agroforestry systems with maximum content in top soil due to the availability of more organic matter from trees (Table 4). The continuously addition of leaf 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; Reza *et al*., 2018) which conforms with the present report.

The current study found that the MBC was higher in *Eenset-coffee-avocado* (1601.3±206.31) followed by *enset-coffee* (812.26±408.43) and *Enset* (288.24±39.06) (Table 4) because of the presence of a litter layer better than *enset* or mono cropping that promotes microbial activity. Wu *et al*., (2016) reported a higher MBC in afforested soils with higher litter inputs which agree with the present findings. A significant positive correlation between soil organic matter and soil microbial biomass carbon (Table 6) in my 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 (Chen *et al*., 2017). Further, high soil N in the *Enset-coffee-avocado* soil and *Enset-coffee* land use type due to higher microbial biomass C in these sites. MBC was more in the surface soil layer and less in the sub-soil in all the land-use types (Table 4). This pattern is because of lower microbial biomass carbon and nitrogen content in the lower subsoil and more organic matter (OM) in the top humus soil that promotes microbial activity, reported similar findings (Soleimani *et al*., 2019). 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 availabilit, and plant composition (Van Leeuwenet *et al*., 2017). A similar trend was reported by several studies in various ecosystems (Soleimani *et al*., 2019).

The present study along the soil depth, the microbial biomass was significantly affected (Table 4), the same finding was reported by (Yohannes, 2017). The soil microbial biomass was

decreased along the soil depth in all agroforestry systems. The same result was observed by (Limbu *et al*., 2020), the concentration of MBC was decreased with increase in depth. Surface soil contains large pool of organic matter that supports a uniquely large and active soil microbial community as reported by (Lehmann & Kleber, 2015). Because of the high nutrients concentration in the topsoil, soil microbial biomass increased at the surface layer and decreased with the increase in depth (Limbu, *et al*., 2020).

The soil microbial property is determined by the ratio of MBC and MBN, the size of the microbial biomass is mainly potentially related to C inputs (Smith *et al.,* 2017). As reported by (Joergensen, 1995) SMBC/SMBN ratios varying from 5.2 in an arable soil to 20.8% in a forest soil. The SMBC/SMBN ratio has often been used to describe the structure of the microbial community.

(Moore *et al*., 2000). A lower SMBC/SMBN ratio indicates that the microbial biomass contains a higher proportion of bacteria whereas a higher value suggests that fungi predominate in the microbial population (Campbell, 1991). The highest ratio was obtained from *Enset-coffee-avocado*, 11.01 and 6.03 in the upper and lowest soils, respectively (Table 5). This indicates the predominance of fungi in *Enset-coffee-avocado* land use type. Whereas in *Enset* land use type the ratio of SMBC/SMBN was 9.2 and 9.16 in the upper and sub soils; *Enset-coffee* land use type SMBC/SMBN 10.64 and 10.41 in the upper and sub soils, respectively.

Similarly, the soil microbial biomass ratio indicates the distribution of the microorganisms in the soil. The ratio (SMBC and SMBN) mainly indicates the fungi and bacteria community in the soil. Values of the soil microbial biomass (SMBC) and (SMBN) in all the land use type was significantly affected by along the soil depth (Table 5). The surface soil had relatively higher ratios of SMBC/SMBN than the deeper parts of the soil in all land use type. In the *Enset-coffee-avocado* land use type the surface soil had higher SMBC/SMBN ratio than other land use type. This ratio was deceased by half along the soil depth Soil depth. This may be an indication of a shift from fungal to bacteria population at the lower depth (Wheatley *et al*., 1990). Therefore, the surface of the soil predominant by fungal community and shifting to the bacterial community with depth increased in all study land use type soil.

The size of the microbial biomass is mainly potentially related to C inputs (Mc Daniel *et al.,* 2014). The SMBC and SMBN when expressed as percentages of SOC and STN respectively give an estimation of the quantities of nutrients in the microbial biomass, organic matter dynamics and

substrate availability in soils (Sparling, 1992). In upper and lower soil depths, the SMBC was between 1.3%-4.7% of SOC whereas SMBN was 0.2%-0.50% of STN, respectively (Table 5). The range of SMBC as a percentage of SOC obtained in this study is comparable to the range of 0.99%- 4.30% reported by (Adeboye *et al.,* 2011). The SMBC as a percentage of SOC were higher in the surface soil than in the deeper parts of soil in all land use type. These results may be due to greater C and N inputs, which are of a quality stimulating greater soil microbial biomass production, into the surface soil (Sparling, 1992).

5. Conclusion and Recommendation

5.1 Conclusion

- The results of this study indicate that land use type and soil depth significantly influence soil physico-chemical and microbial properties.
- Most of the selected soil physico-chemical properties were significantly ($p<0.05$) affected by land use type and soil depth, except for soil pH.
- Soil microbial biomass carbon (MBC) and nitrogen (MBN) were significantly ($p<0.05$) influenced by both land use type and soil depth.
- Soil physico-chemical and microbial properties decreased with increasing soil depth across all land use type.
- The *Enset-Coffee-Avocado* land use type had the highest levels of soil microbial biomass (MBC and MBN), soil organic carbon (SOC), and total nitrogen (tN), whereas the lowest values were recorded in the *Enset* based monoculture land use type.
- The *Enset-Coffee-Avocado* agroforestry system had the highest MBC/MBN ratio among the studied agroforestry systems, suggesting that it supports a more diverse and active microbial community.

5.2 Recommendation

- Encourage the adoption of *Enset-Coffee-Avocado* agroforestry systems, as they enhance soil nutrient content, improve physical soil conditions, and support a higher microbial biomass, which contributes to overall soil health and productivity.
- Introduce and strengthen sustainable land management practices to minimize soil degradation, particularly in monoculture agroforestry systems where lower soil quality and microbial activity were observed.
- Introduce and strengthen sustainable land management practices to minimize soil degradation, particularly in monoculture agroforestry systems where lower soil quality and microbial activity were observed.
- Increase organic inputs such as compost, mulch, and green manure to improve soil organic carbon (SOC) and total nitrogen (tN), particularly in monoculture agroforestry systems.

- Encourage the use of organic amendments and agroecological practices that support microbial diversity, as seen in the *Enset-Coffee-Avocado* agroforestry system, to sustain soil fertility and enhance ecosystem resilience.
- Conduct long-term studies to explore the impact of various agroforestry combinations on soil health, crop productivity, and climate resilience, with an emphasis on sustainable land use strategies.

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Annex 1: Soil sampling, Sampling site and analysis

Agroforestry system

Soil sampling

Laboratory soil analysis