EFFECT OF LAND USE TYPES AND SOIL MACROFAUNA ON SOIL AGGREGATE STABILITY AND CARBON STORAGE IN THE CENTRAL HIGHLANDS OF KENYA

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A56/77793/2017

Thesis submitted to the university in partial fulfilment of the requirements for the award of degree of Masters of Science in Soil Science

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2021

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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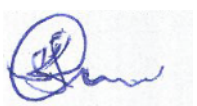
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DEDICATION

I dedicate my thesis work to my family and friends; to the Almighty God for giving me good health, patience and strength.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

ACRONYMS AND ABBREVIATIONS

ANOVA   Analysis of Variance

C   Carbon

CAVS   Collage of Agriculture and Veterinary Sciences

LARMAT   Land Resource Management and Agricultural Technology

LUT   Land use type

SOC   Soil organic carbon

FAO   Food and Agriculture Organization of the United Nations

IPCC   Intergovernmental Panel on Climate Change

ABSTRACT

Changes from natural forests and bush lands to various land use types has been shown to have great influence on soil structure and carbon storage. However, little is still known about how these changes affect soil aggregate stability and C storage over the long-term, especially in Africa. A study was conducted in central highlands of Kenya to evaluate the influence of four land use types on soil aggregation and aggregates C content, as affected by the abundance of earthworms and termites. The four land use types included: (1) an undisturbed natural bush land dotted with *Lantana camara* L. shrubs and *Croton macrostachyus* Hochst. ex Delile. trees, (2) over 50 years old grazed pasture dominated by a mixture of Bermuda grass (*Cynodon dactylon* (L.) Pers.) and Kikuyu grass (*Pennisetum clandestinum* Hochst. ex Chiov.), (3) over 80 years old coffee plantation with *Coffea arabica* L. cv. SL 28, and (4) a maize field that had been cultivated continuously for the last 3 years. Sampling for soil aggregates and soil macrofauna was done using monolith method to a depth of 0.3 m. Separation of stable soil aggregates was done through wet sieving process, where seven aggregate fractions were obtained; large macroaggregates (LM), small macroaggregates (SM), microaggregates (m), silt and clay (s+c), coarse particulate organic matter (cPOM), microaggregates-within-macroaggregates (mM) and silt and clay within macroaggregates (s+cM). The aggregate C content was analyzed using wet oxidation method. Soil macrofauna were handpicked from the monolith soil and preserved in 75% ethanol and identified to genera or species where possible, using morpho-anatomical keys and comparison with reference collections. It was hypothesized that soil aggregates and aggregate C would decrease with increasing level of soil disturbance, with the magnitude of these effects being reduced by the abundance of earthworms and termites. Generalized linear models (GLM) were used to test the effects of land use types on aggregate fractions and aggregate-associated C using R statistical

software However, to test the effects of the two factors on soil macrofauna data, negative binomial regression was chosen as an extension of the Poisson distribution. When analysis of variance (ANOVA) showed significant effects, Tukey's post-hoc tests were performed at $\alpha = 0.05$. Results showed that land use types had significant influence on soil aggregation. Notable differences were observed in large macroaggregates (LM) fraction, with higher weight in bush land (14.4 g 100 g^{-1} soil) and grazed pasture (12.4 g100 g^{-1} soil) compared to coffee plantation (3.9 g 100 g^{-1} soil) and maize field (0.6 g100 g^{-1} soil). On the contrary, microaggregate fraction weight was higher in maize field (41.2 g 100 g^{-1} soil) and lowest in bush land (18.8 g 100 g^{-1} soil). Land use type also had significant effects on soil aggregate-associated C, with the differences similar to those of the aggregate fractions. Among the soil macrofauna recovered, myriapods was the only group that showed significant differences, where *Spirostreptidae sp*. showed the highest abundance in bush land (149.3 individuals m^2) compared to grazed pasture (42.7 individuals m^2) coffee plantation (53.3 individuals m^2) and maize field (5.3 individuals m^2). Soil macrofauna considered 'ecosystem engineers' (earthworms and termites), and have been suggested to have significant effects on soil aggregation also showed no significant differences. Their abundance also showed weak or no correlation with soil aggregates and aggregate-associated C content across the land use types. This study shows the significance of land use change in shaping soil aggregation process and soil C content which could have far-reaching implications on the long-term C storage in the soil. This could be important especially in the tropics where mitigation and adaptation to climate change are ideally closely linked due to the critical importance of soil C for crop production.

CHAPTER ONE: INTRODUCTION

1.1 Background of the study

Conversion of native forests and bush lands to agricultural lands has been shown to accelerate the loss of soil organic matter (SOM) as described by Kamau et al. (2020a). This has partly contributed to increased atmospheric carbon (C) and the associated greenhouse gases, which have been linked to the global climate change. With soil being one of the major sinks of C, the balance between SOM storage and breakdown in agricultural soils is important in controlling the amount CO_2 emitted to the atmosphere (Swift, 2001). Apart from its role in nutrient cycling, SOM is essential for soil aggregate formation and stabilization as it plays an important role as transient or temporary binding agents. In turn, stable soil aggregates protect SOM from further decomposition (Tisdall and Oades, 1982; Six et al., 2002; Kamau et al., 2020a). Among SOM stabilization mechanisms, physical protection of SOM within soil aggregates is key (Tisdall and Oades, 1982). However, physical protection of SOM is significantly affected by land management options applied (Solomon et al., 2000; Mbau et al., 2015; Kamau et al., 2020a). Though studies by Gitari et al. (2018), Kamau et al. (2019) and Nyawade et al. (2019) have shown how land use change can affect productivity of soils in the central highlands of Kenya, little is known about the changes in soil aggregation and soil aggregate-associated C content, brought about by long-term cultivation and grazing. This can be a starting point towards understanding how land use change from native forests and bush lands to cultivated lands affects soil structure and C storage in the long-term.

The importance of soil fauna, especially those termed 'ecosystem engineers' (earthworms and termites), in affecting soil structure has been widely published (Shipitalo and Protz, 1989; Barois et al., 1993; Six et al., 2004; Pulleman et al., 2005; Ayuke et al., 2011). Therefore, the dynamics of soil aggregation cannot be dissociated from soil biological activity. Earthworms, for example, can stabilize the structure by mixing ingested soil with humified organic materials during passage

1

through their guts (Tisdall and Oades, 1982). Shipitalo and Protz (1989) also suggested that the ingestion of soil by earthworms may disrupt the existing bonds in soil aggregates and that the reorganization of the particles may form nuclei which act as sites for the formation of new microaggregates. In addition, Barois et al. (1993) showed that earthworms may reorganize soil structure by grinding and intimately mixing soil mineral and organic materials in their guts, thus changing the amount as well as the orientation of clay particles. However, land use change from native to cultivated soils has been shown to play a key role in determining the patterns of soil macrofauna distribution and consequently affects soil aggregation and C storage. For example, increased tillage operations in conventional farming systems predispose soil macrofauna to physical damage thus, reducing their abundance and diversity (Mbau et al., 2015). Therefore, tillage operations may have significant direct and indirect effects on soil aggregation. This study aimed at assessing influence of four land use types on soil aggregation and C content.

1.2 Statement of the problem

There is an increasing interest in soil quality and belowground biodiversity conservation due to their importance in promoting soil functions (Bommarco et al., 2013). On the other hand, stability of soil aggregates and soil organic carbon (SOC) are key indicators of soil quality in natural and managed ecosystems, and thus important in advancing sustainable management of the environment (Amezketa, 1999; Zhao et al., 2017; Kamau et al., 2020a). However, changes in soil aggregation can be attributed to the physical and chemical properties due to soil management applied (Dexter, 1988). In addition, studies (Tisdall and Oades 1980; Oades 1984; Haynes et al., 1991; Carter 1992; Angers et al. 1993) have also shown how biological processes can contribute to short-term changes in soil aggregation through production or fragmentation of labile organic binding compounds and physical binding in soil particles. Nonetheless, there is limited information

on how permanent changes in land use types influence soil aggregation and carbon protection. Furthermore, there is a need to understand the relationship between soil macrofauna and soil aggregation on the overall C sequestration given their role in mitigating against climate change.

1.3 Justification

An understanding of the quantitative effects of land use practices on soil physicochemical and biological properties and their interactions is required for sustainable utilization of soil resources. For instance, the stability of soil aggregates and SOC protection mechanisms under different land use practices are of great importance for determining effects of human interventions and therefore, on importance of the efforts that aim at improving soil quality. However, due to the intimate relationship between soil aggregation and SOC content, the choice of soil management practices put on the land has a great impact on the resilience of such land use types on climate change and mitigation. Thus, this study will bring a better understanding of how soils respond to disturbance and ultimately, its influence on the overall C storage.

1.4 Objectives

1.4.1 General Objective

Contribute to better management of present land use types through enhancing soil structure stabilization and C storage.

1.4.2 Specific Objectives

1. Evaluate effects of land use types on soil aggregates and aggregate-associated C content.
2. Evaluate soil macrofauna abundance as affected by land use types.

3. Determine the relationship between soil aggregation, aggregate-associated C content and soil macrofauna abundance.

1.5 Study hypotheses

1. Soil aggregate stability and aggregate-associated C content would decrease in more disturbed land use types.

2. Soil macrofauna abundance would decrease with increasing levels of soil disturbance.

3. Soil aggregate stability and aggregate-associated C content will increase with increasing soil macrofauna abundance.

CHAPTER TWO: LITERATURE REVIEW

2.1 Soil aggregate formation and stabilization

According to Tisdall and Oades (1982), the hierarchical order of soil aggregates begins with the binding together of free primary particles and silt sized aggregates (2-20 µm) into microaggregates (20-250 µm). The binding agents in this process include organic matter, polyvalent metal cation complexes, oxides and highly disordered aluminosilicates (Six et al., 2004). It is assumed that the various binding agents act simultaneously at different hierarchical stages of aggregate formation (Six et al., 2004; John et al., 2005). The stable microaggregates, are in turn bound together into macroaggregates (>250 µm) by temporary (i.e., fungal hyphae and roots) and transient (i.e., microbial and plant derived polysaccharides) binding agents (Jastrow et al., 1996; Six et al., 2004; John et al., 2005; Kamau et al., 2020a). Thus, macroaggregates greater than 250 µm, and especially those greater than 2 mm in diameter, appear to be held together largely by fine roots and fungal hyphae. Wetting and drying in the semi-arid and sub-humid regions and the mechanical action of soil fauna (mainly earthworms, termites and ants) are also key to the macroaggregation processes. However, it is important to note that, aggregates of different size classes will have different stability. Dexter (1988) concluded that microaggregates are denser and have a higher internal strength than macroaggregates, and therefore, if the lowest hierarchical order of soil structure is destroyed, the other hierarchical orders are simultaneously destroyed. Consequently, looking at the characteristics of the aggregate fractions is key in understanding soil aggregate stability.

2.2 Factors affecting soil aggregate stability

Electrical conductivity and soil pH controls clay flocculation/dispersion, since clay dispersion increases with increasing soil pH (Rengasamy and Olsson, 1991; Chorom et al., 1994). The role

of organic matter in stabilizing soil aggregates varies with the quality. Some studies have shown clay dispersion increased significantly with the addition of citric and fluvic acids whereas addition of aromatic acids have a flocculating effect (Kennedy et al., 1996; Essington, 2015). Overall however, most studies have reported positive correlations between water stable aggregates and soil organic matter content (Ayuke et al., 2011; Garcia et al., 2019; Kamau et al., 2020b).

As a soil forming factor, climate also has significant influence on the extent of soil aggregate formation and stabilization. Several studies have reported seasonal variations in aggregate stability and seasonal processes that affect aggregate stability (Miller et al., 2018; Yang et al., 2019). Changes in soil moisture content and temperature also affect the formation and destruction of soil aggregates. Dor et al. (2019) showed that wetting and drying cycles decreased the stability of macro-aggregates. The authors reported that during wetting, the soil aggregates can either crumble completely (slake) or stay intact with loosening at the points of weakness (Dor et al., 2019). When the soil is drying, precipitation of soluble minerals and deposition of colloids around the contact points between soil particles increases soil cohesion, consequently creating and stabilizing the soil aggregates (Amezketa, 1999; Totsche et al., 2018). When soil temperature is above 30 °C, its aggregate stability increases (Fox et al., 2007). This occurs as a result of thermal transformation of iron and aluminum oxides, causing them to react as cementing agents for clay particles that form strong silt-sized particles in the soil (Terefe et al., 2008; Kamau et al., 2020a). However, according to Lavee et al (1996), aggregate stability decreases with the level of increasing aridity which influence soil properties that have evolved over a period of time.

Plant roots, soil microbes and soil fauna also play a critical role in stabilization of the soil structure. Tisdall and Oades (1982) considered plant roots as transient binding agents. The presence of roots increases the stability of macro-aggregates chiefly through the trapping of fine particle into stable

macro-aggregates, drying the rhizosphere, sustaining microbial populations within the rhizosphere, and providing food for soil fauna (Tisdall and Oades, 1982; Monreal et al., 1995; Mondal et al., 2019). Root configuration and physiology influences crops' differential abilities in transforming soil structure (Chan and Heenan, 1996). For instance, grasses are said to be superior in stabilizing soil aggregates due to their higher root biomass compared to other plants (Lynch and Bragg, 1985; Miller and Jastrow, 1990; Murugan et al., 2019). Studies have also reported positive relationship between macroaggregate stability and microbial biomass (Carter, 1992; Franzluebbers and Arshad, 1997; Elmholt et al., 2008; Naresh et al., 2018). Microorganisms release extracellular carbohydrates such as starch and cellulose, (Chaney and Swift, 1986) and microbial mucilage may glue soil particles into stable soil aggregates (Tisdall and Oades, 1982). According to Dorioz et al. (1993), the adsorption of water by microorganisms and their growth, develop stress and enhance restructuring and reorientation of clay particle around them thus stabilizing microaggregates. Soil fauna such as earthworms can stabilize soil by intimately mixing ingested mineral and organic matter particles in their guts (Tisdall and Oades, 1982; Shipitalo and Le Bayon, 2004; Pullemann et al., 2005). The burrowing of earthworms also supports root growth, gaseous exchange and infiltration further enhancing the process of aggregate stabilization (Lal, 1988; Bronick and Lal, 2005; Stockdale et al., 2019). Nevertheless, some research indicates that earthworm activity can also contribute to soil degradation. Six et al. (2004) argued that the ingestion of soil by earthworms disrupts existing bonds in micro-aggregates and reorganization of clays resulting into more dispersible casts than the undigested soil. In a recent study, Kamau et al. (2020a) reported that increased number of earthworms (*Nematogenia lacuum*) led to fragmentation of large macroaggregates to microaggregates, thus causing a loss of soil C. Other soil fauna such as termites and ants may enhance soil aggregation through nesting and borrowing habits (Ayuke et al., 2011).

Tisdall and Oades (1982) concluded that water stability of microaggregates is independent on soil management, while water stability of macroaggregates strongly depends on soil management. Studies comparing the relationship between farming systems (low input, organic and conventional systems) and water stability of macroaggregates showed that aggregates in organic systems were significantly stable than those in conventional system (Kong et al., 2005; Minick et al., 2017). Soil tillage indirectly influences soil aggregate stability chiefly through its effect on soil moisture, organic matter, microbial activity and population of soil fauna (Minick et al., 2017; Naresh et al., 2018; Murugan et al., 2019; Obalum et al., 2019). Watts et al. (1996) reported that aggregates collected subsequently after tillage have higher amounts of dispersed clay than those collected immediately preceding tillage. The role of SOC in macroaggregate stabilization and soil productivity has led to the concerns to manage and enhance it.

2.3 Methods of assessing soil aggregate stability

The quantification of stable soil aggregates depends on both the adhesive forces that attach the soil particle together and the type and degree of disturbance applied to it. Tisdall and Oades (1982) concluded that that the hierarchy of soil aggregates consists of three main classes: clay (<2 µm), microaggregates (<250 µm) and macroaggregates (>250 µm). Each of the three aggregate classes has a unique stabilization mechanism and responds differently to environmental conditions such as tillage or management practices, wind and rain. Macroaggregates are disrupted easily by low energy agitation or wetting, while microaggregates are more stable and require prolonged shaking for complete dispersion. Characterization of aggregates requires the analysis of macroaggregates through stability tests, whereas dispersion tests are conducted for clay and silt-sized aggregates (Amezketa, 1999). Therefore, depending on the objective of the study, aggregate distribution may

be determined either wet or dry. Dry sieving is the common method to study the effects of wind erosion and tillage treatments and is conducted using the rotary sieve method (Kemper and Rosenau, 1986). Wet sieving is the most popular method used to study effects of water and stability of aggregates to water, with the wetting process being considered as the disruptive force (Amezketa, 1999). Hence the distribution of water stable aggregates is basically an assessment of macroaggregate stability since the aggregates retained on various sieves must have remained stable during the wetting and sieving process (Kemper and Rosenau, 1986; Jastrow and Miller, 1991). Nonetheless, results obtained using the single and multiple-sieve methods are comparable but the multiple-sieve method is said to be time consuming and more laborious.

2.4 Importance of stable soil aggregates

Polysaccharides are believed to mostly exert their binding capacity on a scale < 50 µm within the macroaggregates (Christensen, 2001; Six et al., 2004). The macroaggregates are further stabilized through the action of microorganisms. Soil aggregates can be biologically stabilized by chemical bonding between organics and soil mineral particles, or by physical binding (Tisdall and Oades, 1982; Lützow et al., 2006; Singh et al., 2018). Occasionally, the formation and stabilization of aggregates occurs simultaneously, but formation often precedes stabilization (Oades, 1993). During macroaggregate stabilization, the intra-aggregate organic matter (consisting of the fresh plant material incorporated in the macroaggregates during aggregate formation) is further decomposed by microorganisms into finer particulate organic matter (POM) (Six et al., 2004). This fine POM becomes increasingly encapsulated with minerals and microbial products forming new microaggregates within the macroaggregates (Six et al., 1999). This formation of microaggregates within macroaggregates has been found to be crucial for the long term sequestration of C (Six et

al., 2004), as microaggregates have a greater capacity to protect C from decomposition compared to macroaggregates (Hernandez et al., 2017; Minick et al., 2017).

Aggregate stability refers to the capability of soil aggregates to resist disintegration following exposure to external destructive forces like tillage, water, and wind (Awale et al., 2017). Generally, the proportion of water-stable aggregates may increase or decrease depending on land use, type of tillage practice, soil type and clay mineralogy (Denef et al., 2001). High soil aggregate stability and greater amounts of stable aggregates are desired for sustaining agricultural productivity and environmental protection. Stable aggregates favor high water infiltration rates (Boyle et al., 1989), provide adequate soil aeration (Carter, 2002), reduce soil erosion by wind and water (Lal, 2015), and enhance root growth (Rillig et al., 2015). Stable soil aggregates also provide physical protection of soil organic matter from microbial decomposition, particularly when the proportion of large (> 0.25 mm) aggregates increases. Conversely, disintegration of aggregates leads to the formation of surface crusts, resulting in higher surface runoff and erosion and lower water infiltration (Arjmand and Mahmoodabadi, 2015; Lal, 2015).

2.5 Effect of land use and soil management on soil aggregation

Land use and soil management systems affect aggregation due to the direct influence on organic resources inputs which play a major role in aggregate formation. Addition of organic matter, such as crop residues and other organic substrates to soil generally enhances soil aggregate formation and stability as the residues serve as the nucleation centers for the formation of new aggregates (Ayuke et al., 2011; Awale et al., 2017). However, soil aggregate formation and stability is not only influenced by the quantity but also by the quality of organic matter inputs (Arjmand and Mahmoodabadi, 2015; Kamau et al., 2020a), with polysaccharides and humic substances being the

key organic components (Elliott, 1986). Consequently, it has been suggested that improvement and maintenance of soil aggregate stability depends on the capacity of organic amendments to produce humic substances (Zhao et al., 2017). In an incubation study, Annabi et al. (2007) showed a rapid effect of composted organic matter on aggregates, which they attributed to the diffusion of humic material within the aggregates, and consequently increased aggregate cohesion. The incorporation of green materials leads to a flush of microbial activity and the production of such effective but ephemeral bonding agents as extracellular polysaccharides (Pagliai et al., 2004). If highly decomposed materials are incorporated, the effect is lesser but last longer. Finally, if highly decomposed materials rich in humic compounds are incorporated, an even smaller but very long lasting stabilizing effect is obtained (Lavelle and Spain, 2001). Several studies have demonstrated the effect of organic resources on aggregate formation. García-Orenes et al. (2005) reported that the addition of organic matter to soil increased the number and size of water stable macroaggregates. However, other studies have also shown that the aggregating effect of organic resources depends on the nature and rapidity of their decomposition, with those with a rapid decomposition rate showing a greater binding effect (Harris et al., 1996; Blair et al., 2005; Alvarez et al., 1998). High quality organic resources (high in N but low in lignin and polyphenol contents) which decompose rapidly have the highest ability to increase aggregate formation.

Under cultivated systems, Tiemann and Grandy (2015) observed that annual crops and no-till systems increased the proportion of dry aggregates of size above 1 mm, whereas conventional tillage had greater proportion of soil aggregates of size below 0.25 mm (Tiemann and Grandy, 2015; Acar et al., 2018). The addition of organic materials and retention of crop residues promote soil aggregation by enhancing soil biological activity and the production of various binding agents such as fungal hyphae, polysaccharides, and mucilages (Sarker et al., 2018; Kamau et al., 2020a).

Addition of organic materials as well as retention of crop residues also protects soil aggregates from direct physical disintegration by the impact of raindrops or by wind.

Tillage affects aggregation by mechanical disruption of aggregates and fragmentation of roots and microbial hyphae, which are major binding agents for macroaggregates (Tisdall and Oades, 1982; Jiao et al., 2006). Tillage also increases the decomposition of SOM and reduces the soil carbon content by increasing access to SOM upon macroaggregate destruction (Six et al., 1999; Balesdent et al., 2000). The clay content of soils has also been found to influence soil aggregation, such that increased clay content is often associated with increased aggregation or aggregate stability (Bronick and Lal, 2005). In increasing soil aggregation, soil clay content indirectly affects soil C storage by occluding organic matter, making it inaccessible to degrading organisms and their enzymes (Bronick and Lal, 2005). Other factors which may affect aggregate proportions include the number of wetting and drying cycles (Utomo and Dexter, 1982).

2.6 Soil organic carbon and its sequestration

Soil organic matter comprises organic detritus in various states of decomposition, such as tissues of living soil organisms, plant and animal residues, and excretions from plant roots and soil microbes. The SOC is an important component of SOM and a key indicator of soil health due to its influences on soil structure, aggregate stability, water storage and availability, water infiltration, nutrient storage and availability, soil biological activity, adsorption of metals and agrochemicals, and pH buffering (Mbau et al., 2015). In addition, sequestering SOC in the soil mitigates the emission of CO_2 to the atmosphere (Kalbitz et al., 2000). Changes in SOC results from the imbalance between inputs (crop residues, manure, and other organic sources) and outputs (from decay, leaching, and erosion) (Brodin, 2016). Land use practices that increase SOM inputs and

optimize the rate of SOM decay play an integral role in the sustainability of SOC. Conversely, reduced input of SOM or its rapid decomposition depletes SOC stocks. For instance, conventional tillage incorporates crop residues into soil and facilitates rapid decay of SOM by microbes due to the introduction of oxygen and greater soil-residue contact (Brodin, 2016).

The SOC pool is an important component of the global C cycle as it stores an estimated 2,500 Pg of both organic and inorganic C in the surface 1 m (Page et al., 2011). This pool plays a crucial role in sustaining crop production and provide essential environmental soil services. Consequently, the loss of SOC due to poor land use or soil management practices can affect soil properties and lead to CO_2 emissions into the atmosphere (Lal, 2004), and cause degradation of soil and water resources (Lal et al., 2006). The sequestration of organic C in soil reduce the risk of climate change by reducing the atmospheric concentration of CO_2. In addition, SOC sequestration has numerous ancillary benefits such as the improvement of soil quality, agronomic/biomass productivity, and advancing global food security as well as mitigation against climate change effects.

The up-scaling of land use and soil management practices that improve soil aggregation is considered to be an important way through which SOC can be sequestered. Studies have shown that SOC sequestration through enhanced soil aggregate stabilization is an important strategy of judicious soil management to mitigate the increasing concentration of atmospheric CO_2 (Bronick and Lal, 2005). The SOC associated with aggregates is protected from mineralization because it is protected from physical, microbial, and enzymatic degradation (Bajracharya et al., 1998). Soil aggregation also reduces the loss of SOC via erosion (Razafimbelo et al., 2008). The protection of SOC in soil aggregates can therefore turn degraded soils from carbon sources into carbon sinks, resulting in increased soil fertility and reduced adverse environmental effects.

2.7 Effects of land use and management on SOC

Regardless of the region or climatic zone, the conversion of native ecosystems to agriculture contributes to the tremendous loss of SOC (Kamau et al., 2017). For instance, several studies have shown that significant amounts of C have been lost from the soil as CO_2 when forests are converted to agriculture (FAO, 2010; IPCC, 2000), as a result of the release of physically protected soil C (Denef et al., 2007; McConkey et al., 2003; Six et al., 1999). Nonetheless, the extent to which a land use or agricultural management influences changes in SOC depends on several factors, including initial levels of SOC before the management was implemented, duration of management imposed, duration of conservation practice adopted, soil and environmental conditions, and crop productivity (Lal, 2004, 2015). Therefore, it may take several years to observe significant changes in SOC stocks (Kalbitz et al., 2000; Lal, 2004). Determining SOC fractions sensitive to management practices and that predict SOC changes and future trends will allow early decisions for management changes that lead to the build-up and maintenance of SOC. However, changes in total SOC take a long time to detect. Different pools of SOC and soil enzymatic activity are typically more sensitive and provide early signs of the effect of management changes than total SOC alone. Hence, there is a growing interest in assessing early indicators of SOC storage such as particulate organic matter (POM), mineralizable C, microbial biomass carbon (MBC), dissolved organic carbon, permanganate oxidizable carbon, and potentially mineralizable nitrogen (PMN) for evaluating differences land uses and changes in management (Kaur, 2012). Studies have shown that these indicators are strongly correlated with each other and with total SOC.

2.8 Role of soil macrofauna in aggregation and C storage

Earthworms, nematodes, and insects, are essential in soil structural development; water, air, and nutrient cycling, SOC turnover, suppressing harmful pests and enhancing beneficial microorganisms in the soil profile (Thies and Grossman, 2006). For instance, the burrowing activity of earthworms creates a network of connected tunnels which increase air permeability and water infiltration rates. In addition, burrowing activity also promotes soil mixing and increases animal and plant residue contact that favors organic matter decomposition and nutrient release (Awale et al., 2017). The feeding and casting activity of earthworms improve aggregate stability and enhance microbial activity (Ayuke et al., 2011; Kamau et al., 2020b). Thus, earthworms are considered ecosystem engineers for their role in modifying the soil environment and availing resources for other organisms (Jouquet et al., 2008), through their impact on soil structure and SOM storage (Lavelle et al., 2001). Apart from speeding up initial breakdown of organic residues, they also incorporate organic matter into their casts and can thereby protect it against rapid decomposition (Bossuyt et al., 2004; Pulleman et al., 2005). Termites also make channels in the soil and influence soil aggregation by mixing organic matter with soil particles and thereby modify the physical properties of soil (Jouquet et al., 2002). They can form stable microaggregates by mixing soil with saliva for nest constructions or, in the case of soil-feeding termites, by excreting faecal pellets that are enriched in organic matter (Jungerius et al., 1999).

Soil aggregates, especially microaggregates (53–250 m) formed within macroaggregates (≥250 m) protect SOC against microbial decay (Tisdall and Oades, 1982; Kamau et al., 2020a). Earthworm activities have been linked to not only macroaggregate formation but also microaggregate formation (Shipitalo and Protz, 1989; Barois et al., 1993) and more specifically the formation of microaggregates-within-macroaggregates with associated C stabilization within this fraction

(Bossuyt et al. 2004; Pulleman et al., 2005; Fonte et al., 2007). Soil fertility and C stabilization are therefore mediated by the interactions between soil organic matter, soil structure and soil macrofaunal abundance and diversity, which in turn depend on soil management (Six et al., 2004). For example, Pulleman et al. (2005) showed that formation of organic C-enriched aggregates was reduced under arable systems compared to pastures, probably due to differences in earthworm abundance or species composition, quality of organic matter and mechanical disturbance.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Description of the study site

This study was conducted at the University of Nairobi Upper Kabete Field Station during the long rain season of 2019. The area lies between 1° 15′ S and 36° 74′ E at an altitude of 1860 m above sea level. The climate in the area is sub-humid and falls in agro-climatic zone III (Sombroek et al., 1982). Rainfall in the area is bimodal with the long rains occurring between March and May while the short rains occur between October and December. The mean annual rainfall is 1006 mm (Gachene, 1997), with about 50% and 28% of the rain occurring during the long and short rainy seasons respectively. The estimated mean annual temperature and evapotranspiration of the area is 17.6 °C and 1152 mm, respectively. The soils in Kabete are classified as humic Nitisols (locally known as Kikuyu red loams) (Gachene, 1997; Jaetzold et al., 2006). These soils are very deep, well drained, dark red friable clay soils showing an ABC sequence of horizon differentiation with clear and smooth boundaries. The top soil is relatively high in organic matter content and overlies an argillic B horizon. The population in the area predominately depends on agriculture and livelihoods are dominated by small-scale subsistence farming. The main land use types in the area include, but not limited to: coffee as the main cash crop and subsistence crops production, with the main crops being maize, beans, potatoes, and assorted vegetables (Ovuka, 2000).

3.2 The study design, soil sampling process and the analysis protocol

Soils were sampled from four land use types: (1) an undisturbed natural bush land dotted with *Lantana camara* L. shrubs and *Croton macrostachyus* Hochst. ex Delile. trees, (2) a grazed pasture land that was established over 50 years ago and is dominated by a mixture of Bermuda grass (*Cynodon dactylon* (L.) Pers.) and Kikuyu grass (*Pennisetum clandestinum* Hochst. ex Chiov.),

(3) a coffee (*Coffea arabica* L. cv. SL 28) plantation that was established 82 years ago. The block sampled had only received 230 kg ha^{-1} Calcium Ammonium Nitrate (CAN – 26% N) as top-dress fertilizer. The understorey of coffee bushes are mulched by prunings from the bushes with only occasional weeding to keep the field clean, and (4) a maize field that had been cultivated continuously for the last 3 years as described by Kamau et al. (2019). Soil for aggregate analysis was sampled using monolith method (Plate 1), in order to minimize unintentional disintegration of the aggregates as described in Kamau et al. (2020a). The monoliths measured 0.25 m × 0.25 m × 0.30 m (length, width and depth, respectively). Common hand tools such as shovels, machetes and hand hoes were used in the sampling process. In each land use type, three 50 m transects were laid out in a zigzag pattern, at least 10 m from the edges of the fields. Along each transect, three sampling points were laid 25 m apart and soil samples collected at three depths; 0–0.05 m, 0.05–0.15 m and 0.15–0.3 m. This produced a total of 27 samples (3 sampling points per transect x 3 depths per sampling point x 3 transects) for each land use type. About 500 g subsample of the soil was placed in sampling bags for soil aggregate and aggregate-associated C analysis.

Plate 1: Soil sampling for aggregate analysis (**a**) and air-drying of the soil in the laboratory (**b**).

3.2.1 Determination of water stable aggregates

Wet-sieving process was used to separate the soil samples into four aggregate fractions namely; large macroaggregates (> 2000 µm), small macroaggregates (250–2000 µm), microaggregates (m) (53–250 µm), and silt + clay sized aggregates (< 53 µm) as describe by Elliott (1986). These fractions have been abbreviated as LM, SM, m and s+c hereafter. Briefly, 100 g of air-dried soil was transferred to a 2000 µm sieve with a recipient at the bottom filled with deionized water (Plate 2a), and left to slake for 5 min. After this, the sieve was manually moved up and down 50 times in about 2 min. This procedure was repeated using the material that passed through the 2000 µm

sieve, now using a 250 µm sieve and finally, a 53 µm sieve using the material that passed through the 250 µm sieve. Soil aggregates retained on each sieve size were backwashed into pre-weighed containers and oven-dried at 60 °C for 48 h. Each aggregate fraction was sand-corrected, by dispersing a sample of these aggregates separately with 5 g L^{-1} sodium hexametaphosphate.

3.2.2 Fractionation of macroaggregates

Due to the fact that some large macroaggregate samples were in small quantities, this fraction was combined with small macroaggregates to make a single sample, hereafter called total macroaggregates. Macroaggregate fractionation procedure was followed as described in Kamau et al. (2020a). Five (5) g of oven-dried total macroaggregates sample was weighed and placed into a microaggregate isolator unit (Plate 2b) with a 250 µm sieve at the bottom and enough deionized water to saturate the sample. The unit was attached to a mechanical shaker. The shaker was allowed to run for 3 minutes, after which the sample was flushed with more deionized water and the slurry poured into a 53 µm sieve inside a larger container such that all aggregates <53 µm in diameter were collected in the container, while those that were >53 µm were retained on the sieve (Kamau et al., 2020a). Three aggregate fractions were produced at the end of the process: coarse particulate organic matter and sand (> 250 µm), microaggregates-within-macroaggregates (53–250 µm) and silt and clay sized fraction within macroaggregates (< 53 µm). These fractions have been abbreviated as cPOM, mM and s+cM hereafter. The aggregates retained in each sieve size were backwashed into pre-weighed containers and oven-dried at 60 °C for 48 h. Like in the wet-sieving process, each of these aggregate fractions were sand-corrected.
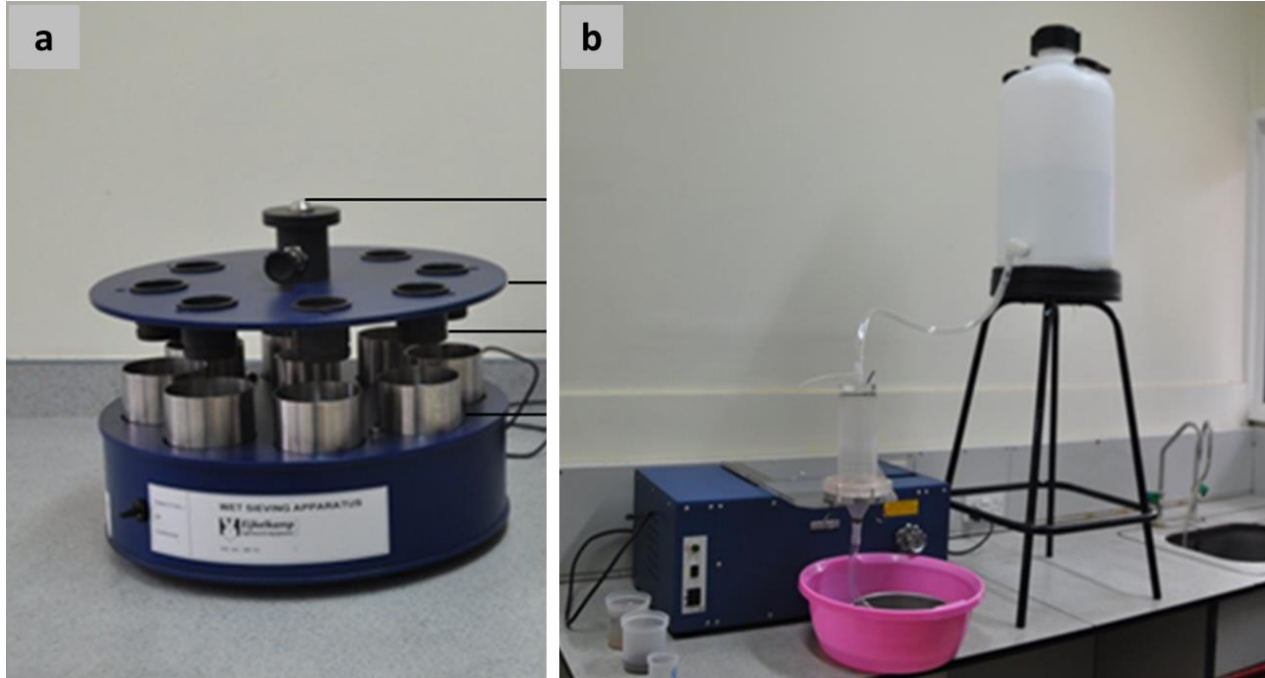
Plate 2: Wet-sieving apparatus (**a**) and microaggregate isolator (**b**) used in aggregate analysis.

3.2.3 Determination of whole soil and aggregate-associated C content

Total OC was determined using the wet oxidation method (Nelson and Sommers, 1982). Five grams (0.5 g) of air-dried soil was transferred into 500 ml Erlenmeyer flask followed by 10 ml of 0.1667 M potassium dichromate ($K_2Cr_2O_7$) solution. The mixture was gently stirred to disperse the soil. Twenty milliliters (20 ml) of concentrated H_2SO_4 (95%) was added to the suspension, shaken gently and allowed to stand for 30 minutes. Distilled water (250 ml) was added followed by 10 ml of concentrated phosphoric acid (H_3PO_4) and 1 ml of diphenylamine indicator. The suspension was titrated with 1.0 M $FeSO_4$ until the color changed to pale green. The percentage organic carbon (OC) was calculated using Equation 1.

$$OC\ (\%) = \frac{[M*(V1-V2)]}{S} \times 0.39 \times mcf \qquad \text{[Equation 1]}$$

Where;

M = molarity of FeSO$_4$ (from blank titration)

V1 = volume of FeSO$_4$ required for the blank

V2 = volume of FeSO$_4$ required for the soil sample

S = weight of soil sample in gram

mcf = Moisture conversion factor

$0.39 = 3 \times 10^{-3} \times 100\% \times 1.3$

3.3 Soil macrofauna sampling

Soil macrofauna sampling was conducted adjacent to the same spots and concurrently with soil sampling for aggregate analysis also using soil monolith method (Plate 3a & b), following the Tropical Soil Biology and Fertility method (Anderson and Ingram, 1993). The monolith dimensions were same as those used to obtain samples for aggregate analysis. Sampling was conducted towards the end of the long rain season in the month of June 2019. The excavated soil was placed in plastic trays and all the soil macrofauna were handpicked and placed in 75% ethanol (Plate 3c). At the end of the sampling exercise, all the macrofauna (except earthworms) were transferred into fresh 75% ethanol while earthworms were transferred into 4% formaldehyde. The soil macrofauna were separated into broad taxonomic units, namely; Araneae (spiders), Blattodea (cockroaches), Chilopoda (centipedes), Coleoptera (beetles), Diplopoda (millipedes), Hemiptera (Bugs), Hymeneoptera (ants), Isoptera (termites), Lepidoptera (moths), Oligocheata (earthworms), and Orthoptera (crickets). The soil macrofauna were later separated to family and genera or species where possible. Identification of the soil macrofauna was done at the Department of Invertebrate Zoology of the National Museums of Kenya using morpho-anatomical keys and comparison with reference collections as described in Kamau et al. (2020a). Soil macrofauna abundance was calculated as the number of individuals per square meter.
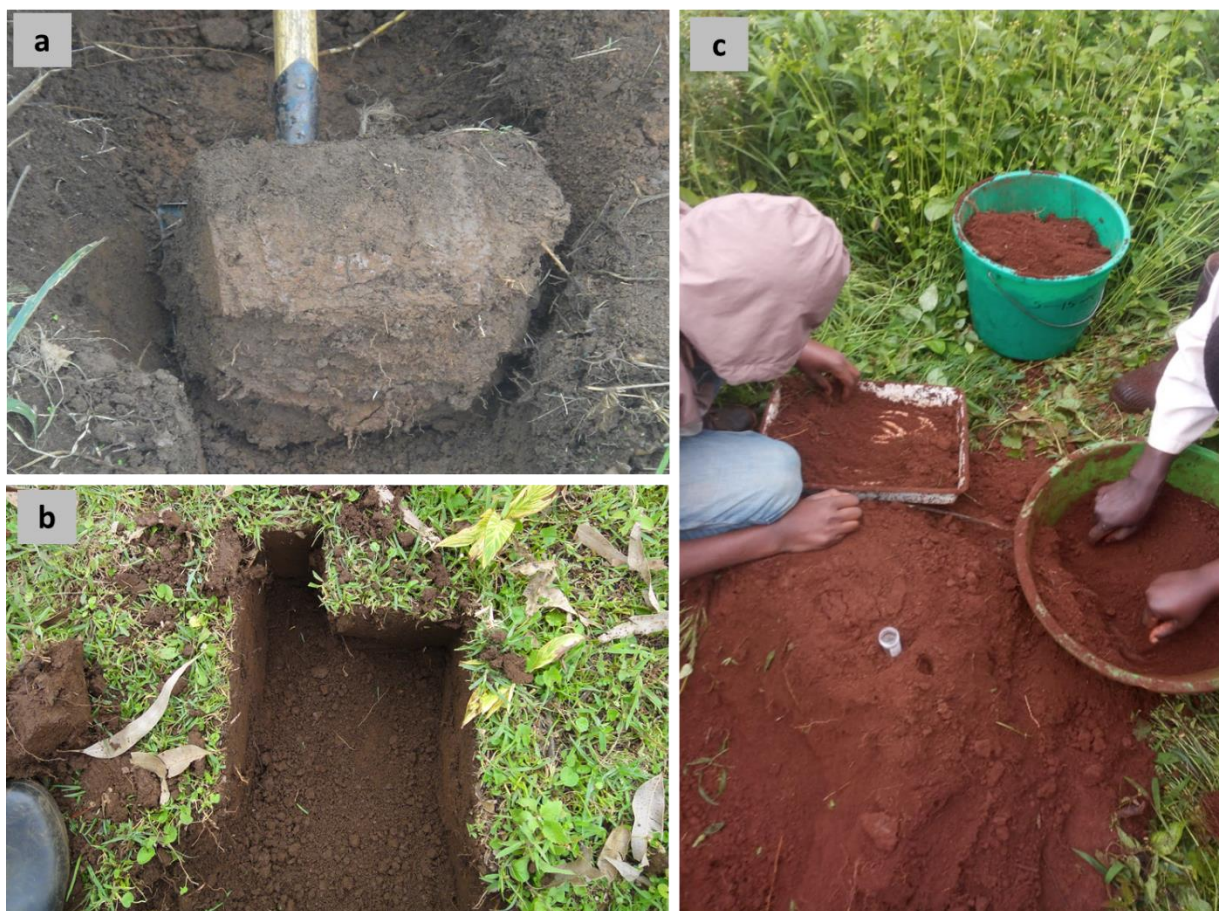
Plate 3: Soil macrofauna sampling process (**a** & **b**) and hand-sorting of the soil macrofauna (**c**).

3.4 Statistical analysis

Generalized linear models (GLM) were used to test the effects of land use types and sampling depth on aggregate fractions and aggregate-associated C using R statistical software (R Core Team, 2019). Land use types and depth were considered as the fixed factors. However, to test the effects of the two factors on soil macrofauna data, negative binomial regression was chosen as an extension of the Poisson distribution. When analysis of variance (ANOVA) showed significant effects, Tukey's post-hoc tests were performed at $\alpha = 0.05$. Pearson correlation analysis was conducted to determine the relationship between soil aggregate fractions from the two stage process and aggregate-associated C content and earthworm and termite abundance.

CHAPTER FOUR: RESULTS

4.1 Effect of land use type on soil aggregate fractions

Though land use type had significant effects on soil aggregate fractions, outright differences were observed between bush land and maize field (Table 1). The weight of LM fraction was higher in bush land (14.4 g 100 g^{-1} soil) and grazed pasture (12.4 g100 g^{-1} soil) compared to coffee plantation (3.9 g 100 g^{-1} soil) and maize field (0.6 g100 g^{-1} soil). On the other hand, microaggregate fractions were higher in maize field (41.2 g 100 g^{-1} soil) and lowest in bush land (18.8 g 100 g^{-1} soil). The fractions SM and s+c were not significantly affected by the land use type. Depth had little influence on aggregate distribution, though the differences depended on the land use type. For instance, in bush land, the weight of LM fraction was higher (22.7 g 100 g^{-1} soil) in the topsoil (0-0.05 m) but low (9.5 g 100 g^{-1} soil) in subsoil (0.15-0.3 m). Similar differences were observed in grazed pasture and coffee plantation, whereas maize field showed no differences. SM fraction showed significant differences in maize field only, where the weight increased from 47.2 g 100 g^{-1} in the topsoil to 62.6 g 100 g^{-1} in the subsoil. Microaggregates showed significant differences in bush land only where the fraction weight increased with depth, whereas s+c fraction showed significant differences in bush land and maize field, with the fraction decreasing with depth.

After fractionation of macroaggregates, only microaggregates-within-macraggregates showed significant differences based on the land use type, with higher aggregate weight in bush land (60.4 g 100 g^{-1} soil) and low maize field (42.0 g 100 g^{-1} soil). Based on depth, only cPOM and s+c showed significant differences, but this depended on the land use type (Table 1). For instance, cPOM fraction showed significant differences in maize field only, where the weight increased with depth from 1.2 g 100 g^{-1} soil in the topsoil to 4.4 g 100 g^{-1} soil in the subsoil. Similarly, s+cM fraction increased with depth in maize field, but showed no specific trend in bush land.

Table 1: Soil aggregate fraction weight distribution (means ± (SE)) as influenced by the land use type and soil depth.

Soil aggregate fraction (g 100 g^{-1} oven-dry soil)†	Land use type (LUT)											
	Bush land			Grazed pasture			Coffee plantation			Maize field		
	Soil depth											
	0-0.05 m	0.05-0.15 m	0.15-0.3 m	0-0.05 m	0.05-0.15 m	0.15-0.3 m	0-0.05 m	0.05-0.15 m	0.15-0.3 m	0-0.05 m	0.05-0.15 m	0.15-0.3 m
LM	22.7 (4.0)[a]	11.0 (3.6)[b]	9.5 (3.7)[b]	18.0 (3.7)[a]	14.0 (0.4)[a]	5.1 (0.3)[b]	8.9 (3.0)[a]	1.6 (2.7)[b]	1.2 (1.5)[b]	0.6 (0.3)	0.7 (0.4)	0.5 (0.1)
SM	63.8 (6.1)	58.4 (5.8)	68.9 (5.7)	49.3 (11.4)	60.1 (2.9)	62.4 (3.4)	54.9 (4.4)	60.6 (7.3)	61.8 (3.9)	47.2 (1.6)[b]	49.7 (5.6)[ab]	62.6 (2.3)[a]
m	11.5 (2.3)[b]	25.4 (5.5)[a]	19.6 (5.5)[ab]	18.8 (7.9)	24.2 (4.2)	29.6 (2.9)	31.6 (4.2)	31.7 (6.1)	32.1 (5.8)	45.8 (1.8)	44.2 (5.0)	33.6 (12.5)
s+c	2.0 (0.1)	5.2 (3.5)	2.1 (0.8)	13.9 (2.1)[a]	1.8 (0.3)[b]	2.8 (0.2)[b]	4.7 (1.6)	6.1 (2.1)	5.0 (1.6)	6.5 (0.2)[a]	5.4 (1.7)[ab]	3.3 (0.2)[b]
cPOM	8.4 (2.4)	6.6 (5.5)	5.4 (4.0)	3.2 (0.8)	4.6 (1.0)	4.8 (3.6)	2.7 (0.7)	1.8 (0.6)	3.2 (0.4)	1.2 (0.6)[b]	1.4 (0.4)[b]	4.4 (1.2)[a]
mM	64.6 (1.0)	54.1 (5.9)	62.5 (3.0)	50.2 (3.2)	59.0 (2.8)	53.0 (2.4)	54.6 (5.0)	55.1 (9.1)	51.4 (1.9)	39.4 (1.3)	39.2 (3.4)	47.3 (10.7)
s+cM	13.5 (1.8)[a]	8.8 (1.2)[b]	10.5 (0.8)[ab]	14.0 (5.0)	10.5 (1.9)	9.8 (1.3)	6.4 (1.1)	5.4 (2.0)	8.4 (2.9)	7.2 (0.9)[b]	9.8 (2.2)[ab]	11.4 (0.7)[a]

Mean aggregate weight of the fractions where significant LUT effects were observed

LM	14.4 (2.8)[A]			12.4 (3.0)[AB]			3.9 (2.6)[BC]			0.6 (0.2)[C]		
m	18.8 (3.1)[B]			24.2 (3.1)[B]			31.8 (2.7)[AB]			41.2 (4.4)[A]		
mM	60.4 (2.5)[A]			54.1 (1.9)[A]			53.7 (3.1)[A]			42.0 (3.5)[B]		

LM = large macroaggregates (> 2000 μm), SM = small macroaggregates (250-2000 μm), m = microaggregates (53–250 μm), s+c = silt and clay (<53 μm), cPOM = coarse particulate organic matter (>250 μm), mM = microaggregates-within-macroaggregates (53–250 μm), s+cM = silt and clay within macroaggregates (<53 μm). Within rows, means followed by different superscript letters (uppercase letters indicate differences based on land use type, while lowercase letters the means across soil depths) are significantly different at $p < 0.05$.

4.2 Effect of land use types on aggregate-associated C content

Similar to aggregates, land use type had significant effects on soil aggregate-associated C, with outright differences observed only between bush land and maize field (Table 2). In LM, the C was higher in bush land with a mean of 4.6 mg g^{-1} soil and low in maize field (0.2 mg g^{-1}). Similarly, C in SM was higher in bush land (17.7 mg g^{-1}) and lowest in maize field (11.8 mg g^{-1}). The C in microaggregates fraction showed opposite trends to that of LM and SM fractions, with higher C content in maize field (9.0 mg g^{-1}) and lowest in bush land (4.1 mg g^{-1}). Based on soil depth, whole soil and aggregate-associated C generally declined with increasing depth in bush land, grazing field and coffee plantation. For instance, C content in whole soil declined from 43.1 mg g^{-1} in the topsoil of bush land to 19.2 mg g^{-1} in the subsoil. Similarly, C content in whole soil declined from 37.0 g and 34.8 mg g^{-1} in the topsoil to 22.2 g and 23.6 mg g^{-1} in the subsoil of grazed pasture and coffee plantation, respectively. The C content in LM and SM fractions of the three land use types showed the same trend as whole soil C. In maize field however, aggregate-associated C showed irregular differences, decreasing with depth at times and increasing in other occasions.

Upon fractionation of macroaggregates, land use type did not show significant influence on all the aggregate fractions (Table 2). Nonetheless, depth seem to have had stronger effects on C content, especially of mM and s+cM fractions. The C content in the fraction cPOM showed significance in maize field only where it increased from 0.2 mg g^{-1} in the topsoil to 0.7 mg g^{-1} in the subsoil. In mM fraction, C content increased with depth in all land use type, except coffee plantation where there was no significant differences. On the other hand, aggregate-associated C in s+cM fraction declined with depth in bush land and grazing field, but increased with depth in maize field.

Table 2: Soil aggregate-associated C (means ± (SE)) as influenced by the land use type and soil depth.

Soil aggregate-associated C (mg g^{-1} oven-dry soil)[†]	Bush land			Grazed pasture			Coffee plantation			Maize field		
	0-0.05 m	0.05-0.15 m	0.15-0.3 m	0-0.05 m	0.05-0.15 m	0.15-0.3 m	0-0.05 m	0.05-0.15 m	0.15-0.3 m	0-0.05 m	0.05-0.15 m	0.15-0.3 m
WS	43.1 (0.9)^a	21.4 (0.6)^b	19.2 (2.6)^b	37.0 (3.9)^a	26.1 (1.3)^b	22.2 (1.3)^c	34.8 (1.7)^a	28.5 (1.8)^b	23.6 (3.3)^b	25.0 (2.1)	25.8 (1.5)	24.5 (1.7)
LM	8.1 (1.4)^a	3.0 (1.0)^b	2.5 (1.0)^b	6.2 (2.5)^a	3.7 (1.1)^{ab}	1.0 (0.3)^b	3.5 (1.5)^a	0.4 (0.1)^b	0.3 (0.0)^b	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)
SM	23.0 (2.1)^a	14.8 (1.9)^b	15.2 (1.9)^b	15.9 (3.8)	15.1 (0.7)	12.2 (0.5)	14.4 (0.8)	11.5 (1.3)	12.3 (1.8)	10.8 (0.9)	11.0 (2.8)	13.7 (2.8)
m	3.8 (1.0)	5.1 (0.5)	3.4 (0.9)	5.3 (2.2)	4.9 (0.7)	4.7 (0.5)	8.9 (0.9)	7.5 (1.4)	6.7 (1.8)	10.0 0.3)	10.0 (0.9)	7.2 (2.7)
s+c	0.7 (0.1)	0.9 (0.5)	0.4 (0.1)	4.0 (2.4)^a	0.4 (0.0)^b	0.6 (0.1)^b	1.4 (0.5)	1.4 (0.5)	1.2 (0.4)	1.5 (0.1)^a	1.3 (0.4)^{ab}	0.7 (0.1)^b
cPOM	1.2 (0.4)	1.4 (1.2)	0.9 (0.7)	0.3 (0.1)	0.8 (0.4)	1.1 (0.9)	0.4 (0.1)	0.2 (0.1)	0.3 (0.0)	0.2 (0.1)^b	0.2 (0.1)^b	0.7 (0.1)^a
mM	9.9 (1.4)^b	5.5 (1.8)^c	14.0 (0.7)^a	7.4 (1.8)^b	11.7 (3.9)^{ab}	12.3 (2.1)^a	12.1 (2.5)	13.2 (1.4)	13.2 (2.9)	9.6 (1.2)	8.7 (1.5)	14.4 (6.5)
s+cM	2.4 (0.3)^a	1.2 (0.2)^b	1.7 (0.3)^{ab}	2.3 (0.1)^a	1.5 (0.2)^{ab}	1.3 (0.1)^b	0.7 (0.2)	0.8 (0.2)	1.0 (0.4)	0.9 (0.1)^b	1.6 (0.5)^{ab}	1.9 (0.2)^a

Mean aggregate-associated C of the fractions where significant LUT effects were observed

	Bush land			Grazed pasture			Coffee plantation			Maize field		
LM	4.6 (1.1)^A			3.6 (1.0)^{AB}			1.4 (1.1)^{AB}			0.2 (0.1)^B		
SM	17.7 (1.7)^A			14.4 (1.3)^{AB}			12.7 (0.8)^B			11.8 (1.2)^B		
m	4.1 (0.5)^C			5.0 (0.7)^{BC}			7.7 (0.8)^{AB}			9.0 (0.9)^A		

[†] WS = whole soil, TM = total macroaggregates (> 250 μm), m = microaggregates (53–250 μm), s+c = silt and clay (<53 μm), cPOM = coarse particulate organic matter (>250 μm), mM = microaggregates-within-macroaggregates (53–250 μm), s+cM = silt and clay within macroaggregates (<53 μm). Within rows, means followed by different superscript letters (uppercase letters indicate differences based on land use type, while lowercase letters the means across soil depths) are significantly different at $p < 0.05$.

4.3 Effect of land use types on soil macrofauna

Among the soil macrofauna recovered from the four land use types, ants were the most dominant group (Table 3). *Tetramorium* was the most numerous of the five ant genera recovered, with more than 80% of the total ants belonging to this genus. The other four genera accounted for less than 20% of the total ant abundance. Only eight species of beetle genus were recovered across the four land use types. *Genocephallum* was the most dominant genus of eight beetle genera with almost 50% belonging to this genus, with most being recovered from coffee plantation. Only *Microtermes sp*. of termites were recovered from these four land use types. The abundance of crickets, cockroaches, true bugs, and moths was very low, often with less than 50 individuals m^2. Only five earthworm species were recovered across the four land use types. *Nematogenia lacuum* was the most dominant species, with over 50% of the total earthworm count being derived from this species. The rest of earthworm species did not show major differences. Nonetheless, though most of these earthworms were recovered from bush land, the differences based on the land use types seems to have been insignificant. Among the myriapods, only *Spirostreptidae* sp showed significant difference, with higher abundance in bush land (149.3 individuals m^2) compared to grazed pasture (42.7 individuals m^2) coffee plantation (53.3 individuals m^2) and maize field (5.3 individuals m^2). *Araneae* sp. was the most dominant of the two arachnid species, though the differences were not significantly different based on land use types.

Table 3: Effect of land use types on soil macrofauna abundance (means ± SE) and diversity.

Taxa	Family	Genera/Species	Common name	Land use types (LUTs)			
				Bush land	Grazed pasture	Coffee plantation	Maize field
Insects							
Hymenoptera	Formicidae	*Dorylus molestus*	Ants	5.3 (5.3)	5.3 (5.3)	21.3 (21.3)	0.0 (0.0)
		Monomorium		0.0 (0.0)	0.0 (0.0)	48.0 (33.3)	5.3 (5.3)
		Mymicaria		0.0 (0.0)	0.0 (0.0)	16.0 (16.0)	10.7 (10.7)
		Pheidole		85.3 (56.4)	250.7 (164.3)	32.0 (16.0)	85.3 (21.3)
		Tetramorium		864.0 (502.2)	304.0 (84.7)	922.7 (341.0)	1557.3 (1454.6)
Coleoptera	Carabidae	*Agonum*	Beetles	16.0 (16.0)	0.0 (0.0)	0.0 (0.0)	42.7 (23.3)
	Elateridae	*Elateridae*		10.7 (10.7)	5.3 (5.3)	0.0 (0.0)	0.0 (0.0)
	Scarabaeidae	*Copris*		32.0 (9.2)	16.0 (16.0)	5.3 (5.3)	5.3 (5.3)
	Staphylinidae	*Paederus*		0.0 (0.0)	32.0 (18.5)	0.0 (0.0)	0.0 (0.0)
		Staphylinidae		21.3 (17.0)	21.3 (17.0)	0.0 (0.0)	10.7 (10.7)
		Gonocephallum		10.7 (5.3)	0.0 (0.0)	224.0 (192.9)	42.7 (14.1)
	Tenebrionidae	*Lagria*		5.3 (5.3)	0.0 (0.0)	0.0 (0.0)	5.3 (5.3)
		Zophosis		0.0 (0.0)	5.3 (5.3)	74.7 (37.3)	0.0 (0.0)
Isoptera	Termitidae	*Microtermes sp.*	Termites	5.3 (5.3)	21.3 (21.3)	117.3 (80.7)	0.0 (0.0)
Orthoptera	Gryllidae	*Gryllus*	Crickets	16.0 (16.0)	0.0 (0.0)	0.0 (0.0)	21.3 (21.3)
Blattodea	Blattelidae	*Herbardina*	Cockroaches	10.7 (10.7)	26.7 (10.7)	0.0 (0.0)	0.0 (0.0)
		Pseudoderopeltis		32.0 (18.5)	0.0 (0.0)	90.7 (66.8)	0.0 (0.0)
Hemiptera	Cydnidae	*Aethus*	True bugs	32.0 (24.4)	0.0 (0.0)	0.0 (0.0)	53.3 (19.2)
		Geocnethus		10.7 (10.7)	0.0 (0.0)	0.0 (0.0)	10.7 (10.7)
	Reduviidae	*Rhinocoris*		5.3 (5.3)	10.7 (10.7)	0.0 (0.0)	0.0 (0.0)
Lepidopetera	Noctuidae	*Noctuidae*	Moths	5.3 (5.3)	10.7 (10.7)	0.0 (0.0)	0.0 (0.0)
Earthworms							
Oligochaeta	Acanthodrilidae	*Dichogaster bolaui*	Earthworms	74.7 (74.7)	5.3 (5.3)	5.3 (5.3)	0.0 (0.0)
		Dichogaster saliens		26.7 (26.7)	26.7 (19.2)	10.7 (10.7)	0.0 (0.0)
		Polytoreutus annulatus		21.3 (21.3)	10.7 (5.3)	0.0 (0.0)	16.0 (16.0)
	Eudrilidae	*Nematogenia lacuum*		202.7 (112.9)	32.0 (18.5)	101.3 (55.7)	186.7 (41.7)
		Gordiodrilus wemanus		42.7 (28.2)	0.0	5.3 (5.3)	5.3 (5.3)
Myriapods							
Chilopoda	Not identified	Not identified	Centipedes	5.3 (5.3)	0.0 (0.0)	5.3 (5.3)	10.7 (10.7)
Diplopoda	*Oxydestidae*	*Oxydestidae*	Millipedes	10.7 (10.7)	69.3 (35.0)	0.0 (0.0)	0.0 (0.0)
	Spirostreptidae	*Spirostreptidae*		149.3 (37.3)[a]	42.7 (10.7)[b]	53.3 (5.3)[b]	5.3 (5.3)[b]
Arachnids							
Araneae	Araneae	*Araneae*	Spiders	112.0 (40.3)	112.0 (40.3)	74.7 (29.7)	26.7 (19.2)
	Theridiidae	*Theridiidae*		5.3 (5.3)	0.0 (0.0)	0.0 (0.0)	5.3 (5.3)

Within rows, means followed by different letters in superscript are significantly different at $p < 0.05$, based on Tukey's honest significant difference (HSD) test.

4.4 Correlation of earthworms and termites abundance with aggregates and aggregate C

Earthworms and termites generally showed weak or no correlation with soil aggregates across the land use types (Table 4). For instance, only the fraction mM showed significant positive correlation with endogeic earthworms in soils under coffee plantation. No other significant correlation between earthworms (epigeic and endogeic groups) and soil aggregate fractions across the other land use types. Termites only showed significant positive correlation with s+cM faction in soils under bush land and under coffee plantation. Similar to aggregate fractions, the abundance of earthworms and termites showed weak correlation with C content aggregates. Only endogeic earthworms showed significant negative correlation with C content in whole soil (WS) and microaggregate fraction. Termites and epigeic earthworm species did not show significant correlation with aggregate-associated C content of WS and of any of the soil aggregates.

Table 4: Coefficients of correlation between aggregates, aggregate-associated C content and earthworms and termites abundance.

Land use type	Soil macrofauna	Ecological group[†]	Soil aggregate fractions							Aggregate-associated C content							
			LM	SM	m	s+c	cPOM	mM	s+cM	Whole soil	LM	SM	m	s+c	cPOM	mM	s+cM
Bush land	Termites	G II (FWLG)	-0.16	0.26	-0.41	0.23	0.10	-0.22	**0.58****	0.25	-0.16	0.37	-0.38	0.16	0.05	-0.44	0.46
	Earthworms	Epigeic	0.45	-0.48	0.49	0.15	-0.42	0.47	-0.36	0.12	0.45	-0.49	-0.05	0.26	-0.39	0.15	-0.42
		Endogeic	-0.26	0.19	0.00	-0.49	0.35	-0.25	-0.28	**-0.50***	-0.25	0.06	**-0.50***	0.46	0.38	0.45	-0.20
Grazing field	Termites	G II (FWLG)	0.36	-0.47	-0.35	0.48	0.46	-0.49	-0.12	-0.09	0.50*	-0.39	-0.39	0.48	0.48	-0.39	-0.18
	Earthworms	Epigeic	-0.11	0.58	0.08	-0.25	0.36	0.05	0.43	0.40	-0.10	0.39	0.12	0.25	-0.24	-0.26	0.49
		Endogeic	0.48	-0.40	-0.49	0.42	-0.31	-0.49	0.02	0.05	0.47	-0.29	-0.48	0.42	0.42	-0.43	-0.04
Coffee plantation	Termites	G II (FWLG)	-0.45	-0.26	0.26	0.48	-0.46	0.10	**0.50***	-0.49	-0.48	-0.37	0.04	0.39	-0.25	-0.46	0.45
	Earthworms	Epigeic	0.34	-0.48	0.48	0.01	0.32	0.45	0.11	0.10	0.28	0.43	-0.49	0.41	0.49	-0.05	0.07
		Endogeic	-0.11	0.35	0.35	-0.26	-0.09	**0.50***	0.14	0.16	-0.04	-0.25	-0.48	0.21	-0.37	0.29	-0.31
Maize field	Termites	G II (FWLG)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Earthworms	Epigeic	0.44	-0.45	-0.23	-0.22	0.49	0.09	0.49	0.46	0.44	-0.02	0.14	0.20	0.49	0.39	0.16
		Endogeic	0.30	-0.33	-0.42	-0.42	0.46	0.32	0.47	0.49	0.33	-0.27	0.32	0.40	0.37	0.49	0.37

[†] Adopted from Ayuke et al. (2011). G II = Group two, W = wood, L = leaf litter, F = fungus grower, G = dead/dry grass. LM = large macroaggregates (> 2000 μm), SM = small macroaggregates (250-2000 μm), m = microaggregates (53–250 μm), s+c = silt and clay (< 53 μm), cPOM = coarse particulate organic matter (> 250 μm), mM = microaggregates-within-macroaggregates (53–250 μm), s+cM = silt and clay within macroaggregates (< 53 μm), WS = whole soil, TM = total macroaggregates (> 250 μm). Coefficients marked in bold are significant: $^{*} p < 0.05$, $^{**} p < 0.01$.

CHAPTER FIVE: DISCUSSION

5.1 Effect of land use type and soil macrofauna abundance on soil aggregation

Generally, macroaggregates decreased while microaggregates increased with increasing soil disturbance. Tillage, the most common form of soil disturbance in conventional management systems, enhances soil-residue contact which increases the rate of organic matter loss through oxidation (Awale et al., 2013). Less persistent (transient and temporary) organic matter fractions are known to stabilize macroaggregates and therefore, increased oxidation rates may result in breaking macroaggregates to microaggregates (Kamau et al., 2020a). Tillage also physically disrupt, and exposes the existing aggregates to increased frequency of wet-dry cycles (Balesdent et al., 2000 Denef et al., 2001). Therefore, in the current study, the decrease in macroaggregates with increasing soil disturbance (especially in maize field) confirms the negative effects of cultivation on soil aggregation. On the other hand, animal trampling in grazed pastures exert pressure on the surface soil which causes compaction especially in the upper 0.05–0.15 m of the soil (Greenwood and McKenzie, 2001). Soil compaction converts macropores into mesopores and micropores, which negatively affects the functioning of soil organisms and plant root systems which are vital in macroaggregation (Kooistra and Tovey, 1994), and this is accompanied by a decrease in the size of soil aggregate fractions (Vidrih and Hopkins, 1996). However, the differences between bush land, grazed pasture and coffee plantation or between maize field, coffee plantation and grazed pasture were not outright, an indication that the changes in aggregate fractions due to increased level of disturbance were only gradual. Besides tillage and grazing, the type of vegetation could also have had significant influence on the observed differences in soil aggregate fractions. Trees and shrubs, for instance, enhance shading, thus reducing temperature and evapotranspiration rates and increase relative humidity below the canopy (Belsky et al., 1989;

32

Vandenbeldt and Williams 1992). Lower heat load beneath the trees and shrubs reduces water stress compared to the adjacent open sites. Some studies have also reported direct increase in moisture content of the surface soils under certain tree species, which has been linked to hydraulic redistribution of subsurface moisture (Kizito et al., 2012; Diedhiou-Sall et al., 2013). This creates microclimatic conditions below the tree canopy, which may directly affect soil aggregation through production of root exudates or indirectly by favoring increase in rhizospheric microbes that may produce mucilages which enhance macroaggregation. Though sampling was not done directly under the canopy and soil temperature and moisture were not measured, there is a possibility of these contributing to the observed differences, given that some trees and shrubs can extend their influence beyond their canopy as reported by Kamau et al. (2017).

Soil macrofauna, especially earthworms and termites which are known to move or ingest substantial amounts of soil, also play a critical role in soil aggregation process. The original soil structure of ingested soil may change completely in the gut of earthworms to produce nuclei for the formation of new microaggregates (Shipitalo and Protz 1989; Barois et al., 1993). Thus, numerous studies have linked soil aggregation process to the abundance of earthworms. Nonetheless, the effects on soil aggregation is largely dependent on their ecological categories and/or functional attributes as described by Kamau et al. (2020a). Anecic species, for instance, make permanent or semi-permanent burrows that can extend deep into several soil horizons, while endogeic earthworms are known to make extensively branched, sub-horizontal networks of burrows in search of organic matter rich soil (Shipitalo and Le Bayon, 2004; Barrios et al. 2018; Kamau et al., 2020b). Epigeic species, on the other hand, generally forage on surface litter and rarely burrow into or ingest soil. Thus, while anecic and endogeic earthworms may be important in soil aggregation process, epigeic species are usually weakly correlated with soil structure (Rossi, 2003; Shipitalo and Le Bayon, 2004; Six et al., 2004). A recent study by Kamau et al. (2020a)

showed significant reduction in large macroaggregates and an increase in microaggregates due to proliferation of *Nematogenia lacuum*. The authors proposed that the species, owing to its small size, could have been contributing to the fragmentation of large macroaggregates into microaggregates fractions, as the soil goes through their gut. In the current study however, despite *N. lacuum* being the dominant species, there was no significant influence of earthworms on soil aggregate fractions as shown by insignificant correlation of earthworms and soil aggregates. This could perhaps be caused by the high variation of earthworms from transect to transect as shown by the large standard errors in Table 4. On the other hand, influence of termites on soil structure is mainly driven by selection of particles and mixing soil with saliva during nest construction (Lavelle, 1997) or for the soil-feeding termites, by excreting fecal pellets that are enriched in organic matter (Jungerius et al., 1999). Thus, their influence on soil aggregation may be more restricted mainly to their nesting spots or the galleries and sheetings they make while gathering food, owing to their mobility (Kamau et al., 2020a). As such, their role, as noted by Ayuke et al. (2011), become clearer in low-C soils where the activity of other soil macrofauna groups is relatively low. However, like earthworms, there was no significant influence of termite abundance on soil aggregate fractions distribution, which could have been caused by the high variation in termite abundance within the four land use types.

5.2 Effect of land use type and soil macrofauna abundance on aggregate C

In this study, there was a significant decline in LM and SM associated-C content with increasing level of disturbance, with the lowest C content in maize field, whereas microaggregates-associated C content showed opposite trend to that of LM and SM fractions. Disturbances of natural forest or bush lands due to changes in land use does not only reduce stability of aggregates fractions, but also lead to loss of C associated with various aggregates (Ayoubi et al., 2012). Organic matter

plays a critical role in soil aggregation process when bound to soil primary mineral particles into stable organo-mineral complexes (Chenu and Plante, 2006). In addition, the free organic matter is important in macroaggregation as explained by the soil aggregate hierarchy concept. The concept states that, soil primary mineral particles are bound into microaggregates by persistent binding agents, oxides, and highly disordered aluminosilicates (Six et al., 2004); the microaggregates are then glued into macroaggregates by transient (plant- and/or microbial-derived polysaccharides) and temporary (fungal hyphae and roots) organic binding agents (Tisdall and Oades, 1982; Jastrow et al., 1996; Six et al., 2004). It is therefore expected that the C content would increase with increasing aggregate fraction size. Nonetheless, these transient and temporary organic binding agents are most vulnerable to tillage or any other form of soil disturbance (Xiao et al., 2017; Corsi and Muminjanov, 2019) and can be redistributed after the collapse of the macroaggregates (Six et al., 2002) or completely lost from the soil with time (Kamau et al., 2020a). In the current study, however, it can be suggested that the lower LM and SM associated C content in maize field relative to the bush land could be an indication that SOM that was once protected inside of macroaggregates may have been redistributed to aggregate fractions of lower size, rather than lost to decay over time. This is due to the fact that microaggregates-associated C increased with increased level of disturbance (the highest amounts being in maize field), and that whole soil C content was not significantly different between the four land use types. This could further be confirmed by the distribution of aggregate-associated C content across the soil depth, where a definite trend could be observed in bush land but not in maize field. Specifically, the greatest proportion of the LM and SM associated C in bush land was observed in the first 0.05 m and declined with depth, but in maize field, the C content in these two soil aggregate fractions was evenly distributed across the three depths. However, the redistribution cannot account for all these changes in entirety, since the cultivated crops may have returned a proportion of the aggregate C

35

over the years. Decrease in aggregate C with soil depth in the natural bush land could be attributed to the pattern of litter and organic residue accumulation, with higher amounts being found in the upper soil layer. On the other hand, constant mixing of the top and subsoil through tillage in maize field could have led to insignificant differences in aggregate C with increasing depth.

Several soil fauna species have been shown to incorporate considerable amount of organic matter in their excretions and could therefore have significant effects on aggregate-associated C content. For instance, selective ingestion of mineral and organic particles by earthworms has been demonstrated to affect C content of casts or cast-derived soil aggregates as demonstrated by Zhang and Schrader (1993), Bossuyt et al. (2004), Fonte et al. (2007), Jouquet et al. (2008) and Van Groenigen et al. (2019). Thus, Jones et al. (1994) referred earthworms and termites as 'ecosystem engineers' due to their ability to ingest or move large amounts of soil through their soil-feeding, nesting and burrowing habits, which may affect soil structure at micro and macro scales. Their activities could nonetheless, be highly influenced by management options applied and this could indirectly affect soil aggregate-associated C content (Lavelle et al., 1994). In their study, Kamau et al. (2020a) reported that the fragmentation of large macroaggregates to microaggregates by *Nematogenia lacuum* under specific agroforestry trees could have significantly reduced the overall aggregate C content. In this study however, despite *N. lacuum* being the most dominant earthworm species, there was weak correlation between aggregate-associated C content and the earthworms. As noted previously, this could have been caused by the high variation of *N. lacuum* abundance within the four land use types. Similarly, termites did not show any significant correlation with aggregate C, which could also have been caused by the high variation in termite abundance.

5.3 Effect of land use types on soil macrofauna

Soil macrofauna that are found within soil and litter plays major role in processes such as nutrient cycling, organic matter decomposition and improvement of physical attributes such as aggregation, porosity and water infiltration (Dangerfield et al, 1996; Rossi and Blanchart, 2005). Therefore, they are key elements in the development of sustainable agriculture and forestry. Generally, in this study, ants were the most dominant group compared to other soil macrofauna groups, especially in maize field. This increased ant numbers could probably have been caused by increased population of insect pests associated with cultivation of maize. On the contrary, other soil macrofauna groups which are more sensitive to disturbance such as millipedes and earthworms seemed to favor the undisturbed native bush land or less disturbed land use types such as grazed pastures. This could be due to a reduction in extreme fluctuations of soil moisture and temperature, and availability of food substrates as shown by the high C content. The activity of soil fauna has been reported to be especially sensitive to changes in soil temperature and moisture (Gongalsky et al, 2008), presence of above ground leaf litter (Rombke et al, 2006), soil fertilization regimes (Geissen et al, 1999), substrate quality (Hamel et al, 2007; Osler and Sommerkorn, 2007) and soil community composition (Helling et al, 1998). Agricultural intensification has also been shown to increase the rate of soil compaction as a result of soil structure collapse. Traditionally, soil tillage is done by the use of agricultural machinery made with disc plows, harrows and sub-soilers (Portilho et al., 2011; Tavares et al., 2015; Surendran et al., 2016) that negatively affect the aggregation of soil, which also leads to a reduction in soil macrofauna abundance and diversity through physical destruction (Benito et al., 2008; Mbau et al., 2015; Ayuke, 2010). On the other hand, though soils in coffee plantation are frequently disturbed by occasional weeding, the litter left after coffee pruning may favour soil macrofauna that may otherwise be negatively affected by decreased substrate as a result of crop residue removal which is common in maize field. Therefore,

organisms could have been able to survive, despite the occasional disturbance. This when compared to the maize field, where the level of disturbance is higher, and where crop residues are often removed in preparation of the land for the successive seasons. However, land use type seems to have little influence, which could perhaps be caused by the high variation of many of the soil macrofauna groups from transect to transect as reported in section 5.1 above. Nonetheless, the significant role of soil macrofauna on soil properties cannot be discounted here (Lavelle, 1997; Ayuke et al., 2009). Earthworms and termites, for example, have been recognized as "ecosystem engineer" due to the immense role they play on soil structure and function at micro level through their soil-feeding, and at macro level through nesting and borrowing habits (Kamau et al., 2020a). In addition, soil fauna play significant roles such as nutrient cycling, organic matter decomposition and soil structure stabilization (Brussaard et al, 2007; Ayuke et al., 2009; Mbau et al., 2015).

5.4 Conclusions and recommendations

This study has shown that land use change from bush land to cultivated land had significant effects on soil aggregate stability and aggregate associated-C content, which could have resulted from the fragmentation of macroaggregates to microaggregates and the loss or redistribution of SOM that was once protected inside of the macroaggregates. The magnitude of these effects were higher in more disturbed land use types. Increased soil disturbances as a result of conversion of the bush land also had significant negative effects on soil macrofauna abundance and diversity. Thus, if conversion of native forests or bush lands is inevitable, farmers should consider adopting land use types that have lower soil disturbance levels or those that encourage higher organic matter inputs to minimize the negative effects. This may not only improve soil structure and carbon sequestration, but would also enhance the ability of such soil to mitigate against climate change.

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