EFFECTS OF SOIL FERTILITY MANAGEMENT PRACTICES ON SOIL AGGREGATION, CARBON AND NITROGEN DYNAMICS IN A LONG-TERM EXPERIMENT AT KABETE

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DECLARATON

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

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This thesis has been submitted for examination with my/our approval as university supervisors.

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DEDICATION

To my parents Mr. and Mrs. Joshua Chumo who made it possible for me to go to school.

To Juliana, my wife and our daughters who supported, encouraged and provided a peaceful studying environment.

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ABSTRACT

Poor resource farmers cultivate steep slopes without soil conservation measures and apply insufficient plant nutrients thus degrading the soils. Integrated soil fertility management using organic and inorganic sources of nutrients is one of the approaches being advocated to farmers as a way of improving soil health and increasing production.

This study was conducted to determine the effects of long-term use of inorganic fertilizer (NPK); inorganic fertilizer (NPK) with manure (F + M); inorganic fertilizer (NPK) with residue (F + R); residue and manure application on soil aggregates, organic carbon, and nitrogen and macrofauna in a humic nitisol soil under annual maize-bean crop rotation. Macrofauna (soil invertebrates) and soil samples were collected from the 31 year-old long-term experiment and assessed for stable aggregate size distribution, total soil organic C and N contents. The diversity, abundance and biomass of soil macrofauna (termites and earthworms) were also determined.

The results of the study showed a significant increase in large (p=0.01) and small macroaggregates (p=0.002) in the 0-15 cm and 15-30 cm depths under inorganic fertilizer (NPK) combined with manure (F + M) treatment. Also, significant increase (p=0.003) in mean weight diameter (MWD) of soil aggregates, soil organic carbon in small macroaggregates (p=0.005) and microaggregates (p=0.044) in soil that received inorganic fertilizer (NPK) with manure (F + M) compared to control. In terms of biodiversity, use of inorganic fertilizer (NPK) and inorganic fertilizer (NPK) combined with manure led to increase in earthworm biomass which was positively correlated with large macroaggregates (r=0.397, p=0.017), silt and clay (r=0.385, p=0.020), C in small macroaggregates (r=0.474, p=0.003) and microaggregates (r=0.493, p=0.002). Long-term use of combined inorganic fertilizer (NPK) with manure improved the stability of the macroaggregates and increased mean weight diameter (MWD) in both 0-15 cm and 15-30 cm compared to all other treatments. Thus integration of fertilizers and animal manures would result in build up of soil organic matter in the long-term, thus contributing to C sequestration.

ABBREVIATIONS AND ACRONYMS

AMF	Arbuscular Mycorrhizal Fungi
C	Carbon
Cm	Centimeter
CT	Conventional Tillage
F+M	Inorganic Fertilizer with Manure
F+R	Inorganic Fertilizer with Residue
FAO	Food and Agriculture Organization
fPOM	Free Particulate Organic Matter
FYM	Farm Yard Manure
ICRAF	International Centre for Research in Agroforestry
iPOM	Intra-aggregate Particulate Organic Matter
Kg	Kilogram
Mm	Millimeter
MWD	Mean Weight Diameter
N	Nitrogen
NT	No till
OM	Organic Matter
POC	Particulate Organic Carbon
RCBD	Randomized Complete Block Design
S+C	Silt and Clay
SOC	Soil Organic Carbon
SOM	Soil Organic Matter

TSBF	Tropical Soil Biology and Fertility
μ	Micron
IRSM	Isotope Ratio Mass Spectrometer

CHAPTER ONE

1.0 INTRODUCTION

1.1 General

Increased population growth coupled with limited resources in many developing countries have contributed to increased level of poverty leading to land subdivision and environmental and land degradation (FAO, 2008). The net result is small farms, low production and increasing landlessness (Mbagwu, 2003; FAO, 2008). Until the first quarter of the twentieth century, the problem was not serious, since many countries had arable lands which could be opened for cultivation. It was expected that cultivable lands could expand by only 20% (Tisdale *et al.,* 2002) but, due to rapid population growth, it has become difficult to feed the growing numbers based on such an increase in cultivable land (Chrispeels and Sadava, 1977).

Increasing population pressures and widespread food deficits in Sub-Saharan Africa (SSA), and Kenya in particular (Thaxton, 2007; Population and Housing Statistics, 2007) have compelled national programmes and international donors to place a high priority on increased agricultural productivity and alleviation of poverty among the small-scale farmers. A major response to these problems has been the emphasis on integrated soil fertility management (ISFM) using organic and inorganic sources of nutrients as a means of increasing production and reducing land degradation (ICRAF, 1997a). A balanced approach is required that addresses both human needs and environmental concerns (Woomer *et al.*, 1994; ICRAF, 1997b).

In the smallholder farming systems, continuous nutrient mining with inadequate or no mineral or organic fertilizer application, coupled by soil erosion on steep slopes (Mati, 2005) and the disappearance of shifting cultivation with its long periods of fallow has exacerbated soil fertility decline (FAO and ACFD, 1999). Inorganic fertilizer which could be used as a remedy are inaccessible due to their high cost (Smaling, 1993), while use of animal manure as an alternative for maintaining soil fertility and crop productivity are available in inadequate amounts and usually of low quality due to poor handling and poor quality livestock feeds (Lekasi *et al.*, 1998; Jackson and Mtengeti, 2005). Farmers are thus left with the option of using a variety of low external input practices to maximize crop yields. Although organic resources used alone offer insufficient nutrients to sustain crop yields and soil fertility (Palm *et al.,* 1997), they do continue to play a critical role as nutrient sources to small scale farmers who are unable to access adequate quantities of mineral fertilizers. As such, in low external input systems, the challenge is to develop ways of managing organic matter decomposition to optimize short- and long-term release of nutrients and the maintenance of soil organic matter (SOM) as well as soil structure.

Soil structure is expressed as the degree of stability of aggregates and is the major factor that determines the physical, chemical and biological processes of the soil dynamics (Bronnick and Lal, 2005). Soil aggregates results from the binding of primary mineral particles with organic and inorganic materials. This process is influenced by the interaction of several factors namely; climate, environment, soil management, and plant effects and largely by soil properties. The soil properties that play a greater role include; soil moisture availability, mineral composition, soil texture, the quantity and quality of soil organic matter, microbial and enzyme activities and mineral nutrients. Soil aggregation thus begins with consolidation of particles into microaggregates (<250 μm) followed by formation of macroaggregates (>250 μm) (Rillig and Mummey, 2006; Bronnick and Lal, 2005; Six *et al*, 2000a)

1.2 Problem statement and justification

Inorganic fertilizers are key to maintain soil fertility and productivity (Bolwig, 2002). However, their use is associated with a number of soil problems, among them: (i) deterioration of soil structure, (ii) soil crusting, (iii) poor infiltration, (iv) accelerated wind and water erosion, (v) loss of organic matter and (vi) loss of biodiversity (FAO, 1999). Soil structure defines the physical environment within the soil in terms of its degree of aeration and the amount of water it will absorb, retain and transmit. Soil structure also determines the suitability of a soil as a habitat for invertebrates and for plant growth (Lavelle and Spain, 2001). Serious land degradation has been taking place and approximately 24 billion tons of topsoil are lost annually, an equivalent of 9.6 million hectares of land which translates into 15-30% of productive land which will be lost by the year 2020 (Steiner, 1996; Sherr, 1999). Therefore, soil degradation and/ or changes in soil quality that result from wind and water erosion, losses of organic matter and nutrients, or soil compaction are of great concern in every agricultural region in the world, because this would impact on food supply, vegetative cover, biodiversity and on climate change (Steiner, 1996). Soil stores significant amount of carbon as soil organic matter (Lal, 2004), but most of it is lost through biological processes like respiration and through erosion.

Soil macrofauna (earthworms and termites) play vital roles in soil aggregation, soil organic matter (SOM) stabilization, soil porosity improvement and water flow into and through the soil (Mando and Miedema, 1997; Lavelle and Spain, 2001; Bossuyt *et al.*, 2005). Although numerous studies have shown that agricultural activities such as conventional tillage and use of agrochemicals depress and/or lead to loss of soil macrofauna population and biodiversity causing a negative effect on the soil structure (Decaëns, 1999), there is limited information on ecological interactions between crop management, soil macrofauna, soil organic matter (SOM) and soil structure in low-input cropping systems. This study was undertaken to ascertain the effect of soil fertility management practices on the soil structure, soil macrofauna and consequently on soil organic carbon and nitrogen dynamics as influenced by long-term crop production.

1.3 Research questions

(i) How do the soil fertility management practices influence soil aggregate stability?

(ii) Does soil aggregate turnover influence carbon and nitrogen dynamics in the soil?

(iii) What are the effects of soil fertility management practices on the soil macrofauna abundance?

(iv) Does soil macrofauna abundance influence the stability, carbon and nitrogen dynamics of the soil aggregates?

1.4 Objectives

Broad Objective

To evaluate the effect of soil fertility management practices on stability of soil structure, organic carbon and nitrogen in a long-term experiment.

Specific Objectives

(i) To determine soil aggregate classes in soil sampled from selected soil fertility management treatments in a long-term experiment.

(ii) To determine carbon and nitrogen contents from different soil aggregate classes.

(iii) To assess the interactions between soil aggregate classes, carbon and nitrogen dynamics and soil macrofauna (earthworms and termites).

1.5 Hypotheses

(i) Integration of organic and inorganic inputs enhance macroaggregate stability.

(ii) Soil fauna abundance enhances macroaggregate stability.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Soil Aggregates and Plant Growth

Lavelle and Spain (2001) defined aggregate as an association of soil particles that has a greater degree of internal cohesion than to the particles surrounding it. A well developed soil structure has high porosity, water stable aggregates and is considered important both in protecting the soil surface against erosion and promoting a satisfactory level of plant growth. Lavelle and Spain (2001) observed that plant growth is favored by well developed structure due to improved aeration and water flow into and through the soil. Soil structure is negatively impacted on by rain drops (Hillel, 1982; Kauwenbergh, 2006), deflation by wind (Hillel, 1982), ploughing and tilling of the land and crushing by heavy machinery movement on the soil surface (Hillel, 1982; Kauwenbergh, 2006).

When soil aggregates that are not water stable are exposed to the impact of rain drop and surface water flow, they are slaked and dispersed leading to excessive erosion of the surface soils (Hillel, 1982). This seals the soil pores and leads to formation of the surface crusts (Lavelle and Spain, 2001). Such surface crusts do reduce water infiltration rate and leads to an increased run-off. Ghildyal and Tripathi (2005) pointed out that soil crusting is one of the major detrimental factors affecting emergence and early growth of seedlings and largely determines the crop stand. Tisdale *et al.*, (2002) observed that, respiration and the normal functioning of the roots are strongly dependent on an adequate supply of oxygen. Ghildyal and Tripathi (2005) observed that the distribution and arrangement of compound aggregate in a given soil gives soil the porous nature. Therefore, total porosity and pore size distribution are closely associated with the aggregate size distribution which in turn affects the entry and movement of fluids (air and water) in soil. Tisdale *et al.,* (2002) reported that the cropping systems that are detrimental to soil tilth and that cause reduced soil porosity and increased compaction have been found to impair nutrient uptake, especially Potassium (K^+).

2.2 The Role of Soil Organic Matter

Soil organic matter consists of a variety of components in varying proportions and many intermediate stages and these include: raw plant residues and microorganisms; active pools of SOM and resistant or stable organic matter (humus) (Woomer and Swift, 1994). These can be separated physically into two main groups namely; (i) particulate organic matter (POM) fractions of size >53 μm and (ii) fine amorphous organic matter (FOM) consisting of fractions of particle size <53 μm associated with organo-silt and organo-clay (Cambardella and Elliott, 1992; Vanlauwe *et al.*, 1998). Particulate organic matter fraction responds to management and is easily decomposed, rapidly lost and act as a source of plant nutrients (Mando *et al.*, 2005; Vanlauwe *et al.*, 1998; Cambardella and Elliot, 1994) and is a good indicator of soil supplying capacity. Fine amorphous organic matter fraction is much slower to mineralize due to better physical protection and is generally considered to contribute to soil stability (Vanlauwe *et al.*, 1998; Mando *et al.*, 2003).

The active pools of the SOM (Six *et al.*, 2002a) and some of the resistant soil organic components together with microbial products are involved in binding of the soil particles into macroaggregates. Improvement of soil organic matter contributes to improved soil physical properties, biological and chemical properties (Woomer *et al.*, 1994). Soil organic matter contributes to soil fertility through cementation of soil within aggregates, retention of cations and conservation of nutrients in organic forms (Lavelle and Spain, 2001).

2.2.1 Soil Organic Matter Fractions

The difference in position within the soil matrix and the resultant accessibility of SOM to soil organisms leads to pools that differ in stability and dynamics (Golchin *et al.*, 1994b). Intra-aggregate organic matter is incorporated and physically stabilized within macroaggregates, while free organic matter is found between aggregates and that aggregate protected pools of carbon are less labile than unprotected pools (Cambardella and Elliot, 1992, 1993). Beare *et al.*, (1994a) noted that protected pools are less exposed to microbial decomposition. In their studies, Golchin *et al.*, (1994a, b) isolated two fractions of free and occluded (intra-aggregate) particulate organic matter. They found out that occluded particulate organic matter had more C and N concentration as compared to free particulate organic matter.

Christensen (1986) described the importance of differentiating the free and intra-aggregate SOM in conceptual models of physically based SOM pools. Three different SOM pools are recognized: active, passive, and slow pools. The active pool is composed mainly of plant residues in different stages of decomposition. This is the smallest and youngest of organic matter and has a turnover time of days to weeks, while the slow pool has a turnover time of years to decades (Paul *et al*, 2001). The active pool is the most labile organic matter pool (Cambardella and Elliot, 1993) and is most involved in microbial mediated processes such as N mineralization. The free particulate organic matter (fPOM), an indirect measure of the active pool, is not associated with mineral particles and consists mostly of incompletely decomposed organic residues (Jastrow, 1996 and Six *et al.*, 2002a).

2.3 Formation of Soil Aggregates

Three major mechanisms are responsible for the formation of aggregates, namely: biological, chemical and physical (Ghildyal and Tripathi, 2005; Six *et al.*, 2004). These mechanisms work together to form aggregates.

2.3.1 Physical factors involved in soil aggregate formation

The factors responsible for the initial formation of small aggregates include expansive and contractive forces associated with wetting-drying cycles (Taboada *et al.*, 2004) or freezing and thawing (Ghildyal and Tripathi, 2005) and colloidal clay effects. Decreased water content typically increases contact points between primary particles and organic matter resulting in increased soil cohesion and strength, thus promoting cementation of soil particles (Ghildyal and Tripathi, 2005). As soil mass shrinks, cracks open along planes of weakness which overtime they become extensive and the aggregates between the cracks are formed (Six *et al.*, 2004). Wetting and drying may also result in aggregate breakdown (Hillel, 1982; Six *et al.*, 2004). Freezing of water drawn from the surrounding soil mass to the ice crystal causes dehydration resulting in aggregation (Ghildyal and Tripathi, 2005). Cracks and surfaces of weakness that separate natural aggregates are largely caused by movements of soil in shrinking and swelling (Hillel, 1982; Six *et al.*, 2004). Water flows between these cracks depositing clay and other materials in suspension to form cutans, which provide zones where naturally occurring aggregate are separated from one another (Marshall and Holmes, 1988).

Water repellency (WR) is an intrinsic and fundamental physical property which refers to the ability of a soil to slightly repel water (contact angle <90°). Partly decomposed organic materials, fungal hyphae, and soil organic matter-derived products such as polysaccharides, humic and aliphatic substances and waxes (Chenu et al., 2000; Ellerbrock *et al.*, 2005) induce hydrophobic properties to soils with a magnitude depending on the quantity and quality of soil organic matter. Excessive water repellency can increase surface runoff loss due to reduction in water infiltration (De Bano, 2000). Moderate repellency can be beneficial to many soil processes. For example, it can increase soil aggregate stability and strength (Blanco-Canqui *et al.*, 2007) and promote long-term SOC sequestration particularly in no-till soils. Rapid water entry into the aggregates causes air compression and slaking. Stabilization of aggregates not only would improve soil structural properties and reduce soil erodibility but also can protect intra-aggregate-occluded organic materials from rapid decomposition and promotes long-term C storage and reduction in C turnover.

2.3.2 Chemical processes involved in soil aggregate formation

Soil aggregation is caused by exchangeable cation interaction and cementing action of inorganic colloidal substances (Oades, 1984). In these mechanisms, aggregate formation involves an interaction between the exchangeable cations on the clay particles and water in the soil pores. The clay particles are negatively charged and surrounded by electrical double layer consisting of fixed and mobile cations. The water dipoles orient along the lines of force in the electrical field of each ion and the force of the charged clay particle. In this way, cation-dipole linkage is strengthened (Ghildyal and Tripathi, 2005; Oades, 1984) with divalent cations such as calcium (Six *et al.,* 2004; Ghildyal and Tripathi, 2005). Soils containing calcium as dominant cation are better aggregated than soils with sodium. As water is removed, the thickness of the film of water around the particle is reduced and the two clay particles are held together by ions (Lavelle and Spain, 2001; Six *et al.,* 2004). In this way, calcium linkage between clay and organic colloids results into the formation of stable aggregates (Ghildyal and Tripathi, 2005). The cementing action of inorganic compounds, such as iron oxides, produces very stable microaggregates (Lavelle and Spain, 2001; Six *et al.,* 2004) in certain clayey soils (Ultisols and Oxisols) of hot, humid regions.

Nwadialo and Mbagwu (1991) observed that mineralogy of the clay fraction is an important factor in microaggregation. Oades and Waters (1991) pointed that in soils that are high in 2:1 clay, SOM is a major binding agent because polyvalent metal-organic matter complexes to form bridges between the negatively charged SOM and 2:1 clay platelets. Part of the stability in 1:1 clay mineral dominated soil is induced by the binding capacity of oxide and 1:1 minerals. Mbagwu (1989) showed that the role played by SOC as an aggregating agent tended to diminish with an increase in other aggregating agents such as silicate and polyvalent metals. Oades (1984) pointed out that where Iron (Fe) and Aluminium (Al) oxides are high; the contribution of organic carbon to aggregate stability is diminished.

2.3.3 Biotic processes and soil aggregate formation

The main biological processes responsible for aggregation include; burrowing and molding activities by earthworms (Brown *et al.*, 2000) and termites, enmeshment of particles by roots and fungal hyphae (Six *et al.*, 2004; Oades, 1993), and production of organic binding agents by microorganisms (Bossuyt *et al.*, 2001).

Earthworms ingest organic matter and mix it with inorganic soil material in their gut which is later excreted as casts which contributes to soil aggregation (Bossuyt *et al.*, 2005). Plant roots and fungal hyphae exude polysaccharides and other organic compounds which bind together individual soil particles and microaggregates into macroaggregates. Microorganisms produce polysaccharides and other organic binding agents as they decompose plant residues, these bind microaggregates into macroaggregates (Six *et al.* 2004). Fungi are the most important agents involved in soil aggregation, their role can be due to physical network of hyphae maintaining soil particles together or by cementing capacity of the extracellular compounds released. Among this, are the arbuscular mycorrhizal fungi (AMF) which seem to play a predominant effect on aggregates formation (Borie *et al.*, 2008). In the rhizosphere, mycorrhizal hyphae may contribute to the aggregating effect as they grow into small pores and bind soil particles together (Miller and Jastrow, 1990). Recent studies have shown that soil particles are bound not only by mycorrhizal hyphae, but also by mycorrhizal polysaccharide. Glomalin is a fungal protein from AMF that is operationally quantified from soil as Glomalin-Related Soil Protein (GRSP). GRSP has recently received increased attention in the context of soil aggregation (Rillig and Mummey, 2006) owing to the frequently observed correlation between GRSP and soil aggregate stability (Wright and Upadhyaya, 1998). However, evidence linking GRSP to soil aggregation remains correlative, and the mechanisms involved are still unclear. Glomalin is hypothesized to act as 'glue' with hydrophobic properties, but direct biochemical evidence is lacking. Glomalin has recently been shown to be tightly bound in the fungal mycelium amounting to about 80%, rather than being secreted into the medium (Driver *et al.*, 2005). Thus, the functionality of Glomalin in the soil would then be only secondarily arising, perhaps by virtue of its relatively slow turnover rate in the environment (Steinberg and Rillig, 2003). Although this may be the case, work by Wright and Upadyaya (1998) showed that there was strong correlation between aggregate stability and glomalin.

Many of the plant roots and microbial organic binding agents resist dissolution by water and hence enhance the formation of soil aggregates thus ensuring their stability. Exudates from plant roots provide carbon which stimulates the formation of aggregates; for example root mucilages can stick particles together, leading to the short-term stabilization of aggregates. Additionally, rhizodeposited carbon and decomposable organic residues from plant roots support a large microbial population in the rhizosphere. The activity of the rhizosphere microbial organisms in turn contributes largely to aggregate formation (Rillig and Mummey, 2006; Tisdall and Oades, 1982).

2.3.3.1 The role of soil macrofauna in aggregate formation and stability

The two most important macrofauna organisms in aggregate production are earthworm (Blanchart *et al.*, 1997) and termites (Lee and Wood, 1971; Jouquet *et al.*, 2005). Their role in aggregate formation in most soils is through promotion of primary binding mechanically or intestinal mixing (Barois *et al.*, 1993). Termites and earthworms produce a wide range of organo-mineral structures, such as crop galleries, casts, crop sheetings, nests, cocoons and wall plasters which are termed as biogenic structures (Mando *et al.*, 1999). Since the structures result from an intestinal transit such as earthworm casts or are mixed and impregnated with saliva e.g. termite sheetings, they constitute microsites where a number of particular physico-chemical changes occur. The soil fauna also produces macro-scale structures ranging from faecal pellets (250 µm to several centimeters) to large subterranean structures such as termite and ant nests (Lavelle and Spain, 2001). Barros *et al.*, (2000) identified three structures and aggregates as follows: earthworm casts as rounded aggregates of 2-8 mm diameter of brown colour, which were often darker than the matrix; termite pellets as rounded aggregates of 60-500 µm diameter, dark brown or of the same colour as the matrix and angular or sub-angular aggregates of 180-800 µm diameter with a range of different colours corresponding principally to fragments of larger aggregates transported by ants.

Soil fauna influence physico-chemical and biological characteristics of the soil through their activities, for example, earthworm structures affect fundamental process such as the organic material cycle (Brown *et al.* 2000), the activation of the microflora and changes in structural properties (Blanchart *et al.*1993). These structures would ultimately disintegrate and integrate into the soil where they influence physico-chemical and biological characteristics (Mora *et al.*, 2003).

2.3.3.1.1 Earthworms

From an ecological point of view, earthworm communities are divided into three functional groups, namely epigeic, endogeic and anecics. These classifications are based on their habitat, food choice, feeding behaviour and ecophysiology (Lavelle et al., 1998; Swift and Bignell, 2001).

a) Epigeic earthworms are those that live in the superficial soil layers (surface) and feed on undecomposed plant litter.

b) Endogeic earthworms are those which forage below the soil surface in horizontal, branching burrows. These species ingest large amounts of soil and dead roots in the soil, showing a preference for soil rich in organic matter.

c) Anecic earthworms build permanent, vertical burrows that extend deep into the soil. This type of worm comes to the surface to feed on manure, leaf litter, and other organic matter (Lavelle and Spain, 2001; and Swift and Bignell, 2001).

Earthworms are known to select organic and mineral soil components that they ingest. As a result, their casts often have much higher content of SOM and nutrients than the surrounding soil (Lee, 1985). Earthworms ingest leaf and root litter debris as well as fungi (Neilson *et al.*, 2000), feacal pellets of other invertebrates and clay minerals. There is evidence that some endogeic species of earthworms ingest only aggregates that do not exceed the diameter of their mouths, whereas other species may feed on larger aggregates and split them into smaller aggregates (Blanchart *et al.*, 1999). Complementary patterns have been observed between larger earthworm species and the smaller species. The compacting species e.g. *Millsonia anomala* (endogeic) produce large globular casts which are surrounded by thin cortex made of clay minerals and organic particles, this reduce aeration thus inhibiting microbial activity, hence such soils are prone to compaction while a species like *Hyperiodrilus africanus* (epigeic) produces fine granular casts after ingesting the larger globular casts of the compacting species with root litter thus decompacting the soil (Blanchart *et al.*, 1993).

Earthworms have played a key role in the formation and stabilization of soil aggregates and in nutrients cycling (Scullion and Malik 2000) through removal of litter and other organic materials from the soil aggregates. This may lead to the formation of relatively stable macroaggregates that tend to be enriched in soil organic matter compared with uningested soil (Scullion and Malik, 2000). The ingestion and digestion of soil and litter by earthworms is also known to induce the formation of new microaggregates. Formation of microaggregates occurs when organic particles are fragmented and pre-existing aggregates are dispersed in the earthworm gut. The dispersed clay is then brought into intimate association with mucilage-coated, decomposing organic fragments and rearranged into newly formed microaggregates that are excreted in casts (Barois *et. al.*, 1993). During burrowing, earthworms exert pressure on the surrounding soil and external mucus is deposited on the burrow walls. They also line the walls of the burrows with organic mucilage leading to formation of a stable structure aggregates (Jeanson, 1964; Six *et al.*, 2004). Satchell (1983) suggested that aggregate stability is induced by cementing of soil particles in the earthworm's gut by calcium humate formed from decomposing organic material and calcite excreted by the worm's calciferous glands. Also, fungal hyphae that proliferate in earthworm burrow help to bind soil particles into stable aggregates. Bossuyt *et al.*, (2005) found that earthworms had a direct and fast impact on microaggregate formation and the stabilization of new carbon within the microaggregates.

Other mechanisms that may explain the increase of micro- and macroaggregate stability by earthworms include; mechanical binding by vascular bundles from ingested plant (Mummey *et al.*, 2006), fungal growth after excretion of the casts (Marinissen and Dexter, 1990) and, microorganisms which proliferate in ingested material in the gut and in the earthworm cast (Brown *et al.*, 2000) and in the earthworm burrow (Mummey *et al.*, 2006). Lavelle and Spain (2001) pointed out that earthworm (endogeic) species are considered major agents of aggregation and soil organic matter stabilization.

Earthworm casts are generally enriched in organic matter and cations, their particle size distribution generally have a higher proportion of the smaller size classes and their density is often higher than bulk density. All these properties have important consequences on aggregate stability and erosion (Le Bissonnais, 1996).

2.3.3.1.2 Termites

Termites are a major component of soil fauna in the tropics (Lee and Wood, 1971); they can improve soil physical properties in a degraded soil within a short time (Mando and Miedema, 1997) while playing a significant role in the decomposition process, soil nutrient availability and cycling through interactions with other soil organisms such as bacteria and fungi (Jouquet *et al.*, 2002). Their food include a wide range of plant materials such as living wood, dead wood in various stages of decomposition, herbaceous plants and grasses, plant debris, fungus, dung and soil rich in organic matter. Termites are classified on the basis of their food choice, feeding habits and nesting behaviour (Jones and Eggleton, 2000; Swift and Bignell, 2001). These include: a) Feeding group I: feed on wood, litter and grass feeders (lower termites), b) Feeding group II: feed on wood, litter and grass feeders (some of the higher termites), c) Feeding group III: feed on very decayed wood or high organic content soil (all higher termites), d) Feeding group IV: feed on low organic content soil (true soil-feeders-all higher termites).

As termites forage on living plant tissues and organic debris over extensive areas, the collected materials are transported into mounds, nests or subterranean galleries and subjected to intense degradation (Lee and Wood, 1971). Termites species such as mound builders use clay-rich soil particles to construct nests (Jouquet *et al.*, 2002), while humivorous termites use oral pellets as building materials, but they stabilize it with faecal droppings that are produced (Kooyman and Onck, 1987). Termites form stable microaggregates by mixing soil with saliva for construction or in case of soil feeding termites, by excreting organo-mineral complexes as faecal pellets, the latter are enriched in organic matter (Jungerius *et al.*, 1999). Pathak and Lehri (1959) studied the degree of aggregation of soil from mounds of *Odontotermes obscuriceps* and compared it with that of sample of adjacent soil. They found out that, aggregates from undisturbed soil were more easily dispersed in water than those from mound samples (Pathak and Lehri, 1959).

Some termites have the ability to cement soil particles into massive structures that are extremely resistant to erosion. Most termite mounds and other structures have organic matter contents higher than the soil from which they were constructed due to use of organic materials to cement soil particles or aggregates (Lee and Wood, 1971; Mando and Miedema, 1997).

In semi-arid soils that are prone to surface sealing or crusting, termite activities contribute towards increasing soil porosity through creation of macro-voids. Termite-induced change of soil structure may favour soil parameters such as hydraulic conductivity and infiltration (Mando and Miedema, 1997).

2.3.4 Soil aggregate size classes and Mean Weight Diameter

Aggregates occur in hierarchies of increasing size whereby small aggregates are bound together to form larger compound aggregates (Tisdall and Oades, 1982; Oades, 1984).

The soil aggregates are classified as follows: large macroaggregate (LM) class with diameter of more than 2000 μm and small macro-aggregates (SM) with diameter between 250 and 2000 μm. Microaggregate (Mi) class comprise aggregates between 53 and 250 μm in diameter, while mineral fraction class, are soil particles of less than 53 μm in diameter (Tisdall and Oades, 1982; Degryze *et al.*, 2004; Bossuyt *et al.*, 2004 a, b; Grandy and Robertson, 2007). Mean weight diameter (MWD) is an index that characterizes the structure of the whole soil by integrating size class distribution into one number (Six *et al.*, 2000d). MWD is a measure of aggregates that remain on each sieve, 2000-8000 μm, 250-2000 μm, 53-250 μm and <53 μm all must be stable to the wetting and sieving processes (Amezkeka, 1999). It has often been used to indicate the effect of different management practices on soil structure (Six *et al.*, 2000d) and calculation of MWD through the following equation:

$$\mathbf{MWD = \sum x_i w_i}$$

Where: x_i is the mean diameter of any particular size range of aggregates separated by sieving, and w_i is the weight of aggregates in that size range as a fraction of the total dry weight of soil used (Blair *et al.*, 2005; Six *et al.*, 2000d).

2.4 Management of Soil Structure

Soil structure mediates both soil physical and biological processes and controls soil organic matter decomposition, while it forms the basic unit of the primary particles binding agents (Tisdall and Oades, 1982).

Studies have shown that cultivation destroys soil structure resulting in loss of soil organic matter (Elliot, 1986; Al-Kaisi, 2001; Mikha and Rice, 2004; Grandy and Robertson, 2007). Lack of soil organic matter leads to poor soil structure, enhanced run-off, erosion and low productivity. Soil organic matter is critical component of soil productivity and therefore the level of SOM is considered to be a function of the net input of organic residues by the cropping system (Gregorich *et al.*, 1996). Under mixed cropping systems, a variety of compounds would be produced, some that could have a rapid and large effect on soil aggregation along with others that might contribute to the long-term stability of the aggregate produced. Incorporation of organic material both in form of crop residues or manure enhances the organic carbon level of the soil (Christensen, 1986), and this has direct and indirect effects on soil physical properties and processes Mishra and Sharma (1997) found out that application of farmyard manure in combination with inorganic fertilizers improved hydraulic conductivity of the soil. The availability of organic residues such as maize stovers are limited because they are mostly used for fuel or fodder, hence under such situations, mixed application of both organic and inorganic nutrients might be appropriate (Sarkar *et al.*, 2003). Grandy and Robertson (2007) pointed out that mixing of residues, legumes, cereal straw and forage crops could result in greatest stabilization of soil structure.

The ability of a soil to supply nutrients, store water, release greenhouse gases, modify pollutants, resist physical degradation and produce crops that are affected by the quality and quantity of the OM that it contains (Hao *et al.*, 2003; Bronick and Lal, 2005).

2.4.1 Effect of tillage on soil aggregates

Tillage is the mechanical manipulation of the soil with a purpose of improving soil conditions affecting crop production (Hillel, 1982). Tillage is done to control weeds; incorporate organic matter into the soil, prepare the seedbed for optimum growth conditions and improvement of soil structure (Unger, 1984). There are different types of tillage practices based on the method used and their objectives and they include; conventional and conservation tillage (Sumner, 2000). Disruption of aggregates through conventional tillage transfers C from slow into active pools. During tillage, macroaggregates are rapidly destroyed and intra-aggregate C pools are released from protected sites and may be rapidly lost thereafter (Oades, 1984; Elliot, 1986; Grandy and Robert 2006a; Grandy and Robertson, 2007).

The studies conducted by Grandy and Robertson (2006b) found that aggregate size class 2000-10000 µm declined after ploughing a previously uncultivated field and CO_2 emissions from the soil doubled over a period of 3 years. Conventional tillage practiced over along period of time decreases soil aggregate size which allows organisms to access to carbon that was once physically inaccessible within aggregates. Thus organic C that took centuries to store is released as CO_2 within few years (Paustian *et al.*, 1997). Under no tillage, macroaggregates tend to stay stable as Beare *et al.* (1994a) reported that macroaggregates protect soil organic matter from decomposition particularly under minimum tillage practices.

For instance, it was estimated that 30-35% of carbon originally present in the A and B horizons of native temperate forest soils was lost after 30 years of cultivation (Ellert and Gregorich, 1996). Tillage disrupts soil aggregates and exposes protected organic material (in inter- and intra-aggregate zones) (Beare *et al.,* 1994b; Paustian *et al.,* 1997) to microbial decomposition (Elliot, 1996; Beare *et al.,* 1994b) leading to increased CO_2 emission into the atmosphere (Al-Kaisi, 2001; Lal, 2004).

2.4.2 Effects of plant residues on soil aggregates

Incorporating plant residues in soil may affect the soil microclimate leading to increased decomposition and organic matter transformation (Sanchez *et al.*, 1989; Cambardella and Elliot 1993; Paustian *et al.*, 1997). Plant residues with high C:N ratio, lignin and polyphenol contents decompose and release nutrients slowly, (Tian *et al.*, 1992) while, residues with low C:N ratio, lignin and polyphenol contents decompose rapidly and have high direct nutrient effects (Kumar *et al.*, 2003). Such crop residues would have varying influences on aggregation and SOC depending on the crop species (Martens, 2000b) and quality (Kumar *et a*l., 2003). For example, residues from corn have high phenol concentration which is linked to increased water stable aggregate (WSA) formation as well as high C:N ratio, and high SOC and carbohydrate concentrations (Martens, 2000a). On the other hand, soybeans residues have low concentration of phenols (Martens, 2000a, 2000b) thus when incorporated into the soil, microorganisms (bacteria and fungi) involved in the decomposition produce polysaccharides and fungal hyphae which stabilizes soil aggregates (Sanchez *et al.*, 1989; Martens, 2000a; Grandy and Robertson, 2007).

Decomposition of plant residues, results in complex mixtures of aromatic compounds that form the bulk of organic matter. Readily available constituents such as cellulose, peptides and simple organics are mineralized energy for synthesis of microbial biomass. The more resistant compounds such as lignin and other phenolic compounds are decomposed more slowly and combine with products of microbial synthesis to form humus which promote long-term aggregation (Marten, 2000b). In the study done by Blair *et al.*, (2005), they observed that incorporation of plant residues into the soil resulted in an increase in MWD. This was due to release of soil binding agents from the residues which led to a sustained increase in MWD.

Coarse and free particulate organic matter are considered as active soil organic matter (Grandy and Robertson, 2007) which are subjected to decomposition by microorganisms (Bongoivanni and Lobartini, 2006). Grandy and Neff, (2008) observed that majority of recovered carbon (70.18%) in the particulate fraction is derived from lignin, whereas very little carbon derived from lipids, proteins or polysaccharides is present in particulate organic carbon pools. Golchin *et al.*, (1994a) pointed that coarse particulate organic carbon consists primarily of identifiable and young plant litter in various stages of decomposition, that is, chemically similar to its source material. Guggenberger *et al.*, (1994) observed that during initial stages of decomposition, unprotected, monomeric compounds are lost as well as cellulose, resulting in accumulation of lignin in particulate organic matter associated with fine-sand fraction. The particulate organic matter will act as nucleation site for the formation of aggregates (Harris *et al.*, 1996).

2.4.3 Effects of manures on carbon and soil aggregates

Manures are by-products of livestock keeping and comprise a combination of solid fecal matter (Jackson and Mtengeti, 2005) urine and spilled food (Woomer *et al.*, 1999) along with beddings (Woomer *et al.*, 1999; Tisdale, 2002). Nutrient contents of manure depend on the nutritional quality of the animal feed, manure handling and storage conditions (Murwira *et al.*, 1995; Lekasi *et al.*, 1998; Kapkiyai *et al.*, 1998). Studies done by Rose (1991) revealed that continued application of manure for along time increased the organic carbon content of the soil.

Incorporation of manure into the soil results in a decrease in bulk density and consequently increase in soil porosity (Tisdale *et al.*, 2002; Rose, 1991), also increases in N, P and K nutrients (N'Dayegamiye, 1990) leading to improved soil physical properties. As a result, porosity of the aggregates and the proportion of the total pore space that was within the aggregates (Rose, 1991), water holding capacity (Rose, 1991: Tisdale *et al.*, 2002), hydraulic conductivity and infiltration rate in the soil increases (Murwira *et al.*, 1995; Tisdale *et al.*, 2002). In the study done by Aoyama *et al.*, (1999), where cattle manure was applied for 18 years resulted in formation of water stable macroaggregates. Although manure application increased the concentration of organic matter both in macro- and microaggregates, manure derived organic matter accumulated in macroaggregates. Manure application was found to increase soil organic carbon contents, aggregate stability and soil biological activities all of which have been associated with improved soil structure (Nyamangara *et al.*, 1999; Aoyama *et al.*, 2000). It was also found that long-term manure applications increased SOC through direct addition from organic matter (OM) in the manure and from increased OM return from subsequent crop residues biomasses due to increased crop production (Whalen and Chang, 2002). Manuring was found to improve aggregation in coarse-textured soils (Nyamangara *et al.*, 1999), while in fine textured soils it was found to decrease aggregate stability (Pare *et al.*, 1999). In an experiment carried out by Chang *et al.*, (1991) on the use of solid beef manure, organic matter, total N and NO_3-N contents increased with the rise in the rate of manure application.

2.4.4 Effects of inorganic fertilizer on soil aggregates

Despite their gains in food production, inorganic fertilizer shows some negative effects on the soil physical properties. Application of inorganic fertilizers results in decline in soil structure, reduced saturated hydraulic conductivity and bulk density (Hati *et al.*, 2006). Hati *et al.*, (2006) found that the use of NPK fertilizer led to a decline in the mean weight diameter (MWD) as well as a decline in percentage of water stable aggregates (WSA). Use of NPK fertilizers was shown to result in soil acidification and depletion of soil structure (Malhi *et al.*, 2003). There are other reports showing that balanced application of inorganic fertilizers maintained soil productivity with slight increase in soil organic carbon content along with increased crop yields (Shen *et al.*, 2004).

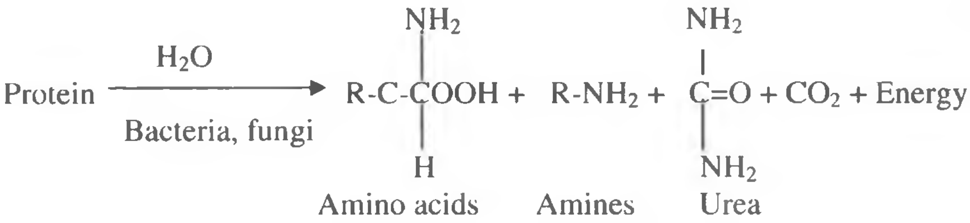
2.5 Carbon Sequestration

Carbon sequestration can be defined as the capture and secure storage of carbon that would otherwise be emitted to or remain in the atmosphere. It is estimated that 2500 gigatons (Gt) of carbon are stored in the soil as soil organic carbon (1550 Gt) and soil inorganic carbon (950 Gt) (Lal, 2004). In a stable ecosystem, release of carbon as CO_2 through oxidation of soil organic matter by microbial activity is balanced by input of carbon into soil in the form of animal manure and/ or plant residues. The direct effects of land use and land-use changes have been estimated to cause net emission of 1.7 ± 0.8 gigatons (Gt) C year ^{1} and 1.6 Gt C year ^{1} during the 1980's and 1990's (Houghton *et al.*, 2000). Maintenance of carbon levels in agricultural soils is advanced through addition of crop residues, manure and nitrogenous fertilizers (Lal, 2004). Besides C input, soil aggregate dynamics influences C sequestration and cycling (Tisdall and Oades, 1982; Jastrow, 1996; Six *et al.,* 1998). The soil organic carbon sequestration is caused by those management systems that added high amounts of biomass to the soil, or those that caused minimal soil disturbance, conserved soil and water, improved soil structure, enhance activity and species diversity of soil fauna and strengthen mechanisms of elemental cycling (Al-Kaisi, 2001; Lal, 2004; Marland *et al.,* 2004). However, carbon from the soil system is lost in the following ways: deforestation, fires, tillage and artificial drainage (Lal, 2004), also through CO_2 emission by rapid oxidation of soil organic matter by soil microorganisms (Al-Kaisi and Yin, 2005). Al-Kaisi and Yin (2005), reported that adopting less intensive tillage alternatives such as no-tillage and strip-tillage and leaving more crop residue cover on the soil surface were effective in reducing soil CO_2 emission, and thus improving soil C sequestration in a corn–soybean rotation. The rate of soil CO_2 emission are normally controlled by several factors, such as CO_2 concentration gradient between the soil and the atmosphere, soil temperature, soil moisture, pore size and wind speed (Raich and Schlensinger, 1992). CO_2 emission was affected by agricultural activities such as tillage and residue management and varies with climatic conditions (Fernandez *et al.*, 1993).

2.6 Nitrogen Mineralization

Nitrogen in the plant root zone is either nitrogen gas (N_2), as a component of the air occupying the soil pore spaces, or organic N present in various forms, including plant and microbial proteins and amino acids in the soil organic matter.

The plant available N is produced through N mineralization, a process where more complex proteins and allied compounds of organic matter are hydrolyzed first to ammonium (NH_4^+) and then to nitrate (NO_3^-) forms as a result of the activities of soil microorganisms, therefore converting them into plant-usable forms. About 98% soil nitrogen (N) is stabilized in the organic matter, and is unavailable to plants. Two processes are involved in conversion of nitrogen to ammonium (NH_4^+) and one process is involved in conversion of ammonium to nitrates (NO_3^-); these two processes are aminization and ammonification, while the other process is nitrification (Thein, 2004). Aminization process involves bacteria and fungi in the decomposition of the organic matter, while in the mineralization, bacteria and fungi decomposes proteins thus releasing amines, amino acids and urea as shown in the equation.



$$\text{Protein} \xrightarrow[\text{Bacteria, fungi}]{H_2O} \underset{\text{Amino acids}}{R\text{-}\overset{NH_2}{\underset{H}{C}}\text{-}COOH} + \underset{\text{Amines}}{R\text{-}NH_2} + \underset{\text{Urea}}{\overset{NH_2}{\underset{NH_2}{C}}=O} + CO_2 + \text{Energy}$$

The amines and amino acids produced by aminization of organic N are decomposed by other heterotrophs i.e. bacteria to release NH_4^+ in the process of ammonification.

$$\begin{tikzcd} R\text{-}H_2 + H_2O \arrow[r] & NH_3 + R\text{-}OH + \text{Energy} \arrow[d, "H_2O"] \\ & NH_4^+ + OH^- \end{tikzcd}$$

Ammonium released into the soil solution during organic matter decomposition, besides mineralization, may follow other pathways like: (i) oxidized by nitrifying bacteria and converted to nitrate or nitrite (NO_2^-), which under flooded conditions can be returned to the atmosphere as N_2 gas through denitrification processes, (ii) held as an exchangeable cation on negatively charged surfaces such as those occurring on clay particles, (iii) lost by conversion to gaseous ammonia (NH_3) under alkaline soil conditions, or (iv) assimilated by soil microorganisms and plants to supply their N requirements; this is called immobilization, because the ammonium is incorporated into tissues by the conversion of inorganic N (NH_4^+, NO_3^-) to organic N (Jonathan, 2006).

Soil organic matter contains about 5% N and during a single growing season, between 1-4 % of organic N is mineralized to inorganic N (Jonathan, 2006; Tisdale *et al.*, 2002). Organic matter is physically protected in the soil by encrustation by clay particles (Tisdall and Oades, 1982) and entrapment in small pores in aggregates inaccessible to microorganisms (Elliot and Coleman, 1988). Organic matter that had been physically inaccessible to microorganisms such as fungi and bacteria, upon physical disruption of soil by tillage, releases the protected N pools for microbial N mineralization (Tisdall and Oades, 1982).

The role of macrofauna in N mineralization was observed by Willems *et al.*, (1996), who found that earthworm activity resulted in N mineralization and Lavelle *et al.*, (1992) reported that in fresh casts of an endogeic earthworms, *Potoscolex corenthrurus* fed on an Amazonian ultisol, concentration of mineral N was up to five times more than in un-ingested soil and most of the N was in the form of NH_4^+. They also found that in humid tropical pasture, 50-100 kg mineral N was produced annually in earthworm casts.

2.7 Laboratory Methodologies

2.7.1 Aggregate separation

There are three methods most commonly used for physical separation of soil namely: sieving, sedimentation and densitometry. Of the three methods, the most commonly used method is sieving. Various sieves sizes are used, but the most common are the following: >2000 µm, 250-2000 µm, 53-250 µm and <53 µm (Tisdall and Oades, 1982; Elliott and Cambardella, 1991; Six *et al.*, 2000c). Sieving is mainly used to measure the stability of aggregates and in most cases, particular class of aggregates are isolated from the soil, exposed to a particular disruptive treatment and re-sieved to determine the proportion of aggregates remaining in that size class. There are two methods of sieving: (i) dry sieving (i.e. sieved in air) and (ii) wet sieving (sieved in water). Samples may either be moist or dry prior to sieving. The most commonly used method is wet sieving. The size distribution of aggregates obtained from wet sieving is very sensitive to the initial water content of the aggregates. When dry aggregates are quickly exposed to water, slaking often occurs. These results from the movement of water from the exterior of aggregates to the interior through capillary pores, as a result, pressure at the center of the aggregates may be considerable and the aggregates will disrupt along the weakest fracture planes causing explosion. Macroaggregates (>0.25 mm) are more susceptible to slaking than microaggregates (0.053-0.25 mm) under most conditions. The water stability of aggregates obtained through wet sieving is greatest when moisture level of the soil is at field capacity prior to wet sieving (Elliott and Cambardella, 1991).

Wet sieving method produces various soil aggregate fractions namely: macroaggregates, microaggregates, silt and clay.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study site

The study was conducted in a long-term experiment based at the National Agricultural Research Laboratories (NARL), Kabete, about 7 kilometers North West of Nairobi (latitude: 1° 15′ S; longitude: 36° 41′ E), at an altitude of 1740 m above sea level (Siderius and Muchena, 1977; Kapkiyai et al., 1998). The area receives an annual mean rainfall of 940mm in two rainy seasons, 'long rains' (mid March-May) and 'short rains' (mid-October-December). The mean annual temperature ranges between 13 °C and 18 °C (Siderius, 1976). The area falls under ecological zone III which is dry sub-humid to semi-arid with a precipitation:evaporation ratio (P/Eo) of about 56%. The soils are classified as humic Nitisol (Siderius and Muchena, 1977) and also referred to as kikuyu red clay loam. The soils are well drained, deep and weathered, dark reddish brown to dark red, friable clay (Jaezold and Schmidt, 1982). The geology of the area is underlain by the Limuru quartz trachytes, an intermediate igneous rocks dating back to the early Pleistocene period.

3.2 Experimental design

The study was superimposed on an on-going long-term experiment established in 1976. The long-term trials aimed at testing different management options for arable crop production such as organic versus mineral inputs in maize-bean rotation, with maize being planted during the long rains and beans in the short rains.

The treatments selected included: control (no organic and inorganic inputs were applied), NPK fertilizer (120 kg N ha^{-1}, 52.6 kg P ha^{-1}), NPK fertilizer with manure (120 kg N ha^{-1}, 52.6 kg P ha^{-1}, 10 t ha^{-1} FYM), NPK fertilizer with residue (120 kg N ha^{-1}, 52.6 kg P ha^{-1}, residue returned), residue and manure (10 t ha^{-1} FYM). These were replicated four times in a randomized complete block design in plots of 7 m long by 4.5 m wide. All the plots had been tilled using hand hoe since the inception of the experiment in 1976.

3.3 Soil Sampling, Pretreatment and Laboratory Analysis

3.3.1 Macrofauna sampling

One soil monolith (25 × 25 × 30 cm) per plot ($n = 3$) was taken for macrofauna sampling eight weeks after planting (June-July 2007). The extracted soil was hand-sorted for macrofauna. Termite specimens were preserved in 75% alcohol and earthworms in 75% alcohol + 4% formaldehyde. The macrofauna samples collected from the field were transported in sealed vials to the laboratory for identification, enumeration and weighing (to determine the biomass) (Moreira *et al.*, 2008; Swift and Bignell, 2001; Anderson and Ingram, 1993). The number and weight of each category of fauna (earthworms and termites) were expressed on an areal basis (per metre square).

3.3.2 Soil pH determination

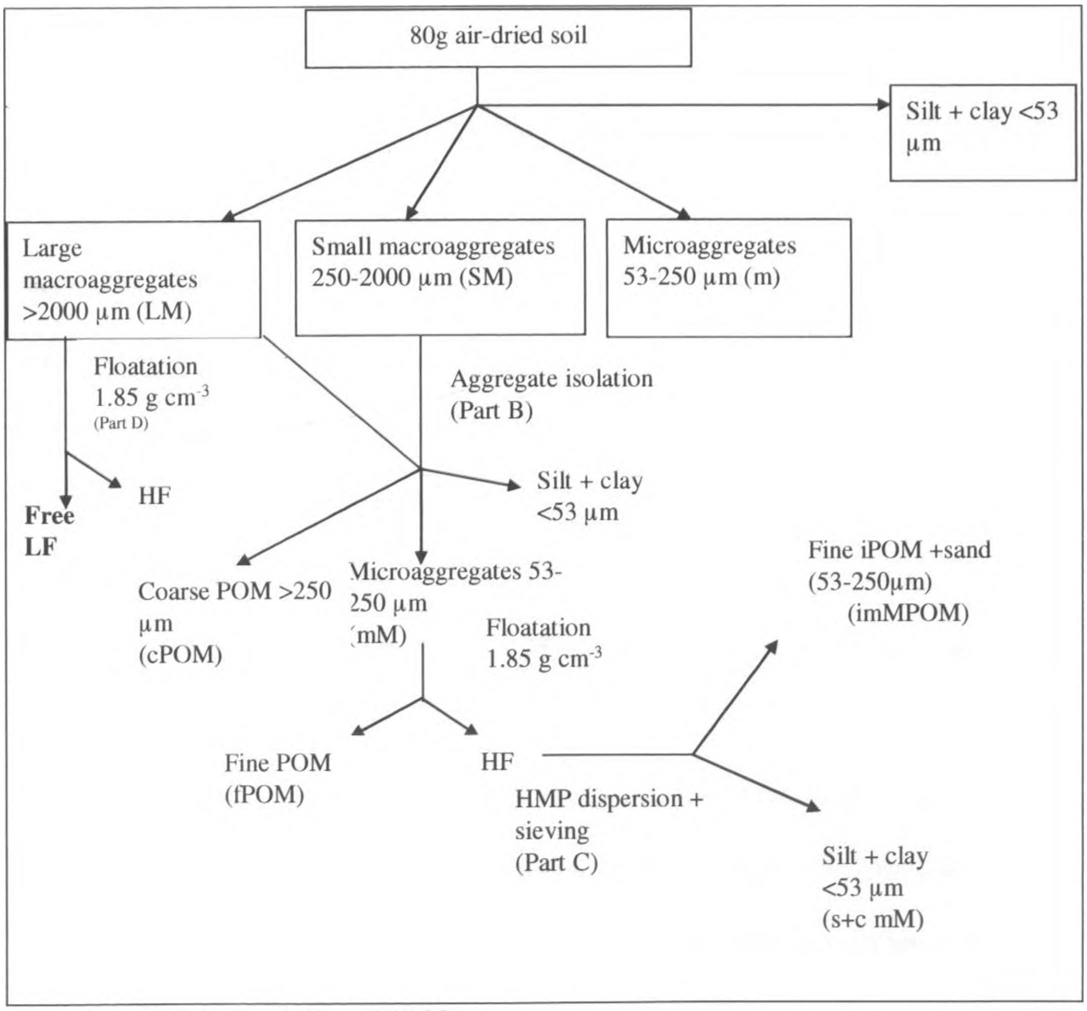
Dry whole soil from each treatment were sub-sampled (in duplicate) from depth 0-15 cm, mixed thoroughly and ground to pass through a 2 mm sieve. Ten grammes of soil sample were drawn from each sub-sample and were put in a separate 100 ml bottle and 25 mls of distilled water was added to each bottle. The mixture were mounted on a reciprocal shaker and shaken for ten minutes at 200 rpm and were left to stand for 30 minutes, then stirred for two minutes. The pH of the supernatant liquid was measured using a pH meter (Anderson and Ingram, 1993).

3.3.3 Aggregate analysis

3.3.3.1 Wet seiving

A representative subsample (about 500 g) of the 0-15 and 15-30 cm depth layers of the monolith was gently passed through 10 mm sieve by breaking up the soil along natural planes of weakness, air-dried and stored at room temperature. The soil was then separated into four water stable aggregate size fractions (Figure 1), (i) large macroaggregates (>2000 µm), (ii) small macroaggregates (250 µm-2000 µm), (iii) microaggregates (53–250 µm), and (iv) silt + clay sized particles (<53 µm), following the method described by Elliot (1986), Pulleman *et al.*, 2004 and Six *et al.*, (2000b). Eighty grammes of air-dried soil were transferred to a 2 mm sieve, placed in a recipient filled with demineralized water, and left to slake for 5 minutes. After 5 minutes, the 2 mm sieve was manually moved up and down 50 times over 2 minutes. The procedure was repeated using the material that passed through the 2 mm sieve, using a 250 µm sieve and subsequently a 53 µm sieve (one by one). Soil aggregates retained on each sieve were backwashed into pre-weighed containers, oven-dried at 60 °C for 48 hours and weighed. A representative 250 ml subsample was taken from the suspension containing the <53 µm silt and clay sized particles to determine the weight of the smallest fraction.

Figure 1 : Fractionation scheme to separate aggregates and particulate organic matter (POM) and determine aggregate



(Source : Six *et al.* 1998 and 2000)

3.3.3.2 Fractionation

Microaggregates (53 – 250 µm) from wet sieving were separated from macroaggregates using aggregate isolator by Six *et al*., (2000a). The devise involved complete break-up of macroaggregates with minimum disruption of microaggregates. Five grammes of macroaggregates were taken to a 250 µm mesh screen on top of the devise and shaken with 50 glass beads (4 mm in diameter) for 3 minutes until all macroaggregates were broken. Where the macroaggregates were not fully broken down, a further shaking for 1 minute was done (to a maximum of 5 minutes) and this was repeated until all the macroaggregates were broken down.

Water was allowed to flow continuously through the aggregate isolator such that the microaggregates were flushed immediately through the 250 µm sieve onto a 53 µm sieve.

Once all the macroaggregates were broken down, the retained sand and coarse particulate organic matter (cPOM) on the top of the 250 µm mesh were backwashed into a pre-weighed container. The sieve holding the microaggregates was moved up and down 50 times in 2 minutes in the suspension containing <53 µm fraction (Six *et al.*, 2000 b, d). A representative 250 ml subsample was taken from the suspension containing the <53 µm silt and clay sized particles to determine the weight of the smallest fraction. Three fractions were obtained namely: (i) coarse POM + sand (> 250 µm), (ii) microaggregates within macroaggregates (53-250 µm), and (iii) silt and clay (< 53 µm), and all were oven dried at 60 °C for 48 hours weighed and analyzed for C and N.

3.3.3.3 Density flotation and dispersion

Free fine particulate organic matter (fPOM) was isolated (Figure 1) from microaggregate within macroaggregates (mM) in 1.85 g cm^{-3} sodium polytungstate (SPT) following a modification from Elliott and Cambardella, (1991) and Cambardella and Elliott, (1993). 3.0 g sub-samples of mM were oven dried overnight at 60 °C. 40ml of 1.85 g cm^{-3} sodium polytungstate [$Na_6(H_2W_{12}O_{40}).H_2O$] were put into centrifuge tubes. Dried sub-samples were added into the centrifuge tubes and weight recorded. The samples were placed under a vacuum (138 kPa = 20 psi) for 10 minutes in order to evacuate the air entrapped within the aggregates. The samples were then centrifuged for 30 minutes at 2200 revolutions per minute (rpm). The floating material (free POM) and SPT were removed using a syringe and passed through a glass fibre filter paper mounted in a millipore filter unit. The collected fPOM were rinsed on the glass fibre filter paper using deionized water.

The heavy fractions that remained in the centrifuge tubes after density floatation were rinsed using deionized water and placed in beakers, and dried overnight at 60 °C. About 3 g of each sample were weighed and placed in centrifuge tubes. 0.5% (5 g/L) sodium hexametaphosphate (NaHMP) and ten beads (4 mm diameter) were added to the samples in the centrifuge tubes and shaken overnight on a reciprocal shaker. After overnight shaking, each sample was poured over a 53 µm sieve. The intra-aggregate POM + sand that remained on 53 µm sieve were rinsed thoroughly and backwashed into a pre-weighed container. Silt and clay fraction (<53 µm) that remained were transferred into pre-weighed containers. All fractions were dried at 60 °C for 48 hours, weighed and analyzed for C and N.

3.3.3.4 Soil carbon and nitrogen analysis

For measurement of soil organic C and N content, about 30 mg of the whole soil samples (before fractionation) and aggregate fractions were taken, and about 10 mg of sand + particulate organic matter fraction (coarse POM and intra-aggregate POM) were weighed in aluminium capsules, which were placed into capsule trays, and sent to University of California at Davis for C and N analysis.

The samples were analyzed using Isotope Ratio Mass Spectrometer (IRSM). Samples were combusted at 1020 °C in a reactor packed with chromium oxide and cobaltic oxide. After combustion, oxides were removed in a reduction reactor (reduced copper at 650 °C) and the helium carrier went through a water trap (magnesium perchlorate). Nitrogen and CO_2 that were produced after combustion, were separated on a Carbosieve column (65 °C, 65 mL/min) before entering the IRMS where readings were taken and reference gas peaks were used to calculate provisional isotope ratios of the sample peaks.

3.4 Data Management and Analyses

All data from the experiment were entered into excel spreadsheet. The data on the effects of soil fertility management practices on soil aggregate fractions, mean weight diameter and soil macrofauna were subjected to analysis of variance (ANOVA) using GenStat 11.1 (2009) statistical package. Regression and correlation analyses were conducted to establish the relationship between soil fauna and soil aggregation and also between soil fauna and carbon and nitrogen dynamics using XLSTAT PRO (XLSTAT, 2009). The statistical significance was determined at $P \leq 0.05$, while means were separated using the Fischer's least significant difference (LSD) test.

CHAPTER FOUR

4.0 RESULTS

4.1 Soil Characterization

After 31 years, all treatments showed a net decline of soil organic carbon, while N contents declined in all except manure (0.18%) and inorganic fertilizer (NPK) + manure (0.20%) (Table1). Crop residue (1.7%) had the least soil organic carbon and inorganic fertilizer (NPK) + manure (2.2%) had the highest, while control (0.15%), inorganic fertilizer (NPK) (0.15%) and crop residue (0.15%) had the least N content and inorganic fertilizer (NPK) + manure (0.20%) had the highest N contents.

At the onset of the experiment in 1976, soil pH was 4.9 (in water), nitrogen content was 0.18% and carbon content was 2.47% at the surface horizon (Siderius and Muchena, 1977).

Soil pH increased across all the treatments above the control with 5.07 and inorganic fertilizer (NPK) + manure with pH 5.45 (Table 1). Soil pH increased by 6.7% and 7.5% in plots treated with manure and inorganic fertilizers (NPK) + manure respectively.

Table 1: Soil characterization for Kabete long–term experiment 31 years since establishment

Treatment	%N	%C	pH (1:2.5 soil:H_2O)
Control	0.15	1.8	5.07
Fertilizer (NPK)	0.15	1.8	5.09
Manure	0.18	1.9	5.41
Residue	0.15	1.7	5.16
Fertilizer (NPK) + Manure	0.20	2.2	5.45
Fertilizer (NPK) + Residue	0.17	1.9	5.23

4.2 Effects of selected soil fertility management practices on soil aggregates

The amount of large macroaggregates (> 2 mm) was significantly higher in the inorganic fertilizer plus manure (F + M) treatment than in all the other treatments at 0-15cm depth ($p = 0.01$). However, at 15-30 cm depth a reverse trend occurred where large macroaggregates were significantly higher in control than all other treatments ($p = 0.01$) (Table 2). At 0-15 cm, small macroaggregates (250 µ - 2 mm) were significantly higher in inorganic fertilizer (NPK) plus manure (F + M) and manure than the other treatments ($p < 0.001$). At 15-30 cm, small macroaggregates were significantly higher in residue (66.3%), manure (63.7%) and inorganic fertilizer (NPK) plus manure (63.1%), and lowest in inorganic fertilizer (NPK) plus residue (44.9%) ($p<0.001$) (Table 2). With respect to depth, small macroaggregates were highly significant ($p=0.002$) in 15-30 cm than 0-15 cm.

Table 2: Effect of soil fertility management practices on soil aggregate fractions

Treatment	Depth (cm)	LM	SM	Mi	S + C	MWD
Control	0-15	0.587^{b}	33.6^{b}	51.19^{a}	14.61^{a}	0.38^{b}
Fertilizer	0-15	0.740^{b}	29.9^{b}	55.86^{a}	13.47^{ab}	0.36^{b}
Manure	0-15	0.700^{b}	37.5^{a}	51.46^{a}	10.30^{b}	0.42^{a}
Residue	0-15	0.473^{b}	36.6^{b}	51.11^{a}	11.84^{b}	0.40^{b}
F + M	0-15	1.197^{a}	43.8^{a}	45.13^{b}	9.83^{b}	0.48^{a}
F + R	0-15	0.480^{b}	34.8^{b}	53.41^{a}	11.32^{b}	0.39^{b}
Control	15-30	1.200^{a}	55.7^{b}	35.45^{b}	7.63^{a}	0.58^{a}
Fertilizer	15-30	0.723^{b}	51.5^{b}	41.12^{a}	6.66^{ab}	0.53^{b}
Manure	15-30	0.413^{b}	63.7^{a}	30.86^{b}	5.04^{b}	0.61^{a}
Residue	15-30	0.497^{b}	66.3^{a}	28.11^{c}	5.06^{b}	0.64^{a}
F + M	15-30	0.960^{ab}	63.1^{a}	30.56^{b}	5.35^{b}	0.63^{a}
F + R	15-30	0.570^{b}	44.9^{c}	45.84^{a}	8.68^{a}	0.47^{b}
SED (Tr.)		0.16	3.47	2.65	1.04	0.03
SED (Depth)		0.09	2.00	1.53	0.60	0.02
SED (Inter.)		0.23	4.91	3.75	1.47	0.04
F-test (Tr.)		0.01	<0.001	<0.001	0.01	0.003
Depth		0.74	0.002	<0.001	<0.001	<0.001
Tr.*Depth		0.11	0.14	0.11	0.28	0.264

Where: LM = Large macroaggregate; SM = Small macroaggregate; Mi = Microaggregate; S+C = Silt and Clay and MWD = Mean Weight Diameter; Tr. = Treatment; Inter = Interaction. Means followed by the same letters within columns are not statistically different at p (<0.05).

At 15-30 cm microaggregates were significantly higher in NPK + residue (45.84%) and NPK (41.12%) than all other treatments ($p < 0.001$) (Table 2).

The quantities of silt and clay at 0-15 cm were significantly higher in control plots than all the other treatments ($p = 0.01$). At 15-30 cm silt + clay contents were significantly higher in inorganic fertilizer (NPK) + residue and control compared to the other treatments ($p=0.01$). There was significant decline in silt + clay contents with depth ($p < 0.001$) where 0-15 cm had more silt and clay compared to 15-30 cm.

Mean weight diameter (MWD) of soil aggregates at 0-15 cm was significantly higher in inorganic fertilizer (NPK) + manure (0.48) and manure (0.42) compared to the other treatments. At the depth 15-30 cm, MWD in the plots treated with crop residue (0.64), inorganic fertilizer (NPK) + manure (0.63), manure (0.61) and control (0.58) was higher compared to inorganic fertilizer (NPK) + residue (0.47) and inorganic fertilizer (0.53). MWD increased significantly with depth, where 15-30 cm had higher MWD compared to 0-15 cm ($p < 0.001$).

4.3 Effects of selected soil fertility management practices on carbon and nitrogen contents

Soil organic carbon in small macroaggregates was significantly higher at both 0-15 cm and 15-30 cm depths in plots treated with inorganic fertilizer (NPK) + manure ($p = 0.005$) compared to all other treatments (Table 3). It decreased significantly ($p < 0.001$) with depth, 0-15 cm had more soil organic carbon compared to 15-30 cm.

Table 3: Organic Carbon contents in aggregate size fractions as affected by soil fertility management practices

Treatment	Depth (cm)	LM	SM	Mi	S+C	cPOM	iPOM
Control	0-15	31.90^{a}	17.59b^{c}	17.47^{b}	20.56^{ab}	10.99^{a}	26.43^{c}
Fertilizer	0-15	12.70^{b}	17.15^{c}	16.93^{b}	19.18^{b}	12.65^{a}	23.27^{cd}
Manure	0-15	19.00^{b}	19.96^{b}	19.01^{b}	21.37^{ab}	14.80^{a}	43.30^{a}
Residue	0-15	12.00^{b}	17.06^{c}	16.94^{b}	19.63^{b}	15.73^{a}	20.08^{d}
F+ M	0-15	22.90^{ab}	22.03^{a}	21.50^{a}	23.17^{a}	13.44^{a}	38.80^{ab}
F + R	0-15	44.90^{a}	19.30^{b}	19.37^{ab}	23.40^{a}	12.34^{a}	33.16^{b}
Control	15-30	16.00^{ab}	15.78^{ab}	15.00^{b}	19.84^{ab}	16.26^{a}	9.57^{a}
Fertilizer	15-30	12.90^{b}	14.84^{b}	14.66^{b}	16.54^{b}	7.73^{b}	9.79^{a}
Manure	15-30	18.20^{ab}	15.38^{b}	15.02^{b}	19.42^{ab}	12.95^{a}	13.44^{a}
Residue	15-30	15.00^{ab}	14.89^{b}	14.90^{b}	17.59^{ab}	13.48^{a}	9.85^{a}
F+ M	15-30	20.20^{ab}	17.41^{a}	16.84^{a}	20.15^{a}	10.48^{a}	13.59^{a}
F + R	15-30	36.50^{a}	15.13^{b}	15.04^{b}	13.95^{b}	10.40^{a}	12.27^{a}
SED (Tr.)		11.28	0.93	1.07	1.62	3.27	2.93
SED (depth)		6.52	0.54	0.62	0.94	1.89	1.70
SED (Inter.)		15.96	1.31	1.51	2.30	4.62	4.18
F-test (Tr.)		0.19	0.005	0.04	0.22	0.75	<0.001
Depth		0.54	<0.001	<0.001	0.002	0.45	<0.001
Tr.*Depth		0.96	0.44	0.71	0.15	0.73	0.028

Where: LM = Large macroaggregate; SM = Small macroaggregate; Mi = Microaggregate; S+C = Silt and Clay; cPOM = Coarse particulate Organic matter; iPOM = Intra-aggregate particulate organic matter; F + M = Inorganic fertilizer + manure (NPK); F + R = Inorganic fertilizer (NPK) + residue; Tr. = Treatment; Inter = Interaction. Means followed by the same lower case letters within columns are not statistically different at p (< 0.05).

At 0-15 cm, nitrogen contents in small macroaggregates was highly significant in plots treated with inorganic fertilizer (NPK) + manure ($p < 0.001$) compared to all the other treatments while at 15-30cm, plots treated with inorganic fertilizer (NPK) + manure, manure and residue were significantly higher compared to other treatments ($p < 0.001$). It was also significantly higher in 0-15 cm than 15-30 cm ($p < 0.001$). SOC in microaggregates was significantly higher at both 0-15cm and 15-30cm in plots treated with inorganic fertilizer (NPK) + manure ($p = 0.04$) compared to the other treatments and depth had a significant effect ($p < 0.001$) in SOC distribution in the soil profile. Plots treated with manure and residue had more N content in microaggregates at 0-15 cm compared to fertilizer (NPK) + manure ($p = 0.008$), while at 15-30 cm, N in microaggregates was significantly higher in inorganic fertilizer (NPK) + manure compared to control ($p = 0.008$). Soil organic carbon content in silt and clay decreased with increasing depth while nitrogen contents were observed to be high in plots that received inorganic fertilizer (NPK) + manure. Nitrogen contents were highest in silt and clay compared to all other aggregate fractions (Table 4). The particulate organic carbon (POC) in intra-aggregate particulate organic matter (iPOM) was highest in surface layers in plots treated with manure and was highly significant between treatments ($p < 0.001$).

The nitrogen content in iPOM in surface soil was significant ($p < 0.001$) in plots treated with manure (3.33%) and inorganic fertilizer (NPK) + manure (3.03%) and was also affected by soil depth where a decline was observed as the profile depth increased.

Table 4: Nitrogen content in aggregate size fractions as affected by soil fertility management practices

Treatment	Depth (cm)	LM	SM	Mi	S+C	cPOM	iPOM
Control	0-15	1.097^{b}	1.361^{c}	1.45^{a}	1.80^{b}	0.76^{b}	1.89^{c}
Fertilizer	0-15	1.160^{b}	1.342^{c}	1.42^{ab}	1.73^{b}	0.77^{b}	1.68^{cd}
Manure	0-15	1.613^{ab}	1.642^{b}	1.58^{a}	1.84^{b}	1.19^{a}	3.33^{a}
Residue	0-15	0.999^{b}	1.393^{c}	1.45^{a}	1.71^{b}	0.83^{b}	1.31^{d}
F + M	0-15	1.910^{a}	1.779^{a}	1.43^{ab}	2.08^{a}	1.01^{a}	3.03^{a}
F + R	0-15	1.472^{ab}	1.477^{c}	1.29^{b}	1.26^{c}	0.71^{b}	2.40^{b}
Control	15-30	1.224^{ab}	1.226^{b}	1.22^{b}	1.64^{b}	0.76^{a}	0.58^{a}
Fertilizer	15-30	0.998^{b}	1.211^{b}	1.21^{b}	1.56^{b}	0.74^{a}	0.63^{a}
Manure	15-30	1.307^{ab}	1.285^{a}	1.29^{ab}	1.61^{b}	0.84^{a}	0.83^{a}
Residue	15-30	1.214^{ab}	1.277^{a}	1.29^{ab}	1.64^{b}	0.85^{a}	0.64^{a}
F + M	15-30	1.491^{a}	1.386^{a}	1.43^{a}	1.87^{a}	0.68^{a}	1.02^{a}
F + R	15-30	1.468^{a}	1.210^{b}	1.29^{ab}	1.26^{c}	0.82^{a}	0.84^{a}
SED (Tr.)		0.23	0.062	0.078	0.11	0.14	0.23
SED (Depth)		0.13	0.036	0.445	0.061	0.084	0.13
SED (Inter.)		0.32	0.087	0.11	0.15	0.20	0.32
F-test (Tr.)		0.07	<0.001	0.008	0.008	0.48	<0.001
Depth		0.49	<0.001	<0.001	0.001	0.27	<0.001
Tr.*Depth		0.71	0.12	0.78	0.42	0.50	0.008

Where: LM = Large macroaggregate; SM = Small macroaggregate; Mi = Microaggregate; S+C = Silt and Clay; cPOM = Coarse particulate Organic matter; iPOM = Intra-aggregate particulate organic matter; F + M = Inorganic fertilizer (NPK) + manure; F + R = Inorganic fertilizer (NPK) + residue; Tr. = Treatment; Inter = Interaction. Means followed by the same letters within columns are not statistically different at ($p < 0.05$).

4.4 Effects of soil fertility management on soil macrofauna

In the study site, six taxa of earthworms (Table 5), two from endogeic and four from epigeic functional groups were found. For termites, two taxa belonging to wood, litter and grass feeders and one belonging to soil, litter and dung feeders were found (Table 5).

Table 5: Earthworm and termite taxonomic richness and functional groups based on monolith methods

Earthworm taxa		Termite taxa	
Taxonomic group	Functional group	Taxonomic group	Food type
Ocnerodrilidae		*Termitiae-Macrotermitinae*	
Nematogenia lacuum	Endogeic	*Microtermes spp*	FWLG
Gordiodrilus wemanus	Endogeic	*Odontotermes spp*	FWLG
		Pseudacanthotermes spp	FLSD
Acanthodrilidae			
Dichogaster (Dt.) affinis	Epigeic		
Dichogaster (Dt.) bolaui	Epigeic		
Euridrilidae			
Polytoreutus annulatus	Epigeic		
Stuhlamannia variabilis	Epigeic		

Where: F = fungus grower; W = wood; L = leaf litter; G = dry grass; S = soil; D = dung/manure

Earthworm and termite abundance as well as biomass were highly variable across the treatments. Earthworms density ranged from 0-485 individuals m^{-2} and biomass ranged from 0.00-5.58 g m^{-2} while termites density ranged from 0-256 individuals m^{-2} and biomass ranged from 0.00-1.07 g m^{-2}. Earthworm species (*Nematogenia lacuum),* was dominant and was sampled in all the treatments. The other five species *Gordiodrilus wemanus, Dichogaster (Dt) affinis, Dichogaster bolaui, Polytoreutus annulatus* and *Stuhlamannia variabilis* were only present in soils that received inorganic fertilizer + manure. For termites, *Microtermes* spp. was distributed across all the treatments and the rest were found mainly in control and inorganic fertilizer (NPK) + manure treated plots (Table 5).

The results in Table 6 showed that earthworm abundance and biomass were relatively higher at 0-15 cm than at 15-30 cm. At 0-15 cm, plots treated with inorganic fertilizer (0.753) and inorganic fertilizer (NPK) + manure (0.749) had significantly higher earthworm biomass (p = 0.042) compared to control. However, earthworm biomass was higher by 0.4% in plots that received inorganic fertilizer (NPK) alone compared to those that received F + M. Inorganic fertilizer (NPK) in F + M led to an increase in earthworm biomass by 36.2% compared to manure alone. Plots that received inorganic fertilizer (NPK) had significantly higher earthworm abundance compared to control (p = 0.009). However, there was an increase in earthworm abundance in plots that received inorganic fertilizer (NPK) compared to those that received inorganic fertilizer (NPK) + manure.

Termite biomasses were higher in control and residue plots as compared to the other treatments (p = 0.048). It was also observed that termite biomass was significantly higher with respect to depth, where 0-15 cm had higher termite biomass compared to 15-30 cm ($p = 0.022$). Termite abundances at 0-15 cm and 15-30 cm were higher in plots that received residue compared to control.

Table 6: Earthworm and termite abundance (individuals m^{-2}) and biomass (g m^{-2}) in different soil fertility management practices

Treatment	Depth (cm)	Termites		Earthworms	
		Abundance	Biomass	Abundance	Biomass
Control	0-15	1.48^a	0.271^a	1.97^{ab}	0.336^b
Fertilizer	0-15	0.77^b	0.040^b	2.59^a	0.753^a
Manure	0-15	1.43^a	0.033^b	1.09^b	0.387^b
Residue	0-15	2.39^a	0.234^a	1.23^b	0.157^b
F + M	0-15	1.50^a	0.083^b	2.27^{ab}	0.749^a
F + R	0-15	0.64^b	0.031^b	1.41^b	0.237^b
Control	15-30	1.41^a	0.047^a	1.24^a	0.250^a
Fertilizer	15-30	0.64^b	0.013^a	1.24^a	0.208^a
Manure	15-30	0.00^b	0.00^a	0.00^b	0.00^a
Residue	15-30	1.93^a	0.086^a	0.92^a	0.062^a
F + M	15-30	0.00^b	0.00^a	1.11^a	0.201^a
F + R	15-30	0.85^a	0.060^a	1.07^a	0.154^a
SED (Tr.)		0.620	0.0571	0.501	0.132
SED (Depth)		0.358	0.0329	0.289	0.760
SED (Inter.)		0.877	0.0807	0.709	0.186
F-test (Tr.)		0.142	0.048	0.124	0.042
Depth		0.131	0.022	0.009	<0.001
Tr.*Depth		0.635	0.300	0.853	0.244

Where: F + M = Inorganic fertilizer (NPK) + manure; F + R = Inorganic fertilizer (NPK) + residue; Tr. = Treatment; Inter = Interaction. Means followed by the same lower case letters within columns are not statistically different at $p < 0.05$.

4.4 Interactions between soil macrofauna, soil aggregate fractions and carbon and nitrogen contents

The quantity of large macroaggregates (r = 0.40), silt and clay (r = 0.38) were positively correlated with earthworm biomass (r = 0.40) and abundance (r = 0.44), while quantity of small macroaggregates were negatively correlated with earthworm biomass (r = –0.33) and abundance (r = –0.37) (Table 7).

Table 7: Pearson Correlation Coefficients between earthworms and termites biomass and abundance with soil aggregate fractions.

Variables	EN	EB	TN	TB
EB	**0.95***			
TN	-0.15	-0.07		
TB	-0.09	-0.11	**0.52***	
LM	0.25	**0.40***	0.01	-0.09
SM	**-0.37***	**-0.33**	**-0.34***	-0.30
Mi	0.33	0.29	**0.33***	0.28
SC	**0.44***	**0.38***	0.31	0.32

EN = Earthworm abundance, EB = Earthworm biomass, TN = Termite abundance, TB = Termite biomass, LM = Large macroaggregates, SM = Small macroaggregates; Mi = Microaggregates, SC= Silt and Clay. Values in bold * are significantly different with a significance level alpha $p < 0.05$.

Aggregate fractions were not correlated with termite biomass, but microaggregates (r = 0.33) were positively correlated, while small macroaggregate (r = -0.34) were negatively correlated with termite abundance.

Small macroaggregate carbon was strongly positively correlated with earthworm biomass (r = 0.47, p = 0.003).

Microaggregate carbon and microaggregate within macroaggregate carbon were correlated with earthworm biomass and abundance, but the correlation was strongly so with earthworm biomass than in earthworm abundance. Overall, there was a very strong positive correlation between TOC and earthworm abundance ($r = 0.41$, $p = 0.012$) and biomass ($r = 0.54$, $p = 0.001$) (Table 8).

Table 8: Correlation Coefficients between earthworms and termites biomass and abundance with carbon contents in soil aggregates

Variables	EN	EB	TN	TB
LM	-0.08	-0.07	-0.13	0.30
SM	0.32	**0.47***	0.25	0.18
Mi	**0.34***	**0.49***	0.28	0.11
SC	0.17	0.26	0.08	0.10
cPOM	-0.06	-0.01	0.07	0.01
iPOM	0.24	**0.38***	0.16	0.05
mM	**0.37***	**0.46***	0.07	0.11
scmM	0.00	0.03	0.20	0.29
TOC	**0.41***	**0.54***	0.29	0.15

(Where: EN = Earthworm abundance, EB = Earthworm biomass, TN = Termite abundance, TB = Termite biomass, TOC = Total Organic Carbon, LM = Large macroaggregates, SM = Small macroaggregates; Mi = Microaggregates; SC = Silt and Clay; cPOM = coarse particulate organic matter; iPOM = intra-aggregate particulate organic matter; mM = microaggregates within macroaggregates; scmM = silt and clay occluded in microaggregates within macroaggregates). (Values in bold * are significantly different with a significance level $p = 0.05$).

Table 9: Correlation Coefficients between earthworms and termites biomass and abundance with nitrogen contents in soil aggregates

Variables	EN	EB	TN	TB
LM	-0.03	0.09	-0.12	0.07
SM	0.23	**0.40***	0.27	0.19
Mi	0.28	**0.44***	0.33	0.17
SC	0.22	**0.34***	0.08	0.10
cPOM	0.02	0.15	-0.06	0.01
iPOM	0.29	**0.43***	0.14	0.01
mM	0.31	**0.44***	0.06	0.02
scmM	0.24	0.29	-0.04	0.00
TON	**0.36***	**0.52***	0.21	0.06

Where: EN = Earthworm abundance, EB = Earthworm biomass, TN = Termite abundance, TB = Termite biomass, LM = Large macroaggregates, SM = Small macroaggregates; Mi = Microaggregates; TON = Total Nitrogen; cPOM = coarse particulate organic matter; iPOM = intra-aggregate particulate organic matter; mM = microaggregates within macroaggregates; scmM = silt and clay occluded in microaggregates within macroaggregates). (Values in bold* are significantly different with a significance level (p = 0.05).

Strong positive correlation existed between total nitrogen (r = 0.52, p = 0.001), microaggregate-N (r = 0.44, p = 0.007), iPOM-N (r = 0.43, p = 0.009) and microaggregate within macroaggregate-N (r = 0.44, p = 0.007) with earthworm biomass. Positive correlations were also observed between small macroaggregate-N (r = 0.40, p = 0.015) and silt and clay-N (r = 0.34, p = 0.015) with earthworm biomass. Total N was positively correlated with earthworm abundance (r = 0.36, p = 0.03), but there was no correlation with all other soil aggregates-N (Table 9).

CHAPTER FIVE

5.0 DISCUSSIONS

5.1 Soil

An increase in pH was observed in the long term experiment at Kabete. The plots treated with manure and inorganic fertilizer (NPK) + manure, had higher N contents and this may account for why the soil pH increased. Other studies have reported a similar effect on soil pH after application of manure. Xu and Coventry (2003) pointed out that the major reasons for soil pH increase could be due to mineralization of N and a decrease in soil pH could be due to nitrification of mineralized N, while Whalen *et al.*, (2000) observed that higher soil pH in manure-amended soils may have been due to buffering from bicarbonates.

5.2 Aggregate Stability

Addition of inorganic fertilizer (NPK) + manure resulted in large and small macroaggregates (> 250 μm). Manure is composed of partially decomposed plant materials and in the presence of inorganic fertilizer there was an increase in available nitrogen in the soil, which was utilized by microorganisms during the decomposition of manure. When inorganic fertilizer (NPK) + manure is incorporated into the soil, organic matter is gradually decomposed to produce microbial biomass, metabolic products, humic substances and release of polysaccharides which were used as binding agents, at the same time, enhancing the growth of fungal mycelia into the soil pores thus binding the soil particles into aggregates (Bossuyt *et al.,* 2004b). Generally the C increase in the macroaggregates and in particular the small macroaggregates constituted up to >90% of the total macroaggregates in this study. Aoyama *et al.*, (1999) reported that manure application had positive effects on macroaggregates and that annual applications of inorganic fertilizer (NPK) + manure could maintain a high level of microbial activity, hence sustainable supply of binding agent could result into stable macroaggregates.

Manure is also a source of energy and nutrients for the soil microorganisms and plant roots that produce extra-cellular polysaccharides known to flocculate soil mineral particles into aggregates (Aoyama *et al.,* 1999). Aoyama *et al.*, (1991) observed that organic matter from cattle manure was largely composed of coarse particles in which carbohydrates of plant origin; fine and water-soluble fractions comprised only a small portion. Manure also contains polysaccharides, aliphatic and aromatic compounds that bind soil particles creating organo-mineral complexes important for flocculating aggregates < 0.2 μm as observed by Tisdall and Oades (1982).

The increase in macroaggregates with depth may be partly due to tillage method. In this study, hand hoe was used and may have resulted in macroaggregates being broken down into smaller sized aggregates in the upper soil layers. This may be attributed to the weak inter-particle bonds that exist between the microaggregates as compared to those within the microaggregates which are strong. These observations were in agreement with the observations by Tisdall (1994) and Tisdall and Oades (1982) who found that bonds within microaggregates were stronger than those between microaggregates and Wegner *et al.*, (2007) and Cambardella and Elliott (1994) who observed that macroaggregates are less stable than microaggregates and are thus susceptible to disruptive forces of tillage, impact of raindrop and wet/dry cycles.

Tisdall and Oades (1982) observed that microaggregates are very stable partly because they are small and also because they contain several types of binding agents whose effects are additive and that particles >250 μm diameter were less stable, and thus were easily broken down into microaggregates. Six *et al*, (1998 and 1999a) also observed that fine intra-aggregate particulate organic matter gradually become encrusted with clay particles and microbial products to form microaggregates within macroaggregates. Six *et al.,* (2000a) suggested that decomposition and fragmentation of coarse POM forms fine intra-aggregate particulate organic matter which were occluded in microaggregates within macroaggregate and that macroaggregate turn-over in conventional tillage (CT) was more than twice as large as that of no tillage (NT). Tillage might have increased the turn-over of macroaggregates in the tillage-depth layer 0-15 cm.

Low aggregation was observed in control plots because no organic materials were applied, hence silt and clay which formed vital part in aggregation was more. Also rainfall drop impact may have played a role in destabilizing and breaking the macroaggregates and microaggregates in 0-15 cm depth into microaggregates, silt and clay (Tisdall 1994). At 15-30 cm, the amount of silt and clay decreased significantly, whereas microaggregates and macroaggregates increased, and this may be due to favorable conditions (such as no disturbance due to tillage and weight from the upper soil layers) for formation of the macroaggregates (Six *et al.*, 2000a, 2004). The lower soil depth is below plough layer where mechanical disturbance due to conventional tillage is minimal. This in conjunction with weight induced compaction and presence of maize roots could have favored binding of silt and clay into macro- and microaggregates. Where manure and inorganic fertilizer (NPK) + manure were applied, silt and clay contents were low due to supply of soil organic matter by the manure and inorganic fertilizer + manure which increased binding agents such as polysaccharides, hence, led to increased microaggregates. The results from Six *et al.*, (1998 and 1999a) support these findings.

The surface layer (0-15 cm) was exposed to raindrop impact, wet/dry cycles, and tillage which influenced aggregate stability and so may have led to aggregate destabilization and disruption and these findings agree with those by Hillel, 1982; Wagner *et al.*, 2007 and Paustian *et al.*, 1997. These processes may have led to a decline in aggregation in the top 0-15 cm of the soil where MWD increased with depth. These findings agree with those by Oades (1984), while Celik *et al.*, (2004) found that in the surface layer (0-15 cm) highest MWD was observed in soils taken from soils that had received organic amendments. Increase in MWD with depth could be attributed to soil compaction forming stable aggregates. An increase in soil organic matter coupled with supply of N from inorganic fertilizer may have led to proliferation of microorganisms such as fungi and especially arbuscular mycorrhizal fungi (AMF) and results from Rillig *et al*., 2001 support these findings. SOM and N supplied from inorganic fertilizer (NPK) + manure are essential for increasing the activities of the fungi (especially AMF) and bacteria leading to increase in the hyphal network (Miller *et al*., 1995) and increase in the production of polysaccharides which act as soil particle binding agents. These findings agree with those by Blair *et al.* (2006) who confirmed that farmyard manure increased MWD.

5.3 Aggregate-associated Carbon and Nitrogen

There was a decline in soil organic carbon and nitrogen contents with an increase in depth. However, organic carbon and nitrogen were high in the plots where inorganic fertilizer (NPK) + manure (F + M) were added at both 0-15 cm and 15-30 cm layers. The incorporation of inorganic fertilizer (NPK) + manure at the rate of 120 kg N, 52.8 kg P and 10 t ha^{-1} manure into the soil may has resulted in high concentration of soil organic carbon and nitrogen compared to the other treatments. The increase in soil organic carbon and nitrogen contents in inorganic fertilizer (NPK) + manure treated plots was attributed to the organic matter from the manure and from the roots of the maize plants. This showed that application of inorganic fertilizer (NPK) + manure had a strong effect on soil organic carbon maintenance and accumulation in cropping systems. An interaction between manure and inorganic fertilizer in inorganic fertilizer (NPK) + manure had additive effects in the release of soil organic carbon and nitrogen. These findings agree with those by Shrestha *et al.*, (2007) and Hao *et al.*, (2003), while Su *et al.*, (2006) observed that application of farmyard manure (FYM) alone or with chemical fertilizers significantly increased SOC by 5.9 Mg ha^{-1} in a 23 year-period. Blair *et al.*, (2006) also observed similar trends where total N increased by 31% from addition of 30 t ha^{-1} of FYM. Gerzabek *et al.*, (1999), observed a 21% increase in total N where application of animal manures had been done for 37 years in comparison with unfertilized control treatment.

Decline of soil organic carbon and nitrogen contents with an increase in soil depth in the microaggregates fraction could be explained by the lack of deep incorporation of organic residues to lower layers through the manually operated hoe. When organic residues are incorporated into the soil, soil organic matter (SOM) accumulates in the formed microaggregates within macroaggregates thus increasing soil organic carbon and nitrogen contents. The microaggregates structure is such that SOM is protected from decomposition by microorganisms (Beare *et al.*, 1994a) and any disruption will breakdown macroaggregates (Six *et al.*, 2004; Brodowski *et al.*, 2006) into microaggregates thus exposing the protected SOM (Grandy and Robertson, 2007) to microbial decomposition. In their studies, McCarthy *et al.*, (2008) observed that organic matter preservation in many soils results from the architectural packing system emerging during microaggregates formation, which encloses colloidal-sized particles of organic matter with minerals creating pores that are filled with organic matter. These minerals serve as physical protective barriers of organic matter from degradation. Thus N and soil organic carbon are preserved in organic matter, on the other hand, microaggregates are very important in the regulation of SOM and this is because of their high stability and low turn-over rates (Six *et al.*, 2002 a, b).

Addition of inorganic fertilizer + manure, manure and residue and the litter from the maize plants may have contributed to the high soil organic carbon and nitrogen contents in 0-15 cm compared to 15-30 cm through an increase in SOM. Organic carbon normally bound to silt and clay fractions are bio-chemically protected and are not available for microbial decomposition (Sleutel *et al.*, 2006; Fonte *et al.*, 2007). In their studies, Mikutta *et al.*, (2006) showed that some plant compounds are transferred directly into the silt and clay fractions, primarily through breakdown, leaching, communition and selective microbial degradation which this study implied that they may have been transferred from silt and clay pools into microbial biomass, but physical protection by mineral surfaces and iron and aluminium hydroxides as well as chemical recalcitrance resulting from secondary reactions, limit this transfer. In this study, organic carbon and nitrogen was higher in the silt and clay fractions compared to all other fractions across the selected soil management practices. Inorganic fertilizer (NPK) + manure and inorganic fertilizer + residue had the highest organic carbon and nitrogen in silt and clay fractions which might have been contributed by the biochemical, chemical and physical protection. In their studies, Sleutel *et al.*, (2006) observed increases in silt and clay size fraction which is associated with organic carbon, coming from farmyard manure, while Six *et al.*, (2000a) also made a similar observation where they associated it to non-acid-hydrolyzable carbon that was found in the silt fraction. Kavoo (2008) also observed similar trends in her study which comprised of organic resources of differing qualities: *Tithonia diversifolia* (high quality), *Calliandra calthyrsus* (medium quality), *Zea mays* stover (medium quality), *Grevillea robusta* sawdust (low quality) and farmyard manure applied at rate of 4 tons C ha^{-1} with or without 120 kg N ha^{-1} mineral fertilizer. She observed that C and N in silt and clay increased across all organic resource treatments compared to the other aggregate fractions due to C and N protection and accumulation in the lower SOM pools. She attributed the high N concentration within silt and clay fraction to be also as a result of N mineralization or due to presence of leached phenolics which bind mineralized N compounds.

Other studies have reported relationships between stabilization of organic C and N in soils and clay or silt plus clay content (Feller and Beare, 1997; Hassink, 1997). Presence of partially decomposed organic materials in the manure and the N from fertilizer enhance proliferation of microorganisms. Puget *et al.*, (1999) reported an enrichment of microbial derived carbohydrates in the silt plus clay fraction compared to the sand fraction of no-tilled and conventional tilled soil. However, Guggenberger *et al.*, (1999) reported an increase in glucosamine than muramic acid under no-tillage at sites with high silt and clay content. Six *et al.*, (2000c) explained that the difference that exists between C and N distribution across different soils was probably due to difference in stability of silt-sized particles (both the 2-20 μm and 20-53 μm sized classes). They further found that a more stable soil contains more C and N in the coarse-silt-sized than in the fine-sized (< 2 μm) particles and their observation was that amounts of C and N increased with particle size (< 2 μm < 2-20 μm < 20-53 μm) suggesting that an increase in concentrations of iron and aluminium oxides would lead to the stability of larger organo-mineral particles.

Particulate organic carbon (POC) in intra-aggregate particulate organic matter (iPOM) decreased with soil depth, while manure contributed significantly ($p < 0.001$) to particulate organic carbon contents, which agrees with the studies by Aoyama *et al.,* (1999) who observed similar trends with application of manure. In the studies done by Sleutel *et al.*, (2006), they observed that cattle manure increased the proportion of protected pools of carbon in small macroaggregates.

In this study, manure had high particulate organic carbon while, inorganic fertilizer + manure and manure had high N contents. Upon incorporation of manure into the soil, the coarse and free particulate organic matter which is considered as active soil organic matter (Grandy and Robertson, 2007) was subjected to decomposition by microorganisms, breaking them down into intra-aggregate particulate organic matter. Intra-aggregate particulate organic matter on the other hand is subjected to further decomposition and fragmented into smaller particles, which become encrusted with mineral and microbial products, leading to formation of microaggregates within macroaggregates and increased physical protection of intra-aggregate particulate organic matter C and nitrogen. As the binding agents generated by decomposition of young particulate organic matter are lost, C and N in particulate organic matter and in stabilized microaggregates is released (Six *et al.*, 2000b). These microaggregates can subsequently be re-incorporated into new aggregates and cycle through the same processes (Six *et al.*, 1999a). During decomposition, microorganisms' biomass builds up and when microorganisms die, the locked-up carbon and nitrogen are then released into the soil.

5.4 Soil Macrofauna

Earthworm biomass decreased with increasing depth, which was expected since substrates and oxygen declined with depth. The fertilizer treated plots had the highest earthworm biomass and this may have been caused by high root biomass. Campbell and Zentner (1993) and Gregorich *et al.*,(1996) found that long term application of mineral fertilizer resulted in increased soil C content due to an increase in plant productivity and residue input. Aoyama *et al.*, (1999) observed an increase in OM content resulting from application of manure and from plant biomass. An increase in organic matter from root biomass may have provided food substrates for the endogeic earthworms which feed on soil organic matter and dead roots. Endogeic, which forage below the soil surface and epigeic earthworms which live and feed at the soil surface were predominantly found within the depth 0-15cm, this may have also contributed to the high biomass at the depth 0-15cm.

Termite biomass decreased with an increase in depth due to decline in substrate. The termite groups which were found here were the fungus growers, wood and grass feeders (Group II feeders). These termites foraged for food hence the high biomass at 0-15 cm. Falling litter and plant residues were high in lignin and polyphenol contents, to help the termites breakdown lignin and the polyphenolic contents are the fungi which live in symbiotic relationship with the termites. According to Lavelle and Spain (2001), these fungi play a role in the nutrition of the grass harvesting and litter feeding termites by partly degrading some of the resistant materials in stored food.

5.4.1 Soil Macrofauna and Aggregate-associated Carbon and Nitrogen

Earthworms while foraging on the soil organic matter do ingest the smaller soil aggregates leading to formation of large macroaggregates. Blanchart *et al.*, (1992 and 1999), in their study observed that aggregates formed with earthworms influence were more stable than those from soils without earthworms activity.

Earthworm biomass were positively correlated with large macroaggregates and negatively correlated with small macroaggregates. Their casts were rich in N and thus provided substrates to the microorganisms which release polysaccharides that act as binding agents, (Mummey *et al.*, 2006).

The observed increase in large macroaggregates may have been due to the movements of the earthworms within the burrows whereby they create axial and radial pressure on the smaller aggregates leading to production of large macroaggregates (Bossuyt *et al.*, 2004a).

Large amount of soil which was moved by the termites may break into microaggregates explained by the significant correlation between termites' abundance and both microaggregates and small macroaggregates in Table 7 which agrees with the findings by Rouland *et al.*, (2003).

In this study, it was observed that earthworm biomass had positive correlation with large macroaggregates and microaggregates within macroaggregates C. They played a significant role in the increase of macroaggregates fraction as well as in the C and N incorporation into macroaggregates. This may be attributed to the endogeic and epigeic earthworms which feed on soil organic matter and litter respectively, thus fixed C and N in their casts, resulting to higher C and N in aggregate fractions. These results agree with Fonte *et al.*, (2007) and Bossuyt *et al.*, (2004) who observed the same trend and also showed that earthworms may help to stabilize SOM via slowing the turn-over of newly added C and that earthworms appeared to increase C in macroaggregates only, but, N increases occurred in all aggregate size fractions. It was observed that there was a positive correlation between earthworm biomass with both C and N. The higher contents of C and N observed in the inorganic fertilizer + manure served as food source and therefore contributed greatly to the greater earthworm biomass. Fonte *et al.*, (2009) also observed that earthworm biomass was positively correlated with total C and N in soil from field under tomato mulch.

There was no significant correlation between termites' biomass and abundances and C and N. This may be due to the fact termites were not soil feeders but litter feeders. This group of termites forages on litter which is taken to the nests far away from the source.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. The long-term use of combined inorganic fertilizer (NPK) with manure resulted in increased water stable aggregates such as large (>2000 µm) and small macroaggregates (250-2000 µm) as well as increased mean weight diameter (MWD) of soil aggregates at 0-15 cm and 15-30 cm depths.

2. Soils sampled from plots that received inorganic fertilizer (NPK) + manure recorded a significant increase in nitrogen in small macroaggregates, microaggregates, silt and clay, and iPOM compared to all other treatments.

3. Application of inorganic fertilizer (NPK) alone and in combination with manure increased earthworm biomass, which in turn influenced the amount of macroaggregates.

6.2 Recommendations

1. Long-term use of inorganic fertilizer (NPK) with manure (F + M) would enhance macroaggregates formation, increased carbon sequestration, nitrogen mineralization and earthworm biomass (macroflora). Hence, continuous application of inorganic fertilizer combined with manure would most likely to improve soil structure, hence soil water holding capacity and farmers need to

be encouraged to adopt integrated soil fertility management strategies in their cropping production systems..

2. Further research is recommended, where a wider selection of integrated soil fertility management practices and rates are used since not many farmers can access the high rate (120 kg N ha^{-1} of inorganic fertilizer and 10 t ha^{-1} of manure) used in this study.

3. Other commonly used organic materials apart from cattle manure need to be included in the study, as well as tillage practices such as conservation tillage. This would give a better picture of the best quantities of organic, inorganic inputs and combinations of the same that would be suitable to sustain soil aggregate stability but also affordable to majority of the small scale farmers.

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APPENDICES

Appendix 1: Kabete NARL Experimental field layout.

Site: Kabete Experimental layout

Entrance

Tarmac road →

N

	Column 1	Column 2	Column 3	Column 4
	REP I	REP II	REP III	REP IV
Row 1	P1 [$N_2P_2FYM_2R$]	P19 [N_1P_1]	P37 [$N_2P_2FYM_1R$]	P55 [$N_1P_1FYM_1R$]
Row 2	P2 [$N_1P_1FYM_1$]	P20 [N_2P_2]*	P38 [$N_2P_2FYM_2R$]	P56 [$N_1P_1FYM_2R$]
Row 3	P3 [FYM_1R]	P21 [$N_2P_2FYM_1$]	P39 [$N_1P_1FYM_1$]	P57 [$N_2P_2FYM_2$]
Row 4	P4 [$N_1P_1FYM_2$]	P22 [$N_1P_1FYM_2$]	P40 [N_2P_2R]*	P58 [$N_2P_2FYM_1$]
Row 5	P5 [FYM_2R]	P23 [CONTR-NIL]*	P41 [N_2P_2]*	P59 [FYM_2R]
Row 6	P6 [$N_2P_2FYM_1$]	P24 [CONTR-R]*	P42 [FYM_1]	P60 [$N_1P_1FYM_1$]
Row 7	P7 [N_2P_2R]*	P25 [$N_2P_2FYM_2R$]	P43 [CONTR-R]*	P61 [FYM_1R]
Row 8	P8 [N_2P_2]*	P26 [$N_2P_2FYM_1R$]	P44 [$N_1P_1FYM_1R$]	P62 [$N_1P_1FYM_2$]
Row 9	P9 [FYM_2]*	P27 [$N_1P_1FYM_1R$]	P45 [N_1P_1R]	P63 [CONTR-R]
Row 10	P10 [N_1P_1]	P28 [$N_1P_1FYM_1$]	P46 [$N_1P_1FYM_2$]	P64 [N_2P_2]
Row 11	P11 [CONTR-R]*	P29 [FYM_1R]	P47 [$N_2P_2FYM_2$]*	P65 [N_1P_1]
Row 12	P12 [N_1P_1R]	P30 [FYM_2R]	P48 [FYM_1R]	P66 [FYM_1]
Row 13	P13 [$N_2P_2FYM_1R$]	P31 [$N_1P_1FYM_2R$]	P49 [FYM_2R]	P67 [FYM_2]
Row 14	P14 [$N_1P_1FYM_2R$]	P32 [N_2P_2R]*	P50 [N_1P_1]	P68 [$N_2P_2FYM_2R$]
Row 15	P15 [$N_2P_2FYM_2$]*	P33 [FYM_2]*	P51 [CONTR-NIL]*	P69 [$N_2P_2FYM_1R$]
Row 16	P16 [$N_1P_1FYM_1R$]	P34 [N_1P_1R]	P52 [$N_2P_2FYM_1$]	P70 [N_2P_2R]
Row 17	P17 [FYM_1]	P35 [FYM_1]	P53 [FYM_2]*	P71 [N_1P_1R]
Row 18	P18 [CONTR-NIL]*	P36 [$N_2P_2FYM_2$]*	P54 [$N_1P_1FYM_2R$]	P72 [CONTR-NIL]

↕ 4.5 m

↔ 7 m

* Selected treatments

Key

N_2P_2= 120 kg N ha-1; 52.8 kg P ha-1

N_1P_1 = 60 kg N ha-1; 26.4 kg P ha-1

FYM_1 = 5 t ha-1

FYM_2 = 10 t ha-1

R= Maize Stover Returned