EFFECT OF LAND USE ON ECOSYSTEM METABOLISM AND DECAY RATE OF EUCALYPTUS SALIGNA AND NEUBOUTONIA MACROCALYX LEAVES IN STREAMS DRAINING UPPER MARA CATCHMENT, KENYA

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A Thesis Submitted to the Graduate School in Partial Fulfillment for the Requirements of the Award of Master of Science Degree in Limnology of Egerton University

EGERTON UNIVERSITY

APRIL 2018

DECLARATION AND RECOMMENDATION

Declaration

This	thesis	is	my	original	work	and	has	not	been	submitted	or	presented	for	examination	in
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DEDICATION

To my father, Andrew (the late) and mum, Hilda, brothers and sisters for their inspiration and tireless moral and financial support throughout the study period. To my dear son Edrian for enduring long period of my absence.

ACKNOWLEDGEMENT

First and foremost, I'm grateful to Almighty God for giving me the opportunity to pursue MSc. in Limnology. Second, I appreciate Egerton University for the great assistance accorded throughout the study. I also recognize Limnology and Wetland Management Program for providing me with facilities, equipment and materials that I used during this research. My sincere gratitude goes to my supervisors: Prof Nzula Kitaka and Prof Charles M'Erimba for their guidance, support and advice during formative stages of proposal writing and defense to the final thesis write-up. I also greatly appreciate the financial support from the MARA FLOWS Project without which the research would not have been done. Special thanks go to Dr. Frank Masese of Eldoret University for his advice and support during fieldwork and thesis writing. I greatly appreciate the support offered by fellow graduate students namely: Emanuel Amuok, Kennedy Ochieng, Risper Ondiek, Elvira Riungu and others not mentioned here, for the field and laboratory assistance.

ABSTRACT

Replacement of native vegetation by exotic species of higher economic value has a potential ecological impact on detritus-based stream ecosystems. Litter processing and metabolism in streams can be used as an indicator of the functional status of streams. This study was conducted in the upper Mara catchment with a sole objective of determining the effect of land use change on leaf litter decay and metabolism in six first order streams namely: Sambambwet, Mosoriot and Chepkosiom that drain forested land use and Masese, Tenwek and Kapsebet that drain agricultural land. Litterbag experiment was used to determine leaf litter breakdown rates in the streams. The study involved collecting, drying and weighing 6 grams of Eucalyptus saligna (exotic) and Neuboutonia macrocalyx leaves (native). A total of 288 litterbags of both plant species were prepared. The litterbags were randomly exposed in the six streams on 27th May 2013 and later retrieved at intervals of 0, 1,3,7,14,28 42, and 56 days, dried and weighed. Macroinvertebrates associated with the litterbags were identified up to the family level. Stream ecosystem metabolism was conducted using the solute-addition experiment in Chepkosiom and Tenwek streams. Decay rates of Eucalyptus leaves were not significantly different between streams draining forested land use (-k = 0.039±0.009, pooled data) and streams draining agricultural land (-k = 0.045 ± 0.009) (t = 0.404, df = 16, p ≥ 0.05). Decay rates for Neuboutonia leaves were however, significantly higher in streams draining agricultural land ($-k = 0.095\pm0.005$) than in streams draining forested land use ($k=0.062\pm0.01$) (t-2.89, df =143, p<0.05). Although macroinvertebrate composition did not differ significantly with land use, diversity was significantly higher in forested land use than agricultural one (t=4.527, df =18, p<0.05). Macroinvertebrate abundance significantly was higher in streams draining agricultural land (t=-3.244, df=18, p<0.05). Neuboutonia leachate enrichment had no effect on metabolism of both streams but Eucalyptus enrichment shifted Tenwek stream from heterotrophic to autotrophic inferring less secondary productivity. This study showed that agricultural land use and replacement of native riparian vegetation with exotic ones such as Eucalyptus has compromised leaf litter decay and metabolism in Upper Mara catchment streams.

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LIST OF ABBREVIATIONS AND ACRONYMS

AFDW Ash Free Dry Weight

APHA American Public Health Association

CPOM Coarse Particulate Organic Matter

DEM Digital Elevation Model

DO Dissolved Oxygen

DOM Dissolved Organic Matter

ER Ecosystem Respiration

ESARPO East and Southern Africa, Regional Programme Office

FPOM Fine Particulate Organic Matter

GLOWS Global Water for Sustainability

Global Information System

GPIS Global Positioning Information system

GPP Gross Primary Production

LVBC Lake Victoria Basin Commission

OM Organic Matter

SRTM Shuttle Rader Topography Mission

TDS Total Dissolved Solids

TSS Total Suspended Solids

WWF World Wildlife Fund

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Organic matter (OM) is labile material of either plant or animal origin and is capable of decaying (Ferreira, et al., 2016). It is grouped into Coarse Particulate Organic Matter (CPOM) >1 mm, Fine Particulate Organic Matter (FPOM) <1 mm and Dissolved Organic Matter (DOM) <0.45 µm. Decomposition is the change in state of organic matter involving loss of mass and changes in chemical composition. It involves a series of three steps that are strongly influenced by time (Ferreira, et al., 2016). The proceses include: leaching (removal of soluble components by water); comminution (physical reduction in particle size through fragmentation) and catabolism (enzymatic breakdown with the release of energy). The decomposition processes lead to the production of fine particulate organic matter and dissolved organic matter that is consumed by fungi, bacteria and macroinvertebrate collectors (Sia and Chauvet, 2017). Hence, stream macroinvertebrates are grouped into different functional feeding groups depending on their role in processing organic matter. Shredder and collector functional groups refer to macroinvertebrates that feed on CPOM and FPOM respectively. The breakdown rates of leaf litter in streams depend on the quality of the litter and composition of the decomposer community such as microbes (bacteria and fungi) and macroinvertebrates shredders. The shredders and collectors are therefore referred to as major primary consumers, providing the main link between the organic inputs and predators in stream ecosystems (Gessner, et al., 1999).

Ecosystem metabolism is a functional metric of ecosystem activity because it is an integrative measure of the processes controlling organic matter dynamics and nutrient cycling in streams (Bernot *et al.*, 2010). Community respiration is thought to mainly reflect microbial community (Bani, *et al.*, 2018) and can therefore provide an important measure of biotic decomposition of OM in the headwater streams such as those in upper Mara catchment. Microbial communities in streams play a role in some components of stream food webs (Hall and Meyer, 1998) and are responsible for important nutrient transformations (Holmes *et al.*, 1996). The role of microorganisms in organic matter decomposition is two-fold: they are primary consumers of organic matter and their presence enriches the palatability and nutrient quality of leaf litter for consumption by macroinvertebrates. Therefore, stream ecosystem metabolism measures the production and respiration of organic matter within a certain reach

of the system and can determine the relative contribution of allochthonous and autochthonous carbon sources of the food web.

In Kenya, the Mau forest has faced a great deforestation pressure due to increased human population. Gereta *et al.*, (2002) report decline in forest cover in Mau from 752 km² in 1973 to 650km² in 1985, 493 km² in 2000 and 390km² in 2009. The loss is attributed to harnessing fuel wood and charcoal, which are the main energy sources for the people in the Mara River Basin as well as for timber and agricultural expansion. Large population of livestock within the catchment has resulted to overgrazing, hence leading to soil erosion. The erosion has been worsened by the poor soil conservation measures within the catchment (Global Water for Sustainability Program- GLOWS, 2007).

Land use activities within the catchment and riparian zone such as agriculture and logging have a great influence on stream communities through changes in both water temperature and energy inputs (Statzner and Kohmann, 1995). Riparian vegetation especially in head water streams such as those in upper Mara influence energy input through supplying organic matter, reducing light availability and thermal energy to stream's primary producers through canopy shading (Triska et al., 1983). Further increase in primary production leads to increase in abundance of macroinvertebrate grazers that in turn have a positive impact on bacterial and nutrient turnover rates (Newbold et al., 1982). Replacement of primary riparian forest with secondary vegetation species lead to changes in the quality and quantity of litter input into the streams. These changes in the community structure affect the nutrient dynamics and the overall stream metabolism (Minaya et al., 2013). Other than land use, other factors that affect stream metabolism and leaf litter decay include climatic conditions, stream morphometry and hydrology. This study however concentrated on the effect of land use because it is the most prevalent in the entire Mara system. Studies done by (Silva-Junior, et al., 2014; O'Driscoll, et al., 2016) showed that catchment land use activities negatively affects both leaf litter decay and metabolism in streams. However, there is inadequate information on how land use and changes in riparian tree species affect the stream functioning especially with respect to leaf litter processing and stream metabolism in Kenyan streams.

1.2 Statement of the problem

Allochthonous organic matter input from the riparian vegetation has proved to be an important source of energy especially in forested streams. The major component of the litter input is leaves that undergo several changes during the decay to provide energy. Participation

of macroinvertebrates and microorganisms is key in litter decomposition. Different land use practices modify riparian vegetation altering the quantity, quality and leaf litter breakdown rates. The modifications also change the overall stream metabolism. These modifications affect the structure and functioning of stream macroinvertebrates due to the changes in the leaf type hence organic matter quality. The rate of leaf litter decomposition and ecosystem metabolism can be used as an indicator of stream ecosystem functioning. Leaf litter decomposition is greatly affected by the leaf species and land use activities in both riparian and catchment area. Parts of the upper Mara sub-catchment are under agriculture that has resulted in substantial modification of the riparian vegetation such as the replacement of native riparian tree species like Neuboutonia with exotic ones e.g. Eucalyptus. The changes of tree species within the catchment and riparian area may affect the in-stream leaf decay rates. However, little is known whether these changes have an impact on macroinvertebrates, leaf litter decay rates and stream metabolism. There is hence a need for a study to be done on the influence of land use on leaf litter decay and stream metabolism. Result from this study will aid in understanding of the impact of agricultural activities within the catchment on the leaf litter decay and metabolism.

1.3 Objectives

1.3.1 General objective

To assess the effect of agricultural activities and forest (land use) on leaf litter decomposition for native (*Neuboutonia macrocalyx*) and exotic (*Eucalyptus saligna*) tree species and how their leachates influences stream metabolism in upper Mara catchment streams.

1.3.2 Specific objectives

- 1. To determine the effect of land use on physico-chemical variables of Upper Mara catchment streams.
- 2. To determine the decomposition rate of *Eucalyptus saligna* and *Neuboutonia* macrocalyx leaves in streams draining agricultural and forested land use.
- 3. To determine abundance, composition and diversity of macroinvertebrates associated with litterbags of *Eucalyptus saligna* and *Neuboutonia macrocalyx* in streams draining agricultural and forested land use.

4. To establish the influence *Eucalyptus saligna* and *Neuboutonia macrocalyx* leaf leachate on ecosystem metabolism of streams draining agricultural and forested land use.

1.4 Hypotheses

- 1. Physico-chemical variables of Upper Mara catchment streams are not significantly different with land use.
- 2. Decay rates of *Eucalyptus saligna* and *Neuboutonia macrocalyx* leaves are not significantly different in streams draining either agricultural or forested land use.
- 3. Abundance, composition and diversity of macroinvertebrates associated with litterbags of *Eucalyptus saligna* and *Neuboutonia macrocalyx* in streams draining agricultural and forested land uses are not significantly different.
- Addition of Eucalyptus saligna and Neuboutonia macrocalyx leachates will not significantly affect ecosystem metabolism of streams draining agricultural or forested land use.

1.5 Justification

Land use changes influence the type and area of vegetation cover within riparian zone. This determines the quantity and quality of organic matter generation to the streams consequently affecting the overall aquatic food web. Increased deforestation in the Mau forest has led to deterioration in the quantity and quality of OM input in upper Mara streams. As a result, metabolism by microbial community that forms the base of the stream food web is likely to have been compromised. The native riparian tree species such as Neuboutonia is being replaced with exotic species such as *Eucalyptus*. The latter is the most preferred tree species in the Mara basin by the people due to its high economic value. The exotic (Eucalyptus) is covering almost 90% of the total tree cover within the riparian areas. Although forested streams remain semi-pristine, agricultural based streams have greatly been affected by the vegetation replacement. These will likely effect the functioning of the streams especially those with different decomposition rates. Input of Eucalyptus leaves in the stream may also affect the trophic status of the stream via altering the organic matter composition. This study will establish whether land use and replacement of the native Neuboutonia with exotic Eucalyptus plantation influence the functioning of the upper Mara catchment streams through leaf litter decay rate and stream metabolism. The results obtained from this study will

contribute to knowledge on the effect of land use on leaf litter processing and how this affects microbial production as an indicator of metabolism in the Upper Mara streams and entire ecosystem. This information will help in the decision making regarding management of the riparian zones and the entire catchment hence complying with the Sustainable Development Goal fifteen. This SDG promotes protection, restoration, and sustainable use of forests and other terrestrial ecosystems.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Effect of land use on allochthonous leaf litter inputs in streams

Land use activities such as clearing vegetation for agriculture or human settlement in the riparian zones lead to changes in the land cover. Changes in land cover modify the physical conditions of streams such as increased sedimentation rate, unstable banks (Heartsill-Scalley and Aide, 2003) and changes in litter inputs to the streams. Forest landscape contributes more litter biomass to the stream than herbaceous vegetation (Afonso *et al.*, 2000; Heartsill-Scalley and Aide, 2003) and hence changes in riparian forests cover greatly influence the timing, quantity and quality of allochthonous Organic Matter inputs in streams (Tuchman and King, 1993). Clearing of forests for agriculture leads to high soil erosion, sedimentation and bank instability (Allan *et al.*, 1997). Movement of sediments, especially during storms, can cause abrasion and physical breakdown of leaf material consequently promoting litter breakdown. However, sediments can also bury leaves resulting in anaerobic conditions, which in turn prevent fungal or macroinvertebrates colonization (Cummins *et al.*, 1980).

Low-order streams are widely viewed as ecosystems fuel through inputs of litter from that enter stream food webs via invertebrate riparian vegetation microorganisms. Litter may reach streams by vertical fall or lateral movement by sliding down the stream banks (Benfield, 1997). The timing of leaf litter inputs to streams differs significantly between temperate and tropical streams (Elosegi and Pozo 2005). North temperate vegetation mainly shed their leaves when day length and temperature decrease in autumn whereas timing of leaf loss in tropical riparian forests is more variable in response to the prevailing dry and wet conditions (Wantzen et al., 2008). However, Magana (2001) noted that litter fall in the tropics is continuous all year round with peaks during the dry season. Moreover, torrential rainstorms, typhoons, and cyclones, as well as human activities such as tree cutting and fruit harvesting, contribute large amounts of fresh green leaves and wood to streams (Wantzen et al., 2008). All of these factors collectively predict timing and magnitude of leaf fall in the tropics although considerably less pronounced than in most parts of the temperate region.

2.2 Leaf litter retention and breakdown in streams

When leaf litter enters a stream, it is subjected to various pathways influencing its retention within the stream (Mathooko *et al.*, 2001). Leaves and small twigs become entangled in various physical structures including rocks, tree roots naturally present within the stream channel (Golladay *et al.*, 1987). The retained leaves and small twigs provide food and habitat for macroinvertebrate shredders such as insects and crabs as well as microbes that colonize the leaves that aid their decomposition process.

Elosegi and Pozo (2005) defined decomposition (breakdown) as the change in state of organic matter involving loss of mass and changes in chemical composition. Decomposition of organic matter (leaf litter) in streams is under the control of a number of interacting processes and their joint effects are usually studied by measuring loss of detrital mass over time. The processes of decomposition are chemical leaching of soluble compounds, aerobic degradation microbial organisms (conditioning), physical abrasion physical by fragmentation by leaf-shredding macroinvertebrates (shredders) (Webster and Benfield, 1986).

Chemical leaching of soluble organic matter may occur during rainfall when senescing leaves are still attached to the tree. Leaching rates generally peak 24-48 h after abscission of the leaves into stream water however, some leaching continues for weeks (France et al., 1997). The leachates (e.g. sugars, amino acids and lipids) are generally energy-rich and easily absorbed by bacteria (Strauss and Lamberti, 2002). Once fallen leaves enter the stream, osmotic breakage of dead cell walls, penetration by fungal hyphae, and softening of the structural elements by microbial enzymes combined with feeding by invertebrate shredders enhance leaching (Wantzen et al., 2008). Evidence suggests that bacterial and fungal biomass increase on leaf litter during decomposition and conversion of CPOM to FPOM (Strauss and Lamberti, 2002). Fungi and bacteria growing on the leaf surface and inside the mesophyll produce enzymes that degrade structural polysaccharides, such as cellulose. The enzymes result in softening of leaf structure hence increasing the food value for shredders (Purahong, et al., 2016). There has been evidence that bacterial and fungal biomass increase during decomposition and conversion of CPOM to FPOM of a leaf litter (Purahong, et al., 2016). According to Gessner and Chauvet, (1994), in streams, fungal biomass and reproduction generally peaks one to two weeks after immersion of the leaves. This process is known as conditioning and it makes leaf litter more readily edible and assimilated by stream

invertebrates coupled with promoting better growth of bacteria (Gessner *et al.*, 1999). Macroinvertebrate shredders and large benthic omnivores (decapods, crabs, and fish) which consume the litter then easily colonize the conditioned leaves. The combined effect of shredders and physical abrasion by the water current reduce the leaf particles to FPOM. Fine particulate organic matter then becomes suspended and is carried downstream where microbes eventually break it down to inorganic nutrients hence contributing to nutrient availability within the stream ecosystem.

The duration of leaf breakdown from abscission to complete decomposition, varies widely, and provides streams with a constant supply of nutrient during the months without leaf litter inputs (Benfield, 1996). Studies conducted in the temperate zone have shown a stream-specific continuum of leaf processing ranging from slow (more than a year) to fast (a month) (Peterson and Cummins, 1974). In tropical streams, the decay rates differ according to the tree species. Studies done by Dobson *et al.*, (2003) in river Njoro showed that leaf breakdown in streams is controlled mainly by litter inputs (litter quality, quantity and timing), biotic and abiotic characteristics among streams (Webster and Benfield, 1986). The species composition of riparian forests determines the quality, quantity and temporal dynamics of leaf litter resources. For example, litter from different tree species decompose at significantly different rates in streams (Webster *et al.*, 1999) and supports different microbial and invertebrate assemblages (Gessner and Chauvet, 1994; Wallace *et al.*, 1997; Graca, 2001). Some species such as *Eucalyptus* whose leaves are less palatable due to their chemical composition and toughness (Canhato *et al.*, 2013). Such leaves are less preferred as food sources for shredders hence have lower decay rate.

Catchment characteristics such as vegetation cover and topography together with water quality such as nutrient levels affect leaf litter decomposition rates (Sponseller and Benfield, 2001). Decomposition experiments undertaken in River Stradomka, Southern Poland showed that upstream reaches have a greater capacity to break down litter than downstream reaches (Fleituch, 2001). This is because breakdown in high-velocity microhabitats, such as riffles dominating upstream is faster than downstream pools (Stout and Coburn, 1989). Leaf litter break down is also faster in streams that have hard waters, alkaline (Jenkins and Suberkropp, 1995), warm temperatures (Dangles and Guérold, 2001) or with high nutrient concentrations (Suberkropp and Chauvet, 1995). In addition, presence of high biomass of decomposers (microbes and macroinvertebrate shredders) increases the leaf breakdown rates.

Aquatic macroinvertebrates play a very critical role with regard to nutrient cycling in the stream since they are the primary processors of organic material. Many streams—greatly depend on allocthonous organic matter as their source of energy. According to Arimoro, *et al.*, (2012), stream macroinvertebrates can process approximately 73% of the riparian leaf litter that enter the stream from the riparian zones. Processing of the nutrient rich leaf litter in turn results to increased growth rates, biomass and survival of these organisms (Cummins, 2016). A part from the perfuming the important role of primary processors of live organic material, they also serve as detrivores. This implies that they aid in processing of the already decomposing organic matter on the streambed. Other macroinvertebrates also serve as predators on other small aquatic organisms. They also act as prey for other animals such as fish, amphibian, reptiles, mammals and birds (Dalu, *et al*, 2017). Macro vertebrates—have developed different feeding adaptations that allow them the effectively feed on different food sources. In general, macroinvertebrates are very important in the stream ecosystem because they aid in energy transfer from the lower to higher trophic levels.

2.3 Stream primary production and respiration

Ecosystem metabolism is an integrative measure of the processes controlling nutrient cycling and organic matter dynamics in streams. Stream ecosystem metabolism includes both gross primary production (GPP), which essentially represents photosynthesis by aquatic autotrophs, and community respiration (CR), which comprises organic matter breakdown by both autotrophs and heterotrophs. One way to determine whether headwater streams is autotrophic or heterotrophic is through analysis of the whole-stream ecosystem metabolism. Ecosystem metabolism (community respiration, primary productivity and net ecosystem production) is a functional metric of ecosystem activity since it integrates processes controlling nutrient cycling and organic matter dynamics in streams (Izagirre *et al.*, 2008; Williamson *et al.*, 2008). This is because DOM is both respired as well as created during photosynthesis.

Dissolved Organic Matter from CPOM processing plays an important metabolic role in streams and rivers by supplying energy and carbon to heterotrophic microbial community (Meyer *et al.*, 1988). The incorporation of DOM into the microbial food web affects the amount and rate at which DOM is supplied to higher trophic levels. Dissolved Organic Matter stimulates both pelagic and benthic microbes in running waters (Bott *et al.*, 1984). DOM is typically the largest and most available active pools of organic carbon on river systems and hence plays an important role in river carbon dynamics (Battin *et al.*, 2008).

Dissolved Organic Matter dynamics are affected by variety of factors that influence the entire stream metabolism.

Factors that control rates of stream metabolism also regulate other properties of the stream, including nutrient process rates such as mineralization and secondary production; for example, carbon cycling drives other nutrient cycles such as nitrates and provides a food-web base via autotrophic production and processing of allochthonous materials (Meyer *et al.*, 2007). Nevertheless, the key regulators of stream metabolism include light availability (Mulholland *et al.*, 2001), nutrient concentration (Grimm and Fisher, 1986), organic matter quantity and quality (Bani, *et al.*, 2018), and hydrology (Roberts *et al.*, 2007). These factors affect both primary productivity and community respirations hence both are good indicators of stream metabolism dynamics.

When primary production is slow or negligible, such as in detritus based community, CR provides a simple measure of heterotrophic activity and the rate of OM decomposition. Community respiration of forested streams is primarily supported by detrital input of OM from allochthonous sources (Baker, 1986). The respiration is thought to mainly reflect microbial community and hence providing an important measure of biotic decomposition (Bani, *et al.*, 2018). Fungi especially aquatic hyphomycetes are considered as main microbial decomposers of leaf litter in streams (Gessner, 1997). However, in their absence the decomposition still occurs but at a slower rate.

2.4 Factors affecting stream metabolism

Headwater streams are particularly influenced by riparian canopy that reduces water temperature and stream phytoplankton primary production due to shading. The riparian vegetation supplies energy in the form of vegetal matter of allochthonous origin (catchment derived) hence supporting the entire stream ecosystem (Vannote *et al.*, 1980). Streams found in unmodified catchments receive significantly higher leaf litter inputs and their litter retention capacity in accumulation zones can be twice that observed in streams in modified catchments (Bunn, 1995). Land use change alters the quantity and supply of stream energy sources from allochthonous to autochthonous (stream-derived).

Land use changes affect stream nutrient dynamics through its influence on nutrient cycling and alteration in the vegetation and soil organic matter. Clearing of the riparian vegetation by cutting or burning increases nutrient to soil directly through combusted organic matter or

indirectly by enhancing the rates of decomposition of OM and reduce plant nutrient uptake (Guggenberger et al., 1996). These enhanced inputs may increase the concentration and fluxes of nitrogen and phosphorus in streams draining disturbed catchments (Williams and Melack, 1997). Nitrogen mineralization and nitrification rates in pasture soils are higher than in forest soils (Neill et al., 1995), hence resulting in high nitrate concentrations in streams draining deforested catchments. Land-use activities besides conversion of forest vegetation to bare land also affects stream nutrient concentrations through runoff input. Wilcock (1986) documented that land use in a stream's catchment can alter the sources and relative importance of different types of organic matter in the stream channel.

Human activities such as deforestation and agriculture in headwater streams riparian zones have an influence on stream metabolism (Maloney et al., 2005). These affect how nutrients are retained or transformed from upper reaches to downstream (Grimm et al., 2005). Agriculture modifies factors controlling stream metabolism through alteration of flow regimes such as change in intensity or timing of flow (Grimm et al., 2005) and increased nutrient, sediment, and pollutant runoff from agricultural and urban sources. High levels of agriculture and deforestation within the catchment result to eutrophication. Eutrophication affects stream biota and ecosystem functioning directly, through increased nutrient concentrations, as well as indirectly through oxygen depletion. High nutrient concentrations in streams potentially stimulate the activity of heterotrophic microorganisms associated with submerged leaf litter and, hence, influence decomposition rates and the availability of detrital resources to invertebrates (Mulholland et al., 1985).

2.5 Status of deforestation in Mara River Basin

Forest encroachment and poor soil conservation measures in Mara basin has promoted soil erosion (Mati *et al.*, 2005) leading to high sediment loading in the river system. There are no current impoundments existing within the Mara system and yet the water levels continue dwindling. The low water levels have had adverse effects on the wetland downstream and the vast Maasai Mara-Serengeti ecosystems (LVBC and WWF-ESARPO, 2010). Study done by Gereta *et al.* (2002) showed that forest cover in this region decreased from 752 km² in 1973 to 650 km² in 1985, 493 km² in 2000. Higher forest cover of 790km² was recorded in 2009. With climatic variability, the Mara River's ability to sustain life downstream is at great peril, since it is the only perennial source of freshwater for wildlife during low flows (UNEP, 2009). The water users have complained of water shortages, poor water quality as well as

advance environmental degradation (UNEP, 2009). This seems to have directly resulted from the perpetual increase in water demands for commercial agriculture, livestock, fisheries, tourism, mining and other industries (Mango, 2010).

Typical tree species that are associated with Mau forest include *Neuboutonia macrocalyx*, *Pouteriaadolf-friedericii*, *Strombosia scheffleri*, *Polyscias kikuyuensis*, *Olea capensis*, *Prunus Africana*, *Albizia gummifera* and *Podocarpus latifoliusis* (Kinyanjui, *et al.*, 2014). The native species are continuously being replaced with exotic species that are thought by the local communities to be of 'higher economic value' (Mutugi and Kiiru 2015). *Eucalyptus saligna* is the most common exotic tree species that has replaced *Neuboutonia macrocalyx* especially along rivers in the upper Mara catchment. *Eucalyptus saligna*, which is commonly known as the Sydney Blue, belongs to family Myrtaceae. This tree is mainly distributed in fertile areas that have well drained soils similar to the upper Mara conditions. *Eucalyptus saligna* is a tall tree, which can grow to a height of between 30 to 60 meters tall. The bark is normally rough and dark brown at the base. The colour however changes to smooth blue grey and white back which extends for about 4 meters from the base as shown in plate 1.

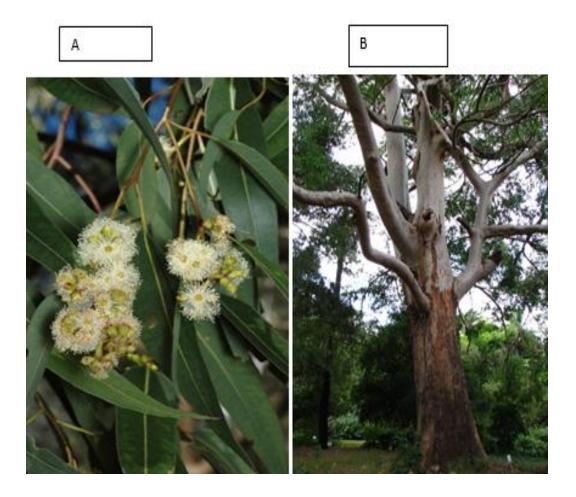


Plate 1: Eucalyptus saligna tree leaves (A) and tree (B)

Neuboutonia macrocalyx on the other hand belongs to family Euphorbiaceae is commonly referred to as croton. Neuboutonia macrocalyx is slender open crowned tree with thin soft and hairy leaves (Plate 2). The tree grows to an average height of between 10-15 meters high. It has straight grey brown stems. Neuboutonia macrocalyx is commonly found along forest edges, openings and regrowth areas. According to Mutugi and Kiiru (2015), Neuboutonia macrocalyx is a characteristic pioneer tree species in almost all the wetland upland forests such those found in the Mara system.

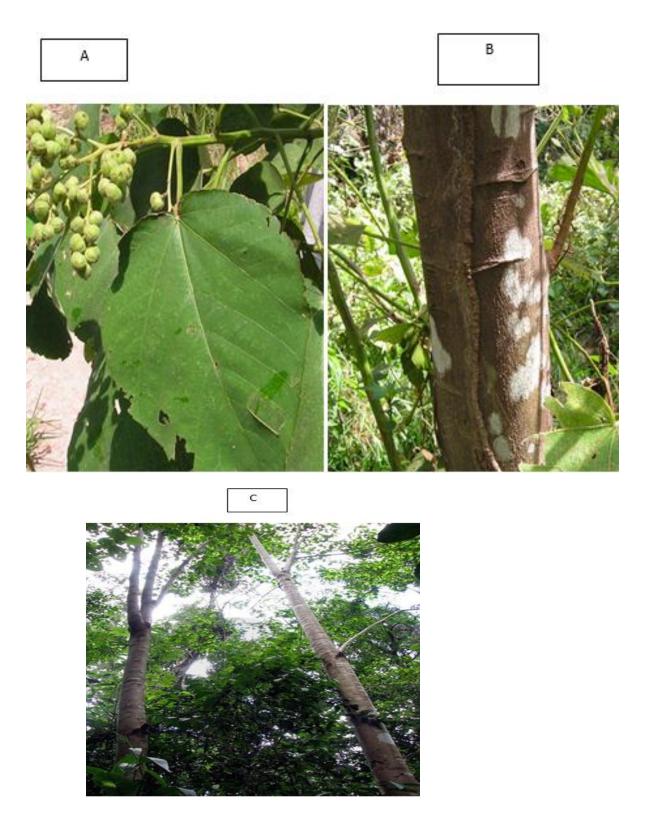


Plate 2: Wide leaves of $Neubotonia\ macrocalyx$ (A), the straight grey stem(B) and the full tree (C)

This replacement of indigenous plant species with exotic ones in the riparian zones most likely alters community structure through changes in stream metabolism. In the upper Mara catchment, the common indigenous riparian tree species such as *Neuboutonia macrocalyx* are

being replaced with exotic ones such as *Eucalyptus saligna*. This is because the later has short maturity period and produces good quality timber providing a high economic value to the Mara people. However, there is inadequate information on how changes in tree species due to land use changes affect organic matter processing and the overall stream ecosystem metabolism in Mara streams.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The Mara River is a transboundary river shared between Kenya and Tanzania with both countries depending on it as source of water for socio-economic activities. The river passes through the Maasai-Mara Game Reserve in Kenya and Serengeti National Park in Tanzania before finally draining into the second largest freshwater lake in the world; Lake Victoria. According to Omengo (2010), the Mara River flows for a length of 395km from a forested zone at an altitude of 2932 - 1134 meters above sea level. The main tributaries of Mara River are rivers Nyangores and Amala; both originating from Mau forest.

The Mara River Basin is located between latitudes 0°38'S and 1°52'S and longitudes 33°47'E and 35°47'E. The basin covers an area of approximately 13,325 km² of which 65% is located in Kenya and 35% in Tanzania. The river flows from the Mau Forest in Kenya, through different landscapes and drains into Lake Victoria at Musoma Bay in Tanzania. The basin is characterized by different types of land cover and land uses because of diverse human activities carried out by the stakeholders in various parts of the basin. Nyangores and Amala sub-basins form the upstream part of the Mara River basin and are located in Bomet County, Bomet central District (Mango, *et al.*, 2011). Mara River is therefore impacted by human activities such as deforestation and subsequent cultivation of land at the headwaters in the Mau Forest Complex. Consequently, loss of vegetation cover, soil erosion, decreased water infiltration capacity, decreased soil fertility, increased sedimentation and pollution of the river water has been reported in the upper catchment. The accelerated loss of vegetation cover in the upper catchment and land degradation, consequently pose a threat to river flows properties and the ecosystem as a whole (Mati, *et al.*, 2005).

This study was done in the upper reaches of the streams in Nyangores sub-catchment that are under the influence of different land uses (agricultural and forested) as indicated in Figure 1. Six first order streams were studied; three under forested land use namely: Chepkosiom, Mosoriot and Sambambwet while Masese, Tenwek and Kapsebet were under agricultural land.

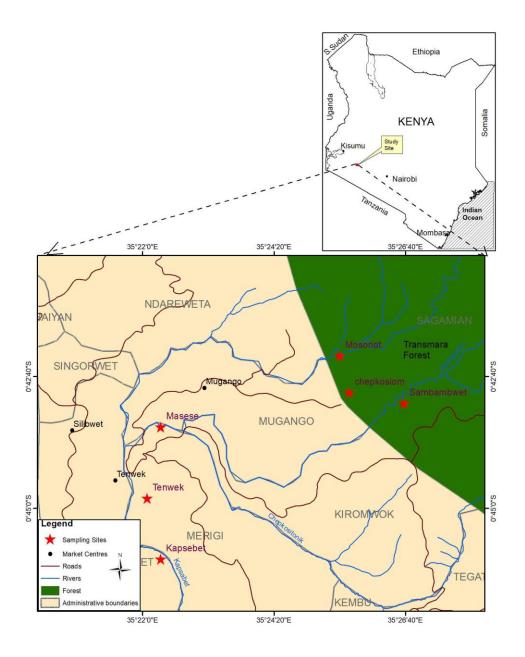


Figure 1: A map showing the samplings sites in Nyangores sub-catchment

Upper Mara Basin climate is greatly influenced by the inter-tropical convergence zone. The temperature of this area varies between 16 °C to 27°C depending on the time of the year. The highland areas have mean temperature of 18°C while the low land areas experience a mean temperature of 25° C (Mwangi, *et al.*, 2016). High rainfall of approximately 1600 mm/year is experiences in the forested uplands while the low lands experience mean rainfall of about 850 mm/year. Rainfall patterns varies both spatially and temporal (Ogutu, *et al.*, 2011). The rainfall seasons are bi-modal with the long rains being experience from mid-March to June while rainfall peak occurs in April. Short rains occur between Septembers to December.

3.2 Description of the study sites

a) Chepkosiom is located at latitude 0° 42' 50.7" S and longitude 035° 2' 26.6" E. It had canopy cover of 98% with overhanging indigenous vegetation and wooden debris as shown in Plate 3. The riparian vegetation comprised of different species of trees (*Neuboutonia sp*, *Dombeya goetzenii*, *Albizia sp*, *Vanguera sp* and sedges among others). Soft sediments, wooden debris, boulders, pebbles and gravel dominated the river bed. The station had an average width of 1.2 ± 0.06 m with mean depth of 0.05 ± 0.0 m. Both river banks were intact with no signs of human impacts. The Chepkosiom was located within a semi-pristine native forest surrounded by tea plantations.



Plate 3: Chepkosiom stream flowing in native forest with closed canopy

(b) Sambambwet is located at latitude 0° 43' 09.4" S and longitude 35° 26'39" E. The stream had canopy cover of 90% of overhanging vegetation and woody debris. Stream bed comprised of detritus, soft sediments, wooden material, boulders, pebbles and gravel. The riparian zones had different species of trees (*Neuboutonia sp* and *Dombeya goetzenii being the dominant tree species*). The station had an average width of 2.12 ± 0.1 m with a mean depth of 0.034 ± 0.02 m. The stream was located in semi- pristine native forest with river banks that have not been impacted by human activities as shown in plate 4.



Plate 4: Sambambwet streambed covered by dense mats of decomposing organic matter

(c) Mosoriot is located at latitude 00° 42′ 19.3″ S and longitude 035° 25′ 28.5″ E. The stream had canopy cover of 95% of overhanging vegetation and woody debris. Streambed comprised of detritus, soft sediments, wooden material, boulders, pebbles and gravel as shown in plate 5. The riparian zone had different native species such as *Croton sp* and *Dombeya goetzenii* among others.



Plate 5: Mosoriot streambed covered with leaves and woody debris

(d)Tenwek lies at latitude 00° 44' 48.9" S and longitude 035° 22' 04.3 "E. The stream had canopy cover of 60% with the riparian vegetation being dominated with *Eucalyptus* tree species. Other tree species such as Neuboutonia mixed with sedges also inhabited the riparian zone although they were few. The station had a mean width of 1.46 ± 0.02 m with an average depth of 0.28 ± 0.01 m. Crop farming was evident on both sides of the river banks, which included maize, onions and grass for fodder. Animal grazing and watering in the river coupled with domestic uses by neighboring communities were also evident. Both river banks were highly eroded due to animal trampling and clay harvesting. *Eucalyptus* logging was also recorded in the riparian zone. Plate 6a shows a point of clay harvesting while Plate 6 b shows point of water abstraction for domestic use.

A B



Plate 6: A point of clay harvesting in Tenwek stream (a) and water abstraction point (b)

(e) Kapsebet lies at latitude 00° 45' 53.7" S and longitude 035° 22' 20.1" E. The station had an average width of 2.78 ± 0.2 m and mean depth of 0.27 ± 0.01 m with a low canopy cover of 25%. Decomposing leaves, sand, mud and small pebbles dominated its bottom. The riparian zone was dominated by *Eucalyptus* tree species on the left bank while the right bank was an open grazing field. A wetland, dominated by sedges, was also observed on the right bank of the stream. The most dominant human activities within Kapsebet stream included: livestock grazing, crop farming, washing of clothes in the stream and riparian settlement (about 10m from the stream edge). Due to intense human activities along the entire stream stretch, both river banks were greatly eroded as shown in plate 7.



Plate 7: Eroded river banks and turbid waters in Kapsebet stream

(f) Masese station (Plate 8), lies at latitude 0° 43′ 32.4″S and longitude 035° 22′20.1″ E. Masese was the smallest with mean width of 0.98 ± 0.04 m and an average depth of 0.27 ± 0.01 m. It had canopy cover of about 60% with the riparian vegetation on the left bank comprising of both *Eucalyptus* and *Neuboutonia* species while the right bank had newly planted *Eucalyptus* plantation. The left river bank was characterized by intact riparian vegetation while the right bank was highly eroded as indicated in Plate 8A. Crop farming was evident on both sides of the stream as indicated in Plate 8B. Farmed crops included maize, potatoes and beans. The water was very turbid due to the intense farming activities together with the newly planted *Eucalyptus* trees. There was human settlement about 10m away from the stream on the left bank while the right bank had tea plantation immediately after the *Eucalyptus* plantation.

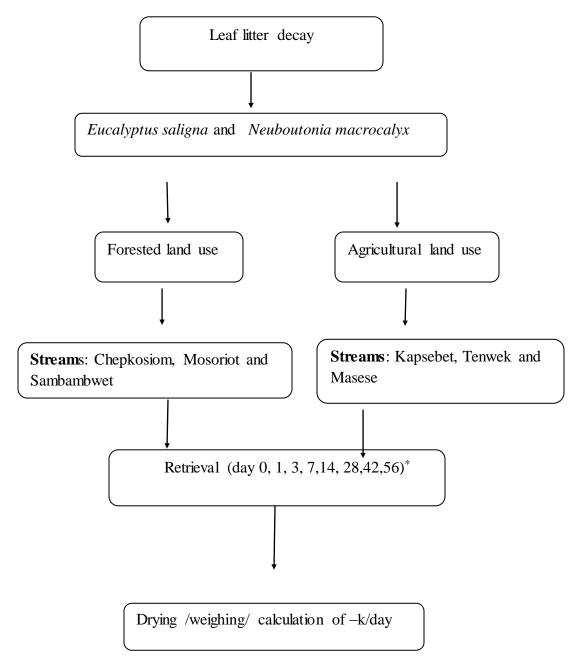


Plate 8: The tea plantation (A) and turbid waters due to the freshly planted tea plantation on the Left bank (B)

3.3 Study design

This study was conducted during the onset of rain for a period of two months during onset of rainfall period. The experiment was run from 27th May to 24th August 2013. It involved conduction of field litterbag experiment to assess the decay rates of *Eucalyptus saligna* and *Neuboutonia macrocalyx* leaves six first order streams flowing in agricultural and forested land use (Figure 2). Three streams (Chepkosiom, Sambambwet and Mosoriot) were flowing in forested land while three (Masese, Tenwek and Kapsebet) were flowing in agricultural land. The litterbags were retrieved from the field and later analysed in the Egerton university laboratory to obtain the decay arte (-k/day). Leachate addition experiment was also carried in

the field to ascertain the impact of *Neuboutonia macrocalyx* and *Eucalyptus saligna* on stream metabolism of Chepkosiom and Tenwek streams. Both experiments aimed at investigating the impact of land use on leaf litter decay rates and stream metabolism.



*Litter bags were retrieved in triplicates for both tree species.

Figure 2: Conceptual framework of the leaf litter decay experiment

3.3.1 Land use analysis of Nyangores catchment

The land use in Nyangores catchment was categorized using a combined digital elevation model, remote sensing images (Landsat 5 Thematic Mapper data of 2008, 30 m resolution) and topographical map (1: 50,000 survey of Kenya 1971) (Minaya *et al.*, 2013). With the coordinates of the sampling points from the GPS, points in the stream network of Mara River were located with Arc GIS software. Based on the Digital Elevation Models (DEM) of Kenya of 90m by 90m resolution for the study area obtained from the Shuttle Radar Topography Mission (SRTM), the sub-catchments were delineated from the upper part of Mara River together with the land use layer. This was followed by calculation of the percentage forested and agricultural land area. The percentage of agricultural and forested land use area was calculated according to Azuma, *et al.*, (2002). Streams were selected in both agricultural and forested land use types. The land use types were defined as: (a) forest sites (FOR, n = 3) draining catchments with >60% forest and (b) agriculture sites (AGR, n = 3) draining catchments with >60% agriculture. The stations were selected based on accessibility, stream size and physical habitat conditions.

3.3.2 Determination of physico-chemical variables and water samples collection

Water physico-chemical parameters, including: pH, temperature, conductivity and dissolved oxygen were measured *in-situ* with appropriate electronic probes during each sampling session. The measurements were done during the entire study period that started on 27th May to 24th July 2013. HACH HQ 40d was used to determine dissolved oxygen concentration and temperature while HACH ECO 40 was used to determine pH, conductivity. Triplicate readings were taken during each sampling time.

Water samples were collected in triplicates for nutrient analysis using 500ml acid washed plastic bottles from the 50m stretch in all the streams during each leaf litterbag retrieval day. The samples were collected before retrieving the leaf litter to avoid the influence of water disturbance during litterbag deployment and retrieval. In the field, the water samples were immediately preserved with 1ml of concentrated sulphuric acid. The samples were then transported to water quality laboratory at Egerton University for analysis immediately on arrival.

3.3.3 Determination of nutrient concentrations in the water samples

Different forms of nitrogen were determined; Ammonium-Nitrogen (NH₄-N), Nitrate-Nitrogen (NO₃-N) and Nitrite-Nitrogen (NO₂-N) using standard methods as described in

APHA (2005). The NH₄-N was determined by sodium salicylate method, where 2.5ml of sodium-salicylate solution and 2.5 ml of hypochloride solution were added to 25ml of filtered water samples from the study streams. The samples were incubated in the dark for 90 minutes after which the absorbance was read at a wavelength of 665nm using a GENESYS 10uv scanning spectrophotometer. Nitrate-Nitrogen was determined using sodium-salicylate method, where 1ml of freshly prepared sodium salicylate solution was added to 20ml of filtered water sample. The processed samples were then placed in the oven and evaporated to complete dryness at 95°C. The resulting residue was dissolved using 1ml H₂SO₄, followed by addition of 40ml of distilled water and 7 ml potassium-sodium hydroxide-tartrate solution respectively. The absorbance was read at a wavelength of 420 nm. Nitrite-Nitrogen was analyzed through the reaction between sulfanilamide and N-Naphthyl-(1) ethylendiamindihydrochloride and absorbance read at a wavelength of 543nm. The final concentrations of NH₄-N, NO₃-N and NO₂-N were calculated from their respective equations generated from standard calibration curves (APHA, 2005).

Soluble Reactive Phosphorus (SRP) was analyzed using the ascorbic acid method (APHA, 2005). The prepared reagents of ammonium molybdate solution (A), sulphuric acid (B), ascorbic acid (C) and potassium antimonyltartrate solution (D) were mixed in a ratio of A: B:C: D= 2:5:2:1 (ml). The resulting mixed solution were added to the filtered water sample at a ratio of 1:10 and the absorbance read at 885nm wavelength using a GENESYS 10uv scanning spectrophotometer after 15 minutes of reaction and concentration determined from known concentrations of standard solutions (APHA 2005). Total phosphorus (TP) was determined through persulphate digestion of unfiltered water to reduce the forms of phosphorus present into SRP. After the digestion, evaporated water was replaced and TP analyzed as SRP using ascorbic acid method. The concentration of TP was determined from a similar processing of known concentrations of phosphorus standard solutions (APHA 2005).

3.3.4 Determination of Total Suspended Solids in the water samples

Total Suspended Solids (TSS) was estimated gravimetrically by filtering a known volume of water samples through pre-weighed Whatman GF/C filters of pore size 0.45 µm. The filter papers were then dried to a constant weight at 95°C for 3 hours. The TSS weight were calculated using modified APHA (2005) formula as shown in equation 1.

Where TSS = Total suspended solids (mg L⁻¹), Wf = Weight of dried filter paper in grams, Wc = Constant weight of filter paper + residue in grams and V=Volume of water filtered (ml)

3.3.5 Determination of Discharge

The water discharge of the study streams was determined using Velocity-Area method. A portable automatic flow meter (Flo-Mate, model 2000, Marsh McBirney) was used to measure the mean water velocity at 60% water depth across the channels and discharge calculated according to Wetzel (2001) as shown in Equation 2.

3.3.6 Determination of litter decomposition rates

Field measurements of litter decomposition were conducted in three streams under forested and three under agricultural lands. Leaves from two different tree species most dominant in the study area were used to indirectly test the effect of land use on leaf litter breakdown. It is well known that stream water quality and function is a product of its catchment processes. One indigenous tree species, *Neuboutonia macrocalyx*, of family Euporbiaceae and one exotic, *Eucalyptus salagna*, of family Myrtaceae were selected. *Eucalyptus* was chosen because it was the most common riparian tree species along the streams draining agricultural land while *Neubotania* dominated the forested land use (Kenya Forestry Service, 2009). Both leaves were of different quality in terms of toughness and chemical composition.

Mature leaves were randomly collected from the trees in the field and transported to the laboratory in Egerton University Njoro. The maturity of the leaves was determined based on the colour and the position on the stalk. The selected leaves were then oven-dried at 60° C for 24 hours. Approximately 6g of the dried leaves were placed into course litterbags (11 x 11cm) with a mesh size of 10×10 mm which were large enough to allow entry of macroinvertebrates as shown in plate 9. The litterbag experiments were set up in triplicates for each plant species. The litterbags were arranged into sets of triplicates (Each set had three *Eucalyptus* litterbags and three *Neuboutonia* litterbags) for each stream (6 streams) in respect to retrieval time (8 retrievals). This resulted to total of 288 litterbags. The bags were then transported to the streams for deployment.



Plate 9: Sample litterbag packed with leaf litter

The litterbags were randomly distributed in a 50m stretch in the upstream part of each study site after recording their initial dry weights. Each stream had 48 litterbags of both plant species tied in two sets to make 24 sets per stream. Each set of litterbags was tied with a manila string and secured in the stream channel by tying to tree roots as shown in plate 10. The strings were tied randomly but far apart to avoid bags from different strings coming into contact with each other.



Plate 10: Litterbags immersed in the riverbed secured on tree roots

The strings were then labelled in such a manner that one could read the set numbers without disturbing the other bags during retrieval as shown in Plate 11. Three groups of litterbags were randomly retrieved from each sampling site after 0, 1, 3, 7, 14, 28, 48 and 56 days and transported to the laboratory in Egerton University. Day 0 represents the setting date. In the lab, leaves were carefully cleaned off extraneous material with tap water in 250 µm through mesh size sieve.



Plate 11: Labelling of the strings used to secure litterbags on tree roots

The remnants of the leaves were oven-dried at 60°C for 48 hours and weighed to obtain dry mass (DM). The processing coefficient (- k) of the leaves was determined by fitting the data to the exponential model as shown in equation 3. This was done after natural log transformations of the dry mass at the beginning and at the end of each particular exposure time.

 $DM_{t} = DM_{0}e^{-kt}.$

Where DM_t is the dry mass (g) remaining at time t (days), DM_0 is the initial dry mass, e is the natural logarithm and k is the breakdown rate constant -k/day.

The k values were then interpreted based on Peterson and Cummins (1974) as 'fast' (0.010-0.015 day-1); 'medium' (0.005-0.010 day-1) and 'slow' (<0,005 day-1). The data used for generating the k-values was fitted to a linear model to provide the time required for 25, 50, 75, and 90% of the leaves to be processed in the six streams (Cuffney and Wallace, 1987) as shown in Equation 4

y=b+ax.....4

Where y is the % RDM and x being the number of days, b the slope and a, is the intercept.

The effect of land use on breakdown rates was calculated by the use of ki/kr ratio.

Where ki = decay rate in streams draining agriculture land use (impacted) and kr = decay rate in streams draining forested land use (reference).

The ratio was calculated for both *Eucalyptus* and *Neuboutonia*. The scores were given following Gessner and Chauvet (2002) as shown in Table 1. However, this method should be used with caution because there are no clear boundaries between different scores.

Table 1: Criteria for interpretation of k_i/k_r score

Criteria(k _i /k _r)	Score	Interpretation
0.75-1.3	2	No clear evidence of impaired stream functions
0.5-0.73 or 1.33-2.0	1	Compromised stream functioning
<0.50 or >2.00	0	Severely compromised stream functioning

The invertebrates retained on the sieve were preserved in 10% formalin for counting and identification to family level. Macroinvertebrates sorting was done both visually and under stereomicroscope at 40 x 10 total magnification, identified and stored in 70% ethanol. Identification was carried out under the same light microscope to family level using available identification key (Gerber and Gabriel, 2002) and grouped into their functional feeding groups according to Ramirez and Gutierrez, (2014); Masese *et al.*, (2014). The number of macroinvertebrate abundance associated with the leaf litter was calculated as total number per litterbag. Shannon Wiener diversity index (H') was used to determine the macroinvertebrate diversity associated with decomposing litter.

3.3.7 Stream metabolism (Primary production and respiration) measurements

The main components of stream metabolism are community respiration (CR), gross primary production (GPP) and net ecosystem production (NEP) (Young, *et al.*, 2008). These metabolism components are measured by monitoring the daily changes in oxygen concentration at a site (Fellows, *et al.*, 2006). The GPP/ER ratio is used to determine the equilibrium between primary production and respiration in the stream (Hall and Beaulieu, 2013). According to Hall & Beaulieu, (2013) the ratio is used to classify a system as net autotrophic or net heterotrophic as follows: If P/R < 1, the ecosystem is heterotrophic and also the NEP is less than 0. On the other hand, if P/R>1, the ecosystem is autotrophic and also NEP is greater than 0.

Whole stream metabolism measurement was done in two streams draining different land use by measuring diel changes in DO using the two-station method outlined by Bott (2007) as shown in Figure 3. One stream draining agricultural (Tenwek) and one draining forested land use (Chepkosiom) were selected based on the accessibility and security. This method allows metabolism estimates in a parcel of water flowing down the study reach by attributing the DO difference between the probes to primary production, community respiration and re-aeration processes within the study reach (Bott, 2007). Two hydrolabs were deployed 100m apart (one at 0 m and another at 100m) and calibrated to take readings of DO, temperature, pH,

conductivity and water depth after every 10 minutes over 24 hours. The 100m stretch was chosen to ensure that different aspects of the stream (riffle and pool) are represented. The probes were supported by chaining them on the tree roots to protect them from being swept away.

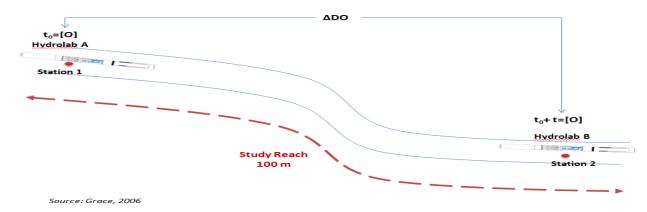


Figure 3: Sketch of the hydro labs deployment in the 100 m stretch (Grace, 2006)

Re-aeration co-efficient k was calculated using the physical characteristics of the stream channel. This was done by using the energy dissipation model (EDM), (Tsivoglou and Neal, 1976) as shown in Equation 5. The model assumes that a parcel of water of water will flow from point

The change in DO (Δ DO) over each study reach was determined by subtracting the O₂ concentration at the downstream site at time ($t_0 + t$) from the O₂ concentration at the upstream site at time t_0 . The O₂ saturation deficit and KO_2 were used to correct Δ DO values for the flux of O₂ resulting from re-aeration (Marzolf, *et al.*, 1994), according to the following model equation 6.

$$\Delta DO = ((Ct - Cto)/\Delta t) - KO_2D))*Z.$$

Where Ct is the oxygen concentration (g O_2 m⁻³), Cto is the O_2 concentration at the upstream site, and Ct is the O_2 concentration at the downstream site with the travel time (time taken by the leachate to travel from upstream to downstream), KO_2 is re-aeration coefficient of O_2 (k/min), D is the saturation deficit or excess, (the difference between the measured DO concentration and the concentration at 100% saturation at a given time) and Z is mean stream depth (m).

From the resulting re-aeration-corrected ΔDO , gross primary production (GPP) and 24-h respiration (R24) were calculated. R24 was calculated by integrating the reaeration-corrected ΔDO from dark hours (from 6.00 pm to 6.00 am), and assuming the respiration rate of night is equal to the day, multiply by 24 to obtain the total daily community respiration. Gross primary production was calculated by the sum of respiration rate of the photoperiod (from 5 am-5 pm). Gross primary production and R24 was used to calculate net ecosystem production.

NEP = GPP - R.

3.3.8 Effect of leaf leachate on stream metabolism

Stream leachate addition experiment was conducted to quantify the effect of leaf leachates on stream primary production and respiration. Leaves of the two dominant tree species in the study area (exotic, *Eucalyptus saligna* and native, *Neuboutonia macrocalyx*) were used for the experimental set up. The leachate addition experiment was done in two streams under different land uses during base flow conditions to avoid the influence of surface run-off (Hoellein *et al.*, (2009). The Leachate addition was done in the field and not in the laboratory due to the associated limitation of oxygen dynamics. Oxygen changes in streams are highly dynamic and are influenced by various environmental and Biological that cannot be mimicked in the laboratory.

Leaves were collected after abscission, transported to the laboratory at Egerton University and dried at 35 °C for 48 hours in the oven for complete dryness to be achieved. Leaves from each tree species were then separately hand fragmented and leached for 24 hrs in distilled water (400 g leaf/ L). Leaching was done by immersing 4000g of leaves in 10ltrs of distilled water. This was done separately for both species hence each tree species had total leachate volume of about 10L. Leachates were filtered through 100 and 50 mm screens and subsequently through a glass fiber filter using a filtration apparatus and kept at 4°C before

being transported to the field. The additions were performed in the field using a leachate of each species separately (with a conservative tracer (sodium chloride) added to assess dilution rates. The leachates were introduced in the thalweg where there is water flow by the use of a peristaltic metering pump at a rate of 30 mL/ min (520N pump; Watson-Marlow). Complete mixing of the solute injections at the first downstream station was verified by taking five measurements of specific conductivity along the study reach. Ecosystem metabolism was measured before and after the leachate was introduced in the streams by measuring diel changes in DO for 24 hours as described in section 3.2.7 above.

3.4 Data analysis

Statistical tests and data analysis were done using SIGMA Plot 11 statistical software. Data on physico-chemical parameters were computed to find their mean values over the entire study period. Variations in mean concentrations of TSS, nutrient: NH₄-N, NO₃-N, NO₂-N, SRP and TP, and *in-situ* measurements: DO, EC, temperature, pH and TDS in the streams were compared using One-way ANOVA followed by Turkey's post-Hoc tests where the results are significantly different. The difference in the decay rate values across the study streams was tested using One-way ANOVA followed by a post-hoc analysis. Student's t-test was used to ascertain any statistical difference of the decay rates in the agricultural and forested land use. The macroinvertebrate data was standardized by adding 1 followed by log transformation before checking for its normality distribution. The transformations were meant to ensure that the data is normally distributed. Significant differences in macroinvertebrates abundance and diversity between agricultural and forested land use were tested using Student's t-test. In all the analyses, 5% level of significance was used as the critical point for rejection of the null hypotheses. The k_i/k_r coefficients (i for impacted (agriculture) and r for reference (forest) streams, respectively) were calculated for *Eucalyptus* saligna and Neuboutonia macrocalyx litterbags (Gessner and Chauvet, 2002) to determine the effect of land use on leaf litter breakdown.

CHAPTER FOUR 4.0 RESULTS

4.1 Land use cover of Nyangores catchment

Percentage forested and agricultural land types were calculated separately. Forest land use covered $94.3 \pm 13.4\%$ of total catchment area as shown in Table 2 while the other land cover included grasslands and shrub lands. Total area under agriculture was approximated to be $93.7 \pm 3.4\%$ while the other land use/cover noted were roads, bare ground, urban areas that comprising <10% of the land area. Appendix 1 shows the image of the land use characterization of the entire upper Mara catchment.

Table 2: Mean (\pm SE) values for different proportions of land uses in the upper Mara catchment. Agriculture, n=3 and Forest, n=3.

Land use	Stream Type			
	Agricultural	Forested		
%Forest	-	94.3±13.4		
% Agriculture	93.7±4.2	-		
%Other land uses	6.3±4.2	5.7±3.3		

4.2 Physico-chemical and Nutrient concentration in the study streams

4.2.1 Physico-chemical parameters of the study streams

All the physico-chemical variables measured are presented in Table 3. Mean temperature ranged between $12.6\pm0.3-20.4\pm0.4^{\circ}$ C in all the streams. Mosoriot stream recorded the lowest temperature in relation to all the other streams (one-way ANOVA; F_5 , $_{42}$ =63.44, p < 0.05). The pH ranged from 4.0 to 8.6 while mean conductivity ranged between 40 and 107 µS/cm. Significantly higher conductivity values were observed in Tenwek stream (one-way ANOVA.; F_5 , $_{42}$ = 56.21, p < 0.05). Dissolved oxygen (DO) concentration among the streams ranged between 5.6±0.3-7.6±0.1 mg/l. Sambambwet (draining forested land use) recorded significantly higher DO value compared to other streams (one-way ANOVA: F_5 , F_6 =42 =48.43, F_6 =48

When the data was pooled, streams that drained agricultural land recorded significantly higher temperature than those draining forested land use (t =-12.84, df = 47, p = <0.05). Conductivity values also differed significantly with the highest values recorded in streams draining agricultural land (t value = 1.0, df = 47, p <0.05). Likewise, TDS was also significantly higher in streams draining agricultural land (t= 17.596, df = 47, p = <0.05). Streams draining forested land use on the other hand, recorded significantly higher DO (t= 7.757, df =47, p=<0.05).

Table 3: Physico-chemical characteristics of the study sites. The symbol \pm represents standard error of the mean while values with the same superscript letter are not significantly different (Tukey *post-hoc* test).

	Forested land	use		Agricultural land		
Variable	Chepkosiom	Mosoriot	Sambambwet	Masese	Tenwek	Kapsebet
Temperature(⁰ C)	12.8±0.3a	12.6±0.3a	13.0±0.4a	16.7±0.7°	19.7±0.4 ^b	20.4±0.4 ^b
Cond (µS/cm)	45.3 ± 2.5^{a}	$40.4{\pm}1.1^{a}$	47.9 ± 2.7^{a}	73.8 ± 6.15^{cb}	107.1 ± 4.6^{b}	88.74 ± 3.2^{b}
pН	4.0-6.0	6.0-6.1	5.0-6.1	8.0-8.6	7.4-7.6	4.2-5.0
DO (mg/l)	7.5±0.1a	7.6 ± 0.1^{a}	7.6 ± 0.2^a	6.6 ± 0.1^{b}	5.8 ± 0.3^{b}	5.6±0.3 ^b
Saturation (%)	77.3±3.2a	77.6±3.3a	78.0±3.8a	71.1 ± 3.2^{b}	58.7 ± 3.6^{b}	59.4 ± 2.6^{b}
TDS (mg/l)	34.9±0.a	36.21±0.9a	36.5±2.5 ^a	65.0±2.8 ^b	72.9±2.7 ^b	80.8±2.5 ^b
Discharge(l/s)	2.65±0.1	2.3±0.01	1.64±0.2	1.34±0.2	2.15±0.4	4.05±0.2

4.2.3 Nitrogen and phosphorous concentrations of the study sites

The mean nitrogen and phosphorous concentrations during the entire study period are presented in Table 4. Tenwek stream that drained agricultural land had the highest level of nitrate of 10.19 mg/l while Masese stream had the lowest of 4.85 mg/l. However, the mean nitrate concentrations among the streams was not significantly different (one-way ANOVA; $F_{5,30}$ =0.712, p > 0.05). Mosoriot stream had the lowest nitrite concentration of 3.72 µg/l while highest concentration of 16.0 µg/l was recorded in Kapsebet stream. Nonetheless, the difference in nitrite concentration was not statistically significant among the streams (one-way ANOVA, $F_{5,30}$ =1.656, p > 0.05). Likewise, NH₄-N concentration also did not vary significantly among the study streams (one-way ANOVA, $F_{5,30}$ =1.482, p > 0.05).

Table 4: Nutrient and TSS concentration of the study sites. Values present means \pm SE. Values with the same superscript letter are not significantly different

	Fores	ted land use		Agricu	ltural land	
-	Chepkosiom	Mosoriot	Sambambwet	Tenwek	Masese	Kapsebet
Variable						
NO ₂ (μg/l)	4.4±2a	3.7±1.0 ^a	3.9±2a	9.8±3.9a	13.4±4.7ª	16.0±4.4 ^a
$NO_3(mg/l)$	$6.1{\pm}4.5^{\mathrm{a}}$	6.4 ± 2.19^{a}	5.8 ± 2.4^a	$10.2{\pm}3.6^a$	$4.9{\pm}2.2^a$	9.6±3.2a
$TP(\mu g/l)$	$4.3\pm2.^{4a}$	3.9 ± 1.7^a	5.8 ± 7.4^a	3.3 ± 0.1^{a}	13.4 ± 7.4^{a}	5.1±0.7 ^a
SRP (µg/l)	4.3 ± 2^a	2.7±1.5 ^a	2.3±4.3a	$2.7{\pm}1.6^a$	13.1±7.9a	2.3±3.6a
$NH_4(\mu g/l)$	$13.7{\pm}4.3^a$	8.4±3.8 ^a	$3.5{\pm}1.2^a$	9.0 ± 4.4^a	17.6±2.8a	11.3±5.2a
TSS (mg/l)	31.3±2 ^a	32.7±4.3 ^{ca}	31.4±4.3 ^{da}	68.8±13.6 ^b	110.9±19 ^b	118.9±14.8e

Soluble reactive phosphorous concentration ranged between 2 - 13 μ g/l with streams draining agricultural land recording higher concentrations. Masses stream had the highest concentration of 13.1 μ g/l while Sambambwet and Kapsebet streams had the lowest of 2.3 μ g/l. However, SRP concentration did not differ significantly among the study streams (one-way ANOVA, F₅, 30 = 0.906, p > 0.05). Highest TP concentration of 13.4 μ g/l was recorded in Masses stream. There was no significant difference in levels of TP among study streams (one-way ANOVA, F₅, 30 = 1.669, p >0.05). Streams draining agricultural land had the highest TSS concentrations compared to the ones draining in forested land use (see Table 4). The highest TSS concentration

of 118.9 mg/l was observed in Kapsebet stream. Unlike the other variables, TSS concentration differed significantly across the study streams (one-way ANOVA, F $_{5,\ 30}=8.509,\ p<0.05$). Kapsebet stream recorded significantly highest TSS concentration compared to the rest (Tukey post-hoc test: p<0.05).

When the data was pooled, significant differences in terms of nitrite concentration were evident between the two land uses with agricultural land recording the highest value of $12.67\pm2.6~\mu g/l$ and forested land use, $8.5\pm2.9~\mu g/l$, (t = -2.293, df = 34, P = < 0.05). Although Tenwek and Kapsebet (draining agricultural land) streams had exceptionally higher NO₃ levels, the concentrations did not vary significantly with land use (t=-1.125, df = 34, p = > 0.05). Streams draining forested land use had mean nitrate concentration of $6.1\pm0.2~mg/l$ whilst streams draining agricultural land had a mean of $8.3\pm1.7~mg/l$. Both TP and SRP did not vary with land use (t=-0.955, df 34, p = > 0.05 and t = -1.045, df = 34, p = > 0.05 respectively). Streams draining agricultural land had mean concentration of $7.2\pm3.1~\mu g/l$ and $6.03\pm3.5~\mu g/l$ for TP and SRP respectively. On the other hand, in forested land use a mean concentration of $4.6\pm0.6~\mu g/l$ were observed for TP and SRP respectively.

4.3 Leaf litter decomposition in the streams

4.3.1 Decomposition trends of *Neuboutonia* and *Eucalyptus* leaves in streams

There was a general decline in percentage of dry mass of both *Neuboutonia* and *Eucalyptus* leaves with increase in exposure time in all the streams as shown in Figure 3. Decomposition trends were comparable for each leave species with respect to land use. *Eucalyptus* leave species decayed faster in agricultural land than in forested land use. A similar observation was made for *Neuboutonia* leave species.

When decomposition of *Neuboutonia* leaves were compared to *Eucalyptus* leaves, it was observed that the former processed faster (Figure 4). *Neuboutonia* reduced exponentially from day 0 to day 56 while *Eucalyptus* recorded minimal reduction of about 1% between day 3 and day 27 which was then followed by a rapid reduction up to day 56. In forested land use both species showed a steady reduction in % DM remaining throughout the study period (Figure 3 b).

4.3.2 Decay rates of *Neuboutonia* and *Eucalyptus* leaves

The decay rates of *Neuboutonia* and *Eucalyptus* leaves in streams draining agricultural and forested land uses are presented in Table 5. Day one retrieval of both species recorded a significantly higher -k/day values than the other retrieval days regardless of the land use.

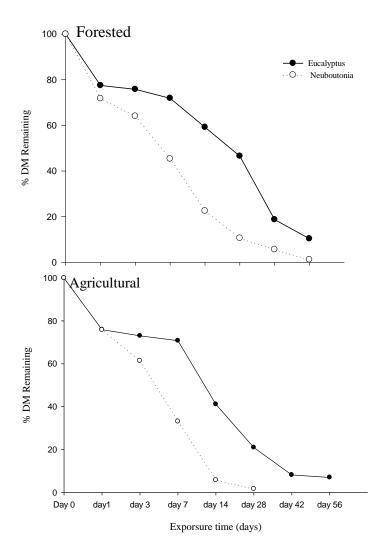


Figure 4: Percent dry mass remaining against exposure time in streams draining forested and agricultural land.

Neuboutonia leaves took lesser days than *Eucalyptus* leaves in all the study streams. Streams draining in agricultural land recorded less days for both species than those draining in forested land use.

Table 5: Decay rate (-k/day) of *Eucalyptus* and *Neuboutonia* species in streams draining forested (For) and agricultural (Agric) land use.

Land use	Species	1	3	7	14	28	42	56	MEAN
For	Eucalyptus	0.211	0.086	0.034	0.017	0.019	0.028	0.022	0.067
		0.01	0.00	0.01	0.01	0.00	0.00	0.00	
	Neuboutonia	0.290	0.105	0.050	0.042	0.060	0.029	0.022	0.087
		0.02	0.00	0.01	0.02	0.01	0.00	0.00	
Agric	Eucalyptus	0.188	0.092	0.045	0.027	0.036	0.029	0.022	0.063
		0.02	0.00	0.01	0.02	0.01	0.00	0.00	
	Neuboutonia	0.248	0.094	0.07	0.10	0.113	*	*	0.125
		0.01	0.05	0.02	0.01	0.02			

^{*} means data missing because the leaves had completely decomposed, unbolded values represent (\pm SE)

Streams draining agricultural land had higher decay rates as opposed to those draining forested land use (Figure 5). From the pooled data, the difference in processing rates of *Eucalyptus* leaves were not significant between streams draining forested land (-k = 0.06 ± 0.03) and those draining agricultural areas (-k = 0.06 ± 0.02) (t = 0.404, df=143, p= >0.05). For *Neoboutonia* leaves, decay rates differed significantly between streams draining agricultural areas (-k = 0.12 ± 0.03) and streams draining forested land use (-k= 0.09 ± 0.04) (t = 2.89, df=143, p= <0.05).

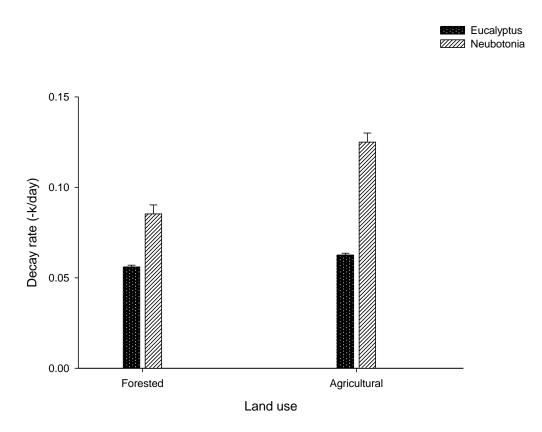


Figure 5: Decay rate (-k) of *Eucalyptus* and *Neuboutonia* leaves in streams draining agricultural and forested land use.

Based on the extrapolation of the decay rates obtained, it would take 63 and 69 days for 90% of leaves of *Eucalyptus* to be processed in streams draining agricultural and forested land use respectively whilst *Neuboutonia* leaves would take 24 and 53 days as indicated in Table 6.

Table 6: Time (days) for 25%, 50%, 75% and 90% of *Eucalyptus* and *Neuboutonia* leaves to decompose

Land use	Species	25%	50%	75%	90%
Forested	Eucalyptus	6	20	41	69
	Neuboutonia	2	12	30	53
Agricultural	Eucalyptus	6	17	37	63
	Neuboutonia	3	7	14	24

ki/kr is a ratio which is used to indicate how land use has impacted on the decay rate of given leaves. In this study, both *Eucalyptus* and *Neuboutonia* leaves had *ki/kr* co-efficient of less than 2 as shown in Table 7 below. The highest value was evident for *Neuboutonia* leave species.

Table 7: *ki/kr* coefficient for *Neuboutonia* and *Eucalyptus* leaves

Tree Species	ki/kr co-efficient
Eucalyptus	1.14
Neuboutonia	1.54

According to the correlation done between the physical chemical variables and the decay rates, the effect of various physical chemical variables differed with land use (Table 8). Among the nutrients, only SRP was significantly related to decay rate in all the streams. Decay rates were faster with an increase in SRP concentration. Nitrate, nitrite and ammonia did not have significant relationship with decay in all the study streams. Temperature did not have significant correlation in Chepkosiom and Kapsebet streams. On the other hand, conductivity did not have significant effect on decay rate in Mosoriot and Chepkosiom streams while DO did not significantly affect decay rate in all the study streams.

Table 8: Pearson correlation coefficients between decay rate and physico-chemical variables. The values with superscript * have a significant correlation (p=0.05).

	For	rested streams		Agricultural streams			
Variable	Mosoriot	Chepkosiom	Sambambwet	Masese	Kapsebet	Tenwek	
Temperature	0.94*	-0.51	0.85*	-0.90*	-0.61	-0.91*	
Conductivity	0.41	0.40	0.91*	0.90*	0.93*	0.93*	
DO	-0.26	0.31	-0.60	0.11	0.22	0.29	
TP	0.15	0.67	0.88*	0.58	0.48	0.29	
SRP	0.77*	0.90*	0.9*	0.77*	0.82*	0.85*	
NO_3	0.29	0.33	0.34	0.18	0.04	0.11	
NO_2	0.69	0.67	-0.59	-0.26	0.77	-0.70	
NH ₃	-0.14	-0.76	0.78	0.22	0.08	-0.58	

4.4 Macroinvertebrate litterbag colonization

4.4.1 Macroinvertebrates taxon composition and abundance

The colonization of Neuboutonia and Eucalyptus leaves in the litterbags was diverse, with all functional feeding groups (collectors, predators, scrapers, grazers, filter feeders and shredders) and major taxa (Ephemeroptera, Plecoptera, Trichoptera, and Diptera) being represented. 5,973 macroinvertebrates individuals were identified during the study. Streams draining agricultural land recorded higher numbers of 4,640 individuals while 1,333 belonged to streams draining forested land use. In all the streams except Masese, Neuboutonia leaves macroinvertebrate had higher individuals than Eucalyptus. All the identified macroinvertebrates belonged to 6 orders and 23 families as shown in Table 9.

Table 9: Presence or absence of various macroinvertebrate taxa and abundance (individuals/litter bag) within the six studied streams draining agricultural and forested land use. (a- Chepkosiom, b-Mosoriot, c-Sambambwet, d-Tenwek, e-Kapsebet and f- Masese: ++++->25, +++-10-25, ++- 5-10, +- < 5 individuals /litter bag and -means absent)

	Forest	ed land us	e	Agricult	gricultural land		
Taxa	a	В	С	d	e	f	
Ephemeroptera							
Baetidae	+	+	+	++++	++++	++	
Caenidae	-	+	-	-	-	+	
Leptophlebidae	+	+	-	+	+	-	
Teloganodidae	+	-	-	+	+	-	
Tricorythidae	+	+	-	-	-	-	
Trichoptera							
Hydropsychidae	+	+	+	+	+	+	
Hydroptilidae	+	+	-	-	-	-	
Leptociridae	+	+	+	+	+	+	
Sericostomatidae	+	+	+	+	+	+	
Coleoptera							
Hydrophilidae	-	+	-	+	+	-	
Elmidae	+	+	+	+	+	+	
Helodidae	+	+	-	+	+	-	
Hydraenidae	+	+	-	-	-	-	
Odonata							
Coenogrionidae	-	+	-	+	+	-	
Protoneuridae	-	-	-	-	+	-	
Diptera							
Psychodidae	-	+	-	+	+	-	
Ceratopogonidae	+	+	+	+	+	+	
Chironomidae	+	++	++	+++	+++	+	
Simuliidae	+	+	+	+	+	+	
Tipulidae	+	+	-	+	+	+	
Plecoptera							
Pyralidae	_	+	-	+	-	+	

Chironomidae and baetidae were the most abundant with more numbers being recorded in the streams draining agricultural land. Other families such as trichorythidae, hydorptidae, and hydranidae were only found in streams draining forested land.

The abundance of macroinvertebrates colonizing leaves exposed in streams draining agricultural land was significantly higher than those draining forested land use for both *Neuboutonia* and *Eucalyptus* leaves (Figure 6) (t = -3.244, d.f=18, p=<0.05 and (t = -2.074, d.f=18, p=<0.05).

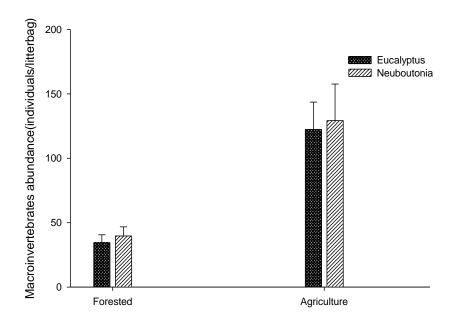


Figure 6: Macroinvertebrate abundance (individuals per bag) of *Eucalyptus* and *Neuboutonia* leaves in streams draining agricultural and forested land use.

The mean colonization abundance (\pm SE) values of major macroinvertebrate taxa in both *Neuboutonia* and *Eucalyptus* are shown in Table 9. Under agricultural landuse baetidae was most abundant in *Eucalyptus* litterbags while chironomidae was dominated in *Neuboutonia* litterbags (Table 10). On the other hand, under forested land use *Eucalyptus* leaves were colonized more by the chironomidae while baetidae dominated the *Neuboutonia* litterbags.

Table 10: Abundance (individuals/litterbag) of five major groups of macroinvertebrates that dominated in the litterbags. a- Chironomidae, b- Hydropsychidae, c- Baetidae, d- Simuliidae, d- Leptoceridae

Land use	Mac	roinvertebra	tes			p – value
	A	В	С	D	E	
Agricultural						
Eucalyptus	7.33±3.7	0.52±0.2	26.81±1.5	0.36±0.1	3.30±1.7	0.027
Neuboutonia	5.01±0.9	1.40±0.1	0.59±0.1	0.27 ± 0.2	1.39±0.4	0.001
Forested						
Eucalyptus	3.5±1.3	2.40±0.1	0.97±0.1	0.34 ± 0.2	1.29±0.4	0.075
Neuboutonia	10.94±5.8	0.46±0.4	14.06±6.0	0.12±0.04	0.97±0.5	0.001

4.4.2 Macroinvertebrate diversity

Macroinvertebrate diversity differed with land use in the streams draining forested land use recording higher diversity index (Figure 7). Although high abundance was recorded in streams draining agricultural land, the diversity was low. The diversity of macroinvertebrate colonizing *Eucalyptus* litterbags did not differ significantly with land use (t = -0.415, df =18, p = >0.05). However, the diversity of macroinvertebrates colonizing *Neuboutonia* litterbags were significantly higher in streams draining forested land use (t = 4.527, df =18, p = <0.05).

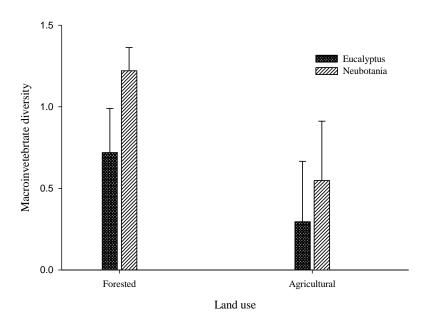


Figure 7: Macroinvertebrate diversity from the Shannon-Weiner diversity index (H'), for *Eucalyptus* and *Neuboutonia* leaves in streams draining agricultural and forested land use

4.4.3 Macroinvertebrate leaf colonization patterns

agricultural macroinvertebrate Generally, in streams draining land, colonization Eucalyptus and Neuboutonia leaves increased with time from day 1 to day 42 (Figure 7). In Eucalyptus, macroinvertebrate mean abundance declined at day 56 from 7.2 to 4.3 individuals per litterbag in streams under forested land use and from 74.3 to 33 individuals per litterbag in streams under agricultural land. Neuboutonia also showed a similar decline pattern at day 56 from 15.1 to 5 and from 53 to 16.7 individuals per litterbag in streams draining forested and agricultural land respectively. Thus, macroinvertebrate leafcolonization showed a similar pattern in both species and land use types.

In streams draining agricultural land, the number of macroinvertebrates per litterbag showed a similar trend for both species. The trend was characterized by steady increase in numbers from day 1 followed by decline from day 42 (Figure 8). Under forested land use *Eucalyptus* and *Neuboutonia* showed different trends as shown in Figure 8. The number of macroinvertebrates colonizing *Neuboutonia* litterbags steadily increased to day 28 followed by sharp decline. *Eucalyptus* on the other hand showed an increase in numbers of macroinvertebrates colonizing the litterbags up to day 7. This was followed by a slight

decline at day 14 that was followed by an increase from day 28 to day 42 after which a decline was observed.

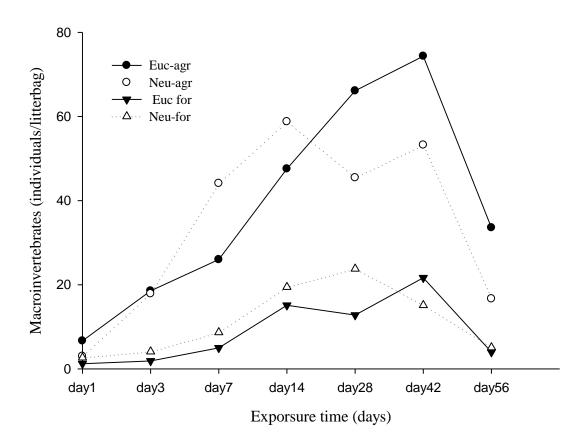


Figure 8: Macroinvertebrate leaf-colonization patterns on litterbags exposed in streams draining agricultural and forested land use area

4.4.4 Functional feeding groups (FFG)

The distribution of FFG among streams draining forested and agricultural land is presented in Table 13.Filter feeders and gatherers dominated in forested and agricultural streams respectively. Shredder abundance was slightly higher in forested sites (13% of the total individuals collected) than agricultural sites (4% of total individuals collected). Predators were the least in forested sites with 3%, and scrappers in agricultural sites with 1%.

Table 11: Summary of percentage composition of macroinvertebrate Functional Feeding Groups (FFG) in streams draining agricultural and forested land

	Percentage c	Percentage composition of FFGs (%)					
Type of stream	Gatherers	Predators	Scrapers	Shredders	Filter collectors		
Forested	16	3	8	13	60		
Agricultural	64	2	1	4	29		

4.5 Stream metabolism measurements

Chepkosiom stream had lower ecosystem respiration (ER) and gross primary production (GPP) than Tenwek stream (Table 12). Both streams had a P/R ratio >1(ER< GPP) making them heterotrophic.

Table 12: Base line GPP, ER and GPP/ER (gO₂/m²/d) in Chepkosiom (Forested) and Tenwek (Agriculture) streams

	TENWEK	CHEPKOSIOM
GPP	8.3	1.9
ER	9.1	4.4
GPP/ER	0.91	0.43
Trophic Status	Heterotrophic	Heterotrophic

The effect of the leachates on Chepkosiom and Tenwek streams are shown in Table 13. In Chepkosiom stream, during *Neuboutonia* enrichments, the ER (4.60 gO₂/m²/d) was higher than GPP (1.6 gO₂m²/d). *Eucalyptus* enrichments also had higher ER of 4.50 gO₂/m²/d compared to GPP of 1.6 gO₂/m²/d. Both enrichments did not change the trophic status of the system.

Tenwek stream followed similar trend as Chepkosiom stream with higher ER (58.3.20 g/O₂m²/d) compared to GPP (28.9 g/O₂m²/d) under *Neuboutonia* enrichments. *Eucalyptus* enrichments in the stream on the other hand had higher GPP (38.4 g/O₂m²/d) compared to ER (23.30 g/O₂m²/d). *Neuboutonia* enrichment did not affect the trophic status of Tenwek stream while *Eucalyptus* shifted the system from heterotrophic to autotrophic status.

 Table 13: Gross Primary Production and Ecosystem Respiration levels under Neuboutonia

 and Eucalyptus leachate enrichment in Tenwek and Chepkosiom streams.

Stream		Leachate enrichment	
	Metabolism	Neuboutonia	Eucalyptus
	$(gO_2/m^2/d)$		
Tenwek (Agricultural)	GPP	28.9	38.1
	ER	58.3	23.3
GPP/ER ratio		0.50	1.64
Trophic status		Heterotrophic	Autotrophic
Chepkosiom (Forested)	GPP	1.6	1.6
	ER	4.5	4.6
GPP/ER ratio		0.36	0.35
Trophic status		Heterotrophic	Heterotrophic

CHAPTER FIVE 5.0 DISCUSSION

5.1 Introduction

Land use activities within the catchment significantly affect both abiotic and biotic processes in streams. According to Baxter et al., (2005) there is strong linkage between terrestrial and riverine ecosystems in terms of litter decay and metabolism. Anthropogenic activities can affect stream biota and ecosystem functioning directly through increased nutrient indirectly through oxygen depletion, increased temperatures concentration or sedimentation (Sweeney et al., 2004). Of major concern in streams are non-point sources of inorganic nitrogen and phosphorus resulting from runoff from agriculture or urban settlements and atmospheric deposition (Carpenter et al., 1998). In upper Mara catchment, human activities along riparian corridors have resulted in replacement of indigenous tree species with exotic ones such as Eucalyptus (Mati et al., 2005; Masese et al., (2014). This has compromised both biotic and abiotic processes within the streams and rivers within the catchment.

5.2.1 Physico-chemical parameters of study sites

The high temperatures recorded in streams draining agricultural land was attributed to reduced canopy cover that increased sunlight insolation into the streams (Omengo, 2010). Kapsebet stream, which had the highest mean temperature, had the lowest canopy cover of 25%. Temperature increases the rate of leaf litter decomposition by enhancing microbial and macroinvertebrate growth and metabolism (Rosemond *et al.*, 2002). The increased growth of microbes on the leaf surface conditions the leaves. Leaf conditioning encourages shredders colonization through increased palatability (Canhoto and Graça, 1999).

The high conductivity recorded in agricultural streams indicates a high amount of dissolved ions in the water due to the increased human activities such as agriculture in the riparian zone of the streams, settlement and urban centers (Minaya *et al.*, 2013). These human activities together with the associated removal of vegetation cover results to increase in loading of sediments rich ions from the catchment into the stream. Forested streams on the other hand were characterized with dense riparian zone that protects the stream from sediment loading hence the significantly lower TSS concentrations of 36±2 mg/l compared to 112±0.2 mg/l for streams draining agricultural land. These results are similar to findings of

Masese, *et al.*, (2014) in upper Mara streams in which TSS concentrations of 200 mg/l and 36 mg/l in streams draining agricultural and forested land use respectively were observed. High TSS level is characterized by large amount of suspended solids such as small soil particles. The suspended particles promote physical breakdown of leaf litter via abrasion as they hit the leaves along with the water current. (Grattan and Suberkropp, 2001).

The concentration of dissolved oxygen in water indicates the level of stream pollution (Yillia $et\ al.$, 2008). Pristine streams are characterized by high dissolved oxygen levels that reduce as the river system becomes polluted. The reduced DO level is due to increase in biological oxygen demand required to breakdown the organic load (Yillia $et\ al.$, 2008). In this study, the low oxygen concentration ($5.6\pm0.3\ mg/l$) recorded in streams draining agricultural land is attributed to human activities within the catchment. Human activities such as agriculture results to increased loading of organic matter into the streams hence increased oxygen demand for decomposition of these organic wastes (Kibichii $et\ al.$, 2007). The lower oxygen levels recorded in streams draining agricultural land may be attributed to the increased organic matter loading from the farmland and urban centers. Kibichii $et\ al.$, (2007) observed similar relationship between organic matter loading and DO levels in the Njoro River catchment. In the study, he attributed the reduced DO to in-stream organic matter input from the agricultural activities within the Njoro River sub-catchment into the streams. Temperature also influences the amount of dissolved oxygen in the water (Ebbert, 2003). Increased temperature levels lower the amount of dissolved oxygen.

5.2.2 Nitrogen and phosphorous concentrations of the study sites

Nutrient concentrations in streams and rivers have strongly been correlated with human land use and disturbance gradients in the catchment and riparian zones (Miller and Wooster, 2010). High nutrient concentrations potentially stimulate the activity of heterotrophic microorganisms associated with submerged leaf litter and hence enhancing decomposition (Grattan and Suberkropp, 2001). Although other_studies have linked in-stream litter decay with nutrient levels, in this study, only SRP was significantly correlated with decay rate in all the sites sampled. Clearing of forested areas for agriculture leads to increased nutrient input into the streams (McKie and Malmqvist, 2009) hence making streams draining agricultural land to have high nutrient levels. In this study, nutrient concentrations were generally higher in streams draining agricultural land than streams draining forested land use. There was higher nitrite (8.5±2.9 µg/l) and nitrate (8.2±1.7 mg/l) concentrations in streams draining

agricultural land compared to the concentrations of the respective nutrients of 12.7±2.6 mg/l and 6.1±0.2 mg/l in streams draining forested land use respectively. This may be attributed to transportation by surface run off from farmland into the streams together with nitrification process. McKie and Malmqvist (2009) stated that high dissolved oxygen levels promote nitrification. The higher ammonium concentration of 12.6±2.5 µg/l in streams draining agricultural land compared to the ones draining forested land use (8.5±2.9µg/l) may be attributed to decomposition of organic matter in the streams. According to Minaya, *et al.*, (2013) catchment land use activities such as agriculture increases the nitrate levels in the streams. The higher TP and SRP concentrations in Masese may be due to input of from the farmland and the settlement areas. Both right and left riparian zone of Masese had a freshly planted tea farm. In Tenwek stream, the evidence soap remnants on in-stream boulders indicated washing of clothes directly in the stream. It has been documented that most detergents contain high phosphate content and thus washing of clothes in the streams directly introduces phosphates into the streams water (Yillia *et al.*, 2008).

5.3 Leaf litter decomposition in the streams

Decomposition process resulted to reduction in remaining dry mass of *Eucalyptus saligna* and *Neuboutonia macrocalyx* leaves. The initial rapid reduction in % dry mas remaining between day 0 and 1 is attributed to the leaching. Study done by Peterson and Communis, (1974) showed that when leaves enter the stream water, there is an initial rapid loss of dry mass due to leaching. One days after leaching, the microorganisms colonize the leaves making it palatable for the macroinvertebrates to colonize (lag phase) (Newman *et al.*, 2015). In this study, the lag phase was evident between day 1 and 7 that was characterized by slow reduction in the % dry mass remaining. The low reduction rate evidenced in *Eucalyptus saligna* between day 3 and 27 could be attributed to the quality of the leaves. Unlike *Neuboutonia*, tough leaves that have inhibitory compounds that hinder microbial and macroinvertebrate colonization characterize *Eucalyptus* (García-Palacios, *et al.*, 2016). This in turn reduces the decay rate of the leaves hence a slow reduction in the percentage dry mass

In all the streams both *Eucalyptus* and *Neuboutonia* had processing coefficients ranging between 0.05 and 0.1 -k/day that indicated relatively fast leaf litter processing according to Peterson and Cummins (1974) classification. The decay rate was highly dependent on the quality of leaf litter and was highest in *Neuboutonia* and lowest in *Eucalyptus*. *Eucalyptus* leaves have a waxy cuticle, phenols and essential oils that negatively influence fungal and shredder colonization hence reducing decay rate (Pozo *et al.*, 1998). Canhoto and Graça

(1999) demonstrated that fungi could only penetrate into the leaf mesophyll of *Eucalyptus* through stomata and waxy cuticle cracks thus limiting fungal colonization on the leaves. In addition, the presence of oils located in glands in the *Eucalyptus* leaves have anti-biotic properties which hinder microbial colonization. These features of *Eucalyptus* reduce or suppress growth of aquatic hyphomycetes *in vitro* (Canhoto and Graça, 1999) and interfere with microbial enzymes (Canhoto *et al.*, 2002). The fast breakdown of *Neuboutonia* leaves may be related to lower contents of inhibitory compounds and toughness, which facilitate microbial colonization hence increasing leaf palatability to aquatic invertebrates. Studies by Mathuriau and Chauvet (2002) and Rosemond *et al.* (2002) showed that leaves with low levels of inhibitory compounds and toughness facilitate microbial colonization and hence faster decay rates.

The low *Eucalyptus* leaf decay rate recorded in this study agrees with the findings of Masese *et al.*, (2014) on *Eucalyptus* leaf decomposition in upper Mara catchment, Kenya. In the study, *Eucalyptus* had a decay rate of 0.02-k/day while *Neuboutonia* had a decay rate of 0.04-k/day.

The riparian zones of streams draining agricultural land were dominated by *Eucalyptus* species whose leaves end up in the stream channel. Unfortunately, the leaves are decomposed slowly compared to native species such as *Neuboutonia*. This may cause the streams to shift the relative importance of allochthonous organic matter to autochthonous sources of carbon for aquatic food webs (Minaya *et al.*, 2014).

According to this study, both tree species had a ki/kr score of 1 indicating that the decay of the leaves from both species were compromised by agricultural land (see Table 1). This implies that the decay rates of both *Eucalyptus* and *Neuboutonia* have been affected by the agricultural and other land use activities. The high temperatures and nitrates concentration (especially nitrates) could probably have caused high decay rates that were observed in the streams draining agricultural land (Grattan and Suberkropp, 2001). According to study done by Grattan and Suberkropp, (2001) in Payne Creek in USA showed that both phosphorous and nitrate addition stimulated decomposition rates in the stream.

5.4 Macroinvertebrate colonization

Macroinvertebrate colonization of leaf litter increased with time due to the conditioning of the leaves by microbial community. Leaf conditioning occurs within one to two weeks after immersion of the litterbags in stream water. This makes the leaves more palatable hence attracting the shredder-groups. The fact that similar macroinvertebrates families colonized both tree species may be attributed to the fact that the litterbags offered alternative food sources and habitat within the leaf pack (Mathuriau and Chauvet, 2002). Cortes *et al.*, (1997) working in the streams draining the mountains of Villa Real, in North Portugal demonstrated that there is low or limited attractiveness of artificial leaf bags for shredders. In their study, there was no significant difference in the relative abundance of shredders in leaf-bags and in the streambed. Although in the current study benthos was not sampled, it was assumed that the macroinvertebrates that colonized the litterbags originated from the benthos.

According to Masese *et al.*, (2014) the benthic zone of streams draining agricultural land in upper Mara catchment have lower macroinvertebrate abundance than those in forested land use. Conversely, in this study, the abundance of macroinvertebrates that colonized the litterbags was higher in streams draining agricultural land. This could be attributed to the fact that unlike streams in agricultural land, streams under forestland use have their substrate covered with dense mats of leaves and logs. This provides abundant food supply to the macroinvertebrates thus making the artificial litterbags less attractive. According to McCabe and Gotelli, (2000) disturbances lower diversity but increase the abundance. In this study, high abundance was recorded in the streams draining agricultural land that could be as result of the disturbances from agricultural activities. Baetidae dominated in the streams draining agricultural land that agrees with literature that one tolerant group becomes more abundant in the disturbed streams hence increasing the abundance (McCabe and Gotelli, 2000)

Streams draining forested catchment recorded higher macroinvertebrate diversity than streams flowing through agricultural land. Streams draining forested land had Shannon Weiner index of 10 while those under agricultural land had an index of four. High Shannon wiener index implies higher diversity while low index represents reduce divesity. *Neuboutonia* litterbags had higher diversity in both forested and agricultural land. Mbaka *et al.*, (2014), obtained similar results on the macroinvertebrates assemblages found in streams within Mount Kenya region. In that study, highly disturbed streams due to anthropogenic activities had lower diversity compared to the semi-pristine streams. Generally, the diversity of macroinvertebrates highly depends on the substratum particle size and heterogeneity that offers habitat to variety of macroinvertebrates (Brown, 2003).

In this study, the low diversity in the leaf litterbags exposed in streams draining agricultural streams may be due to the increased anthropogenic activities such as livestock grazing,

farming, water abstraction and deforestation within the sub-catchment that compromises the water quality (Pliūraitė and Mickėnienė, 2009). Poor water quality lowers habitat quality hence reducing the diversity of macroinvertebrates. Pliūraitė and Mickenienė (2009) studying the Lithuanian agricultural streams indicated that some macroinvertebrate taxa and diversity were negatively influenced by poor water quality. Masese et al., (2009) also recorded similar results in Moiben River in Rift Valley, Kenya. The study found that low macroinvertebrate diversities were associated with sites exposed to anthropogenic activities that compromised the water quality. Results from this study agree with the available literature on impact of anthropogenic activities on macroinvertebrates diversity. Studies done by Braccia and Voshell, (2007); McInnis and McIver (2009); Maldonado (2010); Virbickas et al., (2011), proved that anthropogenic activities affect benthic macroinvertebrate assemblages. However, the comparison of this study with the above previous studies should be done cautiously due to the difference in the methods used. In their studies, they sampled the macroinvertebrates in the benthic zone while the current study was only restricted to those found inside the a study in the streams of Central Apennines which showed litterbags. Sabatino, (2014) did that macroinvertebrates community of artificial leaf packs was different from the benthic samples. The study showed that benthic samples were richer and more diversified than the leaf pack.

The influence of the anthropogenic disturbance on the macroinvertebrates diversity observed in this study also concurs with the intermediate disturbance hypothesis. The intermediate disturbance hypothesis states that diversity is always highest at moderate levels of disturbance and lowest at high levels of disturbance (Larsen and Ormerod 2014). The human induced disturbance on the streams draining agricultural land hence caused the reduced levels of diversity. The low Shannon and Weiner index recorded in the agricultural streams therefore implies that the human induced disturbance have compromised the biodiversity of the system. The higher index recorded in streams draining forested land use on the other hand shows that the streams are moderately disturbed.

The macroinvertebrates were assigned to functional feeding group (FFG) at the family level according to Ramirez and Gutierrez, (2014). However, it should be noted that assigning macroinvertebrate FFGs to family level is limited. This is because some families are diverse with species within a given family belonging to different functional group. This implies that assigning of an entire family to one FFG may not be realistic. Proper functional group assignment should be done at the genus level to capture variability within a family. In this

study, macroinvertebrates were however assigned to FFGs at family level because currently there is no identification key that is based on the genus level. The FFG results therefore only represented a snapshot on what groups were associated with the litterbags.

The results obtained clearly indicated that not all the macroinvertebrates found in the litterbags were shredders. Different functional feeding groups were represented as shown in Table 11. This concurs with a study in Pannonia basin river system by Hoffmann (2005) in Central Europe. Hoffman, (2005) found out that macroinvertebrates retained in the litterbags belonged to different functional feeding groups. Filter feeders dominated litterbags exposed in streams draining forested land use (60%) while gatherers (64%) dominated those in streams draining agricultural land. The high abundance of filter feeders in forested streams was due to the dense mats of plant material on the streams bed hence more FPOM that attracted the filter feeders (Dangles *et al.*, 2001). According to Dangles *et al.*, (2001), the higher the amount of FPOM trapped in the litterbags the more macroinvertebrates will colonize the litterbag. Whereas, the high percentage of gatherers in litterbags exposed in streams draining agricultural land was due to availability of the already broken down CPOM.

More shredders were found in streams draining forested land use (13%) than in streams draining agricultural land (4%). The fact that the relative abundance of shredder invertebrates was very low (13% and 4% for forested and agricultural streams respectively) is as result of litter-bags acting more as FPOM retention structure for gatherers rather than a direct source of CPOM for shredders. The fact low numbers of shredders recorded in this study may be because generally shredders are few in tropical streams compared to the temperate streams as reported by Dobson *et al.*, (2003) in the Kenyan highland streams. Though shredders primarily feed on detritus, they also consume other food sources, such as algae and FPOM trapped within leaf packs (Dangles *et al.*, 2001). The shredders may therefore colonize the litterbags and feed on the algae and other materials in the litterbag and not necessarily on the leaves.

5.5 Ecosystem metabolism of Chepkosiom and Tenwek stream

The GPP and ER values observed in this study falls within the same range as those found in a study done by Bernot *et al.*, (2010) in temperate headwater streams in the United States that ranged between 0.1-16.2 g O₂ m⁻² d⁻ while ER ranged between 0.4 -23.1 g O₂ m⁻² d. Generally, Tenwek was more metabolically active than Chepkosiom. High canopy cover coupled with low temperatures results low GPP values (McTammany *et al.*, 2007). However,

the intact canopy provides a good source of organic matter for respiration. Human and animal activities on the riparian of Tenwek stream has resulted to reduced canopy cover and increased nutrient levels. According to Masese, et al., (2016) reduced canopy cover coupled with high nutrient level in streams draining agricultural land results to high GPP. This concurs with the result obtained in the current study. Tenwek (agricultural land) recorded a high GPP of 8.3 gO₂/m²/d while Chepkosiom (forested land use) had a GPP of 1.9 gO₂/m²/d. A study done by Elmore and West (1961) showed that increased temperature also increases the rate of primary production in streams. Generally, both streams were heterotrophic hence making them fall within the classification of other headwater streams in studies done in both tropics and temperate regions (Staehr et al., 2012). Heterotrophic systems imply that the streams are acting as a carbon source and not sink since respiration is higher than primary production. However, despite Tenwek being heterotrophic (agricultural land) it had higher GPP/ER ratio of 0.91. A high GPP/ER ratio indicates a high likelihood of a shift from heterotrophic to autotrophic stream status (Masese et al., 2016). Although Tenwek had higher GPP, it was compromised by increased turbidity. High suspended solids reduce light penetration to the primary producers through increased turbidity. The suspended solids also aid scouring the attached primary producers from their substrates (Peterson, 1996).

Both *Neuboutonia* and *Eucalyptus* enrichments caused a slight change in both GPP and ER in Chepkosiom (forested land use) stream. However, the stream remained heterotrophic due to high allochthonous organic matter input into the system. The intact riparian zone and high canopy cover of 98% provided the streams with abundant supply of high quality organic matter hence there was low demand for organic matter supply. The abundant organic matter supply observed supports the River Continuum Concept (RCC). According to RCC, it is predicted that allochthonous input in forested headwater streams is the main fuel of heterotrophic production (Vannote *et al.*, 1980). The enrichment did not affect the system because of low demand of DOC hence, no effect on ER levels. The increased DOC from the leachate enrichment also is likely to block light hence limiting primary production (Griffiths *et al.*, 2013). Unlike Chepkosiom, Tenwek recorded a high GPP/ER (0.91) ratio that indicated that it was almost shifting to autotrophic status.

Eucalyptus enrichment shifted Tenwek stream from heterotrophic to autotrophic while Neuboutonia had no effect. Tenwek stream was characterized by Eucalyptus tree plantation on both right and left riverbank. This implies that the organic matter that was in the streams

was mainly made of *Eucalyptus* leaves and branches. Unlike *Neuboutonia*, phenols, low nitrogen content and essential oils that negatively influence microbial respiration (May and Ash 1990; Canhoto, et al., 2002) characterize *Eucalyptus* leachate. The *Eucalyptus* leachate therefore suppressed the activity of the microbial respiration hence making ER to be lower than GPP. *Neuboutonia* leachates increased the amount of better quality DOC for the microbial community hence increased ER that made the streams to be more heterotrophic. A study done by Lennon and Pfaff, (2005) in New Hampshire, USA showed that bacterial metabolism is influenced by the chemical composition and quality of the DOC source.

Leaf leachate is a form of dissolved organic carbon (DOC) and represents a large proportion of organic matter pool in streams (Wetzel, 1992). Microbial decomposition of dissolved organic carbon (DOC) contributes to overall stream metabolism and can influence many processes in the stream ecosystem. Dissolved organic carbon in streams can be affected by recent environmental and land use changes within the watershed area. A study done by Masese et al., (2016) showed that the quality of the available DOC has direct effect on the metabolism rates. The shift in the quality and the availability DOC occurs as result of change in the land use activities. In this study, agricultural activities resulted to replacement of the native tree species such as *Neuboutonia* with exotic tree species such as *Eucalyptus* hence resulting to a change in the DOC quality. For instance, presence of low molecular weight DOC from allochthonous sources increases the heterotrophic activity and faster degradation of organic matter (Matheson et al., 2012). This may explain why *Neuboutonia* leachates increased ER especially in Tenwek stream unlike *Eucalyptus*.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

This study clearly shows that human land use activities have an influence on stream ecosystem functioning. Streams under agricultural land are exposed to activities such as agriculture, logging, human settlement and grazing. These activities have resulted to degradation of the streams through replacement of indigenous tree species such as *Neuboutonia* with exotic ones like *Eucalyptus*. Fungi and shredders less prefer *Eucalyptus leaves* due to its toughness, hence reduced efficiency of the detritus food web to higher trophic level. In summary:

- 1. Physico-chemical variables of the Upper Mara catchment streams varied significantly with land use, hence the first hypothesis was rejected.
- 2. Neuboutonia macrocalyx decay rate was significantly higher in streams draining agricultural land use while Eucalyptus saligna leaves decay rate did not differ significantly with land use. The second hypothesis was therefore rejected with respect to Neuboutonia macrocalyx but accepted based on Eucalyptus saligna.
- 3. Macroinvertebrates composition, abundance and diversity did not significantly vary with tree species however, abundance and diversity significantly differed with land use. Therefore the third hypotheses was rejected based on land use but accepted with respect to tree species
- 4. Eucalyptus saligna leachate enrichment shifted the trophic status of the stream draining agricultural land use from heterotrophic to autotrophic while Neuboutonia macrocalyx leachate had no effect on both streams. Consequently, the fourth hypothesis was rejected based on Eucalyptus saligna but accepted with respect to Neuboutonia macrocalyx.

6.2 Recommendations

- Agricultural activities in the riparian zones have increased land use pressures on the stream ecosystem resulting to changes in the physico-chemical variables.
 Environmental organizations such as NEMA and Kenya Forest service should therefore increase awareness to farmers on the importance of maintenance of the riparian zone.
- 2. The replacement of the native tree species and practices of agricultural activities within the riparian zone have negatively affected stream processes such as leaf litter decay, consequently influencing systems energy flow negatively. This requires more enforcement and policing implementation of riparian zone protection laws to ensure protection of these zones and minimize replacement of indigenous riparian vegetation.
- 3. Benthic macroinvertebrates play a critical role in the stream food web. Agricultural land use has however affected the abundance and diversity of the benthic macroinvertebrates in the Upper Mara streams hence compromising their functions. Sustainable use of riparian zone such as maintenances of the buffer strip and allowing occurrence of natural vegetation should therefore be encouraged to ensure conservation of the macroinvertebrates biodiversity.
- 4. Information obtained in this research shows that land use change from forest to agriculture has altered stream metabolism from dominance of heterotrophy to autotrophy. The results also indicate that *Eucalyptus saligna* should not be planted within the riparian zones because it shifts the stream metabolism from heterotrophic to autotrophic. Therefore, the local community should be educated on the importance of indigenous tree species (*Neuboutonia*) to stream ecosystem as opposed to exotic species (*Eucalyptus*).

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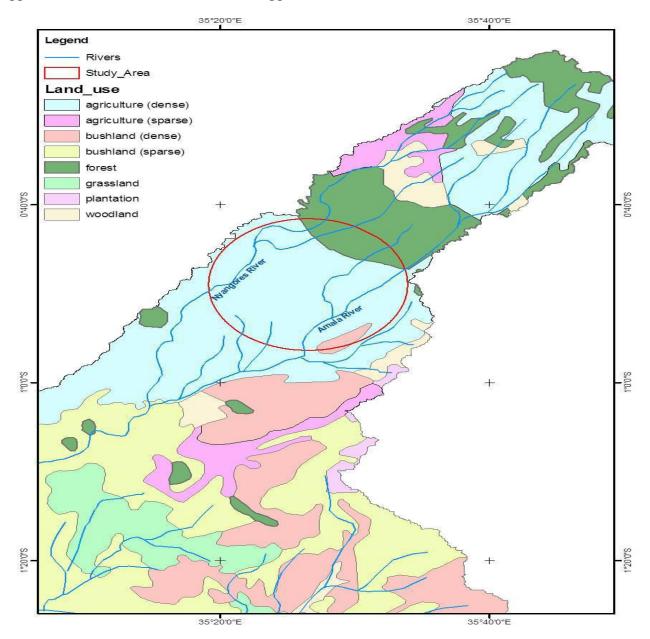
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APPENDICES

Appendix 1: land use characterization of upper Mara catchment



Appendix 2- k/day values of *Eucalyptus* and *Neoboutonia* leaves calculated from exponential decay equations for streams draining in forested and agricultural land (E = Eucalyptus and N = Neoboutonia. * means data missing).

land use	<u>stream</u>		<u>Day 1</u>	Day 3	<u>Day 7</u>	Day 14	<u>Day 28</u>	Day 42	Day 56	Mean
Forested	Mosoiot	<u>E</u>	0.229	0.094	0.019	0.005	0.020	0.027	0.022	0.059
		<u>N</u>	0.292	0.108	0.026	0.006	0.034	0.029	0.022	0.074
	Chepkosiom	<u>E</u>	<u>0.219</u>	0.086	0.042	0.024	0.020	0.029	0.022	0.063
		<u>N</u>	0.253	<u>0.109</u>	0.050	0.053	0.072	<u>*</u>	*	0.107
	<u>Sambambwet</u>	<u>E</u>	<u>0.186</u>	0.079	0.040	0.022	0.017	0.029	0.022	0.057
		<u>N</u>	0.326	0.098	0.073	0.066	0.074	<u>*</u>	*	0.128
Agricultura	<u>I</u>									
	<u>Masese</u>	<u>E</u>	0.205	<u>0.100</u>	0.052	0.026	0.018	0.029	0.022	0.064
		<u>N</u>	0.265	0.088	0.058	<u>0.110</u>	0.118	<u>*</u>	*	0.128
	<u>Kapsebet</u>	<u>E</u>	0.200	0.094	0.038	<u>0.026</u>	0.033	0.029	0.022	0.063
		<u>N</u>	0.244	<u>0.124</u>	0.067	<u>0.110</u>	0.115	*	*	0.132
	Tenwek	<u>E</u>	<u>0.161</u>	0.082	<u>0.046</u>	0.027	<u>0.054</u>	0.029	0.022	0.060
		<u>N</u>	0.234	0.070	0.087	0.071	0.107	*	<u>*</u>	0.114