



Impact of Land use on water quality, sediment composition and functional response of microbial communities in three streams of Nzoia catchment.

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Declaration.

I hereby declare that I am the sole author of this work; no assistance other than that permitted has been used and all quotes and concepts taken from unpublished sources, published literature or the internet in wording or in basic content have been identified by footnotes or with precise source citations.

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List acronyms and Abbreviation

AFDW	Ash Free Dry Weight
APHA	American Public Health Association
AWCD	Average Well Colour Development
BOD	Biological Oxygen Demand
Chl <i>a</i>	Chlorophyll <i>a</i>
cm ²	Centimetres Squared
COD	Chemical Oxygen demand
DO	Dissolved Oxygen
EC	Electrical Conductivity
m ³ /s	Cubic meter per second
ml	Milelitre
mm	Molar mass
NH ₄	Ammonium
NH ₄ CL	Ammonium Chloride
nm	Nanometer
NO ₂	Nitrite
NO ₃	Nitrate
O ₂	Oxygen
°C	Degree Celsius
OD	Optical Density
OM	Organic Matter
PCA	Principal Component Analysis
pH	Potential of Hydrogen
SRP	Soluble Reactive Phosphorous
TDS	Total Dissolved Solid
TP	Total Phosphorous
µg/cm ² /h	Microgram per Square centimetres per hour
µg/gDW/h	Microgram per Gram dry weight per hour
µg/l	Microgram per litre.

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ABSTRACT

Land use change from natural forests to crop land has changed catchments worldwide. The world growing population that needs more space for economic and industrial development is a key driver to this change. The massive deforestation in the recent years is majorly to acquire productive land for crop production, to meet the nutritional needs of the growing population. Yet these changes in land use have impacts on environment especially the aquatic ecosystems and their related biota. This study in three tributaries of the Nzoia system in Mt Elgon western Kenya, aimed at investigating the influence of the shift from forested to agricultural land use on stream functioning in relation to water quality, bacterial abundances, diversity and their responses to stress from the changed land use.

Parameters (Temperature, discharge, dissolved oxygen, electrical conductivity and pH) of the streams (Kapkateny, Kimurio and Teremi) sampled sites, where measured insitu and 60 water samples were collected for analysis of nutrient properties, (SRP, TP, TN, NH₄, NO₂, NO₃ and chlorophyll *a*). Sediment samples were collected for analysis of, sediment grain size distribution (30 samples), (160) samples for microbial oxygen consumption rate, system productivity, bacteria abundances and functional group diversity. Sediment grain size distribution within the streams was classified and grain size <2 mm was considered as fines. Microbial oxygen consumption rate and primary production were measured as a change in oxygen concentration in incubated chambers subjected to dark and light conditions at room temperature for respiration and primary production processes. Bacteria abundances were investigated using the fluorescent staining method and functional group diversity was assessed using carbon substrate utilization.

Several limnological parameters were significantly different between the agricultural and forested stream sections, with high nutrient concentrations and more fine sediment accumulation recorded in agricultural stream sections, as well as high oxygen consumption rates, system primary productivity and bacterial abundances. However, functional group diversity was low in agricultural stream sections. It's worth noting that agricultural land use compared to forested land use, heavily, negatively influences high nutrients concentrations, alters microbial activities (respiration rate), affects biota numbers and diversity in aquatic ecosystem.

CHAPTER ONE

1.0 INTRODUCTION

Pronounced clearing and conversion of forests to other land use types has highly impacted aquatic ecosystems biota as well as their functions in nutrient recycling and distribution (Qu et al., 2017). Up to the early 1980s, land transformation was mainly for industry and infrastructure development (Vitousek et al., 1997). By the 1990s, the tropical forest loss was estimated at 15.2 million hectares (FAO 2013), due to socioeconomic pressure (Rounsevell et al., 2003). Currently, the rapid changes in land use is as result of agriculture, with about 6 million square kilometers of forest and grass lands being converted yearly to crop production fields, leading to a global extinction (Ramankutty and Foley, 1999; Tsai, 2019). Africa's dominant ecosystems and the mountainous hills are one of those facing rapid conversion as a result of fast-growing population and need for more crop production units (Chenje, 2000; Lambin et al., 2003, Ramankutty and Foley, 1999). These conversions have disproportionate impact on aquatic ecosystems functioning and their biota (Myers et al., 2000; Underwood et al., 2009). As well as a change in environmental patterns and its capacity to retain soil, nutrients, water, organic matter and self-purification especially in rivers and streams (Permatasari et al., 2017).

Denude land cover predisposes disturbances to soil surface, loosening its cohesive and adhesive structure, reducing water retention capacity, thereby accelerating soil wash down and deposition into aquatic systems during rain events (Davies-Colley et al., 2015). The changed land cover further increases system pollution, contamination and biodiversity loss (Pienkowski and Beaufoy, 2002; Xiaolan, 2009), together with alteration of stream flows, habitat loss, increased water temperature, and increased terrestrial inputs (Burdon et al., 2013). Many studies have shown clear correlation between agricultural land use and poor water quality (Holden, et al., 2015), because most agricultural inputs including nitrogen and phosphorous get into the aquatic system through surface run off (Ferrier et al., 2001). Sediments and pollutants deposition, nutrients and organic matter loading, from agricultural sites lead to oxygen depletion, eutrophication, habitat degradation, increased habitat loss, impairment of microbiota ecological functioning and diversity (Baker, 2006; Burdon et al., 2013, Zhange, 2015).

In East Africa, Kenya is one of the countries, whose larger population entirely depends on natural systems like streams, rivers and lakes for domestic and agricultural production. Yet there is a limited number of such resources and the available ones are increasingly faced with anthropogenic disturbances such as catchment degradation through agriculture. Which directly increases high nutrient loading, sedimentation in streams and alteration of the hydrological patterns, morphological structures and functioning. Though studies have been carried out in most aquatic systems in Kenya (Nadir et al., 2012; Tenge et al., 2015; Achieng and shikuku, 2019), focus was limited to water

column changes. Little attention has been given to sedimentary microbial functional groups and their processes.

1.1 Problem Statement

Rapid population growth is highly being experienced in Africa. Currently, the growth rate is at 3% annually and projections have been made that by 2025 the African population will be 1.5 billion at 19% increase. The increase is highly observed among the youth (UN-library 2019). East Africa is equally faced with the same challenge as other countries within the continent. The population growth rate between 2013-2017 was at 6.7%. Though the region is experiencing rapid economic growth, challenges such as unemployment still persists (Paul et al., 2017), leaving societies with no economic option rather than high exploitation of natural resources and engagement in agriculture for livelihood.

Mt Elgon catchment natural forests are one of those that have largely been converted to agricultural fields. The acquisition of the land is either through massive forest logging or burning. The land is deeply ploughed to remove the tree root remains. The opened gardens are cultivated throughout the year majorly for maize, cabbage, onion and Irish potatoes growing. The farmers in the area use chemicals and fertilizers for pest and disease control and soil fertility maintenance to boost high yields. The farmers have little or no knowledge of soil erosion control measures and the area experiences high surface run off, which has noticeably increased soil erosion.

In response to some of the problems associated with the negative impact of these anthropogenic activities, scholars have carried out more studies in aquatic systems but mainly focusing on the water column, which apparently has been well understood by many scientists. However, less attention has been paid to sedimentary processes and microbial functions, despite the fact that, sediments are habitat for microbial organisms and they provide a mat for major microbial processes that ensure ecological health and stabilization of aquatic ecosystems through carbon and nutrient recycling, nutrient flow to higher trophic levels, organic matter decomposition and whole river respiration (Hall and Meyer, 1998; Hieber and Gessner, 2002). Therefore, the goal of this study was to assess the impact of land use on microbial functional responses and diversity in stream ecosystems.

1.2 General objective.

To assess influences of land use change on the sediment microbial composition and their functional responses in three streams within Nzoia catchment, Mt. Elgon area.

1.3 Specific objectives.

- i. To determine the influence of land use on water quality and sediment composition.
- ii. To assess variation of sediment microbial oxygen consumption rate and primary production along the stream continuum in relation to forest and agricultural land uses.

- iii. To determine stream microbial respiration responses and primary production to nitrogen addition.
- iv. To compare microbial abundances and functional groups diversity in sediments exposed to different land use practices.

1.4. Hypothesis.

There are significant differences in stream sediment composition and nutrient concentrations between agricultural and forested land uses with high fine sediment and nutrient accumulation in agricultural stream sections.

There is a significantly higher sedimentary microbial oxygen consumption rate and stream primary productivity in stream sections under agricultural land use compared to sections with dominant forested land use.

Stream ecosystem sedimentary oxygen consumption rate and primary production will increase with nutrient addition in both agricultural and forested stream sections.

Functional group diversity and microbial abundances will significantly be higher in stream sections under agricultural land use than in forested land uses.

1.5 Justification of the study.

The fast-growing population in East Africa is faced with a challenge of food scarcity due to dependence on rain fed agriculture and exhaustion of the limited available cultivatable area. Other options such as fisheries have been considered to bridge the gap, but the state of the food basket remains alarming. This pressure has led to encroachment and destruction of natural environments such as grass land and natural forests to create space for agricultural production.

In East Africa, Mt Elgon region forested areas is rapidly being converted to agricultural production units, yet the area has importance streams and rivers that are major water supply sources to both Ugandan and Kenyan communities. Society needs to understand how these changes may impact on aquatic ecosystem functions and effects they have on the related microorganisms, which play a key role in maintaining the river system health. Therefore, this research seeks to increase understanding of how land use change can impact rivers/stream systems and further provide evidence for better policy making, need for restoration and management of such important resources.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1 Effect of land use change on stream water quality

Land use is an anthropogenic act of continuous interaction of man and environment, while using and altering the natural resources and available space for agricultural production, economic development, conservation and recreation (Paul and Rashid, 2017). The different land use types have been noted to have impacts on aquatic systems (WWAP, 2017).

Agriculture production being one of the common land use type, has been for a long time noted for water abstraction (Bharati et al., 2002). Recently, it has been recognized as one of the biggest contributors of inland water contamination with about 70 percent pollutants emanating from such land use (UNEP, 2016). The observation has been made in both, developing and developed countries (WWAP, 2017). In European water bodies, 38 percent of the freshwater pollution is from agricultural activities (WWAP, 2017), the same trend has been observed in US river systems (US EPA, 2016) and in China, water nitrogen pollution is mainly from agricultural land use (FAO, 2013).

Agriculture production in the recent years has shifted from conventional methods to intensification of cropping and livestock systems with high levels of chemical input use, so as to meet the food gap faced by the increasing population, but this has put more pressure on water quality and general aquatic health (FAO, 2013). The increased use of new agricultural chemicals such as hormones (growth promoters), vaccines, antibiotics and pesticides have come a long with the intensification of the system (Lambin et al., 2003). The residues from these intensive agricultural production units finally get drained into adjacent water bodies through nonpoint or point pathways (Stonestrom and Zhang, 2009; Yu et al., 2013).

The intensified agricultural practices which have led to clearing of land vegetation cover to increase production units for specific crops (mainly produced by mono cropping), with high fertilizers and chemical use (Angima et al., 2003), do not take into account soil erosion control measures (Blanco and Lal, 2008), thus increasing surface runoff, alter hydrological patterns, increase sediment, pesticides and pathogenic loading into aquatic systems (Nafziger, 2009). These accumulations affect the oxygen concentrations, temperature, aquatic organisms and general water quality (Malone, 2009).

These agricultural residues especially phosphorous, nitrogen and their forms may result into toxic organisms like toxic algal blooms and bacteria (Holden et al., 2015), that disrupt the functioning of aquatic organisms and deteriorate water quality (Tsegaye et al., 2006; Zhange et al., 2012). A survey carried out by (Bricker et al., 2008), reported that most of the national estuaries eutrophication was a result of nutrient input from agricultural areas. Agricultural crop residues,

animal manure and pesticides were found to be persistent with long residence period in the Midwest and Mississippi river (Goolsby et al., 1999).

The accumulated agricultural residues which may include minerals and dissolved salts for example *selenium* leached from agricultural production inputs as observed in the southern plains of pacific, cause biological effect and deformation of body parts of aquatic organism and affect their performance of nutrient recycling and system purification which impacts the system water quality (Lenat and Crawford, 1994; Boëchat et al., 2011; Lyautey et al., 2015).

Large quantities of terrestrial soil deposition have equally been noted to be higher in agricultural areas and have been reported to cause high suspended solids, turbidity (Davies et al., 2001, 2015; De-stigter et al., 2007) and limit light penetration, therefore affecting photosynthesis and other processes (Giri, 2013, 2016; Haygarth et al., 2002; Meyers and Teranes, 2002). These deposits and other terrestrial inputs normally get into aquatic systems with biologically active solutes which cause high biological oxygen demand (BOD)(Garnier et al., 1999, 2001) and chemical oxygen demand (COD) (Uriarte et al.,2011, Lee, 2003, 2009), which lead to anoxic conditions both in the water and sediment columns (Droppo et al., 2009).

Other practices such as clearing of forested areas for agricultural production alter hydrological patterns (Odira et al., 2010), increase surface runoff which affects the rivers drainage design, increase riverbank erosion, facilitates increased downstream sediment loading (Holden et al., 2015). Cleared catchments expose stream and river waters to direct solar heat thus increasing the water temperature and alter system chemical composition. These coupled with high turbidity affect gas solubility and reduces gas concentrations held in the system, especially dissolved oxygen which is important for system processes like decomposition (Holden et al., 2015; Bailey et al., 2000). A survey carried out by (FAO, 2013) between forested and agricultural land uses, showed that, the root network, under growth and leaf litter within the forested areas offer effective soil erosion control, filtering and retention potential of terrestrial pollutants, reduce surface runoff and prolong retention time, which allows uptake, break down and utilization of elements like potassium, nitrogen and phosphorus (Broadmeadow and Nisbet, 2004; Gundersen, 2007).

2.2 Influence of land use on sediment composition and functions.

Sediments are an important part of the aquatic environment. Their heterogeneous composition provides various ecological niches to microbes and microbial activities (Zhang Ran and Chen, 2015). According to (Brenner and Mondok, 1995; Surian, 2002), land use types such as agriculture do not affect only water quality, but equally alters the sediment budgets (Van Rompaey et al., 2001; Angima et al., 2003; Valentin et al., 2008) by increasing fine sedimentation and altering fluvial grain size distribution.

Ultimate grain size distribution is vital in riverbed stabilization, habitat provisioning, regulating water physicochemical properties and aquatic processes (Bakkar et al., 2008). Clogging of interstitial spaces, burial of coarse bed material (Bailey et al., 2003) reduce substrate surface for periphyton and microorganism attachment.

Continuous sediment deposition and accumulation alters the intrinsic properties of the sediment structures, hydraulics and morphology of the rivers and streams (Di Stefano and Ferro 2002). The high enrichment of silt and clays from the agricultural runoff is equally a source of pollutants (Rhoton et al., 2011) and this affect the fining process downstream. The type and magnitude of sediment fluxes into river systems directly impacts aquatic habitat (Syvitski et al., 2005) and facilitates biodiversity extinctions (Watson et al., 2000).

High deposition increases nutrient retention and occasional resuspension of pollutants in the water column (Newcombe & MacDonald, 1991), thus increasing turbidity (Wood and Armitage 1997) and affecting growth and functional ability of microbial communities (Newcombe and MacDonald, 1991; Zaidi et al., 1999). According to (Likens, 1995; Angima et al., 2003) soil erosion and soil delivery into river and streams was reduced under the conserved forests and grass land but high in agricultural areas. Other studies suggested that reintroduction or conversion of agricultural land to perennial vegetation has been recorded to influence delayed and reduce delivery of sediment, water and pollutants (Hill, 1996; Bharati et al., 2002; Lee et al., 2003; Schultz et al., 2005; Randall, et al., 2013). Forested land cover minimizes erosion and sediment transportation into river systems, reducing the effects of turbidity and alteration of sediment (Brown and Binkley, 1994; Seitzinger et al., 2010). Further studies by (Adhikari et al., 2002; Sikka and Selvi, 2005; Nainar et al., 2017) noted that minimal human interferences reduce adverse effects on the water quality, due to limited sediment deposition and inflow into water bodies.

2.3. Impact of land use change on river system primary production and respiration

Primary production and respiration are a basis of river health. The ratio between the two is used to characterize the ecological functioning in the river and stream continuum. A balance between production (autotrophic metabolism) and respiration (heterotrophic metabolism) ensure system health stability (Vörös, and Padisak, 1991; Hill et al., 2002).

In natural undisturbed stream systems, production to respiration ratio P/R varies longitudinally, in forested head waters a value <1 has been observed and $P/R > 1$ in the mid reaches followed by adown stream decrease with the P/R being <1 (Vannote et al., 1980; Vörös, and Padisak, 1991). Observations by (Garnier et al., 1999), reveal that, in low order streams with less human perturbation, more heterotrophic activity is observed due to less growth of algae and macrophytes as a result of less light penetration but rather allochthonous organic matter input and its breakdown leading to high oxygen thus $P/R < 1$ (Garnier et al., 2001). A similar trend is observed in the lower reaches, but this is mainly due to high influx of particulate material that increase

system turbidity and limit light penetration. In the mid reaches production to respiration is greater than 1 (Stout, 2003).

Primary production directly relates to the amount of organic matter produced at a given surface area (Cloern et al., 2014) and its majorly carried out by phytoplankton and regulated by the availability of nutrients (phosphorous and nitrogen). Photosynthesis to a small extent is carried out by some autotrophs in the category of benthic autotrophs and to a larger extent by photosynthetic bacteria (cyanobacteria), blue green algae and macro vascular plants (Wetzel, 2001; Thomas et al., 2005). The quality, quantity of organic matter and nutrients recycled and available by microorganisms for primary production are dependent on catchment land use type and inputs (Malcoln and Stanley 1982; Ward et al., 1990).

A direct effect of land use change on stream water nutrients, light penetration and oxygen amounts held in the system, are transformed to primary production resource alteration. Nutrient concentrations normally determine system biomass although other stream system factors such as oxygen, temperature and light also play a big role (Opdyke et al., 2006; Church, 2006; Allan Castillo, 2007; Schiller et al., 2007). System productivity may shift from heterotrophic production to autotrophic when nutrient concentrations exceed threshold levels (Liboriussen and Jeppesen 2002; Sabater et al 2011), and stream carbon processing of both bound and particulate forms entirely done by microbial metabolism may be affected (Butman et al., 2016).

System respiration like other aquatic processes is very important especially in the break down and distribution of materials, energy flow in streams, regulation of dissolved organic carbon and organic matter in sediment (Seiki et al., 1994; Caldwell and Doyle, 1995; Pascoal et al., 2005; Cloern, et al., 2014). This process is influenced by sediment structure and particle sizes (Kaplan and Bott 1985; Santmire and Leff, 2007), because sediments structure provide colonization surface for microorganism and give a bed mat to facilitate the organic particle breakdown (Petersen et al., 1989; Vance and Chapin, 2001). The quality of the sediment structure and its distribution in stream is highly dependent on land use type from which the different inputs with diverse chemical composition are released. (Qu et al; 2017; Schimel et al., 2007; Silva-Junior et al., 2014).

2.4 Influence of land use change on microbial functional diversity and abundancies.

Microbial diversity is of great importance, due to their association with energy and organic matter transformation. Clear knowledge about their community structures and diversity with an insight of the relationship between environmental factors like perturbation, pollution, global changes and the ecosystem function need to be well understood (Torsvik et al., 1996). Stream sediment microorganisms play a very important role in nutrient and organic matter recycling as well as ecosystem stability, functionality and energy flow through different processes within the system (Singh et al., 2011). They mobilize organic or chemical energy source and mediate elemental fluxes (Orcutte et al., 2011). Other processes such as denitrification, nitrification and

rem mineralization of organic matter with electron acceptors are equally carried out by microorganisms (Thamdrump and Dalsgaards 2002; Mrozik et al., 2014).

Degradation of organic phosphorous to soluble forms (PO_4), nitrogen cycling,(Bernot et al., 2010) degradation of dissolved and particulate organic matter that have a high influence on the nutrient balance in the water column (Gächter and Meyer, 1993) are done by microbial communities. Other important roles played by microorganisms in aquatic ecosystem structures and processes are system purification (Silva-Junior et al., 2014; Mrozik et al., 2014; Bernot et al., 2010). The microorganisms also facilitate oxygen exchange between the water-sediments inter phase (Davies-Colley et al., 2015). Fundamentally, their extracellular enzymatic activity within the system provides information about the source and amount of organic matter available in the system (Boschker and Cappenberg, 1998).

However, their distribution, composition and diversity are endangered because the microbial habitat is highly degraded as large amounts of soil and plant residues from agricultural areas are deposited and stored in sediments and can cause clogging (Kolten et al., 1997). The lost terrestrial soils deposited in river systems result into sediment addition, accumulation and alteration of its structural composition, degradation of the habitat quality for invertebrates and microorganisms (Lenat and Crawford, 1994). A shift or disturbance of their habitat and compositional structure effects the entire ecosystem functioning and their functional diversity (Zhange, 2015). Sediment accumulation affects microbial nutritional mat quality and biofilms composition which affects their diversity and distribution in a system (Boëchat et al., 2011, 2014).

Pesticides, heavy metals and other toxicants bound in silt and clay coming from agricultural land end up polluting the system and affecting the microbial community composition and their activities. Such pollutants are absorbed into their bodies and can cause damages and death (Freel et al., 2012). According to (Allison and Martiny, 2008; Sun et al., 2012), the most common threat to ecological health of streams is environmental sediments pollution because it influences the functional diversity and abundance of microorganisms. Other observations on microbial diversity decrease were made by (Wang et al., 2012; Giri and Qiu, 2016; Kochling et al., 2017), who reported that environmentally polluted tropical rivers had limited microbial communities and variance in water quality dissolved oxygen amounts, pH and water temperature that were influenced by agricultural land use also influenced their diversity.

Accumulation of pesticides and other chemicals with long residence time and degradation processes change the structural community and microbial diversity in sediments (Ito et al., 2016). The accumulations may lead to system eutrophication, resulting into ineffective and inefficient breakdown of organic matter, high oxygen depletion which causes anoxic conditions in the sediment bed and may result to microbial death (Muturi et al., 2017; Mrozik et al., 2014). These degradations and loss of quality habitat in ecosystems results into reduced microbial growth and

functioning which may later impact on their diversity (Karatayev et al., 2005; Hepp and Santos, 2009; Nalepa, 2007).

Studies have shown that, structural composition and diversity of aquatic microbiota can be controlled by stochastic dispersal and recruitment of terrestrial species (Logue and Lindström, 2008; Battin et al., 2016; Niño-García et al., 2016). Further suggestions were made by (Crump and Hobbie, 2005; Widder et al 2014; Niño-García et al., 2016) that hydrological patterns, stream flows, and physicochemical properties that are influenced by land use type may shape microbial community structuring , dispersal and habitation time.

CHAPTER THREE

3.0 Materials and methods

3.1 Description of the study site and land use pattern

The research was carried out in three third order wadable streams with similar land use pattern originating from Mt Elgon in Kenya. The streams; Kapkateny, Teremi and Kimurio are in the western part of Kenya (Figure 1) and the drain into the Nzoia river. All the three streams are located at an altitude between 1878m to 2239m above sea level and lie between $00^{\circ} 47' 37.23''$ N- $00^{\circ} 53' 50.31''$ N, and $34^{\circ} 33' 50.4''$ E- $34^{\circ} 45' 45.54''$ E. The area has volcanic soils and receives bimodal pattern of rain fall with over 1,270 mm annually. Minimum and maximum temperature range between 15° c and 25° c. The upper reaches of all three streams had over 85% forest cover with different trees, shrubs, herbs and grass species while the lower catchments have been converted to settlement and agricultural production units. In-streams of the forested sections were narrow with fallen tree branches, riffles, pools and shallow clear water, while those in agricultural stream sections had highly eroded banks, widened channels, plants and terrestrial residues, animal droppings, pools with high suspended matter and highly turbid water.

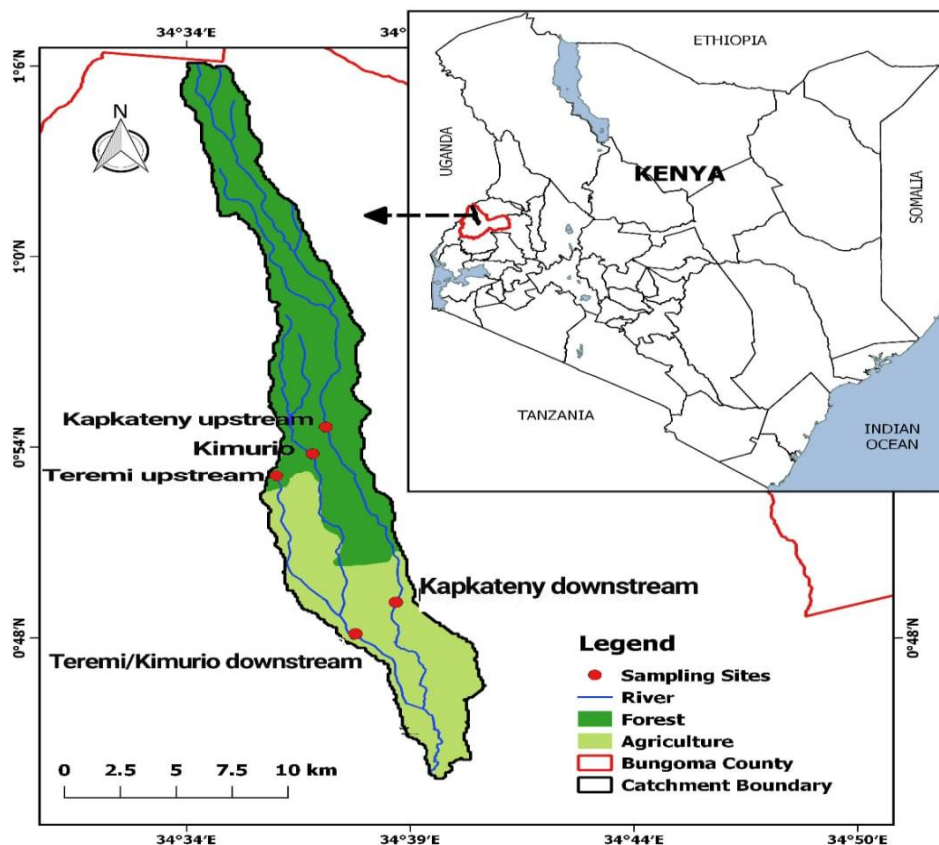


Figure 1. Map showing land use and sampled streams of the Nzoia catchment Mt Elgon Kenya

The sampled areas basically had two dominating land uses :- forested and agricultural. The forested areas had a mixture of different tree species and grasses, while the agricultural areas were dominated by poor agricultural methods such as continuous ploughing on slopes, poor soil erosion control measures, eroded fields, gullies, rills (surface runoff passages), poorly managed crop residues, high use of chemicals and fertilizers. The major crops grown in the mid and lower catchments include Irish potatoes, onion, tomatoes, cabbage and maize, with an integration of cattle keeping and grazing.

3.1.1 Sampling design.

Sampling of some samples was done on weekly basis in the month of November (2019), at five sites, three upstream (forested) and two down stream (agricultural land use) sites of Kapkateny, Teremi and Kimurio. A total of 60 measurements, 12 per site were done for physico parameters and 12 water samples were collected per site for nutrients analysis. A total of 160 sediment samples, 32 per site, 8 per sampling time were collected for measurement of microbial oxygen consumption rate and primary production, while for sediment grain size analysis, bacteria abundancies and functional group diversity, samples were collected once. A total of 30 sediment samples, 6 per site were collected along a stream stretch of 50 meters, at a depth of 10cm for grain size and triplicates samples were collected at a depth of 5cm for bacterial abundancies and functional group diversity analysis.

3.2 Field measurements and water samples collection for limnological parameters analysis.

The physicochemical parameters (Temperature, Dissolved oxygen, pH, Electrical conductivity) were measured insitu using a multimeter probe (HACH 40d). Stream width, depth and discharge were measured using tape measure, deep stick and flow meter (OTT MF Pro- OTT Hydrometer) respectively. Water samples for analysis of chemical parameters were collected using acid washed (10% H₂SO₄) bottles in triplicates and stored in the cool box for transportation to the laboratory for analysis. The samples were filtered immediately on arrival with GF-F filters and analyzed for total phosphorous (TP), Soluble Reactive phosphorous (SRP), dissolved inorganic nitrogen (DIN), according to (APHA, 2005).

3.3. Nutrient analysis

Different nutrients were analyzed; Ammonium-Nitrogen (NH₄-N), Nitrate-Nitrogen (NO₃-N) and Nitrite-Nitrogen (NO₂-N) concentrations were determined using standard methods as described in (APHA, 2005). The NH₄-N was determined through sodium salicylate method, where 2.5 ml of sodium-salicylate solution and 2.5 ml of hypochloride solution were added to 25 ml of filtered water samples from the studied streams. The samples were then incubated in the dark for 90 minutes, after which, the absorbencies were read through scanning spectrophotometer at a wavelength of 665 nm using a (GENESYS 10 uv). Nitrate-Nitrogen was determined through

sodium-salicylate method, where 1ml of freshly prepared sodium salicylate solution was added to 20 ml of filtered water sample. The processed samples were then placed in the oven to evaporated to complete dryness at 95°C. The resulting residues were dissolved using 1ml H₂SO₄ acid, followed by addition of 40 ml of distilled water and 7 ml of potassium-sodium hydroxide-tartrate solution respectively, and their absorbencies were read at a wavelength of 420 nm. Nitrite-Nitrogen was analyzed through a reaction between sulfanilamide and N-Naphthyl-(1) ethylenediamin-dihydrochloride and absorbencies read at a wavelength of 543 nm. The final concentrations of NH₄-N, NO₃-N and NO₂-N were calculated using respective equations generated from their 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 was added to the filtered water sample at a ratio of 1:10 and the absorbencies were read at 885 nm wavelength using a (GENESYS 10 uv) scanning spectrophotometer after 15 minutes of reaction and concentrations were determined from known concentrations of standard solutions (APHA, 2005). Total phosphorus (TP) was determined by persulphate digestion of unfiltered water to reduce the forms of phosphorus present into soluble reactive phosphates (SRP). After the digestion, evaporated water was replaced, and TP was analyzed as SRP using ascorbic acid method. The concentration of TP was determined from a similar process of known concentrations of phosphorus standard solutions (APHA, 2005).

3.4. Sediment grain size analysis by dry sieving method.

A total of 30 samples were collected with a representation of six replicates per site. Sediment samples were collected using a shovel at about 10 cm depth from forested and agricultural stream sections in all the three streams, packed in clean polythene bags for transportation to the laboratory for analysis of sediment grain sizes. The samples were dried at 90°C for 24 hours. The initial weights of the dried samples were recorded, samples were sieved through 12 mm, 10 mm, 8 mm, 6 mm, 4 mm, 2 mm, 1 mm, 0.5 mm stack sieve and the retained weight in each sieve were recorded. The granular material that passed through sieve size 2 mm were considered as fines according to the standard classification of sediment grain sizes by (Singh and Müller, 2007). The relative weight fractions of different sediment sizes were determined following the equation below as % retained weights.

$$(W_r / W_s) \times 100. \dots\dots\dots \text{Eq 3.1}$$

Where W_r is the weight of sediment retained in a sieve size, W_s is the initial dry weight of the whole sediment sample.

3.5. Assessing oxygen consumption, primary productivity and system responses to nutrient addition

The experiment was performed with epipsammic and epilithic biofilms. A total of 160 samples, 8 replicates per site were taken from stream sections under forested and agricultural land uses. Sediment samples were collected at a depth of 5 cm from depositional sites and sieved through a 4 mm sieve to ensure sediment homogeneity. The sieved sediment samples were put in clean containers and kept at 4 °C for transportation to the laboratory for analysis. In addition, epilithic biofilms (stones) with an approximate diameter of 3 cm were collected from each site and placed in plastic beakers with insitu water for transportation to the laboratory. Approximately 3 liters of site water was collected for epilithic and epipsammic biofilms replicates incubation.

3.5.1 Experiment set up and laboratory analysis.

A weight of 10 g of collected epipsammic biofilms was added into each incubation tube with the oxygen sensors (PreSens Oxygen Sensor Spots). The tubes were filled-up with a known amount of insitu water of each site. These were measured by weighing the tubes and adding the required amount of sediment, and in situ water was topped up with no space left, then closed airtight. The tubes were gently shaken and initial oxygen concentration within the chamber was recorded. The samples were then incubated at constant room temperature in the dark for four hours. Subsequent temperature and oxygen concentration were measured using PreSens polymer optical fiber. Four hours were considered basing on observation from earlier research results that reported very low oxygen consumption rates beyond that time (Hill et al., 2002) For assessment of system response to nutrient addition, a solution of 50ml NH₄Cl salt with a concentration 250 µg l⁻¹ was mixed with insitu water before adding to the incubation tubes as it was done by (Beardall, 2001). The samples were incubated in the dark for four hours at room temperature to measure microbial oxygen consumption rate. Then, the same samples were exposed to light under same temperature conditions to measure primary production (oxygen production). Oxygen measurements were taken at an interval of an hour. After the final oxygen measurement, the water was decanted carefully, sediment was transferred to aluminum pan for drying at 120 °C for 2 days, the dried sediment sample weights were recorded, then burnt at 550 °C for 3 hours to obtain the final weights as ash free dry weights. While for the epilithic biofilms, stones of about 3 cm were collected and placed into beakers with oxygen sensors, then filled up with in-situ water leaving no space and closed airtight. Controls were set up with only in situ water and incubated in the same conditions. Oxygen concentration measurements were done as in the previous biofilms. After the experiment, the algal biofilms were scrapped from a predetermined surface area using a toothbrush and distilled water to detach all algal particles from the surface. The solution was adjusted to a known volume for analysis of organic matter and chlorophyll-*a*. The oxygen concentration change in the tubes was approximated as :-
$$\Delta c = (C_i - C_t) / \Delta t \dots\dots\dots \text{Eq 3.2.}$$

And consumption rates were computed and expressed as.

$$\Delta c/\Delta t = ((C_i - C_t)/\Delta t) \times DW \dots\dots\dots \text{Eq 3.3}$$

$$\Delta c/\Delta t = ((C_i - C_t)/\Delta t) \times \text{cm}^2 \dots\dots\dots \text{Eq 3.4}$$

Where C_i is the initial oxygen, C_t is the final oxygen concentration, t is time, cm^2 is the surface area from which the biomass was scraped and DW is the dry weight. The oxygen consumption rate was approximated per dry weights or surface area and expressed as ($\mu\text{gO}_2/\text{g DW/h}$) for epipsammic biofilms and ($\mu\text{gO}_2/\text{Cm}^2/\text{h}$) for epilithic biofilms.

3.5.2 Chlorophyll *a* analysis

The analysis was done with water samples, epilithic and epipsammic biofilms extracts using the spectrophotometric method. Chlorophyll *a* was extracted by acetone extraction method following the (APHA, 2005). Where 25 ml of water samples, 25 ml of epilithic scraped biofilm suspension were filtered through $0.47\mu\text{m}$ GF/F Whatman filters using a vacuum filtration unit. The filter papers were folded and transferred into a test tube, followed by addition of 5 ml of 90% acetone and stored in the freezer overnight to allow extraction of chlorophyll *a* pigment. The filter papers in the tube were sliced, grinded at 5000 rpm for 1 minute to burst open the chlorophyll *a* cell then sample volume was adjusted to 10 ml with acetone and incubated for 24 hrs. in the dark at 40°C . while for epipsammic biofilms, 10 ml of acetone were added to 10 g sediment then kept in the freezer for 24 hrs. All the samples were centrifuged for 10 minutes at 2500 rpm and a clarified extract was decanted into clean glass cuvette for optical density (OD) reading at wave length 750 nm and 663 nm using (DR 3900-Hach Lange) photometer. The reading at 750 nm wavelengths was considered to correct turbidity (Steinman et al, 2017). Chlorophyll *a* concentration was then calculated according to (Talling and Driver, 1961).

$$\text{Chla} = 11.40 \frac{(E_{664} - E_{750})}{V_2 * L} * V_1 \dots\dots\dots \text{Eq 3.5}$$

Where: V_1 is the volume of extract in ml, V_2 is the volume of the filtered water sample in liter, L is the light path length of cuvette in cm, E_{663} and E_{750} are the optical densities (OD) of the sample and 11.40 is the absorption coefficient for chl-a in $\mu\text{g l}^{-1}$.

3.5.3 Organic Matter (OM) analysis in epilithic and epipsammic biofilms.

The organic matter was analyzed by filtering 25 ml of the scrapped epilithic biofilm suspension through pre combusted weighed $0.7\mu\text{m}$ GFF filters (Whatman), and for epipsammic biofilm, 10 g of sediment was used. These were dried at 100°C for 3 hours to obtain sample dry weight, the samples were then combusted in the marvel furnace at 550°C for 3 hours, to obtain ash free dry weight. The surface area of the biofilms was normalized to square centimeters of the sand and stones

surfaces as described by (Marxsen and Witzel ,1991). The organic matter content was estimated as a difference in the weights and was expressed in grams per square centimeter.

$$OM=W_d-W_a \dots\dots\dots Eq 3.6$$

where W_d is the dry weigh after drying, W_a is the weight after burning (ash free dry weight).

3.6 Functional diversity of microbial communities.

The functional group diversity was assessed using carbon sources with ecological meaning, built up in (Biolog Ecoplates). Extracts from biofilms were inoculated into the plates and incubated from 24 to 216 hours under room temperature as described by (Feigl, 2017), and optical density reading were done every 24 hours. The eco plates comprised of 96 wells with 31 individual carbon sources with a defined ecological meaning and one control containing water (Garland, 1996).These were preferred because of the fast results and wide range of use by earlier researchers (Zhang et al, 2013; Nautiyal, 2010).

3.6.1. Sample collection and laboratory analysis

Samples were collected in replicates from each site, 1.3 g sediment and 3 ml of water samples were collected into sterile vials, frozen and transported to the laboratory for further analysis. Laboratory analysis was done under sterile conditions with pre-sterilized equipment and reagents. Frozen samples were thawed at room temperature for 3-4 hours and transferred to sterile 15ml tubes, where 10 ml of sterile sodium pyrophosphate solution was added to detach microorganisms from the sediment. The samples were sonicated in the sonication both for 10 minutes and later centrifuged for 1 minute at 800 rpm. Then 400 µg of the centrifuge suspension was pipetted into 15ml tube followed by addition of 10 ml sterile sodium pyrophosphate solution to have 1:100 end dilution and 130µg of the suspension was pipetted and inoculated quickly into the eco plates. The plates were then incubated at room temperature (25°C) in the dark for 9 days with 24-hour readings using microplate reader (Varioskan Flash- Thermofisher Scientific), at a wavelength of 590nm. The obtained optical density values were corrected by subtracting absorbance values of the 31 wells from the control well. Values <0.0001 were corrected to 0 and the average well color development (AWCD)was calculated per plate and per time as:-

$$AWCD=\sum(C_i-R)/31 \dots\dots\dots Eq 3.7$$

Where R is the OD_{590nm} of the control well and C_i is the OD value in the 31 carbon wells

and the Shannon diversity index was calculated for every 24hours time as

$$H = -\sum Pi * (lnPi) \dots\dots\dots Eq 3.8$$

Where, P_i is OD_{590nm} value in i well divided by mean OD_{590 nm} value of 31 wells.

3.7 Analysis of bacteria abundancies in epipsammic and epilithic biofilms.

Samples were collected in triplicates at a depth of 5 cm for the epipsammic biofilms and were sieved through 4 cm sieve, then ~1.35 g of sediment and 3 ml of unfiltered in-situ water was added. The samples were immediately fixed with 0.75 ml formalin (2% final concentration). While for epilithic biofilms, 3ml of scrapped suspension were transferred into vials and fixed immediately with 0.75 ml formalin (2% final concentration). All the samples were kept at 4°C and transported to the laboratory for analysis. Laboratory analysis was done using fluorescent staining with DAPI (4,6-diamidino -2 phenylindole) method. A working solution was prepared by diluting 5 µg of Sybr Green II RNA gel stain in 995 µL of Dimethyl Sulphoxide 99.5%. Pyrophosphate solution (10 mM pyrophosphate)-4,461 g Tetrasodium pyrophosphate 10 was diluted to 1litre with filtered distilled water (0,22 µm). Tween 80: 10% Tween 80 solution was diluted with small amounts of distilled filtered water, then filtered through 0.22 µm filter and 5 µl were added to the samples, followed by 1ml pyrophosphate solution and 4 ml sterile water. The mixture was sonicated in water bath at 14 rpm for 3 minutes, with intermittent shaking after every minute. The samples were then incubated in ice for 15 minutes, followed by centrifugation for 1minute at 14000 rpm. From the supernatant, 2 ml were drawn and filtered through 5 µm membrane, then 50 µL of the filtrate was pipetted into 15 ml tubes. These were then diluted by addition of 9950 µm of distilled water, and 995 µl of the diluted sample were pipetted into separate tubes to obtain samples for staining with 5 µl staining of solution and the unstained. The resulting solutions were mixed and kept in the dark (under tin foil) at room temperature for at least 15 minutes, then incubated at 75 °C in a water bath for 10 minutes. The samples were then analyzed for bacteria counts using flow cytometry method following (Porter and Feig, 1980) procedure.

3.8. Data analysis.

The obtained data was organized and stored using the excel spread sheets and statistically analyzed using the R statistical program (version 3.6.3) as applied by (Tufto and Cavallini, 2005). Shapiro-Wilk's test was used to examine the data normality, then nonparametric test (Kruskal Wallis test) used to test for significant differences in parameters and Wilcoxon rank sun tests comparisons was used to test significant differences among the sites measured parameters.

Descriptive statistics were used to present the variation in water quality variables and nutrient concentrations. Bar graphs, box and whisker plots were used to present results on bacteria abundances, sediment grain size distribution, microbial oxygen consumption rates and primary production between forested and agricultural stream sections. Principal component analysis (PCA) was used to establish microbial functional groups in different sites and to obtain functional diversity of the benthic microorganisms.

Linear correlation analysis was used to establish correlations between oxygen consumption rates, primary production and bacterial abundancies with selected water quality parameters, and sediment

grain sizes. Land use maps were developed using QGIS software (QGIS Development Team, 2015) and CORINE landcover database (EEA, 2018).

CHAPTER FOUR

4.0 RESULTS.

4.1. Land use.

Land use data and land map (Fig. 1) were extracted from Landsat TM images and land cover data of Kenya environmental planning unit using QGIS 3.45 with Grass 7.6.0. The two predominant kinds of land use types were forested and cultivated land. The land use image was interpreted at of 1:10km and the overall interpretation accuracy of the land use was at 90% basing on the provided coordinates of the sampled areas. The sampled streams (Fig 1) and stream basins were divided into three watersheds. The total sub water shed areas and proportions of each land use were calculated (Table 1).

The total catchment area of the sampled sites ranged between 68.46 to 81.71 km², with Kimurio forested section covering the largest area (81.71 km²) and Kapkateny agricultural sites having the least (68.46 km²). Teremi had a full tree vegetation cover (100%) in the forested stream section and kimurio had the least tree cover in the forested stream section (89.19%). Teremi/Kimurio had the largest area covered to agricultural land use and Kapkateny had the least area under agricultural land use (Table 1.)

Table 1. Total sampled site areas and percentages of land use type coverage.

Site name	Total area (km ²)	Vegetations cover	Shrub cover	Crop cover	others
Teremi forested	70.66	100	0	0	0
Kapkateny forested	70.66	93.75	0	6.25	0
Kimurio forested	81.71	89.19	0	10.81	0
Kapkateny Agric	68.46	7.90	3.23	83.88	5
Teremi/kimurio Agric	70.65	0	0	95.0	5

The studied streams were flowing through areas covered with two predominant land use types (agriculture and forests) that had different vegetation cover. The forested stream sections of Teremi having (100%), Kapkateny (93.7 %) and Kimurio (89.19%) tree dominance. The agricultural stream section of Kapkateny consisted of (83.88%) open and cultivated agricultural land, (3.23%) shrub cover, (5%) other land uses such as homes and roads. Teremi/Kimurio was (95 %) dominated by crop farming and (5%) other land use types (Fig. 2).

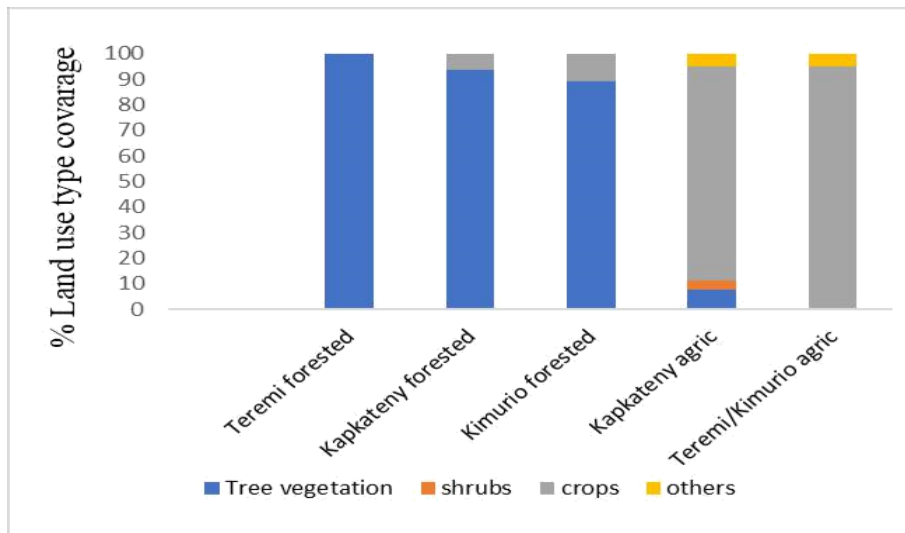


Figure 2. Graphical representation of sampled streams catchment land use types.

4.2. Comparison of physico-chemical parameters between agricultural and forested stream sections.

The limnological parameter data sets were not normally distributed, (Shapiro test, $p < 0.05$). Stream discharge varied among stream sections, with arrange between 0.068 ± 0.12 to 3.66 ± 0.29 m^3/s and significantly higher discharge rates recorded in Teremi/kimurio agricultural stream section (Kruskal-Wallis test, $df = 7.26$, $p < 0.05$). The mean temperatures in both forested and agricultural streams sections ranged between 14.22 ± 0.46 – $21.34 \pm 0.17^\circ\text{C}$ with significantly lower temperatures recorded at Teremi forested stream section and highest in Kapkateny agricultural section (Kruskal-Wallis test, $df = 13$, $p < 0.001$). Dissolved oxygen (DO) concentrations among the streams ranged between 6.07 ± 0.18 to 8.02 ± 0.17 mg/l , with Teremi forested stream section having significantly higher DO value compared to other stream sections (Kruskal-Wallis test, $df = 11.6$, $p < 0.05$). Total phosphorous (TP) concentrations ranged between 57.51 ± 3.69 to 203.79 ± 10.23 $\mu\text{g/l}$. Teremi/Kimurio agricultural stream section registered significantly higher TP concentrations (Kruskal-Wallis test, $df = 8.41$, $p < 0.001$). For Total nitrogen (TN), concentration ranged between 1.15 ± 0.25 to 2.27 ± 0.16 $\mu\text{g/l}$ with significantly higher values recorded in Teremi/Kimurio agricultural stream section compared to others (Kruskal-Wallis test, $df = 17.57$, $p < 0.0001$). Stream $\text{NO}_2\text{-N}$ concentration ranged between 0.78 ± 0.11 to 5.28 ± 0.06 $\mu\text{g/l}$ was noted to be higher in Kapkateny agricultural stream section (Kruskal-Wallis test, $df = 7.17$, $p < 0.002$). Nitrate (NO_3) was significantly higher in Teremi/Kimurio agricultural stream section (Kruskal-Wallis test, $df = 7.69$, $p < 0.0001$) with arrange between 0.15 ± 0.06 to 387.59 ± 21.8 $\mu\text{g/l}$. Stream conductivity ranged between 54.45 and 120.2 $\mu\text{S/cm}$. Significantly higher conductivity values were observed in Kapkateny agricultural stream section (Kruskal-Wallis test, $df = 10.42$, $p < 0.001$). SRP concentrations ranged between 15.37 ± 0.97 to 20.19 ± 0.45 and chl *a* 180.69 ± 0.2 to 589 ± 0.01 $\mu\text{g/l}$. Both were significantly higher in Kimurio forested stream sections ($p < 0.05$). The mean TDS and

TSS ranged between 0.04 ± 0.01 – 0.14 ± 0.01 ; 20.40 ± 0.05 to 450 ± 2.351 mg/l, with streams in agricultural land use recording significantly higher values than those in forested land use. However, pH ranged between 7.1 to 7.87 and $\text{NH}_4\text{-N}$ that ranged between 23.96 ± 7.09 to 30.49 ± 6.93 $\mu\text{g/l}$ respectively, did not show any significant difference among all sites (Table 2) and a general representation between agricultural and forested land use is shown in figure 3.

Table 2. Mean (\pm SE, N=12 per site) measurements with Kruskal -Wallis test results for limnological parameters per sites. (Sites denoted ‘a’ were not significantly different).

Parameter.	Units	Agricultural sites		Forested sites			df	p-value
		Teremi/kimurio	Kapkateny	kimurio	Teremi	Kapkateny		
TP	$\mu\text{g/l}$	203.79 \pm 10.23	193.38 \pm 18.11	86.08 \pm 6.15 ^a	57.51 \pm 3.69	80.23 \pm 4.51 ^a	8.41	0.0003
SRP	$\mu\text{g/l}$	20.05 \pm 1.37	16.29 \pm 2.56	20.19 \pm 0.45	17.44 \pm 0.98	15.37 \pm 0.97	9.5	0.05
$\text{NO}_3\text{-N}$	$\mu\text{g/l}$	387.59 \pm 21.85	569.21 \pm 44.57	0.15 \pm 0.06 ^a	0.15 \pm 0.06 ^a	34.71 \pm 20.40	7.69	2.84E-05
$\text{NO}_2\text{-N}$	$\mu\text{g/l}$	5.28 \pm 0.16	7.95 \pm 1.56	0.78 \pm 0.11	1.06 \pm 0.15	1.24 \pm 0.9	7.17	0.002
$\text{NH}_4\text{-N}$	$\mu\text{g/l}$	24.76 \pm 4.41	30.49 \pm 6.93	22.29 \pm 6.37	26.00 \pm 7.96	23.96 \pm 7.09	17.4	0.67
TN	$\mu\text{g/l}$	2.27 \pm 0.16	2.24 \pm 0.10	1.15 \pm 0.25 ^a	1.16 \pm 0.26 ^a	1.176 \pm 0.25 ^a	17.5	0.0002
Chl α	$\mu\text{g/l}$	415.25 \pm 0.05	252.2 \pm 0.02	589 \pm 0.10	180.69 \pm 0.02	224.15 \pm 0.02	18	0.06
DO	mg/l	6.07 \pm 0.18	6.61 \pm 0.27	7.92 \pm 0.17	8.02 \pm 0.17	7.94 \pm 0.18	7.93	0.0001
TSS	mg/l	450 \pm 23.51	265.38 \pm 11.5	20.40 \pm 0.05	31.94 \pm 0.04	43.15 \pm 0.23	7.76	0.0004
TDS	mg/l	0.14 \pm 0.01	0.09 \pm 0.01	0.06 \pm 0.01 ^a	0.04 \pm 0.01	0.06 \pm 0.01 ^a	7.23	0.002
TEMP	$^\circ\text{C}$	21.44 \pm 0.17	21.74 \pm 0.27	15.4 \pm 0.12 ^a	14.22 \pm 0.46	15.4 \pm 0.04 ^a	17.8	8.40E-05
EC	$\mu\text{S/cm}$	107.12 \pm 40.90	120.2 \pm 2.66	53.4 \pm 0.87 ^a	51.86 \pm 32.	54.45 \pm 0.78 ^a	10.4	0.0005
Discharge	m^3/s	3.66 \pm 0.29	0.68 \pm 0.12	0.85 \pm 0.04	0.69 \pm 0.08	0.38 \pm 0.04	7.26	0.03
pH		7.31 ^a	7.87 ^a	7.19 ^a	7.28 ^a	7.21 ^a	13.4	0.112

The streams water quality parameters differed significantly between agriculture and forested stream sections (Wilcoxon test, $p < 0.001$). Total phosphorous (TP), nitrates (NO_3), nitrites (NO_2), total nitrogen (TN), total suspended solids (TSS) and water temperature were highly significantly different, with higher values recorded in the agricultural stream sections (Wilcoxon test, $p < 0.001$), soluble reactive phosphorous (SRP) were equally higher in agricultural sections at significant levels (Wilcoxon test, $p < 0.05$). Dissolved oxygen (DO) was significantly lower in agricultural stream sections. While, no significant differences were noted in the systems' chlorophyll a , ammonia (NH_4) and PH (Wilcoxon test, $p > 0.05$) (Fig. 3).

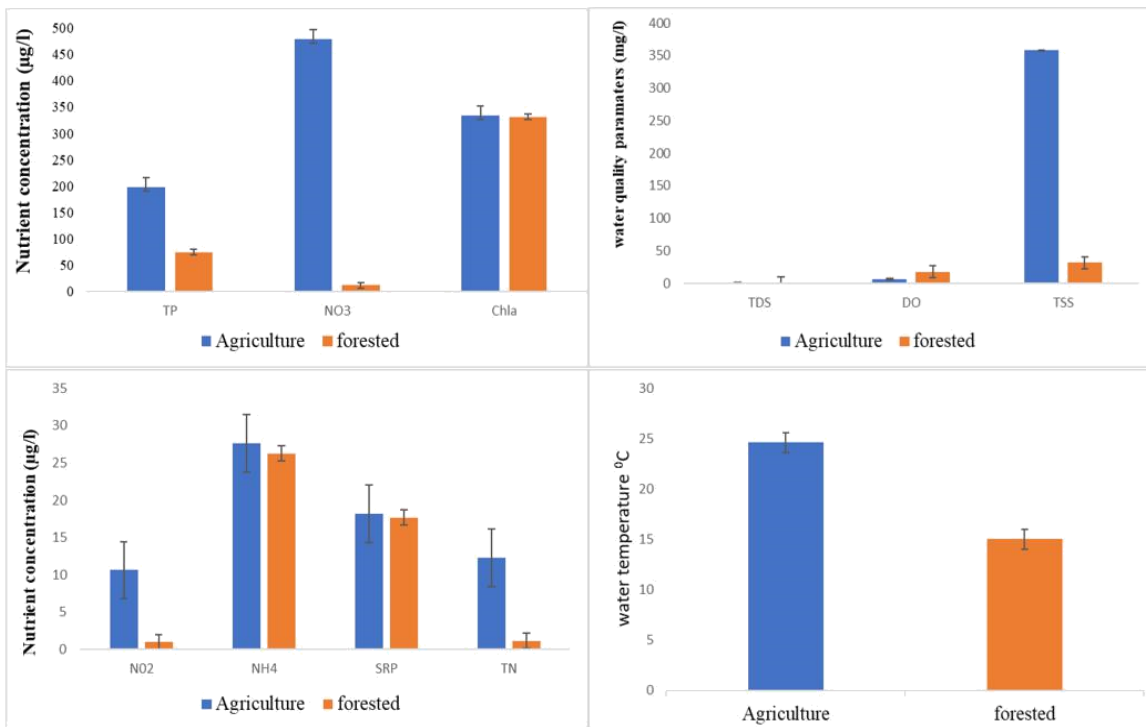


Figure 3. Comparison of mean and standard deviation values of different water quality parameters between forested and agricultural land uses (N= 60).

4.3. Land use influence on sediment grain size distribution in streams.

The stream sediment grain sizes were grouped into three textual classes according to Q25, Q50 and Q75 of their particle distribution. For this study, grain size particles < 2mm were considered as fines, those >2 mm were considered as a mixture of pebbles, gravel and cobbles. Specific site representation of sediment grain distribution is shown in (Fig.4 a). Teremi/kimurio and Kapkateny agricultural sections have a higher dominance of grain sizes below 2mm. A general overview of the distribution pattern between the forested and agricultural stream sections, is represented by the (median Q50) shown in (Fig.4 b), which revealed a highly significant variation in grain size distribution between the two land use types (Wilcoxon test, $p < 0.001$), with a large dominance of grain particles below 2 mm in agricultural stream sections and 6-12 mm in the forested stream sections. The influence of land use in fine sediment deposition and accumulation in stream systems, a general representation of fine sediment accumulation in stream sections between the two land use types (Fig.4 c) was tested. The statistical results revealed a highly significant contribution of agricultural land use to fine sediments deposition (wilcoxon test, $p < 0.001$), with over 40 % of the total sediment structure in stream sections under agricultural land use being dominated by fines (Fig.4 d).

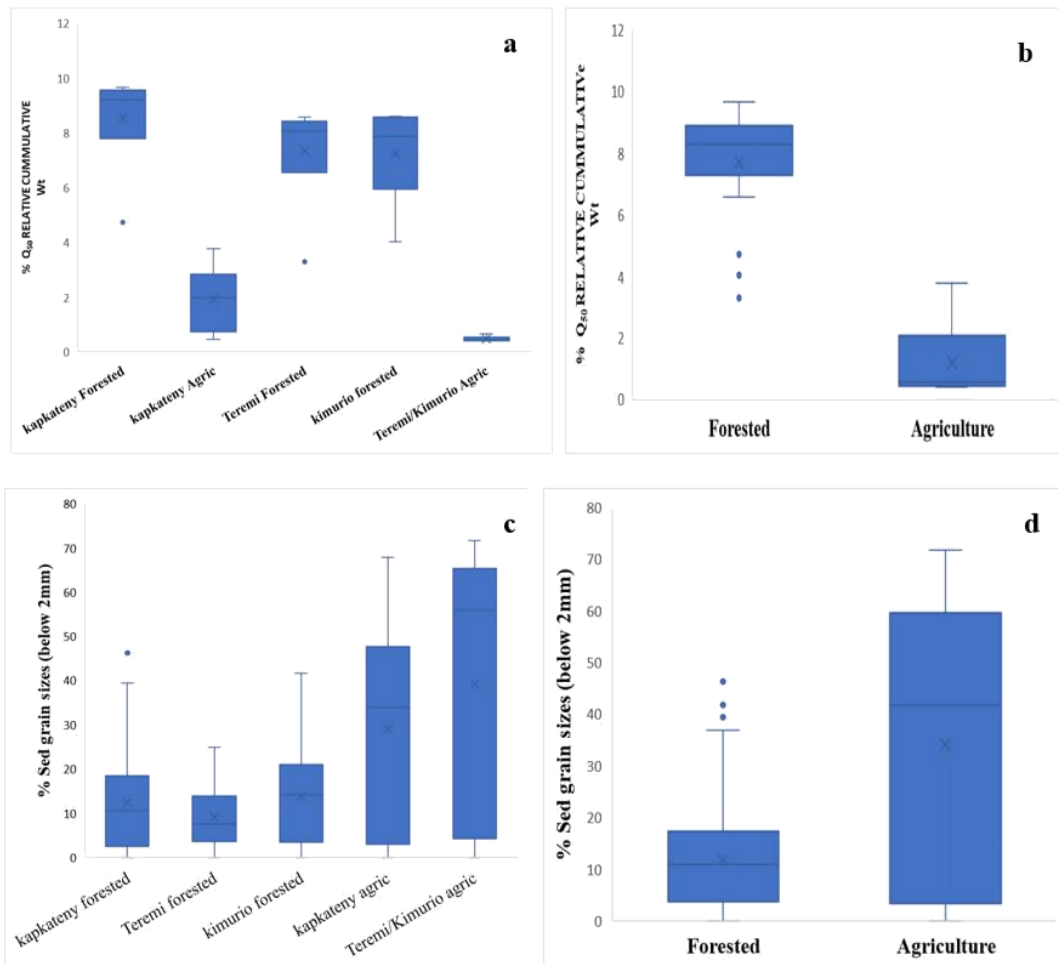


Figure 4. Boxplots with median (Q50) grain size proportions, 25% and 75% quartiles, with whiskers representing the lowest and highest values per site (Fig. 4 a), with a general representation of median Q50 variation between forested and agricultural stream section (Fig. 4 b). Fine sediment (<2mm) accumulation in respective sites (Fig. 4 c) and general overview of variation between agricultural and forested stream sections (Fig. 4 d). (N=30).

4.4. Impact of Land use on epipsammic biofilm oxygen consumption rate and their response to nutrient additions.

Oxygen consumption rate was different between the sites. Significant higher oxygen consumption was noted in agricultural stream sections and low consumption rates in forested stream sections (Fig. 5 a), (Wilcoxon test, $P < 0.001$). System response to nutrient addition was done by addition of $\text{NH}_4 \text{Cl}$ salt solution. The obtained results showed different responses in both stream sections (Fig. 5 b). Highly significant increase in consumption rate in forested section was noted (Wilcoxon test, $P < 0.005$). While no significant response was shown by agricultural stream sections (Wilcoxon test, $p > 0.5$).

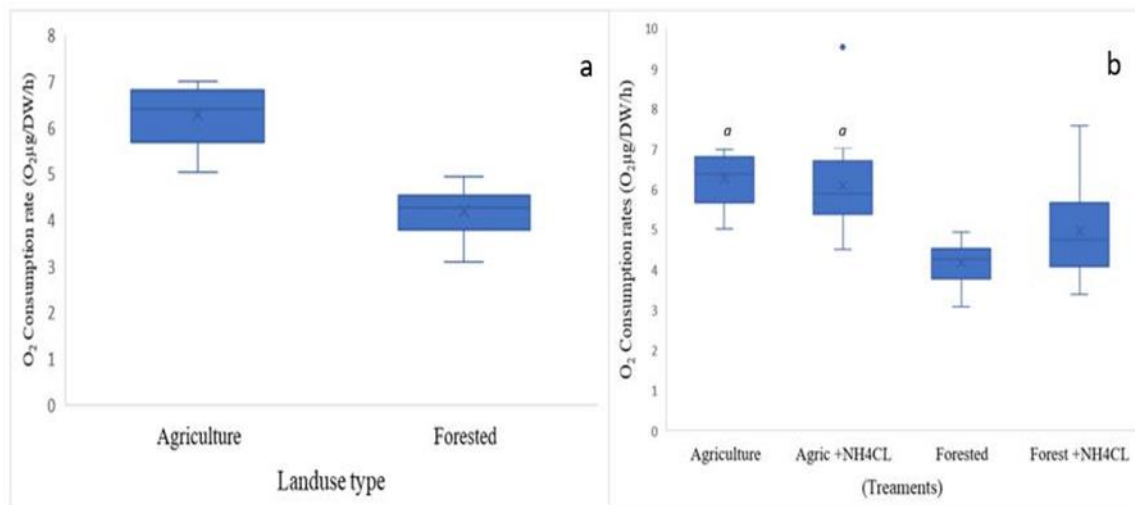


Figure 5. A representation of boxplots with the median values, 25% and 75% quartiles, with whiskers representing the lowest and maximum values of streams epipsammic biofilm oxygen consumption rates between agricultural and forested stream sections (Fig.5a) and a representation of variations in systems responses to nutrient addition (Fig. 5b), (N=160). Those denoted with same letter showed no significant differences.

4.4.1. Influence of land use on epipsammic biofilm primary productivity and their responses to nutrient addition.

Stream primary production was measured by the amount of oxygen produced. The epipsammic biofilm production in the agricultural section was significantly higher than the forested sections (Fig.6.a), (Wilcoxon test, $p < 0.05$). In assessment of their response to nutrient addition, opposite responses were shown for the different stream sections. The epipsammic biofilm primary production of the forested section significantly increased with addition of nutrients (Wilcoxon test, $p < 0.001$), while a highly significant reduction was noted in the agricultural section (Wilcoxon test, $p < 0.0001$ in agricultural sections (Fig. 6 b).

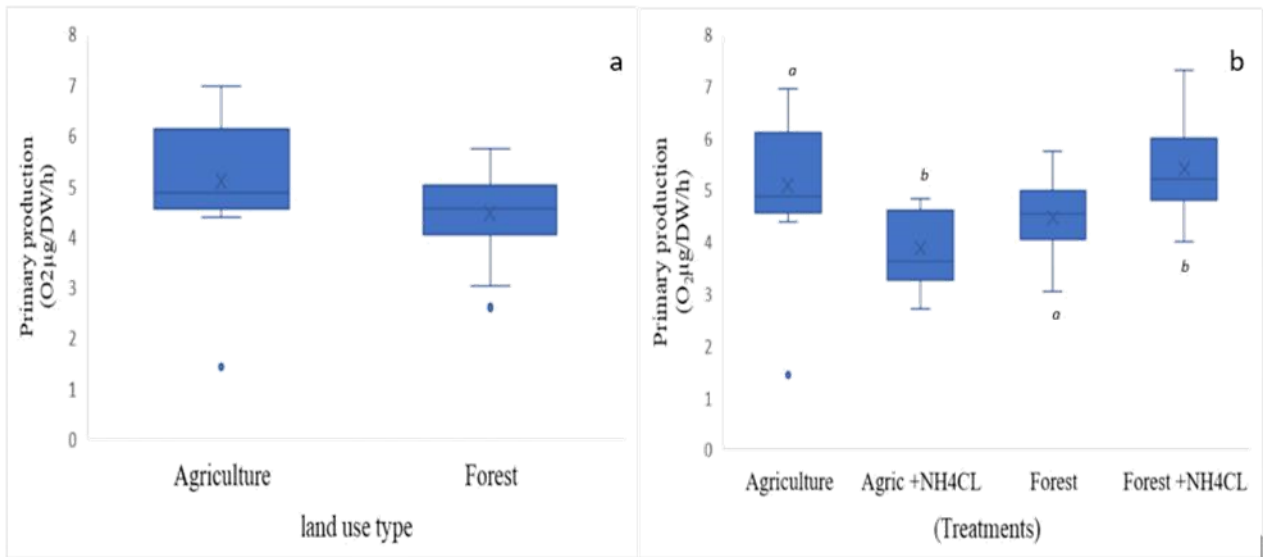


Figure 6. A representation of variations in median values, 25% and 75% quartiles, with whiskers representing the lowest and maximum values of streams epipsammic biofilm primary production between agricultural and forested stream sections (Fig. 6 a) and a representation of their varying responses to nutrient addition (Fig. 6 b), (N= 160).

4.4.2. Variation in stream epilithic biofilm oxygen consumption rate between agricultural and forested stream sections and their responses to nutrient addition.

The observed results in (Fig. 7a) showed differences in the epilithic biofilm oxygen consumption rates between agricultural and forested stream sections. A highly significant difference (Wilcoxon test, $p=0.003$), between the two land use types was observed with higher respiration rates noted in forested stream sections. A response to nutrient addition (Fig. 7 b) showed significant increases in oxygen consumption rates in the forested sections (Kruskal-Wallis, $p<0.001$), While no response was observed in agricultural sections with nutrient additions. (Kruskal-Wallis test, $p>0.05$).

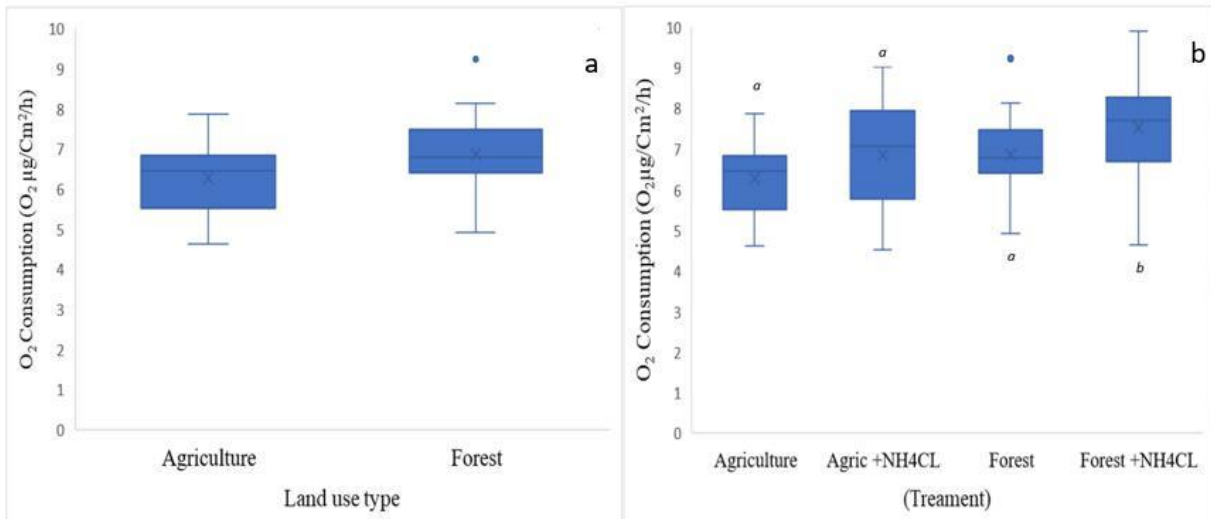


Figure 7. Comparison of median values, 25% and 75% quartiles, with whiskers representing the lowest and maximum values of streams epilithic biofilm oxygen consumption rates between agricultural and forested stream sections (Fig. 7 a) and a representation of their responses to nutrient addition.(Fig. 7 b) (N=160).

4.4.3. Epilithic biofilm primary production and response to nutrient additions.

Epilithic autotrophic biofilm productivity was different in stream sections under different land uses, with significantly higher productivity recorded in epilithic biofilms from forested stream section (Fig. 8 a), (Kruskal-Wallis test, $p < 0.001$). A manipulation of the system with nutrient addition did not show any significant difference (Kruskal-Wallis test, $p > 0.05$) in agricultural stream sections, but a highly significant increase in primary productivity was noted in forested stream section (Kruskal-Wallis test, $P < 0.001$), (Fig. 8 b).

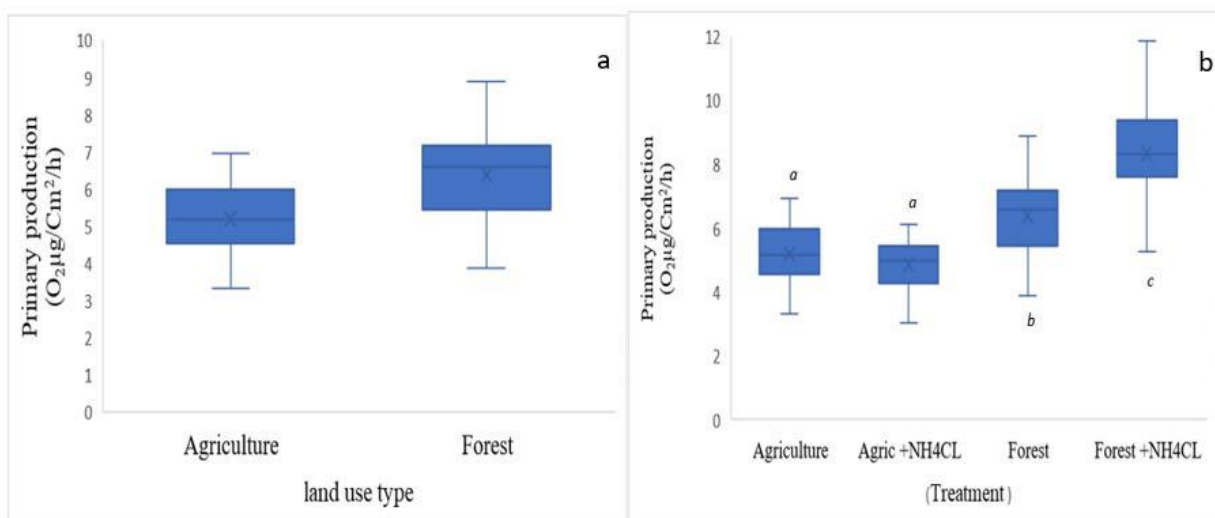


Figure 8. Comparing median values, 25% and 75% quartiles, with whiskers representing the lowest and maximum values of agricultural and forested stream section epilithic biofilm primary production (Fig. 8 a) and a representation of their responses to nutrient addition (Fig. 8 b), (N=160).

4.5. Bacteria abundances

Bacteria abundances were analysed in both epipsammic and epilithic biofilm of the agricultural and forested stream sections. The mean values ranged between (65 ± 7.5 to 200 ± 11.8) cell cm^2 in the epipsammic biofilms and (20.5 ± 0.8 to 68.5 ± 6.3) cell cm^2 in the epilithic biofilms. Significant differences (Kruskal Wallis test $p < 0.001$) in abundances between the biofilm type were observed, with epipsammic biofilms having higher abundances than the epilithic biofilms. Variation in bacterial abundances between agricultural and forested stream sections show significantly higher abundancies in epipsammic biofilms from agricultural stream sections (Kruskal-Wallis test, $p < 0.001$) and significantly lower in epilithic biofilms. Wilcoxon test revealed significant differences between epipsammic biofilms of agricultural sites (Kapkateny and Teremi/Kimurio $p < 0.05$) with Teremi/Kimurio having higher abundance. While for the forested sites (Kimurio and Teremi $p < 0.001$), higher abundances were recorded in Kimurio. Further differences in epilithic biofilms of the agricultural sites Teremi/Kimurio and Kapkateny ($P < 0.001$) revealed low abundancies for Teremi/Kimurio (Fig. 9).

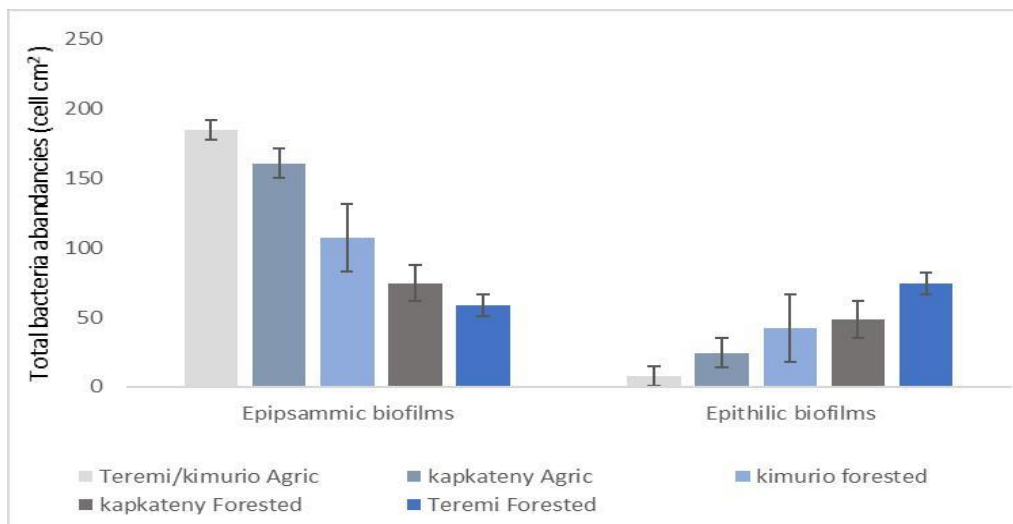


Figure 9. A representation of mean values and standard deviation of bacterial abundancies in epipsammic and epilithic biofilms from forested and agricultural stream sections. (N=30).

4.6. Variation in microbial functional group diversity between stream sections under agricultural and forested land uses.

The average well colour development (AWCD) for each site increased with time forming a sigmoid shape (Fig. 10). The AWCD in all sites was significantly different between 48- 216 hours with a faster change in stream sediments from Teremi forested stream section and the slowest from Teremi/kimurio agricultural stream section (Fig. 10). The AWCD in the first 48 hours showed no significant difference in all sites (Kruskal-Wallis test, $p>0.05$). However, a significant increase after 48 hours was observed in all sites (Wilcoxon test, $p<0.05$), with a faster increase observed in sediment from Teremi forested stream section and slowest from Teremi/Kimurio agricultural stream section sediments (Fig. 10), (Wilcoxon test, $p<0.05$). Shannon diversity index was calculated for every 24 hours and highly significant differences in daily diversity were observed (Wilcoxon test, $P<0.001$), (Table 3). Considering the 196 hours which was used to develop PCA output (Fig. 10), the diversity index was significantly different among sites (Wilcoxon test $p<0.001$), with Kapkateny forested stream section having the highest diversity and the lowest was recorded in Teremi/Kimurio agricultural stream section (Table 3).

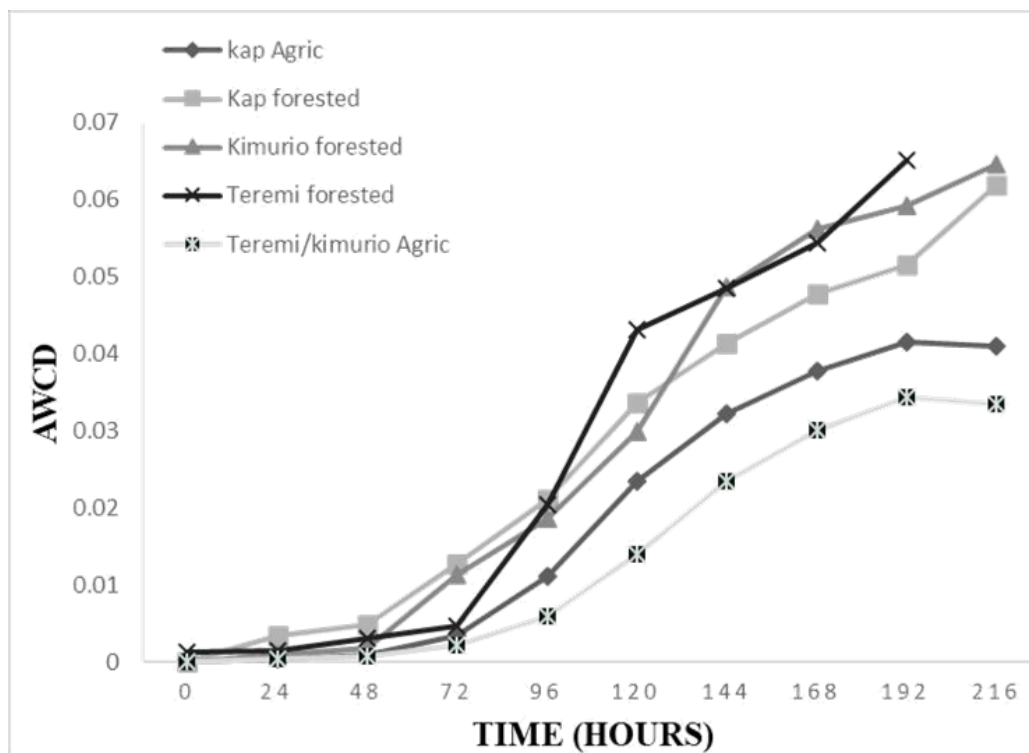


Figure 10. The average well colour development (AWCD) of metabolized carbon sources.

The diversity index calculated at every 24 hours significantly differed in all sites (Wilcoxon test, $p < 0.001$). The 196 hours (bolded column) was used to assess carbon substrate utilization by microbials and the diversity index of that time was considered to give a general overview of site functional group diversity (Table 3).

Table 3. Variation in Shannon diversity index for the entire incubation period (24 -216) hours.

Sites	Shannon diversity indices calculated after every 24 hours								
	24	48	72	96	120	144	168	196	216
Kapkateny Forest	2.95185	2.663662	1.697856	1.33619534	0.948283	0.81018	0.743407	0.697304	0.676813
Kapkateny Agric	0.442225	0.627159	0.608496	0.81798608	0.730212	0.681608	0.534025	0.501888	0.514719
Kimurio Forest	1.028276	1.193967	1.559402	1.21231245	0.871072	0.773747	0.72681	0.693271	0.699787
Teremi Forest	1.361533	0.944484	0.539304	0.40777843	0.661564	0.621696	0.605748	0.616946	0.64444
Terem/kimurio Agric	0.55779	0.565459	0.419069	0.50165765	0.495492	0.439947	0.439544	0.426711	0.459279

The principal component analysis (PCA) was used to visualize the differences of the variety of substrates utilization in investigated sites. Considering substrate utilization in the early hours, no significant differences were observed though there were significant differences in the diversity index. However, 196 hours, showed significant differences in substrate utilization (Kruskal-Wallis test, $p < 0.001$) and were plotted as arrows in the PCA output. The utilized carbon substrates were different at every site (Fig.11). A detailed list of utilized carbon sources per site is given in (Table 4). The general diversity index at 196 hours indicated highly significant differences (Wilcoxon test, $p < 0.001$), with the highest diversity recorded in Kapkateny forested stream section (0.697) and the lowest (0.042) in Teremi/kimurio agricultural stream section (Table 3).

The eco plate output at 196-hours showed a distinct classification of utilized substrates per site. The PC1 explained 40.2 % variance in the utilized substrate with a larger range observed in Kapkateny and Kimurio forested stream sections, which had about (10) carbon sources(H, D3, D4, G1, F1, A4, D2, B2, C4 and B3) utilized. Kapkateny and Teremi/kimurio agricultural sections had seven substrates (H4, H3, C2, G4, E4, C3 and F2) utilized (Fig 11). The PC2 explained the variance between Teremi/kimurio, Kapkateny agricultural and Teremi forested stream sections, with the variance explained by 28.5% with Teremi having 5 carbon sources (G2, D2, F4, H3 and B4) utilized. Refer to (Table 4) for full description of the abbreviations.

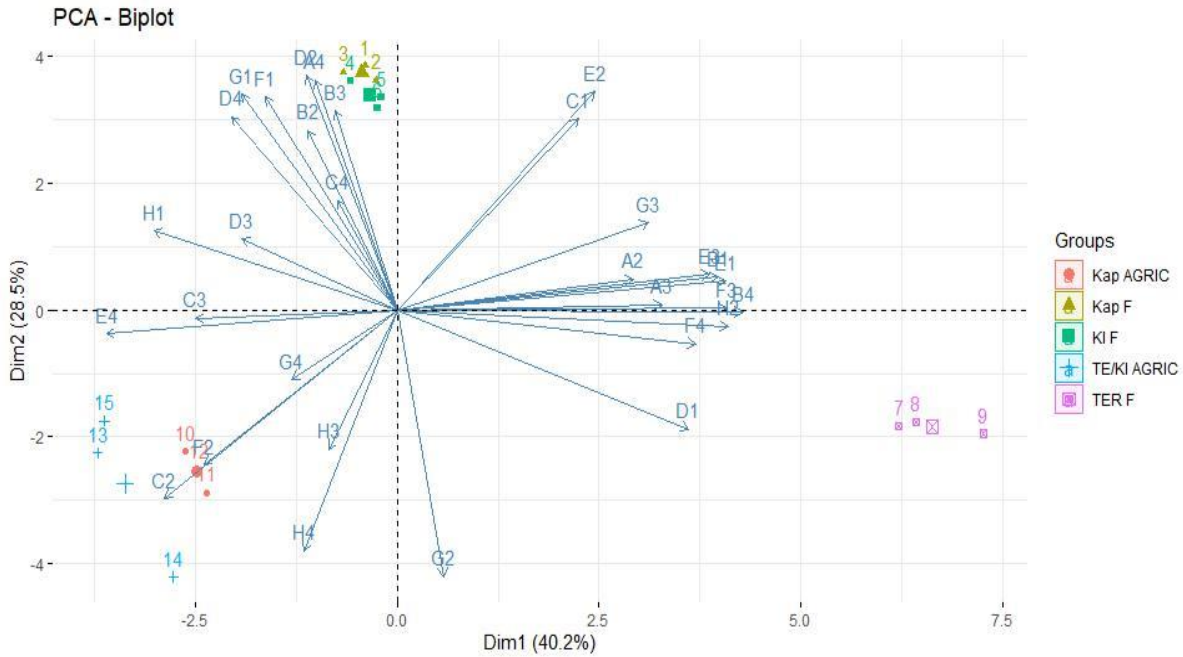


Figure 11. Principal component analysis (PCA) for the normalized OD data of 196 hours of carbon substrate utilization by sediment microbial communities.(N=15).

4.6.1 Microbial carbon utilization of agricultural and forested sites

The microbial community physiological potential for carbon source utilization was distinctively different in all sites. (Wilcoxon test, $p < 0.001$, $F = 105$) with Kapkateny and Kimurio forested stream sections having wide range of utilization of carbohydrates, polymers, amino acid and miscellaneous chemical guilds except the amines. Teremi, mainly dominated by miscellaneous guild, Kapkateny and Terem/Kimurio agricultural section, showed high utilization of amines/amide (Table 4).

Table 4. Individual utilized carbon substrate per site.

Site	PCA-Cord	Name	Chemical guild	
Kapkateny & Kimurio Forested	D2	D- Mannitol	Carbohydrates	
	A2	L-Arginine	Amino acid	
	G1	D-Cellobiose	Carbohydrates	
	F1	Glycogen	Polymers	
	D4	L-serine	Amino acid	
	H1	α -d-Lactose	Carbohydrates	
	E2	N-Acetyl-d-glucosamine	Carbohydrates	
	C1	Tween 40	Miscellaneous	
	Teremi Forested	G2	Glucose-1-phosphate	Miscellaneous
		D1	Tween 80	Miscellaneous
F4		Glycyl-L-Glutamic acid	Amino acid	
H2		d, l- α -Glycerol phosphate	Miscellaneous	
Kapkatenyi & Teremi/Kimurio Agric	E4	L- Threonine	Amines/amide	
	C2	i-Erythritol	Carbohydrates	
	H4	putrescine	Amines/amide	

Supportive correlational tests between different investigated parameters

From the linear regression analysis, there were significantly strong positive relationships between oxygen consumption rate and fine sediment grain size (<2mm), ($R^2=0.74$, $P<0.001$), (Fig. 12. a), as well as with bacterial abundances ($R^2=0.75$, $P<0.001$), (Fig. 12. b). A significant relationship was observed between respiration and organic matter ($R^2=0.63$, $P<0.05$), (Fig. 12. c) and with total phosphorous (TP) ($R^2=0.62$, $P<0.001$), (Fig. 12.d).

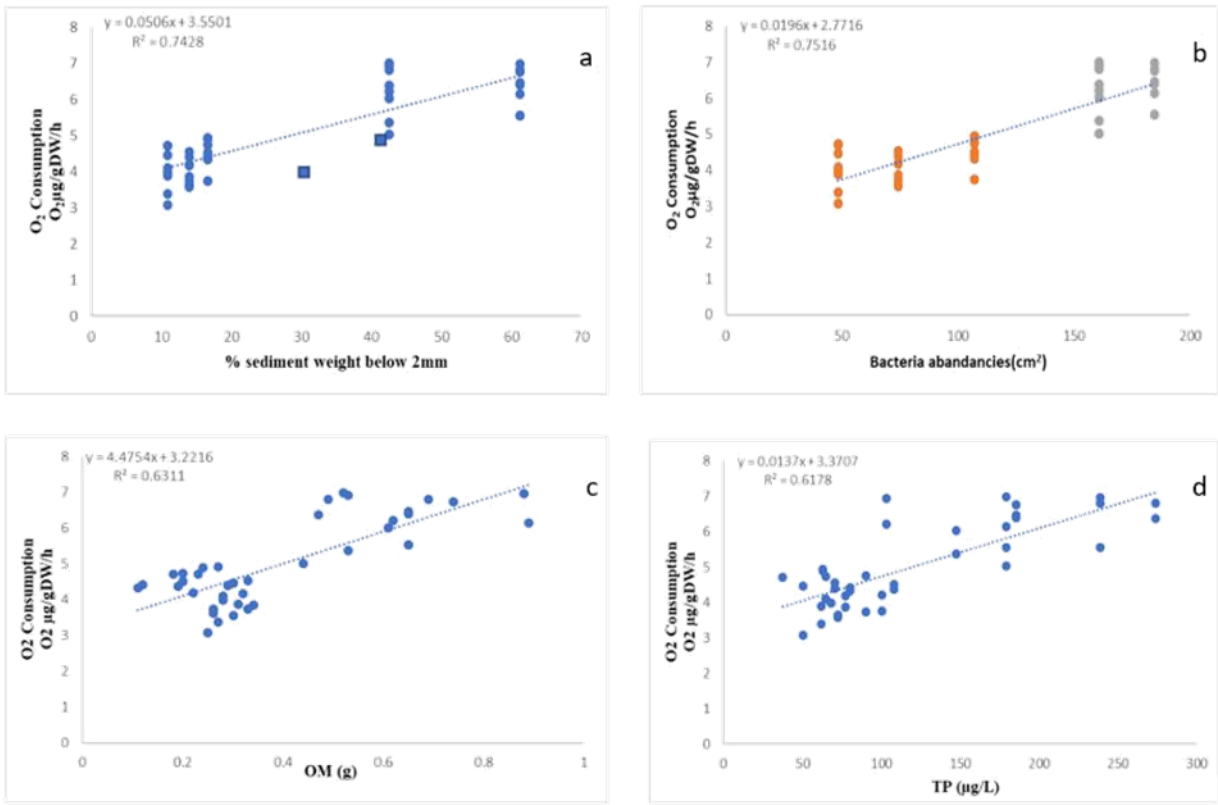


Figure 12. Relationship between oxygen consumption rate with fine sediment grain size, (Fig. 12 a), ($R^2 = 0.74$, $p < 0.001$). Oxygen consumption rate with bacteria abundances (Fig. 12 b) ($R^2 = 0.75$, $p < 0.001$), Oxygen consumption rate with Organic matter (OM), (Fig. 12 c) ($R^2 = 0.63$, $P < 0.05$) and (Fig. 12 d) oxygen consumption rate with Total phosphorous (TP), ($R^2 = 0.62$, $p < 0.05$).

CHAPTER FIVE

5.0 Discussions

Land use and land cover pattern influence both physical and chemical as well as biological characteristics of streams. Agricultural land use compared to other land use types has more negative impacts on the water quality and system processes due to their high contribution of terrestrial, anthropogenic inputs and alteration of the hydrological regimes (Tang et al., 2005). This study hypothesized that there are significant differences in stream sediment composition, nutrient concentrations, system oxygen consumption rates, primary production, microbial abundances and functional group diversity between agricultural and forested land uses in the same stream system and the obtained results revealed variations in all investigated parameters.

5.1. Stream limnological parameters

The significant variations observed in water limnological variables (TP, NO₃, NO₂, TN, TSS, TDS, DO, EC, Temperature and discharge (Table 2) between forested and agricultural stream sections could be attributed to differences in the catchment vegetation cover characteristics and prevailing activities. The differences in the catchment land cover, - forested with high vegetation cover and agriculture with barely any vegetation cover was clearly reflected by the significant variation in discharge, fine sediment accumulation, water temperature, organic matter, TDS and TSS amounts. These demonstrated to be potential indicators of land use change from forest to agriculture. For instance, Teremi/ kimurio with the highest values in most parameters had a larger area converted to agricultural land. According to (Allan et al., 1997), conversion of forested land to agriculture reduces the riparian vegetation of rivers and stream systems, leading to alteration of; flow regimes, in-stream structure composition and functioning. Observations by (Norris, 1993; Anbumozhi et al., 2005; Wilkerson et al., 2006), suggest that, a reduction of riparian vegetation reduces terrestrial organic matter trapping and retention potential, thus increasing their accumulation in adjacent streams. Consequently, these accumulations increase stream/river turbidity (Maitre et al., 2014) and temperature (Allan, 2004; Dallas, 2008; Davies-Colley and Smith, 2001). Supportive observations between land use change from forested to agricultural were made by, (Nayak and Mandal, 2012) in their research in tropical rivers proved that most streams with a well-developed tree distribution and canopy cover had temperature range between 12-18°C, which was similar to this study values of the forested stream sections.

Similarly, variations in TP, NO₃, NO₂, TN, EC, DO concentrations reflected increased terrestrial inputs such as chemical residues, fertilizers, plants and animal waste, which are typically from agricultural land use. Due to vegetation cut down, poor and continuous cultivation, the sampled areas experience high soil erosion and uncontrolled surface runoff

(picture 2 annex), which have led to soil fertility loss. According to the farmers from this area and from my own observation during data collection, application of inorganic fertilizers is practised to boost productivity and different chemical application to reduce the effects of pests and associated losses. According to (Matsumoto and Yamano, 2011), and from my own study findings, commonly applied fertilizers and chemicals (picture 1 annex) within the studied areas contain phosphorus and nitrogen compounds. During rain events, these get washed into and retained within stream systems. Possibly, this accounts for the high concentration of TP, TN, NO_3 , NO_2 and EC in agricultural stream sections. In natural systems, nutrients such as phosphorus, nitrogen and organic matter are important elements in primary production and they are constantly utilized (Rajasegar et al., 2002; Buck, et al., 2004), thus, little amounts would be expected in the system, as it was in the forested stream sections. However, the high values in agricultural stream sections could have been a result of continuous supply from anthropogenic inputs (agriculture), which get deposited, adsorbed and stored in stream systems (Droppo et al., 2009). Coupling the high accumulations with high TSS and TDS (turbidity) (picture 9 annex), nutrient uptake and utilization is reduced. Consequently, this results into low DO amount which was evident for agricultural stream sections. A decrease in dissolved oxygen concentration in the water and sediment column as total suspended solid input increased was noted by (Sujitha et al., 2012; Pitchaikani et al., 2010), in Karamana river. It's also evident in one of the study correlational results between organic matter and oxygen consumption rate ($P < 0.05$, $R^2 = 0.63$). According to (Das et al., 2005 and Prabu et al., 2008), high inorganic input from agricultural areas alters the nutrient budget of freshwater system and further affects their utilization. This study results, high TSS, low DO, high turbidity and resulting Chlorophyll *a* biomass in agricultural stream sections are similar to the findings of (Vörös, and Padisak, 1991; Jones and Knowlton, 2005; Karlsson et al, 2009), who noted that, increased suspended solids and turbidity reduced periphyton, planktonic and bacterial productivity, processes by which nutrients are utilized. Other suggestions for high concentration is fine sediment deposition and accumulation from agricultural land use. Correlations showed a link between total phosphorus (TP), fine sediments ($p < 0.001$, $R^2 = 0.74$), organic matter (OM) concentration and fine sediments ($p < 0.001$, $R^2 = 0.95$), which were higher in agricultural stream sections as a result of high soil erosion and surface runoff from agricultural land use (picture 2 annex).

Surprisingly, though high nutrient concentrations were observed in the agricultural stream sections, a higher chlorophyll *a* biomass would be expected as well, however, there were no significant differences in chlorophyll *a* concentration in stream sections under different land uses. In natural systems with a balanced nutrient budget and sufficient DO amounts, productivity is possible even with minimal light penetration (Filstrup, 2017). While an altered nutrient budget may inhibit uptake of some nutrients, thus reducing chlorophyll *a* biomass. This supports the observation of non-significant differences in chlorophyll *a* concentration.

According to (McCauley et al., 1989; Watson, et al., 1997; Filstrup, 2017), high nutrient concentrations, for example at high TP concentration, uptake of other nutrients such as nitrogen is limited.

5.2. Variation in sediment grain size accumulation and distribution in streams under different land uses

The stream sediment grain size distribution differed in both agricultural and forested stream sections. Mainly as a result of varying land cover patterns (table 1), soil erosion, riparian buffering structures and erodibility ease of the soil structure within different land uses. These dictate on the transportation, sorting and distribution of coarse and fine sediment in a given system, as well as their particle exposure, interstitial space and the sediment chemistry (Opdyke et al., 2006). According to Church, (2006); Allan and Castillo, (2007), change in land cover from forested to crop land has detrimental effects, that range from terrestrial soil loss, heavy transportation and deposition into aquatic systems. While forests compact the soil structure, act as sponge and barriers to soil transportation to adjacent stream/river systems (Schottler et al., 2014), the reverse is observed in the cultivated areas (Blanco and Lal, 2008) where high surface runoff and high sediment transportation is observed.

From my study results, high depositions were observed in stream sections under agricultural land use. This is because the area was continuously ploughed with poor soil and soil erosion management practices (picture 2, 3 annex), which exposed soil structure to high chances of getting eroded and eventually deposited into the recipient stream system. According to Mati et al, (2000); Angima et al, (2003), a high soil loss was estimated from agricultural land use. Similar observations were made by the Kenyan ministry of planning and national development (1994) after their assessment of land use pattern effects on aquatic systems. Other study findings revealed that agricultural dominated land use faced high soil erosion and contributed to large sediment transportation and deposition into adjacent water bodies during high precipitation (Knox, 2006; Belmont et al., 2011). Referring to the studied sites under different land uses, the agricultural land use had poor land management practises, poor agricultural methods (such as monocropping, ploughing along hills, bare plots) (picture 2 and 3 annex) that exposed the area to increased surface runoff and soil erosion during rain events. Absence of buffer riparian vegetation, as it was in the agricultural stream sections, increased accumulation of fine sediment fractions and reduced coarse particle retention and exposure within the stream beds. These findings are similar to those of previous researchers (Bakker et al., 2008; Vigiak et al., 2012), who recorded higher sedimentation in cultivated land during their assessment of land use change. High soil erosion, sediment transportation and deposition from poorly cultivated soils with impervious cover, during rain seasons (Nelson and Booth, 2002; Kevin et al. 2008). These sediment accumulation and distribution may have influenced bacterial abundances.

5.3. Impact of Land use on epipsammic and epilithic biofilm oxygen consumption rate and their response to nutrient addition.

The study results revealed significant differences in oxygen consumption rate in stream sections under different land uses as well as between different biofilm types. The high oxygen consumption rate observed in epipsammic and low rate in epilithic biofilm of agricultural stream sections could be attributed to various factors influenced by the catchment, such as sediment loads that clog the interstitial spaces, sediment grain size distribution, high terrestrial organic inputs, which require enough oxygen for their decomposition, system turbidity that may impair light penetration for photosynthesis and high temperature due to less/no canopy cover thus reducing dissolved oxygen. A correlation test between respiration (oxygen consumption) and fine sediment grain size particle accumulation was run. A strong correlation between oxygen consumption and sediment grain size ($R = 0.74$; $p < 0.001$) is similar to observations that oxygen consumption taken as a measure of microbial activity in sediment, increased with a decrease in sediment particle sizes (Hargrave, 1994; Rysgaard et al., 1994).

Smaller particle sizes offer large surface area to volume ratio for microbial colonization and microbial activities (Santmire and Leff, 2007). The results obtained from this study are in line with other study results which observed that smaller grain sized sediment presented greater surface for bacteria biomass attachment than the larger grain sized sediment (Yeager et al., 1998; Lyautey et al., 2005; Santmire et al., 2007). Besides the grain size, TP which was high in agricultural land use positively correlated with fine sediment grain size ($p < 0.05$, $R^2 = 0.62$). The high TP value in the agricultural stream section could be used to explain the high oxygen consumption rate because phosphorous stimulates microbial activity (decomposition rate). Thomaz et al., (1997), observed that a reduction in phosphorus concentrations reduced microbial activities in the flood plain water bodies.

Another factor to consider, which is in line with out-puts from other researchers, is the high OM content in the agricultural stream sections. Outputs by Thomann and Mueller, (1987); Adhikari et al, (2002); Dorcherty et al, (2006); Dowing et al, (2009); Bernot et al, (2010), reported increased oxygen consumption rate with increasing inorganic matter deposition. Other studies by (Hill et al., 2002; Young et al., 2008; Battin et al., 2008) reported the dependence of respiration rate on particulate organic matter availability. This study results show that organic matter content was high in agricultural stream sections and correlational analysis between organic matter content and oxygen consumption rate revealed a strong positive correlation ($R = 0.63$, $p < 0.001$). Besides OM increasing microbial respiration, its high input and availability in stream systems offers attachment surface to microorganisms (Seiki et al., 1994; Caldwell and Doyle, 1995; Pope et al., 1999; Pascoal et al., 2005;). A test between OM and bacteria abundance showed a positive correlation ($R = 0.68$, $p < 0.05$) and could explain the high

oxygen consumption rate in epipsammic biofilms. Further, strong positive correlation observed between bacteria abundance and oxygen consumption rate ($R=0.75$, $p<0.001$), could explain the observed results. Šantr and Sirašicraba (1991), found a proportional increase in microbial activity with increase in their numbers, that increased with diverse and large surface for attachment in the predominant fine sediment grain size. However, low oxygen consumption rate in the epilithic biofilm of the same stream section (agricultural) was noted and this majorly is explained by high fine sediment deposition and accumulation that could have clogged, covered and dislodged microorganisms that could have a ttached to epilithic biofilms. While, results by Mori et al, (2017) on stream bioflim respiration, showed that oxygen consumption rate in stream riffles that had high substrate heterogenity (such as those observed in the forsted stream section sites) was higher due to their exposure to light and increased microbial activity, and could account for the high respiration in epithilic biofilms of forested stream sections. Sediment exposure, arrangement, interstitial space, hyporheic exchange and sediment chemistry are influenced by land use type and flow velocity (Church 2006; Opdyke et al., 2006; Allan and Castillo, 2007).

Further assessment of system responses to nutrient addition was done. Where the agricultural stream sections showed no significant response, revealing that, addition of excess nutrients to alr eady saturated systems may retard or impair microbial activities. while the observed increase in the forested areas suggest that, nutrient additions stimulated microbial activities in these sections. Results by Gulis et al, (2004), showed increase in microbial respiration after nutrient addition in forested streams. Studies such as those conducted by (Grattan and Suberkropp, 2001; Rosemond et al., 2002; Ferreira et al., 2015), equally showed a positive increase of heterotrophic microbial activity with increase in inorganic nutrient input as it was in the forested stream section. Similarly, (Craft et al., 2002) in their study in the Flat noted an increase in respiration of the hyporheic sediment after nutrient addition. According to (Allan et al., 2002) in an experimental set up using epipsammic and epilithic biofilms with periodic nutrient additions, their results confirmed that algal and bacterial activity increased. This could be used to explain why the epilithic biofilm oxygen consumption rate was higher.

5.3.1 Land use impact to epipsammic and epilithic biofilm productivity and their response to nutrient addition.

System primary productivity is influenced by various factors such as solar light availability, temperature, amount of dissolved oxygen and nutrient availability within a given system and its encompassed within metabolic activities that provide a measure for stream functioning (Niyongi et al., 2004; Bornet et al., 2010; Covino et al., 2012). Quantities of nutrients such as nitrogen, phosphorous and carbon within the stream/river systems may be influenced by, allochthonous inputs (Meyer et al., 2002), land use type, and other natural atmospheric or in

stream factors (Houser et al., 2005; Wipfli et al., 2007). The high primary productivity of epipsammic biofilms from agricultural stream sections (Fig. 6 a) in relation to land use are associated with high nutrient accumulation and availability within the agricultural stream sections. Most studies on stream productivity have reported high productivity in agricultural streams. Study results from (Klose et al., 2009) revealed that, river productivity and algal blooms resulted from agricultural plant nutrients that get eroded into the water course during rain events. In stream processes like decomposition triggered by terrestrial inputs, organic carbon and phosphorous provide more nutrients for periphyton growth (Ensign and Doyle, 2006). Though high production was noted in epipsammic biofilms, of the agricultural stream sections, primary production in the epilithic biofilm of the same stream section was low because these were covered by the fine sediment. Infilling of interstitial spaces according to (Walling and Amos, 1999; Collines and Walling, 2007), increased with increase in fine sediment deposition. Such accumulations reduce chances of microorganisms and periphyton attachment (Yamada and Nakamura, 2002). The high deposition rates as those observed in the agricultural sites cause burials and shading of the epilithic biofilms and physical abrasion of attached autotrophic organisms. On the contrary, high primary production was noted in epilithic biofilm from forested stream section (Fig. 8 a), reason being, the stream bed had a heterogeneous substrate distribution, which offered conducive and more attachment area for biofilm attachment and development. According to Tarkowska and Mieczan, (2012), cobbles, pebble, gravel and rocks offer suitable substratum for biofilm colonization since they are less dislodged by water movement. Study results from Vörös and Padisak, (1991); Singer et al, (2010) suggested that periphyton growth and attachment is influenced by stream flows and turbulent regimes. In relation to the stream velocity result, this study recorded low flow velocities in the forested stream sections. This could have facilitated increased attachment of periphyton biomass, that carryout primary production (photosynthesis). According to Giorgi et al, (2010), in their assessment of factors affecting periphyton biomass and productivity in a pampean stream, they observed that sediment biomass productivity increased in streams with gentle flow velocities and wave surge water flows. Further, (Nikora et al., 2002) reported that periphyton growth and productivity was highest at a flow velocity of less than 0.15 m/s, which is similar to the observed velocity values in the forested stream section. Other factors that could have contributed to the observed result could be the shallow stream water depth and transparency which facilitated light penetration to bottom layers (picture 8 annex). Biggs et al., (1998) reported that, periphyton productivity was highest on substrate that had full light exposure at shallow depths.

The response of algal primary production to N enrichment was assessed through nutrient manipulation with NH_4Cl additions in an experimental setup. The obtained results:- a decrease in agricultural stream section revealed that, system productivity is much more dependent on the

stoichiometry of carbon, nitrogen and phosphorous in suitable proportions for algal uptake. Studies by Mulholland and DeAngelis, (1999); Dodds et al, (2002); Carmichael et al, (2004); Payn et al, (2005), reported that alteration of the C:N:P stoichiometric composition in streams affects periphyton, phytoplankton and primary productivity, as observed in the studied agricultural stream sections. Further explanation by Dodds et al, (2002), suggest that system productivity in excess nutrient conditions, becomes limited due to biotic inability of microorganisms to uptake and accumulate excess nutrients. The forested sites positively responded to nutrients (NH_4Cl) addition, with a highly significant increase in primary production ($p < 0.0001$). Interestingly, this could be an indicator of nutrient limitation in the forested stream section. Earlier studies such as those done by Barton and Johnson, (1978); Gibson, (1971), found out that phosphorous was the most primary production limiting factor in temperate systems but further research in the tropical freshwater ecosystems show that nitrogen plays a key role in tropical stream productivity and microbial activities (Howarth 1999; Drake, 2010). This study results on nitrogen concentrations are in line with study results by (Lombardozi, 2003) in Lake Tanganyika surround by forested land, and those obtained by (Allan, 2007), who typically recorded small fraction of $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}$ in natural head waters. When nutrients were added to these systems, nutrient uptake was increased. Nutrient addition coupled with ambient oxygen levels and less turbidity of the forested stream sections could have enhanced primary production in studied sites as also shown by (Havens, 1999).

5.4. Bacterial abundances and functional group diversity

Bacteria and other micro organisms mainly colonize sediment substrate and are important components of the aquatic ecosystems that ensure ecosystem stabilization, nutrient recycling and terrestrial input degradation (Iozopone, 2007; Madsen and Urban, 2011). Their abundances and distribution are influenced by various factors such as habitat quality, organic matter quality and quantity, salinity, nutrient availability, pH, temperature, redox potential, nature and quality of substrate (Gobet, 2011; Vanbeelen, 2003). These results show distinct differences between microbial abundances of epipsammic and epilithic biofilm depending on the land use. One possible reason for higher abundances in epipsammic biofilms is the large surface area to volume ratio offered by epipsammic biofilms that enhances colonization, as it was also observed by (Pope et al., 1999; Schimel et al., 2007).

However, the overall differences of higher bacterial abundances for stream sediments in agricultural land stretches may be due to fine sediment distribution for sediment grain size < 2 mm, as shown by the correlation test result ($R = 0.92$, $p < 0.001$). Another possible argument could be high accumulation of terrestrial organic matter that offered structural conduciveness and material availability for microbial activities (Garzio et al., 2010). Although studies by

Vrede et al., 2002 suggested that high nutrient concentration especially TN (25-50) $\mu\text{g/l}$ and TP (>200) $\mu\text{g/l}$ suppress bacteria growth, this study result contradicts theirs, because even with higher nutrient concentration in the agricultural stream sections, bacterial abundances were noted to be high. The observed results could be attributed to organic matter content, which equally provided food and habitat. A strong positive correlational output between bacterial abundances and organic matter support the argument ($R=0.85$, $p<0.001$). Relating my findings to the two land uses, agricultural land use offered high allochthonous inputs such as plant and animal residues, which contributed to high organic matter build up, that offered diverse habitat and food that enable bacteria growth and multiplication, thus higher abundances.

However, the microbial diversity in agricultural streams sections was low. Basing on various study results, foreexample those by Vance and Chapin, (2001); Royer-Tardif et al, (2010), showed that plant cover significantly influenced the functional microbial diversity because microbes depend on a variety of external carbon sources. Zhange et al, (2013) results showed a clear influence of terrestrial litter quality on microbial structuring and functional stability. This study results from eco plates assays that showed utilized substrates, were distinctively different based on land use type. Microbial community of Kapkateny and Kimurio were able to utilize a wide range of carbon sources in the group of carbohydrates, amino acids polymers and miscellaneous compounds. The results also showed that forested stream section had microorganisms that were able to utilize more complex compounds such as those in the carbohydrates D-Mannitol, D-cellobiose, N-Acetyl-d-glucosamines, which was similar to (Lyons and Dobbs, 2012; Romaní et al., 2014) result in a comparison of carbon source utilization of the up and down stream of Llobregat river. According to (Grover and Chrzanowski, 2000; Tiquia, 2010), natural system microorganisms are adopted to and are able to produce enzymes that biodegrade complex organic matter through natural processes. And this is related to my findings, where high utilization of complex carbohydrates (D-mannitol, D-Cellobiose, a d-lactose and N-Acetyl-d glucosamine) and the miscellaneous compound were observed in forested stream sections, which could be associated to their presence in those natural sites (Lyons and Dobbs, 2012). Other studies suggest that, carbon sources such as glucose can only be converted by few bacteria such as *bacillus macrerans* (Nam et al., 2001, Garland and Mills, 1991), that have the adoption of highly resistant endospores that allow them to survive in nutrient deprived environments like head waters of most systems, in this case the forested stream section.

However, a shift in the metabolic fingerprints in agricultural stream sections was characterized by higher utilization of amines. Microbial community composition can change in streams depending on the dominating catchment land use type (Wang et al., 2011). Utilization of amines is reliant on the presence of oxidized electron acceptor such as NO_3^- (Jung et al., 2007), which

in my case was high in agricultural stream sections and highly controlled by the amount and quality of nutrient and organic matter exported from the catchment (Abell et al., 2011). Moreover, increased accumulation and loading of such nutrient C, N and P alter the microbial structure and composition (Kohler et al., 2012). From this study findings, suggestions can be made that, high TN, NO₃ and TP concentration affected functional microbial diversity and this coincides with the finding of (Tank & Dodds, 2003; Van et al., 2011), where high amounts of nutrients especially N > 5mg/l and P>200µg/l limited biofilm growth of heterotrophs and autotrophs. Therefore, high utilization of amines in the agricultural stream section might be associated with the presence of organisms that can survive best at high nutrient concentrations, especially NO₃ in agricultural stream sections. (Salomo et al., 2009) found that in the presence of inorganic electron donors as energy sources, chemolithoautotrophic bacteria are able to utilize both easily and non-easily degradable substrate such as amines. A change in utilized carbon sources from a wide range as observed in the forested stream sections to dominance of amines in the agricultural section may be a result of pollutants such as pesticides, (Muturi et al., 2017), heavy metals and herbicides, which are purely originating from agricultural land use.

CHAPTER SIX.

CONCLUSIONS AND RECOMMENDATIONS.

Fine sediment accumulation in the investigated streams was recorded highest in sites under agricultural land uses. This provided evidence that change of land use type from forested to other land uses especially agriculture, increases soil erosion, high surface runoff and terrestrial organic matter deposition into nearby streams, thereby altering riverbed sediment distribution. The drained terrestrial inputs carried along with sediments get deposited, increase pollutants and nutrients accumulation especially TN, TP, NO₃ and NO₂, which were significantly higher in agricultural stream sections.

Though nutrients are important for photosynthesis and autotrophic growth, its necessary to note that their concentrations should be in the right proportion. Natural systems have the ability to balance and utilize naturally existing nutrients. However, anthropogenic alteration such as agricultural inputs lead to high concentration that may affect the system production and the entire food web. It is equally necessary to note that primary production may be limited by some nutrients or nutrient concentrations. This study out comes reveal that, primary production in the streams is facilitated and supported by correctly balanced nutrient amounts for uptake.

Bacterial abundancies varied in stream sections under different land uses with higher abundances in stream sections under agricultural land use. A link between land use and bacterial abundancies is explained by sediment grain size distribution and organic matter since areas dominated with sizes <2mm and high organic matter (OM), offered more surface area for attachment for microbial communities that are tolerant to high pollution, high nutrient concentration and are adopted to live in low oxygen concentrations. However, this limited the functional group diversity of organisms from being able to consume a wide range of carbon compounds to a small easily degradable substrates like amines.

The core study objective was to assess the impact of land use on general stream health, sediment structuring, distribution and functionality of microbial groups. The results affirm that agricultural land use negatively influenced water quality, sediment distribution, microbial functional group diversity and abundances compared to forest land use type.

Recommendations.

Basing on the fact that agriculture is a back born of economic activities in Kenya and East African countries, a major source of food and employment for the fast-growing population, a comprehensive understanding and continuous sensitization is required for society to understand the importance of preserving natural forests and grasslands, regulating terrestrial inputs from getting into aquatic systems, having well developed soil erosion control measures and agricultural ecofriendly practices.

Developing and implimeting policies and advocacy on pesticides, fertilizers, and plant residue management with further restricted use of such pollutants in areas adjacent to water bodies.

Management and restoration strategies of the highly impacted/converted land should be initiated for example reforestation, buffer zone increase to reduce influxes of terrestrial inputs.

It may be worth trying to remove high accumulated fine sediment and foster structures that can encourage microbial colonization and growth to enhance diversity since microorganisms play key roles in ensuring a healthy aquatic system.

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APPENDIX



Picture1. Farmer applying fertilizer and common pesticide used in the sampled catchment.



Picture 2. Surface runoff after rain event in one of the sampling days and open agricultural field along kapkateny agricultural section.



Picture 3 . Agricultural practices along the sampled streams (Mono cropping) with poor soil control measures.



Picture 4. Measurement of the physicochemical parameters of the agricultural and forested stream sections.



Picture 5 . Field sampling of the epipsamic, epithalic biofilms and water sample collection.



Picture 6. Laboratory analysis and experiment set up for primary production measurements.



Picture 7. Laboratory analysis for different water nutrients. (SRP,TP ,TN, NH₄ NO₂, NO₃ and chlorophyll *a*).



Picture 8 Physical appearance of the forested sampled stream section sites with clear water.



Picture 9. Physical appearance of the agricultural sampled stream section sites with highly turbid water.