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Carbon cycling in the Mara River system – Influence of land use and location in the fluvial network on organic matter processing and CO_2-production

Omengo O. Fred

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Master of Science Thesis
by

Fred O. Omengo

Supervisors

Prof. Tom Battin (Austria, University of Vienna- Department of Limnology)

Prof. Michael McClain (Netherlands, UNESCO – IHE, Department of Water Engineering)

Mentor
Dr. Gabriel Singer (Austria, University of Vienna- Department of Limnology)

Examination committee
Prof. Jay O'Keeffe (UNESCO-IHE), Chairman

Prof. Michael McClain (UNESCO – IHE)

Prof. Tom Battin (University of Vienna, Austria)

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Dedication

To my beloved parents in rural Africa, who never lived to see my potential; to all those who love me, whose lives I have changed and who look at me as an example

Abstract

Riverine transport of organic matter (OM) from the continents to the oceans and its processing en-route has attracted the attention of ecologists trying to understand energy flows between aquatic and terrestrial habitats and how this structures aquatic communities. Recently, metabolism of OM in lotic ecosystems has also been recognized as an important source of CO_2 to the atmosphere.

This study has endeavoured to quantify the impact of landuse change on the quantity and quality of suspended particulate organic matter (SPOM) and dissolved organic matter (DOM) in headwater streams in the Mara River catchment, Kenya. For this study 56 streams with stream orders ranging from 1 to 5 were sampled and three main categories of landuse identified: pure forest, agriculture and catchments with a mixture of the two landuse types in varying percentages. A combination of techniques was used, namely: measurements of dissolved organic carbon, estimation of respiratory metabolism by CO_2 determination, quantification of SPOM, isotopic signatures of SPOM, and optical properties of DOM. Bulk DOM concentrations and respiration estimates from CO_2 measurements did not significantly differ among the landuse types. However, using conventional indices derived from optical properties of DOM such as the fluorescence index, slope ratio, humification index, β/α ratio and EEM peaks all indicate that, forested catchment streams are dominated by allochthonous DOM as opposed to the primarily autochthonous agricultural streams. Mixed catchment streams on the other hand had a variable signal that largely overlapped the two distinct landuses. The isotopic signature $\delta^{13}C$ of both SPOM and CO_2 (DIC) clearly separated the agricultural and forest catchment streams by 5 ‰ and 3 ‰, respectively, with agricultural streams being more enriched in the heavy ^{13}C-isotope as opposed to the more depleted forest streams. This indicates a difference in organic matter source for both SPOM and respiratory metabolism. With regard to the majority of other parameters, such as geophysical and geochemical descriptors, and reactive nutrient concentrations, no particular trends were identified along the fluvial network, nor among landuse categories. These results suggest that the present landuse change in the upper catchments of the Mara River is affecting the production and export of OM, with a gradual shift from high molecular weight to low molecular weight OM as landuse changes from forest to agriculture. These dynamics may affect the energy base and community structure of the biota within these streams and the larger Mara River, though not necessarily the overall respiratory metabolic activity and the role of these ecosystems as sources of CO_2 outgassing to the atmosphere.

Key words: Landuse change; organic matter; carbon dioxide; stream; stable isotopes; excitation emission matrix

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Finally this study would not have been a success without the financial and technical support from Nuffic, Department of Limnology of the University of Vienna, Wasser Cluster Lunz and USAID (through the Global Water for Sustainability Program).

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List of abbreviations

ANOVA	Analysis of Variance
C	Carbon
CO_2	Carbon Dioxide
DIC	Dissolved Inorganic Carbon
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
EC	Electrical conductivity
EEM	Excitation Emission Matrix
EFA	Environmental Flow Assessment
FI	Fluorescence Index
GF/F	Glass Fiber Filter
GLOWS	Global Water for Sustainability
HI	Humification index
N	Nitrogen
NH_4	Ammonium
NMDS	Non-Metric Multidimensional Scaling
OM	Organic Matter
P	Phosphorous
POM	Particulate Organic Matter
SD	Standard deviation
SPOM	Suspended Particulate Organic Matter
Sr	Slope ratio
SUVA	Specific Ultraviolet Absorbance
TOC	Total Organic Carbon
TSS	Total Suspended Solids
UV	Ultraviolet
YSI	Yellow Spring Instruments

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1 Introduction

1.1 Background

Mau forest is one of the five main water catchment areas in Kenya covering approximately 400,000 ha. The Mau forest complex is divided into five units: the eastern Mau, western Mau, south western Mau, Transmara and Ol Posimoru. The Mau forest complex constitutes a significant part of the upper Mara River catchment and supports critical economic sectors in the East African region. The streams from the forest support major conservation areas and world famous national parks like the Mara-Serengeti, Lake Nakuru and Lake Natron.

In the recent past, the forest has been under threats and continuous destruction. Encroachment, excision for settlements and logging for timber and charcoal production has escalated. This has led to reduction in the forest cover and fragmentation. Typical examples include land divisions in the 1970s, degazettement and settlements in the 1990s and excisions in the last decade, which all led to a loss of more than 10% of total forest cover (Gereta, Chiombola et al. 2003). This has already impacted major economic industries including power generation (Sondu Miriu), water supply to urban areas (Nakuru, Narok and other towns), agriculture (cash and subsistence crops), and tourism; and furthermore also threatens wildlife in lake Nakuru and Mara-Serengeti reserves by the inconsistent water flows and reductions in discharge. There are increasing concerns over the unabated land use change. For instance, with a loss in 23% of forest cover during the 1980s to 2000 and an increase of 55% and 82% in agricultural land and tea plantations, respectively (Mati, Mutie et al. 2005), the basin has been severely and recognizably modified. The area under agriculture in the Amala subcatchment for instance has increased from less than 20% in the 1960s to more than 51% at the moment. Middle reach catchment areas (Mulot, Longisa and Kaboson) were sparsely populated but have now attracted settlements (Mati, Mutie et al. 2005).

The two main tributaries Amala and Nyangores drain the upper Mara catchment and converge to form the Mara River. Other tributaries include the Talek from the Loita plains, Engare Engito from the Ilmolyookoit and the Sand River at the boarder of Kenya and

Tanzania. The total catchment of the Mara River is 13,750 km^2. The Mara originates in the Mau forest at 2932 m a.s.l. and flows for a total length of 395 km until it drains into Lake Victoria (1,134 m a.s.l.) in Tanzania. The Amala and Nyangores flow through various land uses: a forested escarpment, various tea plantations, settlements and small-scale agricultural farms. In the middle reaches the Mara River flows through Maasai Group Ranches with livestock and wildlife and two internationally renowned conservation areas, the Maasai-Mara National Reserve in Kenya and the Serengeti National Park in Tanzania. The remaining stretch of the river flows through the savannah grasslands common in the Serengeti National Park and later into the Mara Swamp before the river discharges into Lake Victoria.

This study focuses on the headwater streams of the Mara River (western Mau) in the Transmara and Ol Posimoru forest of the Mau forest complex. Here, the streams of the Amala and Nyangores form a complex dendritic pattern draining the Mau forest. Some of the smaller catchments of these streams have been completely deforested and converted to different land uses, mainly agriculture. The remaining parts are partially or fully forested. Extensive field sampling efforts focused on streams characterized by catchments with two distinct land use types (agriculture and forest) and ranging from first to third stream order. Further, rivers up to 5th stream order with mixed catchment land use were included.

1.2 Hypotheses and key questions

Based on the recent developments within the Mau forest, covering an important part of the Mara River catchment, I hypothesize that the intensive deforestation results in a change in the delivery of eroded sediment and allochthonous organic matter into the rivers and streams. Land use changes potentially also affect in-stream processes such as autochthonous primary production. Together, these changes are hypothesized to affect ecosystem metabolism, especially the respiration of organic carbon. This is an important ecosystem process and could increase fluxes of CO_2 to the atmosphere.

Specifically, my hypotheses are:

1) **Deforestation and changes in land use from forest to agriculture alter the quantity and quality of POM and DOM in streams and rivers of the upper Mara river system.**
2) **Subsequently, altered quantity and quality of POM and DOM affects ecosystem metabolic performance (based on estimated fluxes of CO_2) of the impacted streams and rivers.**

These hypotheses were tested by comparing sets of forested, deforested and mixed subcatchments. Covariation with stream size (order) was taken into account by sampling a range of sites with different stream orders representing the riverine network to control for this factor. I address these hypotheses by specifically measuring effects of catchment land use and location in the fluvial network on (i) the concentrations of suspended particulate and dissolved organic matter (SPOM and DOM), (ii) the concentration of gaseous CO_2 as a metabolic response of the ecosystem, and (ii) the quality and origin of OM as indicated by stable isotopic composition of SPOM and optical properties (absorbance and fluorescence) of DOM.

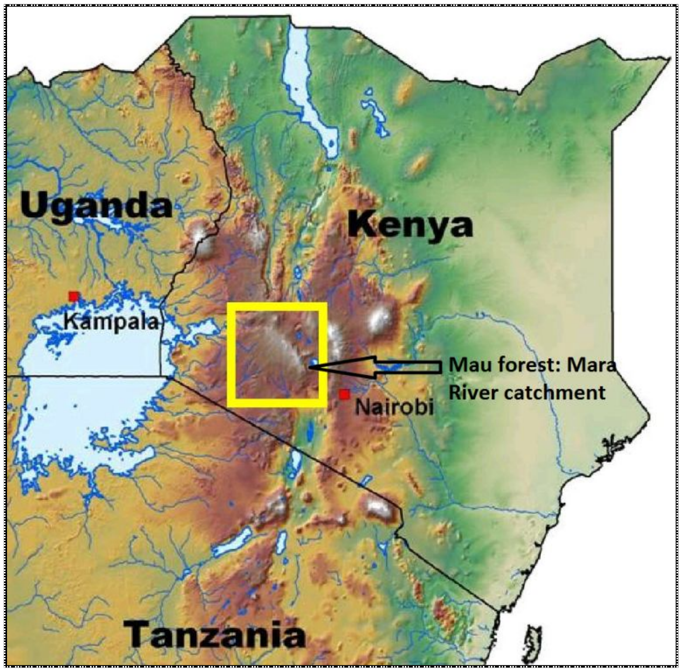


Figure 1: Location of Mau forest: Mara River catchment in Kenya

2 Literature review

2.1 Mara River general background

The Mara River is a world renowned ecosystem that supports diverse forms of life and world-famous conservation areas. The River originates from the Mau escarpment, eventually draining into Lake Victoria. Mau forest is the largest remaining forest in Kenya, it functions as a major water catchment for Kenya and the other East African countries, feeding many rivers, helping to regulate rainfall, and supporting many human activities. The average annual rainfall within the upper catchment of the Mara River ranges from 2000 mm to 1200 mm annually with two rainy seasons, the long rains in March- May and the short rains in October-November.

The upper reaches of the basin, bordering the Mau forest have recorded the highest population densities with an agricultural lifestyle. In general, the population densities range from 10 to 95 persons/km^2 for the less densely populated areas in Narok district, and up to 200-350 persons/km^2 for the densely populated areas like Bomet district (CBS 2008). The population is in scattered homesteads within the basin with very minimal if any planning. Given the increasing human population in the area, pressure on natural resources is high and is anticipated to increase.

Through the *Global Water for Sustainability* (GLOWS) program, a USAID funded initiative supporting site-based initiatives by the ministry of water and irrigation in Kenya, an initial water quality assessment was done for three consecutive years; 2005, 2006 and 2007 at 21 stations within the basin. Based on the analysis of the study, the rainfall of Mara Basin has remained relatively constant over the years whereas the flow characteristics (peak and base flow) of the Mara River are changing. The peaks from the river flow hydrographs for instance occur in around March, April, May and June during the long rains (EFA 2008). The report also notes that the period the river flows at minimum (1m^3/s) has increased over the years.

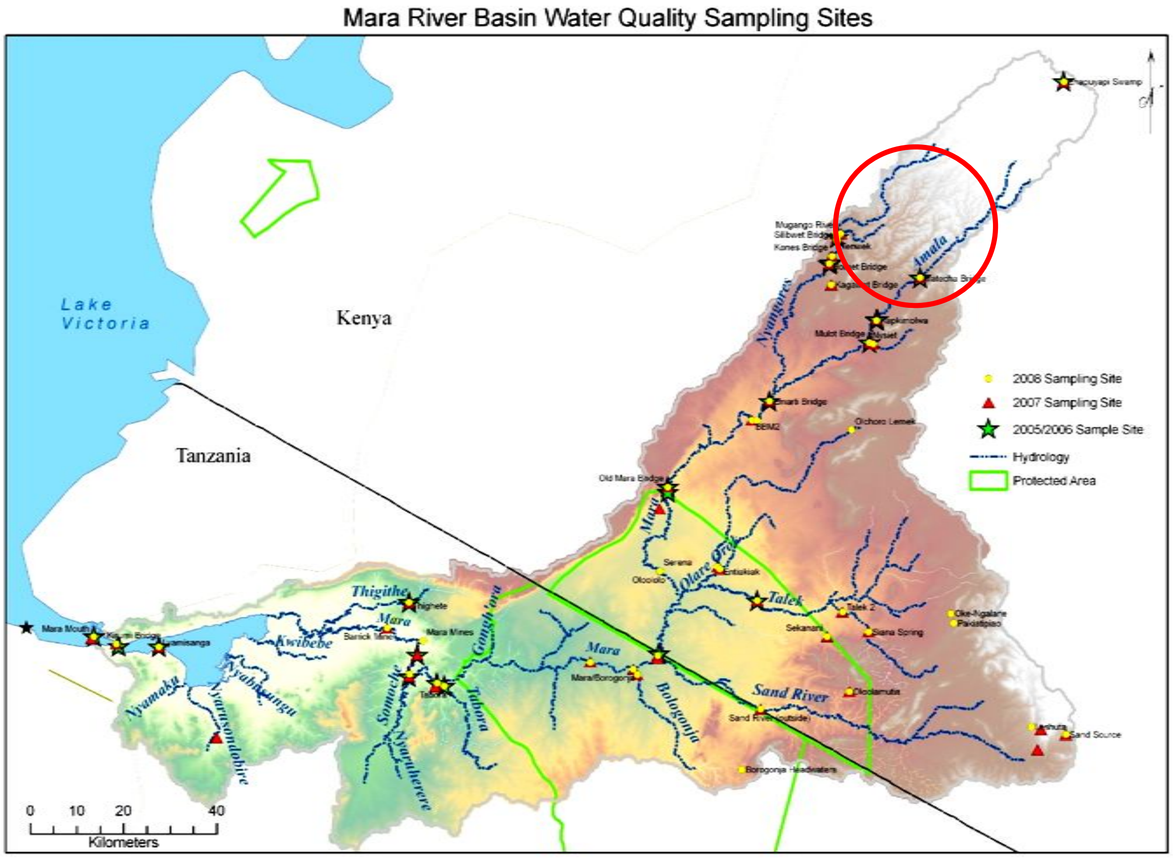


Figure 2: Location of the previous water quality monitoring stations and the current study site (red circle) (Source: (GLOWS 2007))

2.2 Potential threats to water resources within the basin

Pearce et al. (1989) observed that ecological integrity is maintained when the productivity, stability and resilience of systems are sustained; this means that when a system is ecologically healthy it has the capacity to perform all essential ecological processes, including ecosystem services important to humans. An indicator of ecological integrity is the degree to which the ecosystem has been altered from its natural state (Ramsar 1993). The Ramsar convention describes ecological integrity as the sum of the wetland's functions, products, and attributes that are derived from the individual biological, chemical, and physical components of the ecosystem and their interactions; (Ramsar 1993). Changes in ecological character occur when the biological and physical components, and the interactions between them, are enhanced or diminished as a result of both human and natural processes.

Based on the initial water quality assessment within the Mara basin (EFA, 2009), the water quality within the Mara was described to be good and no parameters exceeded national or international water quality standards. However several potential threats to water quality were noticed and this varied from the high human population growth rates, deforestation, potentially unsustainable agricultural expansion and irrigation to untreated wastewater release, and uncontrolled water abstractions. The risk of excess fertilizers, pesticides and herbicides from agricultural run-off was equally obvious in this study and incidences of eutrophication from increased inputs of nitrogen and phosphorous were expected.

Figure 3: Convergence of various landuses Agriculture (tea) in the foreground and cleared forest and intact forest in the background

2.3 Water quality within the basin

Water quality refers to the set of concentrations, speciations and physical partitions of chemical, organic and inorganic substances within a water body (Chapman 1996). Water quality analysis looks at the chemical structure and composition based on the use the water is intended for. Water quality parameters are an important measure of the health or state of any aquatic ecosystem. Human induced activities result in the emission and production of substances some of which are toxic and make the water unsuitable for drinking or as a habitat for flora and fauna. The most common standards used to assess water quality are either in reference to drinking water, safety of human contact or for the health of ecosystems. The existing assessment done under GLOWS was based on the health of the ecosystem and the parameters were compared based on the existing standards and guidelines. Though the scope of this study has only minimal overlap with the initial water quality assessment (GLOWS 2007), it is important to make reference to these parameters in order to get a complete picture of the water quality within the basin.

2.3.1 Temperature, electrical conductivity, alkalinity and pH

Temperature is an important controlling variable for metabolic processes within an aquatic ecosystem. It changes with other parameters and with the season or time of the day. Water temperatures have a substantial effect on the functioning of aquatic ecosystems and physiology of the biota (Thorp and Gibbons 1978). Physiological processes have thermal optima, and alterations to ambient temperatures may affect the species exposed in a variety of ways. These effects may be direct through the changes in metabolism or indirect, e.g. through the influence of oxygen solubility in water.

Electrical conductivity (EC) is a measure of the ability of water to conduct an electric current. High EC implies that there is a high concentration of ions in the water. EC is affected by geology and soils, as well as agricultural runoff. Alkalinity on the other hand is the buffering capacity of the water or the ability of water to resist change in pH at addition of acidic substances. This is affected by geology, soils, and sewage outflow. Alkalinity can vary with river flow, where low flow typically has increased hardness values relative to high flow/flood values. Water pH is an important parameter; most guidelines give a pH range of between 6 and 9 for fresh water ecosystems. Extremely high or low pH has adverse effects on the quality of the water and may kill aquatic organisms.

Based on a previous study (GLOWS 2007), along the Amala River, EC increased from the headwaters in the Enupuiyapi Swamp to the point of convergence of the Nyangores and Amala Rivers. The Nyangores River however, maintained constant levels of conductivity at 50 μS/cm^2 in Silibwet, Tenwek and Bomet just before the convergence with the Amala. Temperature, conductivity, and salinity increased with stream order from the source to the confluence within the Amala, whereas within the Nyangores these parameters were found to be consistently stable. Regarding alkalinity, total hardness ranged from 15 ppm at Enupuiyapi (source swamp) to 68 ppm at Tabora, and calcium hardness ranged from 8 ppm at Enupuiyapi swamp (source swamp) to 44 ppm at Talek Bridge (GLOWS 2007).

2.3.2 Dissolved oxygen

Dissolved oxygen is an indicator of a water body's ability to support life. It is affected by volume and velocity (turbulence) of a water body, climate and season as well as type and number of organisms in the water. Dissolved oxygen is among the most important water quality parameters due to its strong influence on aquatic organisms. It is of significant importance to the respiration activities of aquatic organisms, and continuously low DO hence has a negative impact on aquatic life. In most aquatic ecosystems DO exhibits variation following a diurnal cycle and therefore the values are dependent on the time of the day and productivity of a water body. To preserve biological diversity DO below 6mg/l or 80% saturation may be dangerous, this being determined over at least one diurnal cycle (USEPA 1990). Based on the 2007 study (GLOWS 2007), relatively low DO was measured in the Enupuiyapi swamp (source), while the remaining sampling sites along flowing headwater streams and rivers had high DO levels compared to the source swamp. The low DO level at the source was attributed to intense respiration and high levels of DOM.

2.3.3 Turbidity and total suspended solids

Turbidity and total suspended solids (TSS) give an indication of suspended matter (seston) which may be mineral or organic material. Suspended matter may arise from stream bank erosion or loose soils via surface and subsurface runoff. Increased seston may stem from erosion of farmland soils which typically have lower erosion resistance. When a stream is turbid due to increased suspended matter, light penetration is inhibited and therefore photosynthesis of submerged plants and autotrophic biofilm components is reduced. In May, 2005, turbidity was recorded at an average of 7.1 NTU in most of the headwater forest streams of the Mara River, but more than 1000 NTU were recorded further downstream at the confluence with the Sand River before the Mara flows into Tanzania (GLOWS 2007). However, high turbidity was mostly recorded after rain storms.

2.3.4 Nutrients and other inorganic parameters

The other important inorganic parameters that were considered in the initial studies in the Mara basin include phosphate, ammonium/ammonia, nitrite and nitrate. These nutrient ions deliver N and P, essential elements for plant growth and life in general. Problems only arise when they exceed the natural levels required, leading to surface water eutrophication. Nitrogen and phosphorus are the most abundant nutrients in aquatic systems and both exist in organic and inorganic forms. The concentration of ammonia as NH_3 for instance, is influenced by both pH and temperature; increase in pH causes a shift in the equilibrium from NH_4 to NH_3 which may be toxic to fish. Increased primary productivity may also lead to elevated pH and the likelihood of toxicity. Increased concentrations of phosphate and nitrate most likely originate from fertilizer runoff and/discharge of detergents, and are often responsible for water body eutrophication.

In the year 2005, total dissolved nitrogen ranged from of 0.3 mg/l at Talek Bridge to 15.0 mg/l at Silibwet Bridge in the Nyangores (GLOWS 2007). On the other hand dissolved organic nitrogen was in the range of 0.9 mg/l in the Tigite River (a tributary to the Mara) to 5.58 mg/l at Silibwet Bridge. These concentrations of nitrogen at Silibwet were thus nearly seven times those detected at other sites, and were speculated to have originated from the surrounding tea plantations. Further downstream at Tenwek dam concentrations similar to other stations in the middle portion of the basin, around 1mg/l were recorded. Ammonium dominated the inorganic nitrogen in Enupuiyapi Swamp (River source) and in the rest of the catchment ammonium concentrations were below the WHO standard of 0.5 mg/l in all stations except Silibwet. Just like nitrogen, highest concentrations of total dissolved phosphorous and orthophosphate in the Mara basin were found in the tea-producing area of Silibwet Bridge with 1.21 and 1.15 mg/l, respectively, in 2005 (GLOWS 2007). As a general trend, the nutrient content of Mara River water increased downstream and nutrient concentrations were found to be higher in the agricultural sections of the basin.

2.4 Previous trends in DOM in Mara Streams

Dissolved organic matter (DOM) is an important component of the carbon cycle and energy balance in streams. It is a primary food source in the aquatic food web and an important part of the acid-base chemistry of many low-alkalinity freshwater systems. It is significant in the mobility of trace metals and affects light attenuation in streams. The first survey of DOC concentrations in the Mara was done in 2005. DOC levels were found to be variable and ranged from 19.0 mg/l in Enupuiyapi Swamp station 1 (Fig 4) to 1.92 mg/L downstream of Tenwek Dam station 6 (Fig 4); considered as comprising the headwaters. Further downstream at stations 7 to 21, DOC levels are also variable.

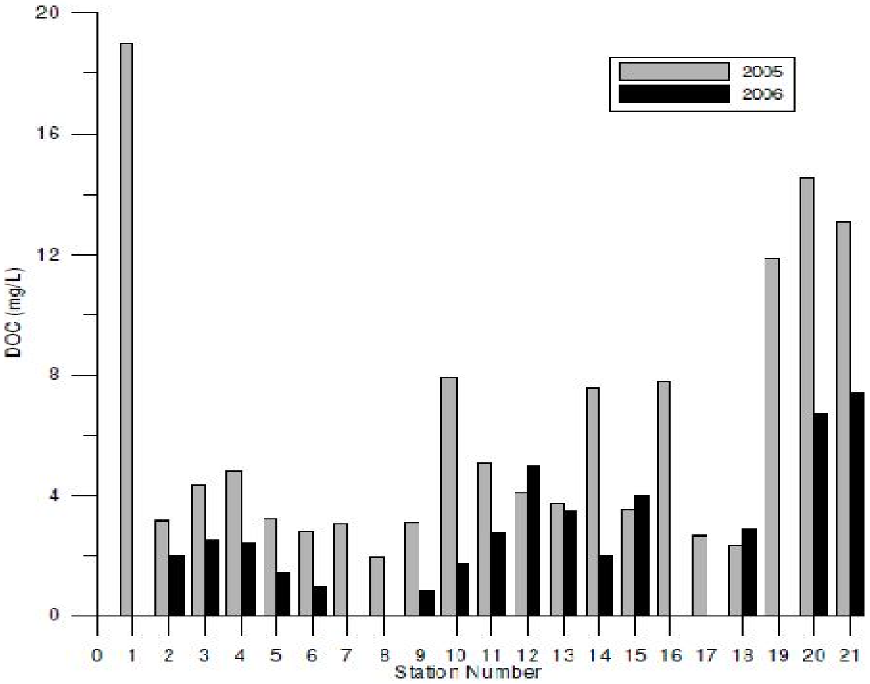


Figure 4: DOC in the Mara River during the 2005 and 2006 sampling campaigns, station 1 is Enupuiyapi Swamp(GLOWS, 2007).

2.5 DOM in the aquatic ecosystem

Dissolved organic matter (DOM) represents a diverse assemblage of structurally complex compounds with aromatic and aliphatic hydrocarbon structures. It consists of a mixture of organic materials such as humic acids, fulvic acids, hydrophilic acids, proteins, lipids, amino acids, carboxylic acids and hydrocarbons. Various chemical species may dominate depending on the type and source of the water or DOM. These compounds arise from different processes mainly the chemical and biological degradation of animals and plants (Thurman 1985). DOM is very difficult to measure and in many cases dissolved organic carbon (DOC) is the preferred measurable expression due to analytical reasons. Humic substances (humic and fulvic acids) are known to comprise a high percentage of DOM and have a higher molecular weight (Woelki, Friedrich et al. 1997). Humic material causes a yellowish-brown color and gives water a muddy taste and odor. This color becomes visible especially if the DOC concentration is higher than approximately 5mg/l (Thurman 1985). Humic substances are refractory anionic macromolecules containing both aromatic and aliphatic components with primarily carboxylic functional groups (Aiken and Leenheer 1993).

The non-humic hydrophilic fractions of DOM are composed of transphilic acids, proteins, amino acids and carbohydrates, and account for about 20-40% of the DOC in natural water. They are less hydrophobic than the humic fractions and form the lower molecular weight DOC (Aiken and Leenheer 1993), (Owen 1995). The transphilic fraction makes up a lower percentage of DOM in natural water and its compounds have a molecular weight in between hydrophobic and hydrophilic fractions (Thurman 1985). The acidic functional group of DOM contains carboxylic acids, phenols, enolic hydrogen and quinone. The carboxylic acid subgroup is considered the most important in the acid group due to its contribution in the solubility and acidity of the organic molecule. Natural waters at a pH between 6 and 8 contain organic acids as ions and these are also the largest dissolved organic compounds. The conventional benchmark of DOM with colloidal molecules is 0.45µm, although 0.22 µm is equally common. In aquatic ecosystems, DOM plays many roles including its usage as an energy source and its enhancing effect on metal solubility and transport. Humics and hydrophilic substances contain the majority of the carboxyl groups. The oxidation of soil and plant organic matter by bacteria generates carboxylic acid functional groups and increases aqueous solubility (Thurman 1985).

Carbohydrates are an important reactive fraction of DOM in water. They exist in several classes known as monosaccharides, oligosaccharides, polysaccharides and saccharides. They are bound to humic substances and linked together into polymers to form several important polymeric sugars. Another important basic group is amines and amides derived from amino acids, polypeptides and aquatic humic substances. They form hydrogen bonds with water and increase its solubility; however they are easily adsorbed by sediment (Thurman 1985).

DOM is known to be important as a substrate for heterotrophs, this is mainly attributed to the fraction of DOM with lower molecular weight substances. The low molecular weight organic acids represent the degradation products of organics released by algae and bacteria as a result of chemical and biological oxidation processes (Owen 1995). The low molecular weight neutrals are uncharged compounds with poor biodegradability. The biodegradable organic matter (BDOM) is made up of the fraction of DOM which can be used by microorganisms as a source of energy and nutrients. This mainly includes the non-humic substances constituting 10 to 20 % of the bulk DOM. Two conventional measurements are common for BDOM: BDOC, biodegradable organic carbon, is mineralized by heterotrophic organisms and can be measured by the difference in the initial and final concentration of DOC after incubation with the native or a standard microbial population. Alternatively, AOC is the assimilable organic carbon which constitutes 1-10 % of the fraction of BDOC and represents the fraction that can be converted to cell mass by a single organism or a defined bacterial community (Camper 2004).

Characterization of DOM has to date remained difficult. It is for instance not easy to identify all organic molecules in water due to the great complexity of the organic matrix, constituted by a mixture of hundreds of simple molecules or hetero-polymers, and the very low concentrations (Thurman 1985). The main breakthrough in characterization has been through various fractionation and measurement techniques that give partial information on organic matter levels, and chemical functions or molecule structures, depending on the methodology employed (Amy 2004).

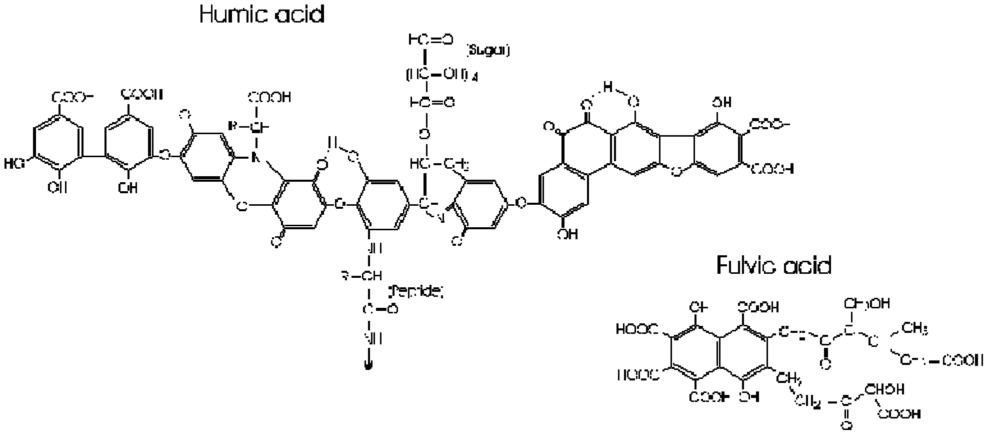


Figure 5: Example of structures of DOM showing the humic and fulvic acid parts (source: (Thurman 1985))

2.6 Importance of DOM in ecology

Dissolved organic matter is attracting the attention of ecologists due to its effects on the physical, chemical, and biological properties of freshwater systems (Gergel, Turner et al. 2002). By attenuation of solar radiation for example, DOM is known to provide UV-B protection to microscopic flora and fauna within the aquatic ecosystem (Morris 1997; Schindler 1997)), but it may also reduce primary productivity by decreasing transparency in an aquatic ecosystem. Fulvic and humic acids of DOM have been reported to influence the acid–base chemistry of freshwaters (Stephenson 1988). This in turn affects the cycling of metals like mercury, copper and aluminum, thereby influencing the amount of trace metals found in aquatic organisms. DOM can also support bacterial secondary production and influence the availability of some forms of phosphorus to phytoplankton. Within the river DOM has several origins. Phytoplankton releases a large portion of its cell material to the open water as extracellular DOM (Cole and Caraco 2001). This DOM pool is colorless and is composed primarily of carbohydrates and amino acids that are immediately metabolized by bacteria. Aquatic macrophytes within the littoral zone can also secrete DOM but the decomposition of these labile, secreted compounds is often very rapid, may happen within about 48hours, and these compounds therefore constitute only a small proportion of DOM in natural waters (Gergel, Turner et al. 2002). Opposed to these autochthonous DOM sources, allochthonous DOM can enter the stream through various pathways from the terrestrial environment: precipitation, leaching or decomposition. Productive wetlands can produce massive amounts of colored DOM made up of fulvic and humic acids. These forms of DOM are considered as products of the degradation of lignin and cellulose (Engstrom 1987). A good part of DOM in natural freshwaters can be composed of these colored, allochthonous compounds. DOM can thus be used as an indicator of organic loading in streams and terrestrial processing and a high degree of variability is seen across different ecosystems. Although most DOM is natural in origin, concentrations and major characteristics including molecular composition are therefore largely a function of catchment landscape features (Bernardes, Martinelli et al. 2004).

The loss of organic carbon from terrestrial ecosystems and its burial in inland waters has been identified as an important redistribution of carbon that ought to be accounted for in carbon sinks and climate change mitigation strategies (Battin, Luyssaert et al. 2009). Streams and rivers have been identified as important sites for respiration of terrestrially derived organic carbon (Battin, Kaplan et al. 2008; Battin, Luyssaert et al. 2009), which has led to the shift from the traditional view of these systems as mere conduits of organic matter from the continents to the oceans (Cole 2007). Though previous studies have confirmed that streams, rivers and estuaries produce remarkable amounts of CO_2 outgassing to the atmosphere, a better understanding of the mechanisms by which fluvial ecosystems achieve this high metabolic performance based on relatively persistent terrestrial and soil organic matter is still lacking (Battin, Kaplan et al. 2008; Battin, Luyssaert et al. 2009).

2.6.1 DOM, ecosystem structure and function

Streams and rivers were traditionally considered as nutrient transporting canals connecting land to oceans. Successive studies have endeavored to explain the distribution and source of nutrients and organic matter supporting the riverine biota and the important connectivity with terrestrial habitats (Cole 2007). Landuse change impacts stream nutrient chemistry partially through the effects on nutrient cycling in vegetation and soil organic matter (Richey 1997). It also alters the metabolism of streams through loading with sediments, nutrients, and contaminants. Streams and adjacent riparian zones are intimately linked by the flow of resource subsidies between terrestrial and aquatic habitats (Williamson, Dodds et al. 2008). As natural transporters, their flowing waters reflect the biogeochemistry of their watersheds. Thus, higher order streams and rivers are considered to be good integrators of both natural and anthropogenic processes in their drainage basins (Bernardes, Martinelli et al. 2004), and they therefore have the potential to offer a broad view of the magnitude of biogeochemical changes over a landscape.

River metabolism has been identified as an integrative metric of cross-ecosystem connectivity. Metabolism measures the rates of production and respiratory use of organic carbon, and provides a direct estimate of the food base and the way energy moves through the river food web (Young, Matthaei et al. 2008). Many tropical and subtropical catchment areas suffer from intensive deforestation in upland areas, resulting in an increased delivery of eroded sediment to the river system which can be expected to affect the ecosystem metabolic processes.

Landscape-level changes in land use can have profound impacts on riparian structure and stream health, and may alter the flow of resource subsidies across the stream-riparian boundary (Houser, Mulholland et al. 2005). Cutting and burning of forest vegetation adds nutrients to soils directly via combusted biomass and indirectly by enhancing the rates of decomposition of organic matter and reducing plant nutrient uptake (Guggenberger, Haumaier et al. 1996), (Vitousek and Hooper 1993). However, as an indicator of cross ecosystem connectivity, river metabolism should be seen as complementary to traditional monitoring tools that rely on structural measures.

As terrestrial organic matter is often the dominant carbon input into river systems (Martinelli, Piccolo et al. 1999); (Coynel, Etcheber et al. 2005), landuse changes can in some cases be rapidly reflected in riverine carbon and nutrient pools (Bernardes, Martinelli et al. 2004);(Raymond, Neung-Hwan et al. 2008). Agricultural land use seems to have variable effects on the concentration and export of organic carbon. Some studies have hinted that agricultural systems export compounds of lower molecular weight (Cronan, Piampiano et al. 1999).

2.6.2 DOM and aquatic food webs

The role of DOM in structuring food webs should not be underestimated. While traditionally food webs have been described for communities in spatially discrete habitats defined by recognizable ecosystem boundaries (Elton 1927),(Lindeman 1942), ecologists have recently shifted their focus towards energy flows across these boundaries and how these affect food webs and ecosystem processes at larger spatial scales (Polis, Hurd et al. 1997). Resource subsidies in the form of living prey, nutrients or detritus exchanged between habitats have been shown to have direct measurable consequences on the consumer dynamics and productivity of the recipient ecosystems (Polis, Hurd et al. 1997). Similarly, microbial communities along a riverine continuum exploit energy which escapes from upstream ecosystems, thereby increasing the overall energy utilization at the stream network level

(Battin, Kaplan et al. 2008).The availability of DOM to heterotrophic bacteria likely depends on its biochemical composition and molecular size, inorganic nutrient concentrations and other environmental factors, such as temperature (Amon and Benner 1996) and all these are essential for the support of aquatic life.

The importance of resource subsidies is particularly evident at the terrestrial-aquatic interface where subsidy fluxes between terrestrial systems and aquatic systems have been well studied (Baxter, Fausch et al. 2005),(McClain, Boyer et al. 2003). Landscape-level changes in land use alter the flow of resource subsidies across the terrestrial-aquatic boundary. For example,(Houser, Mulholland et al. 2005) report that natural or anthropogenic disturbance of upland soil and vegetation can affect instream processes, ecosystem respiration rate and gross primary productivity. Similarly, where agricultural practices extend to the stream margin and the natural riparian forest is removed or replaced with a vegetated buffer, stream macroinvertebrate consumer’s were found to shift from relying on deciduous litter inputs to relying largely on autochthonous algal production, and shredding macroinvertebrates were lost from the aquatic community (Delong and Brusven 1998). Yet, beyond this examination of the loss of basal food resources with different land use practices, terrestrial-aquatic linkages have not been well-studied in human-impacted landscapes.

The understanding of the ecological functions of DOM can lead to our improved understanding as to how geochemical processes function. According to (Zsolnay 2001), this understanding can then be used to develop intervention strategies to mitigate ecological problems. In addition, one can envision that DOM is a link between various ecological compartments. The dynamic nature of organic matter enables it to serve as a sensitive indicator of shifts in ecological processes (Zsolnay 2003) and terrestrial pressures (Bernardes, Martinelli et al. 2004), (Williamson, Dodds et al. 2008), (Bouillon, Abril et al. 2009).

2.7 Human influence and global implications

The detection of human influence on biogeochemical processes is at times complicated by natural variability in nutrient cycling and stream chemistry. Stream concentrations of cations for instance vary with soil cation status (Biggs, Dunne et al. 2002), and stream carbon, nitrogen, and phosphorus levels are known to vary with soil nutrient content (Pote, Daniel et al. 1999), and C:N ratios (Creed and Band 1998), complicating the separation of natural and human effects on nutrient and carbon concentrations.

The effects of land use and climate on the character of riverine dissolved organic and inorganic carbon have important implications for global carbon cycling, owing to their potential to control rates of microbial carbon processing like uptake, retention and outgassing in agricultural systems (Wilson and Xenopoulos 2008b). Metabolism is considered as a primary response variable that assists us to understand the impacts of climate change, land use, and nitrogen deposition (Houser, Mulholland et al. 2005). This is based on the fact that metabolism is a fundamental ecosystem processes and is influenced directly by changes in climate, land use, and atmospheric deposition. A better understanding of terrestrial-aquatic linkages is not only important to improve global estimates of carbon processing in rivers, but is also fundamental to our understanding of the impact of on- going and future land use changes (Bouillon, Abril et al. 2009) and the dynamics of climate change (Williamson, Dodds et al. 2008).

Other factors known to control organic carbon include site-specific factors, such as vegetation and seasonality of meteorological parameters which control DOM composition in soil and soil solution properties and therefore also affect its biodegradability (Marschner and Bredow 2002). Changes in organic matter sources, water levels and associated microbial degradation processes control the observed seasonal and spatial variations in net stable carbon isotopic composition of methane (Moura, Martens et al. 2008).

Another important consideration is the river geomorphology which changes longitudinally, and has been linked to shifts in organic matter supply, river processing rates, and the relationship between primary production and respiration (Vannote, Minshall et al. 1980), (Naiman, Melillo et al. 1987). In catchments dominated by open grassland, the influence of terrestrial energy sources on riverine structure and function may be reduced, and autochthonous inputs may be a significant energy source (Delong and Brusven 1994). However, autochthonous production may also become reduced by the cumulative effects of agricultural activity. For example, increased turbidity and deposited sediments may suppress algal production (Young and Huryn 1996).

2.8 Characterization of DOC

As initially indicated, characterization of DOC has remained a challenge due to the complexity of its structural components. However, with technological advances several analytical techniques have been developed, and found to offer potential advantages for characterizing DOC in waters. This is important because a measurement indicative of fulvic acid source for example, could provide useful supporting data in determining carbon budget for a fresh water ecosystem. Below are some of the techniques currently available and adopted for this study.

2.8.1 Fluorescence excitation-emission matrices (EEMs)

EEM is a simple and rapid technique used to characterize dissolved organic matter. Molecules are excited by light and release energy in the form of light, i.e. fluorescence, that is measured. Fluorescence occurs when these molecules, having been excited by a high energy light source that raised the energy levels of the electrons within the molecules, release energy in the form of light. The intensity of this light depends on the amount of each of these structures present in a water sample. During fluorescence, the absorption of light of a given wavelength by a fluorescent molecule (fluorophore) is followed by the emission of light at longer wavelengths. Distribution of wavelength-dependent intensity that causes fluorescence is known as the fluorescence excitation spectrum, and the distribution of wavelength-dependent intensity of emitted energy is known as the fluorescence emission spectrum. These spectra can be used to fingerprint DOC. It is also possible to create a three dimensional picture by measuring the fluorescence intensity at each point in a matrix of excitation and emission wavelengths, yielding an excitation-emission-matrix (EEM). Fluorescence data can indicate the chemical properties of the humic substances and distinguish DOM from different origins, for example microbial from terrestrial. Microbially derived fulvic acids for instance have fluorophores with a more sharply defined emission peak occurring at lower wavelength than fluorophores in terrestrially derived fulvic acids (McKnight 2001).

Using the fluorescence data, the Fluorescence Index (FI) can be computed by dividing emission intensity at a wavelength of 450 nm to that at 500 nm using an excitation wavelength of 370 nm (Mc Knight et al. 2001). The FI is a simple index that represents a greater decline in emission with increasing wavelength for microbial derived samples. This can serve as a simple index to distinguish sources of isolated aquatic fulvic acids. For example an FI between 1.3-1.4 is characteristic for allochthonous origin, while an FI within 1.7-2.0 points to autochthonous origin. In general, a difference in FI of at least 0.1 may be indicative of a difference in source of fulvic acids (McKnight 2001).

In EEMs, the wavelengths used for excitation typically vary from around 200 to 450 nm, and emission from 250 to 600 nm. In typical spectra three peaks commonly appear. First, a peak at low excitation and low emission wavelengths excitation (<300 nm and < 350 nm, respectively). This peak is typically indicative of proteins. Another peak at low excitation and high emission wavelengths (at about 250 nm and > 350 nm for excitation and emission, respectively) is indicative of humic-like substances. And a peak at high excitation and emission wavelengths (> 300 nm and > 350 nm, respectively) is indicative of fulvic-like substances (Parlanti, Wörz et al. 2000).

2.8.2 UV absorbance

The UV absorbance measurements are known to provide a quick estimate of the organic carbon content of water samples. This measurement is at times used as a substitute for DOC. The UV/OC (Organic Carbon) ratio is useful in determing the humic fraction in the organic matter or the aromaticity (Helms et al 2008). In general a large part of allochthonous DOC is refractory and therefore physico-chemically protected from microbial degradation. It has been hypothesized that upon entrance into the stream this fraction of DOC is photo-bleached and converted into low molecular weight compounds which improve microbial degradation (Helms et al., 2008). Autochthonous DOC on the other hand has a lower aromaticity and is more labile and therefore rapidly metabolized by bacteria. The photo-labile fraction of DOC shows a strong absorbance in the visible light range, the UVA and UVB region (Osburn et al., 2001). The SUVA (Specific UV Absorbance) is defined as the UV absorbance of a water sample at a wavelength of 254 nm normalized for dissolved organic carbon concentration (DOC in mg/l) (Weishaar 2003). This ratio measures the electron richness and thus indicates aromatic carbon and double and triple-bonded non-aromatics. It also measures the contribution of aromatic structure to DOC (USEPA 1986). Low SUVA values indicate non-humic substances such as proteins, while high SUVA values indicate more humic DOC.

2.8.3 Direct measurement of DOC concentrations

Accepted and commonly used methods for measurement of dissolved organic carbon are based on either wet chemical oxidation, direct catalytically supported high-temperature oxidation (combustion), or photo-oxidation procedures. With these techniques, oxidation involves the digestion of organic carbon to carbon dioxide by oxidizing reagents (persulphate), heat or U.V. radiation. The carbon dioxide produced is measured by a non dispersive infra-red gas analyzer.

2.8.4 Carbon isotopes

Carbon isotopes are atomic nuclei that contain six protons plus neutrons that varying in number from 2 to 6. Two stable isotopes of carbon exist; carbon-12 (^{12}C) that makes up 98.93% of carbon on earth and carbon-13 (^{13}C) which makes up 1.07%. One radioactive isotope carbon 14 (^{14}C) exists in small quantities: 1part per trillion. The various sources and sinks of carbon involve different mechanisms with different affinity for ^{12}C and ^{13}C isotopes which allows distinguishing between the various sources by $^{13}C/^{12}C$ ratio. This ratio, once standardized to a reference material (PeeDee Belemnite-PDB or Vienna-PDB: VPDB), is called $\delta^{13}C$ and can be easily determined by an isotope ratio mass spectrometer. $\delta^{13}C$ values range between -100 to +50 ‰ (the natural abundance range). Negative values represent relatively light carbon on average, while samples with high $\delta^{13}C$ values are relatively enriched in the heavy isotope(s). However, it should be noted that isotope values rarely provide a truly unique fingerprint (Fry 2006).

The $\delta^{13}C_{DIC}$ of DIC begins with atmospheric CO_2 with $\delta^{13}C$ of –7% (relative to VPDB). Photosynthetic uptake of $CO_{2(atm)}$ is followed by a significant depletion in ^{13}C. This occurs during CO_2 diffusion into the leaf stomata and dissolution in the cell sap, and during carboxylation (carbon fixation) by the leaf's chloroplast, where CO_2 is converted to carbohydrate (CH_2O). The combination of these fractionating steps results in a 5 to 25‰ depletion in ^{13}C. The amount of fractionation depends on the pathway followed. Three main photosynthetic cycles are recognized: the Calvin or C_3 cycle, the Hatch-Slack or C_4 cycle, and the Crassulacean acid metabolism (CAM) cycle. The C_3 pathway is common in almost 85% of plant species and is the dominant pathway in most terrestrial ecosystems (Ehleringer 1991). Most C_3 plants have $\delta^{13}C$ values that range from -24 to -30‰ with an average value of about -27‰. The more efficient C_4 pathway occurs under low CO_2:O_2 conditions and at higher temperatures, where increased respiration in C_3 plants interferes with their ability to fix CO_2. C_4 plants add an initial step where the PEP carboxylase enzyme acts to deliver more carbon to Rubisco for fixation. The result is a reduction in ^{13}C fractionation during carboxylation. C_4 plants have $\delta^{13}C$ values that range from -10 to -16‰, with a mean value of about -12.5‰ (Vogel 1987). C_4 species represent less than 5% of plant species but are known to be dominant in hot open ecosystems such as tropical and temperate grasslands (Ehleringer 1991). Common agricultural C_4 plants include sugar cane, corn and sorghum.

2.9 Dissolved Inorganic carbon

Another important fraction of dissolved carbon is the inorganic carbon (DIC). (Barnes and Raymond 2009) found that nitrogen additions (e.g. fertilizers and manure) were aiding in the dissolution of lime, increasing the total export of DIC from agricultural watersheds. Their isotopic data suggested excess DIC export from altered systems to originate from increased chemical weathering and enhanced CO_2 production within urban stream reaches. Production of CO_2 from the in-stream processes such as photosynthesis and respiration can equally alter DIC. The in-stream metabolism of organic carbon is often greater than primary production and results in supersaturated dissolved CO_2 making streams and rivers a source of CO_2 to the atmosphere (Cole and Caraco 2001). These processes control the alkalinity and pH of the water and determine the partitioning of DIC into bicarbonate ($HCO_3^{\ -}$), carbonate ($CO_3^{\ 2-}$), and dissolved CO_2 (Barnes and Raymond 2009).

Although current studies have associated increases in the inorganic carbon flux to agricultural activities, it is often difficult to isolate human impacts on riverine constituents and ecology due to the highly correlated nature of land use, geology, soils and climate (Perrin, Probst et al. 2008). However, both urban and agricultural areas export significantly more DIC than their forested counterparts and these increases have been associated to land disturbance activities and enhanced organic matter loading (Barnes and Raymond 2009).

2.10 Carbon dioxide and the terrestrial aquatic interface

This study has taken carbon dioxide (CO_2) as a measure of ecosystem metabolism with an initial hypothesis that changes of land use affect concentrations and availability of organic matter which in turn potentially affect ecosystem metabolism. Alterations of this important ecosystem process could also increase fluxes of CO_2 to the atmosphere.

Inland waters have been shown to contribute significant amounts of carbon dioxide to the global carbon cycle. While carbon enters the biological compartment mainly through photosynthesis, its assimilation supports the respiration of all terrestrial and aquatic organisms. Concentration of CO_2 within the stream is often greater than primary production resulting in saturation of CO_2 and making streams a net source of CO_2 to the atmosphere (Cole and Caraco 2001), (Jones, Shannon et al. 2004). It should be noted that the rate of exchange of gas with the atmosphere depends on two main factors; the concentration gradient between water and air, and the gas exchange coefficient at a given temperature. The gradient is therefore expressed as the difference between the actual concentration and the concentration that is expected at equilibrium with the atmosphere (Wanninkhof 1992).

Another important form of carbon is methane (CH_4). Although the global mass emitted is small compared to CO_2, it has a larger effect on global warming and since aquatic ecosystems are occasionally anoxic some of the carbon gas efflux occurs as methane.

Measurements of CO_2 in streams can give an indication of metabolic performance of a system through the rates of carbon processing. This measure has been proposed as a common integrative metric of cross-ecosystem connectivity that may adequately describe environmental changes across a broad range of landscapes and spatial scales (Williamson, Dodds et al. 2008).

Aspects of ecosystem structure and function have also been identified as important in improving the understanding of aquatic ecosystems and their response to changes in landuse (Young, Matthaei et al. 2008). Being the lowest point in the landscape, rivers represent an exceptionally integrative signal of environmental change. And the origin and fate of carbon in the water provides critical signs of past and present disturbance of the drained landscapes, giving a foundation for forecasting the future (Williamson, Dodds et al. 2008), (Bernardes, Martinelli et al. 2004), (Raymond, Neung-Hwan et al. 2008). A better understanding of the terrestrial aquatic linkage is important in addressing the impact of current and future land use changes and other human induced stressors (Bouillon, Abril et al. 2009). Studying the exchange of subsidies across the terrestrial-aquatic interface may lead to useful insights regarding the conservation and restoration of impacted habitats including ecosystem services. The rates of production and use of organic carbon in river ecosystems as a whole provides a direct estimate of the food base that establishes the life-supporting capacity of an ecosystem. This study has endeavored to characterize the quantity and quality of organic matter in the Mara river basin (Kenya) along the flow path and within various landuse types in the high-altitude perennial headwaters catchment areas. Measurements involved employment of EEM, absorbance, direct measurement of DOC concentration and determination of stable carbon isotopes.

3 Materials and Methods

3.1 Location and time of field sampling

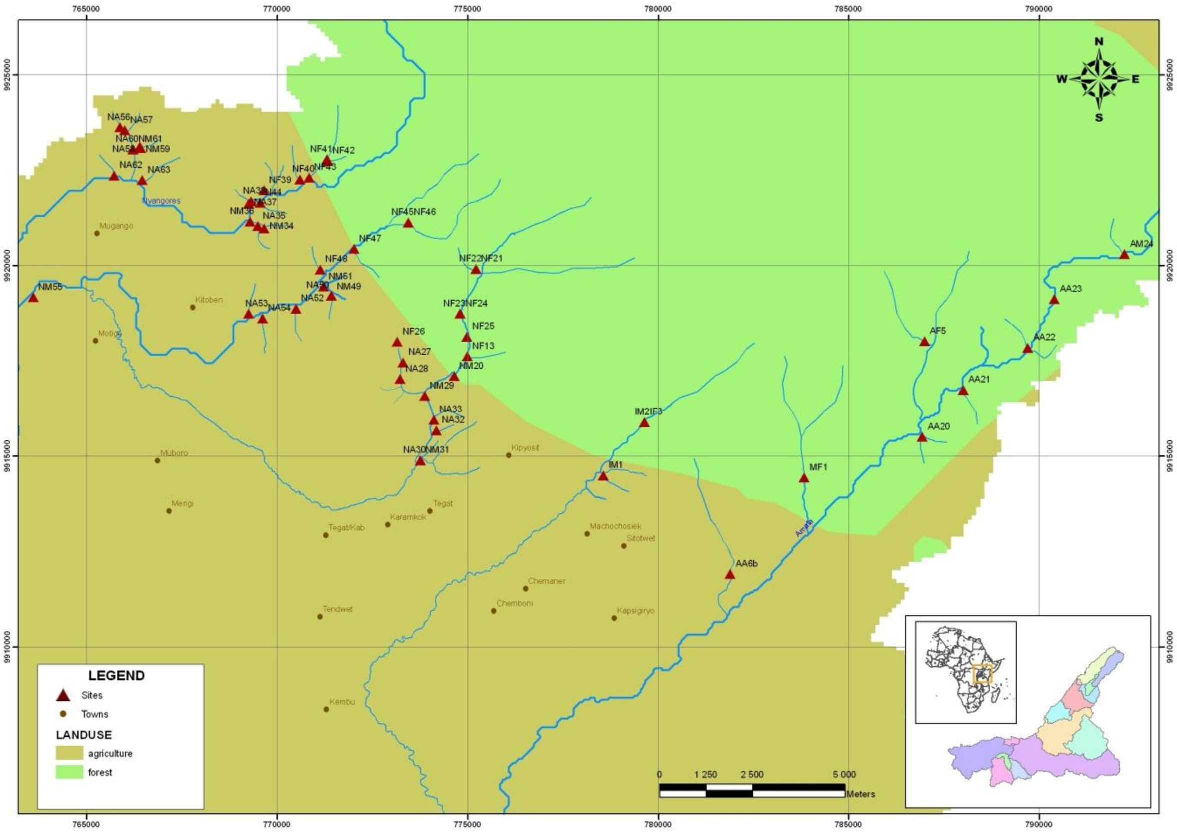


Figure 6: Map of the study area, major towns and sampling sites based on this study

The Mara River basin is located between longitudes 33°47' E and 35°47'E and latitudes 0°38' S and 1°52' S, with the upper 65% of area (8,941 km^2) in Kenya, while the remaining lower portion is in Tanzania. This study focused on Kenyan head water streams in the Mau forest at an altitude of 2000-3000 m a.s.l. Tributaries of the two main rivers forming the Mara River (the Amala and Nyangores) were sampled once in November and December 2009. Sampling was done during the dry season when a majority of the streams were at base flow. However, occasional rainfall was experienced during this time.

Selection of sampling sites, identification of major landuse type and determination of catchment characteristics were done as follows; Delineation of sub-catchments and watershed boundaries and categorization of different landuses within watersheds using digital elevation model. Topographic maps from survey of Kenya, spatial analysis tools (ESRI-GIS) and visual observation and mapping using GPS. Landuse and land cover map from previous studies under GLOWS provided additional support. Stream order was determined using topographical maps 1:50,000 from Survey of Kenya (1969) and navigation to the selected streams was done using a GPS (Garmin etrex HCx) in combination with assistance from the local guides and forest rangers.

During navigation to sampling points, landuse type and major vegetation or crop within each sub-catchment was noted; streams were tracked to the points of confluence and change of stream order. Water samples were collected at the hydro junctions of the selected streams at an average of 100 to 200 meters before the confluence (Fig 7). Selection of stream networks based on a single landuse was undertaken by surveying the river network from the first order tributaries and identifying catchments with a single landuse. An attempt was made to cover entire networks and reach the highest stream order with a single landuse. This however proved elusive in many occasions.

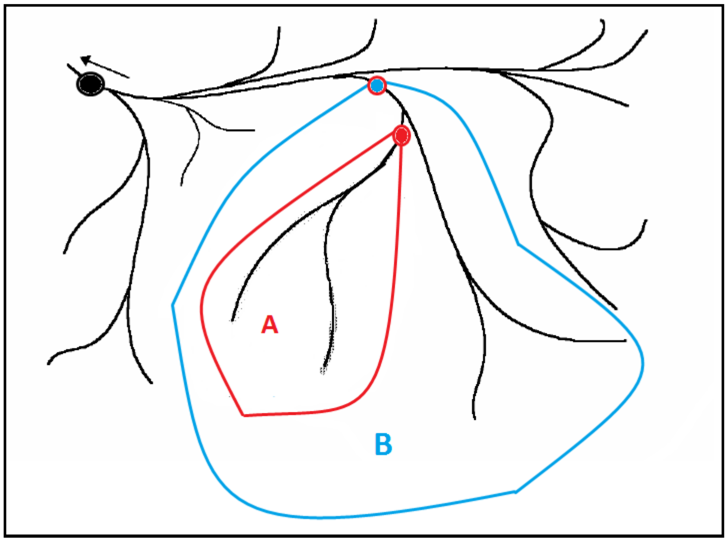


Figure 7: Selection of sub catchments and sampling points; catchment B 3rd stream order and catchment A 2nd stream order. Sampling was done just before the hydro junctions

3.2 On–site measurements

Stream size and morphology were determined using a measuring tape and meter rule and involved measurement of width and depth at specified intervals. Water velocity was measured at 40% depth in different defined intervals based on stream size. Velocity was measured using a Marsh Mc Birney Flow Mate portable flow meter model 2000. The morphological and flow measurements were later used to estimate discharge.

Measurement of physico-chemical parameters: pH, dissolved oxygen, % saturation, conductivity and temperature were done onsite using a YSI professional plus combined electrode meter. Turbidity was measured using a HACH 2100P ISO Turbimeter turbidity meter.

Figure 8: Filtration on site for suspended solids (left) and DOC right

3.3 Geochemistry and nutrients

Clean and sterile falcon tubes were used for collection of water samples for ion analysis. For determination of cations, anions and nutrients, water samples were filtered onsite through pre-combusted (at 450°C) pre-washed Whatman GF/F filters and then using a clean syringe filtered through a sterile Whatman membrane filter (0.22µm pore) into sterile falcon tubes. These samples were stored in a mobile AC/DC cooler box and later stored in the lab in a fridge at 4°C until analysis.

Ion concentrations were determined by Ion Chromatography using a Metrohm 761 Compact IC. The method involves the use of 1mM Na-hydrogencarbonat / 4 mM Na-carbonate eluent, a metrosep A Supp 5 - 150 mm length / 4.0 mm ID / 5 µm particle size column at a flow of 0.8 ml/min and a detection using conductivity after suppression for the anions. For the cations a 4 mM tartaric acid/ 0.75 mM dipicolinic acid (pyridine-2,6-dicarboxylic acid) eluent was used. With a flow of 1.1 ml/min and a Metrosep C 2 - 150 mm length/ 4.0 mm ID / 7 µm particle size column. All these were fitted with an autosampler 788 IC filtration sample processor after online-filtration and sample-/eluent- degassing with a 837 Combi-Degasser. The following Ions were measured; sodium(Na^+), potassium(K^+), calcium(Ca^{2+}), magnesium(Mg^{2+}), fluoride(Fl^-), bromide(Br^-), chloride(Cl^-), nitrite(NO_2^-), nitrate (NO_3^-), phosphate(PO_4^{3-}), sulphate(SO_4^{2-}).

Samples for Ammonium (NH_4-N) were collected from the stream using a plastic jug filtered on site through pre-combusted Whatman GF/F filter and stored in clean plastic drinking water bottles at 4 °C until laboratory analysis. Determination of Ammonium (NH_4-N) was done using a mixed solution (1 part NaOH, 1 part Nitroprussid Salicylat and 1 part Aqua deion) prepared and mixed with 1 ml Dichlorisocyanuracid (0.1g Dichloroisocyanuric acid, sodium salt 98% 1-Sodium-3,5-dichloro-s-triazine-2,4,6-trione, Dichloro-s-triazine-2,4,6-($1H,_3H,_5H$)-trione sodium salt, Sodium dichloroisocyanurate) and 5 ml of sample. A calibration curve was prepared using ammonia standard series. The absorbance of the mixed solution was then measured at 690nm using a Hitachi U-2000 spectrophotometer.

Samples for analysis of orthophosphate were collected and treated like the ammonium samples. The molybdate blue method was used to analyze orthophosphate. A mixed reagent was made by mixing 6 grams of ammonium molybdate dissolved in 250ml of MilliQ water and 0.1454 grams of antimony potassium tartate in 500ml of sulfuric acid. A color developing reagent was similarly prepared by adding 0.37g of ascorbic acid to 70 ml of the mixed reagent and mixing properly. 1 ml of the color developing reagent was added to 10 ml of the sample and color allowed to develop in the dark for 30min. Standards and blanks were prepared and the color developing reagents added. Blanks, standards and samples were then read using a Hitachi U-2000 spectrophotometer at 880nm.

3.4 Particulate and dissolved organic carbon

To remove inorganic and organic carbon residues GF/F filters were wrapped in aluminum envelopes and combusted at 450°C for 4 hours. The filters were then rinsed using Milli-Q water on a filtering unit. The rinsed filters were dried at 90°C for 12 hours, weighed and put in separate aluminum envelopes ready for use in the field.

For measurement of TSS water samples were filtered onsite using the pre-combusted pre washed GF/F filters, the volume filtered was noted and the filters were preserved in aluminum envelopes and stored at 4°C for drying in the lab. TSS was established from the dry mass on the pre-weighed GF/F filters and calculations done from the AFDW and the volume of water initially filtered.

All DOC and optical properties of DOC glass ware and septa were treated for removal of inorganic and organic carbon residues. This was done by application of acid and heat treatment. Treatment involved submerging of glassware overnight in 0.1M HCL, rinsing at least 3 times with distilled water, wrapping in aluminum foil and combusting at 450°C for 4 hours. Clean silicon septa and tweezers were equally submerged overnight in 0.1M NaOH; the septa were rinsed 5 times with distilled water and dried at 90°C for 12 hours. Using the clean tweezers, the septa were assembled onto the DOC bottles without contamination.

For determination of DOC, water samples were filtered through the pre-combusted pre washed whatman GF/F filters and then using a clean syringe filtered though a sterile whatman membrane filter 0.22µm pore into the clean 40ml glass vials with silicon septa screw caps. These samples were stored in a mobile AC/DC cooler box and later stored in the lab in a fridge at 4°C until analysis. DOC was analyzed in Austria by chemical oxidation using an automated DOC analysis method with a Sievers portable TOC analyzer (Sievers 900) fitted with an inorganic carbon remover. This method involves wet-chemical UV/persulfate oxidation with potassium hydrogen phosphate using 1ppm as a standard.

3.4.1.1 Measurement of optical properties of DOM

For determination of optical properties of DOM water samples were collected and treated like the DOC samples. These samples were however stored in amber bottles. Optical properties of DOM (fluorescence spectra) were analyzed in Austria using a Hitachi F-7999FL diode array spectrophotometer. All excitation-emission matrices were obtained by a 3D scan measuring the emission spectra in the range of 200 to 450 nm at 2nm intervals, with an excitation range of 250 to 600 nm at 5 nm intervals, a scan speed of 12000nm/min and a drift less than 1%. The samples were adjusted to room temperature before the scan.

The peaks due to water Raman scatter were eliminated from all spectra by subtracting the Milli-Q water blank spectra from the resultant spectra using the statistical package R. Correction for the inner filter effect was similarly done using R and based on (McKnight 2001)with the following formula;

$$A_{excit} = (\alpha * DOC) * 0.5$$

$$A_{emit} = (\beta * DOC) * 0.5$$

$$A_{total} = A_{excit} + A_{emit}$$

$$\text{Correction factor} = 10^{-A_{total}}$$

Where A_{excit} is the absorbance of the excitation light at a given wavelength α, A_{emit} absorbance of the emitted light at a given wavelength β. 0.5 is half the cuvette size or half the pathlight of the 1 cm cuvette used.

The correction factor was then used to reduce the emission intensity on the basis that the real emission intensity of the solution is decreased because light is absorbed in both the excitation and emission light path. The model fluorometer setting used was able to internally perform the lamp spectral correction "corrected spectra" eliminating instrumental response regarding wavelength characteristics of the monochromator or detector (photomultiplier). For the removal of the Rayleigh scatter, a triangle of zeros was set below the Rayleigh band effectively eliminating the negative values. Finally the fluorescence intensities were normalized for DOC concentration as described in (Parlanti, Wörz et al. 2000). The various peaks were extracted based on Table 1.

Table 1: Excitation and emission wavelengths used in identification of the peaks

	Substance	Excitation range	Emission range
1.	Humic like (A)	237-260	400-500
2.	Humic like (C)	300-370	400-500
3.	Tyrosine like (B)	225-237	309-321
		275	310
4.	Tryptophan like(T1)	275	340
5.	Tryptophan like(T2)	225-237	340-381

Based on Helms et al., 2008 and McKnight et al., 2001

For the absorbance spectra a scan was done with a Shimadzu UV1700 spectrophotometer set at the wavelength range of 250 to 700nm using a 5cm quartz cuvette. Absorbance units were converted to absorption coefficients using the following equation

$$a = 2.303A/l$$

Where $\boldsymbol{a}$ is the absorption coefficient/m, $\boldsymbol{A}$ is the absorbance and $\boldsymbol{l}$ is the path length/m. (Helms 2008)

Based on this scan the slope ratio (Sr) was calculated by taking the slopes of log transformed absorbance's for the shorter wavelength as 275 – 295nm and dividing by the longer wavelength as 350 – 400nm. SUVA254 was computed by dividing the specific absorbance at 254nm by the respective DOC concentrations. β/α was calculated based on Wilson et al., 2008, as the ratio of emission intensity at 380nm (β), to the maximum emission intensity observed between 420 and 435 for an excitation wavelength of 310nm. The fluorescence index was calculated as the ration of emission intensity at 450 to that at 500nm for the excitation wavelength of 370nm. The humification index was measured using excitation at 254 and emission at 300nm, 435 nm, 445 nm and 480 nm. E2:E3 was calculated based on (De Hann 1987) as the ratio of the absorption at 250 to 365. E4:E6 was calculated as the ratio of absorption at 465 to 665nm.

3.4.1.2 Measurements of δ^{13}C and δ^{15}N of POM

δ^{13}C and δ^{15}N of POM were determined from the TSS filters by punching holes into GFF filter to form discs of known area with dried particulate matter, the discs were put into 2 ml Eppendorf vials and acidified with concentrated hydrochloric acid (18%) to remove inorganic C. The discs were then oven dried at 60°C overnight, weighed and packed in Zn-tin cups and rolled into small balls using tweezers. The C and N content and δ^{13}C and δ^{12}C plus δ^{15}N δ^{14}N was analyzed at the University of Vienna chemical ecology lab by use of an elemental analyzer fitted with a stable isotope ratio mass spectrophotometer (EA-IRMS) fitted with an elemental analyzer. The results were expressed as permil (‰) relative to Vienna PeeDee Belemnite and atmospheric nitrogen standard values according to the following equation:

$$\delta \times E(‰) = \left[\left(\frac{R\ \text{sample}}{R\ \text{standard}}\right) - 1\right] \times 10^3$$

Where E is element and R is the abundance ratio heavy to light in the standard or the sample. Internal laboratory reference gases for carbon and nitrogen were calibrated against respective international standards NBS-19, atmospheric nitrogen and NBS-127.

3.4.1.3 Measurement of CO_2 and partial pressure of CO_2

Samples for determination of alkalinity were collected from the stream using a plastic jug filtered on site through pre-combusted Whatman GF/F filter and stored in clean plastic drinking water bottles. These samples were later analyzed in the evening by titration of 200ml of GF/F filtered water samples with 0.1M/l HCL (p.a) to a pH of 4.3. Alkalinity was established by the following formulae

2 * Volume of HCl needed in ml to give the alkalinity in mmol l^{-1}

This is also equivalent to the concentration of HCO^{3-} in mmol l^{-1}

The glass vials for collection of aqueous CO_2 were treated for removal of inorganic and organic carbon residues. This was done by application of acid and heat treatment. Treatment involved submerging of glassware overnight in 0.1mHCL, rinsing at least 3 times with distilled water, wrapping in aluminum foil and combusting at 450°C for 4 hours.

Air-free water samples for determination of CO_2 and CH_4 were collected using a syringe a few centimeters to half a meter, depending on the size of the river, below the surface. The collected water was carefully introduced into headspace vials poisoned with $HgCl_2$ with minimal turbulence. The vials were closed tightly onsite and the samples stored in a mobile AC/DC cooler box and later stored in fridge at 4°C in the lab until analysis.

The carbon dioxide (CO_2) from total dissolved inorganic carbon (DIC) was measured using liquid chromatography gas extraction interface (Abramson, Wirick et al. 2009). During preparation of the samples, a syringe and needle was used to introduce argon gas into the vial while at the same time sample was sucked using another needle and syringe. The extracted sample was slowly introduced into 2 ml glass crimp-seal vials with minimum turbulence. This process was done to reduce equilibration of the sample with atmospheric gas. The vials were over filled and sealed with red butyl rubber/PTFE septa aluminum ring tops without trapping any air bubbles. The samples were analyzed with a Thermo Electron Surveyor lite autosampler equipped with a MS HPLC pump, a LC Isolink interface and Thermo Scientific

Delta V plus stable isotope mass spectrophotometer as described in (Abramson, Wirick et al. 2009).

3.5 Statistical analysis

All the statistical analyses were done using R (2.9.0) statistical package with the activation of MASS and Vegan packages. Graphing was equally done using R in combination with Sigma plot 10.0. Analysis of variance (ANOVA) using stream order, landuse or both was used to test the difference among landuse with regard to the geochemical parameters, ions and nutrients and estimates of DOM and CO_2. When the data were abnormally skewed or variances were not homogenous as indicated by the Bartlett test, log transformation was done.

A multivariate ordination technique was used to assess metavariables and the composite differences in three landuses were evaluated by computing Euclidean distance matrices using geochemical, nutrients or the EEM indices. The triangular dissimilarity (distance) matrix then served as input for non-parametric MANOVA (i.e. “PERMANOVA”) which assesses between-group distances over within-group distances by computation of pseudo-F-values and permutational assessment of significance (Anderson 2001); (McArdle and Anderson 2001). Homogeneity of multivariate dispersion among groups was assessed similarly by permutational analysis (Anderson 2006). The main effects and interactions were tested for the factors stream order and and landuse. The Euclidean distance dissimilarity matrix was further subjected to cluster analysis and non-metric multidimensional scaling (NMDS).

4 Results

4.1 Geochemical parameters and other abiotic factors

For purposes of this study Na^+ K^+, Ca^{2+},Mg^{2+},Fl^-,Cl^-,Br^-,SO_4^{2-} pH, electrical conductivity and alkalinity were measured and have been used as a conservative fingerprint for the geochemical characterization of the study sites.

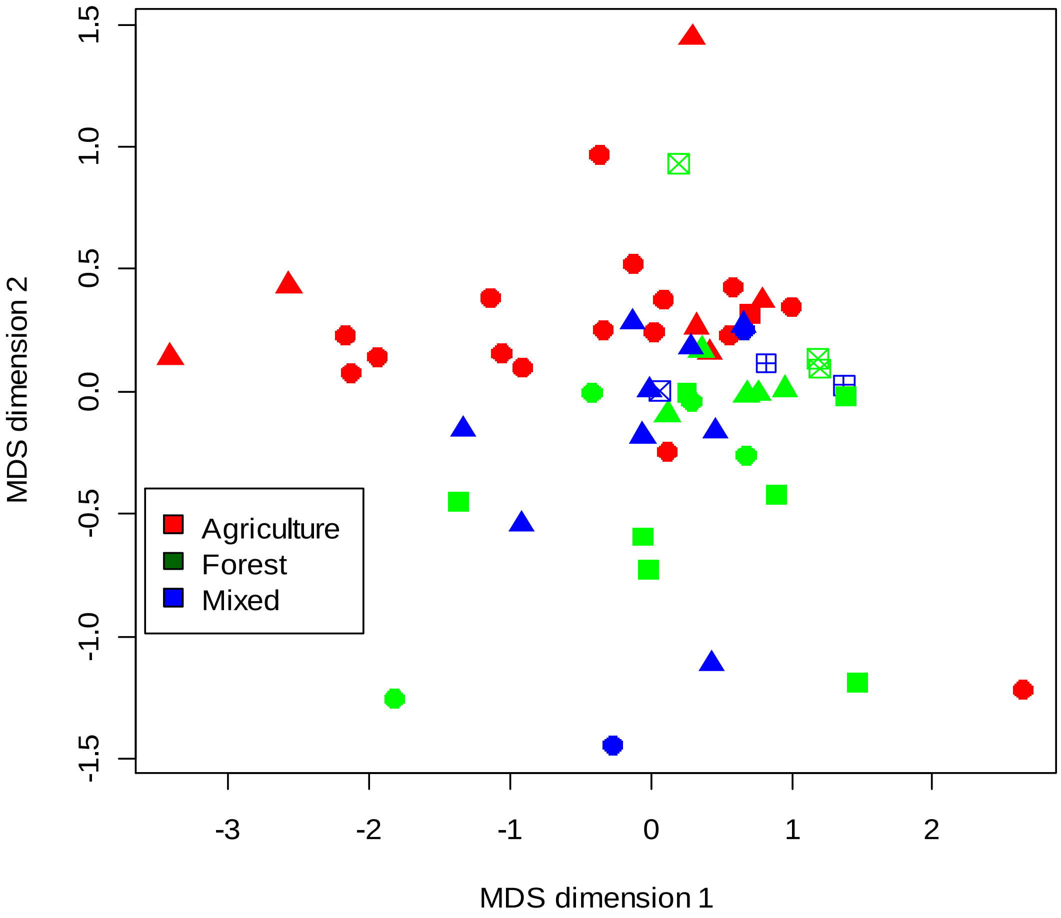
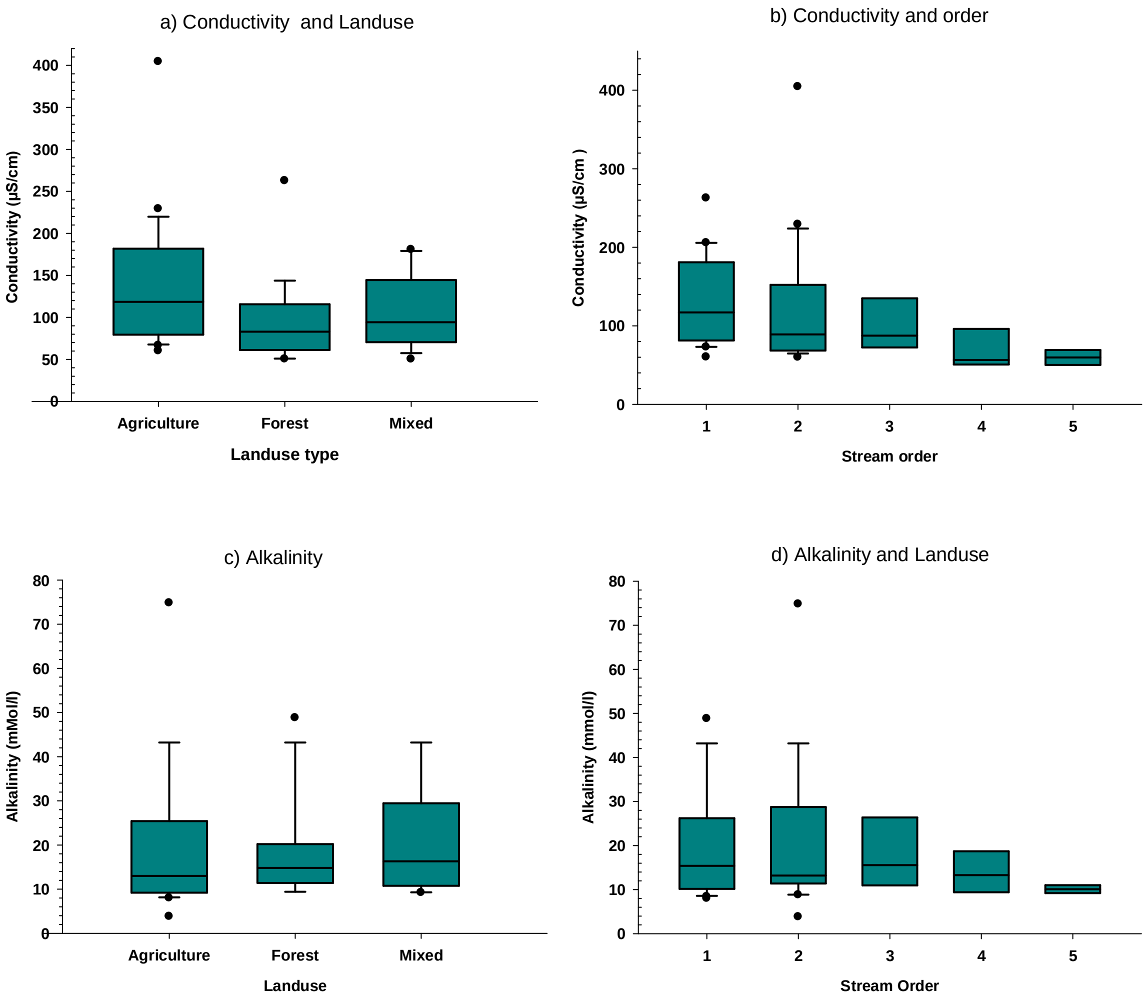


Figure 9: MDS based on major ions (geochemical fingerprint) measured in Mara River streams; Clustering seen with land use (PERMANOVA, p<0.05) and not with stream order (PERMANOVA, p= 0.082) Similar shapes represent similar stream orders and similar colors are similar landuses

Minimal variation in the geochemical parameters was observed throughout the sampling sites. Alkalinity, conductivity and pH did not show significant differences between landuses or stream orders. Conductivity remained fairly stable at 80-150µS/cm for first second and third stream orders and less than 50 µS/cm for fourth and fifth stream orders. There was however a weak trend showing relatively lower conductivities and higher pH in the forest streams with a relatively lower variability as compared to agricultural and mixed catchments (Fig 10, (a) and (e)). The trends in alkalinity showed a strong similarity with the conductivity with the 1st 2nd and 3rd stream orders averaging 10-25mmol/l and slightly lower values in 4th and 5th stream orders. pH was fairly stable with an exception of one agricultural and one forest sight where a pH of 8.6 and 8.5 was recorded, respectively. Differences in the means between the landuses were less than 0.2 pH units. Forested catchments had a slightly higher mean pH value (7.89±0.05SD).



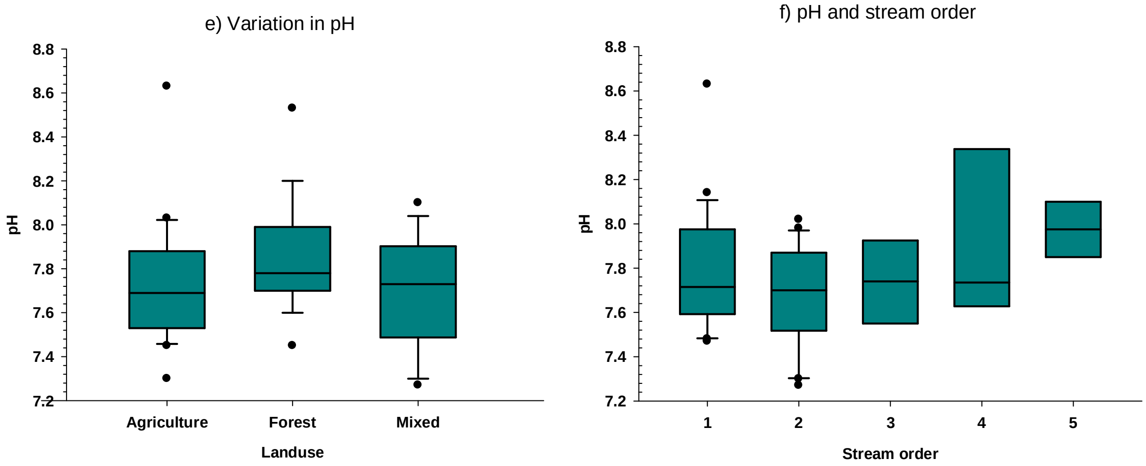
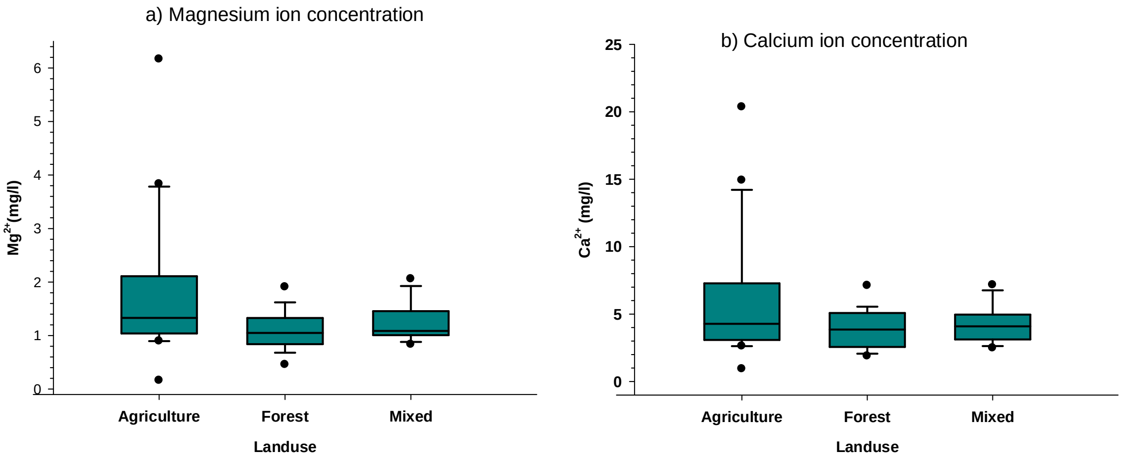


Figure 10: Examples of variation in the physicochemical parameters among landuses and stream orders in the Mara River streams. No significant differences were observed in the individual elements. (Box 95%, whisker max-min values)

Ions Mg^{2+}, Ca^{2+}and K^{+} were in very low concentrations (less than 20mg/l) and did not show significant differences with stream orders or among landuses (ANOVA, $p>0.05$). They strongly correlated with TSS and turbidity. Sodium ions (Na^{+}) on the other hand had relatively higher concentration (maximum 30 mg/l) than the other elements but did not significantly vary between the landuses or stream orders (ANOVA, $p>0.02$) (Fig 11, c). SO$_4^{2-}$, Cl^{-}, and Br^{-} concentrations in streams draining the forest catchments were significantly lower (ANOVA, $p< 0.05$) than their agricultural and forested counterparts. In addition, they showed a decreasing concentration with increase in stream order. However, this was not significant among the landuses. Fluoride on the other hand did not show any significant differences with land use or stream order.



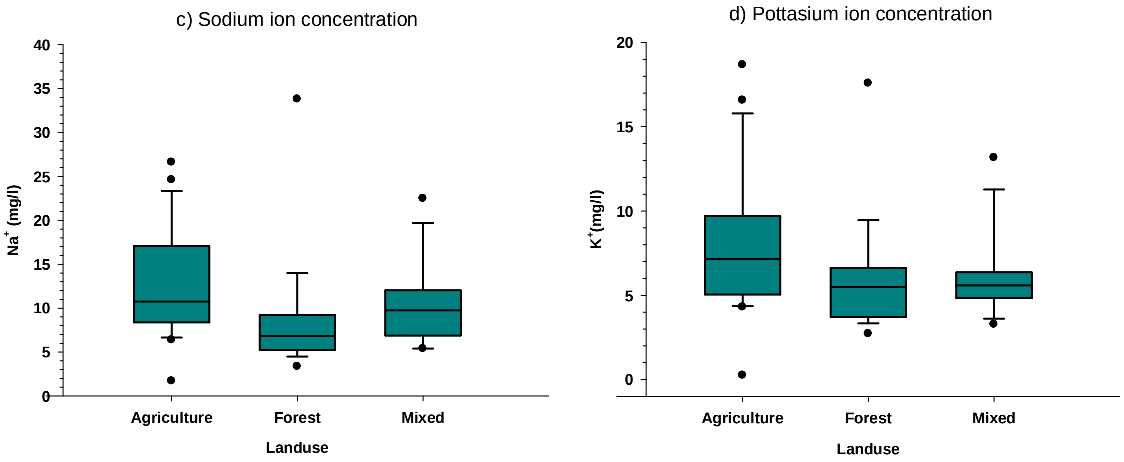


Figure 11: Examples of variations in cation concentrations with landuse and stream order in the Mara headwater streams. No significant differences were found in the majority of the variables describing the abiotic conditions. (Box 95%, whisker max-min values)

4.2 Biotic and abiotic conditions based on suspended particulate matter

4.2.1 Total suspended solids

Within the Mara River streams, TSS was highly variable with the forest streams having significantly (ANOVA, P< 0.001) lower mean TSS concentrations (20±6.5mg/l) and the agricultural streams with a mean of more than 60mg/l (Fig 12,a). However, TSS did not change significantly with stream order (Fig 12, b) (ANOVA, P=0.1304) indicating a strong influence of land use as opposed to location in the fluvial network. Turbidity levels were equally significant with land use (ANOVA, p<0.05) and not with stream order.

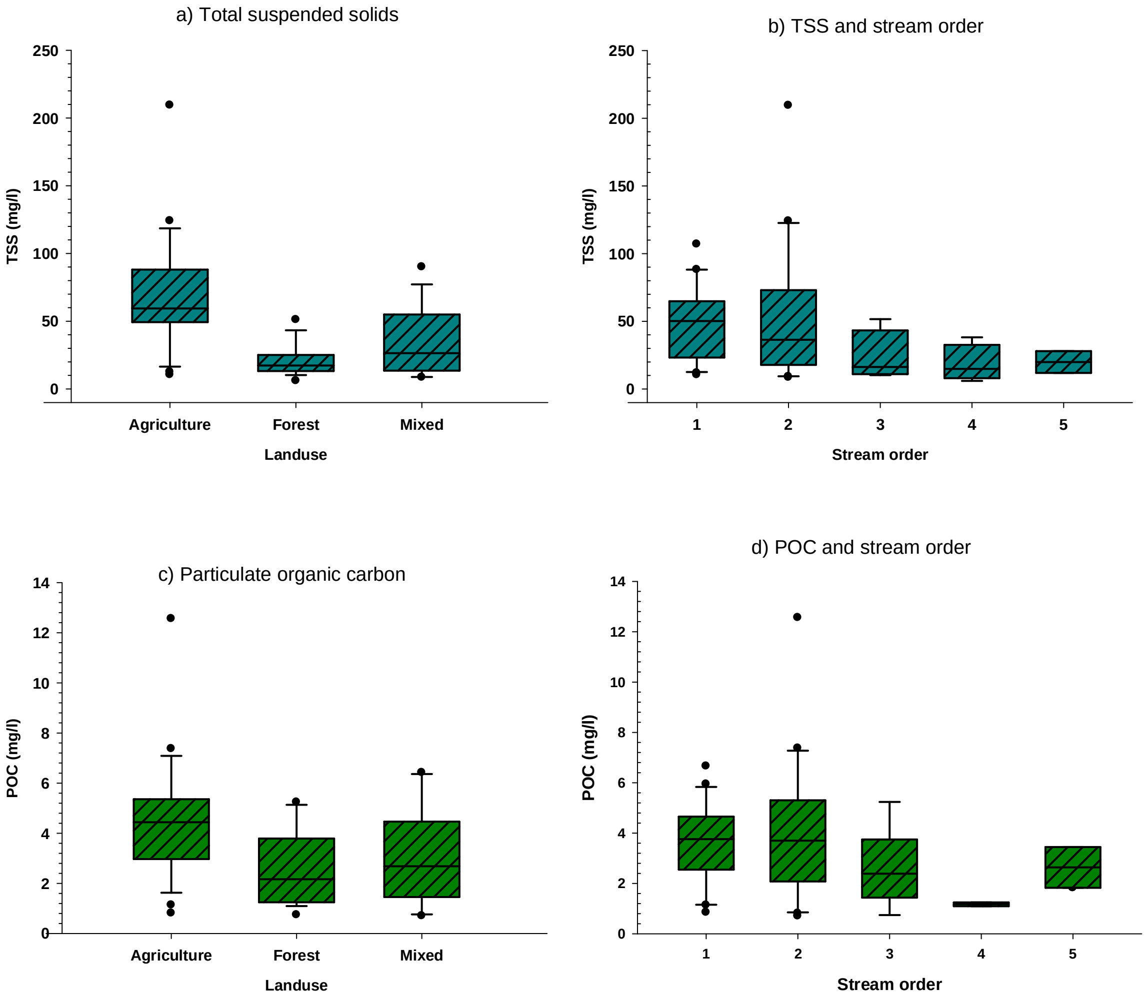


Figure 12: Variations in TSS and POC in Mara River streams; low TSS in forest streams and extreme variability in the mixed Landuse and agricultural streams. Both POC and TSS were significantly different among the landuses (Box 95%, whisker max-min values)

Particulate organic carbon (POC) showed significant differences with land use (ANOVA, $P<0.01$) and a strong correlation with the TSS ($R^2=0.76$). The significance was however relatively lower than that measured for TSS. While agricultural and forest streams behaved the same on a relative scale, the forest streams showed higher concentrations of POC in comparison to the amount of TSS indicating a more POC enriched TSS from the forest catchments. There was no particular trend or significance down the fluvial network.

4.2.2 Stable Isotopic signature of POC

Carbon $\delta^{13}C$

Within the Mara streams the isotopic composition of the seston was used as an additional tracer of the influence of landuse change on OM. The $\delta^{13}C_{POC}$ values measured were within the C3 photosynthetic pathway range of -32‰ and -20‰. It is however important to note that there was a clear significant separation of the two land uses with a more enriched agricultural catchment mean of -23‰ ±1.7 as opposed to the forest mean of - 28‰±1.0 (Fig 13, a) (ANOVA, $p<0.001$) indicating a difference in carbon source. Along the fluvial network, some similarity was noticed in the first and second stream orders (more enriched) as compared to third and fourth stream orders which were more depleted ($p<0.05$) (Fig 13, b).

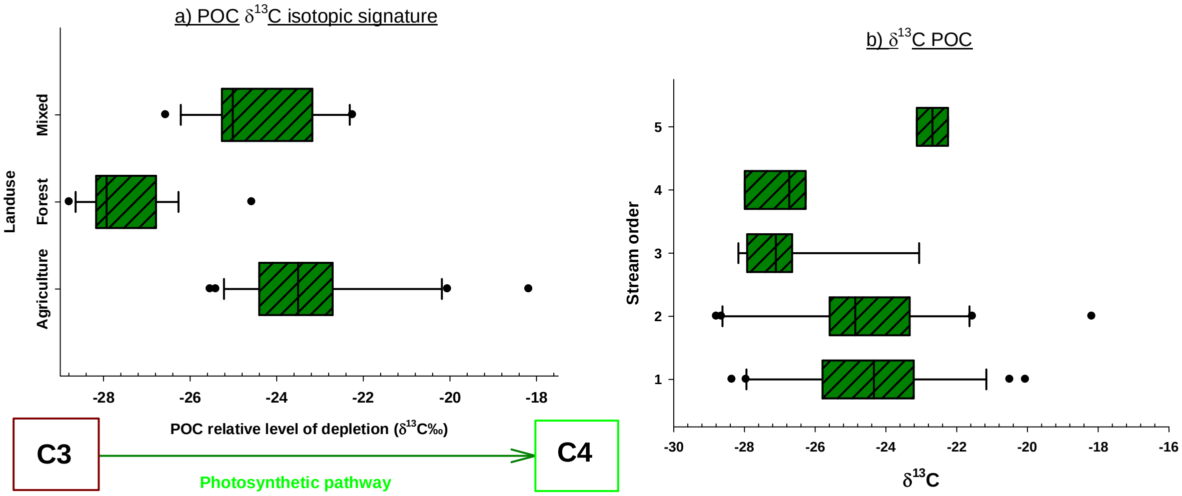


Figure 13: $\delta^{13}C$ carbon isotopic signature of POC in Mara River streams. Significant difference among landuse was observed with the agricultural catchment signatures having a tendency towards the C4 photosynthetic pathway (Box 95%, whisker: max-min values)

Nitrogen isotopic analysis

In the Mara streams, positive δ^{15} N values indicative of a fairly enriched system were measured. While forested catchments were relatively depleted with a mean of 3.9±1.2‰, the agricultural catchments were significantly ($p<0.001$) lower at 6.0±1.3‰ and relatively more enriched than the forested and mixed catchments (Fig 14, a).

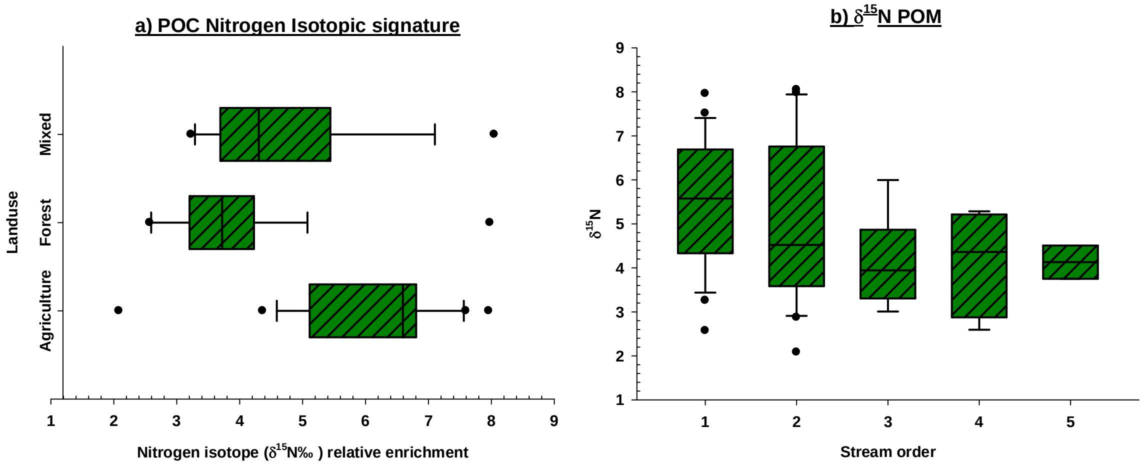


Figure 14: δ^{15}N and δ^{14}N nitrogen isotopic signature of Mara River streams, significant differences were seen among landuse types but not with stream order (ANOVA, $P<0.001$ and $P=0.062$, respectively) (Box 95%, whisker: max-min values)

Mixing model based on carbon and nitrogen isotopes among landuses

The isotopic ratios of δ^{15} N to δ^{13}C were significantly low in the forest streams (ANOVA, $p<0.0001$) for both isotopes with barely no overlap on the δ^{13}C axis and some overlap in the δ^{15} N axis (Fig 15).

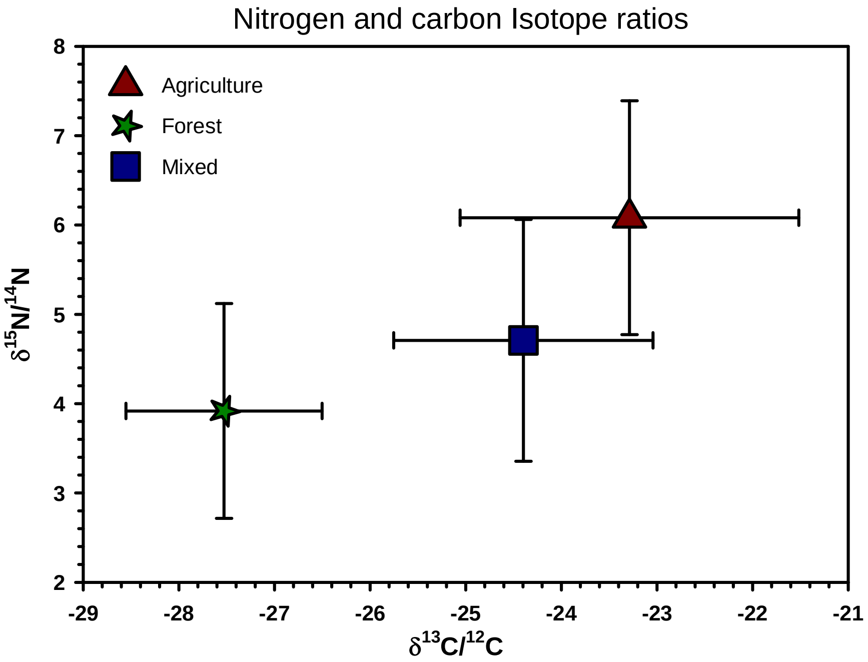


Figure 15: Similarity in the nitrogen source (overlap) and a clear difference in the carbon source as shown by the isotopic signatures for fractionation of δ^{13}C and δ^{15}N among landuses in Mara River streams

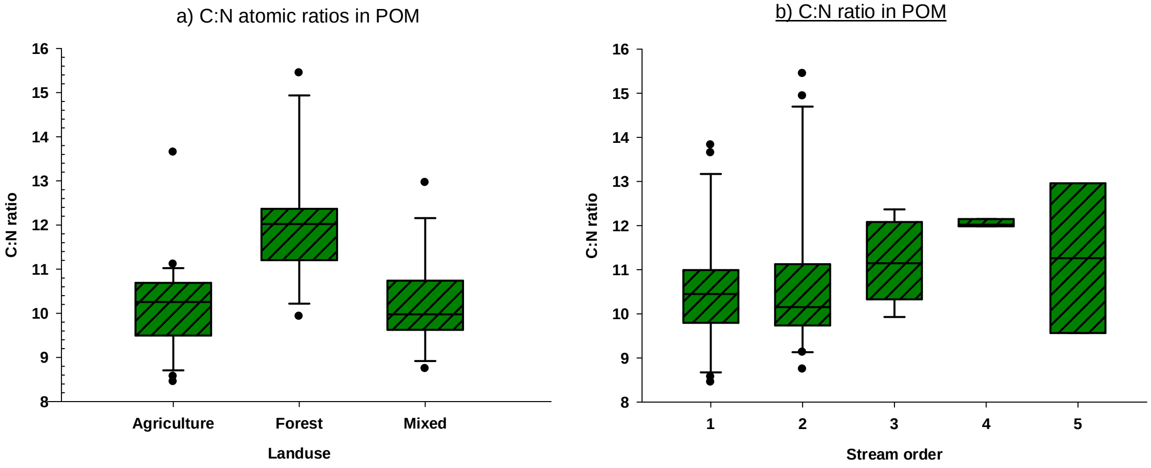


Figure 16: POM C: N atomic ratio variations with landuse types, significant differences seen among landuse types but not with stream order (ANOVA, $P<0.001$ and $P=0.2461$, respectively) (Box 95%, whisker: max-min values)

4.3 Reactive nutrient; biotic fingerprints

A total of six reactive nutrients were measured. NO_2^-, NO_3^- were found to be significantly higher in the agricultural streams ($P<0.05$) while PO_4^{3-} and NH_4-N were showing a trend with higher values in the agricultural streams but this was not significantly different among the three landuses (Fig 18, a, b, c, d). A non parametric multidimensional scaling combining all the reactive nutrients showed clustering with regard to these reactive fingerprint of the three landuses, these differences were significant (PERMANOVA, $p< 0.001$).

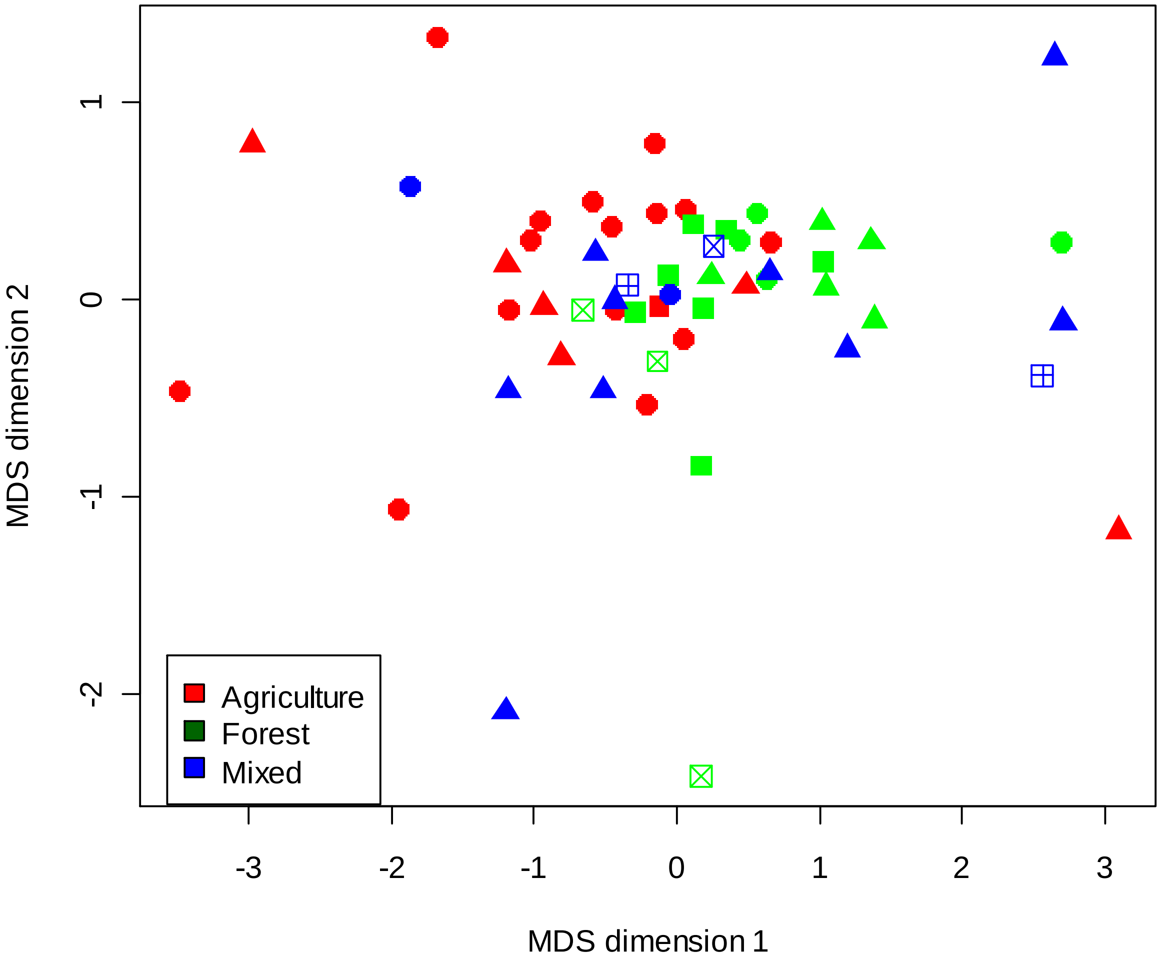


Figure 17: MDS based on (NO_2^-, NO_3^-, PO_4^{3-}, NH_4-N and DOC) i.e the reactive fingerprint. Significant clusters with landuse type was seen in the reactive fingerprint. Similar shapes represent similar stream orders and similar colors are similar landuses

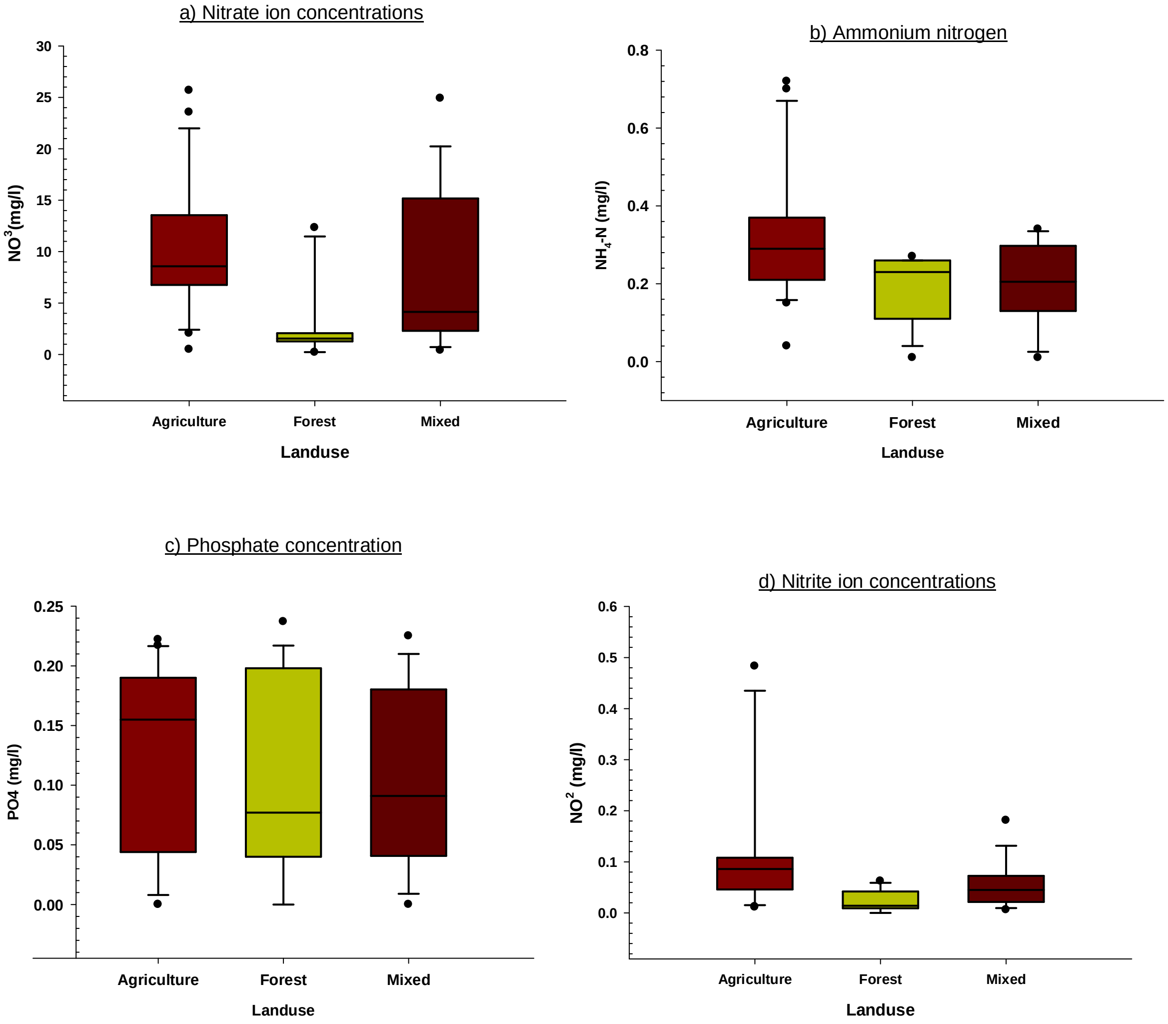


Figure 18: Examples of variations in the ion and nutrient concentrations with different landuse types in the Mara streams. (Box 95%, whisker: max-min values)

4.4 Characterization of dissolved organic carbon

4.4.1 Dissolved organic carbon(DOC)

Measurements of DOC concentration did not differ much with the land use but showed significance ($p<0.05$) (Fig 19, a). The agricultural catchment had relatively higher DOC concentrations 5.2±2.5mg/l as opposed to forest and mixed catchment streams with 4.7±1.1 and 4.3±2.0 mg/l, respectively. There was a weak trend suggesting a reduction of DOC concentrations with an increase in stream order (Fig 19, b), but this was not significant ($p>0.05$). The variability in the DOC concentrations in the first stream order was evident, and this was more pronounced in the agricultural watersheds.

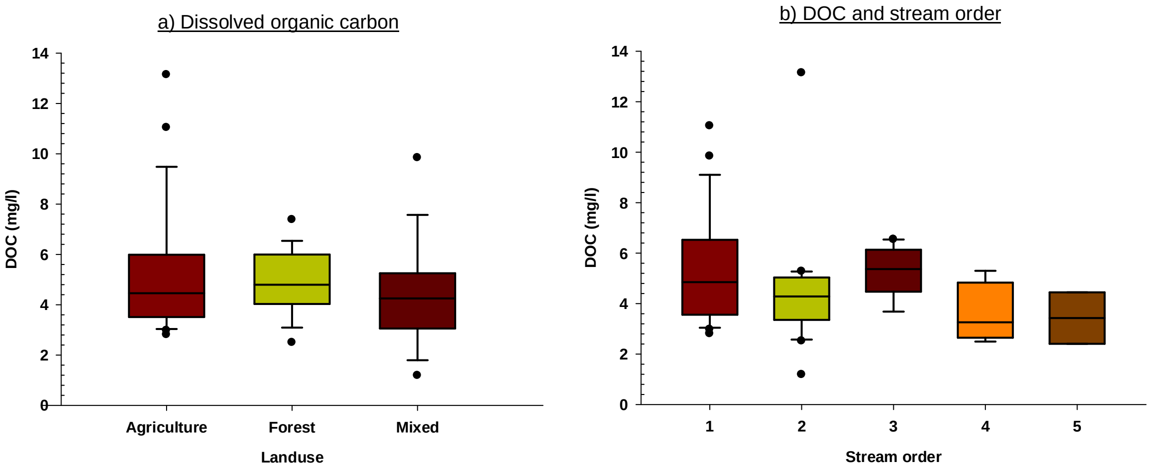


Figure 19: Variations in DOC concentrations in the mara River streams (a), with landuse and (b) with stream order (Box 95%, whisker: max-min values)

4.4.2 Absorbance and EEM Indices

This study employed measurements of absorbance and excitation emission matrices (EEM) and the interpretation of the data using standard techniques slope ratio(Sr), SUVA254, fluorescence Index (FI), humification index (HI), β/α, E2:E3, E4:E6 and EEM peaks (A,C,B and T as described in Helms 2008 to characterize the DOC. The MDS (Fig 20) shows a 2 dimensional ordination based on these indices. Significant differences between the three land uses were seen in FI, β/α, and SUVA254 (ANOVA, $p<0.01$) and E4:E6 ($p<0.05$). Agricultural sub-catchments had significantly high fluorescence indices and β/α values as compared to forest and mixed catchment streams. The forest streams on the other hand had higher SUVA254 and humification indices (Fig 21, a, d). The E2:E3 ratio and the slope ratio showed characteristic trends with landuses but were not significantly different among landuse types or stream orders (Fig 21 c, g).

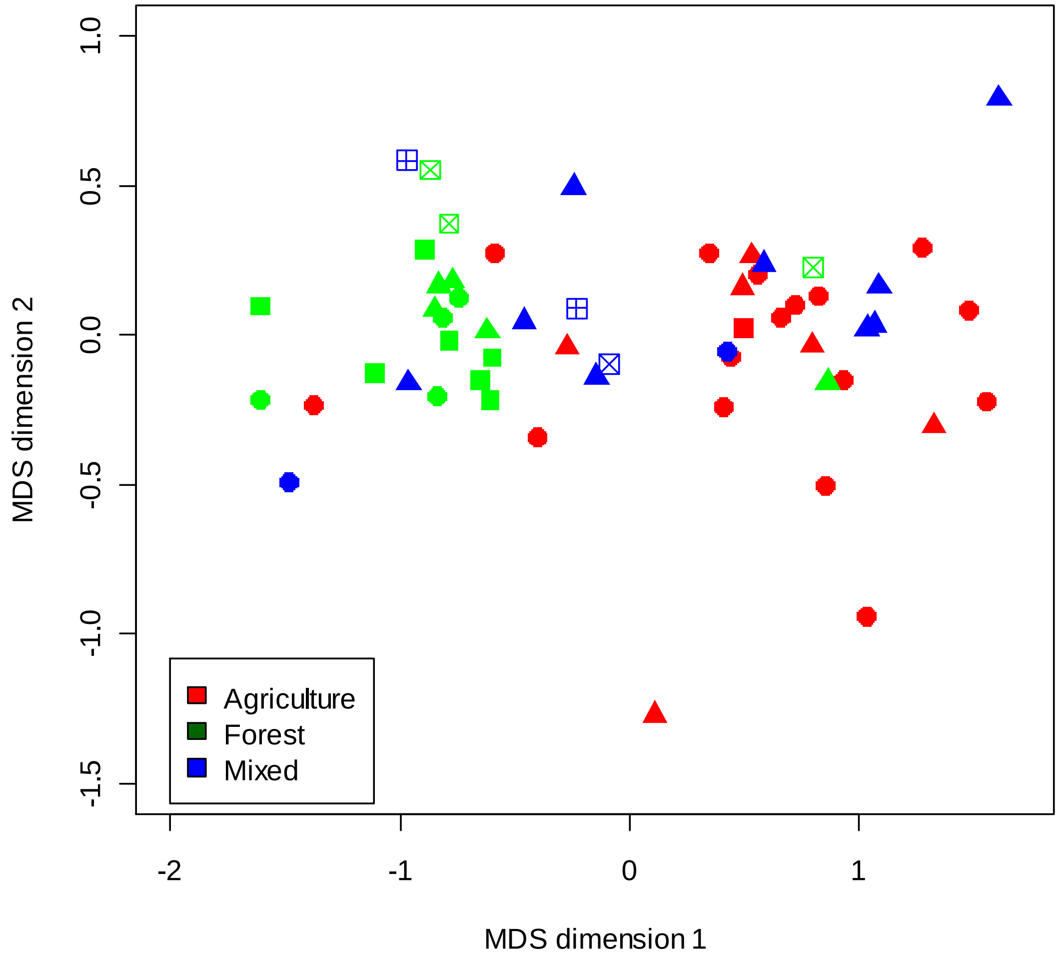
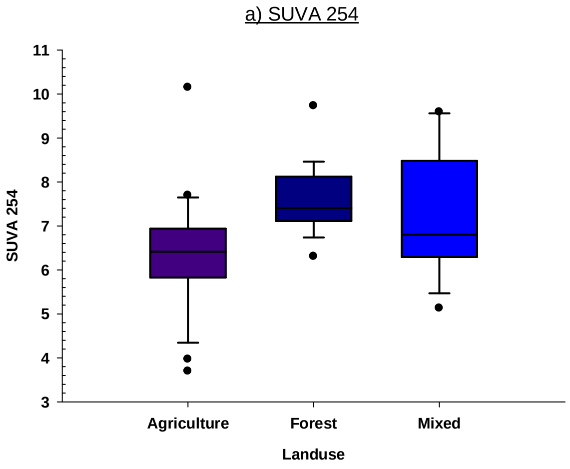
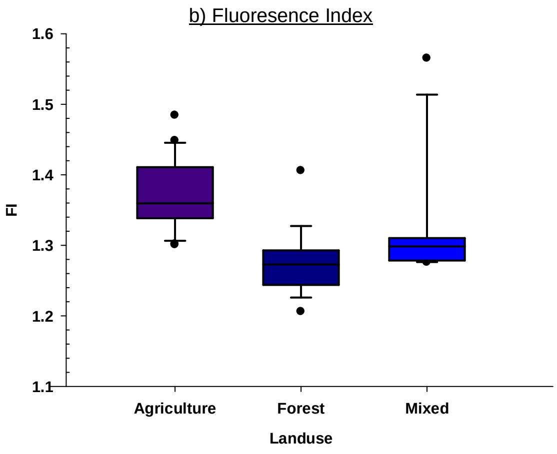
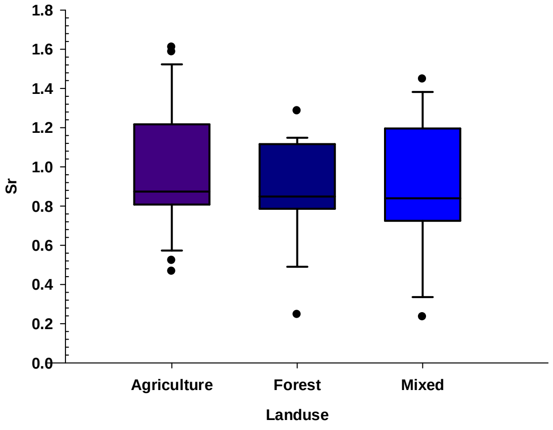


Figure 20: MDS based on major DOC characterization indices Sr, SUV254, FI, HI, β/α, and EEM peaks (A, C, B and T) PERMANOVA identified significant differences among landuses. (PERMANOVA, $p < 0.001$). Similar shapes represent similar stream orders and similar colors are similar landuses

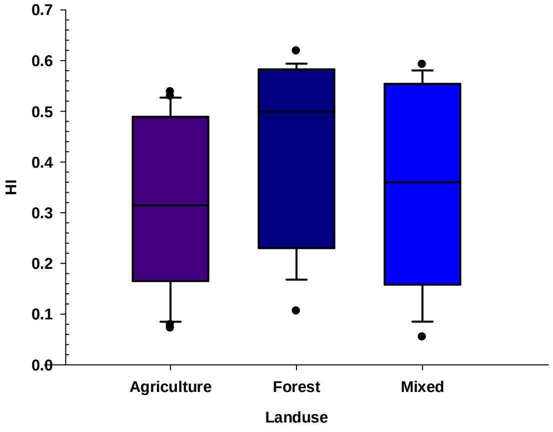




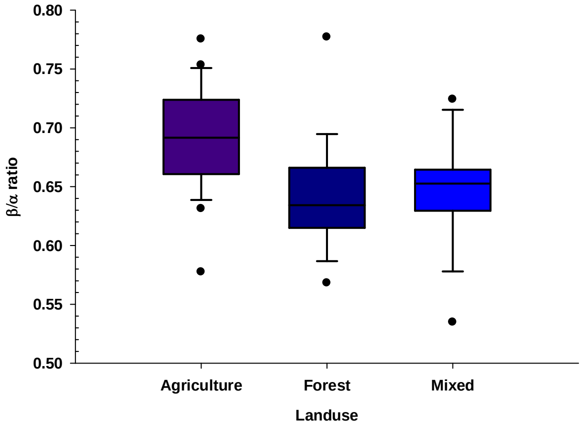
c) Slope ratio



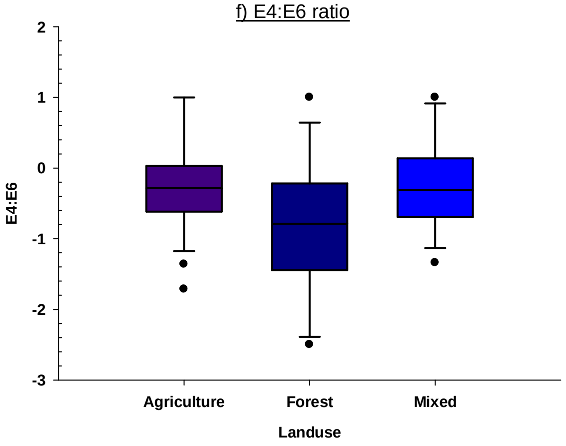
d) Humification Index



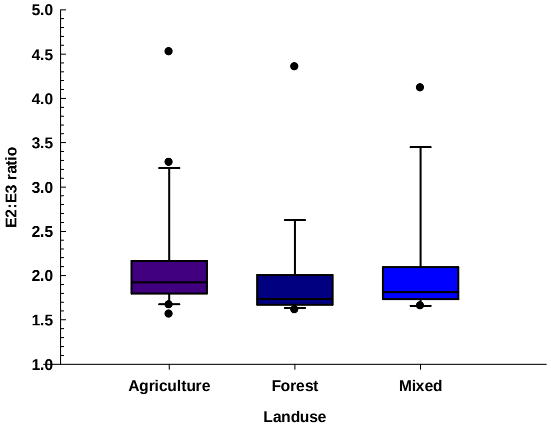
e)β/α ratio



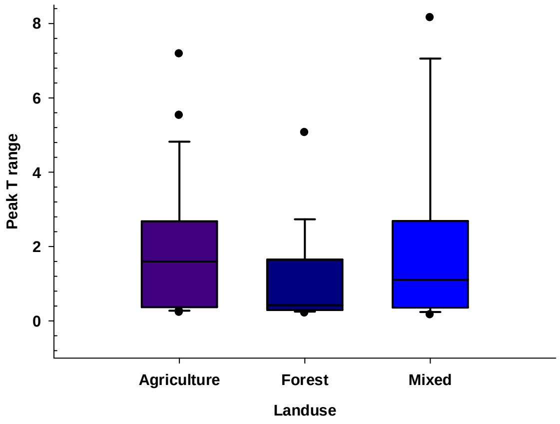
f) E4:E6 ratio



g) E2:E3 ratio



h) Peak T



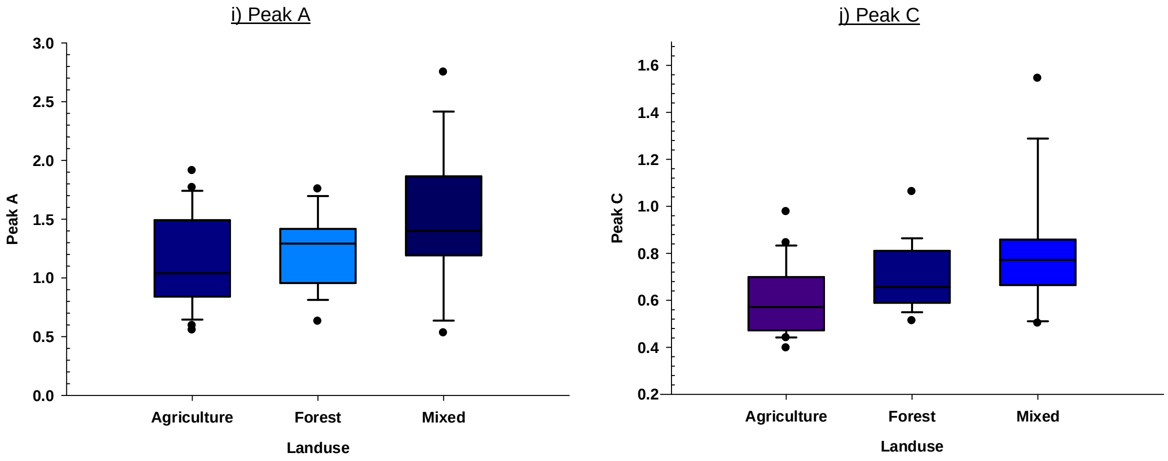
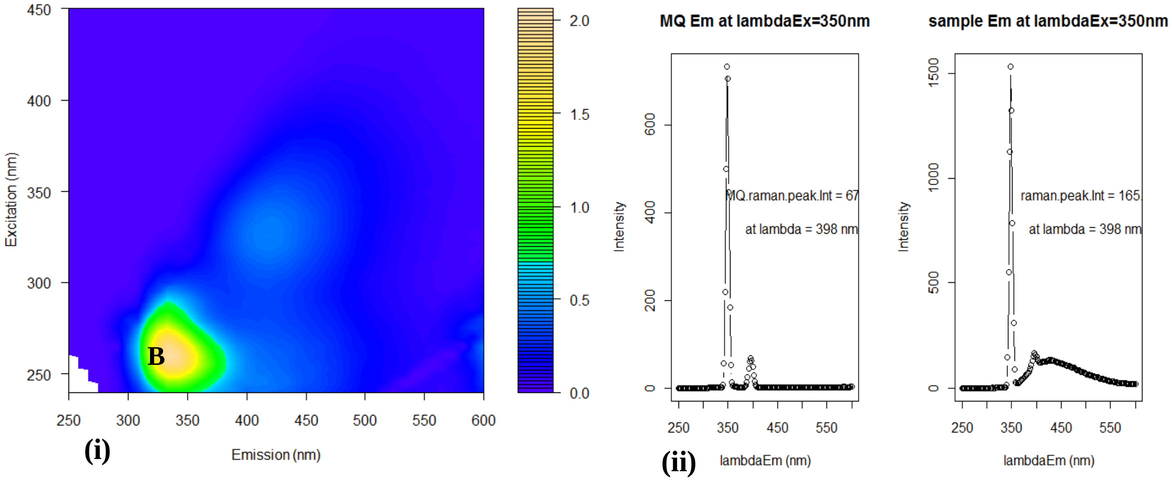


Figure 21: Variations in the optical properties of DOC from the Mara River streams with Landuse. Significant differences seen in FI (b), β/α (e), SUVA254 (a) and peaks A (i) and C (j) (Box 95%, whisker: max-min values)

4.4.3 EEM peaks and contour plots

Of the many peaks that have been identified by synchronous fluorescence scanning and used in the characterization of DOC in water, this study extracted intensities of four characteristic peaks (C, A, B and T) from the excitation emission matrices as detailed in the methods section. Significant differences was seen among landuses for peak C ($p<0.01$) (fig 21, j) and peak A ($p<0.05$) (fig 21, i), thus indicating differences in humification. Protein-like peaks B and T showed trends regarding their frequency of appearance; 54% of agricultural EEM scans showed conspicuous B and T peaks (fig 22, i, v) as opposed to 36% of EEM scans of the forest streams. However, this difference was not significant ($p=0.3$).



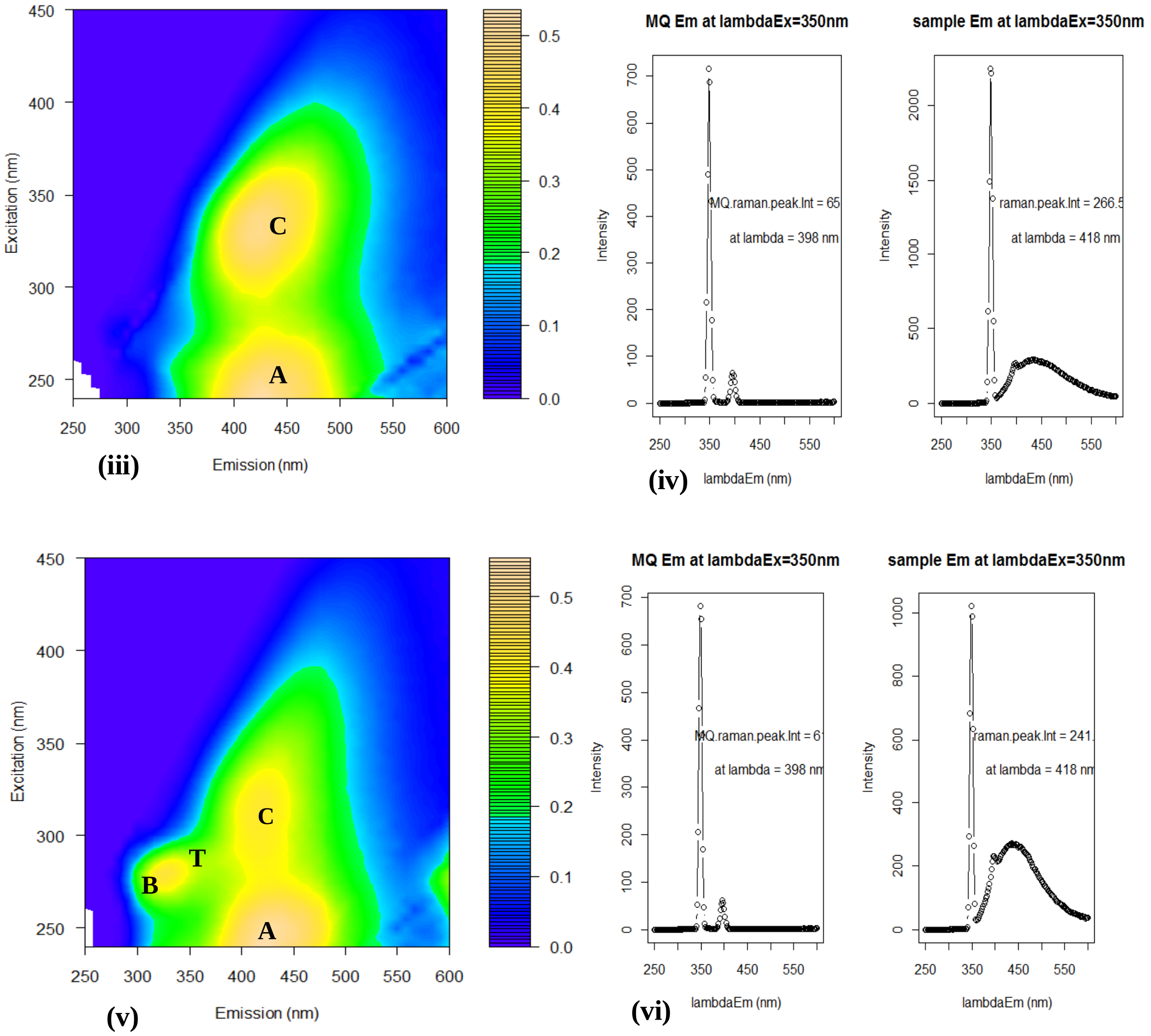


Figure 22: Examples of EEM contour plots and intensity graphs from an agricultural site (i,ii) as opposed to a forest catchment site (iii,iv) and a mixed catchment (v,vi) showing the common peaks C and A in forest streams and B and T in agricultural streams and all in the mixed catchment stream sample.

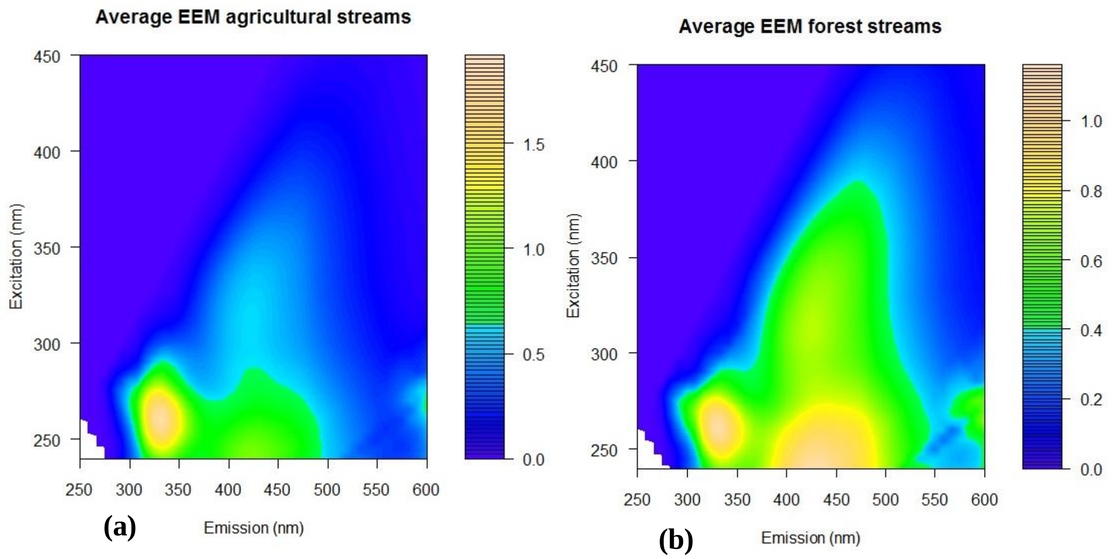


Figure 23: Average EEM image of all the agricultural (a) and all the forest streams (b)

Table 2: Summary of analysis of the optical properties used to characterize Mara River DOM, column headed "Landuse effect" indicates result (p-value) of an ANOVA testing for differences among landuses.

	Parameter	Indicator	Landuse effect	Reference
1.	Slope Ratio(Sr)	DOM molecular weight Ratio of Fulvic and Humic acids	0.874	Helms et al., 2008
2.	SUVA254	Aromaticity	<0.01	Weishaar et al., 2003
3.	Fluorescence Index	Microbial Vs terrestrial DOM	<0.00001	McKnight et al., 2001
4.	Humification Index	Aromaticity	0.24	Zsolnay et al., 1999
5.	β /α ratio	Autochthonous Vs allochthonous	<0.001	Wilson et al., 2008
6.	E2:E3	Molecular weight	0.98	De Haan & De Boer, 1987
7.	E4:E6	Aromaticity	<0.005	Chin et al., 1994
8.	Peak A	Humic Indicator	<0.05	Chin et al., 1994
9.	Peak B	Protein like OM	0.38	Coble, 1996
10.	Peak C	Humic Indicator	<0.01	Coble, 1996
11.	Peak T	Protein like OM	0.31	Coble, 1996

4.5 Ecosystem response; respiration estimates from CO_2

Agricultural stream water samples had a relatively higher CO_2 (as DIC) and more variable concentrations at a mean of 12.5 ±10.7 mg/l. This, however, was not significantly (p=0.26) different from the forest and mixed catchments with concentrations of 9.1 ±6.7 and 7.9 ±4.5 mg/l, respectively (fig 24, a). The isotopic signature on the other hand showed agricultural streams to have DIC significantly enriched with the heavy isotope at a mean $\delta^{13}C$ of -6.7 ±2.1 as opposed to forested and mixed stream catchments at mean $\delta^{13}C$ of -9.5 ±2.6 and -7.4 ±2.8 ($p<0.01$) (fig 25, a). Neither the absolute CO_2 concentrations, nor the isotopic signatures did significantly differ with stream order. However, a trend showing decreasing CO_2 concentrations with increasing stream order can be seen (Fig 24, b).

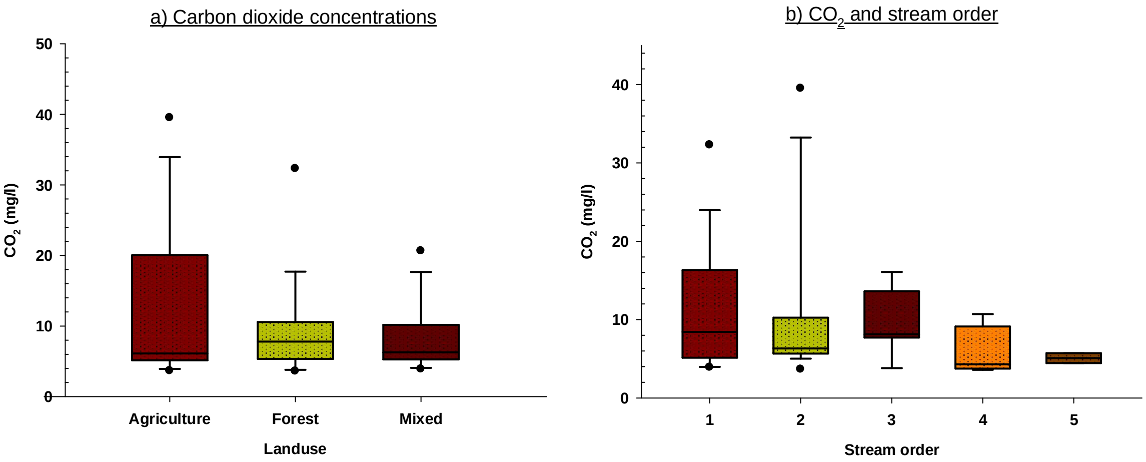


Figure 24: CO_2 concentration in the Mara River streams with landuse (a) and with stream order (b). No significant differences seen with landuse or stream order ($p= 0.2638$)

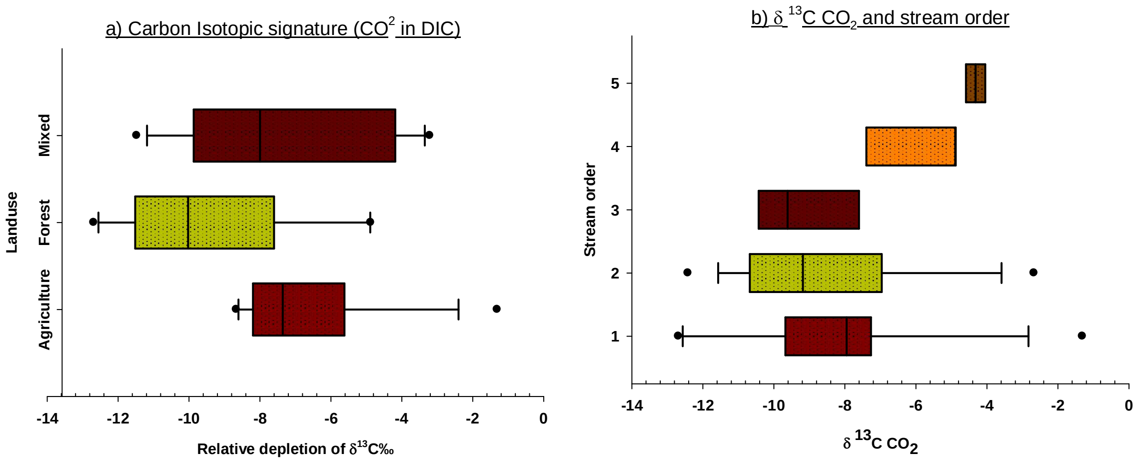


Figure 25: Variations in CO_2(DIC) isotopic signature of the Mara River streams with landuse (a)and with stream order (b). Significant differences seen with landuse ($p< 0.01$) but not with stream order ($p=0.09$) (Box 95%, whisker: max-min values)

5 Discussions and conclusions

Quantity and quality of particulate and dissolved organic matter in streams are known to be influenced by many factors which include soils, geology, topography and vegetation among other things. This study aimed at identifying effects of landuse on carbon dynamics in the upper Mara River system while simultaneously taking into account stream order as a known controlling variable. This setup took into account the concept of continuously changing physical conditions and biological components along the river channel, especially regarding the allochthonous and autochthonous inputs and the processing of organic matter in the flowing water along the river continuum (Vannote, Minshall et al. 1980).

5.1 Geochemical characteristics

Measures of abiotic conditions are diverse and vary from geochemical parameters to climatic conditions including altitude and atmospheric pressures. Geochemical parameters form a key component of the abiotic factors and are known to influence the solubility of major gases, the rate of growth and metabolic performance of all aquatic organisms. They may also affect the structure and composition of DOC for example through changes in ionic strength, redox potentials and pH.

The analysis of the geochemical characteristics within the study area revealed only minor differences among landuse types with regard to the various stream´s geochemical fingerprint, i.e. a suite of geochemically characteristic but biologically conservative ions. The concentrations of Na^+ K^+, Ca^{2+}, Mg^{2+}, Fl^-, for instance did not show any significant difference among landuse types. Likewise conductivity which integratively represents the dissolved solutes did not show any significant difference among landuse types or stream orders. The ions which were found to differ significantly among landuse types were Cl^-, Br^- and SO_4^{2-}. Also, the integrative effect of landuse types on the conservative fingerprint was significant (PERMANOVA, $p<0.05$). This could be associated with erosion, which is indicated by the significant differences in sediment loads among the landuse types, and the characteristic nature of adsorption of most elements to suspended particles. This was further illustrated by the trends in individual conservative ion concentrations that were identical to the trends in TSS (Fig 11 and Fig 12, a). Geologically, the Department of Mines and Geology of Kenya classifies the whole study area to have similar geological properties, i.e. dark grey basalt associated with meta-volcanic rocks of Precambrian age plus sediment from regional metamorphism that has been granitized (Mines and Geology Kenya, 1990). Importantly for the major focus of this study (i.e., carbon dynamics) pH, which is a major indicator of the carbonate balance system and the buffering capacity of a system, did not show significant variation among landuse types.

These inconspicuous results with regard to the geochemical background established a good base for the more relevant comparison of organic matter properties and fluxes, and the chemistry of reactive ion species.

5.2 Reactive nutrients

Reactive nutrients form one of the essential components in the biotic structure of aquatic communities. They are essential for growth and productivity and form an important requirement for organic matter processing, however high concentrations of nutrients can have adverse ecological effects. Regarding the reactive nutrients measurements of Mara streams, the nitrogen species NO_2 and NO_3 showed significant differences among landuse types with the agricultural catchments having higher concentrations. The two nitrogen species have been attributed to allochthonous inputs from human activities (Barnes and Raymond 2009). This can be expected to positively influence primary productivity. However, elevated pH levels, usually a sign of increased primary productivity, were not observed in the agricultural streams. It is also known that nitrogen enrichment is a possible mechanism for increased production of autochthonous DOC (Paul, Stasser et al. 2006) and might be a factor in the variability of DOC seen in the agricultural streams. NH_4-N and PO_4^{3-}, the two nutrient species considered of importance and usually very high reactivity in streams did not show differences among landuse types. This may be due to their high reactivity and their relatively low ambient concentrations in these streams creating a high demand for the nutrients essential for primary productivity and bacterial breakdown of organic matter. Their uptake is therefore very fast upon entrance into the stream, and potentially enhanced levels due to a specific landuse may still be well below saturation levels, so that no effect of landuse is visible.

5.3 Suspended POM

Suspended particles and organic matter may arise from point sources such as farmlands, stream bank erosion, loose soils, catchment and surrounding riparian vegetation or re-suspension from the water column. They form an important link between the biotic and abiotic compartments of the stream ecosystem and a significant contribution to DOC and other important resource subsidies that structure the communities in streams. TSS is also reflected in the stream as turbidity, which shows the optical properties based on light reflected by suspended particles. Among the most pronounced differences among the landuses was the quantity of TSS and of POC. While the TSS in agricultural streams were 3 times higher than in the forest streams, a similar difference was not reflected in the POC concentration (forested streams: 2.5±1.9 mg C/l; agricultural streams: 4.5±mgC/l).

Different carbon sources and sinks can be distinguished using their isotopic signatures. Fractionation during photosynthesis results in either depletion or enrichment of organic matter with the **^{13}C** isotope. The typical pathways are the C3 and C4 for most terrestrial plants resulting in a mean δ^{13}C of -27‰ and -13‰ respectively. However, aquatic macrophytes have extremely variable δ^{13}C that can range between -30‰ to -12‰ and this has been attributed to the carbon source as either HCO_3^- or CO^2 (Gearing 1991). Riverine phytoplankton communities are also known to be depleted in δ^{13}C and range from -30‰ to -25‰. An interesting trend was observed in POC δ^{13}C which clearly separated the three landuses with means of -23±1.7 ‰ (mean ± SD), - 28±1.0 ‰ and -24±1.3 ‰ for agricultural, forest and mixed streams, respectively (Fig 13, a). Although the three values are within the typical C_3 photosynthetic range, the forest catchments are shifted towards the lighter C isotope, while the agricultural catchments produce isotopically heavy carbon. Two main mechanisms may explain these trends: Isotope fractionation and mixing which are being reflected in the end members. There were no C4 plants in the forest catchments and the relatively high canopy cover and limited nutrient availability allowed only moderate algal productivity. On the other hand, parts of the agricultural catchments are covered by maize plantations, a C4 photosynthetic plant and a contribution from the C4 vegetation in the catchment could already increase stable isotope signatures. However, it is difficult to estimate the relative contribution of these two photosynthetic pathways. Also, the microbial and algal contribution to the POC pool and the resultant mixing can additionally alter isotopic signatures in the agricultural streams.

The nitrogen isotope signature is a useful tracer of human influence on aquatic ecosystems and is commonly used as a tracer of the origin of organic matter. Nitrogen enters the biotic environment primarily through the fixation of atmospheric nitrogen by terrestrial plants mediated by soil bacteria. Aquatic phytoplankton on the other hand assimilate dissolved inorganic nitrogen mainly as nitrate into their biomass. The margin of nitrogen isotope values in natural waters is wide especially for ammonium and nitrate in precipitation that range from -20‰ to 10‰. High $\delta^{\mathbf{15}}$ N values are indicative of enriched N inputs from watershed and human activities like agriculture and urban development. Being an essential element, N is quickly taken up by algae and acquires a high ^{15}N signal. Within the Mara streams, land use had a significant effect on the nitrogen isotope ratios of suspended POC. The δ^{15}N signature in agricultural catchments was significantly higher compared to mixed and forest land use, suggesting either inputs from manures which are known to have low nitrogen levels but high δ^{15}N values (Fry 2006), agricultural soil producing isotopically heavy residual organic nitrogen (Bremner & Tabalabai, 1972), or microbial respiration of nitrogen (denitrification) which may be removing nitrate while fractioning on ^{15}N (Peterson 1993). These patterns are expressed in the biplots (Fig 15).

The POM results suggest that the different landuses produced and exported organic matter of differing origin and quality to the streams. Generally, based on these POM isotopic signatures, the mixed catchment is more similar to the agricultural catchment and both can be opposed to the forest catchments. As a good part of the landuse change seemed recent (<5 years, personal observation), there seems to be a very rapid effect of the landuse change on the POC isotopic signal with regard to δ^{13}C. (Bernardes, Martinelli et al. 2004)) and

Raymond, Neung-Hwan et al. (2008) also found that landuse changes can in some cases be rapidly reflected in riverine carbon and nutrient pools.

The C:N ratio of materials can vary with nutrient loads and the type of vegetation within the catchment. The C:N atomic ratio of suspended POM in the Mara revealed some interesting and significant trends over the three landuses. They showed higher ratios in the forest than the other two catchments (Fig 16, a). Thus, organic matter in the forest streams was depleted in nitrogen and of poorer nutritional quality. This could potentially translate into a more refractory POM in the forest streams as opposed to a more labile POM in the agricultural streams.

5.4 DOM and optical properties of DOM

Apart from particulate organic matter, bacterial respiration may also rely closer on available dissolved organic carbon. Although on average DOC concentrations did not differ among landuse types, an increased variability due to single high-DOC streams in agricultural and mixed catchments was observed (Fig 19, a). This can be attributed to various reasons which may include: the diversity of available DOC in these catchments as compared to forested catchments and the diversity in processes (autochthonous production) due to variable nutrient supply (runoff) and other abiotic conditions. This increased variation of bulk DOC concentrations was particularly pronounced in the first order catchments (Fig 19, b) where the stream-catchment integration can be considered highest.

Measurements of absorbance and EEM provide high detail data that has been used to classify DOC. The two techniques are based on the analysis of the photochemically active colored DOC that influences the spectral regime in aquatic environments. Although proper characterization of DOC has remained elusive, various techniques exist that have continued to provide new insights into DOC composition.

A number of indices and tools were used to characterize the DOC. As described above, the results were variable, but most descriptors of DOC quality showed significant differences among landuses, and these results were mostly consistent with published literature. E.g. the fluorescence index (FI) can serve as a simple index to distinguish sources of isolated aquatic fulvic acids, or microbial from terrestrial sources of DOM. Purely autochthonous fulvic acids have a FI between 1.7-2.0, while the allochthonous materials range between 1.3-1.4 (McKnight 2001). According to these authors, a difference in the FI of at least 0.1 may be indicative of a difference in source of fulvic acids. In the case of the Mara streams, the FI was in the range indicating a mix of both autochthonous and allochthonous DOM origin. The mean FI for all agricultural streams was 1.37±0.04 while that for the forest streams was 1.27±0.04 with an intermediate behavior of the mixed landuse streams at 1.32±0.08 (Fig 21, b). Based on this, a clear signal indicating a difference in the source of fulvic acids and DOM in general for the agricultural and forest streams is evident, with autochthonous DOC being more prevalent in the agricultural as opposed to the forest streams. Previous studies have successfully correlated FI with aromaticity based on ^{13}C-NMR and have reported an inverse relationship between FI and aromaticity (McKnight 2001). Based on this correlation we can say that DOC from Mara forest streams has a relatively higher aromaticity than that from agricultural streams. SUVA254 normalized for DOC concentration is another indicator of aromaticity for a large number of humic substances (Weishaar 2003). Helms et al. (2008) also related SUVA254 to molecular weight and concluded that it can be semi-quantitatively used to assess molecular weight and can indicate the relative importance of plant/soil and microbial precursor material. Within the Mara streams, forest catchments produced the highest SUVA values (Fig 21, a) which were significantly different from the agricultural streams. This indicates that both the aromaticity and the average molecular weight of the DOC from the forest streams are higher than of DOC from the agricultural streams. This potentially indicates that agricultural streams have relatively more labile DOC. There was generally an overlap in the concentrations recorded in the mixed catchment both with the forest DOC characteristic and the agricultural ones. This may be due to the diverse nature of DOC available in these landscapes from both forest and agricultural lands.

The beta alpha ratio (β/α), another metric that was used to characterize DOC from Mara streams is a measure of β for more recently derived DOM against α for a more decomposed DOM (Wilson and Xenopoulos 2008b). The β/α ratio should increase with increasing autochthonous carbon production and vice versa for the allochthonous inputs. In line with the findings of Wilson et al. (2008), the agricultural streams in the Mara had a significantly higher β/α values than the forest catchment streams ($p<0.01$), indicative of a more microbially derived DOM in agricultural as opposed to forest and mixed catchment streams.

The E2:E3 and the E4:E6 ratios are indicative of molecular size and CDOM aromaticity (Chin et al., 1994). E4:E6 has also been correlated with oxygen carbon ratios and carbon nitrogen ratios. E2:E3 is inversely related to molecular weight while E4:E6 is inversely related to aromaticity. Higher E2:E3 and E4:E6 values in the Mara were recorded in the agricultural streams (Fig 21, f, g), supporting the conclusion of prevalence of low molecular weight and less aromatic DOC from agricultural catchment streams. However, this was not statistically significant.

Other indicators of DOC structure, the slope ratio (Sr) and the humification index (HI), which are indicative and inversely related to molecular weight and a ratio of known fluorescing components, respectively, showed characteristic but insignificant trends (Fig 21, c, d) with landuse type or stream order. The trends were also in line with the findings of (Helms 2008) who reported streams in forested catchments to have relatively low Sr ratios.

Fluorescence and emission scans were also employed to characterize the DOM. They are known to reveal more details about the structure of organic matter. Several peaks have for instance been used and are associated with different components of organic matter. As explained in the methodology and results sections, the peaks A, C, B, and T were used in this study. Peaks A and C are indicative of the presence of humics and humic-like structures while B and T indicate protein-like substances. The results show that peaks A and C had significantly higher values in the forest streams as compared to agriculture. Also, there was indication of higher concentrations of humic and humic-like compounds in the DOC of these streams. Peaks B and T on the other hand (Fig 22, i, v :) were higher in the agricultural streams as opposed to the forest streams. This indicates higher levels of protein-like and probably more bio-available substances in these streams. It should also be noted that both the protein-like and humic-like peaks were considerably high in the mixed landuse (Fig 22, v, Fig 21, (i, j)) as opposed to agricultural and mixed catchment streams indicating a higher diversity of DOC in the mixed catchment streams.

5.5 Respiration and CO_2

Measurements of CO_2 in streams can give an indication of metabolic performance of a system through the rates at which carbon is processed. The in stream processes that control the concentrations of CO2 are photosynthesis and respiration. Measured CO_2 (DIC) in the Mara streams indicated that the three landuse types did not differ significantly with regard to their metabolism. This study used CO_2 as a proxy for respiratory activity. This seems reasonable, as geochemical fingerprints were highly similar among landuse types, and the measured CO_2 can therefore be directly related to respired CO_2. In contrast to the initial expectations that agricultural catchments would respire more organic carbon due to increased organic matter availability, changed quality, and potentially favorable abiotic conditions such as abundant sunshine, the mean amount of respired CO_2 did not differ much among landuse types. However, the agricultural catchment had a more variable CO_2 concentration, while mixed and forest catchments showed less variability (Fig 24, a). In an aquatic environment, the concentrations of CO_2 are affected by the DIC, respiration by aquatic organisms and the exchange of gas between surface waters and air. The latter mechanism is higher in streams due to the usually high turbulence and low water depth. Also, this rate of exchange with the atmosphere is affected by the concentration gradient or the difference between actual concentration of CO_2 in water and the concentration expected at equilibrium with the atmosphere. Indeed, the isotopic signature $\delta^{13}C_{(DIC)}$ revealed heavy isotopic signatures for all landuse types between –6.7‰ to -9.5‰, indicative of intense equilibration with the atmosphere. Atmospheric CO_2 usually has a $\delta^{13}C$ signature of about -8‰ (Fry, 2006) which compares well to the average $\delta^{13}C_{(DIC)}$ of the streams in the three landuses. However, the stable carbon isotope results still revealed the prevalence of significantly lower $\delta^{13}C_{(DIC)}$ from the forest streams compared to the agricultural streams. The mean difference of 3‰ (Fig 25, a) in $\delta^{13}C_{(DIC)}$ between forested and agricultural catchments compares well with the results from the POC, where the $\delta^{13}C_{(POC)}$ signatures from forest streams were likewise significantly depleted in the heavy isotope by a difference of about 5‰. The direct correlation between the isotopic signature of the POC and CO_2 points to the utilization of POC as an energy source.

5.6 Conclusions

In conclusion, this study has successfully demonstrated differences in organic matter sources and quality in streams draining basins with three landuse types, for both particulate as well as dissolved organic matter. Conversion of forest to agriculture seems to be affecting the quality of DOM in the Mara streams and there is a trend indicating a gradual shift from high molecular weight to low molecular weight organic matter. In contrast, quantities of organic matter and respiratory activity seemed to be similar in streams of forest, mixed and agricultural catchments. Based on the assumptions that geochemical background and atmospheric exchange of CO_2 is similar for streams of the three landuse types, it can therefore be safely concluded that an almost similar amount of mainly allochthonous DOC is respired in the forest streams as compared to the mainly autochthonous DOC from agricultural streams.

While estimates of this study regarding impacts of landuse change on DOC quality and export did not take into account all the compartments of the stream, they indicate that the current landuse changes in the Mara catchment by conversion of forest to agriculture have a significant and measurable impact on dissolved and particulate organic matter. As DOC is the primary substrate fuelling microbial heterotrophs and an important energy base in the aquatic food web (Wetzel 1992), understanding its dynamics is fundamental to understanding the dynamics of the aquatic populations within the Mara River. The outputs of this initial pioneer study are a major pillar and step towards achieving this. In addition, the landuse change patterns currently occurring in the Mara River catchement are fairly typical for the region and it is likely that these results can be applied to most high altitude watersheds within the eastern Africa region.

6 References

Abramson, L., S. Wirick, et al. (2009). "The use of soft X-ray spectromicroscopy to investigate the distribution and composition of organic matter in a diatom frustule and a biomimetic analog." Deep Sea Research Part II: Topical Studies in Oceanography 56(18): 1369-1380.

Aiken, G. and J. Leenheer (1993). "Isolation and Chemical Characterization of Dissolved and Colloidal Organic Matter." Chemistry and Ecology 8(3): 135 - 151.

Amon, R. M. W. and R. Benner (1996). "Bacterial utilization of different size classes of dissolved organic matter." Limnology and Oceanography 41(1): 41-51.

Amy, G., N Her (2004). "Size exclusion chromatography (SEC) with multiple detectors: a powerful tool in treatment process selection and performance monitoring " Water science and technology: water supply 4(4): 19 - 24.

Anderson, D. R., W A Link, D H Johnson and K P Burnham (2001). "Suggestions for Presenting the Results of Data Analyses." The Journal of Wildlife Management 65 (No. 3): 373-378

Anderson, M. J. (2006). "Distance-based test for homogeneity of multivariate analysis." Biometrics 62: 245-253.

Barnes, R. T. and P. A. Raymond (2009). "The contribution of agricultural and urban activities to inorganic carbon fluxes within temperate watersheds." Elsevier 266: 318-327.

Battin, J. T., L. A. Kaplan, et al. (2008). "Biophysical controls on organic carbon fluxes in fluvial networks." Nature Geoscience 1: 95-100.

Battin, T. J., S. Luyssaert, et al. (2009). "The boundless carbon cycle." Nature Geoscience 2(9): 598-600.

Baxter, C. V., K. D. Fausch, et al. (2005). "Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones." Freshwater Biology 50(2): 201-220.

Bernardes, M. C., L. A. Martinelli, et al. (2004). "Riverine organic matter composition as a function of land use changes, Southwest Amazon." Ecological applications 14(4): S263-S279.

Biggs, T. W., T. Dunne, et al. (2002). "Relative influence of natural watershed properties and human disturbance on stream solute concentrations in the southwestern Brazilian Amazon basin." Water Resources Research 38(10.1029).

Bouillon, S., G. Abril, et al. (2009). "Distribution, origin and cycling of carbon in the Tana River (Kenya): a dry season basin-scale survey from headwaters to the delta." Biogeosciences Discussions 6: 5959-6023.

Bremmer, J. M., and M. A. Tabalabai (1972). Use of the ammonia electrode for determination of Kjeldahl analysis of soils. Commun. Soil Sci. Plant Anal. 3:159-165.

Camper, A. K. (2004). "Involvement of humic substances in regrowth." International Journal of Food Microbiology 92(3): 355-364.

CBS (2008). Statistics year book. 2007-2008, Central Bureau of statisticts Kenya.

Chapman, D. (1996). Water quality assessments: A guide to the use of biota, sediments and water in environmental monitoring. Gland.

Cole, J. J. and N. F. Caraco (2001). "Carbon in catchments: connecting terrestrial carbon losses with aquatic metabolism." Marine and Freshwater Research 52(1): 101-110.

Cole, J. J., Prairie, T.Y., Caraco, F.N., McDowel, H. W., Travink, J.L., Striegel, G. R., Duarte, M.C., Koltelainen, P., Downing, A. J., Middelburg, J. J. and Melack, J. (2007). "Plumbing the global carbon cycle: integrating inland waters into the terrestrial carbon budget." *Ecosystems 10*: *171-184*.

Coynel, A., H. Etcheber, et al. (2005). "Contribution of small mountainous rivers to particulate organic carbon input in the Bay of Biscay." Biogeochemistry 74(2): 151-171.

Creed, I. F. and L. E. Band (1998). "Export of nitrogen from catchments within a temperate forest: evidence for a unifying mechanism regulated by variable source area dynamics." Water Resources Research 34(11): 3105-3120.

Cronan, C. S., J. T. Piampiano, et al. (1999). "Influence of land use and hydrology on exports of carbon and nitrogen in a Maine River Basin." J. Environ. Quality 28: 953-961

De Hann, H., T De Boer (1987). "Applicability of light absorbance and fluorescence as measures of concentration and molecular size of dissolved organic carbon in humic Lake Tjeukemeer." Water Research 21: 731:734.

Delong, M. and M. Brusven (1998). "Macroinvertebrate community structure along the longitudinal gradient of an agriculturally impacted stream." Environmental Management 22(3): 445-457.

Delong, M. D. and M. A. Brusven (1994). "Allochthonous input of organic matter from different riparian habitats of an agriculturally impacted stream." Environmental Management 18(1): 59-71.

EFA (2008). Assessing Reserve Flows for the Mara River, Kenya and Tanzania. Environmental flow assesment, Lake Victoria Basin Commission of the East African Community.

Ehleringer, J. R., S L Phillips, W S F Schuster, D R Sandquist (1991). "Differential utilization of summer rains by desert plants." Oecologia 88(3): 430:434.

Elton, C. S. (1927). "The nature and origin of soil-polygons in Spitsbergen." Quarterly Journal of the Geological Society 83(1-5): 163.

Engstrom, D. R. (1987). "Influence of Vegetation and Hydrology on the Humus Budgets of Labrador Lakes." Can. J. Fish. Aquat. Sci. 44(7) 1306-1314

Fry, B. (2006). Stable Isotope Ecology. Germany, Springer Science.

Gearing, J. N., DC Coleman, B Fry (1991). Carbon isotope techniques, Academic Press San Diego.

Gereta, E. J., E. A. T. Chiombola, et al. (2003). "Assesment of the Environmental Social and Economic impacts on the Serengeti ecosystem of the development in the Mara River Catchments." African journal of ecology.

Gergel, S. E., M. G. Turner, et al. (2002). "Landscape indicators of human impacts to riverine systems." Aquatic Sciences - Research across Boundaries 64(2): 118-128.

GLOWS (2007). Water Quality Baseline Assessment Report: Mara River Basin, Kenya- Tanzania. . F. I. University: 61.

Guggenberger, G., L. Haumaier, et al. (1996). "Assessing the organic phosphorus status of an Oxisol under tropical pastures following native savanna using 31 P NMR spectroscopy." Biology and Fertility of Soils 23(3): 332-339.

Helms, J. R., A Stubbins, J D Ritchie, E C Minor, D J Kieber, K Mopper (2008). "Absorption spectoral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter." Limnol. Oceanogr 53(3): 955-969.

Houser, J. N., P. J. Mulholland, et al. (2005). "Catchment disturbance and stream metabolism: patterns in ecosystem respiration and gross primary production along a gradient of upland soil and vegetation disturbance." North American Benthological Society 24(3): 538-552.

Jones, D. L., D. Shannon, et al. (2004). "Role of dissolved organic nitrogen (DON) in soil N cycling in grassland soils." Soil Biology and Biochemistry 36(5): 749-756.

Lindeman, R. L. (1942). "The trophic-dynamic aspect of Ecology." Ecology 23.

Marschner, B. and A. Bredow (2002). "Temperature effects on release and ecologically relevant properties of dissolved Organic carbon in sterilized and biologically active soil samples." Soil Biology and Biochemistry 34: 459-466.

Martinelli, L. A., M. C. Piccolo, et al. (1999). "Nitrogen stable isotopic composition of leaves and soil: tropical versus temperate forests." Biogeochemistry 46(1): 45-65.

Mati, M. B., S. Mutie, et al. (2005). Land Use Changes in the Transboundary Mara Basin: A Threat to Pristine Wildlife Sanctuaries in East Africa. 8th International River Symposium. Brisbane, Australia.

McArdle, B. H. and M. J. Anderson (2001). "Fitting multivariate models to community data: A comment on distance based redundancy analysis." Ecology 82(1): 290-297.

McClain, M. E., E. W. Boyer, et al. (2003). "Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems." *Ecosystems* 6: *301-312*.

McKnight, D. M., E W Boyer, P K Westerhoff, P T Doran, T Kulbe, D T Andersen (2001). "Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity " Limnol. Oceanogr 46(1): 38-48.

Morris, D. P., B R Hargraeves (1997). "The role of photochemical degradation of dissolved organic carbon in regulating the UV transparency of three lakes on the Pocono Plateau." Limnol. Oceanogr 42: 239:249.

Moura, J. M. S., C. S. Martens, et al. (2008). "Spatial and seasonal variations in the stable carbon isotopic composition of methane in stream sediments of eastern Amazonia." Tellus 60B: 21-31.

Naiman, R. J., J. M. Melillo, et al. (1987). "Longitudinal patterns of ecosystem processes and community structure in a subarctic river continuum." Ecology 68(5): 1139-1156.

Owen, D. M., G L Amy, Z K Chowdhury, R Paode, G McCoy, K Viscosil (1995). "NOM Characterization and Treatability." AWWA 87 (1): 46-63.

Parlanti, E., K. Wörz, et al. (2000). "Dissolved organic matter fluorescence spectroscopy as a tool to estimate biological activity in a coastal zone submitted to anthropogenic inputs." Organic Geochemistry 31(12): 1765-1781.

Paul, A., R. Stasser, et al. (2006). "Nature and Abundance of Organic Radicals in Natural Organic Matter effect of pH and Irradiation." Environmental Science & Technology 40(19): 5897-5903.

Perrin, A. S., A. Probst, et al. (2008). "Impact of nitrogenous fertilizers on carbonate dissolution in small agricultural catchments: Implications for weathering CO2 uptake at regional and global scales." Geochimica et Cosmochimica Acta 72(13): 3105-3123.

Peterson, B. J., B Fry, L Deegan, A Hershey (1993). "Trophic significance of epilythic algal production in fertilized tundra river ecosystem." Limnol. Oceanogr 38: 872-878.

Polis, G. A., S. D. Hurd, et al. (1997). "Elnino effects on dynamics and control of an Island ecosystem in the Gulf of California." Ecology 78(6): 1884-1897.

Pote, D. H., T. C. Daniel, et al. (1999). "Relationship between phosphorus levels in three ultisols and phosphorus concentrations in runoff."

Ramsar (1993). Wetland ecological integrity.

Raymond, P. A., O. Neung-Hwan, et al. (2008). "Anthropogenically enhanced fluxes of water and carbon from the Mississippi River." Nature 451(24).

Richey, J., S R Wilhelm, M E Mcclain, R L Victoria, J M Melack, C Araujo (1997). "Organic matter and nutrient dynamics in river corridors of the Amazon Basin and their response to anthropogenic change " Ciencia e Cultura (Sao Paulo) 49(1-2): 98-110.

Schindler, D. W., P J Curtis (1997). "The role of DOC in protecting freshwaters subjected to climatic warming and acidification from UV exposure." Biogeochemistry Volume 36(1): 1-1.

Stephenson, M., G L Mackie (1988). " Total cadmium concentrations in the water and littoral sediments of Central Ontario Lakes." Water, Air, & Soil Pollution 38(1): 121: 136.

Thorp, J. H. and J. W. Gibbons (1978). Energy and environmental stress in aquatic ecosystems. National technical information center. Springfield, US.

Thurman, E. M. (1985). Organic Geochemistry of Natural Waters. Dordrecht, Dr W. Junk Publishers.

USEPA (1990). Biological criteria: National program guidance for surface waters. Washington D.C, US environmental protection agency.

USEPA (1986). Quality criteria for water - 1986. U. e. p. agency. Washington D.C.

Vannote, R. L., G. W. Minshall, et al. (1980). "The River Continuum Concept." Canadian Journal of Fish Aquatic Science 37: 130-137.

Vitousek, P. M. and D. U. Hooper (1993). "Biological Diversity and Terrestrial Ecosystem Biogeochemistry." Biodiversity and ecosystem function: 1.

Vogel, J. S., D E Nelson, J R Southon (1987). "14C Background levels in an accelerator mass spectrometry system." Radiocarbon 29: 323-333.

Wanninkhof, A. (1992). "Relationship between wind speed and gas exchange over the ocean." Journal of geophysical research 97(5): 10.

Weishaar, J. L., G R Aiken, B A Bergamaschi, M S Fram., R Fugh, K Mopper (2003). "Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon." Environmental Sciences Technology 37: 4702:4708.

Wetzel, R. G. (1992). "Gradient-dominant ecosytems: Sources and regulatory functions of dissolved matter in freshwater ecosystems." Hydrobiologia 229: 181-198.

Williamson, C. E., W. Dodds, et al. (2008). "Lakes and streams as sentinels of environmental change in terrestrial and atmospheric processes." The Ecological Society of America 6 (5): 247-254.

Wilson, H. F. and M. A. Xenopoulos (2008b). "Effects of agricultural land use on the composition of fluvial dissolved organic matter." Nature Geoscience 2.

Woelki, G., S. Friedrich, et al. (1997). "HPLC fractionation and structural dynamics of humic acids." Fresenius' Journal of Analytical Chemistry 357(5): 548-552.

Young, G. R., C. D. Matthaei, et al. (2008). "Organic matter breakdown and ecosystem metabolism: functional indicators for assessing river ecosystem health." North American Benthological Society 27(3): 605-625.

Young, R. and A. Huryn (1996). "Interannual variation in discharge controls ecosystem metabolism along a grassland river continuum." Canadian Journal of Fisheries and Aquatic Sciences 53(10): 2199-2211.

Zsolnay, A. (2001). The prediction of the environmental function of the dissolved organic matter (DOM) in ecosystems.

Zsolnay, A. (2003). "Dissolved organic matter: artefacts, definitions, and functions." Elsevier (Geoderma) 113: 187-209.

7 Appendices

Table 3: Summary of the parameters measured in the Mara river study showing the means with landuses and statistical significance

	Parameter	Catchment landuse type									ANOVA	
		Agriculture			Forest			Mixed			Landuse	Stream Order
		n	mean	SD	n	mean	SD	n	mean	SD	p Value	p Value
1	Discharge (l/s)	23	4.32	6.12	19	67.78	148.34	14	227.27	617.96	0.124	<0.0001
2	Temperature (^{0}C)	23	16.35	1.56	19	15.16	2.27	14	16.64	2.02	0.060	0.550
3	Dissolved oxygen (mg/l)	23	5.68	0.73	19	6.22	0.93	14	6.01	0.91	0.120	0.345
4	Dissolved oxygen (% sat)	23	73.92	8.73	19	79.32	10.69	14	78.64	9.10	0.149	0.223
5	Conductivity (µS/cm)	23	139.29	76.86	19	94.02	49.78	14	104.77	44.32	0.085	0.211
6	pH	23	7.73	0.28	19	7.85	0.25	14	7.72	0.25	0.230	0.335
7	Turbidity NTU	23	112.71	211.53	19	2.50	6.83	14	29.73	39.41	0.033	0.702
8	Alkalinity (mMol/l)	23	20.90	17.34	19	18.24	11.00	14	20.03	11.95	0.830	0.763
9	Suspended solids (mg/l)	23	68.81	42.63	19	21.27	12.16	14	33.84	24.68	<0.001	0.130
10	Ammonium Nitrogen (mg/l)	23	0.56	1.25	19	0.18	0.08	14	0.20	0.10	0.249	0.766
11	Ortho Phosphate (mg/l)	23	0.30	0.19	18	0.37	0.25	14	0.38	0.21	0.430	0.680
12	Phosphate ion (mg/l)	23	0.12	0.08	19	0.11	0.09	14	0.11	0.07	0.710	0.013
13	Nitrite ion (mg/l)	23	0.15	0.20	19	0.02	0.02	14	0.05	0.04	<0.01	0.230
14	Nitrate ion (mg/l)	23	10.60	6.56	19	2.56	3.34	14	7.72	7.34	<0.01	0.013
15	DOC (mg/l)	23	5.15	2.53	19	4.87	1.26	14	4.30	2.02	<0.05	0.349
16	Bromide ion (mg/l)	23	0.09	0.05	19	0.03	0.01	14	0.05	0.02	<0.001	0.030
17	Chloride ion (mg/l)	23	5.65	2.50	19	3.02	1.64	14	3.12	1.08	<0.001	0.083
18	Sodium ion (mg/l)	23	12.98	6.19	18	8.52	6.71	14	10.28	4.72	0.06	0.156
19	Potassium ion (mg/l)	23	8.86	7.48	18	5.93	3.31	14	6.16	2.48	0.16	0.393
20	Calcium ion (mg/l)	23	6.29	4.94	18	3.92	1.38	14	4.24	1.33	0.061	0.314
21	Magnesium ion (mg/l)	23	1.84	1.37	18	1.10	0.36	14	1.24	0.35	0.052	0.286
22	Fluoride ion (mg/l)	23	0.61	0.22	19	0.50	0.37	14	0.54	0.25	0.480	0.375

	Parameter	Catchment landuse type									ANOVA	
		Agriculture			Forest			Mixed			Landuse	Stream Order
		n	mean	SD	n	mean	SD	n	mean	SD	p Value	p Value
23	Sulphate ion (mg/l)	23	2.61	1.78	19	1.69	0.87	14	1.59	0.51	<0.05	0.847
24	Carbon dioxide: DIC (mg/l)	17	12.60	10.78	18	9.14	6.70	12	7.95	4.58	0.263	0.681
25	$\delta^{13}CO_2$ in CO_2	17	-6.71	2.15	18	-9.55	2.68	12	-7.45	2.87	<0.01	0.099
26	POC (mg/l)	23	4.50	2.39	19	2.53	1.40	13	3.05	1.94	<0.01	0.091
27	$\delta^{13}C$ POM (‰)	23	-23.29	1.77	19	-27.53	1.03	13	-24.40	1.36	<0.0001	0.040
28	% of carbon in TSS	23	7.37	3.25	19	12.37	4.01	13	10.77	6.39	0.002	0.045
29	$\delta^{15}N$ POM(‰)	23	6.08	1.31	19	3.92	1.20	14	4.71	1.35	<0.0001	0.112
30	% of nitrogen in TSS	23	0.86	0.38	19	2.03	3.75	14	1.18	0.58	0.025	0.050
31	C:N atomic ration	23	10.18	1.05	19	12.05	1.43	13	10.23	1.04	<0.0001	0.516
32	Slope ratio	23	0.91	0.45	19	0.89	0.28	14	0.89	0.35	0.870	0.674
33	SUVA254	22	6.37	1.31	19	7.58	0.79	14	7.27	1.42	<0.01	0.290
34	Fluorescence Index	23	1.37	0.05	19	1.27	0.04	14	1.32	0.08	<0.0001	0.288
35	Humification index	22	0.32	0.17	19	0.42	0.18	14	0.36	0.20	0.240	0.675
36	β/α	22	0.69	0.04	19	0.64	0.05	14	0.65	0.04	<0.01	0.714
37	E2:E3 ratio	22	2.33	1.39	19	1.97	0.63	13	2.06	0.66	0.980	0.399
38	E4:E6 ratio	23	-0.94	3.85	19	-0.79	0.90	14	-0.24	0.64	<0.050	0.612
39	Peak A	22	1.15	0.39	19	1.20	0.31	14	1.52	0.57	<0.05	0.060
40	Peak C	22	0.60	0.15	19	0.70	0.14	14	0.80	0.26	<0.01	0.220
41	Peak B	22	1.65	1.62	19	1.06	1.24	14	1.83	2.26	0.380	0.815
42	Peak T	22	1.79	1.81	19	1.12	1.26	14	2.04	2.43	0.320	0.675

Table 4: Overview of the sampling sites, main Rivers and catchment landuse

Stream #	Main river catchment	Catchment landuse	Stream order	GPS Co-ordinates	
				X	Y
1	Amala	Agricultural	2	786943	9915486
2	Amala	Agricultural	1	788020	9916724
3	Amala	Agricultural	1	789712	9917833
4	Amala	Agricultural	1	790414	9919110
5	Kisilbei (Amala)	Agricultural	2	781900	9911897
6	Bondet (Amala)	Forest	3	787000	9918000
7	Amala	Mixed	2	792255	9920288
8	Issey (Amala)	Forest	3	779649	9915874
9	Issey (Amala)	Mixed	2	778567	9914476
10	Issey (Amala)	Mixed	2	779651	9915876
11	Mangoite (Amala)	Forest	3	783846	9914421
12	Nyangores	Forest	5	769563	9921639
13	Nyangores	Agricultural	1	773309	9917450
14	Nyangores	Agricultural	1	773233	9917013
15	Nyangores	Agricultural	2	773770	9914872
16	Nyangores	Agricultural	1	774185	9915662
17	Nyangores	Agricultural	1	774130	9915937
18	Nyangores	Agricultural	1	769497	9921025
19	Nyangores	Agricultural	1	769262	9921618
20	Nyangores	Agricultural	2	769324	9921681
21	Nyangores	Agricultural	1	771435	9919202
22	Nyangores	Agricultural	1	770500	9918861
23	Nyangores	Agricultural	1	769258	9918726
24	Nyangores	Agricultural	1	769629	9918605
25	Nyangores	Agricultural	1	765882	9923618
26	Nyangores	Agricultural	1	766015	9923538
27	Nyangores	Agricultural	1	766398	9923117
28	Nyangores	Agricultural	2	766227	9923036
29	Nyangores	Agricultural	3	765727	9922352
30	Nyangores	Agricultural	2	766472	9922232
31	Nyangores	Forest	3	774997	9917610
32	Nyangores	Forest	3	775230	9919901

Stream	Main river catchment	Catchment	Stream	GPS Co-ordinates	
33	Nyangores	Forest	3	775230	9919903
34	Nyangores	Forest	1	774818	9918730
35	Nyangores	Forest	4	774818	9918730
36	Nyangores	Forest	1	774995	9918126
37	Nyangores	Forest	2	773162	9917991
38	Nyangores	Forest	2	769666	9921967
39	Nyangores	Forest	2	770604	9922250
40	Nyangores	Forest	2	771320	9922779
41	Nyangores	Forest	1	771330	9922733
42	Nyangores	Forest	2	770849	9922302
43	Nyangores	Forest	4	773400	9921100
44	Nyangores	Forest	3	773456	9921119
45	Nyangores	Forest	1	772026	9920427
46	Ainabng'etunyek(Nyangores)	Forest	4	771137	9919888
47	Kechirbek(Nyangores)	Mixed	1	774657	9917091
48	Nyangores	Mixed	2	773884	9916570
49	Nyangores	Mixed	4	773770	9914872
50	Nyangores	Mixed	2	769674	9920971
51	Nyangores	Mixed	2	769303	9921144
52	Nyangores	Mixed	2	771435	9919202
53	Nyangores	Mixed	2	771228	9919437
54	Nyangores	Mixed	5	763606	9919159
55	Nyangores	Mixed	1	766426	9923080
56	Nyangores	Mixed	2	766227	9923036