NITRATE SOURCE APPORTIONMENT, FRACTIONATION, AND REMOVAL MECHANISM USING STABLE ISOTOPES - RIVER SONDU – LAKE VICTORIA CATCHMENT, KENYA

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Gent, August 2018

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ABBREVIATIONS AND NOTATIONS

Ca$^{2+}$ calcium

$Cl^-$ chloride

Class CT commercial tea farming

Class MF mixed farming

Class TF tea farming and forest

Class U urban

DO dissolved oxygen

EC electrical conductivity

HCA hierarchical cluster analysis

$HCO_3^-$ inorganic carbonates

K$^+$ potassium

LUC land use change

LVEMP Lake Victoria Environmental Management Project

Mg$^{2+}$ Magnesium

M&S manure and sewage

N nitrogen

Na$^+$ sodium

NEMA National Environmental Management Authority

$NH_4^+$ ammonium

$NO_2^-$ nitrite

$N_2O$ nitrous oxide

$NO_3^-$ nitrate

$NO_3^-\text{-}N$ nitrate nitrogen

$NO_2^-\text{-}N$ nitrite nitrogen

$NH_4^+\text{-}N$ ammoniacal nitrogen

NF&R $NH_4^+$ in fertilizer and rain

NF $NO_3^-$ fertilizers

NP $NO_3^-$ in precipitation

PO$_4$-P phosphate- phosphorous
PCA  principal cluster analysis
$SO_4^{2-}$  sulphates
$^{15}N/^{14}N$  isotope ratio of nitrogen
$^{18}O/^{16}O$  isotope ratio of oxygen
‰  per mil
δ  delta
ε  enrichment factor
α  fractionation factor
mixSIAR  stable isotope analysis in R
Tukey HSD  Tukey Honest Significant Difference
TSS  total suspended solids
Soil N  soil nitrogen
TDN  total dissolved nitrogen
VSMOW  Vienna Standard Mean Ocean Water
ABSTRACT
Nitrate(NO$_3^-$) pollution remains a global phenomenon affecting surface water, disrupting aquatic ecosystems, and threatening human health. Nutrient monitoring of concentrations accompanied by NO$_3^-$ source identification is essential for developing efficient nitrate management programmes as well as conserving surface water catchment basins. Sondu River basin is faced with a complex uneven distribution of nitrogen on a spatial scale creating a too much, too little nitrogen paradox. Too much nitrogen is due to nitrogen losses which cause eutrophication. Too little nitrogen is due to the nutrient deficit present in tropical small-scale holdings from minimal fertilizer use. In this study hydro-chemistry and dual isotope approaches were incorporated to evaluate the sources of nitrate in Sondu catchment based on 49 surface water sampling sites monitored for 9 months from December 2016 to September 2017. A multivariate statistical analysis was used to group the data and determine the parameters causing variance in the water quality. Through Hierarchical cluster analysis the sampling sites were classified into four groups characterised by mixed farming, commercial tea farming, tea zones dominated by forests and urban centres. Nitrate-nitrogen concentrations (0.33-3.94 mg N $^{-1}$) were influenced by geogenic and anthropogenic activities. The highest mean concentrations were recorded in the commercial tea farming zones while the lowest mean concentrations were recorded in the tea and forest zones. Mean $\delta^{15}N - NO_3$ and $\delta^{18}O - NO_3$ ranged from 5.19 ‰ to 10.59 ‰ and -0.32 ‰ to 13.81‰ respectively. A mixing model mixSIAR was used to determine the proportional contribution of five potential NO$_3^-$ sources (NO$_3^-$ in precipitation, NH$_4^+$ fertilizer and rain, NO$_3^-$ fertilizer, soil N and manure and sewage). mixSIAR output indicated that “manure and sewage” and “soil N” were the highest contributors whereas “NO$_3^-$ in precipitation” contributed the least. The contributions varied with season and land use class, the dry season recorded higher “manure and sewage” contributions whereas the wet season indicated higher contributions from “soil N”. The denitrification potential of selected river sections was investigated using incubation lab set-up. The denitrification rates ranged from 0.2 mg l$^{-1}$ hr$^{-1}$ to 2.1 mg l$^{-1}$ hr$^{-1}$ indicating nitrate, available carbon and soil texture as the controlling factors for denitrification. The enrichment factors for denitrification ranged from-19.2 ‰ to -9.0 ‰. The integration of hydrochemical and isotopic data can be used as an effective tool to understand nitrate trend, sources and transformations within a multi-tributary and mix land use watershed.
1. INTRODUCTION

1.1 Background and context
Lake Victoria is a paramount water resource in East Africa. It is the largest tropical fresh water body with a surface area of 68,800 km². The lake has a wide land catchment area extending over five countries mainly Kenya (6%), Uganda (45%) and Tanzania (49%) with sub-catchments from Burundi and Rwanda draining into the lake waters (Ouma et al., 2016). Over the past four decades the lake ecosystem has undergone dramatic changes marked by key environmental pressures such as declining fish stocks, eutrophication, proliferation of water hyacinth and deterioration of water quality (Lowe-McConnell, 1994; Odada et al., 2004).

A previous study on Lake limnology by Njuru et al., (2013) attributes this changing lake dynamics to increasing nutrient fluxes via sediments from river basins draining into the lake, surface run-off, biomass burning, urban and industrial point source pollution. Marshall et al., (2014) reported a rise in nutrient concentration of the lake from 21.9 µg/l NO₃-N and 41.8 µg/l PO₄-P in 1989 to 98.7 µg/l NO₃-N and 57.0 µg/l PO₄-P in 2008. Elevated nutrient levels are linked to eutrophication which leads to dense algal blooms of cyanobacteria, rapid growth of phytoplankton, aquatic microphytes and depletion of dissolved oxygen especially in deeper lake layers. The resulting deoxygenation of hypolimnetic layers may lead to hypoxic conditions which have been associated with fish kill and declining fish population in the lake (Hecky et al., 1993). Elevated nitrate concentration in drinking water adversely affects human health causing diseases such methemoglobinemia; nitrate is converted to nitrosamines with hydrochloric acid in gastric juice. Nitrosamine is preferentially bound to haemoglobin instead of oxygen endangering human and animal health. Due to factors above, the World Health Organization (WHO) has set a limit for NO₃–N concentration at 10 mg N/l for drinking water (Wang et al., 2016).

Sondu-Miriu is the fourth largest river basin draining into the Kenyan Lake Victoria with a catchment area of 3,508 km². Earlier studies on lower Sondu–Miriu estimated an average NO₃–N concentration of 0.14 mg N l⁻¹ during baseflow (Mwashote & Shimbira, 1994). According to more recent research by Jacobs et al., (2016), NO₃–N concentration during baseflow is higher at 0.74 mg N l⁻¹. Nutrient input within Sondu catchment has been linked to rapid urbanization. The rapid population growth rate estimated at 6% has caused an increase in demand for arable land triggering land use changes (LUC). LUC indirectly elevates nutrient levels by modifying hydrological processes and increasing soil erosion. Coupled with steep
slopes and a sediment transport capacity index of 0.14, Sondu R. has caused an increase in the nutrient loaded sediment input into L. Victoria (Vuai et al., 2012).

With regard to nutrient loading, the lake basin in Kenya is faced with an uneven distribution of nitrogen on spatial scales. Whereas there is proof of excess nitrogen discharges evidenced by eutrophication of the Winam gulf, nutrient deficits in form of agricultural inputs are present in tropical holdings due to minimal fertilizer use i.e. studies report use of < 20 N kg ha\(^{-1}\) in tropical areas like Eastern Africa compared to 99.76 N kg ha\(^{-1}\) for USA and 73.64 N kg ha\(^{-1}\) for European Union (Kotschi, 2013). This phenomenon creates a too much, too little Nitrogen paradox. Too little nitrogen is due to the low N inputs for feed and food production. Too much nitrogen is exemplified in eutrophic zones such as the Winam gulf of Lake Victoria (Kundu et al., 2017).

Jacobs et al., (2017) attributes 22% - 32.7% of N input into the Lake to riverine origin. While Ali et al., (2013) quantified the nitrate input from atmospheric deposition and river input as 15.3 N kg ha\(^{-1}\) yr\(^{-1}\) and 1.3 N kg ha\(^{-1}\) yr\(^{-1}\). Sondu and Nyando catchments (7,500 km\(^2\)) are the main riverine N contributors to the eutrophic Winam Gulf of Lake Victoria. Previous studies on nitrate source contribution to the lake have been focused on estimates and models rather than measurements from actual nutrient input sources. The available data on the nitrate source contribution for Sondu R. and the lake region is therefore sparse or contradictory. This is also exacerbated by the lack of a systematic monitoring approach which would account for nitrate trends (spatial and temporal variations). However, for effective management strategies, NO\(_3^-\) concentration monitoring alone is inefficient and should be coupled with source identification techniques such as isotopic fingerprinting (a dual isotope approach δ\(^{15}\)N and δ\(^{18}\)O). This technique is applied in the study to provide insight on source and respective contributions of NO\(_3^-\) in Sondu catchment.

1.2 Current challenges
Assessment of water resources reveals both natural factors and human activity greatly influence water quality. Direct linkages between increasing nutrient loads with changing land use have been underestimated as more attention has been directed to other natural factors such as geology, soil type, topography and rainfall (Jacobs et al., 2017). Its estimated that Mau Forest Complex (source of River Sondu) has experienced a 32% loss of forest cover between 1973 and 2000. Furthermore, deforestation has been coupled with increased agricultural activity by both large and small-scale holdings, increasing urban and peri-urban settlements, set-up of
agro-based industries, and a hydroelectric power plant. These LUCs have been observed to cause considerable land fragmentation, destruction of wetlands which increase the release of reactive N into the River basin (Olang & Kundu, 2011). According to Jacobs et al., (2017), Sondu River’s NO$_3$-N concentrations during baseflow revealed a variation of loading with land use, natural forest recorded the lowest concentration, 0.30 mg N l$^{-1}$ and tea and tree plantations recording higher concentrations, 1.62 mg N l$^{-1}$. Evidently, nutrient loading in Sondu catchment is influenced by different land uses.

Point sources such as industrial and domestic effluent are regulated legislatively e.g. industrial effluent/municipal wastewater is limited to 10 mg N l$^{-1}$ NO$_3$ (NEMA, 2002). However, the current infrastructure used to treat point sources for domestic wastewaters is inefficient, only 5% of the sewage is effectively treated resulting in the discharge of raw and partially treated waste into the river ecosystem (Mansour et al., 2017). This is further aggravated by the lack of a comprehensive sewerage network and proper sanitation facilities. Only 31% of urban dwellers and 20.6% rural dwellers have access to improved facilities, 18% urban dwellers and 15% rural inhabitants have access to unimproved facilities, 3% of urban settlers and 16% rural dwellers practise open defecation (GoK, 2015; WHO/UNICEF, 2015). The discharge from leaking septic tanks, pit latrines and open defecation is often directed to surface waters.

Non-point sources from agricultural activities remain difficult to characterize affecting proper evaluation with regard to their contribution to the total nitrate contamination (Banadda et al., 2011). The existing management programmes such as Lake Victoria Environmental Management Programme (LVEMP) are limited to monitoring NO$_3$- concentrations. Initiatives implemented to reduce nutrient loading have been focused on curbing land degradation and reducing soil erosion/sedimentation in the river basin. However, the outcome is pollution persists. This partly due to recommendations based on short term assessment of nutrient dynamics in the basin and the lack of adequate data on nitrate source apportionment. An integrated monitoring approach which accounts for nutrient loading based on i) land use ii) temporal and seasonal nutrient dynamics iii) source apportionment and contribution is proposed to provide insightful data.

Dual isotope approach has successfully been used to apportion and quantify nitrate sources in surface waters globally (Wang et al., 2016; Widory et al., 2012; Xue et al., 2009). The major sources of NO$_3^-$ in agricultural watersheds (atmospheric deposition, ammoniacal fertilizer, nitrate fertilizer, manure, and sewage) can be distinguished from their distinct isotope ratios of
nitrogen ($^{15}$N/$^{14}$N) and oxygen ($^{18}$O/$^{16}$O) (Xue et al., 2012). However, uncertainties in NO$_3^-$ source apportionment arise due to i) temporal and spatial variation in δ$^{15}$N, ii) isotopic fractionation during denitrification which blurs distinct isotopic composition and iii) mixing from numerous sources (Kendall et al., 2007). A mixing model such as the Bayesian stable isotope mixing model has been proposed and used to determine the proportional distribution and contribution of each source to the mixture therefore addressing the uncertainty arising from mixing (Xue et al., 2012). The incorporation of long-term temporal and spatial sampling will help address uncertainties arising from variations of δ$^{15}$N. An integrated approach based on physicochemical, isotopic, and modelling analysis can be a useful tool to identify and quantify potential sources of NO$_3^-$ in Sondu river basin.

Data and information on nitrate sources and transformations in River Sondu catchment can be used by respective county/ national government departments for the development of efficient management strategies and policies to control excess nitrate discharge in Sondu catchment and Lake Victoria Basin.

1.3 Objectives

The overall objective of this study is to identify, quantify and study nitrate fractionation mechanisms in R. Sondu using physicochemical, isotopic techniques and Bayesian isotopic mixing model.

The specific objectives are:

1. To investigate spatial and temporal variation in physico-chemical and isotopic parameters ($\delta^{15}$N, $\delta^{18}$O)
2. To estimate the proportional contribution of potential nitrate sources (manure and sewage, soil Nitrogen, ammoniacal fertilizer, nitrate fertilizer and atmospheric nitrogen deposition) to nitrate mixture in surface water using a Bayesian isotopic mixing model (mixSIAR).
3. To investigate nitrate fractionation (denitrification) for selected upstream water catchment
2 LITERATURE REVIEW

2.1 Outline

This chapter aims to review literature on the identification of nitrate sources in surface waters using isotope tracer approach. The main content highlighted includes i) Nitrogen cycling and transformation in aquatic systems, (ii) Isotopic composition of nitrate in aquatic systems iii) Nitrogen transformation processes affecting isotopic composition iv) Application of stable isotopes in nitrate source identification.

2.2 Aquatic Nitrogen Cycle

Nitrogen release in terrestrial ecosystems influences the magnitude of nutrient input in aquatic systems (Heathwaite, 1993). An estimated 40% of the total nitrogen flux reaches aquatic ecosystems via different pathways including: i) atmospheric deposition on the catchment or directly on the water body, ii) leaching from diffuse sources within the catchment, iii) run-off from inorganic fertilizer application, iv) mining of N soil stocks, v) surface application of manure in catchments, vi) direct input from point sources such as sewage treatment works (Durand et al., 2011; Heathwaite, 1993).

Nitrogen is essential for primary production of macrophytes and diatoms in freshwater ecosystems (Reis et al., 2016). However, excessive nutrient loading disrupts aquatic ecosystems leading to eutrophication. Reactive nitrogen Nr is defined as all chemical forms of N excluding dinitrogen gas N (Sobota et al., 2016). Dominant N species in water include: reduced inorganic N species (NO$_2^-$, NO$_3^-$, NH$_4^+$, NH$_3$), dissolved and particulate organic Nitrogen (DON, PON) (Durand et al., 2011; Heathwaite, 1993). Exchange of Nr in the atmosphere, biosphere, hydrosphere and land is described by nitrogen cycling which is predominantly controlled by microbial activity (Sobota et al., 2016). Customarily, the cycle is broadly categorized into three processes, Nitrogen fixation, nitrification and denitrification (Laursen & Seitzinger, 2005).

2.2.1 Nitrogen fixation

In nature, dinitrogen gas is fixed from the atmosphere into a bioavailable Nr by microorganisms and lightning (Sobota et al., 2016). The microbial processes include:

Ammonification which occurs through two pathways. For the first pathway, nitrogen fixation is accomplished by bacteria and archaea (Stein & Klotz, 2016). Nitrogen gas is converted to
ammonium-nitrogen (NH$_4^+$-N) in an exergonic reduction process. Nitrogen fixation is particularly important in eutrophic lakes with large populations of cyanobacteria. This is primarily due to the algae’s ability to fix nitrogen directly from their atmosphere in nitrogen limited catchment sources (Heathwaite, 1993). Ammonium-nitrogen may be immobilized in the biomass of nitrogen fixers or host however this requires oxygen limited conditions (Green & Kauffman, 1982).

The second pathway is through anaerobic Assimilatory Nitrite Reduction to Ammonium (ANRA) and Dissimilatory Nitrite Reduction to Ammonium (DNRA). In the past, DNRA was used to describe nitrate removal in soils in a form other than dinitrogen gas or assimilation into microbial biomass (Stein & Klotz, 2016). DNRA occurs in optimal conditions of carbon abundance and limited nitrate (Widory et al., 2012; ISONITRATE, 2009). Under anaerobic conditions nitrogen is available to organisms as ammonium. Under conditions of high pH (> 8) and high temperature ammonium is converted to ammonia NH$_3$ (Heathwaite, 1993).

Ammonical N also occurs due to mineralization which is the degradation of organic nitrogen (protein molecules, peptides, amino acids and urea) by heterotrophic bacteria (Stein & Klotz, 2016).

### 2.2.2 Nitrification

**Nitrification** is a two-step oxidation process involving ammonia and nitrite oxidizers. In aerobic conditions, ammonium is oxidized to nitrite through nitrification by *Nitrosomonas* bacteria. Nitrite is oxidized through nitrification into nitrate-nitrogen, NO$_3^-$ N (Eq. 3.1) by nitrifying bacteria, *Nitrobacter*. Temperature and pH influence nitrification such that the growth rate of nitrifying bacteria increases optimally with pH of 8.4-8.6 and temperatures above 15° C (Heathwaite, 1993). Complete ammonia oxidation i.e. comammox oxidizers convert ammonia to nitrate (Stein & Klotz, 2016).

\[
NH_4 + 2O_2 \rightarrow NO_3 + H_2O + 2H^+ \tag{3.1}
\]

**2.2.3 Denitrification**

Whereas nitrogen is immobilized via assimilation and nitrogen fixation, its permanently removed via denitrification (Hernandez and Mitsch, 2007; Hansen et al., 2016). NO$_3^-$-N is very labile and mobile in the soil profile due to its negative charge. Denitrification from NO$_3^-$ to N$_2$ is performed by canonical denitrifiers (Stein & Klotz, 2016). The rate of denitrification is dependent on oxygen availability, temperature, nitrogen, organic carbon supply, nitrate
concentration in stream water overlying stream sediments, stream flow regime and water residence time (Hernandez and Mitsch, 2007).

Nitrate is dominant in highly enriched rivers whereas DON is dominant in less enriched rivers (Durand et al., 2011). Pristine riparian zones ordinarily function as sinks for the main pollutant, NO$_3^-$ in most surface waters (Boulware et al., 2002). Attenuation of NO$_3^-$ occurs through denitrification or dissimilatory nitrate reduction (Heathwaite, 1993). Denitrification occurs either in riparian zones such as wetlands prior reaching surface waters or by anaerobic sedimentary deposits in the river bed itself (Sebilo et al., 2002). It depends on riparian vegetation and microbial community’s ability to process pollutants from surface runoff and ground water flow (Boulware et al., 2002).

2.2.4 Anammox

*Anaerobic ammonium Oxidation, Anammox* describes the simultaneous conversion of NO$_2^-$ and NH$_4^+$ into dinitrogen gas. The process is exclusively performed by *Brocadiaceae* bacteria. Anammox is associated with nitrogen removal in ocean waters and oxygen minimum zones. (Stein & Klotz, 2016).

2.2.5 In-stream Nitrogen transformations

Nitrogen transformations within streams are highly dependent on metabolic processes of stream flora i.e. aquatic and stream bank macrophytes, epilithic algae, micro-heterotrophs and denitrifying bacteria. Nitrate uptake is accounted for by macrophytes (73%) while uptake by stream biota is accounted for in transformation process such as nitrification and denitrification. Inputs of ammonium and urea are converted to nitrate via detritus and bryophytes hence preventing nitrogen losses. Denitrification occurs along the stream water-sediment interface. It causes the transfer of dinitrogen gas into the atmosphere or river sediments. Wetlands act as nitrate sinks aiding the removal of nitrate through denitrification. However, efficacy of nitrogen removal is low when nitrogen loading is high (Heathwaite, 1993).
Figure 2-1: The nitrogen cycle. Nitrate ($\text{NO}_3^-$) and ammonium ($\text{NH}_4^+$) are introduced into the aquatic ecosystem via leaching from the geosphere, input from human activities, atmospheric deposition and biological fixation. Nitrate is converted to $\text{N}_2$ and lost to the atmosphere via denitrification. (Capua, 2013)

Modification of nitrate concentrations varies with seasonality. High storm events are associated with higher river discharges which may lead to elevated nitrate concentrations due to reduced benthic denitrification.

2.2.6 Human impact on Nitrogen cycling.

Human mediated nitrate sources have doubled the annual global $\text{Nr}$ inputs to ecosystems compared to pre-industrial era (Bernhardt, 2006; Zhou et al., 2014). Anthropogenic sources of $\text{Nr}$ such as fertilizer are developed via the Haber-Bosch process (Sobota et al., 2016). The main sources of input are observed from industrial, agricultural, and urban activities including excessive use of fertilizers, atmospheric deposition through biomass burning and the increased release of sewage and animal waste (Jacobs et al., 2017). Adverse effects associated include increased eutrophication of aquatic ecosystems and acidification of freshwater.
Poor land management practices act as an indirect source of physical (increased sedimentation), chemical (inorganic N, heavy metals) and biological stressors (proliferation of water hyacinth and dense blooms of cyanobacteria) of Lake Victoria catchment basin. These activities include wetland reclamations, increased domestic and untreated or poorly treated industrial effluent, fertilizer run-off from agricultural farms and sediments transported by rivers (Kundu et al., 2017). Sediment deposition has resulted in heavy metal pollutants especially lead. While high nutrient input from fertilizers and increased release of human and animal waste elevates eutrophication levels, increases N$_2$O emission and algae biomass growth causing anoxic conditions in the deeper layers of the lake (Zhou et al., 2014; Ouma et al., 2016).

Similarly, nitrogen export from river catchments is influenced by land use and efficacy of chemical cycling. Forest ecosystems have a low nitrogen load however, deforestation affects soil temperature while enhancing mineralisation leading to nitrogen losses. Urban surface water runoff can contribute a high nitrogen load to the aquatic system. In rural areas, the load will vary with annual precipitation and for urban catchments it varies according to the proportion of impermeable surface areas such as paved roads or paths (Heathwaite, 1993).

Lower water tables and N enrichment in urban riparian zones could reduce nitrate consumption by denitrification and increase NO$_3^-$ production by nitrification. Anthropogenic activities which hamper the riparian attenuation of NO$_3^-$ in urban watersheds include engineered structures which alter the hydrological flow path. These alter the volumes of water moving from uplands to streams as surface runoff (Boulware et al., 2002).

The hydrological cycle also determines the transport and export of N. In Sondu river catchment changing rainfall patterns due to climate change have resulted in increased drought spells as well as intensified floods. This is exacerbated by deforestation and encroachment of the Mau (headwaters of Sondu Miriu) which reduces the water catchment area. The variation in hydrology has a two-fold effect, increase or dilution of nutrient levels. Severe floods may influence erosion of top soil increasing the mobility of nutrients stored in the topsoil or decrease solute concentration through dilution. Increased mobility will differ depending on the land use i.e. agricultural activities amplify the availability of N through fertilizer inputs or increased discharge and mixing from different sections of the catchment may result in dilution (Jacobs et al., 2017).
2.3 Isotopic composition in aquatic systems

2.3.1 Principle of stable isotopes

An atom is composed of protons, electrons, and neutrons. Protons and electrons are charged, positively and negatively respectively whereas neutrons have no charge. Both neutrons and protons have similar mass proportions while the mass of electrons is negligible.

Elements, by nature, are defined by the number of protons. For given elements, the number of protons (atomic number Z) is the same however, the number of neutrons (neutrons number N) may vary from atom to atom. The sum of Z + N produces the mass number A. Although the varying neutron number may not produce changes in the gross chemical properties of an element or its compounds, it causes mass changes and produces isotopes. Therefore, an isotope of a given element differs from another isotope of the same element by the number of neutrons in its nucleus e.g. oxygen has three naturally occurring isotopes: $^{16}\text{O}$ with 8 protons and 8 neutrons, $^{17}\text{O}$ with 8 protons and 9 neutrons, and $^{18}\text{O}$ with 8 protons and 10 neutrons. Ultimately, the different masses cause chemical and physical differences which result in isotopic fractionation (Sharp, 2017).

Isotope application is distinguished between radioactive and stable isotopes. Radioactive isotopes have unstable nuclei which disintegrate over time to form other isotopes whereas stable isotopes do not decay to other isotopes over a geological time scale. However, stable isotopes may be a product of radioactive isotopes (Kihumba, 2016).

2.3.2 Abundance of Rare Isotopes

Isotopic abundance denotes the ratio between two isotopes of a given element in its natural state. Conventionally, isotopic ratios are expressed as the dimensionless ratio of the heavy (rare) isotope to the light (abundant) isotope e.g. $R = ^{18}\text{O}/^{16}\text{O}$ or $^{11}\text{B}/^{10}\text{B}$ (Mariotti et al., 1981). The ratio is expressed as shown in (Eq. 3.2)

$$R = \frac{\text{abundance of heavy isotope}}{\text{abundance of light isotope}}$$  \hspace{1cm} (3.2)

The abundance of $^{15}\text{N}$ is expressed in atom per cent as the ratio:

$$a = \frac{^{15}\text{N}}{^{15}\text{N} + ^{14}\text{N}}$$  \hspace{1cm} (3.3)

Atmospheric nitrogen has an a value of 0.3663 ± 0.0004 atom $^{15}\text{N}$% (Mariotti et al., 1981).
Isotopic ratios can be represented as absolute or relative to certified international standards (Nestler et al., 2011). The measuring of absolute abundance with great precision is difficult. However, a higher accuracy is attainable when measuring two compounds with small variations in isotope ratio as shown in Eq. 3.4 (Mariotti et al., 1981). For the relative unit, a notation of delta $\delta$ is used

$$\delta^{15}N = \left( \frac{R_x - R_{std}}{R_{std}} \right)$$  \hspace{1cm} (3.4)$$

Or it may also be expressed per a thousand as

$$\delta^{15}N(\text{%}) = \left( \frac{R_x}{R_{std}} - 1 \right) \times 1000$$  \hspace{1cm} (3.5)$$

Where $R$ denotes the ratio of abundance of heavy to light isotope, $R_x$ is the sample and $R_{std}$ abbreviates the standard. Since natural deviations from the international standards are small, delta values are represented in per mil, or parts per thousand % (Mariotti et al., 1981) as shown in equation (3.5). When the ratio of heavy to light isotope is higher in the sample than in the standard, the $\delta$ value will be positive and the sample is enriched in the heavy isotope. A negative $\delta$ value means that the ratio of heavy to light isotope of the sample is lower than the standard and the sample is depleted in the heavy isotope than the standard (Sharp, 2017).

### Table 2.1 Isotopic abundance and relative atomic masses of elements

<table>
<thead>
<tr>
<th>Element</th>
<th>Abundance (%)</th>
<th>Isotope atomic mass</th>
<th>Isotopic Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen</td>
<td>99.76</td>
<td>15.99</td>
<td>$^{18}$O/$^{16}$O</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>16.99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>18.00</td>
<td></td>
</tr>
<tr>
<td>Nitrogen</td>
<td>99.63</td>
<td>14.00</td>
<td>$^{15}$N/$^{14}$N</td>
</tr>
<tr>
<td></td>
<td>0.37</td>
<td>15.00</td>
<td></td>
</tr>
<tr>
<td>Boron</td>
<td>19.78</td>
<td>10.01</td>
<td>$^{11}$B/$^{10}$B</td>
</tr>
<tr>
<td></td>
<td>80.22</td>
<td>11.01</td>
<td></td>
</tr>
<tr>
<td>Carbon</td>
<td>98.89</td>
<td>12.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.11</td>
<td>13.00</td>
<td></td>
</tr>
</tbody>
</table>
2.3.3 Reference Standards

A set of reference standards was established to allow for comparisons of data from different laboratories (Sharp, 2017). According to Mariotti et al., (1981) the standard selected should embody qualities such as perfect homogeneity, chemical and isotopic stability. As a result, a standard abundant in nature and of simple chemical form is selected. A reliable standard measurement for N$_2$ is equivalent to $\pm$ 0.026 $\%$ (Mariotti et al., 1981). The international standard for $\delta^{15}N$ is air (Eq 3.6) and for $\delta^{18}O$ (Eq 3.7) is the Standard Mean Ocean Water (SMOW) (ISONITRATE, 2009).

\[ \delta^{15}N \ (‰) = \left( \frac{15/14 R}{15/14 R_{AIR}} - 1 \right) \times 1000 \] (3.6)

\[ \delta^{18}O \ (‰) = \left( \frac{18/16 R}{18/16 R_{SMOW}} - 1 \right) \times 1000 \] (3.7)

2.3.4 Nitrogen Isotope Fractionation

According to Mariotti et al., (1981) partition of isotopes between two compounds containing the same element with different isotopic ratio is called isotopic fractionation.

Isotopic fractionation is a result of differences in the masses and thermodynamic properties of isotopes of the same element. (Sharp, 2017). The basic fractionation principle, (Sharp, 2017) is summarised as; In any multiphase system, there is a preferential fractionation of isotopes, with one phase preferentially incorporating the heavy or light isotope relative to other coexisting phases. The differences are present between compounds in chemical and physical equilibrium. Due to isotopic fractionation, composition of isotope in reactant and product compound will vary.

Fractionation occurs in isotope exchange reactions classified as equilibrium and kinetic. Equilibrium processes are reversible. In an equilibrium effect, an isotope will concentrate in one constituent of the reversible system. The component with more of the heavier isotope is regarded as enriched while that with the lighter isotope is considered depleted. Concentration of the isotopes is dependent on the bond strengths of the isotopes. Heavier isotopes concentrate in the component where the element is bound more strongly. Kinetic processes involve irreversible systems where products are swept away from the reactants before they have an opportunity to come to equilibrium i.e. fast, incomplete, or unidirectional processes like evaporation, diffusion and dissociation reactions and biological reactions such as
photosynthesis. Kinetic isotope effect will more likely result in the lighter isotope reacting more rapidly than the heavier isotope thus the product will be lighter than the reactant while the substrate will be enriched with the heavy isotope (Cole et al., 1990).

The magnitude of an isotope effect (equilibrium or kinetic) is expressed as a fractionation factor \( \alpha \). It acts as a measure between two substances A and B it is defined as

\[
\alpha_{A-B} = \frac{R_A}{R_B}
\]  

(3.8)

Nitrogen isotope fractionations are kinetically controlled (Sharp, 2017). When considering a unidirectional reaction, isotopic fractionation is represented in the form \( \alpha_{ph} = R_{ph}/R_s \), where R is the isotopic ratio \(^{15}\text{N}/^{14}\text{N} \) (Mariotti et al., 1981). P and S are the products and substrate source of Nitrogen. If a substrate and a product maintain the same \(^{15}\text{N}/^{14}\text{N} \) ratio, fractionation of \(^{15}\text{N} \) isotope does not occur. The ratio or fractionation factor is unity \( \alpha \approx 1 \). Therefore, any deviations from unity indicate isotope fractionation (Karel, 2004).

Due to kinetic effects of nitrogen isotope in nature, fractionations are often reported using the enrichment factor notation \( \varepsilon \) given as

\[
\varepsilon = (\alpha - 1)
\]  

(3.9)

\( \varepsilon \) signals no reversible equilibrium process (Sharp, 2017) while also allowing fractionation to be expressed on the \( \delta \) scale (Karel, 2004). The effects are small and hence deviations are reported from unity (Kihumba, 2016).

Equivalently,

\[
\varepsilon = \left( \frac{\delta_p - \delta_r}{\delta_r + 1} \right)
\]  

(3.10)

Rayleigh’s equation is used to denote isotopic enrichment or the evolution of isotopic composition in a closed system (Mariotti et al., 1981; Yevenes et al., 2016)

\[
\delta_r = \delta_{r0} + \varepsilon \ln(NO_3 - N)
\]  

(3.11)

Where \( \varepsilon \) is the isotopic enrichment factor whereas, \( \delta_{r0} \) and \( \delta_r \) represent the isotopic compositions of substrate at time 0 and t and \( r_0 \) and \( r \) are concentrations of the substrate at time 0 and t respectively, NO\(_3\) is the remaining fraction of nitrate (Kihumba, 2016). Rayleigh’s
is often used to model change in isotopic composition for nitrate during denitrification (Tsushima et al., 2006).

### 2.3.5 Nitrogen transformation processes affecting isotopic composition

The major processes affecting nitrogen isotopic composition include both biological processes such as N-fixation, assimilation, mineralization, nitrification, denitrification and physical processes such as volatilisation, sorption/desorption (Kihumba, 2016). The availability of nutrients and reaction rates will greatly influence the magnitude of fractionation (Sharp, 2017). Generally, the biological processes cause an increase in δ¹⁵N of the substrate and a decrease in δ¹⁵N of the product (Kendall et al., 2007).

Isotopic fractionation associated with bacterial *nitrogen fixation* is considered a single process measured on the product plant. The fractionation measured for (n=4) (δ¹⁵Nfixed – δ¹⁵Nair) ranged from +3.7‰ to -2.2‰ while the average fractionation between atmospheric N₂ and fixed nitrogen in organic matter was 0‰ (Kihumba, 2016; Sharp, 2017).

Similarly, *mineralization/ammonification*, which is the breakdown of organic matter to ammonium, is linked to a small fractionation δ¹⁵N 0 ± 1‰ between soil organic matter and soil NH₄⁺ (Kihumba, 2016).

*Assimilation* is a process by which organisms transform nitrogen into a bioavailable form. The isotope with the lighter mass is preferentially incorporated and biosynthesised. In anoxic waters the process is dependent on ammonium concentrations, a concentration of 40 µM is associated with a fractionation factor of -20 to 30‰ (Sharp, 2017). Similarly, the assimilation by algae in aquatic ecosystems has also been reported to range from -30 to 0‰. For concentrations of 9µM, fractionations range from -5 to -15‰ (Kihumba, 2016).

*Volatilization* is a physical process which causes the loss of ammonia NH₃, to the atmosphere. It causes enrichment of the heavier ¹⁵N isotope values in the residual NH₄⁺. It occurs as both kinetic and equilibrium processes. Fractionation is largely dependent on factors such as pH, temperature, and humidity. The overall process causes a fractionation of 25‰ in residual NH₄⁺ (Kihumba, 2016).

*Nitrification* is essentially a multi-step oxidation process (NH₄⁺ → NO₂⁻ →NO₃⁻). Isotopic fractionation estimates for the conversion of ammonium to nitrite range from δ¹⁵N values 18‰ to -29‰. The large fractionation is due to formation of various intermediary and by-
products which include hydroxylamine, NH$_2$OH and NO$_2^-$.

The second reaction step is quantitative where all the nitrite is converted to nitrate resulting in no fractionation. Ultimately, the fractionation values are dependent on the proportions of ammonium and nitrate after reactions i.e. the fraction of substrate pool consumed. Fractionations in nitrogen limited systems is small (Kendall et al., 2007).

Fractionation of $\delta^{18}$O is rather dependent on incorporation of H$_2$O and O$_2$ a mixture of two O atoms from H$_2$O and one from O$_2$. For water $\delta^{18}$O values range from -25 %o to +4 %o and soil O$_2$ values +23.5 %. NO$_3^-$ derived from nitrification should have a range of $\delta^{18}$O values from -10 %o to +10 %o (Kihumba, 2016).

**Figure 2-2** Diagram of the nitrogen cycle. The figures in brackets depict average fractionations linked with each process. Nitrogen is converted to bioavailable form (labile organic N) for **assimilation** or **immobilization** by microorganisms via nitrogen fixation. Labile organic N is converted to ammonium through **ammonification**. Ammonium is oxidized to nitrite and nitrate through **nitrification**. Nitrite is converted to dinitrogen gas via **denitrification** (Sharp, 2017).

**Denitrification** is the dissimilatory reduction of nitrogenous oxides to gaseous products often happening in a two-stage process (Tsushima et al., 2006). Denitrification has a large isotope fractionation with enrichment $\varepsilon$ value variations depending on conditions such as substrate composition, concentration and microbial availability of the electron donor, temperature, denitrification rate and type of bacteria involved in the process and their growth conditions (Tsushima et al., 2006). Laboratory experiments yield values of -17 %o to -20 %o for $^{15}$N. Due
to kinetic isotope effects the lighter isotopes of $^{15}\text{N}$ and $^{18}\text{O}$ are converted to dinitrogen and dinitrogen monoxide resulting in enrichment of the heavy isotopes in the remaining substrate of $\text{NO}_3^-$. Other enrichment factors reported for denitrification range from $^{15}\text{N} -40 \, \%$ to $-5 \, \%$ and oxygen isotopic enrichment factors are between $-18 \, \%$ and $8 \, \%$ (Xue et al., 2009). Fractionation from soil samples is often less $-12 \, \%$ to $-14 \, \%$ (Blackmer & Bremner, 1976). Denitrification generally leads to isotope values increasing linearly by factor between 1.3:1 and 2.1:1 for $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ however, deviations from this ratio have been observed mostly due to temperature variability and substrate concentration (Voss et al., 2006; Xue et al., 2009).

Isotopic fractionation associated with benthic denitrification was found to be minimal $\delta^{15}\text{N} \, 1.5 \, \%$ to $-3.6 \, \%$ while riparian denitrification has significant enrichment $\delta^{15}\text{N} \, -18 \, \%$ (Sebilo et al., 2002).

2.3.6 Mixing

Mixing of various nitrate sources control nitrate pollution leading to the modification of the isotope composition of dissolved nitrate. Due to the non-conservative nature of nitrogen as discussed in nitrogen cycling, the isotopic composition of the various sources of nitrate are perpetually changing. Therefore, initial compositions are altered by fractionations before, during and after mixing.

Mixing when coupled with the transformation and conversion processes such as denitrification/nitrification may cause difficulty when distinguishing multiple nitrate sources based on $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$. During denitrification, the light isotopes are preferentially taking part in the reaction causing enrichment of $\delta^{15}\text{N} – \text{NO}_3^-$ and $\delta^{18}\text{O} – \text{NO}_3$ which is difficult to distinguish from mixing of $\text{NO}_3^-$ sources (Sebilo et al., 2002).

Multi-isotope and multi-tracer approach is used to resolve sources of N from cycling transformations (Kendall et al., 2007). under specific conditions. Isotopic analysis must be studied on a spatial and temporal scale while accompanied by multi-isotope and multi tracer approach which includes hydrologic and chemical data Furthermore, the use of dual isotopes is crucial in qualitative analysis of processes such as denitrification (Wang et al., 2016).

Boron isotopes are used to further identify nitrate by the separation of manure and sewage sources as they are ubiquitous, co-migrants of isotopes and unaffected by nitrate conversion processes (ISONITRATE, 2009).
2.4 Application of stable isotopes in nitrate source identification

2.4.1 $\delta^{15}$N and $\delta^{18}$O values of NO$_3^-$ sources

Isotopic compositions of nitrate are a result of mixing and cycling (Kendall et al., 2007). Its effective in distinguishing between five key sources (nitrate fertilizers, ammoniacal fertilizers, manure and sewage, soil N and atmospheric N deposition (Xue et al., 2012).

Fig 2.3 a) displays box plot of different $\delta^{15}$N sources and sinks. The typical $\delta^{15}$N ranges are presented by the 10$^{th}$ and 90$^{th}$ percentiles. Synthetic inorganic fertilizers show low $\delta^{15}$N values (-4‰ to +4‰) whereas organic fertilizers have a range from +6‰ to 30‰ (Wang et al., 2016; Sharp, 2017). Xue et al., (2009) reported ranges of +5‰ and +25‰ for manure and +4‰ to +19‰ for sewage. Other values reported for $^{15}$N for sewage range from +8‰ to +20‰. These enriched values arise from ammonia volatilization in sewage causing $\delta^{15}$N enrichment in the residual NH$_4^+$-N. Through nitrification the residual NH$_4^+$-N is converted to $^{15}$N-enriched NO$_3^-$ (Da et al., 2017)

The range of $\delta^{15}$NO$_3^-$-N values for artificial fertilizers (NH$_4^+$, NO$_3^-$ and urea) include -6‰ to +6‰. The slight variation in $\delta^{15}$N content arises from small fractionation during processing of fixed N while those of soil range from 0‰ to 8‰. It should be noted that some natural factors and biological activities cause small variations in the N isotopic ratios of soil organic N. These include soil depth, vegetation types, mineralization, and nitrification (Xue et al., 2009). The sources of atmospheric N in rain arise from volatilization of ammonia, nitrification and denitrification of soils and anthropogenic sources e.g. forest fires. The values of $\delta^{15}$N–NO$_3^-$ range from -3‰ to +9‰ (Kendall et al., 2007) while (Xue et al., 2009) proposed ranges from -13‰ to +13‰.
Due to the complexity of the biogeochemical N cycle, N isotope composition of NO$_3^-$ could change during transformation leading to overlapping of $\delta^{15}$N–NO$_3^-$ therefore a dual isotope approach (use of $\delta^{15}$N and $\delta^{18}$O) is employed to identify nitrate sources (Widory et al., 2012; Wang et al., 2016).

According to Xue et al. (2009), nitrification derived nitrate should have $\delta^{18}$O ranging from -10 ‰ and +10 ‰ (Fig 2-3 b). $\delta^{18}$O values are used to distinguish atmospheric NO$_3^-$ (+25 ‰ to
75 ‰) from mineralized soil NO$_3^-$. Synthetic fertilizers show a range from $+17$ ‰ to $+25$ ‰. The dominant sources of NO$_3^-$ reported in literature are illustrated in Figure 2.4, a bi-plot of $\delta^{18}O$–NO$_3^-$ against $\delta^{15}N$–NO$_3^-$. 

**Figure 2-4** $\delta^{15}N$ and $\delta^{18}O$ of various nitrate sources. Values for atmospheric $\delta^{18}O$–NO$_3^-$ are divided into the ranges observed for samples analysed using the denitrifier and non-denitrifier methods. The arrows show expected slopes of data from denitrification of nitrate (Kendall *et al.*, 2007).

### 2.4.2 Analytical techniques for the determination of $\delta^{15}N$–NO$_3^-$ and $\delta^{18}O$–NO$_3^-$

Isotopic techniques are most effective when the following conditions are met: (a) potential sources of nitrates can be identified and their isotope compositions analysed (b) potential sources of nitrates have distinctively different isotope compositions with limited isotopic variability and results of isotope data interpretation are complimented or supported by other approaches, such as findings from multivariate analysis of hydrogeochemical parameters (Kendall *et al.*, 2007).

Under this premise, isotopic composition of nitrogen species is widely used to define the sources and fate of N in water. Different techniques have been developed for the determination of $\delta^{15}N$ and $\delta^{18}O$ nitrate isotopes. These include the ion exchange method or silver nitrate method by (Silva *et al.*, 2000). With this technique, samples are concentrated on cation and
anion exchange resin columns, nitrate is eluted from the resin columns using hydrochloric acid, neutralized with Ag₂O, filtered and purified to produce silver nitrate (AgNO₃) salts. The Δ¹⁵N–NO₃ can be measured via conversion of AgNO₃ to N₂ using combustion. ¹⁸O analysis is conducted using combustion method where AgNO₃ is combusted with finely ground spectrographic graphite to produce CO₂ which is cryogenically purified and analysed for its oxygen isotope composition (Silva et al., 2000). Alternatively simultaneous measurement of Δ¹⁵N and Δ¹⁸O may be accomplished using TC/EA-IRMS (thermal conversion(elemental) analyser-isotope ratio mass spectrometer (Kendall et al., 2007).

Other proposed methods include the “bacterial denitrification method” (Sigman et al., 2002) and cadmium reduction method or “azide method” proposed by (McIlvin & Altabet, 2005). “Bacterial denitrification method” allows for the simultaneous determination of Δ¹⁵N and Δ¹⁸O produced from conversion of NO₃⁻ to N₂O by denitrifying bacteria, Pseudomonas chlororaphis and Pseudomonas aureofaciens, which naturally lacks N₂O reductase activity. Nitrous oxide is extracted using helium carrier gas and analysed for its isotopic composition using an isotope ratio mass spectrometer. This involves use of published trace-gas methods, N₂O is extracted from sample vials, undergoes online purification, cryogenic trapping, and chromatographic separation from CO₂ (Sigman et al., 2002).

### 2.4.3 Quantification of nitrate source contributions

Dual isotope approach has successful been used to trace sources of nitrate in watersheds. Previously used to study 16 watersheds in the US, isotopic signatures of NO₃⁻ varied depending on the land use. Forested watersheds NO₃⁻ was derived from Soil N stocks while the predominant sources from agricultural land/watersheds were manure and fertilizer (Voss et al., 2006; Xue et al., 2012). Aside from tracing sources, isotope analysis has also been used to quantify relative contributions from the same sources using mass-balance mixing models. According to Deutsch et al., (2006), riverine NO₃⁻ sources were derived from drainage water 86%, groundwater 11% and atmospheric deposition 3%.

The basic mass-balance mixing model based on two isotopes and three sources can be expressed as follows:

\[
\delta^{15}N_M = f_1 \delta^{15}N_1 + f_2 \delta^{15}N_2 + f_3 \delta^{15}N_3
\]

\[
\delta^{18}O_M = f_1 \delta^{18}O_1 + f_2 \delta^{18}O_2 + f_3 \delta^{18}O_3
\]
\[ f_1 + f_2 + f_3 = 1 \]  
(3.14)

Where \( \delta^{15}N_M \) and \( \delta^{18}O_M \) are the NO\(_3^-\) isotope values from the mixture as the subscripts 1, 2 and 3 represent any of the three sources, \( f \) is defined as the proportional contribution of the respective source.

However, as discussed earlier, mixing models are inaccurate due to uncertainties introduced by temporal and spatial variability in \( \delta^{15}N \) and \( \delta^{18}O \) of NO\(_3^-\), isotope fractionation as a result of denitrification and too many NO\(_3^-\) sources (Kihumba, 2016).

A Bayesian framework model is used to determine the probability distribution of the proportional contribution of each source to a mixture (Xue et al., 2012). The model has been used for five main sources in surface waters (manure and sewage, nitrate fertilizer, ammonium fertilizer, soil N, nitrate in precipitation).

The mixing model can be expressed mathematical as shown:

First a few terms are defined, data comprises N measurements, on J isotopes with K sources (Xue et al., 2012; Parnell et al., 2010):

\[ X_{ij} = \sum p_k (S_{jk} + c_{jk}) + \varepsilon_{ij} \]

\[ S_{jk} \sim N(\mu_{jk}, \omega_{jk}^2) \]

\[ c_{jk} \sim N(\lambda_{jk}, \tau_{jk}^2) \]

\[ \varepsilon_{ij} \sim N(0, \sigma_j^2) \]

(3.15)

Where \( X_{ij} \) is the observed isotope value \( j \) of the mixture \( i \) where \( i=1,2,3, \ldots, N \) and \( j = 1,2,3, \ldots, N \); \( S_{jk} \) is the source value \( k \) on isotope \( j \); normally distributed with mean \( \mu_{jk} \) and variance \( \omega_{jk} \); \( p_k \) = is the proportion of source \( k \), which needs to be estimated by the SIAR model; \( c_{jk} \) is the fractionation factor for isotope \( j \) on source \( k \) and is normally distributed with mean \( \lambda_{jk} \) and variance \( \tau_{jk}^2 \); \( \varepsilon_{ij} \) is the residual error representing the additional unquantified variation between individual mixtures normally distributed with mean 0 and standard deviation \( \sigma_j \). Further analysis with the model will be reviewed under chapter 5.
3 DESCRIPTION OF THE STUDY AREA

3.1 Outline
This chapter aims to provide information on the physical and geographical aspects of Sondu River Basin. The main content highlighted includes i) Geographical location (ii) Geology, and soils iii) Climate, Drainage and Hydrography v) Land use and Pollution

3.2 Geographical location
The study area is located in R. Sondu catchment area, which has headwaters in the South-West block of the Mau Forest Complex, drains into Lake Victoria. Sondu Miriu River, one of the eleven major rivers in the Lake Victoria basin, drains a total area of 3,508 km² in the Western part of Kenya. The basin is located within latitudes 00°23’S and 0°110’S and longitudes 34°46E. The main tributaries are Kipsonoi and Yurith (Masese et al., 2012). The river originates from the western slopes of the Mau Escarpment and flows through a narrow to Nyakach Escarpment. It then meanders into the Odino falls and enters the flood plains of Nyakwere where it drains into the Winam Gulf of Lake Victoria. The elevation of the catchment ranges from 1140 meters above sea level (a.s.l) at the Lake shore rising to 2900 m a.s.l in the north-east region (Wesser et al., 2018).

3.3 Geology and Soils
The study area is classified under the geomorphological unit of the Kericho plateau which is covered by phonolites. Geology is dominated by lava of Tertiary age dating from mid-Miocene times. There exists three main geological units i.e. the Quaternary volcanic rocks, Bukoban system and Alluvium. The Quaternary volcanics are mainly alkaline in nature consisting of basalts, phonolites, trachytes and nephelinites. The Quaternary volcanics are exposed along the Sondu valley (Krода, 1988). Phonolites dominate the lower part of the catchment and phonolitic nephelinites in the upper part (Binge, 1962; Jacobs et al., 2017).

The soils were formed in early Miocene age from the weathered tertiary lavas (Jacobs et al., 2017). Due to the angle of the lavas, the soils are stone free with uniform physical structure for a depth of upto 6 m. These soils are further classified into mollic Andosols, humic Nitosols (towards Kericho town) and humic Acrisols (Kroha, 1988). Cambisols, Greyzems, Lithosols, Phaeozems, Planosols, Rankers, Vertisosls and Xerosols area also located in the catchment (Vuai et al., 2012).
3.4 Climate, Drainage and Hydrology
From Koppen Geiger classes, Sondu basin is categorized as a Tropical wet climate, Af. with annual rainfall of 1700 mm. The temperature and rainfall is modified by elevation of the basin and the highlands indicating a range of annual rainfall from 800 mm – 2000 mm (Fig 3-1) (Da et al., 2017). According to Jacobs et al., (2017) the “seasons” are characterized by a bi-modal rainfall pattern alternating between wet and dry spells (Fig 3-2). The “long rains” occur between April and May while the short rains between August and September. The driest months usually recorded are January and February. The average annual precipitation recorded in a commercial tea farm was 1988± 328 mm.

![Hydrography map](image)

**Figure 3-1** Hydrography map depicting the annual rainfall for study area. Annual precipitation ranges from 800 to 2000 mm.

The extent of variation of diurnal daily depends on the season. Highest maximum daily temperatures are recorded in the drier season of January and February (>25°C) (Jacobs et al., 2017). The coolest months are usually June and July. The time variation of Sondu flow is
influenced by morphometry, lithology, land use/cover and rainfall patterns (Olang & Kundu, 2011).

![Figure 3-2](image)

**Figure 3-2** The average monthly precipitation (left axis), the average monthly diurnal temperatures are illustrated (right axis.) (Jacobs, 2018)

The level of Lake Victoria controls the drainage of the area which flows from north-east to south west. R. Sondu Miriu is rejuvenated by faulting on the southern side of Kavirondo rift valley. In the Sotik area, R. Sondu has eroded southwards along the phonolite-Bukoban contact capturing the drainage from the Mau. The south ward extension of R.Sondu Miriu, along the Bukoban-phonolite contact, resulted in the capture of Kipsonoi, Sisi, Itare and Kiptiget streams whose headwaters spring from the south east (Binge, 1962).

The mean annual discharge rate was estimated at 40.3 - 42.2 m$^3$/yr (LVEMP, 2005; Vuai *et al.*, 2012).

3.5 Land use and pollution

The land use includes agriculture (small holder agriculture, commercial tea and tree plantations) and natural forest which accounts for 64% and 27% of the total basin area respectively (Jacobs *et al.*, 2016; Rwigi *et al.*, 2016). The main industrial activities are agro-based dealing with tea harvesting and processing. Undue pressure from the human settlements (> 225± 148 persons/km$^2$) around South-West Mau has resulted in land use changes from activities such as forest excisions, encroachment of smallholder farms, illegal logging, charcoal burning and poaching.
Commercial tea plantations are located at elevations lower than the natural forests. Most of the farms were established during the colonial era, mid 1950s (Jacobs et al., 2017). Commercial tea farming is categorized by high fertilizer application rates (150-250 kg N ha\(^{-1}\) yr\(^{-1}\)), 4-10 times more N ha\(^{-1}\) yr\(^{-1}\) compared to small scale holdings (< 20 kg N ha\(^{-1}\) yr\(^{-1}\)) (Jacobs, 2018). From a field study survey, the main fertilizers used included urea, ammonium fertilizers (CAN and DAP), organic fertilizers, foliar feed and NPK compound fertilizer.

It is estimated that small scale holdings growing sugar, tea, maize, and cotton mostly in the mid and lowlands are increasing at a rate of 2-3% per annum (Masese et al., 2012; Vuai et al., 2012). This increased intensity of agriculture and deforestation has been linked to higher magnitude and frequency of runoff events and reduced baseflows, increased nutrient contamination, erosion and sedimentation of tributary streams and Sondu river (Masese et al., 2012).

Figure 3-3 Land use map of the study area.
NO₃ loading from small scale holdings arises from intensive farming and minimal fertilizer use. These poor farming techniques result in mining of soil N via soil erosion and leaching. Furthermore, uncontrolled cattle grazing, slash and burn methods farming methods in the midland areas exacerbate land degradation. The slash and burn methods result in the release of organic nitrogen which is deposited into the river via wet and dry atmospheric deposition. It should be noted that substantial private sector investment in fertilizer and maize marketing has resulted in an increase in fertilizer inputs for maize farmers. According to Ariga & Jayne (2014) fertilizer application rates have risen to > 51 kg/acre/yr.

The sedimentation is further concentrated due to steep slopes (2.3) and a sediment transport capacity index of (0.14). The total nutrient discharge flux in Sondu-Miriu was determined as 813 mol y⁻¹ km⁻² estimated to be 5 times higher than discharge from other catchments on the Tanzanian catchment of L. Victoria, the DIN nutrient discharge was estimated at 40 t y⁻¹, the total TN discharge was estimated at 1,821 t yr⁻¹ (LVEMP, 2005; Vuai et al., 2012). According to Katsurada, (2007) the gully morphology of Sondu is characterised by steep sloped igneous rocks which can cause rapid runoff and erodible soft sediments especially in the absence of vegetation cover causing severe gully erosion.

Contamination from urban areas arises from municipal raw or partially treated domestic sewage, industrial waste, leachate from open dump sites and urban surface runoff due to impervious surfaces (LVEMP, 2005).

The Kenyan government approach to catchment conservation and preservation has been centred around conserving Mau, the origins of most headwaters. This includes eviction of people from Mau Complex and key catchment areas. Moreover, the government continues to create awareness of the best land management practises such as agroforestry and minimal tillage among small scale holders (Masese et al., 2012). National Environmental Management Authority (NEMA) activities have also created awareness against polluting the river using agrochemicals (Owiti et al., 2013).
4 METHODOLOGY

4.1 Description of sampling sites and strategy

Land use map was developed using 2016 LANDSAT-8 imagery using ArcGIS version 10.5. The map yielded eight main categories based on the land cover i.e. wetland, bushland, forest, tea and forest, swamp, water body and small-scale farming. Spatial sampling was based on four activities: Forest with tea farming (TF) and anthropogenic activities i.e. commercial tea Farming (CT), small scale holdings (MF) and urban settlements (U).

Temporal sampling was based on the bimodule season of the area i.e. wet seasons (April to May, August to Sept) and dry seasons (Dec to Feb). Sampling was done in four campaigns i) dry season in December 2016 only MF, CT and TF sites were monitored ii) the transitional period from dry to wet season in March 2017 iii) start of the short rains -wet season during the month of July 2017, iii) the end of the wet season in Sept 2017 after fertilizer application in the CT sites.

The water samples were collected as grab samples in 200 ml PVC bottles. The bottles and bottle caps were pre-rinsed three times using the surface water to be sampled and stored in coolers at temperatures below 4˚C to prevent the degradation of the sample. The samples were transported to Lake Victoria Environmental Management Project lab at the end of each day for storage at -20˚C. Prior to transportation to ISOFYS lab at Gent University, the samples were filtered using 0.45 µm Whatman (polyamide membrane filters) and refrozen at -20˚C.

4.2 Hydrogeochemical analysis

In situ measurements of the following parameters were taken, temperature, pH, electrical conductivity (EC), dissolved oxygen (DO) using multi parameter kit (Hanna). Geographical parameters latitude, longitude and altitude of each sample site were recorded in the field using a hand-held Garmin GPS.

The samples were analysed for 13 parameters calcium Ca²⁺, magnesium Mg²⁺, sodium Na⁺, potassium K⁺, nitrate NO₃⁻ sulphate SO₄²⁻, fluoride F⁻, chloride Cl⁻, ammoniacal nitrogen NH₄⁺ – N, nitrate nitrogen NO₃⁻ – N, nitrite nitrogen NO₂⁻ – N, isotopic signatures δ¹⁵N – NO₃⁻ and δ¹⁸O – NO₃⁻. Concentrations of NH₄⁺ – N, NO₃⁻ – N, NO₂⁻ – N were analysed on a continuous flow analyser (Autoanalyzer 3, Bran + Luebbe, Germany).

The major cations (Ca²⁺, Mg²⁺, Na⁺, K⁺) and anions (SO₄²⁻, F⁻, Cl⁻, NO₃⁻) were analysed using an ion chromatography (Ion chromatograph -930 compact IC).
The $\delta^{15}\text{N} - \text{NO}_3^-$ and $\delta^{18}\text{O} - \text{NO}_3^-$ values of the samples were determined using the “bacterial denitrification method” described by Sigman et al., (2001). During the procedure nitrate is converted to nitrous oxide $\text{N}_2\text{O}$ by denitrifier bacteria ($\text{Pseudomonas aureofaciens}$) which lacks $\text{N}_2\text{O}$ reductase activity (cannot further denitrify $\text{N}_2\text{O}$). Simultaneous measurements of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ (analytical precision $\pm 0.2$ ‰) are performed on the extracted $\text{N}_2\text{O}$ gas using trace gas preparation unit (ANCA TCII, SerCon UK). $\text{N}_2\text{O}$ is injected into the system using an autosampler and CO/CO$_2$ is removed using scrubbers. By cryogenic trapping and focusing the $\text{N}_2\text{O}$ is compressed onto a capillary column at 350 °C and analysed by an Isotope Ratio Mass Spectrometer (IRMS). The stable isotope ratios are expressed in delta units and a per mil notation relative to an international standard as shown in Equation 3.2 and 3.5 (Xue et al., 2012).

4.3 Determination of $\delta^{15}\text{N}-\text{NO}_3^-$ fractionation in denitrified soils

$^{15}\text{N}$ isotope enrichment due to denitrification in the riparian zone was determined using a laboratory set up as described by Karel (2004). Superficial river bed sediments were collected from a bridge using grab sampler (fitted with a weight to ensure sinking and a messenger for locking-in the sediment). The fresh soil was air-dried, crushed to pass through 2 mm screens. To study denitrification, dry soil of 20 g for each sample was placed in 500 ml Scot bottles and treated with a solution of 0.2 mM of calcium chloride ($\text{CaCl}_2$) added in a ratio of 1:15 (dry soil: solution). $\text{CaCl}_2$ was added to prevent the breaking of soil aggregates.

The bottles were sealed with caps which had two central holes fitted with tubes connected to an air flashing system to create anoxic conditions by replacement of air with dinitrogen gas. In order to purge both the head space and the slurry mix, one tube was immersed in the slurry mix while the other tube was in the headspace. The flashing was controlled at a pressure of 1 bar. Nitrification was inhibited by addition of nitrapyrin, 120 mg Kg$^{-1}$ of soil (Blackmer & Bremner, 1976). The slurry mix was continually stirred. The setup was preincubated for two days to aid the adaptive process for denitrifying bacteria. After the lapse of two days, prior to the start of sampling 20 mg $\text{NO}_3^-\cdot\text{N}$ L$^{-1}$ for each 20 g of soil was added. The soil suspension was extracted using a syringe at intervals of 0,4,8,24 and 48 hours. The suspension underwent centrifugal oscillation for 20 minutes (Sigma 3-16P); the suspension was settled for 3-5 min then filtered. The samples were filtered using both 0.45 µm and 0.20 µm Whatmann membrane filter(polyamide). Analysis of $\text{NO}_3^-\cdot\text{N},\text{NO}_2^-\cdot\text{N}$ concentrations were analysed using continuous flow (Autoanalyser 3, Bran + Luebbe, Germany).
Similar to the water sample analysis, isotope-ratio analysis of $\delta^{15}\text{N} - NO_3^-$ and $\delta^{18}\text{O} - NO_3^-$ of the filtrate was analysed as described by “bacterial denitrification method” (Sigman et al., 2002)

4.4 Statistical methods
4.4.1 Statistical analyses
The correlation between hydrogeochemical variables was analysed using Spearman’s non-parametric correlation at $p < 0.05$. Both One-way, Two-way ANOVA tests were performed on hydrogeochemical and isotopic data which fulfilled the assumption of normality and equal variance (Levene statistic for homogeneity of variance). These tests were performed to determine both significant differences and interactions based on a spatial and temporal scale.

A pairwise comparison (Post-hoc analysis) was performed using Tukey Honest Significant Difference test at 95% significance level. Non-normal and unequal variance variables samples were tested using Kruskall-wallis rank sum test and a post hoc non-parametric (Wilcoxon rank sum) paired comparison test ($P < 0.01$ and $P < 0.05$).

Statistical analysis was carried out on R studio software (3.4.3)

4.4.2 Multivariate statistical methods
4.4.3 Cluster analysis
Descriptive statistical analyses on raw data and multivariate statistical analysis including Hierarchical Cluster Analysis (HCA) was performed on hydrogeochemical datasets from the four campaigns to determine natural grouping in data spatial variability.

Cluster analysis is an unsupervised pattern recognition technique that uncovers intrinsic structure within a dataset. The objects of the system are classified into clusters based on their nearness and similarity. HCA is a common approach used following a Euclidean distance. The distance referring to the difference between analytical values from samples.

4.4.4 Principal component analysis and Factor analysis
Principal Component Analysis, (PCA) and Factor analysis(FA) was applied to reduce the dimensionality of data set and find a small number of uncorrelated linear compounds of the variables that are the principal components (PCs) (Singh et al., 2004).

The PC is given by
\[ p_{ci} = \sum_{j=1}^{N} e_{oj}^{i} \frac{x_{j} - \bar{x}_{j}}{\sigma_{j}} \]  \hspace{1cm} (4.1)

Where \( x_{j} \) and \( \bar{x}_{j} \) denote the observations and their mean values respectively. \( \sigma_{j} \) is the standard deviation of variable \( x_{j} \). The vector which maps the original variables onto the principal component is called an empirical orthogonal (uncorrelated) function, EOF (Kim et al., 2017).

The working principle of PCA is incorporated into five main steps. The first step is standardizing the raw data as shown

\[ x_{\text{std}} j = \frac{x_{j} - \bar{x}_{j}}{\sigma_{j}} \]  \hspace{1cm} (4.2)

Where \( x_{\text{std}} j \) is a standardizing value of \( x_{j} \).

Equation 4.2 is substituted into Equation 4.1

\[ p_{ci} = \sum_{j=1}^{N} e_{oj}^{i} x_{\text{std}} j \]  \hspace{1cm} (4.3)

The second and third steps involve creating the covariance matrix and calculating eigen values and eigenvectors of the the covariance matrix. The fourth involves choosing the number of components based on significance, Eigen Value One criterion. PCs are calculated based on equation 4.3. These values are useful in explaining the proportion of total variance of all variables in the data set (Kim et al., 2017).

The retained PCs are rotated to allow each component to have a small number of large loadings and many zero (or small) loadings. The loading is a correlation between the component and variable. The output are varifactors VFs (Abdi & Williams, 2010).

Prior to analysis, the data was standardized using Z- Score standardization function on \( r \) to reduce the dimensionality caused by different measuring scale units. Kaiser-Meyer-Olkin (KMO) was used to determine the suitability of the data for PCA/FA analysis. KMO is a measure of sampling adequacy which indicates a commonality in the proportion of variance. High value (closer to 1) implies PCA/FA is useful for the dataset (Ye et al., 2016). Furthermore, Bartlett’s test was applied to whole normalized data set to indicate a correlation between variables. PCA was performed on standardized data to obtain significant PCs. These PCs were subjected to a varimax rotation to factor the contribution of variables with major significance.

Multi-Variate analysis was carried out on R studio software (3.4.3).
4.4.5 Estimation of nitrate source contribution

Quantification of source contribution of NO$_3^-$ sources was determined using a Bayesian isotope mixing model. The Bayesian isotope mixing model was applied using mixSIAR for sample isotope data ($\delta^{15}N$–$NO_3^-$ and $\delta^{18}O$–$NO_3^-$) and 5 potential sources: nitrate in precipitation (NP), Soil N, ammonium fertilizer and rain (NF&R), Nitrate fertilizers (NF) and Manure and sewage(M&S). The $\delta^{15}N$–$NO_3^-$ and $\delta^{18}O$–$NO_3^-$ values of the 5 sources were obtained from Xue et al., (2009).

The output from mixSIAR was plotted as boxplots on Microsoft office Excel.
5 RESULTS AND DISCUSSION

5.1.1 Effect of land use on physicochemical parameters

Descriptive statistics was performed on surface water dataset for 14 variables as shown in (Table 5.1). The summary includes the number of samples analysed and the number of samples below the detection limit. A high coefficient of variability (CV) was most notable for NH$_4$+-N (157.9%) indicating input from anthropogenic sources such as domestic sewage. Some of the measurements of NO$_2$-N were below the detection limit (Table 5.1).

Table 5.1 Descriptive statistics summary of physicochemical parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit</th>
<th>No.</th>
<th>Below detection limit</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg$^{2+}$</td>
<td>mg/l</td>
<td>49</td>
<td>0.28</td>
<td>1.65</td>
<td>0.84</td>
<td>0.33</td>
<td>0.33</td>
<td>39.29</td>
</tr>
<tr>
<td>K$^+$</td>
<td>mg/l</td>
<td>49</td>
<td>-</td>
<td>0.88</td>
<td>6.29</td>
<td>3.20</td>
<td>0.94</td>
<td>29.38</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>mg/l</td>
<td>49</td>
<td>-</td>
<td>1.15</td>
<td>9.35</td>
<td>3.69</td>
<td>1.76</td>
<td>47.70</td>
</tr>
<tr>
<td>Na$^+$</td>
<td>mg/l</td>
<td>49</td>
<td>-</td>
<td>2.35</td>
<td>10.72</td>
<td>5.55</td>
<td>1.98</td>
<td>35.68</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>mg/l</td>
<td>49</td>
<td>-</td>
<td>0.49</td>
<td>4.54</td>
<td>2.28</td>
<td>0.98</td>
<td>42.98</td>
</tr>
<tr>
<td>F</td>
<td>mg/l</td>
<td>49</td>
<td>-</td>
<td>0.13</td>
<td>0.66</td>
<td>0.22</td>
<td>0.09</td>
<td>40.90</td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>mg/l</td>
<td>49</td>
<td>-</td>
<td>0.22</td>
<td>3.60</td>
<td>1.20</td>
<td>0.65</td>
<td>54.17</td>
</tr>
<tr>
<td>NO$_3$</td>
<td>mg/l</td>
<td>49</td>
<td>-</td>
<td>0.36</td>
<td>10.18</td>
<td>4.31</td>
<td>1.76</td>
<td>40.84</td>
</tr>
<tr>
<td>NH$_4$-N</td>
<td>mg N/l</td>
<td>49</td>
<td>-</td>
<td>0.06</td>
<td>1.80</td>
<td>0.19</td>
<td>0.30</td>
<td>157.89</td>
</tr>
<tr>
<td>NO$_2$-N</td>
<td>mg N/l</td>
<td>49</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.01</td>
<td>-</td>
</tr>
<tr>
<td>pH</td>
<td>pH. unit</td>
<td>49</td>
<td>-</td>
<td>6.25</td>
<td>8.08</td>
<td>7.18</td>
<td>0.42</td>
<td>5.84</td>
</tr>
<tr>
<td>Temp</td>
<td>˚C</td>
<td>49</td>
<td>-</td>
<td>15.27</td>
<td>27.30</td>
<td>19.72</td>
<td>2.87</td>
<td>14.55</td>
</tr>
<tr>
<td>EC</td>
<td>μS/cm</td>
<td>49</td>
<td>-</td>
<td>33.20</td>
<td>103.00</td>
<td>59.98</td>
<td>17.97</td>
<td>29.96</td>
</tr>
<tr>
<td>DO</td>
<td>mg/l</td>
<td>49</td>
<td>-</td>
<td>2.46</td>
<td>8.23</td>
<td>6.81</td>
<td>1.36</td>
<td>20.00</td>
</tr>
</tbody>
</table>

5.1.2 Physiochemical characteristics of surface water

The physiochemical parameters for the 49 waters samples is represented in Table 5.2. The pH of R. Sondu was neutral (7.2 ± 0.5, 7.4 ± 0.27, 7.3 ± 0.4, 6.88±0.31 for December, March, July, and September) (Table 5.2). The variation in pH was hardly beyond half a unit from the neutral value of 7.0. The pH values were neutral compared to ranges reported from previous studies (5.4- 8.47) (Mwashote and Shimbira, 1994; Vuai et al., 2012). The EC showed no variation with seasonality (P > 0.1), 57.7 ± 16.9 μs/cm, 72.5 ± 18.5 μs/cm, 57.7 ± 16.8 μs/cm, 72.5 ± 18.5 μs/cm for Dec, Mar, Jul, and Sept respectively (Table 5.2).
The average temperature measured showed variation with seasonality ($P < 0.1$). The mean temperature ranged from 15.7 °C to 27.30 °C with the highest temperatures measured in March (Table 5.2). The slight variation in temperature between the different sampling points is influenced by elevation and the hour of sampling. The lowest temperature was measured during morning hours while the highest temperature in the afternoon. The measured mean DO concentrations ranged from 2.46 mg/l to 8.23 mg/l (Table 5.2).

Na was the dominant cation (Table 5.2) followed by $\text{Ca}^{2+} > \text{K}^+ > \text{Mg}^{2+}$. The range of concentrations for the cations included: $\text{Na}^+(2.4 - 10.7) \text{mg/l}$, $\text{K}^+(0.9 - 6.3) \text{mg/l}$, $\text{Ca}^{2+} (1.2 - 9.3) \text{mg/l}$, $\text{Mg}^{2+} (0.3 - 1.7) \text{mg/l}$. $\text{Na}^+$ and $\text{K}^+$ occurrence in a riverine ecosystem is influenced by natural processes such as dissolution of Na-feldspars (albite dissolution) and anthropogenic sources such as fertilizers, and industrial waste. The physical and chemical weathering of igneous mineral rocks (silicate, ferromagnesium, gypsum) provides $\text{Mg}^{2+}$ and $\text{Ca}^{2+}$. Additionally, $\text{Ca}^{2+}$ is sourced from limestone, feldspar, quartz, silicate mineral rocks which form part of Sondu Basin’s lithology. Therefore, the cations and anions are likely sourced from albite and gypsum dissolution, silicate and carbonate weathering however, anthropogenic input cannot be ruled out.

The seasonal pattern of the cations was governed by higher mean concentrations in March, end of dry season, (7.6 mg/l, 4.3 mg/l, 4.4 mg/l, 1.2 mg/l) for $\text{Na}^+$, $\text{K}^+$, $\text{Ca}^{2+}$, $\text{Mg}^{2+}$ respectively whereas the lowest means were recorded in the month of September, end of wet season, for (4.3 mg/l, 0.7 mg/l) for $\text{Na}^+$ and $\text{Mg}^{2+}$ (Table 5.2). The lowest mean concentration for $\text{K}^+$ and $\text{Ca}^{2+}$ was detected in July (2.7 mg/l) and December (3.23 mg/l) respectively. Based on seasonality, the solute export from the catchment is defined by non-chemostatic relationship where concentrations are affected by river flows, groundwater recharge and anthropogenic input. Higher concentrations of the cations ($\text{Na}^+$, $\text{K}^+$, $\text{Ca}^{2+}$, $\text{Mg}^{2+}$) are present during the dry season due to higher rates of evapotranspiration, lower precipitation and higher temperatures. The low precipitation also results in higher contribution of river flow from groundwater. Lower concentrations are present in the wet season due to dilution effect from precipitation.
### Table 5.2 Summary of seasonal physiochemical characteristics of Sondu R. (Mean, standard deviation, minimum and maximum)

<table>
<thead>
<tr>
<th>Season</th>
<th>Parameter</th>
<th>Unit</th>
<th>Na [mg/l]</th>
<th>NH$_4$-N [mg/l]</th>
<th>K [mg/l]</th>
<th>Ca [mg/l]</th>
<th>Mg [mg/l]</th>
<th>F [mg/l]</th>
<th>Cl [mg/l]</th>
<th>SO$_4$ [mg/l]</th>
<th>NO$_3$ [mg/l]</th>
<th>pH</th>
<th>EC [µs/cm]</th>
<th>Temp ºC</th>
<th>DO [mg/l]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dec 2016</td>
<td>Mean</td>
<td></td>
<td>5.47</td>
<td>0.13</td>
<td>2.88</td>
<td>3.23</td>
<td>0.82</td>
<td>0.22</td>
<td>2.07</td>
<td>1.16</td>
<td>3.66</td>
<td>7.20</td>
<td>57.70</td>
<td>18.87</td>
<td>7.77</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td></td>
<td>2.07</td>
<td>0.05</td>
<td>0.49</td>
<td>1.33</td>
<td>0.29</td>
<td>0.04</td>
<td>0.86</td>
<td>0.82</td>
<td>1.32</td>
<td>0.46</td>
<td>16.88</td>
<td>2.98</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>Min</td>
<td></td>
<td>2.72</td>
<td>0.06</td>
<td>1.99</td>
<td>1.79</td>
<td>0.49</td>
<td>0.17</td>
<td>1.15</td>
<td>0.53</td>
<td>2.23</td>
<td>6.53</td>
<td>35.30</td>
<td>15.27</td>
<td>6.91</td>
</tr>
<tr>
<td></td>
<td>Max</td>
<td></td>
<td>10.72</td>
<td>0.26</td>
<td>3.76</td>
<td>6.15</td>
<td>1.36</td>
<td>0.30</td>
<td>4.14</td>
<td>3.60</td>
<td>6.91</td>
<td>8.08</td>
<td>94.20</td>
<td>23.33</td>
<td>8.23</td>
</tr>
<tr>
<td>Mar 2017</td>
<td>Mean</td>
<td></td>
<td>7.63</td>
<td>0.15</td>
<td>4.35</td>
<td>4.39</td>
<td>1.17</td>
<td>0.32</td>
<td>2.85</td>
<td>1.59</td>
<td>3.58</td>
<td>7.36</td>
<td>72.45</td>
<td>22.00</td>
<td>5.95</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td></td>
<td>1.96</td>
<td>0.03</td>
<td>0.84</td>
<td>1.17</td>
<td>0.31</td>
<td>0.14</td>
<td>0.97</td>
<td>0.67</td>
<td>1.66</td>
<td>0.27</td>
<td>18.47</td>
<td>3.06</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>Min</td>
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<td>4.81</td>
<td>0.12</td>
<td>3.25</td>
<td>2.86</td>
<td>0.77</td>
<td>0.20</td>
<td>1.82</td>
<td>0.71</td>
<td>1.47</td>
<td>6.98</td>
<td>52.00</td>
<td>17.46</td>
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<td>0.21</td>
<td>6.29</td>
<td>6.21</td>
<td>1.65</td>
<td>0.66</td>
<td>4.41</td>
<td>2.62</td>
<td>6.83</td>
<td>7.80</td>
<td>103.00</td>
<td>27.30</td>
<td>7.36</td>
</tr>
<tr>
<td>July 2017</td>
<td>Mean</td>
<td></td>
<td>5.08</td>
<td>0.25</td>
<td>2.67</td>
<td>3.80</td>
<td>0.76</td>
<td>0.18</td>
<td>2.20</td>
<td>0.87</td>
<td>4.42</td>
<td>7.31</td>
<td>56.68</td>
<td>20.35</td>
<td>6.56</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td></td>
<td>1.22</td>
<td>0.47</td>
<td>0.80</td>
<td>2.13</td>
<td>0.27</td>
<td>0.03</td>
<td>1.12</td>
<td>0.35</td>
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<td>0.44</td>
<td>15.14</td>
<td>2.34</td>
<td>1.58</td>
</tr>
<tr>
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<td>0.49</td>
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<td>9.35</td>
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<td>3.06</td>
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<td>2.07</td>
<td>1.24</td>
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<td>0.88</td>
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<td>0.31</td>
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<td>1.47</td>
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<td>7.20</td>
<td>89.30</td>
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</table>
NO$_3^-$ is the dominant anion in Sondu Miriu river basin (Table 5.2). The anion ranges included: NO$_3^-$ (0.4-10.2 mg/l), Cl$^-$ (0.5-4.5 mg/l), F$^-$ (0.1-0.7 mg/l), SO$_4^{2-}$ (0.2-3.6 mg/l) respectively. For F, Cl$^-$ and SO$_4^{2-}$ the highest mean concentrations were recorded in March (0.3 mg/l, 2.9 mg/l and 1.6 mg/l) respectively (Table 5.2). SO$_4^{2-}$ is derived from gypsum and oxidation of sulphide ores, aerosol emissions, fuel combustion (via atmospheric wet or dry deposition), phosphate fertilizers and domestic sewage and manure. Atmospheric wet deposition occurs due to the entrainment of particles by precipitation. Since water droplets are denser than the gaseous particles, the deposition of entrained particles is accelerated during storm events. Cl$^-$ is sourced from silicate (salt soil dissolution) and precipitation. Additionally, Cl$^-$ is sourced from human related activities such as waste water from industries and municipalities and agricultural runoff, and animal waste (Liu et al., 2006). Chen et al., (2009) reported elevated concentrations for Cl$^-$ and SO$_4^{2-}$ in the drier season is due to higher input from sewage and livestock effluent as a result of low flows. Precipitation tends to dilute manure and sewage in the wet season. In Sondu catchment, discharge of sewage is present from diffuse sources. Few households are connected to sewer lines therefore, domestic waste is more likely to be directed to surface water channels without treatment. Municipal domestic sewage is either discharged raw or partially treated from Kericho town treatment plant. Further input is present from leachate of solid waste open dumpsites and leaking sewer lines. US Environmental protection agency EPA set limit of SO$_4^{2-}$ for protection of aquatic water is 500 mg/l while the limit of Cl$^-$ for drinking water is 250 mg/l.

NO$_3^-$ showed significant difference in means between different seasons (P < 0.05). A pairwise comparison revealed a significant difference in the means recorded for December and September as well as March and September. The relatively higher concentration in Sept (5.46 mg/l) and July (4.47 mg/l) is associated with surface run off from agricultural fields and the forest (Table 5.2). Mena-rivera et al., (2017) showed similar findings of higher nitrate levels during the wet season due to increased runoff.

The compositional relation between the ions was further investigated using Spearman’s correlation ranking (Table 5.3) and correlation diagrams (Fig 5-1). The significant correlations were dependent on the coefficient value. Strong coefficient ratios had a positive or negative value ≥ 0.7, plus or minus 0.5 to 0.69 had a moderate correlation and plus or minus ≤ 0.3 had a weak correlation (Mena-rivera et al., 2017). The matrix indicates strong positive correlation between Cl$^-$ and Na$^+$, K$^+$, Ca$^{2+}$, Mg$^{2+}$ and EC ($r = 0.7$, $r = 0.7$, $r = 0.7$, $r = 0.7$, $r = 0.9$) indicating a common geogenic source i.e. albite dissolution and silicate weathering (Table 5.3). SO$_4^{2-}$
indicates a moderate correlation coefficient with $F^-$, $Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$ ($r = 0.6$, $r = 0.6$, $r = 0.6$, $r = 0.5$, $r = 0.6$) suggesting the ions have a common source (water-rock interaction). $F^-$ had a strong correlation with $Na^+$, $K^+$, $Mg^{2+}$ ($r = 0.7$, $r = 0.7$, $r = 0.7$) and a moderate correlation with EC and temperature ($r = 0.6$, $r = 0.5$) which depicts $F^-$ was sourced from a geogenic source.

$NO_3^-$ showed no significant correlation with $Na^+$, $Mg^{2+}$, $Ca^{2+}$, and $SO_4^{2-}$ ($r = 0.0$, $r = 0.0$ and $r = 0.1$) implying that weathering of rock-forming minerals is not the main source accounting for $NO_3^-$ (Table 5.3). According to Kamtchueng et al., (2016), a strong correlation between $K^+$ and $NO_3^-$ indicates input from fertilizer whereas a strong correlation of $Cl^-$ and $NO_3^-$ represents input from human related sources such as sewage and manure. Therefore, bi-plots showing the relationship between $NO_3^-$ vs $K^+$ and $NO_3^-$ vs $Cl^-$ (Fig 5-1 a and b) were used to trace the possible relationship between nitrate concentrations and fertilizers/manure & sewage. $K^+$ and $NO_3^-$ showed no significant correlation ($r = 0$) (Fig 5-1 a) while $NO_3^-$ showed a weak positive correlation with chloride (Fig 5-1 b) ($r = 0.3$) implying fertilizer and manure & sewage may not be the main sources of $NO_3^-$ in Sondu basin. However, these sources cannot be ruled out.

$Cl^-$ is biologically and chemically inert and change in its concentration is caused by mixing processes such as dilution in stream. A plot of $NO_3^-/Cl^-$ molar ratios against $Cl^-$ molar concentration ($\mu$M) (Fig 5-1 c) was used to further study $NO_3^-$ dynamics and $NO_3^-$ sources.
Figure 5-1  Bi-plots showing the relationship among dissolved species a) (K⁺ vs NO₃⁻) and (NO₃⁻ vs Cl⁻) c) NO₃⁻/Cl⁻ molar ratio vs. Cl⁻ µM for the seasons December (Dec) 2016, March (Mar), 2017, July (Jul) 2017 and September (Sept) 2017.

Table 5.3: Spearman’s correlation coefficients for physicochemical parameters of R. Sondu.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cl⁻</th>
<th>SO₄²⁻</th>
<th>F⁻</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Ca²⁺</th>
<th>Mg²⁺</th>
<th>NO₃⁻</th>
<th>pH</th>
<th>Temp</th>
<th>EC</th>
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<tr>
<td></td>
<td>mg/l</td>
<td>mg/l</td>
<td>mg/l</td>
<td>mg/l</td>
<td>mg/l</td>
<td>mg/l</td>
<td>mg/l</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
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<td></td>
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<td></td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
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<tr>
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<tr>
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<td></td>
</tr>
<tr>
<td>Mg²⁺</td>
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<td>1</td>
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<td></td>
</tr>
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<tr>
<td>pH</td>
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<td>0.6</td>
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<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.2</td>
<td>0.0</td>
<td>0.4</td>
<td>1</td>
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<tr>
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<td>-0.3</td>
<td>-0.3</td>
<td>-0.6</td>
<td>-0.5</td>
<td>-0.4</td>
<td>-0.08</td>
<td>0.2</td>
<td>-0.2</td>
<td>-0.6</td>
<td>1</td>
</tr>
</tbody>
</table>

Figures marked in bold (> 0.5 - 1) indicate moderate to strong correlations between variables.

NO₃⁻/Cl⁻ molar ratios were as follows: 1.09, 0.78, 1.92 and 1.64 for December, March, July, and September indicating a seasonal trend of lower ratios in the dry season compared to wet season. Chen et al., (2009) indicated similar findings relating the lower molar ratios of NO₃⁻.
/Cl⁻ in the dry season to increased Cl⁻ from higher input of sewage and livestock effluent. Contribution from manure and sewage is often diluted by precipitation in the wet season.

5.1.3 Multivariate statistical methods

Multivariate statistical methods are used to categorise spatial patterns in water quality data set (Kamtchueng et al., 2016; Mena-rivera et al., 2017; Singh et al., 2004). The multivariate analysis of the river water-quality data set was performed through Hierarchical cluster analysis (HCA) and principal component/Factor analysis (PCA/FA) techniques. HCA is used to determine similar groupings for the sites while PCA/FA is used to determine the main variables causing variance in the river water quality parameters.

5.1.3.1 Hierarchical Cluster analysis (HCA)

HCA is a tool often applied where several parameters are measured for one or multiple samples and is used to group sites of similar characteristics (Kamtchueng et al., 2016). HCA was used to identify the spatial pattern of the sampled sites from Sondu catchment. The analysis was performed on normalized river water quality data set by means of the commonly used Ward’s criterion and Euclidean distances as a measure of similarity.

![Cluster Dendrogram](image)

**Figure 5-2.** Dendrogram of the hierarchical cluster analysis of the water samples from Sondu Basin. The sampling points (Fig 3-3) were grouped into four clusters represented by colours: red – cluster 1, green – cluster 2, blue – cluster 3 and purple – cluster 4.
The Ward’s criterion uses an analysis of variance approach to minimize the sum of squares within the clusters and maximizes the variance between separate clusters (Singh et al., 2004). HCA yielded a dendrogram (Fig 5-2) grouping all 19 sites into four clusters.

The four clusters were statistically significant as each grouping was based on similar characteristic features and natural background source types.

These clusters were characterised as follows: cluster 1 included streams draining commercial tea farms (Uniliver and Finlay’s) (Fig 5-2). Commercial tea farming (CT) is categorized by high fertilizer application rates (150 - 250 kg N ha\(^{-1}\) yr\(^{-1}\)). Approximately 60% of the tea farms are located in the upper and midland reaches of the river. Therefore, the expected impact of human activities, especially with regard to nitrate concentration, is mainly from tea farming. The average concentrations measured included: Na\(^+\) (5.3 mg/l), K\(^+\) (3.0 mg/l), Mg\(^{2+}\) (0.7 mg/l), Ca\(^{2+}\) (3.5 mg/l), Cl\(^-\) (2.2 mg/l), SO\(_4^{2-}\) (0.9 mg/l), and EC (57.1 µS/cm) (Table 5.4). In the natural environment, NO\(_3^-\) can be found in low concentrations in precipitation (wet deposition) however, high concentrations are an indicator of pollution from anthropogenic activities (Kamtchueng et al., 2016). The contamination is likely from fertilizer application, agricultural runoff and soil nitrogen. Based on the nitrate concentrations (6.44 mg/l), CT corresponds to relatively high pollution (Table 5.4). The hydrogeochemical data had relatively moderate concentrations compared to the other clusters. The pH levels were within the expected range of 6.5 - 8.5.

Cluster 2 included sites dominated by forest with tea farming. (TF). These grouped sites exhibited the lowest mean concentrations (Table 5.4) for Na\(^+\) (4.5 mg/l), K\(^+\) (2.8 mg/l), Ca\(^{2+}\) (2.6 mg/l), Mg\(^{2+}\) (0.7 mg/l), Cl\(^-\) (1.6 mg/l) and EC (46.2 µS/cm) and SO\(_4^{2-}\) (1.0 mg/l). Based on NO\(_3^-\) concentration (3.0 mg/l), anthropogenic pollution is considered relatively low. These tea farming zones were located in the upper reaches of the river where human population density is low (Jacobs, 2018; Vuai et al., 2012). Previous studies indicated a similar trend where low N exports from forested areas appeared to buffer the effect of higher N- export from Tea zones suggesting strong capacity for river in-situ purification in the sampled locations (Jacobs et al., 2016).

Cluster 3, represented streams passing through Kericho town and one CT site influenced by residential settlements upstream. Relatively moderate concentrations were recorded for Na\(^+\)
(5.6 mg/l), K⁺ (3.5 mg/l), Mg²⁺ (0.9 mg/l), Cl⁻ (3.6 mg/l) and SO₄²⁻ (1.0 mg/l), EC (73.9 µS/cm) (Table 5.4).

Table 5.4 Summary of physico-chemical parameters of HCA clusters

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Parameter</th>
<th>Na mg/l</th>
<th>K mg/l</th>
<th>Ca mg/l</th>
<th>Mg mg/l</th>
<th>Cl mg/l</th>
<th>NO₃ mg/l</th>
<th>SO₄ mg/l</th>
<th>pH</th>
<th>Temp °C</th>
<th>EC µS/cm</th>
<th>DO mg/l</th>
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<tbody>
<tr>
<td>1</td>
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<td>0.71</td>
<td>2.18</td>
<td>6.44</td>
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<td>7.00</td>
<td>18.26</td>
<td>57.07</td>
<td>7.18</td>
</tr>
<tr>
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<td>0.20</td>
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<td>18.88</td>
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<tr>
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<tr>
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<td>74.36</td>
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<tr>
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<td>0.41</td>
<td>2.19</td>
<td>18.61</td>
<td>1.30</td>
</tr>
</tbody>
</table>

Cluster 1 represents commercial tea farming; Cluster 2 represents tea and forest; Cluster 3 indicates urban areas/densely populated regions; Cluster 4 shows mixed farming areas within the catchment.

The urban centres are characterised by discharge of domestic sewage to the open environment or leaking septic tanks and sewer tanks. Other human activities along the river banks include cultivation, washing of clothes and cars and watering of animals. The latter acts as an additional source for manure while the cultivation leads to increased soil N input into R. Sondu. Domestic sewage is associated with elevated Cl⁻ input (Kamtchueng et al., 2016). This is evidenced by the relatively high Cl⁻ mean concentration (3.60 mg/l) (Table 5.4). The lowest DO values (5th percentile, 3.96 mg/l) were measured in the urban settlement of Kericho town, downstream of a leaking sewer line discharging into the river. Reduced DO values are indicators of high organic pollution. The release of sewage into the riverine ecosystem causes an increased consumption of oxygen by microorganisms breaking down organic matter which causes low DO levels.

Sampled sites for cluster 4 were located in the lower reaches of Sondu R. The areas are characterised by small scale mixed farming (MF) on fragmented plots of less than 2 ha. Cluster 4 included the highest mean concentrations Na⁺ (7.02 mg/l), K⁺ (3.74 mg/l), Mg²⁺ 1.16 (mg/l), and SO₄²⁻ (1.80 mg/l), corresponding to sources such as water mineralization from physical and chemical weathering of rock-forming minerals (quartz Na-feldspar, albite, limestone) as well
as human activity (Table 5.4). In the lower catchment human impacts are associated with increased population and increased soil erosion linked to land degradation and overgrazing. According to Vuai et al., (2012) overgrazing currently exceeds the sustainable grazing rate by the factor of 5. The resulting increased sediment yields from eroded sites may cause relative higher nutrient loading within the catchment.

HCA technique was successfully applied to classify stream water quality in R. Sondu and can be used to develop future sampling strategies through creating a framework for categorising a catchment and reducing the number of sample sites. An advantage of this would be reducing cost while improving the efficacy of long-term monitoring programmes.

5.1.3.2 Principal component analysis (PCA)/Factor analysis (FA)
PCA is a statistical tool used to identify the main parameters that explains data variation from observed groupings of the sample sites. It analyses observations described by several dependent variables, reduces the data set to uncorrelated variables called principal components PCs which can be used to elucidate variance in the original data set (Mena-rivera et al., 2017). Relevant PCs are distinguished according to Eigen value-one criterion which stipulates that only factors with an Eigen value equal to or greater than 1 ought to be considered (Abdi & Williams, 2010).

Kaiser-Meyer-Olkin (KMO) was used to determine the suitability of the data for PCA/FA analysis. KMO is a measure of sampling adequacy which indicates a commonality in the proportion of variance. High value (closer to 1) imply PCA/FA is useful for the dataset (Ye, et al., 2016). The KMO was equivalent to 0.70. Furthermore, Bartlett’s test was applied to the entire normalized data set to indicate a correlation between variables. The test is used to show whether a correlation matrix is an identity matrix which is used to identify relations between variables. The significant level (P < 0.05) from Bartlett’s test exhibits a relationship between variables implying the data set was suitable for PCA/FA.

PCA was performed based on the four clusters separately and compared to PCA based on the four seasons separately & overall standardized data set separately. PCA on four clusters yielded best results. The relevant PCs were selected based on the eigen-one criterion as 3 PCs, 4 PCs, 2 PCs, and 2 PCs for cluster 1 - 4 respectively. The PCs explained 100%, 92%, 100% and, 99% of the cumulative variance for the river water quality data set for cluster 1 - 4 respectively.

However, to further reduce the contribution of variables with minor significance factor analysis (FA) was performed. FA attempts to explain the correlations between the observations in terms
of the underlying factors, which are not directly observable (Li et al., 2010). FA is accomplished through a varimax rotation of relevant PCs loadings to generate varifactors, (VF). The loading is a correlation between the principal component and variable. The rotation increases the absolute values of the larger loadings and reduces the absolute values of the smaller loadings (Abdi & Williams, 2010). According to Singh et al., (2004), the factor loadings are classified as strong, moderate or weak corresponding to absolute values of > 0.75, 0.75-0.50 and 0.5-0.3 respectively.

The factor loadings obtained from the varimax rotation are represented in Table 5.5. Cluster 1, VF 1 accounted for 50 % of the total variance with a strong positive loading (> 0.75) on EC, Na+ and Ca2+ and moderate loading on K+ and Mg2+ implying the main cause of variance in water quality parameters was water-rock interaction through albite and gypsum dissolution, silicate and carbonate weathering (Ye et al., 2016). VF 2 accounted for 32% of the total variance with a strong positive loading > 0.75 on K+, Cl-, temperature, moderate loading on NO3- and a strong negative loading on Mg2+. This VF may signify variance from an anthropogenic source indicating the pollution gradient for cluster 1. The possible sources of K, Cl-, and NO3- include pollution from an agricultural source (fertilizers, NPK), manure and sewage.

Cluster 2; The first varifactor accounted for 49% of the total variance with strong loadings (> 0.85) on EC, Na+, K+, Ca2+, and Mg2+ which can be interpreted as the variation of water quality as a result of the mineral component i.e. dissolution of feldspar, gypsum, or the weathering of silicate and carbonate rocks (Table 5.5). VF 2 explained 20% of the total variance with strong loading (> 0.88) on NO3- and Cl- which can be interpreted as pollution mainly from manure and sewage which was confirmed by the bi-plots of isotopic signatures seen further in Fig 5.3. For VF 3, a strong positive correlation was observed for pH explaining 16% of the total variance. VF 4 explained 16% of the total variance with strong loadings on DO and pH.

Cluster 3, VF 1 accounted for 58% of the total variance with strong positive loadings on pH, Temp, NO3-, Cl- and strong negative loading for DO (> 0.9) (Table 5.5) which suggests variation in water quality parameters are influenced by organic pollution from waste-waters and domestic sewage (Li et al., 2010).
Table 5-5 Factor analysis loading matrix of physico-chemical variables of surface water (Sondu Miriu)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Principal Components (PCs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CLUSTER 1</td>
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<tr>
<td></td>
<td>1</td>
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<tr>
<td>Na</td>
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</tr>
<tr>
<td>K</td>
<td>0.55</td>
</tr>
<tr>
<td>Ca</td>
<td>0.99</td>
</tr>
<tr>
<td>Mg</td>
<td>0.61</td>
</tr>
<tr>
<td>Cl</td>
<td>0.21</td>
</tr>
<tr>
<td>NO3</td>
<td>0.62</td>
</tr>
<tr>
<td>SO4</td>
<td>-0.92</td>
</tr>
<tr>
<td>pH</td>
<td>-0.81</td>
</tr>
<tr>
<td>Temp</td>
<td>-0.08</td>
</tr>
<tr>
<td>EC</td>
<td>0.91</td>
</tr>
<tr>
<td>DO</td>
<td>-0.93</td>
</tr>
<tr>
<td>Eigen Value</td>
<td>5.71</td>
</tr>
<tr>
<td>% variance</td>
<td>50</td>
</tr>
<tr>
<td>% Cumulative Variance</td>
<td>50</td>
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<table>
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</tr>
<tr>
<td>K</td>
<td>0.89</td>
</tr>
<tr>
<td>Ca</td>
<td>0.86</td>
</tr>
<tr>
<td>Mg</td>
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<tr>
<td>Cl</td>
<td>0.21</td>
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<tr>
<td>NO3</td>
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<tr>
<td>SO4</td>
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<tr>
<td>pH</td>
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<tr>
<td>Temp</td>
<td>-0.51</td>
</tr>
<tr>
<td>EC</td>
<td>0.98</td>
</tr>
<tr>
<td>DO</td>
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<tr>
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<tr>
<td>% Proportional Variance</td>
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<td>% Cumulative Variance</td>
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<table>
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<td>Cl</td>
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<td>NO3</td>
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<td>SO4</td>
<td>0.05</td>
</tr>
<tr>
<td>pH</td>
<td>0.93</td>
</tr>
<tr>
<td>Temp</td>
<td>1</td>
</tr>
<tr>
<td>EC</td>
<td>-0.21</td>
</tr>
<tr>
<td>DO</td>
<td>-0.99</td>
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Table 5.5 cont’d

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<tr>
<td>K</td>
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<td>Ca</td>
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</tr>
<tr>
<td>Mg</td>
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<td>Cl</td>
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<tr>
<td>NO₃</td>
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</tr>
<tr>
<td>SO₄</td>
<td>0.21</td>
</tr>
<tr>
<td>pH</td>
<td>0.91</td>
</tr>
<tr>
<td>Temp</td>
<td>0.89</td>
</tr>
<tr>
<td>EC</td>
<td>0.79</td>
</tr>
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<td>DO</td>
<td>0.94</td>
</tr>
<tr>
<td>Eigen Value</td>
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<tr>
<td>% Proportional Variance</td>
<td>56</td>
</tr>
<tr>
<td>% Cumulative variance</td>
<td>57</td>
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</table>

*Figures in bold > 0.75 which signify strong factor loadings and the main variables causing variance for each Principal Component (PCs)*

Raw and partially treated and municipal waste from Kericho urban centre is directed into the river channel. Waste waters constituent organic matter and high nitrogen input in the form of NH₄⁺. Through nitrification, ammonium is converted to nitrate acting as a source for nitrate in streams. The conversion is accelerated in relatively high temperatures hence causing the positive correlation between nitrate and temperature. Organic matter is mineralized to carbon dioxide by microorganisms in the presence of oxygen. The consumption of oxygen causes reduced DO levels in the riverine ecosystem. VF 2 accounted for 42% of the variance in water quality parameters, strongly correlating Na⁺, K⁺, SO₄²⁻ and EC which represents water-rock interaction (albite and gypsum dissolution).

Cluster 4, VF 1 proportionality of total variance was 56%, with a strong loading on (> 0.75) K⁺, Mg²⁺, Ca²⁺, F⁻, Cl⁻, NO₃⁻ and EC indicating partial influence from mineralization and partial influence from human activities (soil N, manure and sewage). VF 2 accounted for 43% of the total variance with strong gradients assigned to K⁺, Ca²⁺, Cl⁻, SO₄²⁻ and moderate loadings for Mg²⁺ (0.59), Na⁺ (0.69) and EC (0.61) describing water-mineral rock interaction (Table 5.5).

From the PCA/FA results, variations in the water quality parameters are explained by both natural geogenic sources and anthropogenic activity (Mena-rivera *et al.*, 2017). Source
identification was further assessed using isotopic signatures and quantification of nitrate source contribution.

5.2 Spatial-temporal variation of stream ammonium and nitrate concentration

There was no significant difference in \( \text{NH}_4^+ - \text{N} \) concentrations \((P > 0.05)\) between seasons. The mean concentrations between different land uses also indicated no significant difference \((P > 0.05)\). \( \text{NH}_4^+ - \text{N} \) concentrations ranged from 0.06–1.80 mg N \( \text{l}^{-1} \). The highest mean \( \text{NH}_4 - \text{N} \) (1.04 mg N \( \text{l}^{-1} \)) was detected in U suggesting input from waste water (Table 5.6). The main N species in urban waste waters is ammonium which is converted to nitrate through nitrification. Further evidence of organic pollution was indicated by the low DO levels detected in U zones during July \((3.1 \pm 0.9)\) and September \((3.9 \pm 1.2)\) (Table 5.6). The concentrations detected were within the range of those previously recorded by Vuai et al., (2012) in the catchment \((0.2 - 0.4 \text{ mg N l}^{-1})\) during intermediate flow (November). However, for urban areas the concentrations were slightly higher \((0.8–1.0 \text{ mg N l}^{-1})\) which could signify higher input in the urban sites sampled. Most of the concentrations for \( \text{NO}_2^- - \text{N} \) were below the detection limit therefore, determining a seasonal trend was not possible \((e.g., \text{ in March only one measurement was detected})\).

\( \text{NO}_3^- - \text{N} \) exhibited no significant interaction effect \((P > 0.5)\) between sampling season and land use. \( \text{NO}_3^- - \text{N} \) was the most abundant inorganic N species in the Sondu Basin ranging from 0.33 –3.94 mg \( \text{N} \text{l}^{-1} \) (Table 5.7). The highest mean value was detected in CT in Sept \((1.4 \pm 0.4 \text{ mg N l}^{-1})\). The lowest mean was detected in TF during March \((0.45 \pm 0.14 \text{ mg N l}^{-1})\). Nutrient trends and transportation, within Sondu catchment, are interlinked with various factors including topography (steep slopes), climate, anthropogenic activity, and sediment transport capacity index. The latter is defined as the maximum flux of sediment a stream is able to transport (Aksoy & Kavvas, 2005). The temporal variation within the catchment is affected by climate, mostly precipitation, indicating relatively higher concentrations \((1.40 \text{ mg N/l}, 1.39 \text{ mg N/l}, 1.17 \text{ mg N/l})\) in September for clusters 1,2 and 4 respectively. The nitrate nitrogen concentrations were higher than those previously reported in Sondu \((0.3 – 1.6 \text{ mg N/l})\) (Jacobs et al., 2018).

Higher concentrations in wet seasons can be explained by various factors. According to Dupas et al.,(2017), a vertical distribution of \( \text{NO}_3^- - \text{N} \) in soil profile reveals higher concentrations of \( \text{NO}_3^- - \text{N} \) in the top compared to deeper soil layers leading to increased contribution of nitrate from soil via run-off during storm events. Nitrate input during high flow is also traced to activation of shallow flow pathways in \( \text{NO}_3^- - \text{N} \) rich soil layer i.e. during storm events, drains
formed in arable land act as source zones and major discharge generation zones (Musolff et al., 2015).

Table 5.6: Summary of seasonal mean NH$_4^+$-N, Cl$^-$ and DO concentrations of River Sondu

<table>
<thead>
<tr>
<th>Season</th>
<th>NH$_4^+$-N (mg/l)</th>
<th>Cl$^-$ (mg/l)</th>
<th>DO (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT (Dec)</td>
<td>0.07±0.007</td>
<td>1.88±0.35</td>
<td>7.63±0.04</td>
</tr>
<tr>
<td>CT (Mar)</td>
<td>0.14±0.01</td>
<td>2.28±0.65</td>
<td>6.50±0.14</td>
</tr>
<tr>
<td>CT (July)</td>
<td>0.18±0.011</td>
<td>2.66±0.02</td>
<td>6.98±0.05</td>
</tr>
<tr>
<td>CT (Sept)</td>
<td>0.18±0.063</td>
<td>1.83±0.22</td>
<td>7.50±0.38</td>
</tr>
<tr>
<td>Cluster 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TF (Dec)</td>
<td>0.12±0.02</td>
<td>1.38±0.24</td>
<td>7.93±0.26</td>
</tr>
<tr>
<td>TF (Mar)</td>
<td>0.14±0.02</td>
<td>2.16±0.34</td>
<td>6.70±0.82</td>
</tr>
<tr>
<td>TF (July)</td>
<td>0.10±0.007</td>
<td>1.16±0.01</td>
<td>7.22±0.25</td>
</tr>
<tr>
<td>TF (Sept)</td>
<td>0.13±0.025</td>
<td>1.38±0.21</td>
<td>7.61±0.23</td>
</tr>
<tr>
<td>Cluster 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U (July)</td>
<td>1.04±1.06</td>
<td>3.91±0.01</td>
<td>3.08±0.88</td>
</tr>
<tr>
<td>U (Sept)</td>
<td>0.77±0.94</td>
<td>3.30±1.29</td>
<td>3.86±1.22</td>
</tr>
<tr>
<td>CT*(July)</td>
<td>0.10</td>
<td>0.60</td>
<td>6.00</td>
</tr>
<tr>
<td>CT*(Sept)</td>
<td>0.11</td>
<td>2.02</td>
<td>6.40</td>
</tr>
<tr>
<td>Cluster 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MF (Dec)</td>
<td>0.17±0.06</td>
<td>2.97±0.95</td>
<td>7.59±0.58</td>
</tr>
<tr>
<td>MF (Mar)</td>
<td>0.17±0.03</td>
<td>3.99±0.46</td>
<td>4.81±0.34</td>
</tr>
<tr>
<td>MF (July)</td>
<td>0.11±0.02</td>
<td>2.03±0.01</td>
<td>7.33±0.31</td>
</tr>
<tr>
<td>MF (Sept)</td>
<td>0.13±0.024</td>
<td>2.41±0.96</td>
<td>7.61±0.23</td>
</tr>
</tbody>
</table>

* Land uses without Min and Max measurements had 1 monitoring point.

TF stands for tea estates with areas of considerable forest cover; CT stands for commercial tea zones; MF stands for small-scale mixed farming characterized by scattered land use; and U stands for streams passing through urban towns for December (Dec) 2016, March (Mar), July (Jul), September (Sep) 2017.

NO$_3^-$-N retention in soils is influenced by natural factors such as mineralogy, soil pH, soil organic carbon and hydrological properties due to soil age. Sondu is dominated by Nitisols with high kaolinite content which can also contribute to NO$_3^-$-N retention acting as a major discharge zone during runoff events (Jacobs, 2018). Spatial variations in concentrations are mostly influenced by land cover and land use. CT nitrate concentration was relatively high in Dec (1.39 mg N l$^{-1}$) and Sept (1.40 mg N l$^{-1}$) probably due to agricultural runoff from wet erosion. Although Dec is categorised as a dry month, unexpected rainfall events may occur especially in the upper reaches of the catchment where weather is modified by topography (higher elevations). Concentrations recorded in U are mostly from manure and domestic sewage (point and diffuse sources) evidenced by the isotopic signatures as illustrated in Fig 5-
3 (d and e) as well as higher Cl\textsuperscript{-} concentrations in the entire catchment (3.9 mg/l and 3.3 mg/l) during July and Sept (Table 5.6).

**Table 5.7** Summary of spatially and temporal NO\textsubscript{3}\textsuperscript{-}N concentrations, δ\textsuperscript{15}N-NO\textsubscript{3}\textsuperscript{-} and δ\textsuperscript{18}O-NO\textsubscript{3}\textsuperscript{-} values of R. Sondu

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Class</th>
<th>Mean ±SD</th>
<th>Min</th>
<th>Max</th>
<th>Mean ±SD</th>
<th>Min</th>
<th>Max</th>
<th>Mean ±SD</th>
<th>Min</th>
<th>Max</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>NO\textsubscript{3}\textsuperscript{-}N</td>
<td>mg/l</td>
<td></td>
<td></td>
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<td>2.02</td>
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<td>6.81</td>
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<td>0.97±0.77</td>
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<td>1.52</td>
<td>8.66±1.33</td>
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<td>9.60</td>
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<td>7.71</td>
<td>9.60</td>
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<tr>
<td></td>
<td>CT (Jul)</td>
<td>0.98±1.48</td>
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<td>8.11±2.16</td>
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<td>10.59</td>
<td>6.64±6.02</td>
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<td>13.81</td>
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<td>CT (Sept)</td>
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<td>1.93</td>
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<td>9.49</td>
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<td>7.66</td>
<td>5.95±3.11</td>
<td>0.58</td>
<td>10.14</td>
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<td>TF (Mar)</td>
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<td>0.71</td>
<td>7.12±1.31</td>
<td>5.26</td>
<td>8.65</td>
<td>4.95±1.46</td>
<td>2.90</td>
<td>6.40</td>
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<td>6.61±1.25</td>
<td>5.73</td>
<td>8.04</td>
<td>7.41±2.35</td>
<td>5.23</td>
<td>9.90</td>
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<td>TF (Sept)</td>
<td>1.39±0.34</td>
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<td>1.74</td>
<td>5.72±0.48</td>
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<td>CT (Sept)</td>
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<td>7.61</td>
<td>6.36</td>
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<td></td>
<td>U (July)</td>
<td>0.87±0.14</td>
<td>0.77</td>
<td>0.97</td>
<td>6.95±1.04</td>
<td>6.21</td>
<td>7.69</td>
<td>7.22±1.36</td>
<td>6.25</td>
<td>8.63</td>
</tr>
<tr>
<td></td>
<td>U (Sept)</td>
<td>1.17±0.23</td>
<td>1.00</td>
<td>1.06</td>
<td>8.41±0.85</td>
<td>7.80</td>
<td>9.01</td>
<td>7.40±0.92</td>
<td>6.75</td>
<td>8.05</td>
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<td>0.95</td>
<td>1.05</td>
<td>7.81±1.53</td>
<td>6.14</td>
<td>9.13</td>
<td>9.71±3.20</td>
<td>6.70</td>
<td>13.70</td>
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<tr>
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<td>MF (Mar)</td>
<td>0.90±0.13</td>
<td>0.76</td>
<td>1.04</td>
<td>9.10±0.39</td>
<td>8.67</td>
<td>9.60</td>
<td>6.32±1.41</td>
<td>5.14</td>
<td>8.30</td>
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<tr>
<td></td>
<td>MF (Jul)</td>
<td>0.72±0.04</td>
<td>0.68</td>
<td>0.76</td>
<td>8.55±0.84</td>
<td>7.94</td>
<td>9.51</td>
<td>7.41±1.79</td>
<td>6.06</td>
<td>9.44</td>
</tr>
<tr>
<td></td>
<td>MF (Sept)</td>
<td>1.17±0.35</td>
<td>1.03</td>
<td>1.74</td>
<td>7.05±0.60</td>
<td>6.65</td>
<td>8.45</td>
<td>9.79±1.88</td>
<td>8.45</td>
<td>11.12</td>
</tr>
</tbody>
</table>

* Land uses without Min and Max measurements had 1 monitoring point.

TF stands for tea estates with areas of considerable forest cover; CT stands for commercial tea zones; MF stands for small-scale mixed farming characterized by scattered land use; and U stands for streams passing through urban towns; CT* represents a commercial tea farming zone influenced by residential settlements for December (Dec) 2016, March (Mar), July (Jul), September (Sep) 2017.

However, during the dry season activated flow paths are reduced as the NO\textsubscript{3}\textsuperscript{-}N source zone is disconnected from the stream resulting in lower concentrations during dry seasons (Dupas et al., 2017; Jacobs, 2018). This was evident in the TF zones where the lowest concentrations were recorded during the drier months of December and March.

Manure and sewage often act as an anthropogenic source for Cl\textsuperscript{-} (Chen et al., 2009). Aside from direct discharge of raw and treated waste-waters into the river, the flow of pollutants within the urban centre is also influenced by impervious surfaces due to human-built environments. Human input was also present from unregulated activities such as washing of clothes and cars within the river. The nitrate-nitrogen concentrations were relatively moderate.
indicating possible sediment sinks, filtration, and denitrification function from existing wetlands.

Variation in nitrate in the mixed farming zones suggests influence from both rainfall and anthropogenic input. MF zones are categorised by land degradation from over farming and over grazing. Coupled with a higher sediment transport capacity index due to a widening river channel in the lower reaches where MF is located the eventual runoff input is high. Sediment transport capacity index increases the nutrient transportation in various forms (dissolved, particulate, organic and inorganic) attached to suspended solids and soil sediment. The sediments can be sources of legacy (long-term) nutrient input through dissolution under appropriate conditions (Vuai et al., 2012). Nitrate concentrations are also influenced by the residential settlements and animal husbandry which are sources for manure and sewage. During the month of March, the $^{15}$N and Cl$^{-}$ concentrations are high however the nitrate concentrations were relatively moderate (0.90 mg N$^{-1}$) which may signify possible denitrification.

NO$_3$-N concentration in Sondu catchment is therefore primarily influenced by agricultural activities (fertilizer input, manure, surface run-off from agricultural fields), domestic sewage and soil N.

5.3 Identification of nitrate sources through isotopic composition of nitrate in Stream Waters

A summary of the descriptive statistics of NO$_3^-$ stable isotope data based on land use classes is indicated in Table 5.7. The land use types as earlier described included: commercial tea farming (CT); tea and forest (TF); mixed farming (MF) and urban centres (U). The $^{15}$N and $^{18}$O values indicated no temporal variation (seasonality) (P > 0.05). However, $^{15}$N showed a significant difference with land use (P < 0.01). A post-hoc analysis showed a difference between TF and MF land uses. This is probably due to the distinctively different NO$_3^-$ sources where TF has lower values likely sourced from soil N while MF is sourced from manure and sewage.

The values of $\delta^{15}N$ – NO$_3^-$ and $\delta^{18}O$ – NO$_3^-$ ranged from 5.1 ‰ to 10.5 ‰ and -0.3 ‰ to 13.8 ‰ respectively (Table 5.7). The highest average $\delta^{15}N$ – NO$_3^-$ value was recorded in MF during March (9.1 ± 0.3 ‰) and $\delta^{18}O$ – NO$_3^-$ was recorded in CT land use during December 10.2 ± 2.0 ‰ (Table 5.7). The cluster TF exhibited lower $\delta^{18}O$ – NO$_3^-$ mean values (5.6 ‰, and 5.0‰) during the dry season (December and March). The measured mean values of
\[ \delta^{15}N - NO_3 \] fall within an overlap of soil N (0\% - 8\%) and manure and sewage sources (4\% - 25\%) as reported in the literature (Kendall et al., 2007; Xue et al., 2009; Sharp, 2017).

First, the identification of the predominant nitrate sources was performed using a classical dual isotope bi-plot approach of \[ \delta^{18}O - NO_3 \] vs. \[ \delta^{15}N - NO_3 \]. The source values were obtained from Xue et al., (2009) as illustrated in Fig 5-3 a, however to increase the resolution of the plot, the axes were reduced (Fig 5-3 b, c, d, e) to feature the source windows which fit the isotopic data represented in Table 5.7.

The isotopic signatures in December (Figure 5-3 b) represented three land uses, MF, TF, and CT. The main source contribution for CT and TF was soil N. The isotopic signature for the MF cluster was clearly sourced from manure and sewage. This is probable due to the high-density population and animal keeping practises of MF in the lower reaches of Sondu. December is a dry season typically not associated with seeding or fertilizer application.

The month of March (Figure 5-3 c) is categorised as a transitional season from dry to wet conditions. The predominant NO$_3^-$ source for MF was manure and sewage. The contribution is probable due to leaching from poor sanitation facilities (pit latrines) into the river channel. The isotopic signatures for TF were predominantly sourced from soil N and a few sites from manure and sewage. The low flows in March may have caused pronounced input from manure and sewage for TF and MF which is supported by increased Cl$^-$ and NH$_4^+$-N concentrations in March compared to Dec (Table 5.6). The NO$_3^-$ sources for CT fall into soil N and manure and sewage. The effect of denitrification from upstream wetlands for one of the CT sites may have caused an enrichment of \[ \delta^{15}N - NO_3^- \] values.

The isotopic signatures in the month of July (Figure 5-3 d) showed a high variability of isotopic signatures for each land use. MF sites had isotopic signatures from manure and sewage. The TF and U sites signature sources also fit into either manure and sewage or soil N source windows. CT* categorised in cluster 3 is a commercial tea zone which appears to be highly influenced by the residential settlements as its isotopic signatures were sourced from manure and sewage. CT in cluster 1 showed similar variations with isotopic signatures registered in soil N and some sites moving into the manure and sewage source window. The Cl$^-$ concentration for CT showed an increase from Mar (2.28 mg/l) to July (2.66 mg/l) indicating possible increased input from manure and sewage as (Table 5.6).
September is categorised as the end of wet season. MF and CT isotopic signatures fit into soil N source box (Fig. 5-3 c). This is probable due to increased surface runoff caused by wet erosion. Isotopic signatures for both TF and U were categorised as soil N and manure and sewage.

Encroachment of forest headwaters due to rapid population growth as well as pressure for arable land has led to deforestation and illegal logging increasing the loading from soil erosion and sediment deposited in the river (Masese et al., 2012). However, TF site categorised in the manure and sewage source may indicate heavier $\delta^{15}N - NO_3^-$ due to the effect of mixing as it was sampled at a confluence of tributaries draining different areas.

a)

b)

c)
The manure and sewage source input present in the U cluster is further indicated by the relatively higher NH$_4^+$-N (0.77 mg/l) and Cl$^-$ (3.30 mg/l) concentrations in September when compared to other land uses (Table 5.6).

The bi-plot approach characterized manure and soil N as the main sources of nitrate in Sondu catchment. However, this approach is limited and yields inconclusive information pertaining to the predominant NO$_3^-$ source (Xue et al., 2012). Therefore, a mixing model (mixSIAR) was applied to assess and quantify the contribution from the five sources (nitrogen in precipitation, ammonium and nitrate fertilizer, manure and sewage, soil N).

5.4 Denitrification

Denitrification is the dissimilarity reduction of NO$_3^-$ to gaseous forms N$_2$O, N$_2$ via NO$_2$. As NO$_3^-$ concentration decreases, denitrification causes an enrichment of $\delta^{15}N - NO_3$ and $\delta^{18}O - NO_3$ in remaining NO$_3^-$ pool (Wang et al., 2016). The $\delta^{15}N - NO_3$ and $\delta^{18}O - NO_3$ linear ratios are expected to increase from 1.3:1 to 2.1:1 respectively (Kendall et al., 2007). Some deviations from this ratio have been observed mostly due to temperature variability and substrate concentration (Voss et al., 2006; Xue et al., 2009). Denitrification is associated with large isotopic fractionation with $\delta^{15}N - NO_3$ enrichment factors ranging from - 40 to - 5 $^{0}/_{00}$ (Kendall et al., 2007).
In this study the observed linear relationship between the δ\(^{15}\)N and δ\(^{18}\)O values (Fig 5.4) for the different seasons were as follows: (Dec: δ\(^{18}\)O = -0.3δ\(^{15}\)N + 11.1; Mar: δ\(^{18}\)O = 0.6δ\(^{15}\)N + 1.2; July: δ\(^{18}\)O = -1.3δ\(^{15}\)N + 17.6; Sept: δ\(^{18}\)O = -1.99δ\(^{15}\)N + 22.1). The isotopic values of δ\(^{15}\)N – NO\(_3\) for December, July and September increased as δ\(^{18}\)O – NO\(_3\) values decreased (Fig. 5.4). The gradient obtained for September (-1.99) indicated a linear ratio of approximately 2:1 for δ\(^{15}\)N – NO\(_3\) to δ\(^{18}\)O – NO\(_3\) signifying possible denitrification in September. However, DO levels in Sept (Table 5.6) were above the optimal conditions for denitrification (<3.1 mg/l) (Xue et al., 2012).

Figure 5-4 Plot of δ\(^{18}\)O–NO\(_3\) vs δ\(^{15}\)N–NO\(_3\) for seasons: Dec 2016, March 2017, July 2017 and September 2017

Although, denitrification within the river watershed as a whole was improbable, denitrification is present in anaerobic microsites within an aerobic system e.g. within river sediments and riparian zones (Koba et al., 1997). The rate of sediment denitrification is dependent on conditions such as substrate composition, concentration, temperature, and microbial availability of the electron donor (nitrate), temperature, and type of bacteria involved in the process and their growth conditions (presence of carbon). According to Garcia-Ruiz et al., (1998) denitrification is further influenced by the physical and chemical characteristics of both water and sediment. Most often industrial and domestic waste released into rivers constitutes organic matter presenting a carbon source for microbial metabolic utilization. In anoxic conditions, microbial communities utilize nitrate as an electron donor causing reduced concentrations of NO\(_3\). Therefore, in optimum anoxic conditions denitrification is possible.
In this study, anoxic conditions were created to investigate the denitrification potential for selected river sediments. The measured carbon content for the river sediments ranged from a 0.56% C to 24.66% C as indicated in (Table 5.8). To increase the rate of denitrification, additional NO$_3^-$ was introduced to provide sufficient substrate for denitrification. The denitrification rate ranged from 0.2 mg l$^{-1}$ hr$^{-1}$ to 2.1 mg l$^{-1}$ hr$^{-1}$ (Table 5.8).

**Table 5.8 Kinetic fractionation (denitrification) of Sondu R. sediments.**

<table>
<thead>
<tr>
<th>Sediment code</th>
<th>% C in sediment</th>
<th>$\delta^{15}$N</th>
<th>Incubation time (Hours)</th>
<th>NO$_3^-$-N mg N/l</th>
<th>Denitrification rate mg N/l/hr</th>
<th>Nitrate removal (%)</th>
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</thead>
<tbody>
<tr>
<td>SD 1</td>
<td>0.56</td>
<td>7.17</td>
<td>0</td>
<td>78</td>
<td>2.13</td>
<td>98</td>
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<td></td>
<td></td>
<td>24</td>
<td>2.6</td>
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<td></td>
<td></td>
<td>36</td>
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<td></td>
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<tr>
<td>SD 2</td>
<td>9.99</td>
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<td>0.56</td>
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<td>64</td>
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<td>51</td>
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<td>SD 3</td>
<td>24.66</td>
<td>4.55</td>
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<td>21.50</td>
<td>0.20</td>
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<td>8</td>
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<td>24</td>
<td>14.49</td>
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<td></td>
<td></td>
<td>36</td>
<td>13.68</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>48</td>
<td>11.77</td>
<td></td>
<td></td>
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<tr>
<td>SD 4</td>
<td>1.06</td>
<td>4.73</td>
<td>0</td>
<td>23.01</td>
<td>0.25</td>
<td>53</td>
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<td></td>
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<td>8</td>
<td>21.37</td>
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<td>24</td>
<td>12.45</td>
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<td>36</td>
<td>11.67</td>
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<td></td>
<td></td>
<td></td>
<td>48</td>
<td>10.68</td>
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</table>

The decrease of concentration as a function of time is indicated in Fig (5-5 a). To observe the effect of higher nitrate levels, SD 1 and SD 2 were treated with higher concentrations of NO$_3^-$ (Fig 5-5 a) which resulted in higher denitrification rates (2.1 mg l$^{-1}$ hr$^{-1}$ and 0.6 mg l$^{-1}$ hr$^{-1}$) as indicated in Table (5.8). Although SD 2 and SD 3 had higher carbon content (9.99% and 24.66%), the denitrification rates were moderate to low which implied that the carbon existed in an unavailable form for microbial metabolism utilization. The enrichment factors were derived from the slope of $^{15}$N against the natural log of remaining fraction of NO$_3$-N as shown in Fig. (5-5 b),ε factors obtained included -8.9 $^{\circ}/_{00}$ and -19.2 $^{\circ}/_{00}$ for SD 1 and SD 2 respectively (Fig 5-5 b) accounting for 34% to 98% of nitrate removal (Table 5.8). The $\delta^{15}N$ = NO$_3$
enrichment factor value for SD 2 is within the ranges derived from experiments -29.4°/00 to -17.0°/00 (Blackmer & Bremner, 1976; Sebilo et al., 2002). Soil texture and fineness increases downstream as was the case for SD 2. According to Garcia-Ruiz et al., (1998) finer sediments may indicate higher denitrification rates due to increasing sites for anoxic environments and microbial attachment.

a)

![Graph showing NO3-N concentration vs time for SD 1 and SD 2. The equation for SD 1 is y = -9.5024x - 4.5081 with R² = 0.984. The equation for SD 2 is y = -19.165x + 5.6217 with R² = 0.9826.]

b)

![Graph showing 15N-NO3 concentration vs Ln NO3-N. The equation for SD 1 is y = -9.5024x - 4.5081 with R² = 0.984. The equation for SD 2 is y = -19.165x + 5.6217 with R² = 0.9826.]

**Figure 5-5:** Determination of denitrification rate and enrichment factors for selected sites SD 1 (downstream Sondu hydroelectric power dam) and SD 2 (wetland) a) NO3- concentration (mg/l) vs time (hours) b) enrichment factors determined from the slope of δ15N-NO3 vs natural logarithm values (ln)for fraction of remaining NO3-N.
This study establishes the potential for denitrification is directly proportional to availability of nitrate and available carbon with higher nitrate levels accounting for higher denitrification rates. However, the nitrate concentrations in the catchment are lower (0.3–4.0 mg N l\(^{-1}\)) (Table 5.7) than those presented for denitrification measurements (21.5-78 mg N l\(^{-1}\)). Therefore, measurement of in situ- denitrification may offer more conclusive insight to the extent of denitrification in the catchment. For this reason, denitrification was considered absent and not applied to the mixSIAR mixing model in section 5.5.

5.5 Probability distribution of NO\(_3^−\) Source apportionment

A mixing model, mixSIAR, was used to approximate proportional contributions of five potential NO\(_3^−\) sources including NO\(_3^−\) in precipitation (NP); NO\(_3^−\) in fertilizer (NF); Soil Nitrogen; NH\(_4^+\) fertilizer and rain (NF&R); manure and sewage (M&S) for two seasons; dry (December and March) and wet season (July and September) in four land use types (CT, MF, TF and U) based on δ\(^{15}\)N-NO\(_3^−\) and δ\(^{18}\)O–NO\(_3^−\) values.

As previously stated in section 5.4, no obvious denitrification was determined during the sampling period. Furthermore, the DO concentrations as shown in Table 5.6 were above the recommended levels for denitrification (< 3.1 mg/l). Therefore, denitrification was assumed to be absent.

The proportional contribution from NP, NF, M&S, Soil N showed no significant variation with land use (P > 0.05). However, source NF&R showed variation (P < 0.05) within the different clusters. A post hoc analysis for pairwise comparisons revealed NF&R contribution varied between tea farming zones TF and mixed farming zones MF.

The proportional contributions are illustrated in Fig 5.6. The mean probability estimate (MPE) source contribution in the CT zones showed variability with seasons. In the dry season, the highest contribution was from M&S (25.2%) followed by soil N (23.0%), NF (19.4%), NF&R (18.8%) and NP (13.8%) (Fig 5-6 a). During the wet season, soil N contribution increased to 24.5% while the manure and fertilizer contribution decreased to 23.98%. Contributions from NF&R increased to 19.3% while NR MPE contribution decreased to 18.6% (Fig 5-6 b). Lower manure and sewage input during the wet season is due to a dilution effect from precipitation. Increased contribution from soil N is likely due to surface runoff due to precipitation.

The order of proportional contributions for MF zone (M&S > soil N) did not vary with season. The highest contribution for both dry (Fig 5-6 c) and wet (Fig 5-6 d) seasons was M&S (25.4%
and 26.3%) followed by soil N (24.1% and 24.6%). NF&R indicated an increase in contribution from 17.9% to 21.2%. Contribution from NF and NP decreased from 18.5% to 15.7% and 14.0% to 13.0% respectively.
Figure 5-6 NO$_3^-$ source contribution from five sources (nitrate in precipitation, manure and sewage, ammonium fertilizer and rain, nitrate fertilizer and soil Nitrogen) for different land uses in dry (Dec and March) and wet season (July and September) a) commercial tea (CT) farming in dry season b) CT in the wet season c) TF dry season d) TF wet season e) MF dry season f) MF wet season g) U wet season. The boxplots indicate the lower, mean and upper quartiles. The whiskers indicate the 10$^{\text{th}}$ and 90$^{\text{th}}$ percentiles.
TF source contributions in the dry season varied as follows: M&S, 23.7%; soil N, 22.7%; NF&R, 21.8%; NF, 18.8%; NP, 12.9% (Fig 5-6 e). An increase in contribution of soil N (24.3%) was observed in the wet season (Fig 5-6 f). M&S contribution reduced to 21.4%, while MPE for NF and NF&R increased to (20.1%) and (20.8%). The contribution from M&S is low compared to other land uses which is likely due to less anthropogenic interference.

The urban sites were sampled in the wet season indicating high MPE from M&S at 24.1%; soil N, 22.3%; NF&R 21.3; NR 18.7%, and NP 13.6% (Fig 5-6 g). The high contribution from manure is probable due to discharge of sewage into the river channel. Banks of streams flowing through the urban centres were also highly eroded increasing the contribution from soil N. Urban surface run off varies with precipitation and proportion of impermeable surface areas. Poor land management practises along river banks and the lack of vegetation cover lead to erosion which increases soil N contribution.

The main sources of nitrate within the catchment include soil N and manure and sewage. According to Xue et al., (2012) mixSIAR output is advantageous as it deduces additional potential NO₃⁻ sources which could not be detected in the bi-plots (Fig 5-3) evident from the contributions from NF&R, NP and NF shown in Fig. 5-6. However, uncertainties arising from the model are due to the use of end member isotopic signatures from other studies rather than those measured from the study.
6 CONCLUSIONS AND FUTURE RESEARCH

6.1 Major research findings

6.1.1 Multivariate statistical techniques for NO$_3^-$ pollution assessment

By using multivariate techniques and multiple data sources, we determined water quality parameters of Sondu River Basin are dependent on water-rock interactions and anthropogenic activities. Correlation matrix indicated the major cations ($Na^+$, $K^+$, $Mg^{2+}$ and $Ca^{2+}$) and anions ($F^-$, $SO_4^{2-}$) were derived from a common geogenic source. Further investigation of the sources of correlation between $Cl^-$ and $NO_3^-$ indicated both anions were traced to an anthropogenic source, most commonly discharge of manure and sewage.

Through the hierarchical cluster analysis, we suggest four main clusters describing the water quality of Sondu. Cluster 1 corresponded to moderate concentrations for hydro-chemical variables while recording high nitrate concentrations; the major land use of each of the sites was commercial farming categorised by use of fertilizers. Cluster 2 indicated sites in tea and forest zones recording relatively lower concentrations of physicochemical parameters. Cluster 3 and 4 had moderate concentrations for both nitrate and hydro-chemical variables, influenced by urban centres and mixed farming respectively. A Principal component analysis was performed on each of the clusters to establish the main variables influencing the water quality parameters. The main causes of variance in water quality parameters of for cluster 1 and 2 was indicated by mainly geogenic source while a smaller variance component was explained by anthropogenic sources. Cluster 3 was mainly influenced by anthropogenic activities from the urban settlement whereas Cluster 4 indicated partial influence from both anthropogenic and geogenic sources exhibiting the influence of mixed farming and residential settlements within Sondu catchment.

Evidently, NO$_3^-$ is sourced from anthropogenic sources from agricultural (surface runoff, fertilizer, manure) and human activities (domestic sewage and industrial waste).

6.1.2 Source identification for NO$_3^-$ sources using dual isotope approach ($\delta^{15}$N-NO$_3^-$ and $\delta^{18}$O-NO$_3^-$)

As with most global surface waters, nitrate pollution remains a challenge within the Lake Victoria Basin, Africa’s largest freshwater body. Initiatives to alleviate the eutrophic status while effectively managing nitrate have been implemented. However, pollution persists as nitrate concentrations continue to rise. In order to support nitrate level monitoring, NO$_3^-$ source identification was carried out using dual isotope approach. Five main sources were considered
including, atmospheric deposition, nitrate fertilizer, ammonium fertilizer, soil N, manure, and sewage. Each source has distinct isotopic $\delta^{15}$N-NO$_3^-$ and $\delta^{18}$O-NO$_3^-$ signatures. The samples isotopic signatures were determined using “bacterial denitrification method”. Using a classical bi-plot approach, the sample values were plotted, and each site assigned to a specific source window. The main sources of nitrate in Sondu catchment include soil N, manure, and sewage.

During the dry season of Dec and Mar, clusters 1, 2 and 4 were mostly sourced from manure and sewage whereas in the wet season cluster 2 and cluster 1 was derived from soil N. Precipitation tends to dilute manure and sewage while increasing surface runoff and sediment concentration due to water erosion. Cluster 3 and 4 indicated a manure and sewage source. Sites from both clusters are located in the lower reaches of the river where population density is high. This is probable due to the poor sanitation facilities, leaching from solid waste disposal sites, leaking sewage pipes and domestic wastewater disposal discharge channelled into the river.

Incorporating an integrated approach using spatial and temporal variations of hydrochemistry and isotopic signatures is a useful tool in identifying surface water nitrate sources in catchments affected by constant land use change.

6.1.3 Isotopic fractionation for selected riparian and downstream site.

The denitrification potential of two selected river sediments was determined using an incubation lab set-up. Both sites indicated denitrification, with higher nitrate removal in the downstream site (Sondu hydro power dam) 98% at a denitrification rate of 2.1 mg N l$^{-1}$ hr$^{-1}$ compared to the wetland site 34% at a rate of 0.6 mg N l$^{-1}$ hr$^{-1}$. Soil texture rather than carbon content appeared to be the controlling factor for denitrification. The downstream site had a lower carbon content and finer sediment.

From this study, we show that nitrate sinks are present within the river system especially in anaerobic microsites present within the aerobic river system.

6.1.4 Bayesian isotope mixing model (mixSIAR) for estimation of NO$_3^-$ source apportionment and quantification

From the study, mixSIAR model output showed the temporal and spatial variability in NO$_3^-$ sources. The mean proportional estimates indicated highest contribution from ‘manure and sewage’ and ‘soil N’. In the dry season, cluster 1(CT) and cluster 2(TF) exhibited higher mean concentrations from manure and sewage and lowest contribution from atmospheric deposition.
During the wet season, the highest contribution for CT and TF zones was ‘soil N’. Cluster 3 and 4 showed higher contributions from manure and sewage in the wet season. Additional sources and water samples should be employed to eradicate uncertainty of the potential NO$_3^-$ sources.

6.2 Future perspectives and recommendations
The findings from this study provide insight into the nitrate pollution within Sondu catchment as well as Lake Victoria Basin. However, several limitations were observed and highlighted, and further improvements are necessary to increase knowledge in this field.

In order to determine spatial and temporal trends for nitrate concentrations an increase of monitoring stations employed over longer periods factoring both groundwater and surface water resources need to be implemented to understand the major processes controlling human sourced nitrate in Sondu basin.

Multiple tracer approach $\delta^{15}$N-NO$_3^-$, $\delta^{18}$O-NO$_3^-$ and $\delta^{11}$B$^-$ would be a useful tool in determining the fate of nitrate in surface water. Signatures $\delta^{15}$N, $\delta^{18}$O are used to distinguish mineral from organic fertilizers while $\delta^{11}$B$^-$ discriminates manure from sewage and mineral fertilizers. The repeated application of fertilizers in land uses such as commercial tea may have an effect on nitrate legacy pollution. This results in observations of continued nitrate pollution despite implemented initiative aimed at reducing the concentrations of nitrate. Tracing the fate of $^{15}$N labelled fertilizers over a long-time scale will also provide insight into legacy pollution present within the catchment.
REFERENCES


