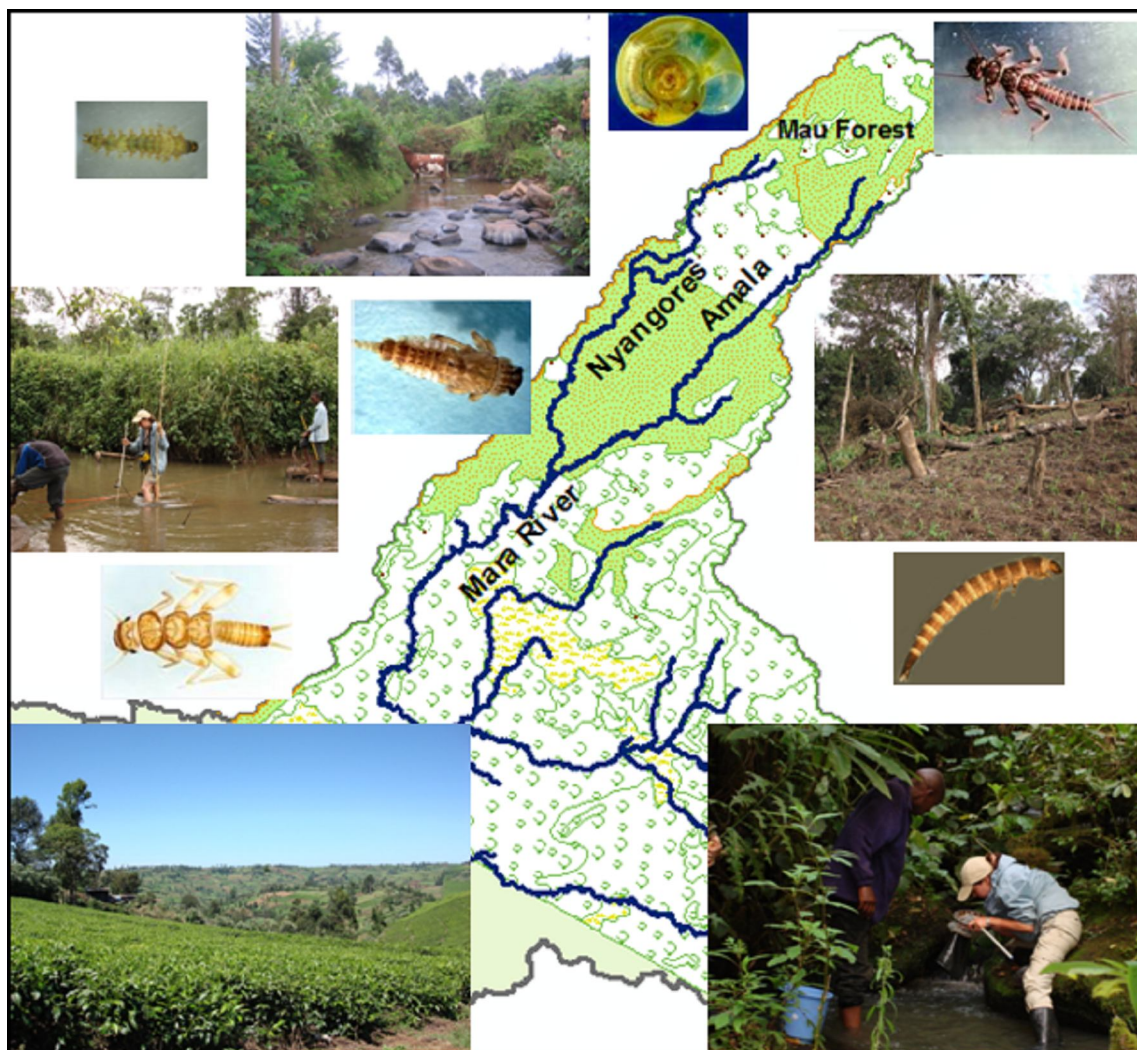


# UNESCO-IHE

## INSTITUTE FOR WATER EDUCATION



**Land use influence on the benthic macroinvertebrate communities of streams in Nyangores and Amala tributaries of Mara River, Kenya.**

**Veronica G. Minaya Maldonado**

**Msc Thesis (ES 10.32)**

**April 2010**

**UNESCO-IHE**  
Institute for Water Education





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Master of Science Thesis

by

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**Delft**

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The findings, interpretations and conclusions expressed in this study do neither necessarily reflect the views of the UNESCO-IHE Institute for Water Education, nor of the individual members of the MSc committee, nor of their respective employers.

## **Dedication**

This work is dedicated to them, who believed in me and were always with me in one way or another, especially..

to Dad, thanks "papito" for keeping me motivated and encouraged to reach my dreams.

To Vicky, Roberto, Alejo for their support and love

## **Abstract:**

Macro invertebrates have remained a key indicator of changes in the physical and chemical conditions of aquatic ecosystems. Due to the increase of human population and changes in land use, the Mara Basin has been under constant pressure which has most likely affected the aquatic ecosystems within the basin. This study was conducted in the upper catchment of the Mara River Basin to test the impacts of the current land use changes on the abiotic conditions and macroinvertebrate community composition. Invertebrate samples were collected in headwater streams with distinct land use types namely forest, agriculture and mixed. A total of 9006 individuals within 75 taxa belonging to 13 orders were identified from the 25 sampling sites. The most dominant orders were: Ephemeroptera 41.28%, Diptera 30.83% and Annelida 17.21%. The results of this study demonstrate that the term "Land use" and its classification into Agriculture, Forest and Mixed were a general and rough way to classify the sampling sites of the Mara streams and predict the effect on local benthic macroinvertebrate community. Although some trends describing the physical chemical conditions were recorded, sites clustered strongly with substrate type, river size and the level of anthropogenic disturbance observed at site. These variables can therefore be used as good indicators of stream degradation as they seemed to reflect the products of the change in land use and macroinvertebrate species composition varied with these in-stream characteristics

**Keywords:** Bioindicators, benthic macroinvertebrates, species composition, rapid field screening, SASS5, land use, river quality, Mara River.

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## List of Abbreviations

GLOWS	Global Water for Sustainability Program
WWF	World Wild Foundation
KNBS	Kenya National Bureau of Statistics
MEFA	Mara Environmental Flow Assessment
EFA	Environmental Flow Assessment
APHA	American Public Health Association
HKH	Hindu Kush-Himalayan
GF/F	Glass Fibre Filters
YSI	Yellow Spring Instruments
SASS_5	South African Score System (Version 5)
SASS_4	South African Score System (Version 4)
SASS (6-15)	South African Score System (sensitive taxa)
SASS (8-15)	South African Score System (most sensitive taxa)
ASPT	Average Score per Taxon
EPT	Ephemeroptera, Plecoptera and Trichoptera
POET	Plecoptera, Odonata, Ephemeroptera and Trichoptera
COPTe	Coleoptera, Odonata, Plecoptera, Trichoptera and Ephemeroptera
Eve	Evenness
FFG	Functional Feeding Groups
WQC	Water Quality Classes
DO	Dissolved Oxygen
NH <sub>4</sub> -N	Ammonia Nitrogen
PO <sub>4</sub>	Orthophosphate
TSS	Total Suspended Solids
BOD	Biological Oxygen Demand
CPOM	Coarse Particulate Organic Matter
ANOVA	Analysis of Variance
ANCOVA	Analysis of Covariance
NMS	Non-Metric Multidimensional Scaling

# 1 Introduction

## 1.1 Background

The Mara River is one of the most important freshwater ecosystems for Kenya and Tanzania. The river has a catchment area of 13504 km<sup>2</sup>, with 65% in Kenya and 35% in Tanzania. With the main source of this trans – boundary river being the Mau Forest, it flows through diverse landscapes with a total length of 395 km discharging into Lake Victoria through Tanzania (Figure 1.1).

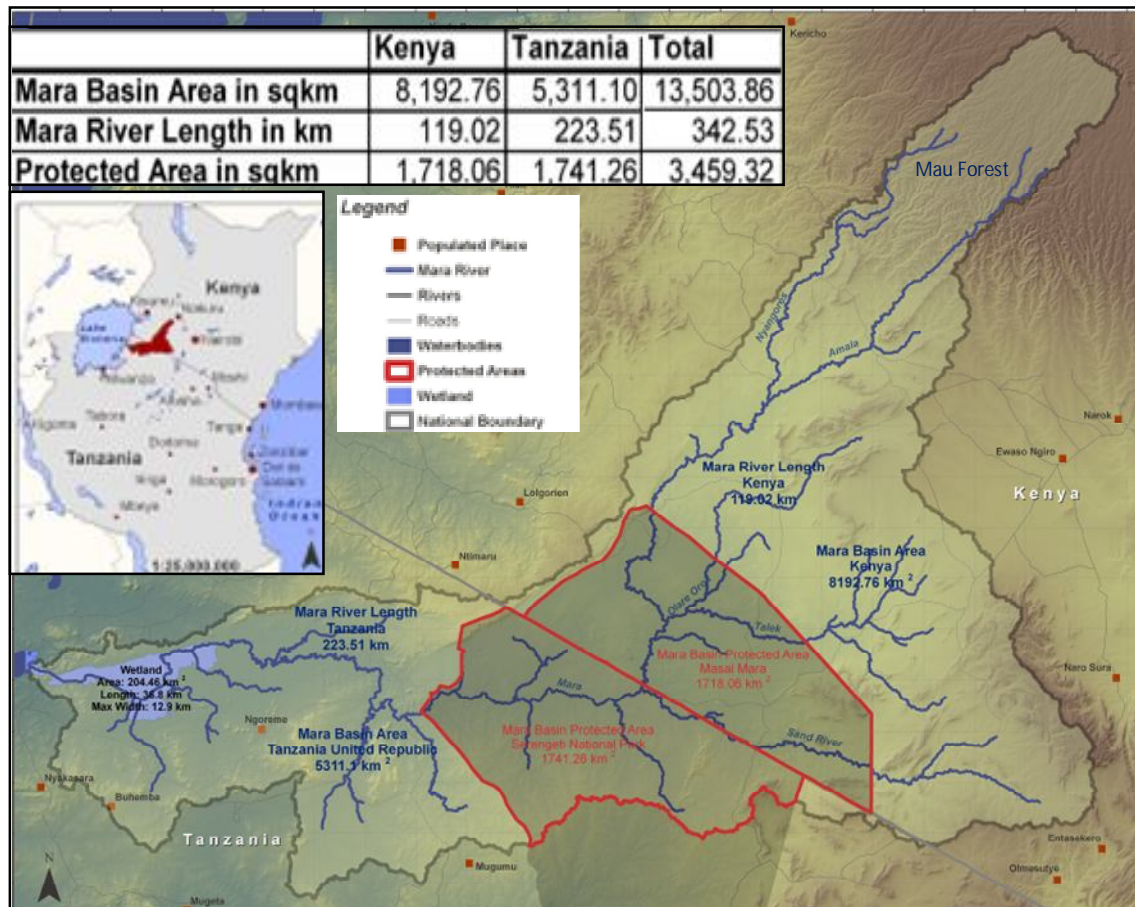


Figure 1.1 Mara River Basin (Source: Courtesy of GLOWS)

The climatic conditions within the basin vary with altitude. At 2915 m a.s.l in the Mau Forest the precipitation in the upper basin has an annual mean of 1400 mm/year and decreases to 600 mm/year at lower altitudes 1140 m a.s.l before discharging into Lake Victoria (Mutie, 2005). Hydrometeorological studies have shown that annual precipitation levels have not changed significantly over time, but there is variation within the seasonal and monthly distribution (Melesse et al., 2008). Temperature is also variable and depends on altitude, but with an average of around 25°C.

The main permanent tributaries in the upper basin are the Amala and Nyangores that originate in the Mau Forest. The optimum conditions in the highlands make livestock farming feasible and different types of agricultural activities and crops such as tea, coffee and maize (Mutie, 2005).

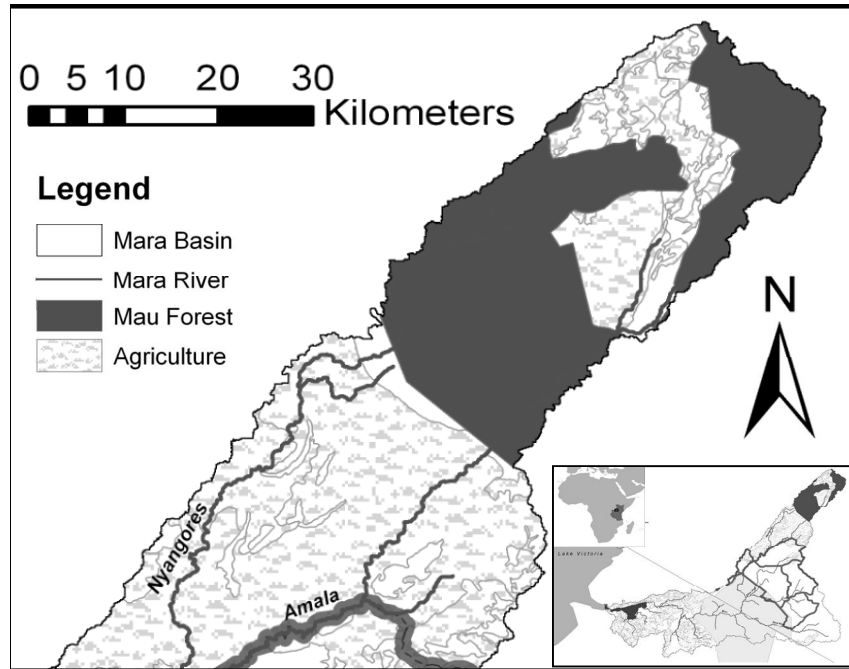
The Mara River flows through the Masai Mara National Reserve and the Serengeti National Park; these parks hold an exceptional diversity of animals and represent the stage of the world-famous Serengeti-Mara Ecosystem and with it the migration of wild beasts from July to October. The importance of this ecosystem in the sustainability of the tourism industry and support of local livelihoods has made it an important resource. With the increase of human population at an annual rate of 7% within the basin and the 55% increase in agricultural land in the last 14 years, the Mara Basin has been under constant pressure (Mati et al., 2005). According to different studies, these anthropogenic activities have led to a continuous trend of degradation and faster loss of vegetation, especially in the upper catchments, and consequently the reduction in the quantity and probably quality of the river within the Mara River and its tributaries (Mati et al., 2005, Mutie et al., 2006, Mati et al., 2008, Dali, 2007). As a result, there is a tremendous need for thorough evaluation of the current ecological, biophysical and hydrological status of the river ecosystem and development of proper protocols for its management.

Despite the fact that local regulations for Environmental flows exist (The Kenya Water Act, 2002), the monitoring and controlling of water resources has not been thoroughly implemented. The different activities such as deforestation, water abstraction of livestock, and agricultural irrigation among others are heavily affecting the ecosystem (Mati et al., 2008; Raburu et al., 2009). In the map of land use coverage (Figure 1.2) it could be noticed clearly that within the Mau Forest in the upper basin an area equivalent to a third of the Forest has been converted to agricultural land and therefore contributing to the degradation of the ecosystem upstream. Downstream the situation is not very different, and even though the existence of the protected areas in the middle basin prevent or minimize human impacts, the impacts from upstream continue downstream (Mara EFA Report, 2008). But the lack of sufficient data has led to an insufficient understanding of the ecosystem functions and therefore poor or inappropriate environmental management has been carried out.

Nevertheless, a group of scientists from Kenya, Tanzania, Netherlands and USA have been trying to determine the environmental flow that must be kept in order to maintain the river quantity and quality for sustainable development. The study used a science-based approach, the Building Block Methodology (BBM), which is one of the most holistic approaches to determining environmental flows (King et al., 2000). The assessment was carried out with a number of multi-disciplinary specialists that recommended different flow levels according to each component of the river ecosystem. The results generated showed that the acceptable flow cannot be reached in the upper and mid reaches within the Mara River Basin during drought years, possibly due to the alterations in the flow regime (Mara EFA Report, 2008).

Based on the Environmental Flow Assessment (EFA) in the Mara River Basin and from the critical indicators used to monitor the ecosystem's health, invertebrates were identified as one of the most sensitive indicator in responding to river quality levels. The results from the study regarding the macroinvertebrate component showed that the benthic compositions from the upper reaches to lower reaches were highly dependent on the increasing degradation due to anthropogenic activities

(Raburu et al., Kibichii et al., 2008). Macroinvertebrates are important indicator species based on their sensitivity to environmental changes and as such are good indicators of an ecosystem's health (Karr & Chu, 2000). They also play an important role in the food chain and nutrient cycling and provide useful information about ecosystem properties and diversity (river quality and trophic status).



**Figure 1.2 Land use coverage in the Upper basin in the Mara River Basin**  
**Source: adopted from FAO Africover Project**

This study seeks to determine the influence of catchment modification and land use on macroinvertebrate community composition in the upper catchment inside and outside the Mau Forest, Mara River Basin

The benthic macroinvertebrate community was sampled at several reaches, and its composition was related to the three land uses dominant in the catchment, as well as other physical and chemical characteristics.

## 1.2 Problem definition

The aim of this study is to identify critical macroinvertebrate indicators within the Mara Basin that could be used in future monitoring, and determination if the water quantity and quality is sufficient to maintain desired ecological processes

### 1.2.1 Hypothesis

The existing land use change has an effect on the stream habitat characteristics

Macroinvertebrates abundance, diversity and composition vary as a function of in-stream and habitat characteristics.

### **1.3 Research questions**

*Has the changes in land use in the upper reaches of the Mara River affected the composition and abundance of in-stream macroinvertebrate communities?*

- What is the variation in abundance and species composition of the benthic community in the different land use types?
- Does the composition and diversity of benthic macro-invertebrates reflect the substrates types?
- What are the existing macro-invertebrates functional feeding groups (FFGs) and how are these spatially distributed in the different land use in the upper basin of the Mara River?
- Is it possible to describe the effects of land use on the benthic community by applying the rapid bio-assessment approach (Moog et al., 1999) and the South African Score System methodology (Dickens & Graham, 2002)?

### **1.4 Scope and limitations**

This study covered the effects of three main land use types, i.e. forest cover, agriculture and mixed cover (forest plus agriculture) in the Amala and Nyangores tributaries of the upper Mara basin. The area is located inside and outside the Mau Forest. The different land cover types and the physical – chemical parameters as indicated by the screening protocol and the geomorphological features were compared with benthic macroinvertebrate species composition as the principal component of the bioassessment approach.

Despite the fact that the macroinvertebrates composition is a good bioindicator for river quality assessment, there are some limitations that must be taken into account prior to data analysis (e.g. seasonal variations and lower taxonomical resolution in identification)



## 2 Literature review

### 2.1 Benthic macroinvertebrates as bioindicators

Bioindicators are defined as living organisms which are sensitive to environmental disturbances and stressors and provide a response that can explain ecological processes (Alam, 2008). They have been used since the beginning of the last century as an important bio – monitoring tool to evaluate river quality and levels of organic waste in rivers. This methodology was developed by Kolkwitz & Marzon in 1908 (Ziglio, 2006).

Biodiversity has been used as a tool to assess and predict some complex interactions between human landscape management and its implications, with some organisms responding faster and more definitively than others (Paoletti, 1999). Undoubtedly, benthic macroinvertebrates are among the most used bioindicators due to their sensitivity to environmental changes (Resh, 1995; Barbour et al., 1999) and in providing scientifically defensible evidence of environmental status (Plafkin et al., 1989; Klemm et al., 2003). However, bioassessments using well-established methodologies are still in a developing stage for some African countries. For the purpose of this study the South African Scoring System (SASS5), which is in its fifth edition, was used (Dickens & Graham, 2002).

Macroinvertebrate community abundance and diversity can be used to assess ecological changes and impacts that might occur due to the change in land use. It recommends the stream protection by maintaining and if it is possible minimizing the urban and agricultural land cover in the catchment (Roy et al., 2001). Several studies have recognized the significant correlation between land use and macroinvertebrate communities, showing the total number of taxa and the percentage of groups like Ephemeroptera, Plecoptera and Trichoptera (EPT) decreasing and Oligochaeta and Diptera increasing as the pollution and changes in the river quality increases (Barbour et al. 1999).

The functional feeding guild is one of the most important behavioural traits that macroinvertebrates have and it is expressed as percentages of single feeding types. The functional feeding groups are shredders, grazers, active filter-feeders, passive filter-feeders, detritus feeders, miners, xylophagous, predators, parasites and others (Ofenbock et al., 2004; Moog, 2002). According with Cummins & Klug (1979) and Barbour et al. (1996) shredders are more sensitive as compared with active and passive filter-feeders which are more tolerant and capable to expand their food variety. Depending on the type of stress, different functional feeding groups can increase or decrease their proportion. For example; the percentage of shredders has a consistent response of declining to organic pollution and RETI index, which is a combination of % shredders and grazers, which responds positively to channel alteration and straightening by increasing their proportion of these feeding types (Ofenbock, 2004).

The distribution and composition of macroinvertebrate taxa is related to the capacity to tolerate the environmental disturbances and stress usually linked to the change of land use (Rios and Bailey, 2006). In many studies the expansion of agricultural land is related with modifications in the macrohabitats, and therefore alteration in the macroinvertebrates community composition (Corkum, 1990 and Quinn and Hickey, 1990). The reduce of allochthonous input, periphyton development, greater sediment input and increasing water temperature are some of the consequences of the agricultural land growth, in order to reduce the impact according with Rios and

Bailey (2006) the riparian vegetation near the streams seem to help increasing EPT taxa, total richness and diversity as a whole.

Freshwater ecosystems are very sensitive to environmental changes due to human activities as compared with terrestrial ecosystems (Sala et al., 2000; Dudgeon et al., 2005; Revenga et al., 2005). This sensitivity makes benthic macroinvertebrates a relevant case study to evaluate the ecosystem's health (Karr & Chu, 2000).

## **2.2 Factors influencing the distribution of macroinvertebrates**

Invertebrate communities can differ upstream and downstream in the same stream due to longitudinal gradients in the physical environment imposed by hydrology. Also, changes in the river quality influenced by land use including intensive agriculture and urbanization, and the allochthonous and autochthonous organic matter input by riparian vegetation (Vannote et al., 1980).

There are many ecological factors that influence the distribution of benthic macroinvertebrates. These factors include substrate types, water velocity, discharge, riparian vegetation, altitude, latitude and land use as a proxy for process level effects (physical – chemical features and the level of pollution in the ecosystem) (Giller & Malmqvist, 1998).

The substrate types play an important role as a main feature of micro habitats where different benthic macroinvertebrates reside. A study done by Iwata et al. (2003) in a tropical rainforest found a strong correlation between deforestation and substrate type that affect and influence the abundance, diversity and composition of benthic macroinvertebrates

There is ample range of different substrate types, which are usually very heterogeneous, although in low-gradient rivers a uniform substrate also can be found (Allan, 1995). According to Allan (1995) diversity and abundance of benthic macroinvertebrates increased with median particle size but also a broad substrate type leads to a larger abundance of benthic invertebrates (Resh et al., 1995).

In addition to substrate type the water velocity determines the particle size, composition and stability (Allan, 1995). The current of water as a force has a high influence on the ecological distribution as well as the invertebrate behavioural and morphological attributes (Allan, 1995). Velocity as a parameter depends on the flow and area of the cross section and varies throughout the river. The mean water velocity in the Amala River for 2007 fluctuated between 0.30 and 0.77 m/s (Mara EFA Report, 2008).

Within the broad features of landscape, land use is a critical characteristic because it leads to erosion and thus geomorphologic change in streams and with it a possible greater chemical contamination and increase in turbidity. Many studies reveal that in tropical Africa the clearing of forested zones and the transformation from forest to cultivation areas give rise to the increase levels of temperature, conductivity, total suspended and dissolved solids, turbidity (Kibichii et al., 2007, Kasangaki et al., 2008; Ndaruga et al., 2004) and with the presence of livestock in these areas leads to the increase of ammonia and nitrite in rivers (Kibichii et al, 2007). Some studies reveal that the over-use by animals and their watering in streams add up to anthropogenic activities in- and near-stream like bathing, laundry washing affect the stream habitat and biotic features (Mathooko, 2001; Malmqvist & Rundle, 2002)

It is important to put into consideration the runoff from land uses in conjunction with other anthropogenic activities that might influence the water condition of rivers and therefore the distribution and abundance of the benthic community (Giller & Malmqvist, 1998; Weigel et al., 2002; Malmqvist & Rundle, 2002). It is important to highlight that, in order to comprehend the organization of benthic communities; one must examine factors existing at multiple spatial scales. According with Heino et al. (2003) the regional and local species richness showed a strong positive relationship with each other. However, it is not clear enough that regional features set the limits to local species richness.

All benthic macroinvertebrates are ectothermic, therefore temperature plays an important role in their ecology and influence in their growth rate, life cycles and other behavioural and morphological attributes (Allan, 1995). Within the Mara Basin water temperature increases as the stream order increases. Water temperature varies from 12.5 °C in the upper reaches to 16 °C in middle reaches (Water Quality Baseline-Mara River, 2007)

Dissolved oxygen is also a main driver for the survival of the aquatic fauna; low oxygen concentrations can heavily impact most of them (Ward, 1992). However some species, especially Diptera larvae and Oligochaeta, have a certain tolerance to oxygen deficiency (Williams & Felmate, 1992). According with Kreuzinger (2009), dissolved oxygen concentration levels less than 50% are a signal of presence of dissolved organic matter that generally comes from domestic manure and agricultural wastes. The dissolved oxygen concentrations for the streams located in the upper basin vary around 70% (Water Quality Baseline-Mara River, 2007)

The pH is also an important parameter because it is influenced by chemical and biological processes and it can be influenced by industrial discharges (Chapman, 1996). According with the water quality baseline for the Mara River, pH values have not changed dramatically-- the range fluctuates between 6.0 and 8.5 (Water Quality Baseline-Mara River, 2007).

From a long time salinity has been considered a key factor that determines benthic community in lotic and lentic systems (Mathi & Dorris, 1968). Electrical conductivity plays an important role on the dissimilarity of the benthic community and it should be carefully compared with other water quality variables like dissolved oxygen, water temperature, pH, NO<sub>3</sub>-N, NO<sub>2</sub>-N, PO<sub>4</sub> and turbidity in order to avoid possible confusions with geomorphological features (Kefford, 2006).

### **2.3 Freshwater ecosystem health assessment based on different biotic approaches**

The health of an ecosystem is based on the ability to supply goods and services required and sustain both human and nonhuman residents (Moog, 2009).

In order to quantify the impact of different stressors on the river ecosystem's health, many bioassessment methods have been developed in the last three decades regarding the use of benthic macroinvertebrates (Dickens & Graham, 2002). For instance: South African Score System (SASS), Rapid Field Screening Method, Biological Monitoring Working Party (BMWP) among others.

The South African Scoring System SASS, which is in its fifth version, is the most common and successful method of scoring sensitivity of macroinvertebrate taxa present at a site used in African countries. The SASS principle resembles the BMWP approach with the difference that the tolerance

value for each family in the SASS varies between 1 (most tolerant) to 15 (most sensitive), for the BMWP score range varies from 1 to 10 (McMillan, 1998). The SASS calculates the Average Score Per Taxon (ASPT), which is explained more in detail in section 3.3.4 within this study. The SASS version 5 scoring sheet used is illustrated in Annex B.

The Rapid Field Screening Method considers the biological condition with the habitat quality, which makes it an ecological assessment approach based on sensoric criteria (natural and non-natural turbidity, colour, foam, odour, waste, ferro-sulphide reduction) and the macroinvertebrate taxa that can be identified in the field (Moog, 2005). This method is mainly used to assess ecological status of rivers when organic pollution exists or another type of environmental stressor. This method classifies the streams in five ecological classes: high, good, moderate, poor and bad. The use of the evaluation table for the rapid field screening is explained later on in section 3.2.4.

The Biological Monitoring Working Party BMWP is the most accepted, and widely used for river quality assessment based on biological monitoring using benthic macroinvertebrates. This approach was developed in UK 1978 without targeting any specific geographical area and has been tried in other tropical rivers with great success (de Zwart and Trivedi, 1992; Thorne & Williams, 1997).

This method consists in a scoring system which calculates the ASPT by adding up the scores for each family present in the sample, the score ranges vary from 1 (most tolerant) to 10 (most sensitive). The number of taxa suggests the diversity of the site and also the condition of the ecosystem, the higher the better (Friedrich et al., 1996).

The disadvantage of the approaches previously mentioned is the effect of sampling effort explained by Barbour et al. (1999) that says that in a prolonged sampling time more macroinvertebrate taxa is expected and the results for ASPT will be higher. It is important to maintain consistency throughout the sampling procedure.

The types of single metrics selected are ecologically relevant for the biological assemblages addressed under the objectives of the study. They are as well sensitive to stressors and give a quick response that can be distinguished from natural disturbance such as noise and signals, and cost – effective to sample (Verdenschot & Moog, 2006; Barbour et al., 1995). Addressing several metric types may reflect different dimensions of the ecosystem (Moog, 2009). As it was mentioned before, the physical-chemical parameters were used broadly in many countries to determine the pollution in the river. Alone, these parameters may not reflect the reality of the entire stream, but in conjunction with evaluation of the benthic macroinvertebrates, they could reflect ecological quality and environmental conditions as a whole.

Within the single-metric macroinvertebrate measures developed so far, the most suitable for this study include the following:

Richness measures:

- # Total Taxa (Total Families)
- # Plecoptera
- # EPT Taxa

Tolerance/intolerance measures:

- SASS5-Scores

Composition measures:

- # of individuals
- % Oligochaeta + Diptera Taxa

Diversity measures

- Shannon index

Loss measures

- Community loss index

Functional measures

- % of Functional Feeding Groups (FFG)

Each of the single metrics and indices were calculated independently for each sampling site in order to identify its response to degradation (Hering et al., 2004) based on the different land uses. Most metrics show distinctive responses purporting that they are effective proxies of ecosystem features (Yoshimura, 2006). This understanding contributes with information for restoration plans, as well as provides an ecological risk assessment in order to minimize impacts in the river ecosystem.

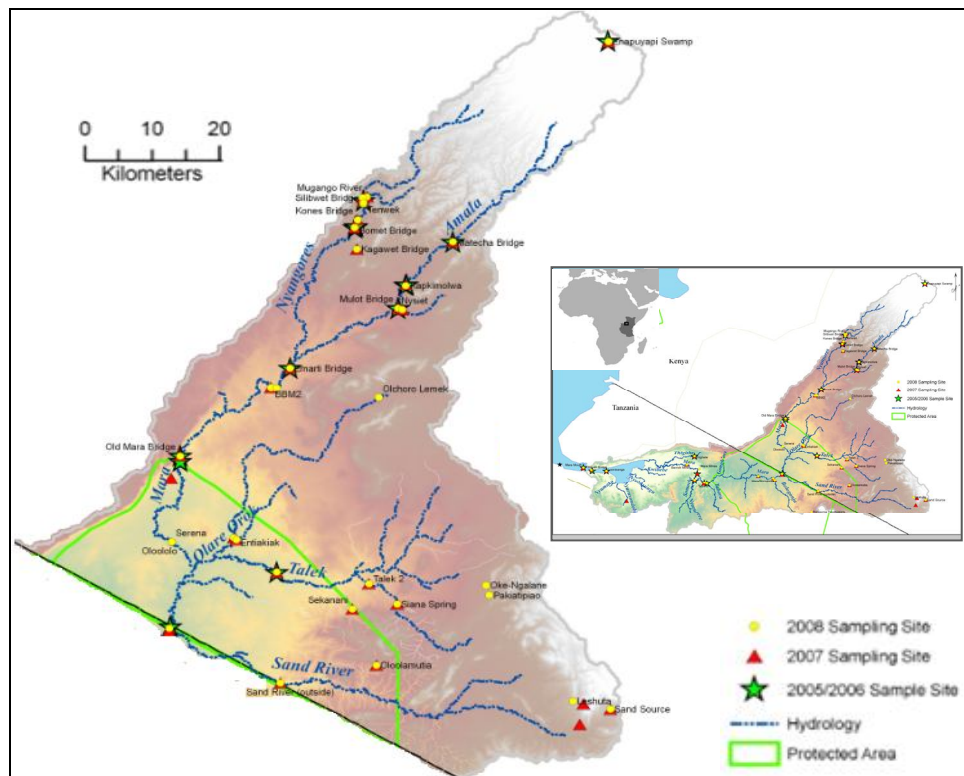
## **2.4 Review on current studies carried out in the Mara River**

The river quality monitoring carried out in the Mara River basin in the Kenyan side dates from initial studies during 2004 that were performed by WWF in conjunction with the Kenyan Ministry of Water and Irrigation, Narok District ([www.globalwaters.net](http://www.globalwaters.net)). This monitoring has been increasing yearly (Figure 2.1). The water quality data collected include temperature, pH, alkalinity, conductivity, dissolved oxygen, total dissolved solids, total suspended solids and turbidity. The parameters were selected mainly to link water chemistry and biology. According to Kreuzinger (2009) the most important set of physical – chemical parameters supporting the biological elements reflecting thermal conditions (temperature), oxygenation conditions (oxygen saturation), acidification status (pH), nutrient conditions (BOD, nitrate and orthophosphate).

In addition to the physical-chemical water quality monitoring within the Mara basin, a macroinvertebrate sampling program was carried out in five of the almost thirty current sampling sites for water quality within the Kenyan side by GLOWS. This provided a base for the current study which also seeks to link the biotic and abiotic components of this ecosystem. The importance of pairing physical – chemical and biological components is that the biological monitoring covers a higher variety of pressures, larger spatial scale and therefore provides the most integrative view of the ecosystem health (Moog, 2009), and it can be related to physical – chemical monitoring in order to establish a complete connection with the benthic community structure and composition (Kreuzinger, 2009).

According to Mara Environmental Flow Assessment (2008), the sites located in the confluence of the Nyangores and Amala River (Middle Mara) found the number of taxa reduced to eight indicating more degradation of the river ecosystem in only 50 km travel distance. At the moment more invertebrate sampling and water quality parameters monitoring are still ongoing. In addition, sampling sites have been increasing to better understand the relationship between macroinvertebrates species composition and water quality status. However, as illustrated in Fig. 3, sampling and monitoring work in the headwaters and upper basin is limited.

The results of this study contributed to the understanding of the changes in the ecosystem due to the land management use in the upper catchment for both inside and outside the Mau Forest within the Mara River basin.



**Figure 2.1 Water quality sampling sites in the Mara River Basin (Kenya)**

**Source: Adopted from GLOWS map**

*Land use and abuse in the upper Catchment*

The Mau Forest located in the upper catchment of the Mara Basin is classified as a montane and moist Forest in Kenya, with one of the highest precipitation rate of around 1400 mm annually (Gereta et al.,2003). Its elevation is around 3000 masl and give rise to the main tributaries Nyangores and Amala, which flow from north-east to south-west and after a few kilometres converge to form the Mara River.

The Mau Forest shortening started with the land division, relocation and settlement plan in 1970 (www.wikipedia.com). Almost 60 000 ha have already been cleared for human settlement and tends



to increase despite government's effort to relocate and compensate people that are being evicted on a daily basis (news, 2009) (Figure 2.2).



**Figure 2.2 Deforestation in Mau Forest, Amala River**

The term land in Kenya can have several connotations, for the government land is the physical space which represents the cultural, political and social power of the Nation; for the people land means a source of livelihood and it determines the level of prosperity or poverty (KLA, 2000). The change in land use from forest to agricultural land started in 1970 when the government tried to create space for crop production and settlement for people which has increased in the last decade considerably. In the Kenyan side of the Lake Victoria basin, land-use practices have produced damage on the aquatic ecosystems (Raburu, 2003; Okungu & Opango, 2005), for instance the increase in nutrient enrichment, pesticide contamination and sedimentation in streams (Osano et al., 2003) and the Lake Victoria itself (Okungu & Opango, 2005). The average household size has increased by 13% since 1999 until 2002 (Mati, 2005). The land use supports about 80% of the population and employs 70% of manpower in Kenya (KLA, 2000).

The high precipitation in the upper catchment makes it suitable for tea, coffee and maize production. It also supports horticulture and floriculture. According with the Economic Survey in 2008, from a period of time of one year (from 2006 to 2007), wheat production increased 6.4%, tea 19%, coffee 10.6%, milk 17.17%, while maize declined 6.1% (KNBS, 2008). The increase of wood extraction from the Mau Forest is notorious; women go there every day to take the resources for timber, fuel wood, charcoal and livestock grazing. The Welfare Monitoring Survey indicates that around 90% of the people in Narok and Bomet districts use firewood collected from the Forest (KNBS, 1996). All these activities are deteriorating the ecosystem and the forest itself (Figure 2.3).

The deforestation leads to negative impacts in the basin, not only water is declining for consumption, irrigation and livestock but vegetation and natural habitats are declining especially for wild elephants and buffalos (KLA, 2000).



**Figure 2.3 Forest land extracted for timber and fuel wood. Mau Forest, Nyangores River**

#### *Water Use and abuse*

Water is the most vital resource for human and livestock (cattle, goat, sheep and poultry) survival . It is essential for agriculture, and other activities such as industry, hence it contributes to rapid economic growth. However, nowadays freshwater ecosystems are threatened by many factors including different kinds of pollution, pesticides, fertilizers and human-related activities (KLA, 2000). The use of poor farming methods is the consequence of the lack of effective laws and regulations; rapid population growth and the lack of good management practices have ecological impacts on the river; however and fortunately, the government in coordination with the Environmental Management Department are working on an institutional framework to provide standards (KLA, 2000).

According with the annual welfare monitoring survey carried out in Kenya, the main sources of water for the rural communities in Narok and Bomet districts within the Rift Valley, are from the river, and in the more developed and populated towns comes from piped water. It is worth mentioning that the access to piped water does not guarantee the availability and reliability of water supply (KNBS, 1996)



## 3 Materials and Methods

### 3.1 Research area

The study area was located in the upper catchment of the Mara River Basin (south western Kenya) and focuses on the main tributaries, Amala and Nyangores. These tributaries originate from the Mau Escarpment and flow from north-east to south-west to feed the Mara River. Several streams from, first to fourth stream order (Strahler system) feed the Amala and Nyangores Rivers in the upper catchment. The Mara River is a sixth order stream at the junction of these two tributaries. The upper catchment of the Mara River basin has an average precipitation of 1400 mm and it varies among years. The evapotranspiration is around 1090 mm per year and the temperature varies from 10.5 °C to 15 °C (Melesse et al., 2008).

The study area has only one large town, Mulot, and smaller town centres, which include Silibwet, Tendwet, Sierraleone, Tenwek, Tegat, Kembu and, Mugango among others. The main activities are large scale livestock production and agriculture. Agricultural produce includes tea, wheat, coffee and small-scale horticulture, fruits and root crops (Figure 3.1).

For the purpose of this study, the representative sampling sites in the upper catchment were located within three main land use types, namely:

- 1) Forest (almost no human intervention)
- 2) Agriculture (e.g. tea, coffee, maize and livestock grazing)
- 3) Mixed (a combination of intervened forest and small scale agriculture)



**Figure 3.1 Agriculture in upper catchment Mara Basin, Nyangores River**

## 3.2 Sampling design and procedure

### 3.2.1 Sampling sites selection

Prior to the field sampling, a reconnaissance survey was carried out in order to select representative sampling sites. For this purpose various maps regarding topography, geology, hydrology, land use and road accessibility were used. On site, the potential sites were carefully analyzed within a 200m stretch in order to avoid considerable tributaries or point sources of pollution that may affect the results of the study (Furse et al., 2006). The selection of the sites was random with the aim of covering both subcatchments Amala and Nyangores to determine only the effect of land use on freshwater ecosystems. Specific stressors in the water bodies, for instance, waste dumping sites and, point-sources of pollution, were avoided.

Due to the limitation on the stipulated duration of the study, roads inaccessibility and the prevailing weather conditions at the time of study, an extensive sampling design of 30 sampling sites could not be carried out. For this reason, the sampling design included 26 study sites not evenly distributed within the three different land use types described above. Figure 3.3 illustrates their respective locations on the map.

The sampling program was carried out during November and early December (base-flow conditions) Eight sites were located in the Amala catchment and eighteen in the Nyangores catchment. Since the main objective was to sample sites according to prominent land use characteristics, the Nyangores catchment upstream offered more sites especially for Forest land use as opposed to the Amala catchment, where most of the forest has been transformed into agricultural land and most of the streams remain dry for most of the year.

The codes of the sampling sites were given according to the catchment and land use type in the surrounding, i.e. first and second letters, respectively. For instance, NA30 means that the sampling site was located in the Nyangores River within an agricultural land use area, AF5 was located within the Amala catchment at a forest area and, NM36 was on the Nyangores River within mixed in the surrounding. For the case of IM1, IF3 and MF1 they have the code of the name of the stream, in this case Issey and Mangoitae rivers, and all of them are located in the Amala catchment.

Most of the sampling sites were located within first to third order stream. Sites NF45, NF48 and NM31 belonged to fourth stream order and site N44 belonged to fifth stream order. This latter was the only sampling site, which was located in the main Nyangores River. The purpose was to select representative samples for which its runoff comes from a specific land use as Figure 3.2 illustrates. This criterion was chosen in order to avoid unreliable results, and attribute the effect of land use to the community composition, diversity and abundance of macroinvertebrates.

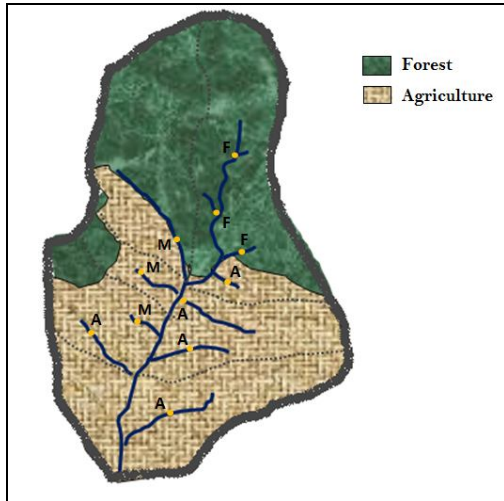


Figure 3.2 Scheme of the sampling design

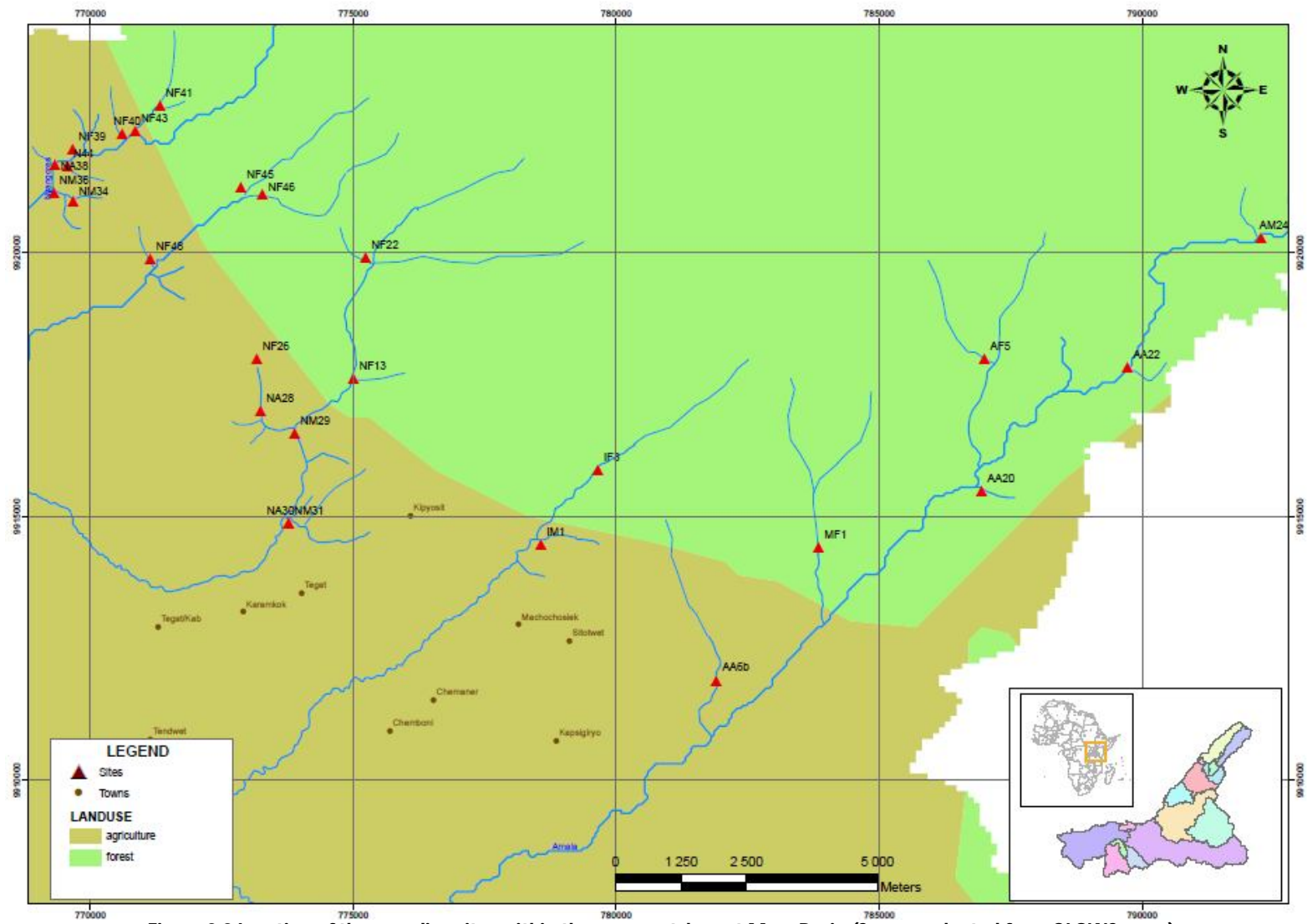


Figure 3.3 Location of the sampling sites within the upper catchment Mara Basin (Source: adopted from GLOWS maps)

The criterion to define the reference sites was based on the rapid field screening methodology (Moog et al., 1999), which roughly determined the water quality class. Usually water quality class I or II are considered reference sites, the main feature of the reference site is that it has none or only very little human intervention or any pollution level. Reference sites are chosen to assess the degree of degradation occurred at impacted sites.

### 3.2.2 Field-work and sampling

The sampling period was during November and December, 2009. This period belongs at the end of the second annual peak in flow levels for an average year (MEFA, 2008). However, this may vary between years, and few heavy rains were present restraining sampling for one or two days.

The potential sampling sites were identified and the local assistance gotten at site helped to optimize time and resources. People in the field were familiar with the community, local conditions and risks that existed on the site due to the presence of wildlife.

An equipment list is an important tool that should be checked prior to the fieldwork: sampling gear, measurement equipment, protocols, camera, communication system, food and first aid kit as the essentials.

#### *Physical and chemical measurements*

Water samples from the sampling site were taken prior to disturbances produced by the measurements of other *in-situ* parameters. A one-litre container was carefully filled with water samples from each site. The water samples were taken for NH<sub>4</sub>-N and PO<sub>4</sub>-P analysis in the laboratory. All procedures were carried out in accordance with QA/QC standard methods provided by the American Public Health Association (APHA, 1999).

A second water sample was taken to measure turbidity. The portable turbidity meter used was Hach 2100P, which gives direct readings in NTU units; range is from 0 to 1000 NTU. The average of two different readings of the same sampling site value was recorded.

A third water sample was taken and filtered using GF/F pre-weighted filters. The volume filtered was recorded in order to calculate Total Suspended Solids (TSS) later in the lab with the dried GF/F filters data. The water sample filtered was kept in a different container for analysis at the laboratory. The procedure for alkalinity determination includes 100 ml of sample and acid (0.1 mole/l HCL) to a pH of 4.3.

After this procedure, other physical – chemical parameters were measured at site with a handheld multiparameter meter YSI Professional Plus. Prior to the readings at the sampling site, the YSI was calibrated with DO saturation measurement. The parameters recorded after the stabilization of values were: pH, DO (%), DO (mg/l), conductivity (µs) and temperature (°C).

#### *Field Protocols*

During the sampling, two protocols were used in order to provide complete information of the sampling site regarding hydrogeomorphological, and the physical and chemical features plus a first feeling of the condition of the ecosystem by the rapid field screening (Moog et al., 1999).

The first protocol that was used at the site contains: geological/ geographical, hydrological/ morphological and physical and chemical information measured at site.

The second protocol used is the rapid field screening that is an adapted version from the project ASSESS-HKH or the old federal rapid field assessment methodology of Austria respectively (Moog et al., 1999). This adapted version was used successfully in Ethiopia in 2006 (Aschalew, 2007) as well as in Benin and Togo in 2008 (unpublished report by IITA). The procedure includes sensory features, ferro – sulphide reduction, bacteria, fungi and periphyton features and a list of benthic macroinvertebrates. All this information was ticked or counted in accordance with the river characteristics (Hartmann & Moog, 2008). The result of this protocol gives an assessment of the condition of the ecosystem; the higher score indicates the category at which the sampling site is placed, the classification goes from Class I (non-polluted, very good condition) to Class V (extremely polluted, bad condition). The screening protocol for assessing the quality of the sampling sites is illustrated in Annex A.

#### *Macroinvertebrates sampling*

Complementing the two protocols explained before and after making a survey of the site, a macrohabitat identification and sampling design was carried out. Due to the time and difficulties in some sites arising from the presence of wildlife the original approach of targeting 20 sampling units was changed to 10 sampling units in order to maintain consistency during the sampling in all sites.

The labels of each container were clearly identified with sampling codes, date, time and additional information- if needed. This information was written down twice- one label inside the container and another outside in order to have a double check in the procedure.

The sampling methodology was based on a standardised multihabitat approach (Hering et al., 2004). A total of 10 unit samples were distributed in proportion to the different number of macrohabitats (Moog, 2009). Macrohabitats less than 10% coverage within the 100m stretch were neglected. All 10 sampling units were pooled together to get a composite sample per site.

The samples were taken within approximately 100 m section of the river and according to the percentage of cover of each macrohabitat. The macrohabitats included mineral (mud, sand, silt, stones) and biotic habitats (floating macrophyte, submerged macrophyte, bank vegetation, algae). The samples were taken from downstream to upstream to avoid disturbance in the sampling area.

The sampling was done using a hand-net (500 µm mesh size) as follows:

- 1) In each macrohabitat samples were taken in a 25 x 25 cm frame for about 1 minute. In case of stony substrate, stones were lifted with a screwdriver or spatula to remove the animals that were hidden in-between. In case of macrolithal or megalithal substrates, a soft brush was used to clean and remove animals gently.
- 2) The net was washed carefully in order to get all the animals sampled.

- 3) All the unit samples were combined to get a composite sample. Afterwards elutriation was carried out; this is a procedure to separate the woody debris, plant material and sediment from the animals.
- 4) Quick identification of taxa was done during sampling in order to apply the rapid field screening method.
- 5) The composite sample was fixed with formaldehyde solution (4% concentration) and kept in containers, correctly labelled according to the quality control procedures.

After each sampling, the gear was carefully checked and rinsed in order to avoid cross contamination.

#### *Hydrological and Morphological Measurements*

The hydro-morphological measurements include flow velocity, stream width and depth. The equipment used was a Marsh-Mc Birney portable flow meter model 2000 for velocity measurements and for width and depth a tape meter and ruler were used respectively (Figure 3.4).

In-stream characteristics were estimated in a range of 100 m upstream from the sampling site, for instance the visual method was used to roughly estimate the percentage of canopy cover. The level of disturbance was estimated based on the visual appreciation and interviews with local people.



**Figure 3.4 : a) Sampling and b) field measurements at sampling sites**

#### 3.2.3 Laboratory work

##### *Physical – chemical measurements*

All the water samples were kept in a cooler at 4 °C at the field. Once in the laboratory, all the filters were put in the oven and weighted in a precise weighing scale. The results of total suspended solids were calculated by subtracting the weight of the filter from the weight of the filter and sediment and divided by the volume filtered.



The samples for the determination of PO<sub>4</sub>-P were analyzed using a calibration curve and the Hitachi U-2000 spectrophotometer at 880 nm following the procedures of the Standard Methods 4500-P Phosphorus.

A calibration curve was prepared using ammonia standard series according to USEPA Method 350.2 and Standard Method 4500-NH<sub>3</sub> Nitrogen (Ammonia). The absorbance of the mixed solution was measured at 690 nm using a Hitachi U-2000 spectrophotometer.

#### *Macroinvertebrates sorting and identification*

The samples needed 2-weeks preservation time with the formaldehyde solution at 4% concentration (Hartmann, 2005) and then transferred to ethanol solution 70% concentration.

All samples were cleaned, sieved and separated in different white trays with bottom grid for easier sorting and for identification.

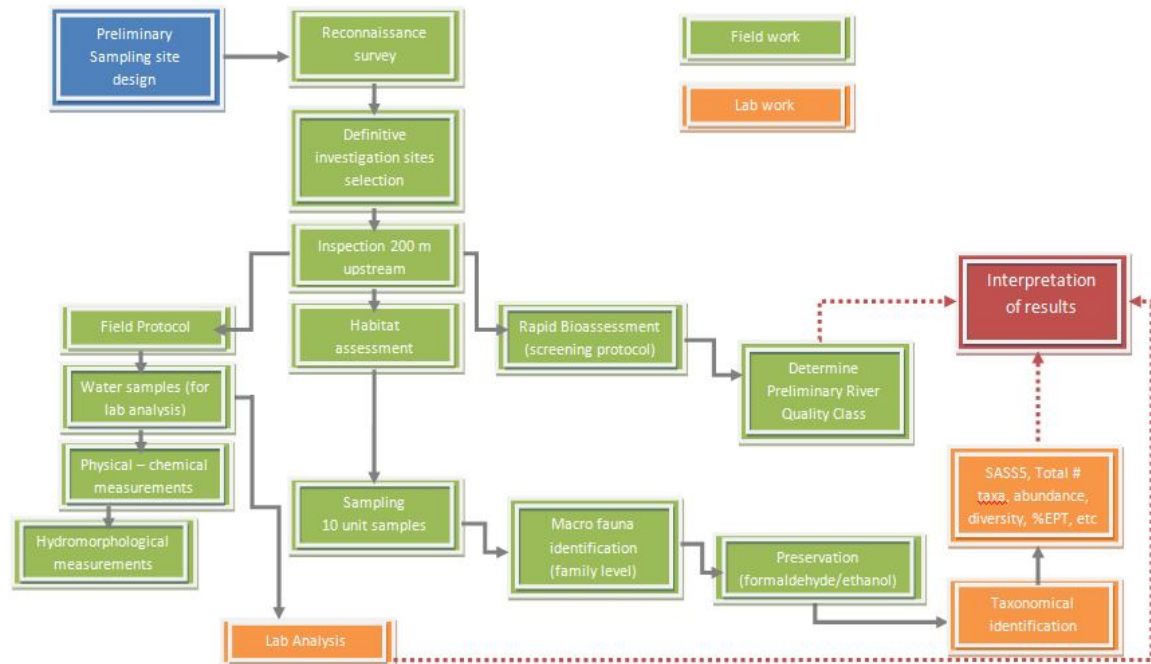
The macroinvertebrates were identified up to family level and some individuals (e.g. Trichoptera, Coleoptera) up to genus and subfamily level (e.g. Chironomidae).

For identification the identification key used was Aquatic Invertebrates of South African Rivers Field manual (Gerber & Gabriel, 2002). Binocular microscope, and dissecting microscopes were used during identification, especially for very small individuals.

The sorted animals were counted, placed in individual vials and preserved with ethanol (70% concentration). Each container was clearly identified by site and taxa. Some specimens that could not be identified and sorted at site were identified to lower resolution later by expert taxonomist at the University of Natural Resources and Applied Life Sciences (BOKU) in Austria; Ephemeroptera and Odonota by Dipl.-Ing. Thomas Huber, Trichoptera and Plecoptera by Dr. Wolfram Graf, Chironomidae by Dr. Berthold Janecek, in the National Museum of Vienna the order Coleoptera was identified by Dr. Manfred Jäch and in Germany the Oligochaeta and Hirudinea by Hasko Nesemann.

The following figure illustrates the field work and laboratory work done during the sampling period (Figure 3.5).





**Figure 3.5 Flowchart of the sampling design and procedure**

### 3.3 Data analysis

The analysis was done considering land use as the main driver and later it was found strong effect on the benthic composition when sites were clustered according to substrate type, river size and the level of disturbance observed at site as the main in-stream characteristics. These variables were used as good indicators of stream degradation as they are main products of the change in land use.

The physical – chemical and biological parameters were analyzed for all the groups. Two methodologies were used for river quality classification: the South African Score System and the Rapid Field Screening.

#### 3.3.1 Physical and chemical parameters

Since sampling was done once per site and no replicate samples were taken, the fluctuations of physical and chemical parameters are not taken into account within the data analysis. All samples are considered under the same conditions.

According to HAMM (1969), the dissolved oxygen saturation can be ranked in different classes regarding its deficit and oversaturation values shown in the Table 3.1.

**Table 3.1 Dissolved oxygen saturation classification (HAMM, 1969)**

WQ Class	Dissolved Oxygen (%)	
	Deficit in %	Oversaturation in %
I	95 - 100	100 - 103
I - II	85 - 95	103 - 110
II	70 - 85	110 - 125
II - III	50 - 70	125 - 150
III	25 - 50	150 - 200
III - IV	10 - 25	> 200
IV	< 10	

One way ANOVA was applied to analyze differences among groups defined by land use, river size and level of disturbance regard to pH, conductivity, dissolved oxygen saturation and concentration, temperature, alkalinity, turbidity, total suspended solids, ammonia-nitrogen and orthophosphate. T-test analysis was performed for the substrate characteristics due to the classification in only two groups.

Similarly, hydro – morphological and physical habitat features measured and recorded at site were analyzed using one way ANOVA. These parameters include: Q (as a function of velocity and area), width, depth, water velocity, substrate characteristics, canopy cover, level of disturbance. The gradient metric was not considered since most of the sites were taken in the upper catchment and have a variation between 2000 to 2300 masl (Barbour et al., 1999)

### 3.3.2 Biological Parameters

Single metrics were calculated with the data of the benthic macroinvertebrates taxa. The single metrics include: #Total Taxa, # Total Families, EPT # Taxa, # of individuals, POET # Taxa, COPE # Taxa, Coleoptera # taxa, Diptera # Taxa, Chironomidae # Taxa, Diversity Shannon index, Evenness, % of Functional Feeding Groups (FFG).

The estimation of species diversity was based on: number of species (species richness) and the distribution of the individuals among the species (evenness).

The diversity index was calculated with Shannon-Wiener index (Pielou, 1969)

$$H' = \sum_{i=1}^S p_i \ln p_i$$

$p_i$  = individuals of a certain species  $i$  from the total number of individuals in a sample of relative abundance.

$S$  = number of species

Evenness also uses Shannon-Wiener:

$$V' = \frac{H'}{\ln S}$$

The functional feeding group was classified based on the Fauna Aquatica Austriaca (Moog, 2002) and it determined the percentage of: shredders, grazers, active and passive filter-feeders, predators, miners and other feeding types that are present in the different land uses (Table 3.2).

**Table 3.2 Functional Feeding Groups**

Feeding Type	Abbreviation	Sources of food
Shredders	SHR	Fallen leaves, plant tissue, CPOM
Grazers	GRA	Epilithic algal tissues, biofilm, partially POM
scrapers, rasps		Endo & epilithic algal tissues, partially tissues of living plants
Filtering collectors		Suspended FPOM, CPOM, prey
Active filter-feeders	AFIL	Food in water current is actively filtered
eddy filterers		Suspended FPOM, micro prey is whirled
passive filter-feeders	PFIL	Food brought by flowing water current
Detritus feeders (gathering collectors)	DET	Sedimented FPOM
Leaf borers, miners	MIN	Leaves of aquatic plants
Piercers		Algae & cells of aquatic plants
Xylophagous	XYL	Woody debris
Predators	PRE	Prey
Parasites	PAR	Host
Other feeding types	OTH	Cannot be classified into this scheme
Omnivorous animals		Diverse

The SASS5 was used as a scoring system to determine the average score per taxon (ASPT) for each site (Dickens & Graham, 2002). The score range between 1 (most tolerant) to 15 (most sensitive). The SASS5 was calculated for the sensitive and most sensitive taxa, SASS 6-15 and SASS 8-15 respectively.

$$ASPT = \frac{SASS5 \text{ scores}}{\text{total number of taxa}}$$

The interpretation of the results are based on the SASS\_5 score and ASPT for each site (Chutter, 1998) and shown in Table 3.3. The SASS 5 water quality classes (WQC) was set according with the interpretation in order to compare with the rapid field screening method.

**Table 3.3 Interpretation of SASS 5 and ASPT scores**

SASS score	SASS ASPT	Interpretation	SASS 5 - WQC
>100	> 6	Water quality natural, habitat diversity high.	I
<100	> 6	Water quality natural, habitat diversity low.	II
>100	< 6	borderline case	III
50-100	< 6	Some deterioration in water quality.	IV
<50	variable	Major deterioration in water quality.	V

### *River Quality classification*

The rapid field screening method was used in order to assess the river quality in each sampling site. The table used is attached in annex section A. The explanation of how to use the table is as follows;

- “+” means that the feature was observed, and “-” not observed. Both + and – are accounted 1 score, ++ for 2 scores and so on.
- Words and percentages are accounted for 2 scores

The value of total scores of each column gave the classification of the sampling site. The scores for the dominance of organisms within the benthic macroinvertebrate list were taken from the SASS 5 scoring sheet.

It is important to mention that site NF22 was not considered for the NMS due to the missed information of Ephemeroptera order that was observed at this site and not during sorting in laboratory.

#### 3.3.3 Statistical Analysis

Statistical analysis was done using the statistical software R version 2.8.1 using packages bbmle MASS and vegan (R-Development-Core-Team 2005). Graphics were done using MS-Excel, SigmaPlot 10.0, R 2.8.1 and PC\_ORD 5. For all statistical analysis the level of significance was set at 0.05.

The number of investigation sites in agricultural land use type was 6, in forest 13 and mixed 6. In case of substrate type in soft bed sediment was 20 and for non-soft bed sediment 5. For the river size the number of investigation sites categorised for small stream was 17, medium 5 and large 3. Finally for level of disturbance category, the number of investigation sites identified for minimum disturbance was 4, occasional disturbance 14 and permanent disturbance 7.

Analysis of covariance (ANCOVA) using stream order as a covariate was used to test for differences among land uses on physical – chemical parameters, hydro – morphological features and single metrics of benthic macroinvertebrates. Oneway-ANOVA was performed to test for differences among level of disturbance and river size categories. In the same way a t-test was performed among the substrate characteristics to test significant differences on the parameters mentioned above.

A Spearman correlation was performed between physical – chemical, hydro-morphological and biological parameters and tested for significance.

Hierarchical cluster dendrogram analysis on standardized physical – chemical data assessed similarity of sampling sites based on a Euclidean distance matrix.

I used non-parametric permutational ANOVA (Anderson, 2006) to test the main effects and interactions of physical – chemical and environmental parameters on the benthic macroinvertebrate composition. These tests were done in three groups: 1) pH, conductivity, DO saturation and concentration, temperature and alkalinity, 2) PO<sub>4</sub>-P, TSS, Turbidity and 3) width, depth, discharge and velocity.

An NMS ordination method was selected for the analysis of benthic macroinvertebrate community composition because it is the most appropriate tool when the number of taxa (variables) exceeds the number of sampling sites (observations) (Anderson, 2006). NMS was based on Bray-Curtis dissimilarity matrices computed from log (x+1)-transformed benthic taxa abundances. The Bray-Curtis dissimilarity matrix was also used for a cluster analysis. The purpose of using MDS is to provide a visual description of how the sites are similar or distant from each other among the biological data set. The configuration in 2-D was poor due to the complex set of data, so it was done in 3-D. The stress value represents the percentage of the data from the matrix that could not be represented in the graphic. Orientation of the axes is random and assigning meaning to the individual axes in the sense of underlying variables is not a trivial task due to the non-linear nature of the analysis. The NMS of the benthic composition was subjected to different layouts to identify possible clusters.

The Bray-Curtis dissimilarity matrix was also subject to testing for effects of the various factors (e.g. Land use). I tested for differences in location and dispersion using non-parametric permutational MANOVA (abbr. PERMANOVA) and a permutational analogue of the Levene-test (abbr. PERMDISP) (Anderson, 2006).

Finally, I tested for association between benthic community structure and abiotic variables by Mantel tests (Manly, 2006). The “Mantel statistic” is computed as a Pearson correlation coefficient between entries of the distance matrices, its significance level is computed by permutation. For this purpose a Euclidean distance matrix was computed from standardized physico-chemical and environmental variables.

**Table 3.4 Summary of the statistical analysis and different parameters**

Statistical Test	Sampling sites grouped by	Parameter
ANCOVA	Land use	Macroinvertebrate single metrics
One-way ANOVA	Land use	Physical – chemical
	River size	Physical – chemical
		Macroinvertebrate single metrics
	Level of disturbance	Physical – chemical
Two-way ANOVA	WQ classes (Rapid Field Screening and SASS)	Macroinvertebrate single metrics
		Physical – chemical
t-test	Substrate type	Macroinvertebrate single metrics
Spearman rank correlation		Physical – chemical
		Hydro – morphological features
		Macroinvertebrate single metrics
NMS	Macroinvertebrate composition	Land use
		River size
		Level of disturbance
		Substrate type
		WQ classes (Rapid Field Screening and SASS)
Cluster Dendrogram		Physical – chemical (Conductivity, TSS, turbidity)

## 4 Results

### 4.1 Sampling sites description

The hydro-morphological measurements were taken during sampling and in base flow conditions for all 25 sites. Measurements of width, depth and canopy area are displayed in Table 4.1 for each of the sampling sites.

**Table 4.1 : Sampling sites and main characteristics in upper catchment Mara Basin**

Land Use	Site	Stream Order	Coordinates		Site specific		T (°C)	Canopy area (%)	Date (2009)
			N	E	Velocity (m/s)	Discharge (l/s)			
Agricult.	AA20	2	9915486	786943	0.04	3.1	13.4	50%-70%	Nov-24
Agricult.	AA22	1	9917833	789712	0.02	1.7	14.5	50%-70%	Nov-24
Agricult.	AA6b	2	9911897	781900	0.1	1.8	17.5	< 50 %	Dec-01
Agricult.	NA28	1	9917013	773233	0.04	1	17.5	50%-70%	Dec-03
Agricult.	NA30	2	9914872	773770	0.03	1	15.4	< 50 %	Dec-03
Agricult.	NA38	2	9921681	769324	0.19	5.6	16.8	< 50 %	Dec-08
forest	AF5	3	9918000	787000	0.15	2	14.5	70%-90%	Nov-23
forest	IF3	3	9915876	779651	0.14	30	18.5	50%-70%	Nov-30
forest	MF1	3	9914421	783846	0.08	7.9	13.7	70%-90%	Dec-01
forest	N44	5	9921639	769563	0.82	2293	16.9	< 50 %	Dec-09
forest	NF13	3	9917610	774997	0.03	10	14.3	50%-70%	Dec-02
forest	NF22	3	9919903	775230	0.23	45	15.6	70%-90%	Dec-02
forest	NF26	2	9917991	773162	0.12	2.5	12.9	70%-90%	Dec-03
forest	NF39	2	9921967	769666	0.07	3.8	12.7	> 90%	Dec-09
forest	NF40	2	9922250	770604	0.01	0.6	13.6	> 90%	Dec-09
forest	NF41	2	9922779	771320	0.26	9.7	14.6	> 90%	Dec-09
forest	NF43	2	9922302	770849	0.08	7.3	14.1	70%-90%	Dec-09
forest	NF45	4	9921119	773456	0.14	356	16.6	< 50 %	Dec-10
forest	NF46	3	9921119	773456	0.31	178	16.2	< 50 %	Dec-10
forest	NF48	4	9919888	771137	0.23	547	16.1	< 50 %	Dec-10
mixed	AM24	2	9920288	792255	0.19	22	13.5	50%-70%	Nov-25
mixed	IM1	2	9914476	778567	0.02	1.5	19	50%-70%	Nov-30
mixed	NM29	2	9916570	773884	0.3	18.9	16.5	< 50 %	Dec-03
mixed	NM31	4	9914872	773770	0.12	61	15.4	< 50 %	Dec-03
mixed	NM34	2	9920971	769674	0.19	3.7	13.7	< 50 %	Dec-08
mixed	NM36	2	9921144	769303	0.07	3.8	16.6	70%-90%	Dec-08

Land cover: Agriculture

Amala site 1: AA20

This sampling site was located on a small stream of variable width that ranges between 0.30 to 0.60 m and soft bed sediments (sandy & muddy bottom substrate). The river banks are low with a depth that averaged 20 cm. The stream runs through agricultural land where farming is undertaken at a

small-scale. At some points, the stream flows through zones of wetland. There was good riparian canopy cover that was estimated as being between 50 –70% at approximately 100m upstream from the sampling site. The stream forms natural meanders and it is occasionally disturbed by livestock grazing in areas closer to the stream.

#### Amala site 2: AA22

This sampling site was situated in a small stream of constant width of approximately 1m and runs through agricultural land, where the riparian cover was estimated as being between 50 to 70% at the sampling site stretch. The bed sediment is soft (mainly muddy bottom substrate) and it changes just before its discharge in the Amala River. The agricultural land varies from small to medium scale.

#### Amala site 3: AA6b

Sampling site located in a very small stream of variable width that ranges between 0.40 to 0.55 m. The sampling site is close to Kisilbei River and goes through Lelkatet community. It has very small percentage of canopy cover being estimated at site less than 50% and soft bed sediment (muddy bottom substrate). The main agriculture close to the sampling site is maize and tea and the landscape is very steep presenting some signs of erosion. The stream is used as a crossing road for livestock and people upstream as well as downstream from the sampling site

#### Nyangores site 1:NA28

Sampling site located in a small stream of variable width that ranges between 0.80 to 1.1 m and goes through agricultural land which produces mainly tea. Its origin is from a spring 2km upstream from the sampling site. It has eucalyptus trees along the banks and its bed sediment is soft (sandy bottom substrate). The canopy cover was estimated as being between 50 to 70 % at site. The stream downstream is permanent disturbed and it is used for livestock dewatering in several sections.

#### Nyangores site 2:NA30

Sampling site situated in a small stream with variable width that ranges from 0.40 to 0.70 with very poor canopy cover estimated in less than 50%. It has soft bed sediment (muddy bottom substratum) and it is considered as an occasional disturbance site due to livestock grazing on the riparian vegetation. The predominant agricultural farming is tea.

#### Nyangores site 3:NA38

The sampling site was located in a small stream of variable width that ranges from 0.40 to 0.60 and it has grass as riparian vegetation. The stream goes through pure agricultural land mainly tea. The stream is used as cattle watering place in section upstream and downstream of the sampling site.

Land cover: Forest

#### Amala site 4: AF5

Sampling site located in a small stream that forms natural meanders of constant width that ranges from 0.70 to 0.95. The canopy cover was estimated as being between 70 and 90%. It has occasional

disturbance especially from elephants, which increased the complexity of habitats by introducing large woody debris . People from close communities collect water from this stream for domestic consumption according with the local guide that helped during fieldwork.

#### Amala site 5: IF3

Sampling site located in a river of variable width that ranges from 1.3 to 1.7 m and canopy cover estimated as being between 50 to 70%. The bed sediment is composed by gravel of different diameters ranging from 5 to 25 cm. This river has occasional disturbance from human activities at site and elephants upstream increasing the level of turbidity and TSS.

#### Amala site 6: MF1

Sampling site located in a river of variable width that ranges from 0.8 to 1.2m and discharges in Mangoitae River. It has occasional disturbance especially of elephants increasing the level of TSS. The site has good riparian vegetation estimated between 70 to 90%. and shows excessive contribution of coarse particulate organic matter (CPOM). The bed sediment is soft composed mainly by silt and sand.

#### Nyangores site 4: N44

Sampling site located in the main Nyangores river and has a width of approximately 13m. It has a high diversity of microhabitats: pools, rifles, runs and eddies. The bed sediment is composed by boulders of different diameters that range from 3cm to 2m. The canopy cover estimated at site was less than 50%.

#### Nyangores site 5: NF13

Sampling site located in a low velocity river of variable width that ranges from 1.5 to 2.3 m. The canopy cover estimated at site as being between 50 to 70% and it has a high content of woody debris. It presented occasional disturbance of livestock and human activities. In this site a high presence of filamentous green algae was found; which indicates signs of eutrophication and allochthonous influence.

#### Nyangores site 6: NF22

Sampling site located in a river of variable width of approximately 2m. Its bed sediment is soft (mainly sand) and canopy cover estimated as being between 70 to 90%. It has occasional disturbance of elephants according with the local guide that helped during fieldwork. This site showed a wide variety of microhabitats where Ephemeroptera order was observed during a quick identification during sampling but later on in laboratory this order was missed. This site was not considered for further analysis due to the missed information.

#### Nyangores site 7: NF26

Sampling site located in a small stream of variable width that ranges from 0.55 to 0.70m and it has soft bed sediment (sandy and muddy bottom substrate). It has disturbance from livestock grazing



and human activities increasing the level of TSS. The canopy cover estimated at site as being between 70 to 90%.

Nyangores site 8: NF39

Sampling site located in a small stream of variable width that ranges from 0.90 to 1.3m and forms natural meanders with soft bed sediment (sandy bottom substrate) and canopy cover estimated more than 90% with a high contribution of large woody debris. Upstream, water is taken for domestic consumption by the people from close communities.

Nyangores site 9: NF40

Sampling site located in a small stream of variable width that ranges from 0.60 to 0.80 m and forms natural meanders. The stream has soft bottom bed sediments and canopy cover estimated more than 90% with high contribution of woody debris. The sample was collected where the riparian vegetation was thick and it goes underground in some sections.

Nyangores site 10: NF41

Sampling site located in a small stream of variable width that ranges from 0.85 to 1.10m. The stream goes underground in some sections upstream the sampling site. It has a soft bed sediment composed mainly of sand. The canopy cover was estimated at more than 90% with a high contribution of woody debris.

Nyangores site 11: NF43

Sampling site located in a small stream of variable width that ranges from 0.90 to 1.10 m and soft bed sediment (sandy bottom substrate). It has a canopy cover estimated from 70 to 90% with a high contribution of woody debris. It is the combination of NF41 and other small tributary. It has some occasional disturbance especially from elephants according with the local guide during fieldwork.

Nyangores site 12: NF45

Sampling site located in a river of constant width of approximately 7m and low velocity. The river has soft bed sediment and it is composed solely of silt. This site is very poor in microhabitats showing a very low number of taxa during sampling. The canopy cover estimated was less than 50% and the riparian vegetation is mainly composed by a secondary forest. The site showed occasional disturbance of human activities and livestock.

Nyangores site 13: NF46

Sampling site located in a river of variable width that ranges from 3 to 3.5 m and discharges in the river where sample NF45 was taken. The stream had soft bed sediment (sandy and silt bottom substratum) and gravels of variable diameters from 6 to 25 cm approximately and presented natural meanders. The canopy cover estimated is less than 50%. Human hunting trails were found close to the river.

#### Nyangores site 14: NF48

Sampling site located in a river of constant width approximately 9m and has non-soft bed sediment (mainly gravel of variable diameters 3 to 20cm). On the right side bank there is riparian vegetation; whereas on the left side there is mainly grass. Downstream the sampling site this river passes by the Ainabngetunyek community. The canopy cover estimated at site was less than 50%. It has some occasional disturbance by livestock and human activities.

Land cover: Mixed

#### Amala site 7: AM24

Sampling site located in a river of variable width that ranges from 1.8 to 2.5m. The stream has non-soft bed sediment with boulders of different diameter that varied between 2cm and 1 m showing a large number of macrohabitats. The river passes by agricultural land upstream and then through forest before it discharges to the main Amala River. According with the local guide that helped during fieldwork the water from this river is taken for domestic consumption. The canopy cover estimated was from 50 to 70%

#### Amala site 8: IM1

Sampling site located in a river of variable width that ranges from 0.50 to 0.75 m and soft bed sediment (muddy bottom substratum). The stream has occasional disturbance especially downstream from the sampling site by livestock. It is a subcatchment composed by 60% agriculture (mainly tea) and 40% forest land cover. The canopy cover was estimated as being between 50 and 70% and in some sections the river goes underground.

#### Nyangores site 15: NM29

Sampling site located in a stream of variable width that ranges from 0.95 to 1.10m and soft bed sediment (sandy & muddy bottom substrate). It presented high disturbance from livestock and human activities. The canopy cover estimated was less than 50%.

#### Nyangores site 16: NM31

Sampling site located in a river of constant width of approximately 3m and non-soft bed sediment (predominantly stony bottom substrate). The river presented high turbidity level with foams and algae in some stretches of the river upstream and downstream the sampling site. It has a permanent disturbance of livestock and human activities. The origin of this river is in forest land and it goes through agriculture land use mainly maize and tea. The canopy cover was estimated in less than 50%.

#### Nyangores site 17: NM34

Sampling site located in a small stream of variable width that ranges from 0.65 to 0.80 m and soft bed sediment (sandy bottom substrate). The stream passes by forest and agriculture land mainly tea with good management practices. It has some occasional disturbances from livestock and human activities. The canopy cover estimated was less than 50%.

Nyangores site 18: NM36

Sampling site located in a small stream of variable width that ranges from 0.70 to 0.85 m and soft bed sediment (sandy & muddy bottom substrate) that forms natural meanders. The stream is a combination of the stream where sample NM34 was taken and other small tributary. It has a high disturbance from human activities and livestock. The canopy cover was estimated as being between 70 and 90%.

### Shade effect

The canopy cover was estimated in 100m stream during sampling and it was divided into four groups: >90%, 70% - 90%, 50% - 70% and < 50%. Canopy cover with less than 50% was observed in sites that belong to the three different land uses while canopy cover more than 90% was observed only in sampling sites within the forest land use. A significant difference in canopy cover was recorded among the land uses types (One-way ANOVA,  $F = 13.8083$ ,  $d.f. = 2$ ,  $p < 0.001$ ) and significant difference between stream order and canopy cover (One-way ANOVA,  $F = 3.1485$ ,  $d.f. = 4$ ,  $p < 0.05$ ).

The sampling sites located in forest that have < 50% of canopy cover were N44 of stream order 5<sup>th</sup>, NF45 and NF48 in 4<sup>th</sup> stream order and NF45 in 3<sup>rd</sup> stream order. The riparian vegetation of these last three sites was secondary forest, with evident human disturbance.

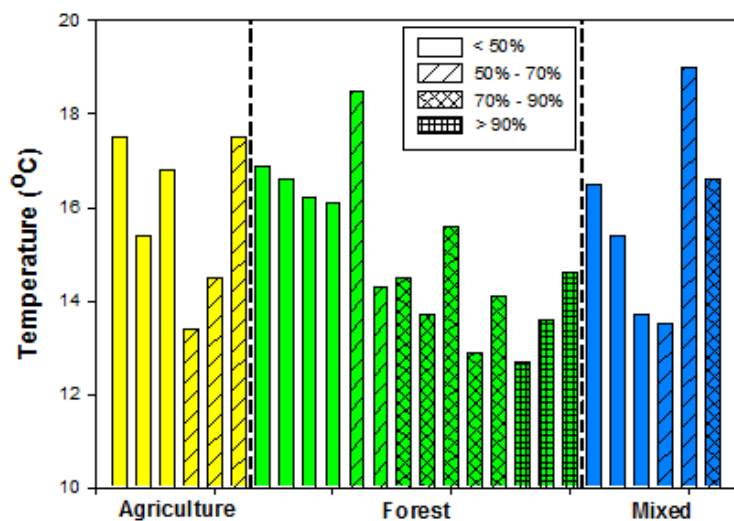


Figure 4.1 Canopy cover and temperature across sampling sites in the upper catchment of the Mara River basin. (Data from fieldwork November – December 2009)

## 4.2 Physical – chemical parameters

### Land use

Physical – chemical parameters were measured on site and in a laboratory and are summarized in the Table 4.2. Significant differences are seen among different land uses only in conductivity, turbidity and total suspended solids. Nevertheless alkalinity and  $\text{NH}_4\text{-N}$  show higher average values in agricultural sites as compared with lower average values in forest and mixed sampling sites.

Table 4.2 Range and mean values of physical-chemical parameters for the sampling sites according with land uses

Parameter	Agriculture			Forest			Mixed		
	mean	max	min	mean	Max	min	mean	max	min
pH	7.67	7.88	7.45	7.87	8.53	7.45	7.64	7.98	7.27
Conductivity	<b>197.63</b>	404.40	79.40	82.53	143.70	50.20	109.23	177.40	64.70
DO (%)	77.03	95.00	57.70	81.10	94.40	65.10	82.40	98.90	73.10
DO (mg/l)	6.02	7.78	4.27	6.37	7.33	5.27	6.41	7.98	5.26
Temp. ( C)	15.85	17.50	13.40	15.02	18.50	12.70	15.78	19.00	13.50
Turbidity (NTU)	<b>114.57</b>	388.00	0.02	0.80	7.95	0.02	38.33	114.50	0.02
Alkalinity (mmol/l)	<b>15.39</b>	43.20	0.62	3.74	43.20	0.46	7.95	43.20	0.77
NH4-N (mg/l)	<b>1.30</b>	6.25	0.18	0.21	0.33	0.01	0.23	0.33	0.10
PO4 (mg/l)	0.36	0.90	0.11	0.38	0.96	0.14	0.31	0.60	0.08
TSS (mg/l)	<b>77.80</b>	199.41	11.14	21.01	51.08	10.21	38.95	90.00	8.53

One – way ANOVA test indicated a significant difference in electrical conductivity among the different land uses (ANOVA, n = 24, F= 7.1433, d.f.= 23, P < 0.01). The highest value was recorded in agricultural areas with a mean of 197.63  $\mu\text{S/cm}$  compared with 109.23  $\mu\text{S/cm}$  in mixed land use and 82.53  $\mu\text{S/cm}$  in forest. (Figure 4.2)

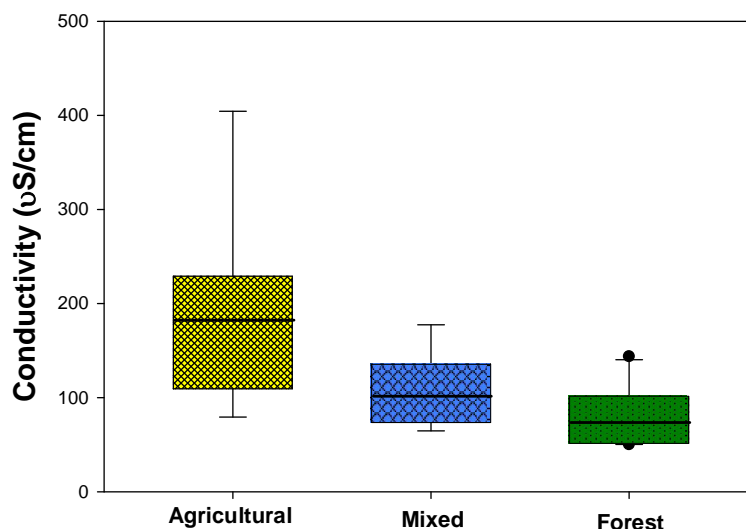
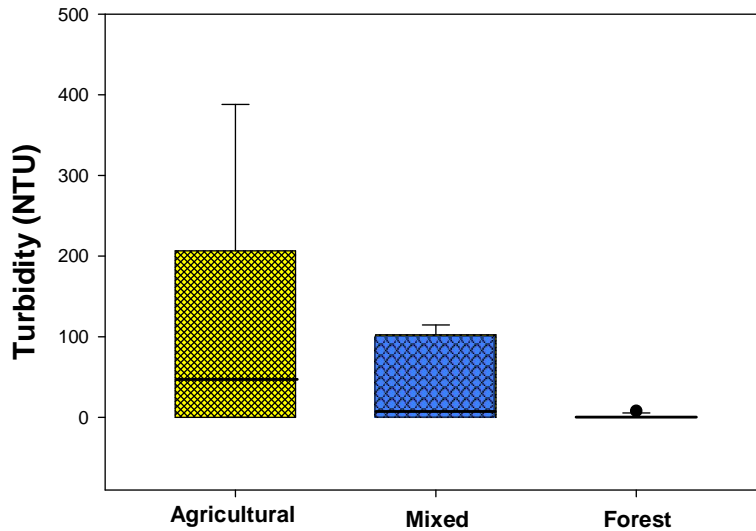


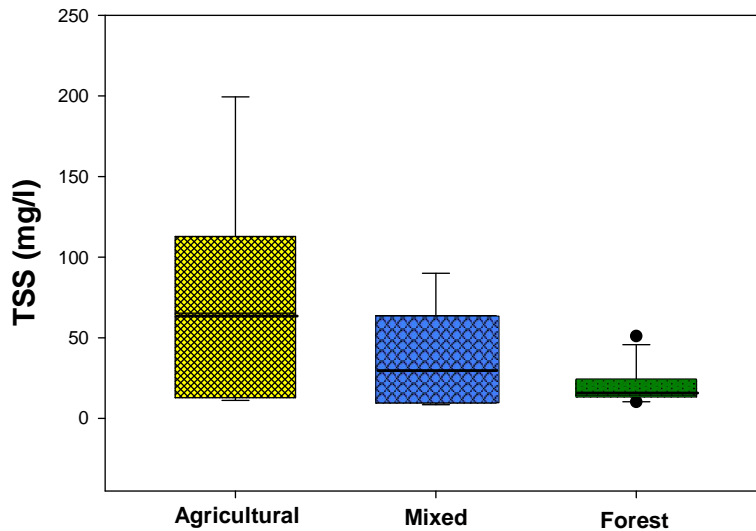
Figure 4.2 Box (95% confidence interval) and whisker (max-min values) plot of the conductivity (November-December 2009) among land cover types, line represents the median value, dots the outliers, and numbers at the bottom the total number of samples

A significant difference in turbidity was seen among the different land uses (One-way ANOVA, n = 24, F= 4.6597, d.f.= 23, P < 0.05) (Figure 4.3). The mean, maximum and minimum values for turbidity in the different land uses are shown in table 4.2. However, it is important to note that the water was disturbed by watering cattle during sampling in site AA6b, which belonged to the agricultural land use (highest value = 388 NTU). Some sampling sites within agricultural land use were being used as cattle watering place.



**Figure 4.3** Box (95% confidence interval) and whisker (max-min values) plot of the turbidity (November-December 2009) among land cover types, line represents the median value, dots the outliers and numbers at the bottom the total number of samples

Total suspended solids (TSS) also showed a significant difference among the different land uses (One-way ANOVA,  $n = 24$ ,  $F = 4.7932$ ,  $d.f. = 23$ ,  $P < 0.05$ ). The high values of TSS in the agricultural land were caused also for the disturbance of livestock during sampling (Figure 4.4).



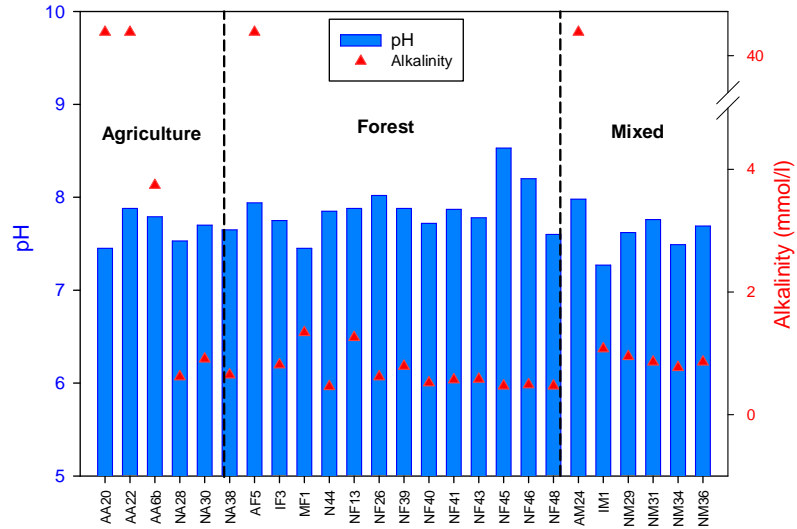
**Figure 4.4** Box (95% confidence interval) and whisker (max-min values) plot of the total suspended solids (November-December 2009) among land cover types, line represents the median value, dots the outliers and numbers at the bottom the total number of samples

### Acidity Level

The average values for pH are shown in table 4.2. The maximum value measured was 8.53 in Forest and the minimum is 7.27 in Mixed land use. There was no significant difference among land use types (ANOVA,  $p > 0.05$ ) and no considerable variation was observed among sites within the same land use type (Figure 4.5).

## Alkalinity

The values for alkalinity varied in a range between 0.46 mmol/l and 1.34 mmol/l, except for the sites AA20, AA22, AF5, AM24 with a value of 43.20 mmol/l and AA6b with 3.74 mmol/l. All these sites that showed high values were located in the Amala subcatchment.



**Figure 4.5** Box (95% confidence interval) and whisker (max-min values) plot of the total suspended solids (November-December 2009) among land cover types, line represents the median value, dots the outliers and numbers at the bottom the total number of samples

## DO%

The maximum Dissolved Oxygen saturation measured during the sampling program was 98.90% in a Mixed land use catchment, while the minimum measured was 57.70% in an agricultural land use. The dissolved oxygen concentration has also the maximum and minimum values in these sites as 7.98 mg/l and 4.27 mg/l, respectively.

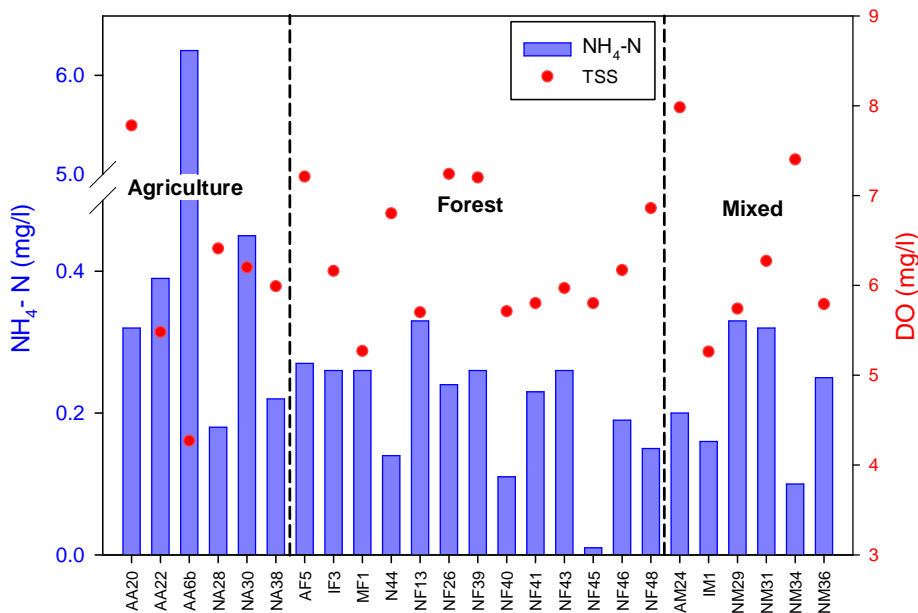
According to the ranking classification for oxygen deficit and saturation (HAMM 1969), explained in subchapter 3.3.1, most of the sites can be categorized between water quality class I and II – III.

## Temperature

The sampling sites maintained a temperature in a range between 12.7 and 19 °C. The maximum and minimum temperatures registered for the three different land uses are indicated in Table 4.2.

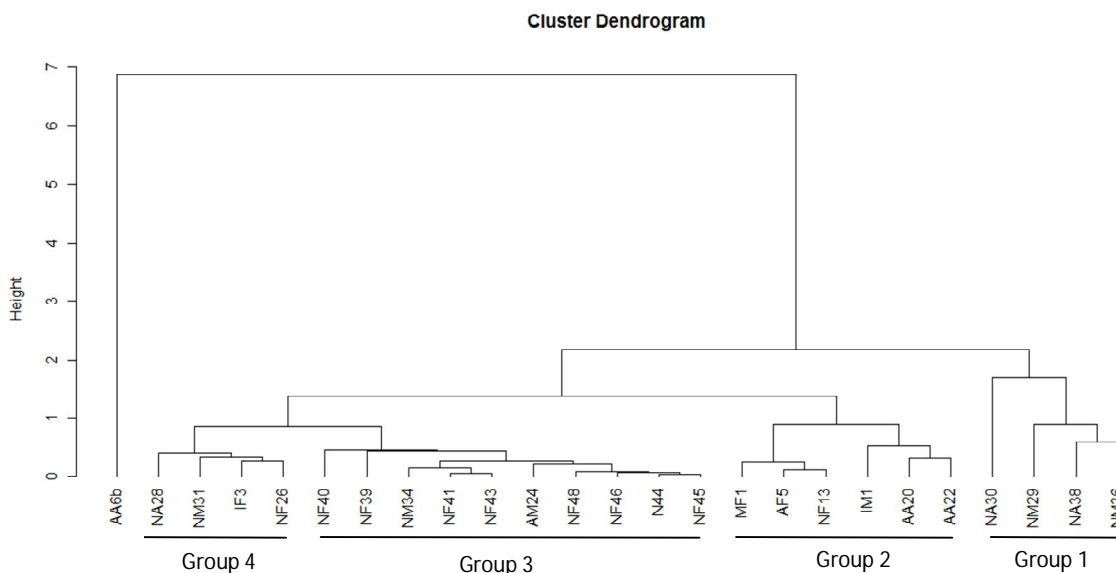
## NH<sub>4</sub> – N

The values of ammonium – nitrogen registered for the sampling sites are recorded in Table 4.2. These values do not show significant difference between land uses (ANOVA,  $p > 0.05$ ). Only one site AA6b shows the highest value of 6.25 mg/l and the lowest DO concentration.



**Figure 4.6** NH<sub>4</sub>-N and DO concentration of the river water at the sampling sites among the land use types in the upper catchment of the Mara River basin (Data from fieldwork November – December 2009)

The following dendrogram shows the similarity among sites according to the physical and chemical data: conductivity, TSS and turbidity. Four groups and one outlier can be distinguished; as expected most of the sampling sites in forest land use type are clustered together. Forest sampling sites that presented high levels of these physical-chemical parameters were clustered with some mixed and agricultural sampling sites.



**Figure 4.7** Dendrogram of hierarchical cluster analysis based on Euclidean distance using standardized transformed data from physical – chemical parameters across 25 sampling sites. Group 1: high concentrations of conductivity, turbidity and TSS; Group 2: high concentration of conductivity, low concentrations of turbidity and TSS; Group 3: moderate concentrations of conductivity, low concentrations of turbidity and TSS; Group 4: moderate concentrations of conductivity and TSS and low concentrations of turbidity. AA6b considered as an outlier, highest concentrations in conductivity, turbidity and TSS.

### In-stream characteristics

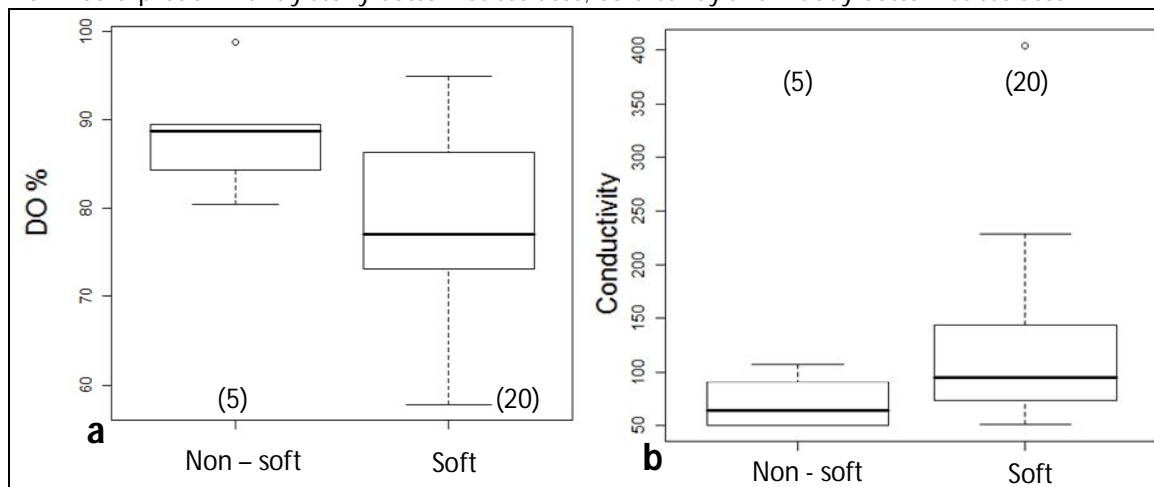
As pointed out in the subchapter 3.3, sampling sites clustered most strongly according to three main indicators of the change in land use being: substrate type, river size and the level of disturbance observed at site. These groups were statistically tested to find out significant differences among them.

#### *Substrate type*

A significant difference in electrical conductivity between the substrate types was demonstrated (t-test,  $n = 25$ ,  $t = -2.483$ ,  $d.f. = 21.43$ ,  $P < 0.05$ ) (Figure 4.8 b) and in the same way for dissolved oxygen saturation (DO%) (t-test,  $n=25$ ,  $t = 2.1146$ ,  $d.f.=24$ ,  $p < 0.05$ ) (Figure 4.8 a).

However, the physical-chemical parameters did not show significant difference between non-soft and soft substrate types (t-test,  $p > 0.05$ ).

Non – soft: predominantly stony bottom substrates; Soft: sandy and muddy bottom substrates.

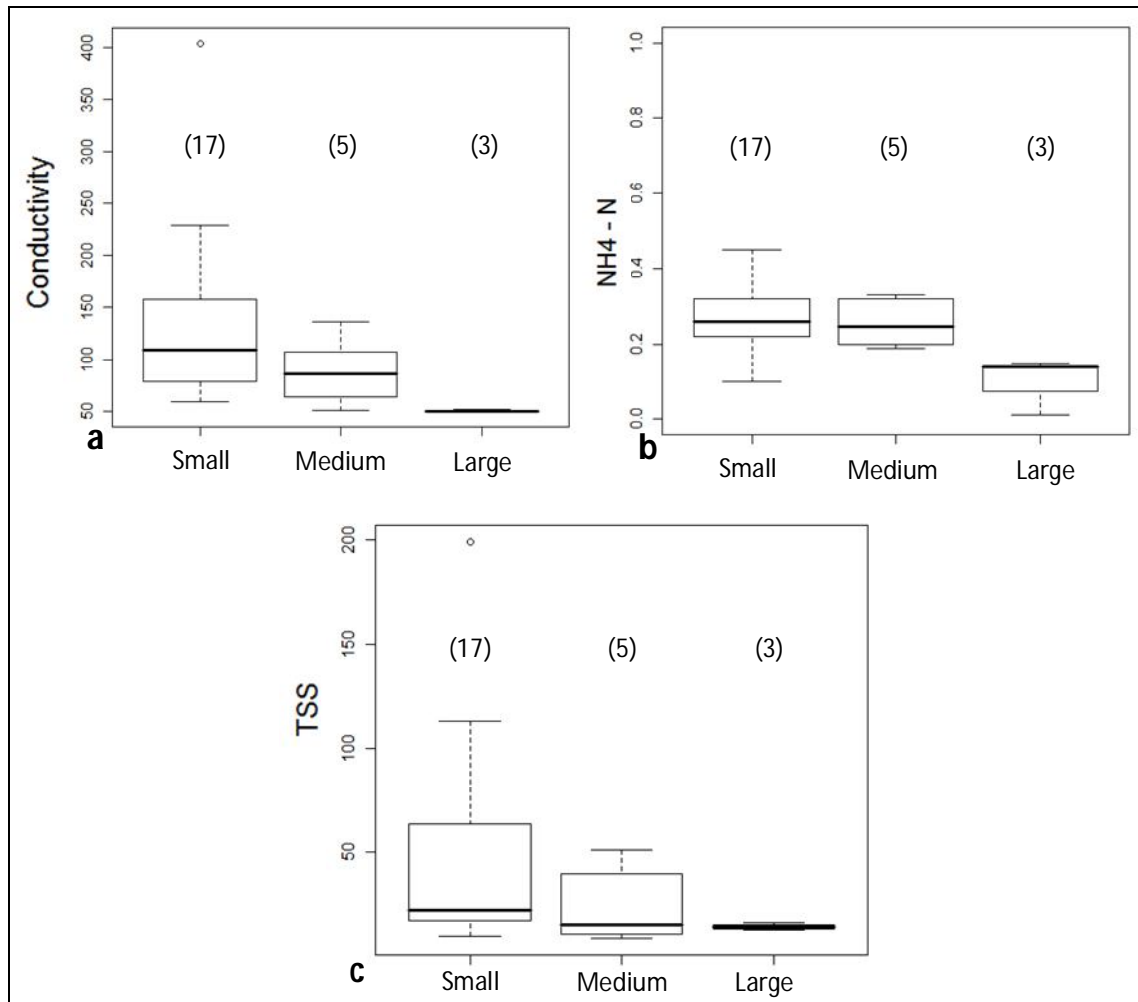


**Figure 4.8** Box (95% confidence interval) and whisker (max-min values) plot of a) DO saturation and b) conductivity (November-December 2009) between substrate type, line represents the median value, dots the outliers and numbers at the top/bottom the total number of samples

#### *River size*

Significant differences in conductivity (One-way ANOVA,  $n = 25$ ,  $F = 12.155$ ,  $p < 0.01$ ),  $\text{NH}_4\text{-N}$  (One-way ANOVA,  $n=25$ ,  $F = 4.7253$ ,  $p < 0.05$ ) and TSS (One-way ANOVA,  $n = 25$ ,  $F = 4.2482$ ,  $p < 0.05$ ) were demonstrated among the different river sizes (Figure 4.9).





**Figure 4.9** Box (95% confidence interval) and whisker (max-min values) plot of a) conductivity, b) NH<sub>4</sub>-N and c) TSS (November-December 2009) among river size of the sampling sites, line represents the median value, dots the outliers and numbers at the top the total number of samples.

#### *Level of disturbance*

Significant differences in conductivity (One-way ANOVA,  $n = 25$ ,  $F = 8.0195$ ,  $p < 0.01$ ), Turbidity (One-way ANOVA,  $n = 25$ ,  $F = 3.3627$ ,  $p = 0.05$ ) and TSS (One-way ANOVA,  $n = 25$ ,  $F = 3.9229$ ,  $p = 0.05$ ) were demonstrated among the different level of disturbances observed at site (Figure 4.10).

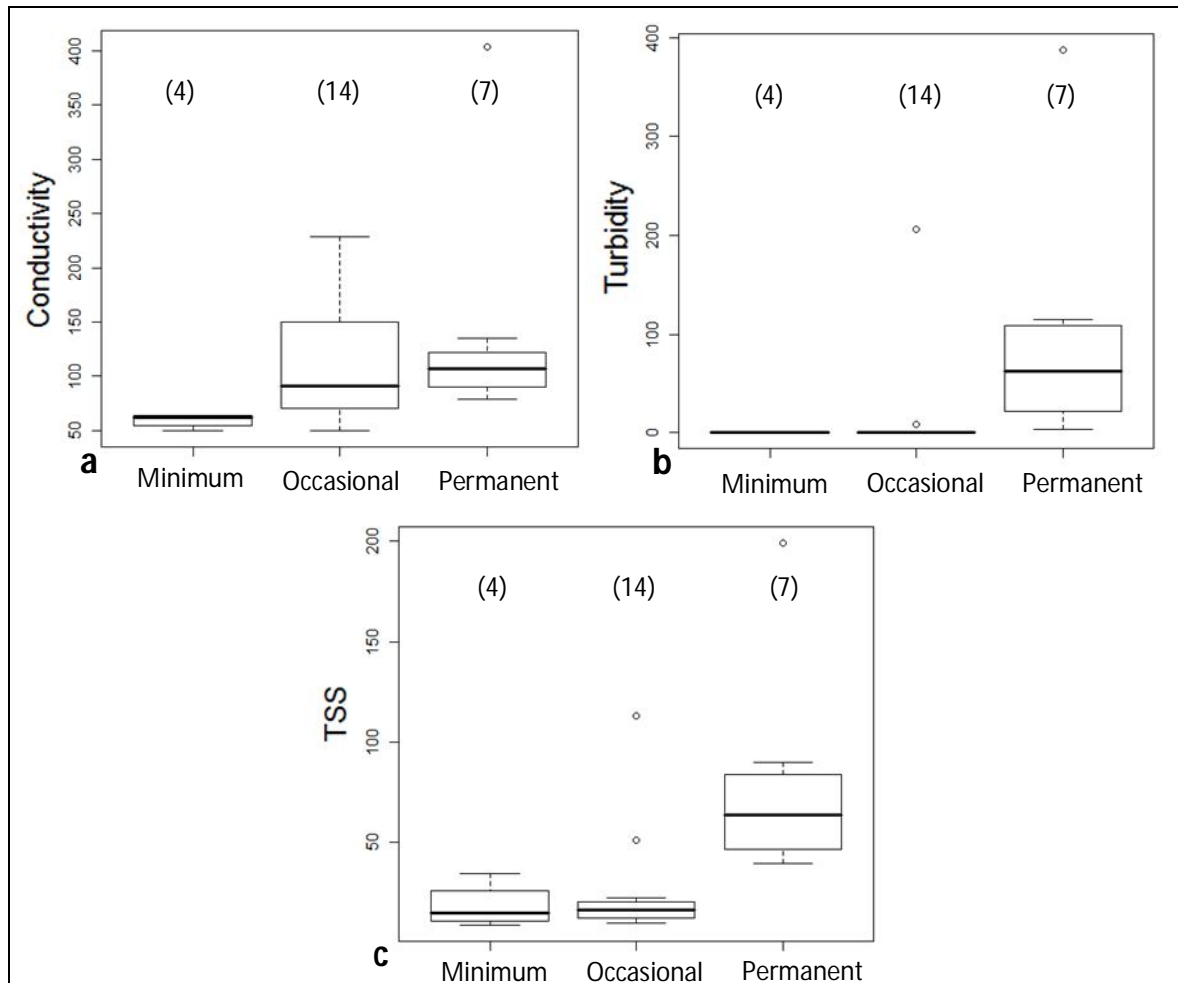


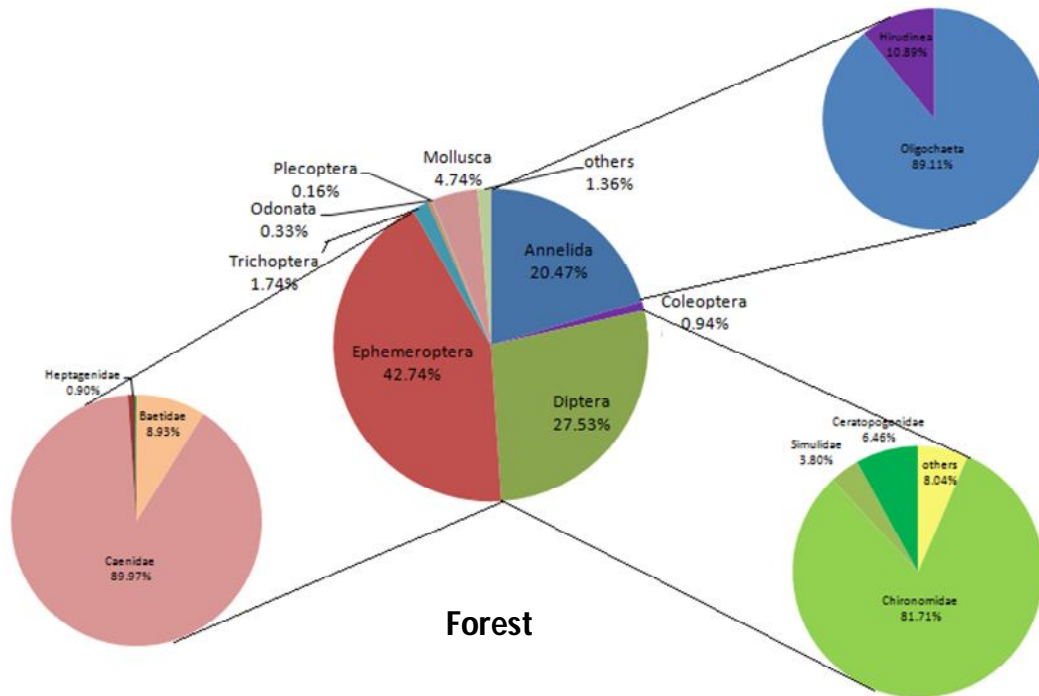
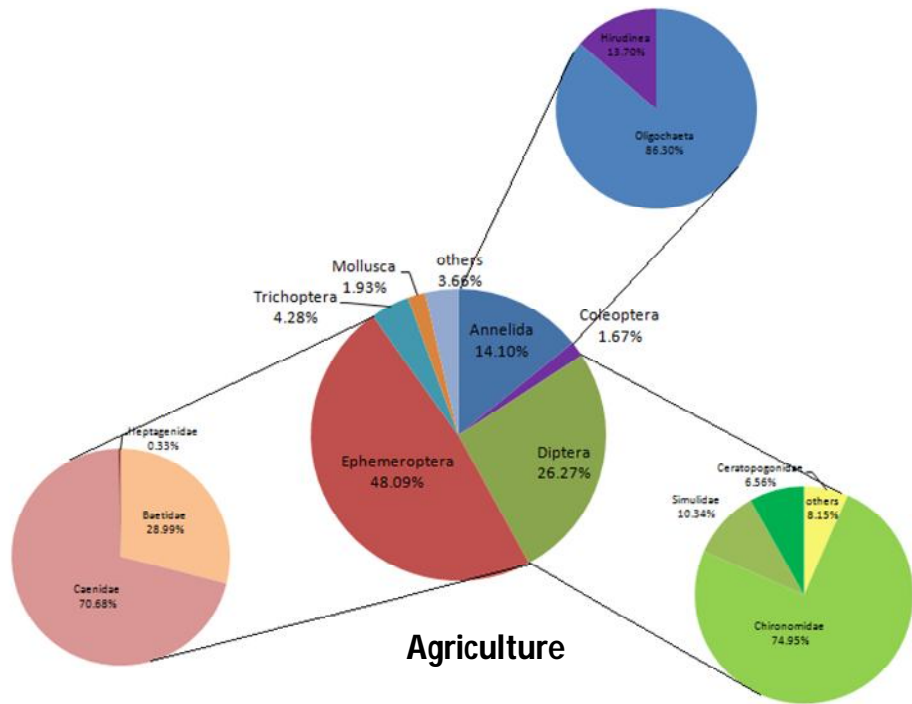
Figure 4.10 Box (95% confidence interval) and whisker (max-min values) plot of a) conductivity, b) turbidity and c) TSS (November-December 2009) among the level of disturbance observed at the sampling sites, line represents the median value, dots the outliers and numbers at the top the total number of samples.

### 4.3 Macroinvertebrate assemblages

A total of 9006 individuals within 75 taxa belonging to 13 orders were identified for the 25 sampling sites. The highest number of taxa (33) and individuals (1559) was recorded at site MF1 and the lowest number of taxa (3) and individuals (41) at site NF45, both belonging to the forest land use type (Figure 4.12 and Table 4.3).

The most dominant orders were: Ephemeroptera 41.28%, Diptera 30.83% and Annelida 17.21% across the sampling sites. The Ephemeroptera order present 12 different taxa, Diptera 18 taxa and Annelida 6 taxa. The percentage of families in each dominant order for the three land uses types are presented in Figure 4.11.

The distribution of various taxa at the sampling sites is presented in Table 4.3.



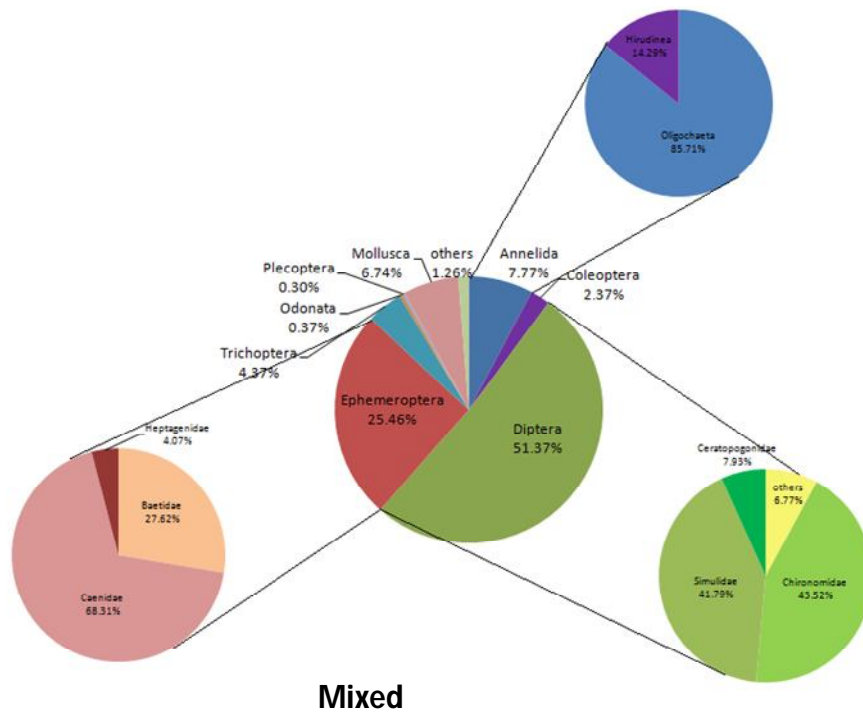


Figure 4.11 Percentage of families' taxa in the three most dominant orders for the land uses types in the upper catchment of the Mara River Basin during fieldwork (November – December 2009).

Table 4.3 Distribution of various macroinvertebrate taxa at the 25 sampling sites in the upper reaches of the Mara River during November and December 2009.

Order	Site	AA20	AA22	AA6b	NA28	NA30	NA38	AF5	IF3	MF1	N44	NF13	NF26	NF39	NF40	NF41	NF43	NF45	NF46	NF48	AM24	IM1	NM29	NM31	NM34	NM36		
Annelida	Oligochaeta																											
	Tubificidae	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	
	Lumbricina								X																			
	Mermithidae		X																						X			
	Hirudinea																											
	Glossiphoniidae																											
	<i>Helobdella</i>																											
	<i>adiastola</i>	X	X			X		X		X	X	X	X			X	X			X				X	X	X	X	
	<i>Placobdelloides</i>																											
	<i>multistriatus</i>																									X		
Turbellaria	<i>Alboglossiphonia</i>																											
	<i>disjuncta</i>	X	X	X	X	X				X		X	X			X			X				X	X	X			
	Dugesia spec.	X	X	X		X				X		X											X	X	X			
	Helodidae	X	X		X	X		X	X			X				X				X	X	X	X			X		
	Hydrophilidae		X	X																								
	Gyrinidae																											
	Orectigyus									X																		
	Chysomelidae												X															
	Dytiscidae		X					X					X										X					
	Agabus	X									X																	
Coleoptera	Hyphydrus									X																		
	Bidessini									X																		
	Copelatus														X													
	Elmidae	X						X		X	X		X	X					X	X	X				X	X		
	Staphylinidae									X																		
	Dixidae	X	X			X		X				X										X						
	Ceratopogonidae	X	X	X	X	X	X	X		X	X	X	X		X	X	X		X	X	X			X	X			
	Chironomidae																											
	Tanypodinae	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X		X	X	
	Macropelopia												X															

Order	Site	AA20	AA22	AA6b	NA28	NA30	NA38	AF5	IF3	MF1	NA44	NF13	NF26	NF39	NF40	NF41	NF43	NF45	NF46	NF48	AM24	IM1	NM29	NM31	NM34	NM36		
Ephemeroptera	Procladius									X			X															
	Pentaneurini							X		X			X								X			X				
	Orthocladinae	X	X	X	X	X		X		X	X	X		X	X	X	X			X	X	X			X	X	X	
	Chricotopus sp.												X												X			
	Chironominae																								X			
	Chironomini	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X			X			X		X		
	Polypedium													X						X					X			
	Chryptochironomus													X											X			
	Tanytarsini	X	X	X	X		X	X			X	X				X					X			X	X	X	X	X
	Culicidae	X									X															X		
	Ephydriidae										X											X						
	Limonidae	X		X					X	X		X	X		X	X	X				X			X		X	X	
	Muscidae																									X		
	Psychodidae	X	X	X		X		X															X			X		
	Simuliidae	X		X	X		X	X			X		X		X		X	X			X	X	X	X	X	X	X	
	Stratiomyidae														X										X			
	Tipulidae		X	X		X		X	X				X		X	X	X	X			X	X		X	X	X	X	
	Tabanidae	X							X		X				X		X	X				X	X			X		
	Baetidae																											
	Afroptilum				X	X		X	X	X			X			X	X	X	X	X	X	X	X	X	X	X		
	Baetis				X					X		X				X						X	X		X		X	
	Unknown genus sp.1											X										X	X		X			
	Unknown genus sp.2																					X	X		X			
	Pseudocloeon												X															
	Xyrodromeus												X															
	Cheleocloeon												X															
	Caenidae																											
	Afrocaenis	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	
	Caenidae genus			X	X																		X	X		X	X	
	Oligoneuridae												X															
	Leptophlebiidae												X															

Order	Site	AA20	AA22	AA6b	NA28	NA30	NA38	AF5	IF3	MF1	N44	NF13	NF26	NF39	NF40	NF41	NF43	NF45	NF46	NF48	AM24	IM1	NM29	NM31	NM34	NM36		
Hemiptera	Heptagenidae					X					X										X	X			X			
	Corixidae									X		X			X							X						
	Gerridae	X						X		X	X													X				
	Mesoveliidae																			X								
	Nepidae		X		X					X		X											X					
	Saldidae							X																				
	Veliidae										X											X						
Homoptera	Cicadellidae	X	X																									
	Ecnomidae																						X					
	Leptoceridae																											
Trichoptera	Setodes	X	X				X	X	X	X		X	X		X	X				X			X				X	
	Ymymia										X																	
	Triaenodes	X																								X		
	Lepidostomatidae	X	X																				X					
	Hydropsychidae																											
	Dipletroninae		X		X		X			X	X	X		X	X	X	X					X	X				X	
	Hydropsychinae										X	X									X	X	X		X			
	Hydroptilidae				X	X															X	X						
	Pisuliidae																											
	Salvatares												X							X			X					
	Polycentropodidae																											
Polycentropodinae												X			X													
Polypectropus										X																		
Psychomyiidae										X												X						
Isopoda	sp1		X																									
	Coenagrionidae											X									X							
Odonata	Gomphidae								X						X	X	X					X	X					
	Zygoptera											X																
Plecoptera	Perlidae																											
	Neoperla																				X	X						
	Nemouridae																				X							

Order	Site	AA20	AA22	AA6b	NA28	NA30	NA38	AF5	IF3	MF1	N44	NF13	NF26	NF39	NF40	NF41	NF43	NF45	NF46	NF48	AM24	IM1	NM29	NM31	NM34	NM36	
Crustacean	Potamonautidae	X								X		X				X					X	X	X				
Mollusca	Sphaeriidae																										
	Pisidium spec.	X						X		X		X			X	X							X			X	
	Limnaeidae				X			X																			

X= present taxa



The number of taxa and individuals for the 25 sampling sites are illustrated in the following Figure.

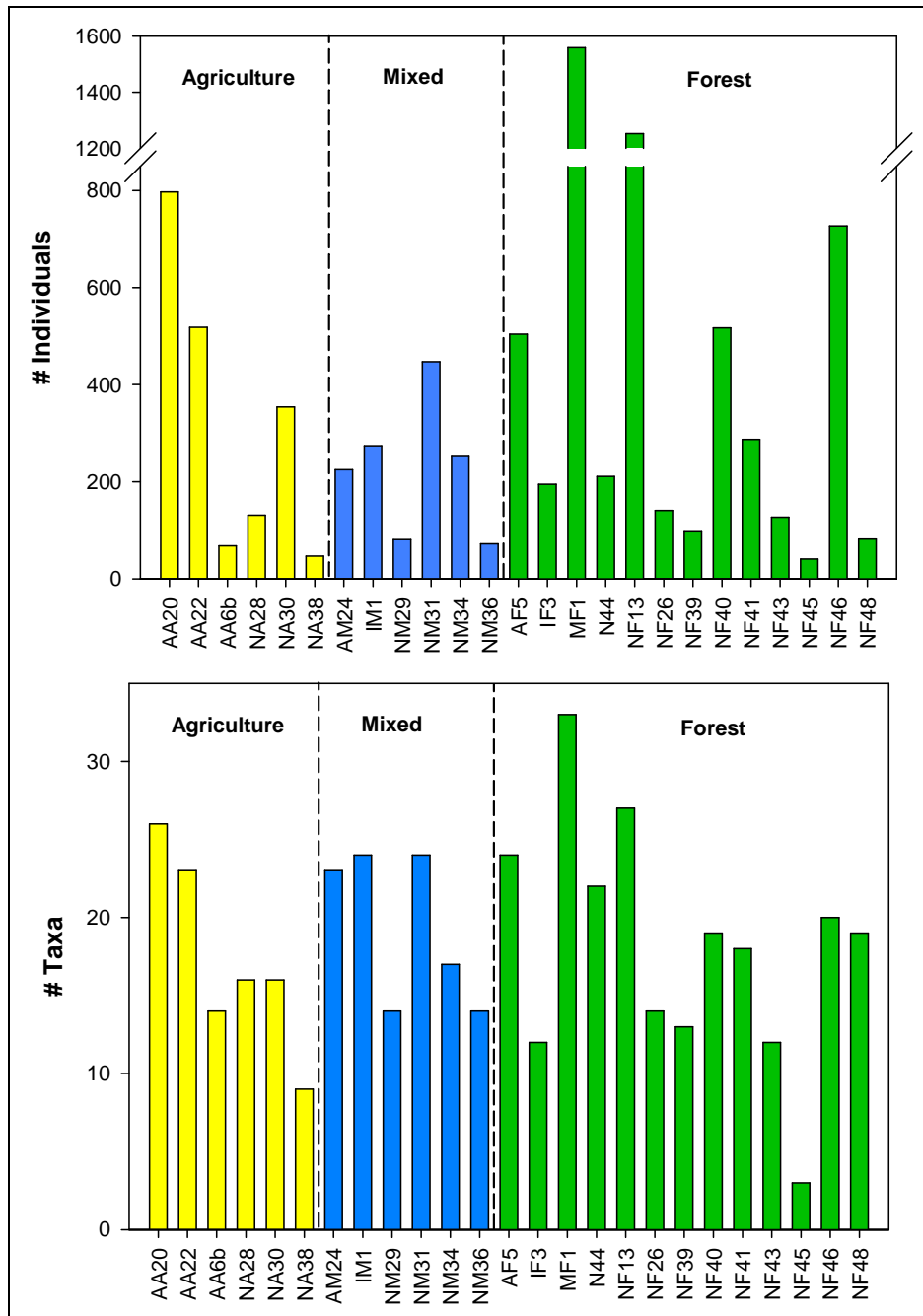


Figure 4.12 a) Number of individuals and b) number of total taxa found in each land cover during fieldwork (November – December 2009).

### Functional Feeding Group (FFG)

The FFG was calculated for each land use as a percentage of relative abundance and are illustrated in Figure 4.13. The group of detritus feeders are predominant in the three land uses followed by predators and grazers in forest and agricultural areas and passive filter feeders and predators in mixed land use.

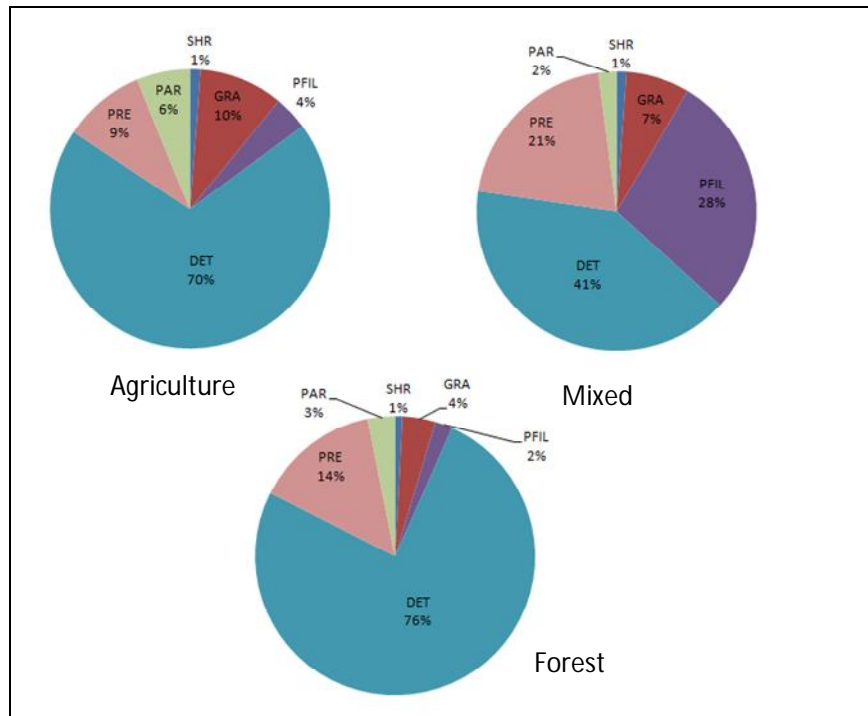


Figure 4.13 Functional Feeding Groups in the different land uses found during fieldwork (November – December 2009).

## Single metrics

### Land use

An ANCOVA test was performed on macroinvertebrate single metrics using stream order as a covariate and did not show any significant difference (ANCOVA,  $p > 0.05$ ). The summary of statistics for each biological index and single metric is shown in Table 4.4. There was, however, another interesting trend, where mean values of scores and indices are higher in mixed land use followed by forest and lower values in agriculture, especially for # of taxa, diversity, Evenness, SASS\_5 score, # of families, EPT taxa, POET taxa, COPT taxa, SASS 6-15 (sensitive taxa) and SASS 8-15 (most sensitive taxa).

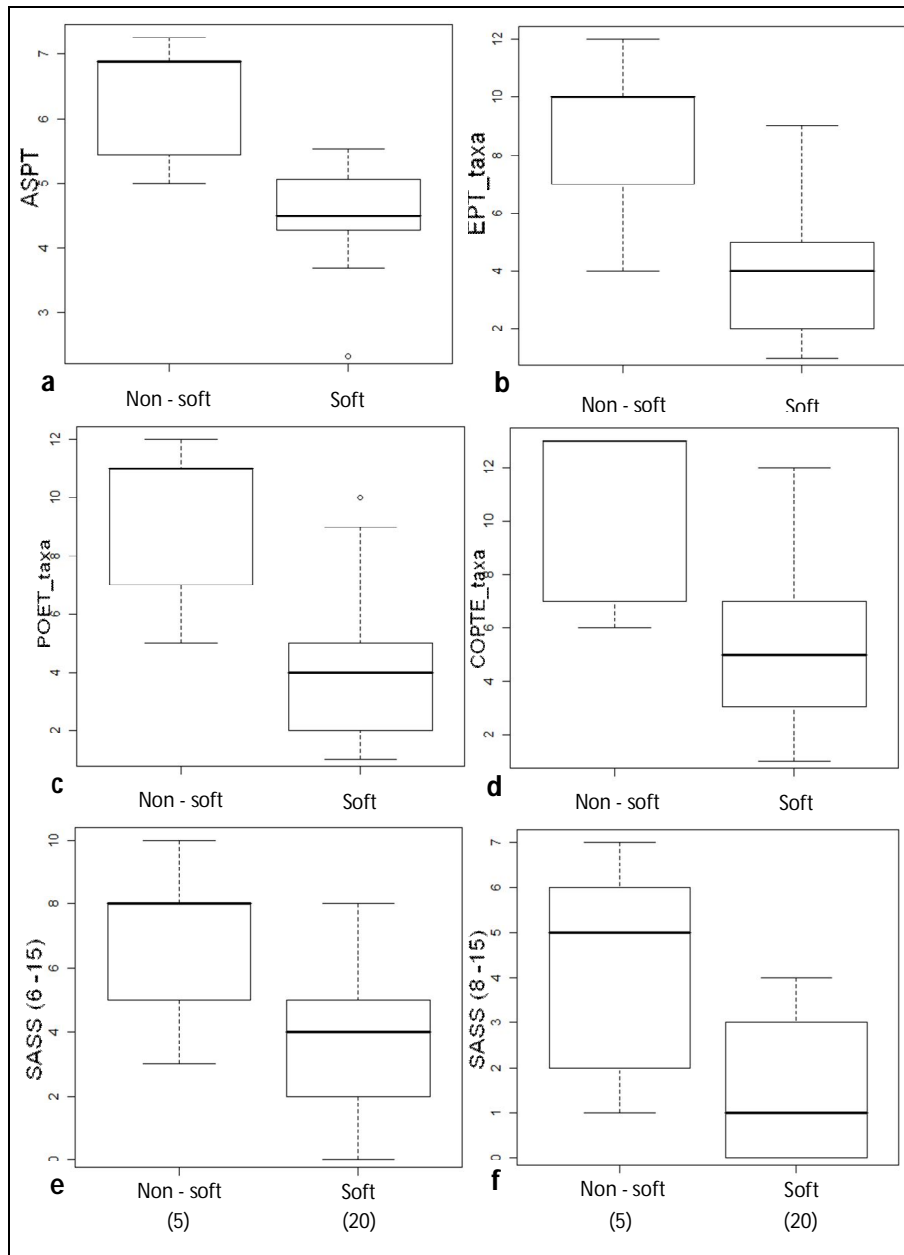
**Table 4.4 Summary statistics for indices and single metrics among land use types.**

Score/Indices	Land Use	n	mean	median	minimum	maximum	std dev.	CV%
total individuals	A	6	319.17	242.50	47.00	797.00	297.20	93.12
	F	13	441.54	211.00	41.00	1559.00	477.16	108.07
	M	6	225.17	238.50	72.00	447.00	138.97	61.72
# Total Taxa	A	6	17.33	16.00	9.00	26.00	6.19	35.69
	F	13	18.15	19.00	3.00	33.00	7.65	42.12
	M	6	19.33	20.00	14.00	24.00	4.89	25.27
Diversity	A	6	1.78	1.76	1.13	2.24	0.42	23.38
	F	13	1.81	1.78	0.78	2.66	0.54	29.69
	M	6	1.96	1.96	1.62	2.31	0.33	17.02
Evenness	A	6	0.64	0.69	0.41	0.81	0.16	24.28
	F	13	0.66	0.69	0.43	0.90	0.16	23.96
	M	6	0.67	0.68	0.52	0.85	0.12	17.48
# Family Taxa	A	6	13.00	12.00	7.00	20.00	4.52	34.74
	F	13	13.77	15.00	3.00	24.00	5.93	43.08
	M	6	14.00	12.50	10.00	21.00	4.29	30.64
SASS_5 score	A	6	62.67	63.00	29.00	99.00	26.03	41.53
	F	13	71.62	81.00	7.00	116.00	34.84	48.65
	M	6	71.33	60.00	37.00	117.00	32.97	46.22
SASS - ASPT	A	6	4.72	4.98	3.73	5.46	0.65	13.77
	F	13	4.99	4.95	2.33	7.25	1.24	24.91
	M	6	4.93	4.82	3.70	6.88	1.08	21.97
EPT_taxa	A	6	3.67	4.00	1.00	6.00	1.63	44.54
	F	13	5.00	4.00	1.00	12.00	3.21	64.29
	M	6	6.00	6.00	2.00	10.00	3.22	53.75
POET_taxa	A	6	3.67	4.00	1.00	6.00	1.63	44.54
	F	13	5.54	5.00	1.00	12.00	3.43	61.94
	M	6	6.33	6.00	2.00	11.00	3.67	57.94
COPTe_taxa	A	6	5.17	6.00	2.00	7.00	2.23	43.13
	F	13	7.23	6.00	1.00	13.00	4.00	55.36
	M	6	7.50	6.50	3.00	13.00	4.14	55.14
Coleo_taxa	A	6	1.50	1.00	0.00	3.00	1.22	81.65
	F	13	1.69	2.00	0.00	6.00	1.60	94.62
	M	6	1.17	1.50	0.00	2.00	0.98	84.27
Dipt_taxa	A	6	7.67	7.50	5.00	11.00	2.16	28.18
	F	13	7.15	7.00	1.00	12.00	2.85	39.88
	M	6	7.50	6.50	5.00	11.00	2.81	37.48
Chiro_taxa	A	6	3.67	4.00	3.00	4.00	0.52	14.08
	F	13	3.54	3.00	1.00	6.00	1.66	47.03
	M	6	3.67	3.00	2.00	7.00	1.75	47.76
SASS_6_15	A	6	3.83	4.50	1.00	6.00	1.94	50.63
	F	13	4.62	5.00	0.00	8.00	2.47	53.47
	M	6	4.83	3.50	1.00	10.00	3.43	70.97
SASS_8_15	A	6	1.83	2.00	0.00	4.00	1.72	93.95
	F	13	2.23	2.00	0.00	6.00	1.96	88.06
	M	6	2.67	2.00	0.00	7.00	2.50	93.87

In-stream characteristics

*Substrate type*

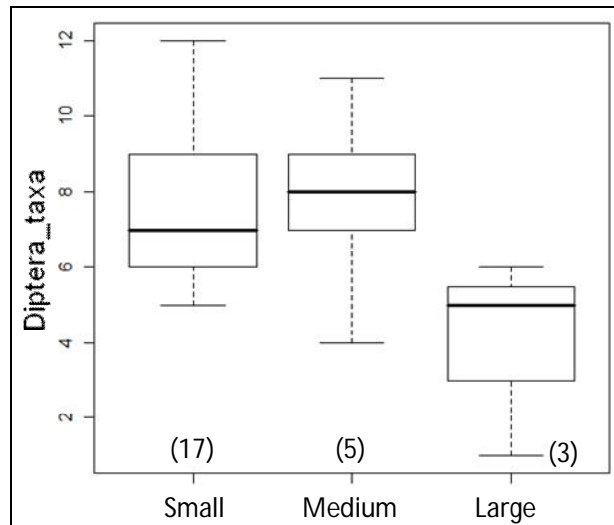
A significant difference among substrate types was demonstrated in SASS - ASPT (t-test,  $n = 25$ ,  $t = 4.4077$ ,  $d.f. = 24$ ,  $p < 0.001$ ), EPT\_taxa (t-test,  $n = 25$ ,  $t = 4.0989$ ,  $d.f. = 24$ ,  $p < 0.001$ ), POET\_taxa (t-test,  $n = 25$ ,  $t = 3.9737$ ,  $d.f. = 24$ ,  $p < 0.001$ ), COPTe\_taxa (t-test,  $n = 25$ ,  $t = 2.8821$ ,  $d.f. = 24$ ,  $p < 0.05$ ), SASS\_6\_15 (t-test,  $n = 25$ ,  $t = 2.6435$ ,  $d.f. = 24$ ,  $p < 0.05$ ), SASS\_8\_15 (t-test,  $n = 25$ ,  $t = 2.9025$ ,  $d.f. = 24$ ,  $p < 0.01$ )(Figure 4.14).



**Figure 4.14** Box (95% confidence interval) and whisker (max-min values) plot of macroinvertebrate single metrics between the substrate type, line represents the median value, dots the outliers and numbers at the bottom of Figure the total number of samples.

*River size*

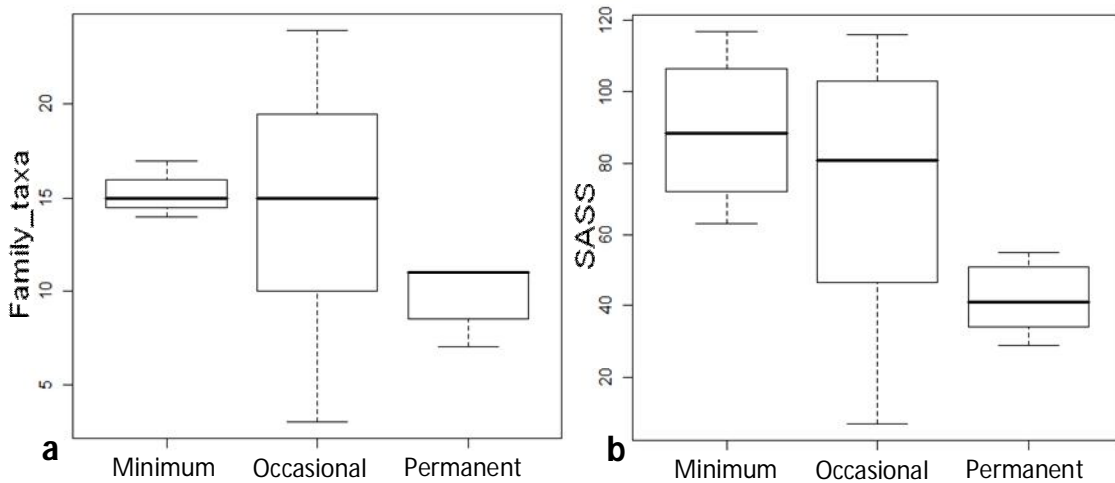
A significant difference among the river size classification was demonstrated just in Diptera\_taxa (One-way ANOVA,  $n = 25$ ,  $f = 3.6234$ ,  $p < 0.05$ ) (Figure 4.15).



**Figure 4.15** Box (95% confidence interval) and whisker (max-min values) plot of Diptera taxa among river size of the sampling sites, line represents the median value and numbers at the bottom of Figure the total number of samples.

*Level of disturbance*

Significant differences among level of disturbance classes was demonstrated in Family\_taxa (One-way ANOVA,  $n = 25$ ,  $F = 16.7377$ ,  $p < 0.001$ ), SASS score (One-way ANOVA,  $n = 25$ ,  $F = 10.8609$ ,  $p = 0.05$ ), SASS - ASPT (One-way ANOVA,  $n = 25$ ,  $F = 3.1905$ ,  $p = 0.05$ ), EPT\_taxa (One-way ANOVA,  $n = 25$ ,  $F = 3.4073$ ,  $p = 0.05$ ), POET\_taxa (One-way ANOVA,  $n = 25$ ,  $F = 3.9569$ ,  $p < 0.05$ ), COPTE\_taxa (One-way ANOVA,  $n = 25$ ,  $F = 3.7875$ ,  $p = 0.05$ ), SASS\_6\_15 (One-way ANOVA,  $n = 25$ ,  $F = 5.2742$ ,  $p < 0.05$ ), SASS\_8\_15 (One-way ANOVA,  $n = 25$ ,  $F = 6.5169$ ,  $p < 0.05$ ) (Figure 4.16).



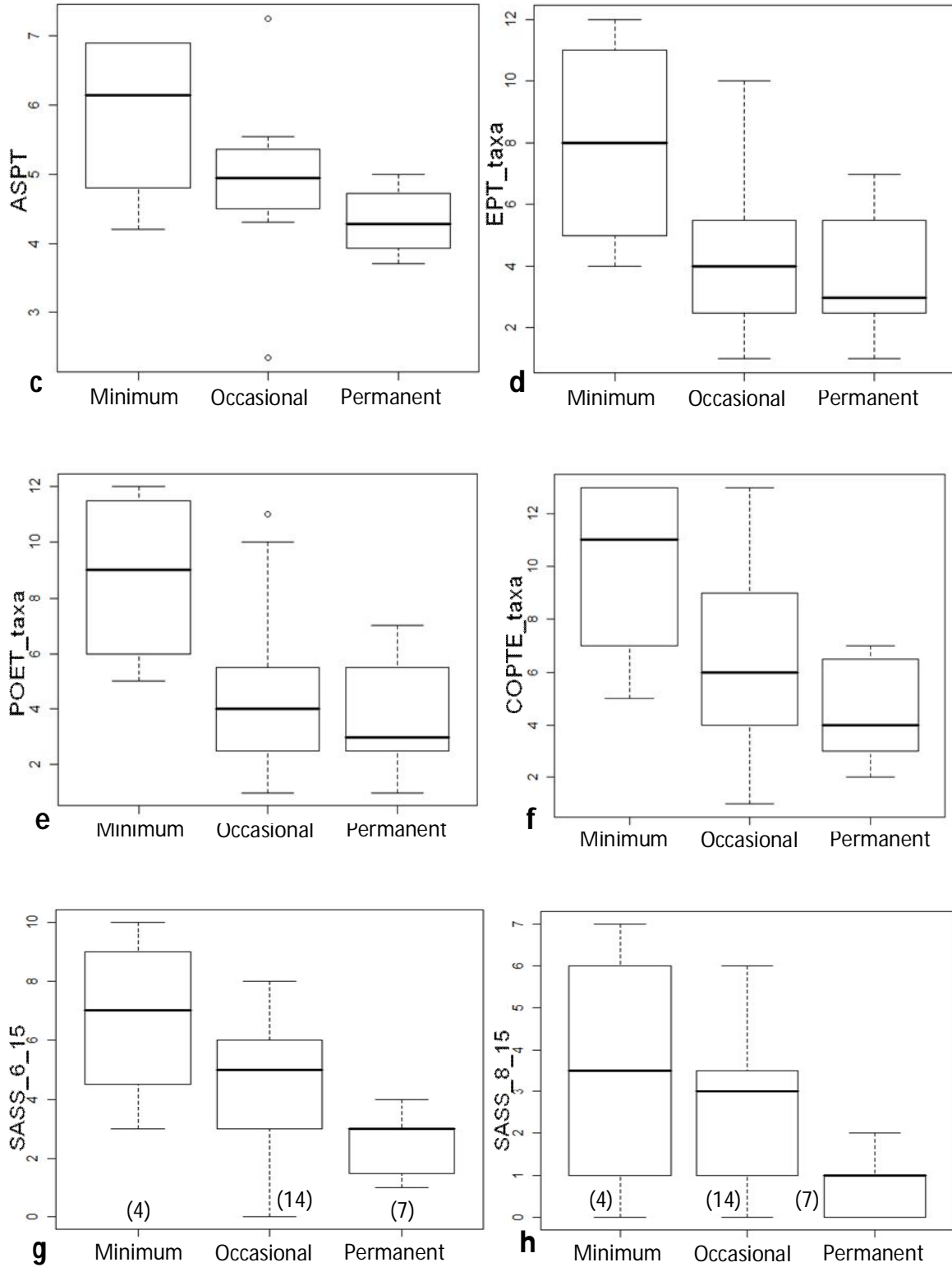


Figure 4.16 Box (95% confidence interval) and whisker (max-min values) plot of macroinvertebrate single metrics among level of disturbance observed at the sampling sites, line represents the median value, dots the outliers and numbers at the bottom of Figure the total number of samples

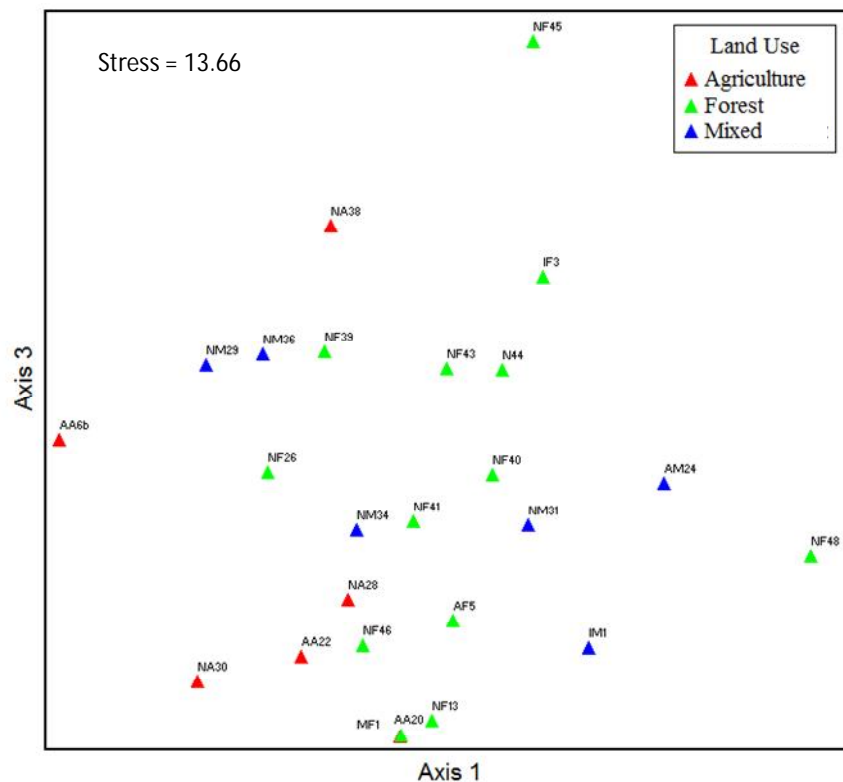
## Non-parametric Multidimensional Scaling (MDS or NMS)

NMS was based on Bray-Curtis dissimilarity matrices computed from  $\log(x+1)$ -transformed benthic taxa abundances.

The NMS of the benthic composition was subjected to different point colour coding to identify possible clusters and later tested for difference in location and dispersion using non-parametric permutational MANOVA (abbr. PERMANOVA) and a permutational analogue of the Levene-test (abbr. PERMDISP) (Anderson, 2006).

### Land use

The following Figure shows the location of the sites according to the benthic community structure, and the layout shows the land use types. It does not show increased similarities among replicates of the same land use type. The stress value is 13.66 which according to Borg & Groenen (2005) represent a fair value. A significant difference could not be demonstrated neither in dispersion (PERMDISP:  $F=0.1145$ ,  $df_1=2$ ,  $df_2=22$ ,  $p=0.89$ ) nor location (PERMANOVA:  $F=1.0890$ ,  $df_1=2$ ,  $df_2=24$ ,  $p=0.34$ ). The mantel statistic did not show a significant correlation between the matrix of benthic community structure and the matrix of physical chemical ( $r=0.1225$ ,  $p=0.16$ ) and environmental parameters ( $r=0.2232$ ,  $p=0.08$ ).



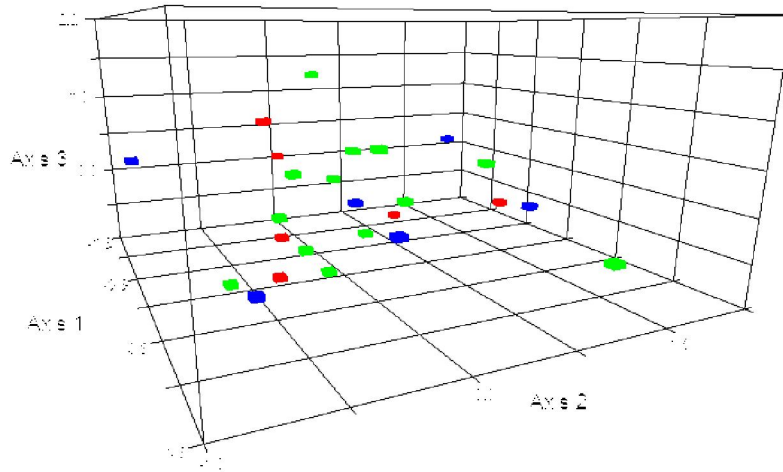


Figure 4.17 NMS 2-D and 3-D of the 25 sampling sites (November – December 2009) using clustering of Bray – Curtis similarities on  $\log(x+1)$  transformed benthic taxa abundances, point colour coding on land use (▲ Agriculture, ▲ Forest and ▲ Mixed land use).

#### In-stream characteristics

##### *Substrate type*

Figure 4.18 shows the location of the sites according with the benthic community structure and the layout shows the substrate type. A significant difference could not be demonstrated in dispersion (PERMDISP:  $F=0.9565$ ,  $df_1=1$ ,  $df_2=23$ ,  $p=0.33$ ) but there is significant difference in location among the substrate type (PERMANOVA:  $F=1.7876$ ,  $df_1=1$ ,  $df_2=24$ ,  $p<0.05$ ).



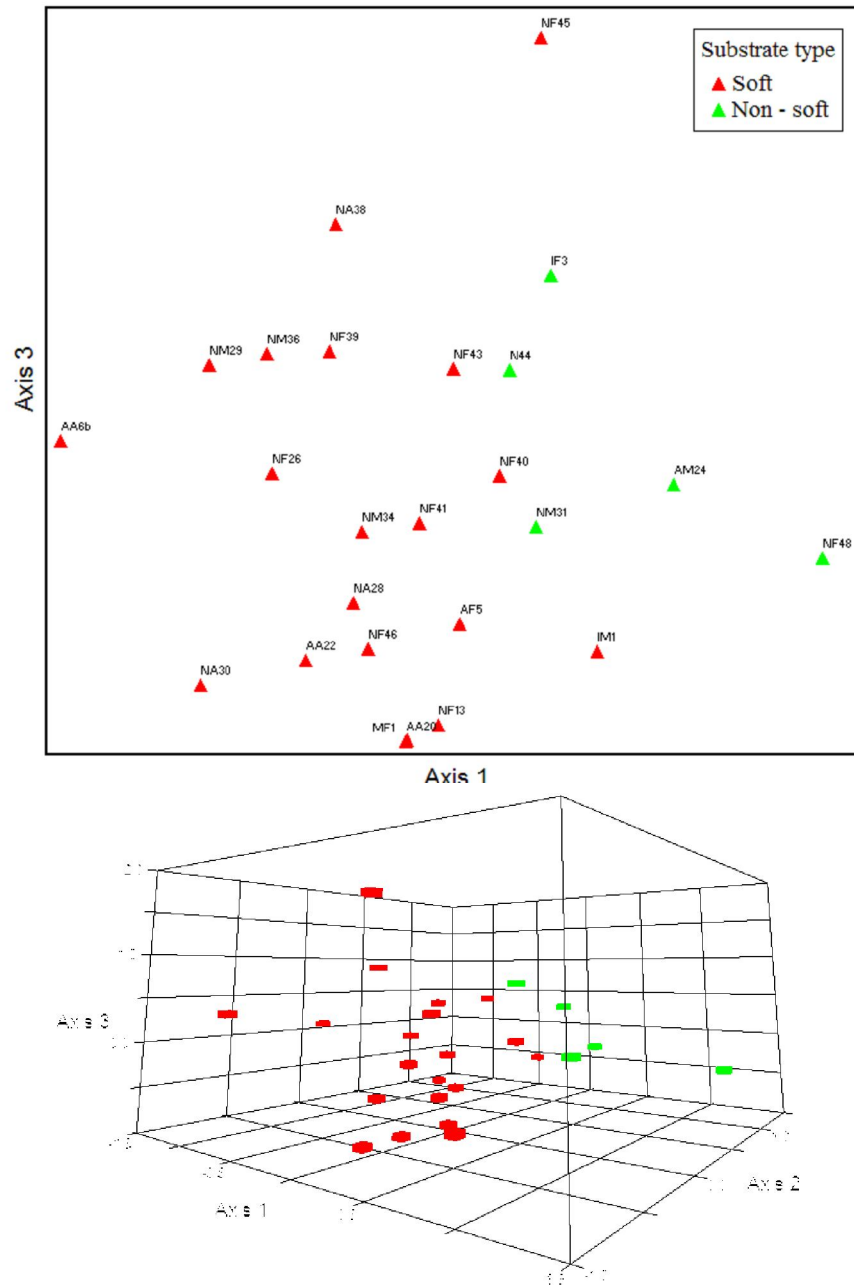
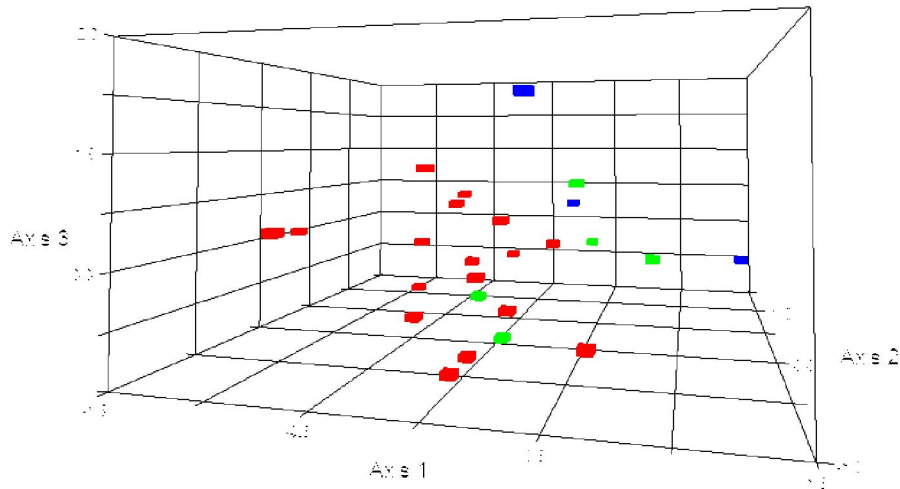
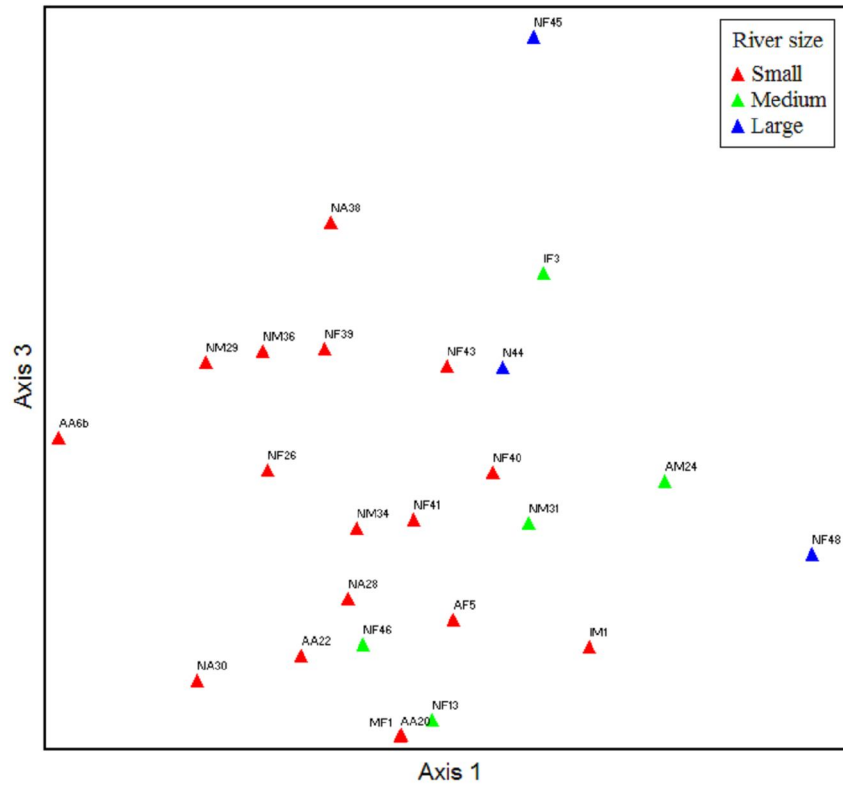


Figure 4.18 NMS 2-D and 3-D of the 25 sampling sites (November – December 2009) using clustering of Bray – Curtis similarities on  $\log(x+1)$  transformed benthic taxa abundances, point colour coding on substrate type (▲ Soft (sandy & muddy) and ▲ non-soft (predominantly stony))

#### River size

Figure 4.19 shows the location of the sites according with the benthic community structure and the layout shows the river size. A significant difference could not be demonstrated in dispersion (PERMDISP:  $F=1.4242$ ,  $df_1=2$ ,  $df_2=22$ ,  $p=0.26$ ) and not significant difference in location among the different river size (PERMANOVA:  $F=1.0639$ ,  $df_1=2$ ,  $df_2=24$ ,  $p=0.35$ ).



**Figure 4.19 NMS 2-D and 3-D of the 25 sampling sites (November – December 2009) using clustering of Bray – Curtis similarities on  $\log(x+1)$  transformed benthic taxa abundances, point colour coding on river size (▲ small, ▲ medium and ▲ large)**

*Level of disturbance*

Finally the level of disturbance was also considered for layout in the NMS of the benthic community structure. A significant difference could not be demonstrated in dispersion (PERMDISP:  $F=1.5919$ ,  $df_1=2$ ,  $df_2=22$ ,  $p=0.22$ ) but it shows significant difference in location among the sampling sites for different level of disturbance (PERMANOVA:  $F=1.6022$ ,  $df_1=2$ ,  $df_2=24$ ,  $p<0.05$ ).

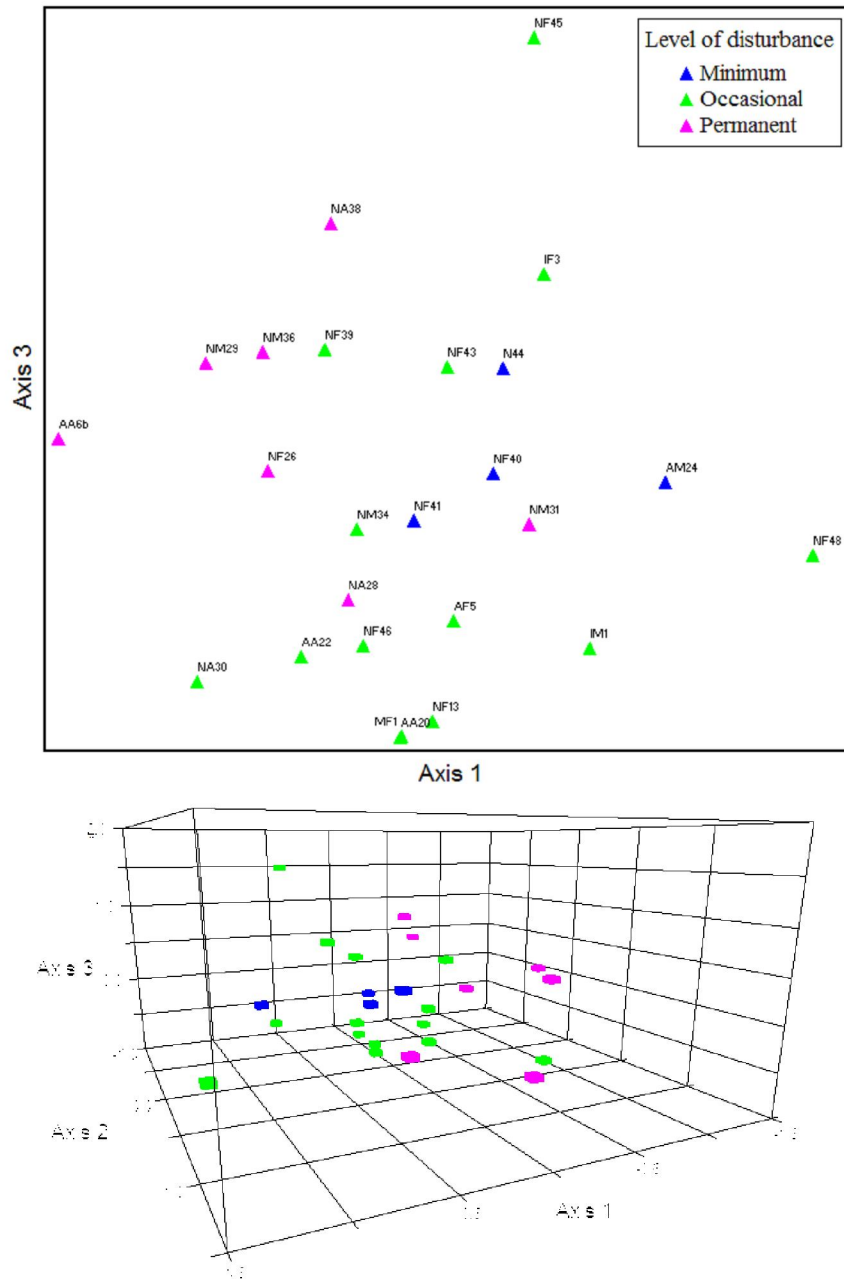


Figure 4.20 NMS 2-D and 3-D of the 25 sampling sites (November – December 2009) using clustering of Bray – Curtis similarities on  $\log(x+1)$  transformed benthic taxa abundances, point colour coding on level of disturbance (▲ minimum, ▲ occasional and ▲ permanent)

### Rapid Field Screening

The rapid field screening based on sensory features, ferro sulphide reductions, presence of bacteria, fungi, periphyton and a list of benthic macroinvertebrates assessed the water quality classes for each of the sampling sites, 6 sites belonged to WQ class I, 8 to class II, 9 to class III, 1 to class IV and 1 to class V (Table 4.5). A significant difference was demonstrated in dispersion (PERMDISP:  $F=12.709$ ,  $df_1=4$ ,  $df=24$ ,  $p<0.001$ ) and it also shows significant difference in location among the sampling sites

for the different water quality classes (PERMANOVA:  $F=2.3585$ ,  $df_1=4$ ,  $df_2=24$ ,  $p<0.001$ ). (Figure 4.21)

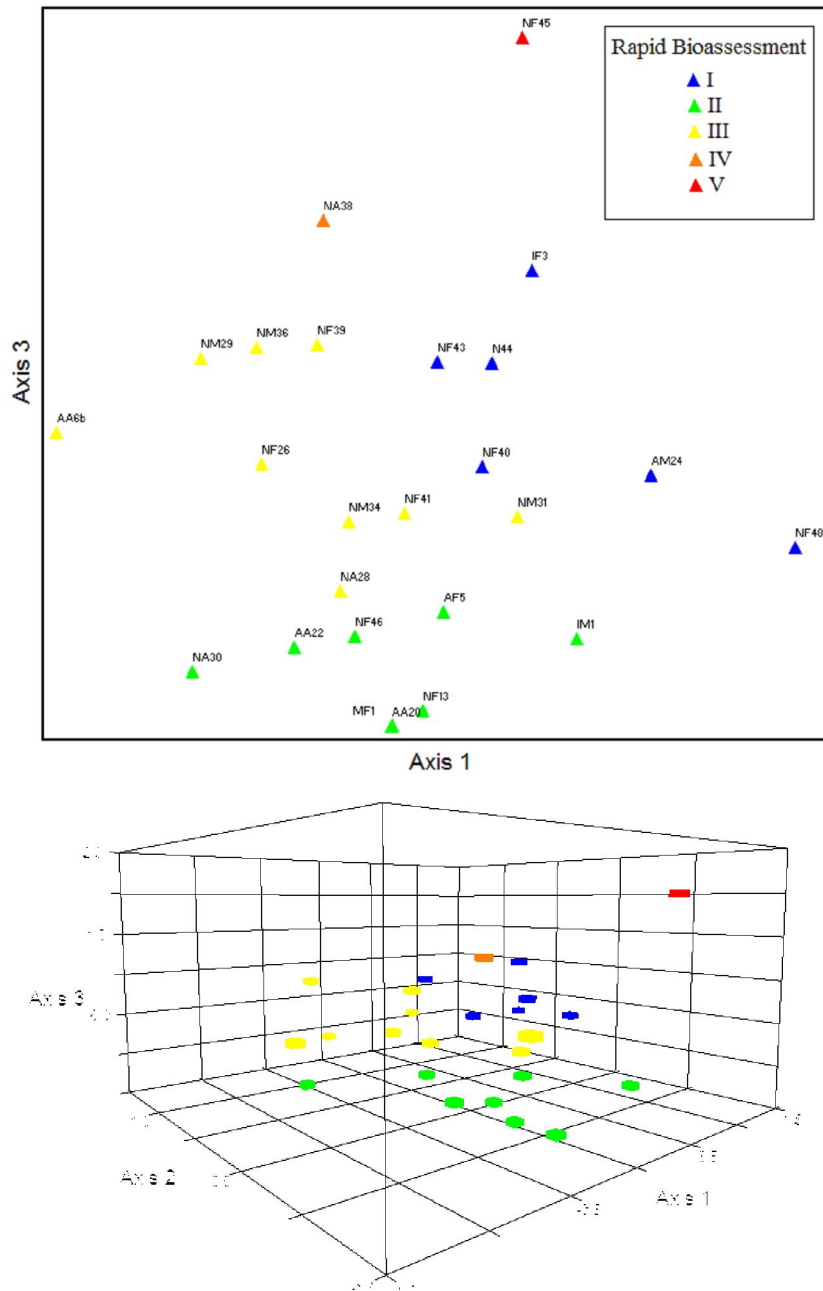
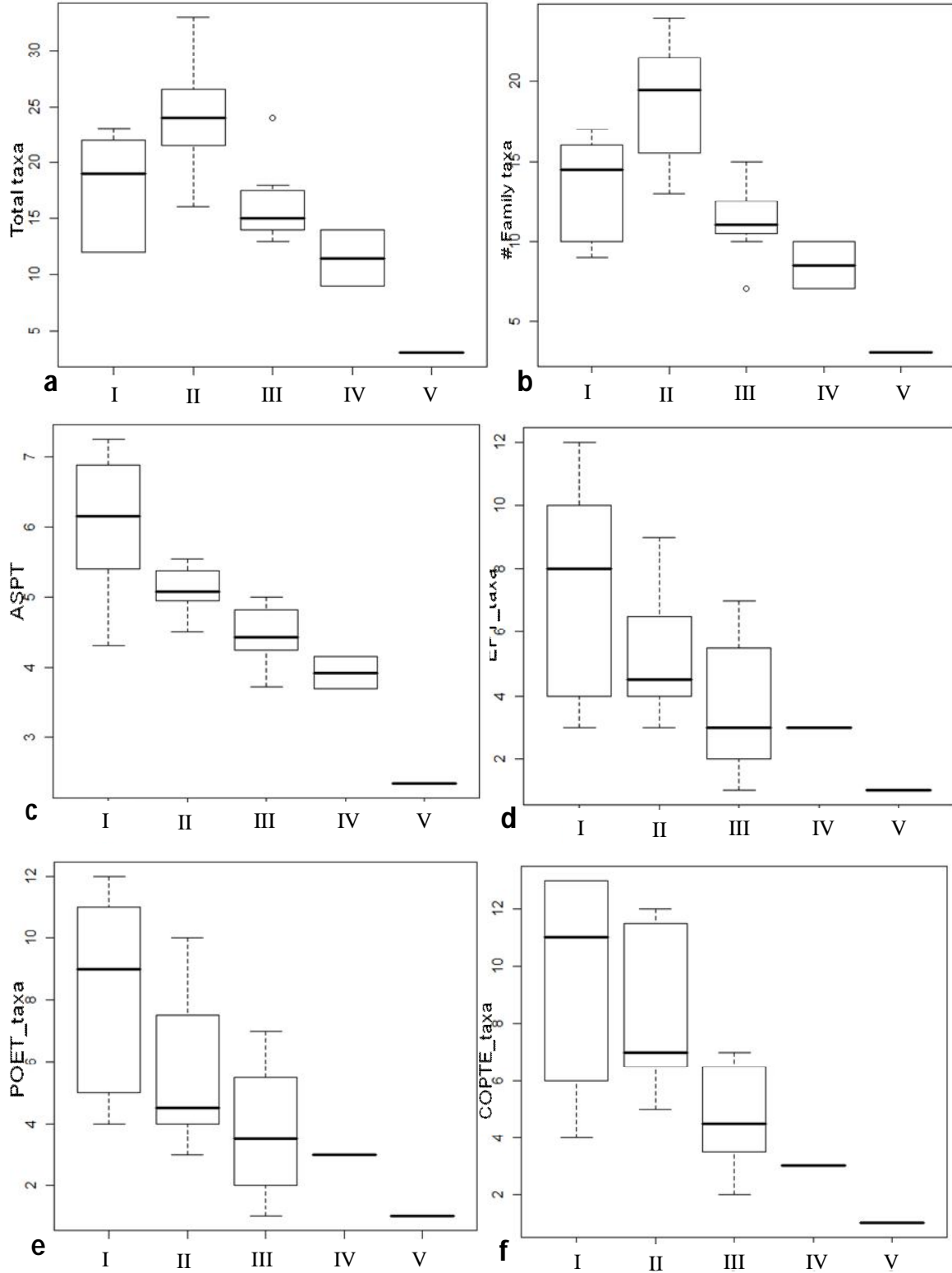


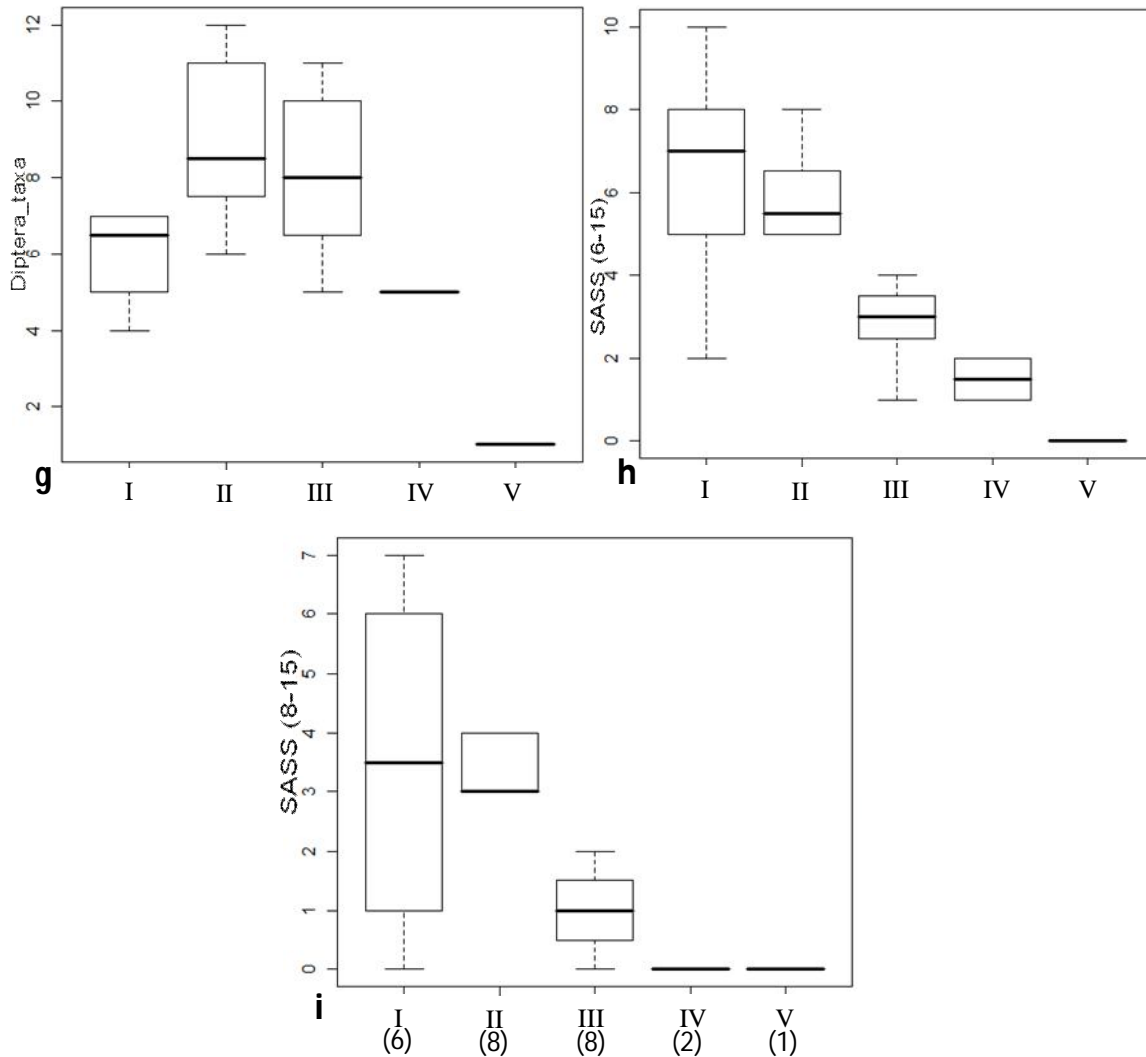
Figure 4.21 NMS 2-D and 3-D of the 25 sampling sites (November – December 2009) using clustering of Bray – Curtis similarities on  $\log(x+1)$  transformed benthic taxa abundances, point colour coding on water quality classes by rapid field screening method (▲ WQ class I, ▲ WQ class II, ▲ WQ class III, ▲ WQ class IV and ▲ WQ class V)

**Table 4.5 Water quality classes of the sampling sites based on the rapid field screening method**

Land Use	Site	Rapid screening
Agricultural	AA20	II
	AA22	II
	AA6b	III
	NA28	III
	NA30	II
	NA38	IV
Forest	AF5	II
	IF3	I
	MF1	II
	N44	I
	NF13	II
	NF26	III
	NF39	III
	NF40	I
	NF41	III
	NF43	I
	NF45	V
	NF46	II
	NF48	I
	Mixed	AM24
IM1		II
NM29		IV
NM31		III
NM34		III
NM36		III

A Two-factorial analysis of variance showed a significant difference among water quality classes (Rapid screening) in total taxa (Two-way ANOVA, n = 25, F= 6.9445, p<0.01), Family\_taxa (Two-way ANOVA, n = 25, F= 6.1610, p <0.01), SASS - ASPT (Two-way ANOVA, n = 25, F= 11.4581, p < 0.001), EPT\_taxa (Two-way ANOVA, n = 25, F= 3.6366, p<0.05), POET\_taxa (Two-way ANOVA, n = 25, F= 3.5672, p < 0.05), COPTE\_taxa (Two-way ANOVA, n = 25, F= 4.2797, p < 0.05), Diptera\_taxa (Two-way ANOVA, n = 25, F= 5.5922, p < 0.01), SASS\_6\_15 (Two-way ANOVA, n = 25, F= 5.7686, p < 0.01), SASS\_8\_15 (Two-way ANOVA, n = 25, F= 5.1295, p < 0.05) (Figure 4.22), Width (Two-way ANOVA, n = 25, F= 3.3837, p < 0.05), Q (Two-way ANOVA, n = 25, F= 3.2996, p < 0.05)





**Figure 4.22** Box (95% confidence interval) and whisker (max-min values) plot of macroinvertebrate single metrics among water quality classes based on the rapid field screening method, line represents the median value, dots the outliers and numbers at the bottom of Figure the total number of samples

### South African Score System (SASS\_5)

The interpretation of the SASS\_5 score in conjunction with the ASPT was explained before in subchapter 3.2.2 and allocated 2 sites in WQ class I, 1 site in class II, 4 sites in class III, 10 sites in class IV and 8 sites in class V. The results are shown in Table 4.6 and Figure 4.23. Sites that show a natural water quality and high diversity habitat are NF48 and AM24 sites that belong to forest and mixed land use respectively.

Table 4.6 SASS\_5 scores and ASPT values for the 25 sampling sites

Land Use	Site	SASS score	# Taxa	SASS ASPT	SASS 5 - WQC
Agricultural	AA20	99	20	5	III
	AA22	81	16	5.1	IV
	AA6b	41	11	3.7	V
	NA28	55	11	5	IV
	NA30	71	13	5.5	IV
	NA38	29	7	4.1	V
Forest	AF5	94	19	4.9	IV
	IF3	49	9	5.4	IV
	MF1	108	24	4.5	III
	N44	96	14	6.9	II
	NF13	116	22	5.3	III
	NF26	31	7	4.4	V
	NF39	44	10	4.4	V
	NF40	81	15	5.4	IV
	NF41	63	15	4.2	IV
	NF43	43	10	4.3	V
	NF45	7	3	2.3	V
	NF46	83	15	5.5	IV
	NF48	116	16	7.3	I
	Mixed	AM24	117	17	6.9
IM1		107	21	5.1	III
NM29		37	10	3.7	V
NM31		55	11	5	IV
NM34		65	14	4.6	IV
NM36		47	11	4.3	V



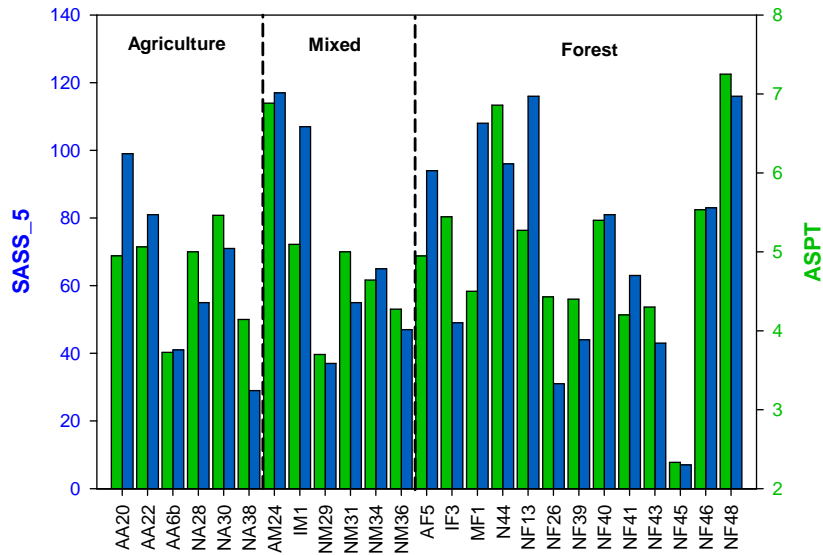
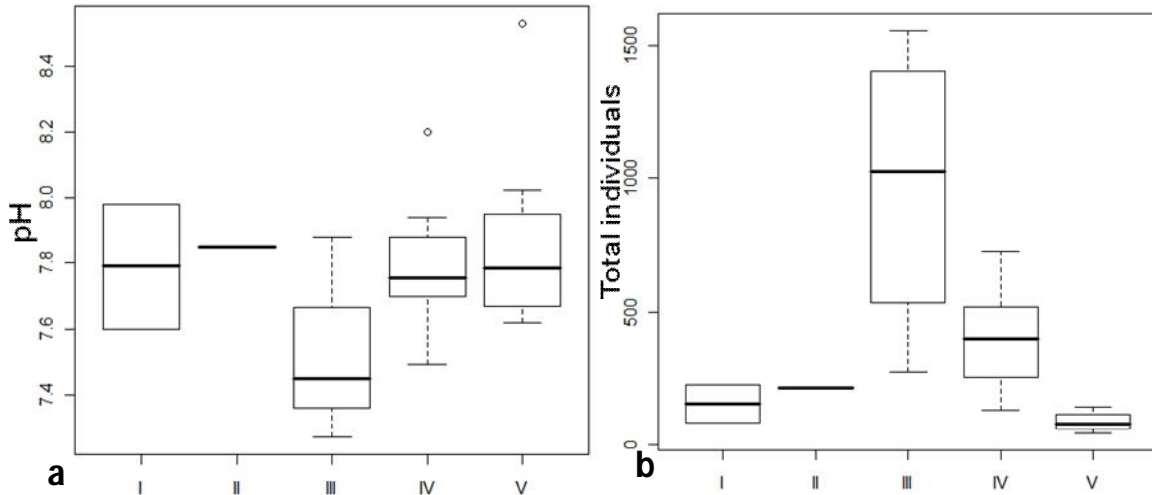
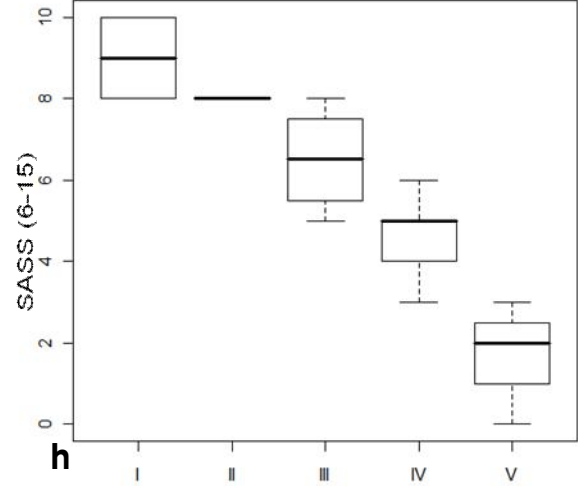
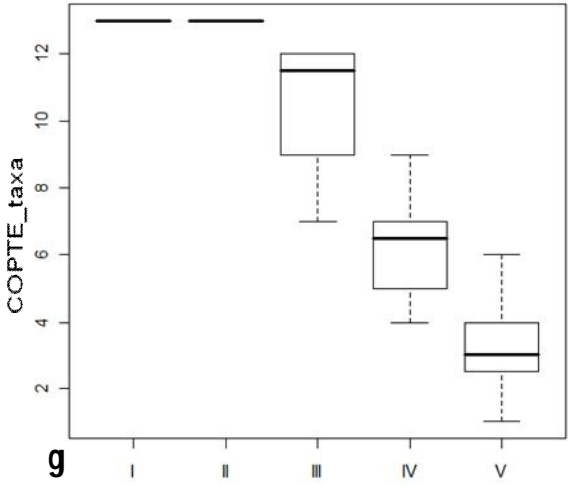
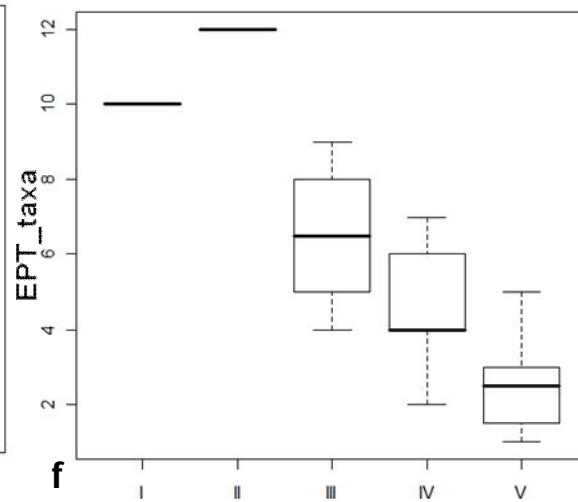
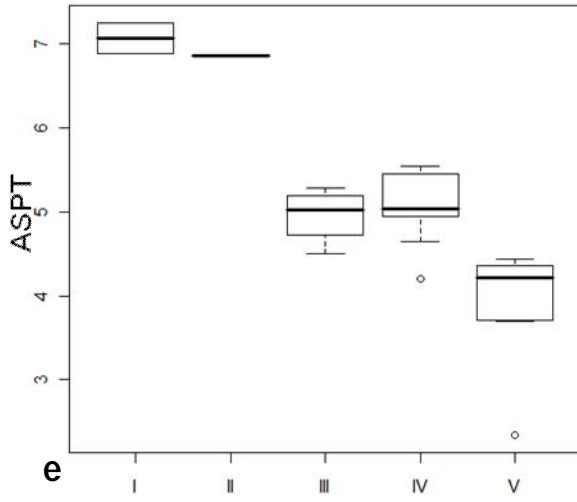
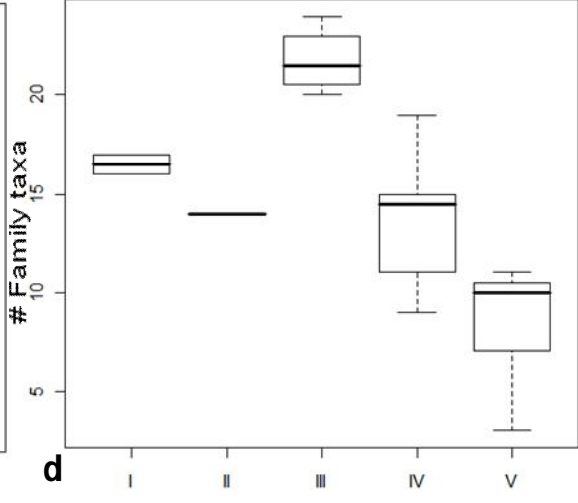
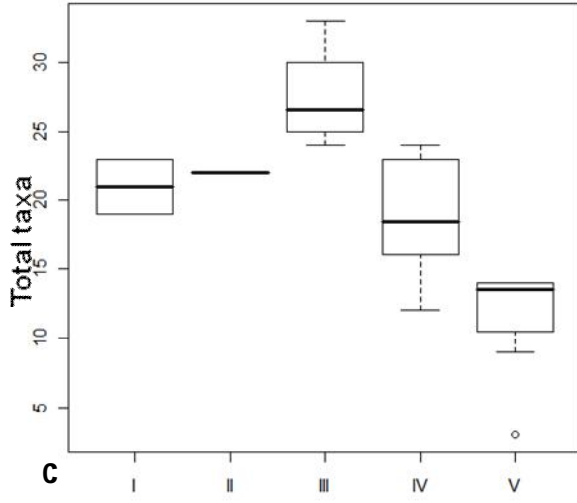


Figure 4.23 SASS\_5 scores and ASPT across the sampling sites among the land use types

A Two-factorial analysis of variance showed a significant difference among water quality classes (SASS\_5 WQC) in pH (Two-way ANOVA,  $n = 25$ ,  $F = 5.0560$ ,  $p < 0.05$ ), total abundance (Two-way ANOVA,  $n = 25$ ,  $F = 11.6184$ ,  $p < 0.001$ ), total taxa (Two-way ANOVA,  $n = 25$ ,  $F = 17.5189$ ,  $p < 0.001$ ), Family\_taxa (Two-way ANOVA,  $n = 25$ ,  $F = 20.8995$ ,  $p < 0.001$ ), SASS - ASPT (Two-way ANOVA,  $n = 25$ ,  $F = 37.3607$ ,  $p < 0.001$ ), EPT\_taxa (Two-way ANOVA,  $n = 25$ ,  $F = 13.5159$ ,  $p < 0.001$ ), COPTE\_taxa (Two-way ANOVA,  $n = 25$ ,  $F = 26.6475$ ,  $p < 0.001$ ), SASS\_6\_15 (Two-way ANOVA,  $n = 25$ ,  $F = 37.5708$ ,  $p < 0.001$ ), SASS\_8\_15 (Two-way ANOVA,  $n = 25$ ,  $F = 27.7183$ ,  $p < 0.001$ ) (Figure 4.24), Width (Two-way ANOVA,  $n = 25$ ,  $F = 12.1061$ ,  $p < 0.001$ ), Q (Two-way ANOVA,  $n = 25$ ,  $F = 11.228$ ,  $p < 0.001$ )





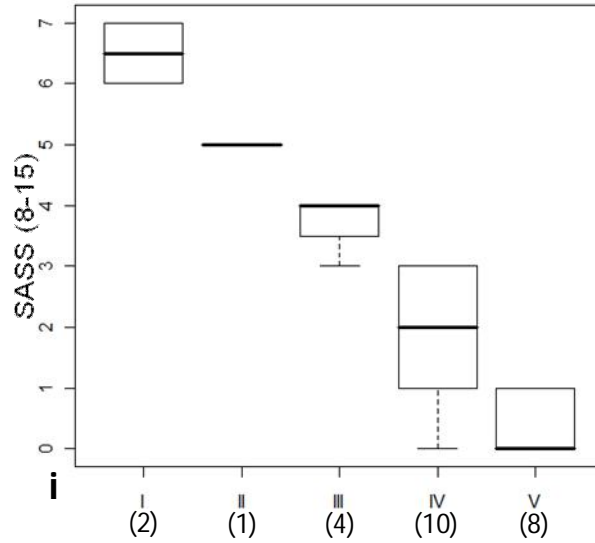
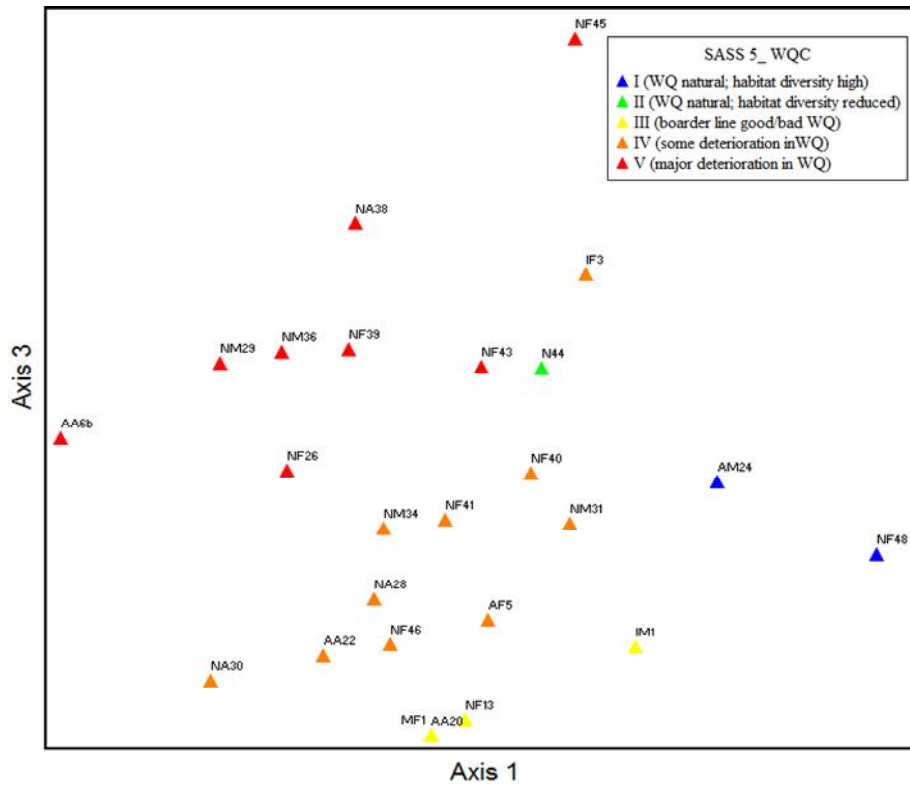


Figure 4.24 Box (95% confidence interval) and whisker (max-min values) plot of macroinvertebrate single metrics among water quality classes based on the South African score system (SASS\_5), line represents the median value, dots the outliers and numbers at the bottom of Figure the total number of samples

Significant differences were demonstrated among water quality classes (SASS\_5 WQC) in dispersion (PERMDISP:  $F=10.542$ ,  $df_1=4$ ,  $df_2=24$ ,  $p<0.001$ ) and it also shows significant difference in location among the sampling sites for the different water quality classes (PERMANOVA:  $F=2.2613$ ,  $df_1=3$ ,  $df_2=24$ ,  $p<0.001$ ). (Figures 4.25)



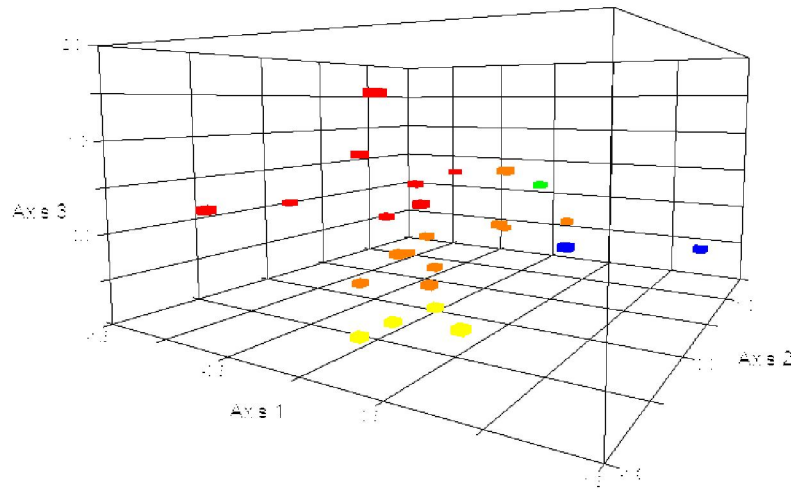


Figure 4.25 NMS 2-D and 3-D of the 25 sampling sites (November – December 2009) using clustering of Bray – Curtis similarities on log (x+1) transformed benthic taxa abundances, point colour coding on water quality classes by SASS method (▲ WQ class I, ▲ WQ class II, ▲ WQ class III, ▲ WQ class IV and ▲ WQ class V)

### Linking biological with physical – chemical and environmental parameters

A Spearman rank-order correlation was performed in order to find possible relationships between single macroinvertebrate metrics and biological scores with physical–chemical and hydro-morphological parameters. Table 4.7 shows the scoring criteria used based on the 1, 3, 5 scoring system, which is commonly used in developing fish and macroinvertebrate Index of Biotic Integrity (IBI) (Barbour et al., 1999).

Table 4.8 shows the correlations calculated and the level of significance (Significance codes: '\*\*\*\*' 0.001 '\*\*\*' 0.01 '\*\*' 0.05).

**Table 4.7 Parameters with scores based on the 1, 3, 5 scoring system**

Parameter	Scoring criteria		
	5	3	1
Land Use	Agriculture (n=6)	Mixed (n=6)	Forest (n=13)
Substrate type	-	Non-soft (n=20)	Soft (n=5)
River size	Large (n=17)	Medium (n=5)	Small (n=3)
Level of disturbance	Permanent (n=7)	Occasional (n=14)	Minimum (n=4)

From Table 4.8 can be inferred that turbidity, total suspended solids and PO<sub>4</sub> have a negative correlation with the number of family taxa, SASS score and other macroinvertebrate single metrics. The size of the river which is a proxy of width and flow has a positive correlation with the SASS - ASPT (average score per taxon). Temperature was found to have a negative correlation on the specific taxa of the Coleoptera and Diptera orders.

Table 4.8 Spearman rank correlation between physical-chemical and hydro-morphological parameters with biological scores and single metrics

	LU	Conduc	DO%	Temp	Turbid.	Alkal	PO4	TSS	Famil	SASS	SASS	EPT_	POET_	COPT	SASS_	Widt	Dept	Q	SO	Subs	Screen
LU	1																				
Conduc	0.59**	1																			
DO_con	0.04	-0.34	0.95***																		
Turbid	0.55**	0.36	-0.15	0.46*	1																
Alkal	0.5*	0.82***	-0.09	0.19	0.13	1															
NH4	0.34	0.76***	-0.26	0.11	0.42*	0.69***															
TSS	0.33	0.21	-0.25	0.23	0.86***	-0.09	0.53**	1													
taxa	0	0.29	0.01	0.26	-0.50*	0.45*	0.50*	-0.6**													
Eve	0.04	-0.37	0.23	0.24	-0.04	-0.34	0.1	-0.07													
Family	0.02	0.26	-0.06	0.24	-0.54**	0.44*	0.50*	-0.6**	1												
SASS	0.12	0.04	0.17	0.21	-0.56**	0.28	0.41	-0.7***	0.93***	1											
SASS	0.11	-0.21	0.35	0.02	-0.31	-0.04	0.04	-0.43*	0.50**	0.75***	1										
ASPT	0.08	-0.16	0.03	0.14	-0.24	-0.03	0.21	-0.38	0.59**	0.75***	0.75***	1									
EPT_	0.17	-0.23	0	0.14	-0.28	-0.09	0.16	-0.38	0.55**	0.73***	0.73***	0.99***	1								
POET_	0.14	-0.1	0.1	0.04	-0.44	0.08	0.29	-0.55**	0.75***	0.89***	0.81***	0.94***	0.92***	1							
COPT	0.14	-0.1	0.1	0.04	-0.44	0.08	0.29	-0.55**	0.75***	0.89***	0.81***	0.94***	0.92***	0.92***	1						
Coleo_	0.05	0.27	0.14	0.42*	-0.50*	0.45*	0.33	-0.5*	0.75***	0.70***	0.50*	0.26	0.2	0.55**							

	LU	Cond	DO%	Temp	Turb.	Alkal.	PO4	TSS	Family	SASS score	SASS ASPT	EPT_taxa	POET_taxa	COPTES_taxa	SASS_6_15	Width	Depth	Q	SO	Subs type	Screening	
Dipt_taxa	0.03	0.35	0.04	0.54**	-0.29	0.44*	-0.21	-0.25	0.51**	0.34	0.03	-0.09	-0.12	0.09								
Chiro_taxa	0.06	0.18	0.03	0.34	0.01	0.18	0.03	0.01	0.19	0.09	0.02	-0.01	-0.07	0.12								
SASS_6_15	-	-	-	-	-0.50*	-	-	-0.62***	0.76***	0.92***	0.90***	0.79***	0.78***	0.91***	1							
SASS_8_15	0.12	-0.11	0.28	0.09	-0.50*	0.12	0.25	-0.64***	0.77***	0.92***	0.83***	0.72***	0.66***	0.86***	0.93***							
Width	0.04	0.02	0.37	-0.2	*	0.25	0.33	***	***	***	***	***	***	***	***	0.2	1					
Q	0.46*	-0.54**	0.14	0.17	-0.3	-0.41	0.22	-0.35	-0.02	0.2	0.36	0.42	0.44	0.34	0.2	1	0.77***	0.56**	1			
SO	0.50*	-0.58**	0.26	0.15	-0.21	-0.38	0.21	-0.24	-0.12	0.07	0.12	0.25	0.27	0.15	0.06			0.58**	0.65***	0.75***	1	
Canopy	-	-	-	0.50*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Disturb	0.38	0.06	-0.19	-0.33	0.12	0.05	-0.15	0.18	0.07	-0.13	-0.02	0.05	0.08	0.02	0.26	0.28	0.37	0.32				
Substrate	0.45*	0.42*	-0.16	0.3	0.75***	0.16	0.22	0.64***	-0.50**	-0.58**	-0.50*	-0.36	-0.42	-0.46*	-0.58**	0.24	0.32	0.16	-0.2			
size_river	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Screening	0.17	-0.40*	0.44*	0.17	-0.04	-0.15	0.07	-0.17	0.01	0.31	0.58**	0.55**	0.57**	0.50*	0.41	0.82***	0.67***	0.81***	0.78***	0.72***	-0.3	
SASS_WQC	0.40*	-0.55**	0.3	0.23	-0.25	-0.37	0.21	-0.36	0	0.27	0.50*	0.44	0.44	0.38	0.36	0.22	0.39	0.08	0.22	*	1	
	0.32	0.20	-0.16	0.15	0.45*	-0.02	0.04	0.46*	-0.50**	-0.68***	-0.78***	-0.59**	-0.64***	-0.71***	-0.77***	0.22	0.39	0.08	0.22	*	1	
	0.08	0.05	-0.26	-0.2	0.5**	-0.15	0.36	0.59**	-0.80***	-0.94***	-0.78***	-0.8***	-0.79***	-0.9***	-0.91***	0.29	0.42	-0.2	0.32	-0.5*	0.66***	

Significance codes: '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05

## 5 Discussion

The results of this study demonstrate that the term “Land use” and its classification into Agriculture, forest and mixed land use were too general and rough way to predict the effect on the local benthic macroinvertebrate community. Land use as a factor is a proxy that may represent an interaction of many controlling variables that describe the in stream characteristics. In trying to examine the manifold aspects, this study observed that the substrate type, level of disturbance and the size of the River, were the best predictors of benthic macroinvertebrate community composition. Statistical analysis could not reflect any direct responses of macroinvertebrate assemblage to changes in land use. However, it is important to note that significant differences among land use types were found at the respective site groups for conductivity, turbidity and total suspended solids which are good integrators of in-stream characteristics. In addition, a Spearman rank correlation test showed a positive correlation between land use and physical-chemical parameters like conductivity, turbidity, alkalinity and the level of disturbance observed at site. These results are in line with the findings of Kasangaki et al. (2008), who found that clearance of forest was endangering freshwater systems in East Africa. Removal of riparian vegetation has also been found to lead to modification of stream hydraulics, substrate features, light and thermal system, water chemistry composition and organic matter contribution which all together affects the riverine communities (Giller & Malmqvist, 1998; Osano et al., 2003; Okungu & Opango, 2005) Based on the findings of this study the three drivers; substrate type, level of disturbance and size of the river can be singled out as the most determinant drivers of macroinvertebrate assemblage.

### 5.1 General hydro-morphological patterns and changes with stream order and land use

Most sampling sites were within first to third order streams with an exception of three forest sites in the fourth order and one mixed site in the fifth order. Based on hydro-morphological measurements and comparing second stream orders for the 3 land use types, agricultural catchments were found to have a smaller width in comparison to the forest and mixed land uses. This may be attributed to human activities that have led to channel modifications and probably over-abstractions within this land use type. Similar results were recorded along the Njoro River on the northern part of Mau forest in Kenya where the watering of livestock was found to influence especially small streams (Yillia et al., 2009)

Another important metric that influences the in-stream processes is canopy cover. Based on the estimates during fieldwork, some agricultural sites had dense riparian vegetation sometimes up to 70%. Although these were composed of medium-size trees and eucalyptus trees which have questionable benefits in water catchment areas, the overall short term effect on the stream abiotic conditions may simulate the forested catchments. In contrast the forest sites had a less variable canopy cover which was estimated to vary from 70% to >90%. Another factor that may have contributed to less impact on some of the agricultural and mixed land use streams in certain stretches was a narrow width with steep sided banks that were not reachable by livestock. However in areas where wide pools existed, human activities and livestock watering dominated. In some forest areas within the Nyangores, where settlements were bordering the forest, the forest was

highly disturbed by human activities like clandestine breweries and illegal logging of timber, fuel wood, and livestock grazing. In some areas, the absence of any form of control by the Forestry Department was evident.

## **5.2 Physical – chemical parameters**

### *Conductivity*

This study identified significant differences in conductivity among the sites when classified by substrate type, size of the river and level of disturbance. Other physical and chemical parameters related to conductivity namely TSS and turbidity were also significant among land use types (Figure 4.2 to 4.4). A significant negative correlation was found between conductivity and substrate type, as well as size of the river and a significant positive correlation with level of disturbance (Table 4.8). This points to a direct link between land use change and substrate type, river size and level of disturbance. It is therefore evident that though land use change is affecting the substrate type, river size and level of disturbance, the level to which land use affects these in-stream characteristics is variable among the land uses. However, based on the correlations, these results suggest that the effects have an impact on the water chemistry. Similar studies by Kibichii et al. (2007) and Kasangaki et al. (2008) attributed high values of conductivity to near and in-stream activities.

At the subcatchment of similar geological composition conductivity was identified as a good discriminator to assess the level of impact and damage of the buffering capacity of the streams from anthropogenic activities. In the Mara streams, the highest conductivity value was found in agricultural site AA6b (404.4 mmol/l), which was 8 times higher than the lowest conductivity recorded at site N44 (50.2 mmol/l) located in forest land use. Conductivity was positively correlated with NH<sub>4</sub>-N, Alkalinity and level of disturbance observed at site and negative correlated with the size of the river which also meets the criteria and the findings described above.

### *Total Suspended Solids and Turbidity (TSS)*

The results demonstrated significant differences in TSS when the sampling sites were sorted by river size and level of disturbance, while turbidity showed significant differences just in the level of disturbance classification. In terms of correlation TSS and turbidity were positively correlated with the level of disturbance observed at site. The highest TSS value was recorded in agricultural site AA6b (199.41 mg/l), which is 23 times higher than the lowest TSS value recorded at site NM24 (8.53 mg/l) located in mixed land use. Without any doubt high levels in conductivity, TSS and turbidity are associated with some level of degradation and disturbance at site as result of the human activities (Kibichii et al., 2007; Kasangaki et al., 2008).

### *Dissolved Oxygen*

This parameter was found to be significantly different between sites of different substrate. The correlation test between them also implied that the dissolved oxygen saturation decreases when the bed sediment changes from stony substratum to soft sediments. Human settlements, urbanization and other pressures have been found to provoke responses in the water chemistry as well as the reduction of dissolved oxygen levels (Ndaruga et al., 2004)



## *Ammonia-Nitrogen*

NH<sub>4</sub>-N was significantly different among sites of different stream sizes. The results showed that higher concentration values of NH<sub>4</sub>-N were found in rivers of small size. The large amount of livestock densities most likely lead to a high amount of ammonia loads in the rivers due to the runoff from grazed fields, which corresponds to some level of degradation of the riparian areas (Kibichii et al., 2007). These trends might be holding the key to variations in the characteristics of the macro-invertebrate assemblages in the three land use types, even though dissolved oxygen and ammonia-nitrogen were not statistically significant.

### **5.3 Linking land use effects and macroinvertebrate single metrics**

I used cluster analysis (NMS ordination) to trace the similarities of the sampling sites. Sites clustered most strongly according with substrate type, river size and the level of disturbance observed at site leading to the conclusion that these variables could be used as good indicators of stream degradation as they are main products of the change in land use.

#### *Substrate type*

Substrate is a determinant of benthic communities' abundance and distribution due to the different morphological features like case-building and behavioural preferences for respiration and food-gathering mechanisms (Moog, 2009). The layout of the different substrate type on the benthic community composition showed clear the scatter plots in the NMS based on the Bray-Curtis similarity matrix. Further statistical analysis showed significant difference in location (PERMANOVA,  $p < 0.05$ ). Similarly, a significant difference between sites of different substrate types was demonstrated in macroinvertebrate single metrics like SASS - ASPT, EPT\_taxa, POET\_taxa, COPTE\_taxa, SASS\_6\_15 and SASS\_8\_15 (Figure 4.14). These results clearly point the effect that the bed sediment and by extension substrate type has on the benthic community composition. Similar results have been recorded by various initial studies (Stark, 1993; Giller & Malmqvist, 1998; Iwata et al., 2003). Likewise, Allan (1995) states that the diversity and abundance of benthic macroinvertebrates increase with median particle size but a broad substrate type may lead to a larger abundance of benthic invertebrates (Resh et al., 1995). In addition, the homogenization of benthic habitats has been shown to reduce species diversity on lotic organisms (Cohen et al., 1993). This may explain the poor number of taxa found in some site in Nyangores (NF45) where the substrate is mainly composed by silt.

#### *River size*

River size as described by discharge and stream width was identified as another important in-stream characteristic. Using this parameter, a positive correlation was found with SASS - ASPT and substrate type. A similar study carried out by Giller & Malmqvist (1998) demonstrated that discharge and physical-chemical features and the level of pollution in the ecosystem influence the benthic species composition. However, the difference in location and dispersion could not be demonstrated among the sites of different river size (PERMDISP and PERMANOVA,  $p > 0.05$ ). By classification of the sampling sites into three broad size categories; small, medium and large as a function of flow and width, a significant pattern indicating decreasing conductivity with increasing river size was seen similar trends were seen in NH<sub>4</sub> and TSS (Figure 4.9).

So far, much attention has been placed on the substrate type and river size and from the results of these two land use products it can be seen that medium and large rivers which have a stony substratum (IF3, N44, NM31, AM24 and NF48) show a high number of EPT taxa due to the preference of the Mayfly family Baetidae for rocks and their active behaviour to cling and dart from stone to stone.

Small rivers with soft bed sediment that have high number of EPT taxa (NF43, NF40 and IM1) had a variety of different habitats suitable for EPT colonization, especially for Caenidae that prefer slow streams and muddy areas. Small soft bottom rivers that contain CPOM and pieces of wood provide suitable habitats for Heptageniidae and Leptophlebiidae, which are normally associated with stony habitats and interstices.

The ideal habitats for Ecnomidae and Hydroptilidae are slow streams and quiet pools and fast flowing habitats for Polycentropodidae and Hydropsychidae. Most of Trichoptera taxa live in head waters of streams. Perlidae and Neumoridae families of Plecoptera have fast flowing streams and CPOM as ideal habitats.

#### *Level of disturbance*

The sampling sites sorted by the level of disturbance observed at site showed significant differences in location (PERMANOVA,  $p < 0.05$ ). The level of disturbance observed and recorded at the site was also reflected in the significant difference found in conductivity, turbidity and TSS (Figure 4.10) and biologic parameters (Family taxa, SASS - ASPT, EPT, POET, COPT, SASS\_6\_15, SASS\_8\_15) (Figure 4.16) in the river. The level of disturbance showed a negative correlation with all the biologic parameters (Table 4.8), confirming once more that human intrusion has negative effects on the whole ecosystem in line with the findings of Sala et al., 2000; Dudgeon et al., 2005; Revenga et al., 2005. The number of family taxa and the decline of taxa richness were the most reliable indicators of degradation and are commonly used in biological assessments (Kerans & Karr, 1994).

From the Figure 8.1 (Annex C) I can identify three clusters of the three level of disturbance observed at site. First, the group of minimum disturbance level represented by N44, NF40 and AM24 have a high number of EPT taxa. Secondly, the group of occasional level of disturbance constituted by AF5, IM1, NF46, AA22, NA30, NF13, AA20 and MF1 have a high number of taxa because it holds both sensitive and tolerant taxa. Lastly the group of permanent disturbance at site: NF26, NM29, NM36 and NA38, are strongly influenced mainly by conductivity and turbidity.

#### **5.4 Application of the Rapid Field Screening**

The figure 8.2 (Annex C) in conjunction with Figures 4.22 (a) and (d) show a high number of EPT taxa in sampling sites that belong to class I, while site in class II contains a high number of total taxa meeting the criterion of the ecological theory, where class II holds taxa that is not extinguished from class I and offer conditions for tolerant taxa from class III that is not able to live in class I due to the high competition.

Figure 4.22 (c) can clearly showed the trend of the SASS-ASPT when the sites are classified by the rapid field screening method. On the contrary figure 4.24 (e) showed variability in the ASPT value when classified by SASS5\_WQC. The ASPT is a good metric and a more reliable measure of good

river quality (Chutter, 2008) but it does not work in the investigation area when it is combined with the SASS 5 score. Dallas (1997) and Chutter (1998) stated that in poor microhabitats the SASS score will be more affected than ASPT, this last one can present few taxa but with the adequate sensitivity.

In general, the sampling design meets the criteria needed for the rapid field screening method as opposed to the SASS 5 methodology.

### **5.5 Application of the South African Score System (SASS\_5)**

The South African Scoring System Version 5 (SASS5) is being applied for bio-assessment of rivers not only in South Africa but in other African countries, e.g. Ghana (Vlek, 2009), Zimbabwe (Gratwicke, 1998/99), among others. This last version is a refinement of the SASS method developed by Chutter (1994) and nowadays is used for the national river monitoring programme in South Africa (Dickens and Graham, 2002) and it is recommended for the determination of flow requirements of rivers (O'Keeffe & Dickens, 2000).

Despite the fact that the SASS methodology states that it can be used in low/moderate flows (Dickens & Graham, 2002), it does not have specific ranges for this consideration and most of the sampling sites investigated belonging to 1st and 2nd stream order are within a range from 0.6 l/s to 22 l/s, showing a behaviour of very low-water lentic soft-bottom systems.

As it was pointed out in chapter 3, the sampling consisted of a composite of 10 unit samples from different macrohabitats according to the percentage of coverage within 100 m stretch, the design was not a water quality study and therefore it does not fully match the SASS sampling conditions and requirements. It is important to mention that one of the adjustments done in SASS\_4 to become SASS\_5 was the change from composite sample to individual macrohabitat samples due to the loss of resolution (Dickens & Graham, 2002), the approach used in this study was more similar to SASS\_4. Another point that must also be considered is that the SASS\_5 methodology indicated that results from preserved (fixed) samples should not be called SASS results (Dickens & Graham, 2002). However, the methodology was applied in the results derived from fixed samples at site with formaldehyde solution. Due to the limitations found during fieldwork (e.g. access to clean water and time spending at each site), it was difficult to carry out this in-situ analysis.

SASS\_5 corrected the weak point that SASS\_4 had at giving a general score to the cased Trichoptera where Leptoceridae was included. This family was wrongly considered as a sensitive taxon when in reality is not (Dickens & Graham, 2002). In this same line of reasoning, the SASS ranking 2 of Chironomidae family should be reconsidered as these group colonizes soft substrates quite dominantly. The study done for 263 Austrian Chironomidae species demonstrated that Chironomidae family is found in all water quality classes but with a high concentration (50%) in very good water quality (Moog et al., 1999). The score for this family should be adjusted or maybe considered if the Chironomidae found belong to the blood-red Chironomids, related to organic pollution and able to resist low dissolved oxygen levels due to their high haemoglobin association (Thorp & Covich, 1991). A problem in applying the indices is that individual taxa vary quite widely in sensitivity, depending on the nature of the particular disturbance. To overcome this problem a suitable index should be developed using sensitivity scores targeted to a particular impact.

In the NMS ordination the sites showed clusters that later with PERMDISP and PERMANOVA test showed significant difference in dispersion and location respectively. Furthermore they demonstrated significant difference in almost all single metrics in regard to physical and chemical parameters with flow, width and pH (Figure 4.24).

From figures 8.3 & 8.4 (Annex C) of an ordination plot for SASS and physical – chemical, it can be seen that the high levels in conductivity and TSS may be responsible for major deteriorations in the water quality of Mara streams. This has been demonstrated elsewhere (Giller & Malmqvist, 1998; Kibichii et al., 2007; Kasangaki et al., 2008).

There is a positive correlation between the two bioassessment methods rapid field screening and SASS 5 ( $r=0.67$ ,  $p<0.001$ ) (Figure 8.5 (Annex C)). This correlation does not show the same scoring criteria for both, the reason was the low score of the Chironomidae family and that the SASS does not target very small streams, while the rapid field screening method uses environmental features and macroinvertebrates to assess the status of the water quality.

If the classification of the sampling sites is now summarised according to the SASS 5 WQC, it can be observed 2 sites in WQ class I, 1 in WQ class II, 4 in WQ class III, 10 in WQ class IV and 8 in WQ class V, while the field screening located 6 sites in WQ class I, 8 in WQ class II, 8 in WQ class III, 2 in WQ class IV and 1 in WQ class V. In short, it can be concluded from the classification that SASS\_5 underestimates the water quality of the sites.

## 5.6 Findings in comparison with other similar studies

A study carried out in Njoro River, Kenya demonstrated that at cultivated sites the dominance of *Baetis sp.* and Simuliidae composed up to 75% of the observed invertebrate abundance (Kibichii et al., 2007). A comparison was done with this study and sites AA6b (agriculture) and NM31 (mixed land use) showed that Simuliidae abundance was 50% and 62% respectively, while the abundance of Baetidae was 72% in site NA30 (agriculture).

The percentage of abundance of Oligochaeta in poor water quality sites (class IV and V) was higher than in good water quality sites (class I and II), this result tallies with the findings from a study carried out in tropical streams in Kenya by Ndaruga et al. (2004). However in the same study the abundance of Chironomidae was found to be positively correlated with poor water quality which is not the same case in comparison with this study. The percentages of abundance of Chironomidae in water quality class I and II are slightly higher than in classes III, IV and V. These results go along with the study done for Austrian Chironomidae species, where 263 out of more than 500 Chironomidae species were studied and allocated in the different water quality classes. The results showed that almost 50% of 263 Chironomidae species were found in WQ class I and the percentage decreases as water quality declines (Moog et al., 1999).

In West Africa, Baetidae and Caenidae were considered to be the most tolerant taxa among the Ephemeroptera order due to their occurrence at deteriorated sites (Thorne & Williams, 1997). This is also true for similar studies in Kenya (Kibichii et al., 2007) and high-altitude streams in Uganda (Kasangaki et al., 2008). These families were found in all the sampling sites within the upper reaches of the Mara River during November and December 2009 with high percentage of abundance in sites

agricultural sites AA22 (63%), NA30 (81%), forest sites MF1 (55%), NF13 (63%), NF26 (63%) and mixed land use site NM34 (59%).

The broad distribution of tolerant EPT families, like Baetidae and Caenidae in Ephemeroptera order and Hydropsychidae in Trichoptera order reduce the sensitivity of the two orders by increasing taxa numbers at degraded sites (Thorne & Williams, 1997). This could be seen in the high percentage of EPT abundance in sites AA22 (68%), NA30 (82%), NM34 (59%) all of them belonging to the water quality class IV and NF26 (64%) and NM36 (50%) belonging to the water quality class V.

A low number of Plecoptera families were found only in sites NF48 and AM24 both of them belonging to the water quality class I. Plecoptera was found absent in the rest of water quality classes as seen in other studies (Raburu, 2003; Ndaruga et al., 2004). This result confirms the usefulness of indicators of pristine conditions. In fact, studies carried out in Kenyan streams reported low numbers of Plecoptera (Dobson et al., 2002; Kibichii et al., 2007)

The lack of percentage of shredders along the sampling sites shows strong evidence of the scarcity of information regarding the functions of the present species in tropical streams (Dobson et al., 2002). The site with the highest percentage of shredders was NF48 (6.46%) followed by IM1 (5.5%), the rest of the sites show shredders percentage less than 5%. On the contrary, the percentage of detritivores seems to be the dominant along the sites, in most of the cases with more than 50%. This last result could be explain with the assumptions of Dobson et al., (2002)of the adaption of detritivores in taking advantage of the abundance of allochthonous detritus in tropical streams.

## 6 Conclusions and Recommendations

This study has shown that the term “land use” as initially conceived blurred the level of detail that was holding the key to predict the determinants of benthic macroinvertebrate composition. Physical, chemical parameters and in- and near-stream variables were identified as adequate predictors of macroinvertebrate community composition within the upper catchment of the Mara River basin. The in-stream characteristics, specifically substrate type and level of disturbance were found to affect the water chemistry leading to an increase in the levels of conductivity, TSS, turbidity and  $\text{NH}_4\text{-N}$ . The high concentrations had a negative effect on the macroinvertebrate community composition as been showed in the outcomes of the single metrics analysis. River size had a positive correlation with most of the macroinvertebrate single metric indicating that species seemed to prefer high flow conditions.

It was found that a high number and diversity of; Ephemeroptera, Plecoptera and Trichoptera were present in higher stream currents and stony substratum Therefore, these conditions are potentially improving the habitat diversity for sensitive taxa which are good indicators of the health of riverine ecosystem.

According to the benthic macroinvertebrate communities in the different land uses a high abundance and diversity in forest and mixed land use was found. At the same time the predominant functional feeding group observed in the three land uses were the detritus feeders mainly in forest followed by agriculture and mixed land use.

In this study the application of the rapid field screening method was found to be more applicable than the SASS as a tool to describe the water quality of the sampling sites. A combination of the SASS scores with the ASPT in the determination of water quality classes seemed to underestimate the quality and health of the streams. This underestimation could be due to the undefined cause-effect basis of the SASS methodology and the fact that it does not define the ranges for its applicability.

This research was carried out after the second peak of in-flow levels and apparently the time might not have been adequate for macroinvertebrates colonization. Therefore it is necessary to conduct further research to cover not only base flow conditions but also rainy seasons in order to understand seasonal population dynamics of macroinvertebrates. From the results of this study, it is highly recommended to continue with the monitoring of benthic macroinvertebrate community and if possible include additional sampling sites in the Amala subcatchment.

This study was an initial approach to understand the interactions of macroinvertebrate communities, land use change and associated processes in the upper catchment of the Mara River basin. The results of this study are a good foundation in the attempt to identify suitable indicators for monitoring headwater streams in the Mara River catchment. In addition they can contribute to similar case studies in other catchments of the region

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## 8 Annex

**Annex A:** Screening Protocol for assessing river quality (adapted version from ASSESS-HKH, revised version for Ethiopia, Moog 2007)

DECISION SUPPORT TABLE – ORGANIC POLLUTION	RIVER QUALITY CLASSES				
	I	II	III	IV	V
<b>Multiple choices possible</b>					
<b>Sensory features</b>	<b>To be ticked/counted if not in accordance with natural river type</b>				
Non natural turbidity, Suspended solids			+	+	++
Non natural colour		+	+	+	++
Foam		+	+	+	+
Odour (water)		+	++	++	++
Waste dumping		+	+	+	+
<b>Ferro-sulphide reduc. – (water velocity &lt; 0,25 m/s)</b>	-				
Mud reduced but with aerobic surface		+	+++	++	
Mud reduced but with anaerobic surface				++	+++
Lower surface of stones (% cover black dots)		< 25 %	25-75 %	75-100 %	100 %
Upper & lower surfaces of stones (-"-)				+	++
<b>Ferro-sulphide reduc.– (water vel.) 0,25-0,75 m/s)</b>	-	-			
Mud reduced but with aerobic surface			+	+++	+
Mud reduced but with anaerobic surface				+	++
Lower surface of stones (% cover black dots)			< 50 %	50-100 %	100 %
Upper & lower surfaces of stones (-"-)					+++
<b>Ferro-sulphide reduc – (water velocity &gt; 0,75 m/s)</b>					
Lower surface of stones (% cover black dots)			< 25 %	25-50 %	50-100 %
Upper & lower surfaces of stones (-"-)					+++
<b>Bacteria, fungi, periphyton</b>					
Sewage fungi & bacteria (visible to the naked eyes)	(-)	(-)	few	medium	many +++
Sulphur bacteria (visible to the naked eyes)	(-)	(-)	(-)	+	+++
Stones with algal vegetation (periphyton) in thin layers	++	++			
% of thick, significant layers of algae	< 25 %	25-75 %	75-100 %	75-100 %	Few
Filamentous green algae	none to few	filaments, tufts	large tufts	(large) tufts	+
<b>Benthic macro-invertebrates</b>					
Species richness	(very) high	High	medium	few	very few
Dominance of very sensitive org. (9 to 10)*	+++				
Dominance of sensitive organisms (6 to 8)*	+	+++	+		
Dominance of medium tolerant org. (4 to 6)*			+++	+	
Dominance of tolerant organisms (3 to 4)*			+	+++	+
Dominance of extremely tolerant org. (1 to 2)*					+++
Plecoptera	++				
Baetidae (Acanthiops)	++				
Libellulidae	+	+			
Coenagrionidae	+	++			
Heptageniidae (Afronurus)	+	++	+		
Simuliidae	+	++	++	+	
Leeches (more than naturally occurring)	-	-	+	+++	+
Caenidae	few/medium	medium/many	medium/many	few/medium	
<i>Hydropsyche</i>	many	Many	medium	few	
Chironomids with red colour		single	few	medium	+++many
Air-breathing animals, e. g. rat-tail maggots					+++
Tubificidae (mud-worms)	single	few	few/medium	medium/many	many**
<b>Sum of columns</b>					

\*) check scores in the SASS\_5 score sheet

**Annex B: The SASS Version 5 scoring sheet**

SASS Version 5 Score Sheet					Taxon					Taxon																		
					S	Veg	GSM	TOT						S	Veg	GSM	TOT											
Date: / / 200__	Taxon								HEMIPTERA									DIPTERA										
Collector:	PORIFERA				5				Belostomatidae*					3					Atheriidae					10				
Grid Reference: WGS-84 Cape datum	COELENTERATA				1				Corixidae*					3					Blepharoceridae					15				
S: E:	TURBELLARIA				3				Gerridae*					5					Ceratopogonidae					5				
Site code:	ANNELIDA								Hydrometridae*					6					Chronomidae					2				
River: .....	Oligochaeta				1				Naucoridae*					7					Culicidae*					1				
Site description: .....	CRUSTACEA								Nepidae*					3					Dixidae*					10				
Weather Condition: .....	Amphipoda				13				Notonectidae*					3					Empididae					6				
Temp: .....°C pH: .....	Potamonautidae*				3				Pleidae*					4					Ephydriidae					3				
DO: .....mg/l Cond: .....mS/m	Atyidae				8				Veliidae/M. velidae*					5					Muscidae					1				
Biotopes sampled:	Palaemonidae				10				MEGALOPTERA										Psychodidae					1				
SIC ..... Time .....minutes	HYDRACARINA				8				Corydalidae					8					Simuliidae					5				
SOOC ..... Time .....minutes	PLECOPTERA								Sialidae					6					Syrphidae*					1				
Average size of stones .....cm	Notonemouridae				14				TRICHOPTERA										Tabanidae					5				
Bedrock .....	Perlidae				12				Dipseudopsidae					10					Tipulidae					5				
Aquatic veg'n ..... Dom. sp. ....	EPHEMEROPTERA								Ecnomidae					8					GASTROPODA									
Mveg/C ..... Dom. sp. ....	Baetidae 1sp				4				Hydropsychidae 1 sp					4					Ancylidae					6				
Mveg/OOC ..... Dom. sp. ....	Baetidae 2 sp				6				Hydropsychidae 2 sp					6					Bulininae*					3				
Gravel ..... Sand .....	Baetidae > 2 sp				12				Hydropsychidae > 2 sp					12					Hydrobiidae*					3				
Mud .....	Caenidae				6				Phlebotamidae					10					Lymnaeidae*					3				
Hand picking/Visual observation .....	Ephemeridae				15				Polycentropodidae					12					Physidae*					3				
Flow: Low/Medium/High/Flood	Heptageniidae				13				Psychomyiidae/Xiphocent					8					Planorbinae*					3				
Turbidity: Low/Medium/High	Leptophlebiidae				9				Cased caddis:										Thiandae*					3				
Riparian land use:	Oligoneuridae				15				Barbarochthoniidae SWC					13					Viviparidae* ST					5				
Disturbance in the river: eg. sandwinning, cattle drinking point, floods etc.	Polymitarcyidae				10				Calamoceratidae ST					11					PELECYPODA									
Observations: eg. smell and colour of water, petroleum, dead fish, etc.	Prosopistomatidae				15				Glossosomatidae SWC					11					Corbiculidae					5				
	Teiganonidae SWC				12				Hydroptilidae					6					Sphaeriidae					3				
	Tricorythidae				9				Hydropsalpingidae SWC					15					Unionidae					6				
	ODONATA								Lepidostomatidae					10					SASS Score									
	Calopterygidae ST,T				10				Leptoeridae					6					No. of Taxa									
	Chlorocyphidae				10				Petrothrinoidea SWC					11					ASPT									
	Chlorolestidae				8				Pisuliidae					10					Sample collection effort exceeds method? .....									
	Coenagrionidae				4				Sericostomatidae SWC					13														
	Lestidae				8				COLEOPTERA										Other biota including juveniles:									
	Platycnemidae				10				Dytiscidae*					5														
	Protoneturidae				8				Elmidae/Dryopidae*					8					Comments:									
	Aeshnidae				8				Gyrinidae*					5														
	Corduliidae				8				Halplidae*					5														
	Gomphidae				6				Helodidae					12														
	Libellulidae				4				Hydraenidae*					8														
	LEPIDOPTERA								Hydrophilidae*					5														
	Pyralidae				12				Limnichidae					10														
									Psephenidae					10														

Procedure: 'Kick SIC & bedrock for 2 mins, max. 5 mins; Kick SOOC & bedrock for 1 min; Sweep marginal vegetation (IC & OOC) for 2m total and aquatic veg 1m; Stir & sweep gravel, sand, mud for 1 min total; \* = airbreathers; Hand picking & visual observation for 1 min . record in biotope where found; Score for 15 mins/biotope but stop if no new taxa seen after 5 mins; Estimate abundances: 1 = 1, A = 2.10, B = 10.100, C = 100.1 000, D = >1 000; S = Stone, rock & solid objects; Veg = All vegetation; GSM = Gravel, sand, mud; SWC = South Western Cape; T = Tropical; ST = Sub-tropical; Rate each biotope sampled: 1 = very poor (i.e. limited diversity), 5 = highly suitable (i.e. wide diversity)

**Source:** The South African Scoring System (SASS) Version 5 Rapid Bioassessment Method for Rivers (Dickens & Graham, 2002)

### Annex C: Discussion graphics

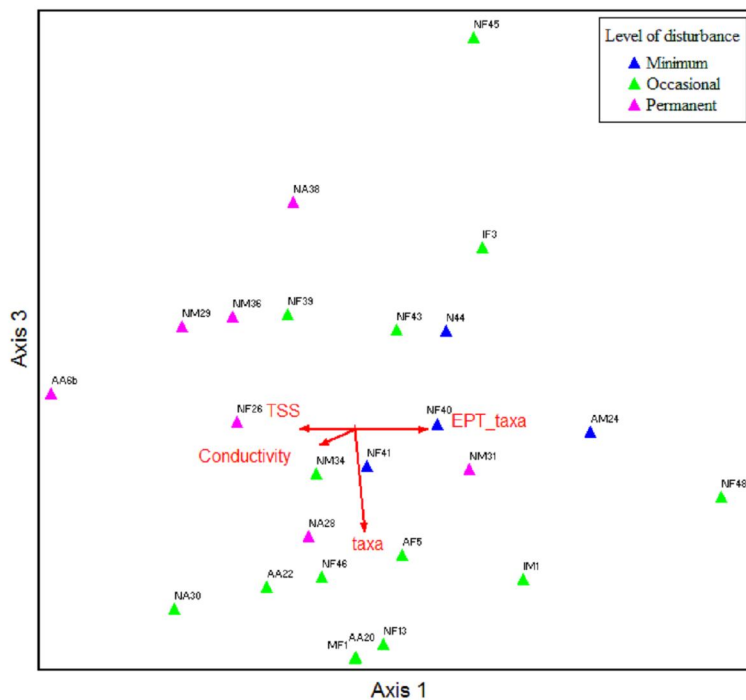


Figure 8.1 NMS 2-D of the 25 sampling sites (November – December 2009) using clustering of Bray – Curtis similarities on log (x+1) transformed benthic taxa abundances, point colour coding on level of disturbance, arrows show the most important variables that describe the sites

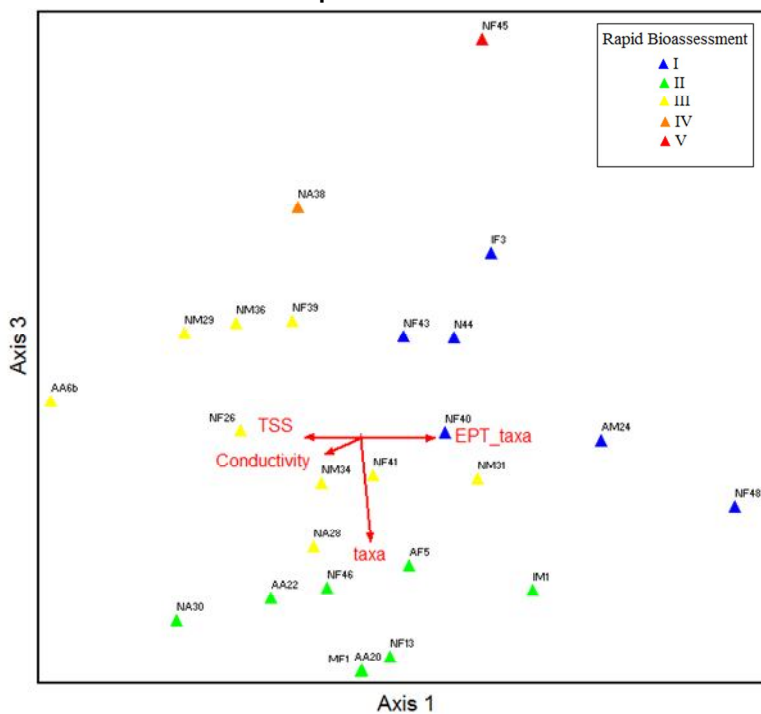


Figure 8.2 NMS 2-D of the 25 sampling sites (November – December 2009) using clustering of Bray – Curtis similarities on log (x+1) transformed benthic taxa abundances, point colour coding on water quality classes based on the rapid field screening method, arrows show the most important variables that describe the sites

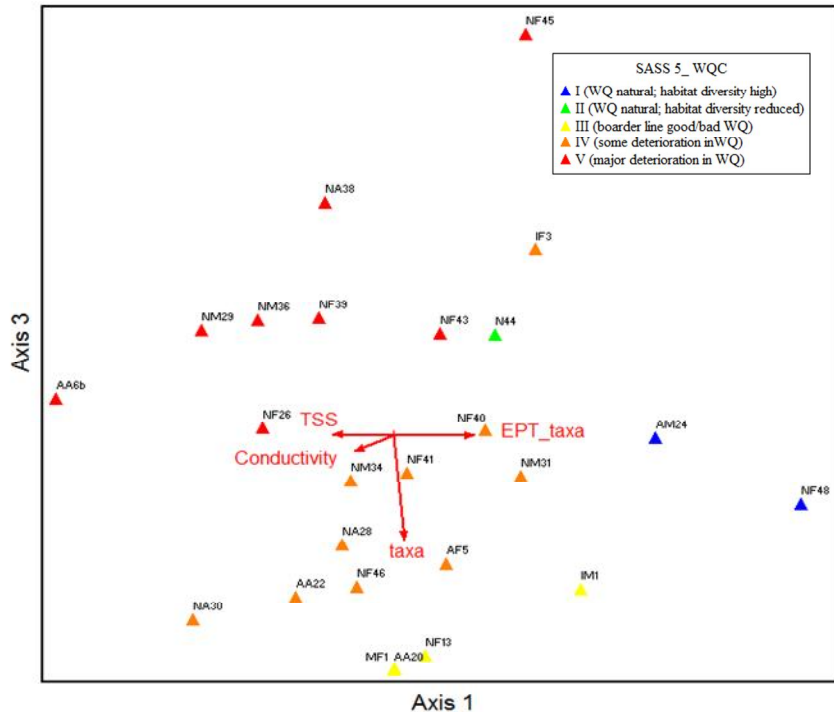


Figure 8.3 NMS 2-D of the 25 sampling sites (November – December 2009) using clustering of Bray – Curtis similarities on log (x+1) transformed benthic taxa abundances, point colour coding on water quality classes based on the SASS, arrows show the most important variables that describe the sites

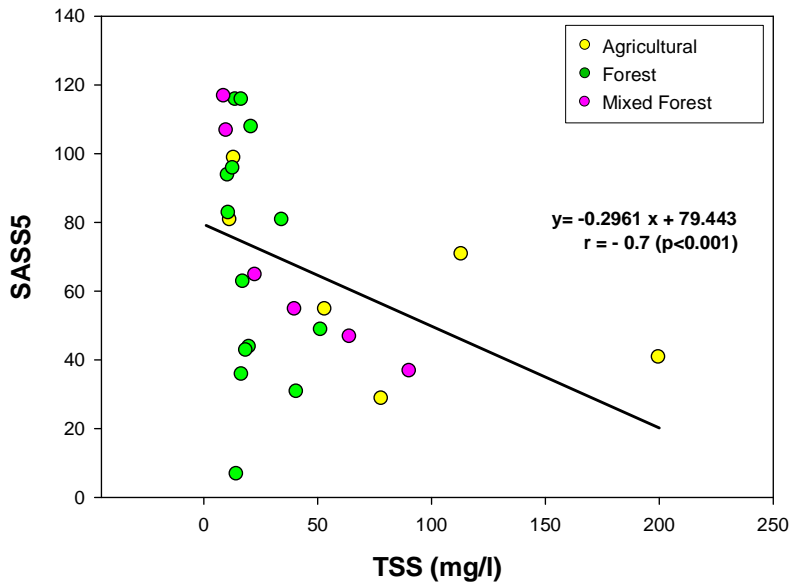


Figure 8.4 Correlation between TSS and SASS 5 score.



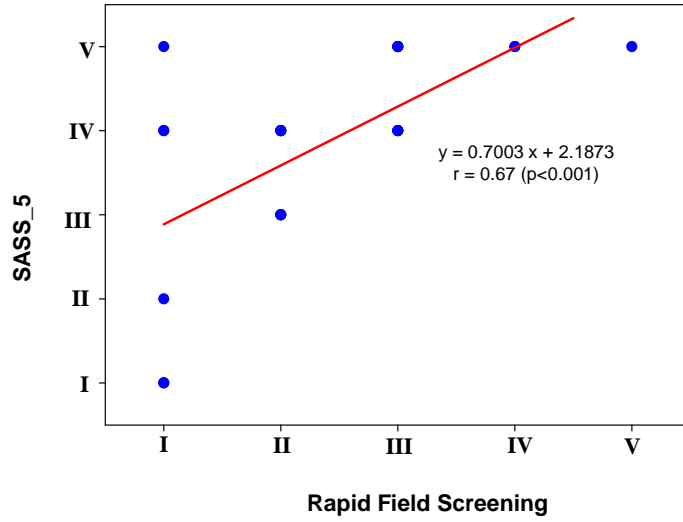


Figure 8.5 Correlation between Rapid field screening and SASS\_5 WQ classification