

**BACTERIAL DIVERSITY IN RELATION TO ANTHROPOGENIC
ACTIVITIES: A CASE STUDY OF KIBALE NATIONAL PARK WETLANDS,
UGANDA**

BY

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**A DISSERTATION SUBMITTED TO THE DIRECTORATE OF RESEARCH
AND GRADUATE TRAINING IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE AWARD OF DEGREE OF MASTER
OF SCIENCE IN CONSERVATION AND NATURAL
RESOURCES MANAGEMENT OF
KYAMBOGO UNIVERSITY**

NOVEMBER, 2024

DECLARATION

I MUNGULENI VINCENT, hereby declare that this dissertation titled "***BACTERIAL DIVERSITY IN RELATION TO ANTHROPOGENIC ACTIVITIES: A CASE STUDY OF KIBALE NATIONAL PARK WETLANDS, UGANDA***" is authentic, original, and has never been submitted to any higher institution or university for an academic award.

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APPROVAL

We the undersigned verify that this dissertation entitled "***BACTERIAL DIVERSITY IN RELATION TO ANTHROPOGENIC ACTIVITIES: A CASE STUDY OF KIBALE NATIONAL PARK WETLANDS, UGANDA***" was done by Munguleni Vincent, under our supervision and is ready for submission with our approval.

Dr. Stephen Buah

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Signature

Date

Dr. Abubaker Muwonge

.....

.....

Signature

Date

DEDICATION

This dissertation is dedicated to all members of my family for their love and endless support physically, spiritually and financially. In particular, I dedicate the study to **Miss Asiru Rosalind, Mrs. Yemima Angucia** and **Mr. Agami Nason Fua** for their cherishing and parental encouragement for me in my pursuit of higher studies.

ACKNOWLEDGEMENT

I would like to extend my gratitude and appreciation to BeZero Carbon for the offer of a scholarship to enable me to undertake this research. I am very much thankful to **Professor Jonathan Davies, Dr. Zihui Wang, Dr. Nick Atkinson** and **Dr. Sadadi Ojoatre**, who provided feedback and support as part of the BeZero Carbon delegation.

My highest appreciation goes to my Supervisors, **Dr. Abubaker Muwonge** and **Dr. Stephen Buah**, who mentored and guided me during this course of study. I appreciate your advice and support during the entire journey. Your effort, knowledge and encouragement will be forever remembered.

Lastly, I would like to acknowledge Kyambogo University, the staff of Biological Department and my classmates whose unity and togetherness brought me this far.

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OPERATIONAL DEFINITION OF TERMS

Anthropogenic activity: Anthropogenic activities refer to any human activities that affect the environment including but not limited to actions such as industrial processes, urbanization, agriculture, and pollution.

Bacterial diversity: Bacterial diversity refers to the variety of bacterial species present in a particular environment in both number and type.

Environmental deoxyribonucleic acid (eDNA): It refers to DNA that is extracted from the environment, which in this case is the water from the wetlands.

Land use and land cover: Land use refers to how land is utilized by humans (e.g., agriculture, urban development, forestry), while land cover refers to the physical characteristics of the land surface (e.g., forests, grasslands, water bodies).

Non-metric multidimensional scaling (NMDS): NMDS is a multivariate statistical technique used to visualize and analyze similarities or dissimilarities between bacterial communities in our wetland water samples.

Physicochemical properties: Physicochemical properties refer to the physical and chemical characteristics of water such as pH, temperature, dissolved oxygen and conductivity.

Shannon diversity: Shannon diversity here refers to the measure of bacterial species richness in an environment.

Wetland: A wetland referred in this study is an area of land covered by shallow water and characterized by its hydrology, vegetation, and soil types. It includes swamps, streams and rivers.

LIST OF ACRONYMS

| | |
|-----------|--|
| AAI | Anthropogenic Activity Index |
| ASV | Amplicon Sequence Variant |
| Mpanga AT | Mpanga After Town |
| Mpanga BT | Mpanga Before Town |
| DADA2 | Divisive Amplicon Denoising Algorithm 2 |
| DNA | Deoxyribonucleic Acid |
| EA Farm | Emmanuel Adegnyira Farm |
| EC | Electrical Conductivity |
| eDNA | Environmental Deoxyribonucleic Acid |
| GPS | Global Positioning System |
| KAFRED | Kibale Association for Environmental Development |
| KNP | Kibale National Park |
| LULC | Land Use and Land Cover |
| MWE | Ministry of Water and Environment |
| NGS | Next Generation Sequencing |
| NMDS | Nonmetric Multidimensional Scaling |
| OTU | Operational Taxonomic Unit |
| PCR | Polymerase Chain Reaction |
| pH | Potential of Hydrogen |
| RNA | Ribonucleic Acid |
| UBOS | Uganda Bureau of Statistics |
| UNEP | United Nations Environmental Program |
| UWA | Uganda Wildlife Authority |

ABSTRACT

Bacterial diversity refers to the vast variety of bacteria in an ecosystem which function in maintaining wetland ecosystem health through nutrient recycling for the trophic status and building symbiotic relationships with other organisms. This system is being threatened in many wetlands of Uganda as a result of an increase in anthropogenic activities such as agriculture and urbanization. The study aimed to examine bacterial diversity in relation to the anthropogenic activities in Kibale National Park (KNP) wetlands. A cross-sectional design was used in data collection. Land Use and Land Cover (LULC) changes were determined in ArcMap 10.8 through unsupervised classification of high-resolution images of twenty years for 2003, 2013 and 2023. The level of wetland degradation was determined using the Anthropogenic Activity Index (AAI) score. Temperature, dissolved oxygen, electrical conductivity and pH were measured with an electrical meter. Meanwhile, composition of bacteria from 50 water samples was determined using Environmental Deoxyribonucleic Acid (eDNA) technologies and metabarcoding. The forest cover of KNP reduced from 78% to 42% in the 20 years while farmland, grassland and built-up all significantly increased. Generally, there was a significant difference in physicochemical properties and AAI between inforest and outforest wetlands of similar types. Outforest wetland were more degraded than inforest wetlands. The dominant bacterial phyla in all wetlands were *Proteobacteria* and *Actinobacteriota* where the former dominated inforest wetlands and the later dominated outforest wetlands. Bacterial diversity significantly varied between inforest and outforest wetlands of the same type except for swamps where they were similar. As a result of differences in environmental conditions, bacteria composition was richer in intact wetlands than in degraded wetlands of similar types. Conditions in degraded wetlands abundantly harboured harmful species such as *Arcobacter cryaerophilus* and those known to thrive in polluted waters like *Thiothrix nivea*. Reduction in forest cover led to increased anthropogenic activities around the wetlands which altered their physicochemical properties that then negatively influenced their bacterial diversity. The wetlands of KNP should therefore be restored and protected through science backed interventions and policies for improved ecosystem health. This would benefit both the community by providing clean-safe water and improved sanitation as well as saving the biodiversity in these wetlands from damage.

CHAPTER ONE

INTRODUCTION

1.0 Overview

This chapter entails the background, statement of the problem, objectives, the null hypotheses, justification, limitations and significance of the study.

1.1 Background of the study

Wetlands are crucial for the world's nutrient cycle, sediment accretion, pollutant filtration, management of floods, and erosion (Ballut-Dajud *et al.*, 2022). Organic matter from plants and animals in these wetlands is broken down by bacteria into absorbable nutrients by the plants hence outlining the critical importance of both the bacteria and the wetland in the trophic status of an ecosystem. These biologically rich and diverse wetland ecosystems provide a range of advantages and services to local communities such as community water supply and food production. The estimated yearly value of wetland ecosystem services for human wellbeing is US\$ 60,000 per acre (Davidson *et al.*, 2019).

Wetlands' amazing capacity to sequester carbon is one of the main ways they benefit humanity, especially swamps. Despite making up only around 3% of the planet's surface, wetlands are storage for 30% of all land-based carbon (Economist, 2022). Wetlands are 55 times more efficient and effective than tropical rainforests at sequestering carbon (Economist, 2022). Wetlands absorb and store carbon form organic matter for hundreds to even thousands of years, which is released when they are disturbed, and the wetlands of the world are at great risk of being disturbed. Since 1970, more than one-third of these

carbon-storing, biodiversity-producing systems have been degraded or polluted, and this destruction rate is three times higher than that of forests (Ballut-Dajud *et al.*, 2022).

After more than 200 years of intense human activity such as urbanization and industrialization, more than 50 % of the natural wetlands have been destroyed worldwide (Davidson *et al.*, 2019). African wetlands cover an area of approximately 1,341,500 km² comprising 3.98 % of Africa's total land area. These include inland wetlands account for 81 %, and coastal wetlands account for 19 % (Beuel *et al.*, 2016). Some of these are important biodiversity spots as well as heritage for the local people. Such as the Bangweulu wetlands in Zambia and the Okavango wetlands of Botswana respectively. The wetlands in Africa are symmetrically distributed around the equator including the countries of East Africa which contain the great lakes and many other swamps and rivers (Mulei *et al.*, 2018).

The Victoria basin for example is surrounded by three countries of Kenya, Tanzania and Uganda. These countries benefit greatly from its resources such as fish and climate modification to suit agricultural production. As of 2019, Uganda's wetlands had a total area of 30,105 kilometres squared (km²), however, their size is rapidly reducing (Kiyawa *et al.*, 2019). Uganda is projected to have only 1.6% of its natural wetlands cover remaining by 2040 if human meddling is not stopped (MWE, 2022). The main cause of this significant decrease in Uganda has been attributed to anthropogenic activities such as urbanization and agricultural practices.

In Uganda, wetland habitats are under increasing pressure because of agriculture, urbanization and economic growth in human settlements. Such anthropogenic perturbations can have a negative influence on the wetland ecosystem, impairing its

ecological functions and posing a risk to the health and well-being of humans and other species using the wetland (Orel *et al.*, 2022). Land use nearby has a significant impact on wetland ecosystems, the land uses are manifested in various anthropogenic inputs from pollution sources like agricultural and industrial deposits, sewage leaks, urban storm water runoff, fires and wildlife interactions (Orel *et al.*, 2022).

As a result of different land uses, the wetland ecosystems are exposed to different bacteria causing various diseases, inorganic chemicals, and organic compounds or are degraded of their natural nutrients. For example in sewage polluted it has been found that harmful *Firmicutes* species became abundant and can easily spread in to the community especially those under the genera of *Clostridium*, *Bacillus* and *Staphylococcus*. However these inputs can also be crucial in the processes that harm the ecosystem and microbes themselves, such as eutrophication, decreased dissolved oxygen rates, and changes in bacterioplankton communities hence altering community compositions. Additionally, these human-made inputs change the bacterial taxonomy and are connected to illnesses and mass extinctions of a variety of ecologically and economically significant wetland species of plants and animals (Ziegler *et al.*, 2016).

The KNP is a forested area and a significant region holding various primate species, including the Chimpanzee, Baboons, Red colobus monkey, Black-and-white Colobus monkey, Grey-cheeked, Red-tailed monkey, Mangabey, Blue monkey, Vervet monkey, and L'Hoest monkey (UWA, 2022). More than 200 kinds of birds live in these forests, including the most well-known bird, the Great Blue Turaco (*Corythaeola Cristata*), as well as other mammals like elephants, otters, and bush bucks (Hungarica, 2014).

Protecting KNP wetlands has actual benefits such as the Bigodi wetland community receiving revenue of \$10 from each visitor (Lepp, 2007). A portion of these profits were utilized to create the Bigodi Nursery and Secondary Schools, pay the wages of the school instructors, as well as build bridges across streams in the community. The Bigodi Women Group was founded by the local women who depend on raw materials from the swamp to make crafts and create attractive bead pieces which are sold (Lepp, 2007). These benefits are best realized when the ecosystem is healthy yet the KNP biodiversity has seen major threats arising from tea plantations and rising urban centers in the area. The biodiversity influenced by these rises not only limits to the trees, mammals and birds but as well as the bacteria diversity of KNP.

In the wetlands of KNP, bacteria are crucial components of as they contribute to the functioning of many different processes including biogeochemical cycles, symbiotic relationships and the trophic status. The distribution pattern of bacteria in water is important in that it can reflect the habitat health status and hence be used as an indicator of healthy ecosystems such as abundant levels of *Proteobacteria* (Feng *et al.*, 2022). It is well acknowledged that the high anthropogenic impacts from home, industrial, and agricultural activities pose a danger to wetland ecosystem health and bacterial biodiversity. Therefore, it is crucial to investigate how the bacterial composition of an area can be an indicator of environmental changes due to increased human activities near wetlands (Horton *et al.*, 2019).

Phyla such as *Actinobacteriota* are known to be indicators of degradation from anthropogenic activities as they are able to survive in polluted environments better than the other phyla's (Wang *et al.*, 2020). Additionally, wetlands impacted by anthropogenic activities such as domestic waste and sewage usually contain pathogenic species which

leads to the spread of diseases to humans or from humans to other wildlife. It is therefore important to understand the bacteria composition within KNP ecosystem and the threats that could alter the normal functioning of this biodiverse ecosystem.

1.2 Statement of the problem

Biodiversity assessment of wetland ecosystems is a very important study for better understanding and management of such an ecosystem. In Uganda, a sharp rise in environmental changes particularly in the agricultural and industrial sectors have both had a significant negative influence on wetland ecosystems. In KNP, the main anthropogenic activity include tea plantations which are known for their extensive use of chemicals such as pesticides and fertilizers (Majaliwa *et al.*, 2015). Urban settlements with inadequate waste management systems also pollute these wetlands. Such activities impact not only the macro biodiversity (plants and animals) of a wetland but the micro-organisms as well including bacteria.

Most of recent studies about environmental changes impacting wetlands focuses on biological community distribution, changes in wetland properties and changes in water quality (Zhou *et al.*, 2020). This is so because the effects of these changes are easier to observe in large organisms (plants and animals) than in bacteria, which require a careful and accurate evaluation. As a result of this few studies have been carried out to document the impacts of anthropogenic activities on wetland bacteria especially in Uganda and KNP wetlands in particular. The microbiome studied in these areas are mainly those of primates due to the potential to cause zoonotic diseases yet many other bacteria taxa perform other functions as well (Krief *et al.*, 2020). Therefore, similar effort is needed to understand

bacterial distribution as an indicator of degradation can result from environmental changes in the wetlands of KNP.

1.3 General objective

To assess the effect of anthropogenic activities on ecosystem health using bacterial diversity in the wetlands of KNP.

1.4 Specific objectives

- i. To evaluate the anthropogenic LULC changes in KNP and the surrounding sub-counties from 2003 to 2023.
- ii. To examine the physicochemical properties and the AAI of selected wetlands in KNP.
- iii. To determine the bacterial diversity of selected wetlands in KNP.

1.5 Hypotheses

- i. There is no significant change in the LULC of KNP and the surrounding sub-counties from 2003 to 2023.
- ii. There are no significant variations in the physicochemical properties and AAI among selected wetlands in KNP.
- iii. There is no significant variation in the bacterial diversity among selected wetlands in KNP.

1.6 Scope of the study

This research concentrated on the diversity of bacteria in the wetlands of KNP as a case study area and covered the districts of Kamwenge, Kabarole and Kyenjojo. The study

sought to identify and map all the environmental changes and land uses around these wetlands. It also looked at how these changes affected the physicochemical properties, AAI and bacterial in the different parts of intact and degraded wetlands. The time scope for data collection was 2 months.

1.7 Justification for the study

Kibale national park has a very biodiverse ecosystem found both inside the forest and outside and is known as the capital of primates. Anthropogenic activities are increasing every day and this will always have a negative effect on biodiversity and ecosystem health. This would best be addressed by understanding both the ecosystem functions and anthropogenic changes. For better conservation of KNP biodiversity, the relationship between its wetland ecosystems and anthropogenic changes happening around it needed to be understood. Few studies in KNP had related bacteria to anthropogenic activities for assessing ecosystem health.

1.8 Significance of the study

This study assessed and documented the bacterial diversity of KNP wetlands while also investigating the effects of environmental changes and common anthropogenic activities in Ugandan wetlands on bacterial diversity. The scientific community should be able appreciate the use of bacteria diversity for a detailed assessment of wetland ecosystem health in developing countries of the tropics as opposed to relying on only traditional methods. The findings of this study will be used to scientifically inform policy reforms in conservation of wetlands of Uganda.

CHAPTER TWO

LITERATURE REVIEW

2.0 Introduction

This chapter discusses the existing findings and theories under the stated problem, the gaps and challenges in such studies using the already existing literature related to studying bacterial diversity, wetlands and environmental changes.

2.1 Land use and land cover changes

Land use and cover changes can be caused by natural processes, as well as by purposeful and inadvertent human activity. However, it is now recognised that human activity is changing the world's natural landscape at previously unheard-of rates and on a variety of spatiotemporal scales (Kayiranga *et al.*, 2016). These changes include forest covers, agricultural landscapes, towns and cities as well grassland vegetation cover. In Uganda, such unprecedented LULC changes are occurring at many geographical and temporal dimensions due to population pressure and other socio-economic and ecological causes. As Uganda is aiming to reach a middle-income economy and by 2040, there will be 80 million people in Uganda, up from 34 million in 2014 (UBOS, 2014), over 80% of these people rely directly on natural resources for their livelihoods, including wetlands, grasslands and forests. It's also critical to recognise how diverse Uganda is in terms of the societal, economic, and geographical contexts that combine to form the changes in LULC but as well keep in mind how unsustainable growth can lead to disasters.

Wetland degradation in Uganda is particularly being accelerated by Uganda's persistently concerning forest decline as cleared forest exposes these wetlands to human access. Apart from climate causes, human activity is to be blamed for the majority of forest loss (Majaliwa *et al.*, 2010). The forest and surroundings of KNP are among the areas impacted by human activity such as deforestation and agriculture. In the past, people who lived in and around KNP did so in a sustainable manner and had access to the forest for necessities including firewood, vegetables, honey, water, and household medications. However, mostly as a result of lumbering and tea plantations, the forest suffered a significant decline in the 1970s and 1980s. The current speed at which forests are being converted and degraded poses a growing threat to the forest, even though it offers significant social and environmental advantages (Kayiranga *et al.*, 2016). Due to agricultural loss caused by wild animal invasions, particularly by elephants, inhabitants in the region have established plantations of pine and eucalyptus trees instead of crops (Majaliwa *et al.*, 2015). New large vegetation covers have been formed by the gradual horizontal expansion of these plantations around the KNP.

Land use patterns have an immediate impact on water quality, which in turn affects biodiversity in the wetlands (Han *et al.*, 2022). For example, within a few years of the initial cutting of the forest, soil erosion increases together with the silting of wetlands (Zi-Ru *et al.*, 2021). Since wetland productivity is determined by the amount of organic matter washed into it from surrounding forests, the conversion of forest to cropland has been linked to a decrease in wetland production and a poor trophic status. After the forest area was converted to agriculture land, Jung *et al.*, (2019) observed a decrease in biodiversity. Chase *et al.*, (2020) and Montràs-Janer *et al.*, (2024) all observed similar findings. This is so because when wetlands are in their natural environment surrounded by vegetation,

it provides a conducive environment for bacterial species such as *Lamia testudinis*, *Rhoduluna limpnophila* and *Rhodobacter sphaeroides* among others. Such bacteria species are able to effectively recycle the nutrients within the wetland that can then be re-absorbed by the plants which are fed on by the animals in the park for example Elephants and Gorillas in KNP hence the cycle is continuous. Therefore, alterations to the characteristics of a wetlands make it more vulnerable to severe forms of degradation as a cascade reaction. The expansion of agricultural land uses around KNP forest has therefore jeopardised the park's conservation efforts and further threatens its integrity. Cleared vegetation for tea plantations means less nutrients to be recycled by bacteria for the plants to grow. When plants are not growing, the animals have nothing to feed on thereby less biodiversity will be seen in the affected wetlands than in those with vegetation cover.

2.2 Wetlands

2.2.1 Characteristics and importance

According to Davidson *et al.*, (2018), wetlands are areas where water covers the soil or is present either at or near the surface of the land for varying periods of time during the year, including during the growing season. They form unique ecosystems that exist at the interface between terrestrial and aquatic environments making them critically important for both the land and the surrounding water bodies (Niu *et al.*, 2012). Wetlands can vary greatly in size, water depth, and plant life, but they all share the common characteristic of being water-saturated for at least part of the year. Wetlands can be classified as either permanent (wet all year round) or seasonal (wet only during certain periods such as after rain or during flood seasons) (Davidson *et al.*, 2018).

The soil composition of wetlands often referred to as hydric soils are typically waterlogged and have low oxygen levels. These unique conditions result in a distinct soil profile that is high in organic material. The vegetation of wetland ecosystems support plants that are specially adapted to survive in saturated soil and low oxygen conditions which are called as hydrophytes (water-loving plants) including papyrus found in many KNP swamps (Apodaca & Chapman, 2004). There are very many types of wetlands but in this study, what is being called a wetland include swamps, rivers and streams. Often referred to as “kidneys of the landscape” due to their ability to filter and purify water, the wetlands found in KNP play numerous ecological, hydrological, and economic benefits such biodiversity hotspots, water filtration, flood control, groundwater recharge, economic and cultural value among others. But these values are being threatened by degradation through agriculture and development, urban pollution, climate Change and invasive species. These usually alter the wetlands biodiversity and other properties as well (Mulei *et al.*, 2018).

Through conservation efforts, such threats can be averted for proper management of wetlands and realize their full potential (Gosling, 2011). For example in KNP the wetlands of Bigodi have been carefully conserved and it is now dominated by various primates like Mangabey, Baboon and birds like Turaco, Hornbill among other mammals hence benefitting both the community and the wildlife even though it is located outside the forested area (Mulindwa, 2016).

2.2.2 Wetland physicochemical properties

Physicochemical parameters have demonstrated significant temporal and geographical variations and are regarded as one of the elements capable of altering the wetland

environment. Two main factors can influence the physicochemical properties of water in wetlands; first is agriculture which releases a variety of pollutants that affect surface water, such as metals, pesticides, pathogens, nutrients, and salts (Akhtar *et al.*, 2021). And for the case of KNP this can be directly attributed to the large-scale tea plantation farms that surround the national park and are known for their extensive use of chemicals such as pesticides and fertilizers. Secondly, hazardous materials released into the water by urban operations can be found in untreated and partially treated sewage, construction debris, home trash and solid/liquid municipal waste components (Han *et al.*, 2022). The main towns where these wetlands were found included Fortportal city, Bigodi town, Kamwenge town, Kyenjojo town which all drain their water into either Mpanga river or Dura river systems (Martínez *et al.*, 2011). These contaminants are extensively distributed in low quantities and contain certain inorganic compounds like zinc, iron, copper and nickel. These are essential for the growth of organisms, but when quantities of these elements exceed permissible bounds, they become toxic to all lifeforms in the wetland.

One of the key elements that modifies how the aquatic environment operates is temperature, which also affects the distribution and growth of various bacterial species (Tinta *et al.*, 2022). Temperature has been shown to affect species composition and production and it varies on the depth, season, weather and location of the wetland. When wetland features are intact, the temperatures are often stable. The temperature of a wetland can be influenced by factors such as the weather of the day, the vegetation cover like the tree shades as well as the waste water being discharge in such a wetland. For example some industries discharge hot water directly into a wetland which could ultimately lead to an increase in its temperature (Ballut-Dajud *et al.*, 2022).

Biological processes and the preservation of the physical and chemical features of the water depend heavily on the pH. It controls how basic or acidic the ecosystem is and is an essential component of any wetland ecosystem. The majority of comparable research suggests that the pH should never exceeds the desired limit of 6.5-8.5, which could otherwise be hazardous and affect bacteria levels. According to (Lammel *et al.*, 2018), although most microorganisms thrive at a wide range of pH level, extreme pH levels can have an impact on all aquatic life. Measured as the hydrogen ion potential it is suspected to be at extremes near the wetlands where there is much chemical use such as the tea plantations and the urban centres as per Cózar *et al.*, (2007).

According to Bilewu *et al.*, (2022), electrical conductivity is a measure of an aqueous solution's capacity to transfer an electric current in aquatic settings. The three primary factors that can easily influence conductivity are home effluents, fertilisation discharge from agriculture, and urbanisation. Such dissolved salts when they become cations or anions they are then small enough to pass through the bacterial cell wall and change the chemistry of its cytoplasm which in most cases can be very fatal (Ma *et al.*, 2020). Of these ions hydrogen is the smallest which is why high pH is rarely a conducive environment for the survival of any micro-organisms. Similarly, a low pH could be due to other chemicals bonding with the hydrogen ions and this as well could be spelling out a disaster for un adapted microorganisms like the *Proteobacteria* (Signori *et al.*, 2014).

The amount of oxygen in a body of water is referred to as dissolved oxygen, which controls ecosystem health. Due to its critical biological and chemical effects on surrounding water, oxygen is a crucial water quality metric to maintain. Both aerial diffusion and the photosynthetic by-product of aquatic plants allow oxygen to reach the water. The water's pressure, salinity, and temperature all affect the level of oxygen

dissolved in it (Beutel *et al.*, 2006). The amount of dissolved oxygen in water bodies can be a measure of pollution as polluted water depletes oxygen which is used in decomposition of organic matter (Gopalkrushna, 2011). Healthy wetland habitats have a high level of dissolved oxygen that can be retained through lower temperatures and large surface area for its dissolution. Many bacteria genera are aerobic and facultative in nature and need oxygen to thrive such as *Pseudomonas* and *Nitrobacteria* while others like *Clostridium* and *Bacteroides* are anaerobic. Therefore, the presence of such species can be used as an indicator for oxygen level within a wetland (Zhang *et al.*, 2020).

The way that humans interact with the environment and with natural processes changes the physicochemical properties of wetlands. Differentiating between how environmental factors affect the level of physicochemical properties is a challenging task which therefore needs to be carefully studied before making assumptions. Many other physicochemical properties do impact bacterial diversity in a wetland as well such as biological oxygen demand, nutrient levels (nitrogen, phosphorous and sulphur), chemical oxygen demand, water density and many others. With each of these, a bacteria phyla would react differently depending on the levels. However, one cannot study all these complexities between these parameters and in this case the most influential as per Peralta *et al.*, (2013) and Sui *et al.*, (2021) were chosen for the study.

2.3 Bacteria

2.3.1 Bacterial diversity of wetlands

As the most active wetland components, wetland bacteria are crucial to the health of the ecosystem in wetlands (Yin & Yan, 2020). Wetland bacteria play important roles in various ecological processes, including wetland regulation, nutrient cycling, wetland

formation, pollution removal, and ecosystem diversity. Bacteria also provide significant opportunities for resolving anthropogenic problems like breaking down waste within the wetland especially for phyla such as *Proteobacteria* (Cavicchioli *et al.*, 2019). The broken waste can then be used by other organisms like plants for growth hence reducing waste accumulation as well as improve the flow of energy along the food trophic levels.

Environmental studies have revealed that wetland bacteria diversity is primarily influenced by pH, wetland types, temperature, vegetation and nutrient availability (Lauber *et al.*, 2009). Bacteria with thick peptidoglycan cell wall are better adapted to survive in such conditions as compared to their counterparts with thinner cell walls. Changes in land use and wetland property, leading to changes in the characteristics, have an effect on the structure and composition of wetland bacteria that is significant. Arroyo *et al.*, (2015) showed that the diversity and richness of bacteria in a wetland are extremely delicate to environmental changes surrounding that wetland.

2.3.2 Characteristics of the main bacterial phyla

The composition of the bacteria community at the level of phylum in wetlands typically shows that the main phylum is *Proteobacteria* within the wetland, followed by *Actinobacteriota*, *Bacteroidetes*, *Chloroflexi*, *Acidobacteria*, *Gemmatimonadetes* (Peralta *et al.*, 2013), (Arroyo *et al.*, 2015). As per Wu *et al.*, (2021) this bacterial abundance alternate depending on the degradation severity in various forms of wetlands use. The main phylum *Proteobacteria* is found in more natural habitats and is involved with biogeochemical processes, oxidizing inorganic and organic complexes and photosynthesis (Mukhopadhyay *et al.*, 2012). This phylum can recycle carbon, nitrogen and sulphur nutrients in the environment thus explaining why it is more abundant in

undisturbed swamp wetlands. This is because such wetland have high levels of organic carbon and nitrogen (Röske *et al.*, 2012). Meanwhile, *Actinobacteriota* becomes more prevalent in a disturbed wetland environment than in a natural one (Eichorst *et al.*, 2018). This implies that the relative abundance of *Actinobacteriota* compared to *Proteobacteria* on the outside of the protected Kibale forest must be greater than that inside the protected forest. This is due to human activities causing degradation outside the forest. The phyla *Actinobacteriota* contains a significant number of oligotrophs that can survive in disturbed environments with low levels of nutrients (Zong & Shi, 2020).

Chloroflexi and *Gemmatimonadetes*, on the other hand, are been known to survive in nutrient-depleted conditions. Previous research has shown that *Chloroflexi* is more abundant in nutrient-limited environments and *Gemmatimonadetes* may prefer oligotrophic environments due to their nutrient independence (Fan *et al.*, 2021). Human settlements and activities like agriculture are more robust on the outside of the national park. Hence, we expect to see low nutrients compared to the inside wetlands that have fewer human interactions resulting from tourism. Phyla associated with the human are more likely to be found in water that is polluted with sewage and this in most cases is attributed to the phyla *Firmicutes*. They predominantly live in the human guts and are passed out during bowel movements. The specific of these is the species *Thiothrix nivea* which is a direct indicator of sewage pollution as it prefers to grow in sewage pipes and survive off of sulphur (Tinta *et al.*, 2022).

2.3.3 Bacterial interactions

There is interaction among bacterial species co-occurring in the same environment (Ding *et al.*, 2017). According to Momeni *et al.*, (2017), changes in bacterial communities

correspond to variations in the relative abundance of some particular major groups of bacteria. This makes interactions potentially common within higher-order of bacterial communities. Cascade extinction occurs when the extinction of one or a few species causes the extinction of other species. Hoek *et al.*, (2016) has shown interaction between bacteria easily shifting from mutualism to competition with increased degradation levels.

Given the importance of mutualism in promoting diversity, the transition from mutualism to competition as a result of human activities such as high levels of pollution, climate change, or habitat destruction may result in a sharp decrease in the diversity of species. This could thereby create an alternative low-diversity community that has its demerits (Aschehoug & Callaway, 2015). Even though interest has been put in understanding bacterial community responses to anthropogenic degradation, it is still unclear how this happens (Hoek *et al.*, 2016). Many studies that focus on bacteria do attribute the differences to physicochemical properties but with low confidence. This means many other interactions are usually still unaccounted for of which interactions among species is given a high possibility. Their complexity requires careful studies through not just eDNA but as well as traditional methods like laboratory culturing (Lazarevic *et al.*, 2016).

The interaction between anthropogenic activity and bacterial trophic status is complex and occurs across various ecosystems, especially in aquatic environments. Bacteria play diverse roles in ecosystems based on their trophic status such as producing their own food. This is often through processes like photosynthesis or chemosynthesis as well as consuming organic carbon for their energy. Certain bacteria species can switch between autotrophic and heterotrophic modes depending on environmental conditions (Mansfeldt *et al.*, 2020).

Agriculture, urbanization, and industry often lead to the excessive release of nitrogen and phosphorus into ecosystems, especially in water bodies (Walton *et al.*, 2020). This nutrient influx boosts primary productivity, which in turn impacts bacteria composition. For example eutrophication promotes heterotrophic bacterial growth because there's an increase in organic material, such as decaying plant matter from algal blooms (Esser *et al.*, 2023). These bacteria decompose organic matter, consuming oxygen in the process, which can lead to hypoxic (low oxygen) or anoxic (no oxygen) conditions. This disrupts aquatic life, favoring certain bacteria over others (Esser *et al.*, 2023).

Industrial activities, pharmaceuticals, and agricultural runoff introduce chemicals, including heavy metals and antibiotics, into ecosystems which can disrupt bacterial communities. This can be done by either selecting for resistant strains like antibiotic-resistant bacteria or by killing sensitive species (Wu *et al.*, 2021). In broader ecosystem, bacteria cycle nutrients necessary for absorption by plants fed on by animals and when these die, they are fed on by bacteria hence the continuous cyclic interaction. This cycle however can be impacted by anthropogenic activities.

2.3.4 Bacteria in Kibale national park

Renowned for its primate diversity, KNP is also a hotbed for microbial life due to its rich ecosystems of tropical forests, wetlands, and grasslands. Studies in the area has focused on the interactions between primates, their environments, and the associated microbial communities, especially bacteria (Goldberg, 2008). KNP is home to many primates, including Chimpanzees, red colobus monkeys, and baboons. Studies have revealed that these primates harbor rich and diverse gut microbiomes, influenced by diet, habitat, and social structures.

The microbial diversity in these animals plays a crucial role in their digestion, immunity, and overall health. For example, the gut bacteria aid in breaking down fibrous plant material that forms a significant part of their diet. *Bacteroides* and *Prevotella* species are common gut bacteria found in herbivorous primates (Glassing *et al.*, 2016). They help in the fermentation of dietary fibers and complex carbohydrates, enhancing nutrient absorption. *Firmicutes* and *Proteobacteria* phyla are abundant and involved in various metabolic processes in the gut, contributing to energy extraction from food and regulating immune responses (Goldberg, 2008). Studies have documented various pathogenic bacteria that can cross between species, raising public health concerns as well. These include *Escherichia coli* (*E. coli*) and *Salmonella* of various strains, including both commensal and pathogenic types, have been isolated from primates in the park (Yinda *et al.*, 2022). Some strains are resistant to antibiotics, likely due to human influences and environmental contamination (Kolinski & Milich, 2021).

The diverse habitats of Kibale National Park provide a suitable environment for a wide range of environmental microbes. Soil and water samples from the park show the presence of bacteria involved in nutrient cycling, such as nitrogen-fixing *Rhizobium* important for soil health. This contributes to nutrient availability for plant growth in the park's ecosystems. For example decomposer bacteria found in soil and known for their ability to decompose organic matter play a role in the breakdown of dead plant and animal materials (Lammel *et al.*, 2018). The park also faces challenges with the presence of pathogenic and antibiotic-resistant bacteria, likely influenced by human activity which begs for further research in this field to understand the ecological dynamics and the potential health implications for both wildlife and humans (Goldberg, 2008).

2.4 Anthropogenic activities' effects on wetlands

Anthropogenic activities can cause large-scale and long-term changes in wetland structure and bacterial distribution (Fang *et al.*, 2019). Changes such as vegetation type, water nutrient content and physicochemical status in a wetland caused primarily by environmental changes can be used to predict the health status of a wetland. Chemicals from agricultural practices are the main cause of this in developing countries such as Uganda and for KNP mainly tea plantations. Furthermore, Papas *et al.*, (2022) found that wetland degradation from human activities results in decreased wetland area, water quality, vegetation types, water sequestration ability and aquatic biodiversity. As towns expand humans clear forested area for settlement and this can be seen in the expansion of Fortportal city on KNP forest.

Alterations in wetland, hydrologic, biogeochemical, biological, physiological and biochemical processes are all part of the wetland degradation process (Han *et al.*, 2022). Changes in water nutrient conditions caused by wetland alteration may cause bacterial assemblies to change composition and structure (Peralta *et al.*, 2013). This explains the importance of examining the changes in water physicochemical composition mainly pH, temperature, conductivity and dissolved oxygen (Arroyo *et al.*, 2015). Therefore, ability to know whether any the variation in bacterial composition is a factor of the water physicochemical properties alone or mainly the anthropogenic land use and land cover changes in KNP wetlands.

2.5 Bacterial communities and anthropogenic impacts

Human activities commonly lead to environmental changes in wetlands structure and these have a significant negative impact on these ecosystems. Microbes, as a primary

feature of a wetland do play a very important part in the restoration of the wetland. Water pH, oxygen, electrical conductivity and temperature are the most important physico-chemical determinants of the growth of wetland bacterial communities. They are heavily influenced by industrial and agricultural activities (Soares & Rousk, 2019).

Different degradation types and dominant vegetation species have a significant impact on the composition of the wetland bacterial community as well (Xu *et al.*, 2018). The relative richness of common genera varies with environmental changes indicating that wetland bacteria with adapted functions can live in various wetlands. Environmental changes cause compositional change in relative abundance of water bacterial communities (Li *et al.*, 2018). For example nitrogen fixing bacteria can be expected to be dominant in areas of high nitrogen and covered with leguminous vegetation (Heijden *et al.*, 2015). Similarly, it can also be expected that wetlands dominated by plants capable of bioremediation such as the papyrus commonly in swamps should be able to curb and absorb the high chemical pollution in these wetlands through filtration. This would therefore create a more conducive environment for bacteria to grow compared to their counterparts without such vegetation (Li *et al.*, 2018).

2.6 Bacteria and electrical conductivity

The relationship between wetland bacteria and electrical conductivity (EC) varies. Studies have found a negative relationship between *Proteobacteria* and EC, but a positive one between *Actinobacteriota* abundance and EC (Yin & Yan, 2020). This is thought to be linked to the fact that *Actinobacteriota* do have a thicker cell wall known as peptidoglycan which protects them from the harmful effects of high EC (Eichorst *et al.*, 2018). Such a layer is very thin in the phyla *Proteobacteria* and therefore can easily be

penetrated by the harmful chemical that end up destroying the bacteria (Yu *et al.*, 2019). Wetlands have a slightly lower EC than other ecosystems such as forests, which is thought to be due to storm water carrying chemicals applied during agricultural practices and food waste from urban centres. This means that in such wetlands only bacteria able to live in highly ionised water can be able to survive such as *Actinobacteria* (Sui *et al.*, 2019).

In general, studies suggests that natural wetlands, as opposed to human-impacted wetlands, have higher wetland nutrient levels and dissolved ions thereby influencing the diversity and distribution of functional bacterial communities (Li *et al.*, 2018). The complex relationship between bacteria and environmental conditions results in a variation of the wetland bacterial community with different types of anthropogenic activities either domestic, agricultural or industrial. Bacteria better built to survive in those conditions are expected to be more dominant (Cavicchioli *et al.*, 2019).

2.7 Environmental deoxyribonucleic acid metabarcoding

Environmental DNA (eDNA) in this context refers to genetic material shed by organisms into their surroundings such as water, soil and even air, which can be used to study biodiversity within that particular ecosystem (Ruppert *et al.*, 2019). eDNA metabarcoding involves collecting environmental samples, extracting the DNA, amplifying target gene regions using universal primers in PCR, and sequencing them through next-generation sequencing (NGS). Then using the different analyses to generate meaningful information from these sequences (Farrell *et al.*, 2019).

This process usually starts with first collecting the samples from the environment which can be inform of water for our case in the wetlands of KNP but others can be sediments as well. Usually depending on the target species the volume of the water to be filtered

varies and for micro-organisms, 100 ml- 1000 ml can give an adequate DNA to be extracted (Williams *et al.*, 2016). In order to prevent contamination and ensure sample reliability, replicates and field controls are used in the sample collection process.

The DNA from most samples can be extracted using certain specialized kits or traditional methods such as the use of phenol-chloroform but these usually vary in efficiency as different taxa are targeted (Williams *et al.*, 2016). For PCR amplification, primers are used to amplify specific regions of the DNA and in the case of bacteria this is the 16S gene which is targeted. This is so because it contains many variable regions to distinguish between species in the kingdom. A Next-Generation Sequencing (NGS) step can then follow on high-throughput NGS platforms such as Illumina so as to generate millions of sequence reads. It should however be noted that sequencing depth is critical for detecting species diversity and ensuring that even rare species are identified within that sample.

In the bioinformatics and data analysis, the resulting sequence reads are grouped into Operational Taxonomic Units (OTUs) or Amplicon Sequence Variants (ASV) purely based on genetic similarity. This is usually set between 97 and 99% to minimize errors (Ruppert *et al.*, 2019). Many specialized software tools exist out there to carry out such an analyses depending on the researcher's choice but the most common are QIIME, mothur and phyloseq (Ruppert *et al.*, 2019). Clustered OTU' are compared to existing databases for species identification (National *et al.*, 2019). The databases include GenBanks, RDA, SILVA and they help match the OTUs to known species, though gaps in the libraries can sometimes limit identification.

The application of eDNA metabarcoding is widely used for biodiversity monitoring in various ecosystems, including freshwater, marine, terrestrial, and estuarine habitats. It

can detect rare, elusive, and invasive species, monitor ecosystem health, and assess community composition. The method is non-invasive, sensitive, and scalable, offering an alternative or complement to traditional monitoring techniques like visual surveys or netting. Although very useful, it is still a developing area and many challenges can be encountered such as contamination, DNA degradation, and skills and resources (Farrell *et al.*, 2019). Despite these challenges, eDNA metabarcoding is a transformative tool for ecological monitoring and conservation efforts, providing a cost-effective and powerful method to assess biodiversity at multiple spatial and temporal scales.

2.8 Conceptual framework

This was used to visualise the relationship between bacterial diversity of KNP wetlands and anthropogenic activities taking place in or around the park which included crop farming, animal grazing and urban activities like brick-making, and construction among others. The anthropogenic activities also influenced LULC, physicochemical properties and AAI of the wetlands (Figure 2.1).

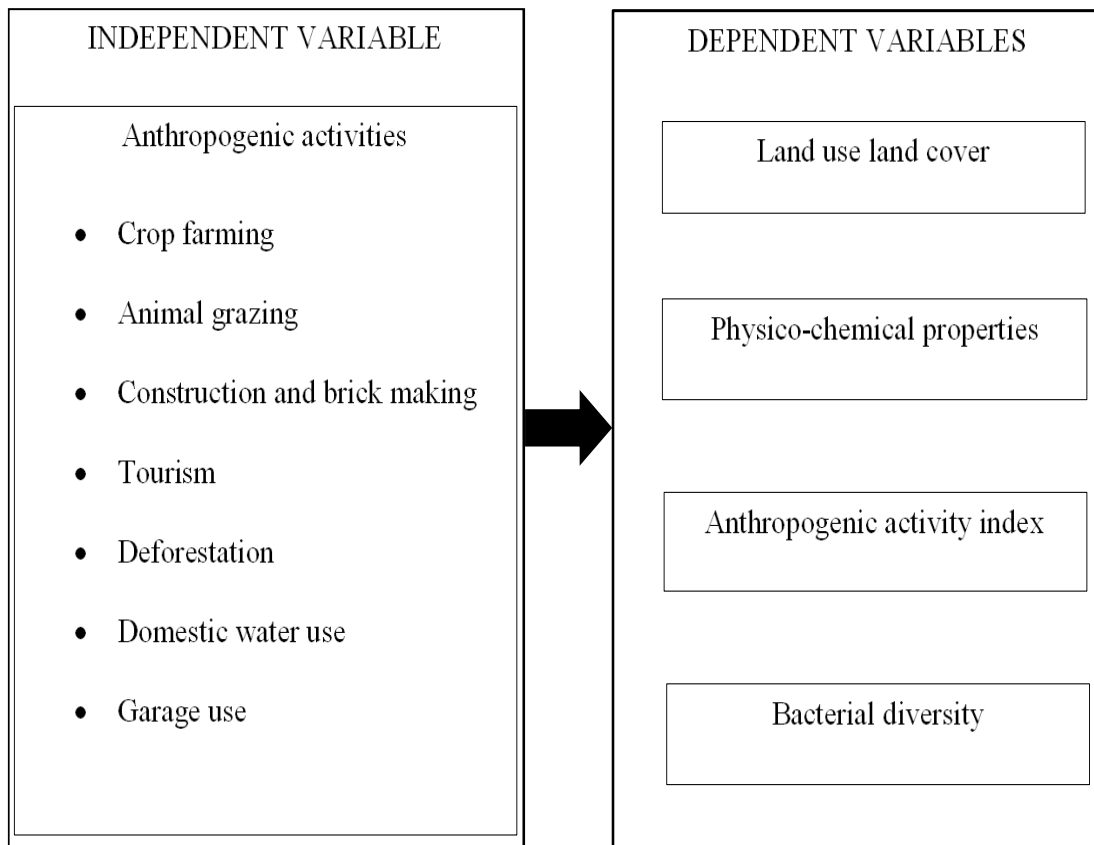


Figure 2.1: The conceptual framework depicting the relationship between anthropogenic activities and the four parameters studied.

CHAPTER THREE

MATERIALS AND METHODS

3.0 Introduction

This section comprises of the study area, the research design, how the environmental changes considered were studied, sampling technique, instruments and data collection.

3.1 Study Area

Kibale National Park (KNP) is a 795 km² mid-altitude rainforest (elevation 1100–1600 m), located in southwestern Uganda (0.436° N, 30.367° E) and is recognized as one of the last few remaining large forest fragments in the country. As one of the few remaining mid-altitude rainforests in East Africa, KNP is situated in the foothills of the Rwenzori Mountains. This is within the Albertine Rift Valley, a region that forms part of the "Eastern Afromontane" biodiversity hotspot as identified by Conservation International (Harter, 2019). The park was originally designated as a forest reserve in 1932 allowing for controlled logging, but was re-gazetted as a national park in 1993 to ensure greater conservation protections (Apodaca & Chapman, 2004).

Approximately 60% of KNP consists of tall and closed-canopy forest with tree heights exceeding 25 meters. The remainder of the park's landscape comprises wetlands, grasslands, and regenerating forests on areas previously cleared for exotic timber plantations. Kibale experiences a mean annual rainfall of 1655 mm, with measurements recorded between 1970 and 2022 ranging from 1205 mm to 2140 mm. The park has two pronounced wet seasons, with drier periods occurring between June–August and December–February. Hydrologically, KNP is drained by two primary river systems, the

Dura River and the Mpanga River, both of which flow into Lake George and extend beyond the park boundaries from where they were tracked. Bigodi borders the southeast corner of KNP, It was a special place since it had preserved a wetland as a popular tourist destination run by the 1992-founded Kibale Association for Rural and Environmental Development (KAFRED), (Hartter, 2019).

KNP is surrounded by a matrix of human-dominated landscapes, including intensive agricultural zones focused on food crops like bananas and cassava, pastures for livestock, timber plantations (pine and eucalyptus), and expansive tea plantations (Gosling *et al.*, 2017). This association of high biodiversity within the park and human land use around its borders highlights the complex challenges posed by conservation in the context of a rapidly growing human population. Previous studies in the park have shown significant effects of land use change on freshwater ecosystems, including alterations in water chemistry and shifts in aquatic community composition and functionality (Gosling *et al.*, 2017). These findings underscore the ongoing pressures on Kibale's ecosystems from both internal and external anthropogenic influences.

In addition to its ecological importance, KNP is also home to a rich diversity of fauna including over 375 bird species and 13 primate species, such as Chimpanzees (*Pan troglodytes*), red colobus monkeys (*Procolobus rufomitratus*), and L'Hoest's monkeys (*Cercopithecus lhoesti*) (UWA, 2022). This biodiversity has made Kibale a focal point for both conservation efforts and ecological research, attracting scientists. They are interested in studying forest ecology, primatology, and the impacts of environmental change on biodiversity. The presence of anthropogenic activities such as tea growing close to the park provided perfect conditions to find the required wetlands for the study. These wetlands were mainly separated into two regions, wetlands surrounded by the

forest and those surrounded by human activities. Swamps, streams and rivers were the different types of wetlands considered for comparison. Mikana stream (Inforest) with Emmanuel Adegnyira farm (EA farm) stream (Outforest) and Rwembaita swamp (Inforest) with Bigodi swamp (Outforest) and the rivers flowing from outside to inside.

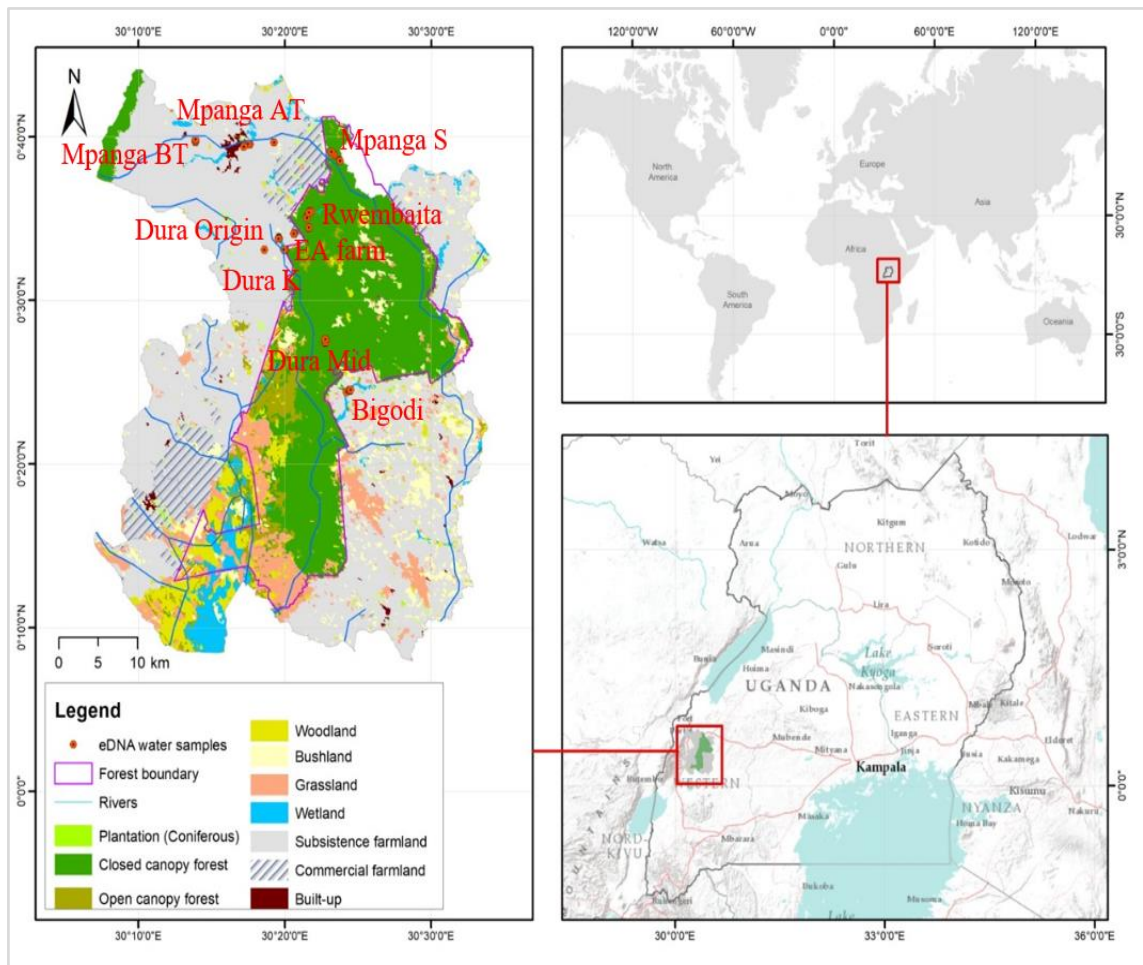


Figure 3.1. Sampling locations on the rivers, swamps and streams inside and outside the protected forest.

3.2 Study design

For a comprehensive examination of the relationship between anthropogenic activities and bacterial diversity in the KNP wetlands ecosystem, a cross-sectional design was used.

In this approach, the researcher examined how anthropogenic activities surrounding KNP

wetlands such as urban settlements and tea plantations through pollution and degradation influence other physicochemical properties of the wetland which then in turn alter the bacterial communities in similar wetland types. These were then measured and recorded using quantitative techniques with numerical values at one point in time.

3.3 Determining study sites and sample size

3.3.1 Site selection criteria

The study employed a rigorous selection process to encompass a range of wetland types with varying degradation levels within the Kibale National Park (KNP) region. Selection criteria focused on wetland sites that offered comparability between those within forested zones and those outside forest boundaries. This was specifically considering differences in land-use impact on wetland ecosystems (Papas *et al.*, 2022). Each chosen wetland was required to share similar basic ecological properties across types. This enabled for comparisons of anthropogenic influence on wetlands inside the forest versus those found outside the forested area.

Accessibility and safety were also critical to site selection, particularly for forest-embedded wetlands where limited access pathways and the presence of large, potentially dangerous wildlife, such as forest elephants, posed challenges to fieldwork (Park *et al.*, 2016). For wetlands situated outside forest protection, selection emphasized observable anthropogenic activities. These included hydrological alterations (e.g., drainage channels), settlements, erosion, and evidence of land-use impact through farming practices, native vegetation removal, domestic water use, and pollution (Clarkson & Sorrell, 2018). Only those activities that were distinctly apparent and measurable in situ were prioritized, allowing for a quantifiable assessment of anthropogenic pressures.

3.3.2 Sample size determination

A total of 50 water samples were collected from the ten wetlands and given that the population size and the population proportion were unknown, the study sample size was calculated using the Cochran formula below:

$$\text{Sample size} = \frac{Z^2 \cdot p \cdot (1 - p)}{e^2}$$

Whereby; Z is the z value at the reliability level, p is the estimated proportion of an attribute present in the population set at 0.5 and e is the acceptable sampling error. Therefore, given an acceptable sampling error of 0.1385 and a confidence level of 95 %, the Z value is 1.96, and the sample size will be;

$$\text{Sample size (n)} = \frac{(1.96)^2 \cdot 0.5 \cdot (1 - 0.5)}{(0.1385)^2} = 50 \text{ samples}$$

3.4 Assessment of land use and land cover changes

Land use and land cover changes (LULC) were determined using high-resolution images downloaded and processed using the same procedure (Sub-sections 3.4.1 to 3.4.4) for the different study periods.

3.4.1 Data source and periods

This environmental land use/cover change study primarily relied on three different periods of multi-spectral Landsat 7 Enhanced Thematic Mapper C1 Level 1 for 2003 images and Landsat 8 OLI C1 Level 1 for 2013 and 2023. Each of these were located within path 173 and row 060 tile that was able to cover the entire study area. These were

downloaded from the USGS Earth Explorer website. The year 2003 was the first year followed by 2013 and 2023 giving us 20 years with 10-year intervals. The study year intervals were thought to be rational for significant LULC changes to have happened in the area (Kusiima *et al.*, 2022). All the images downloaded for the three years were between March and April, which was a dry period hence less cloud-cover on the images. This also allowed the phenology and seasons to be kept consistent across all study years. The images with a cloud cover of less than 10% were filtered and downloaded.

3.4.2 Composite of satellite imageries

The image bands 5, 4 and 3 of the various Landsat images of the same year were processed into single unified composite images and masked with a digital map of KNP and the surrounding sub-counties, representing the study's area of interest. The compositing corrected the atmospheric effects before classification to get a detailed analysis (Song *et al.*, 2001). The surrounding sub-counties were included to show the changes in the anthropogenic activities happening around the protected area.

3.4.3 Classification of images

Masked satellite image classification was used to group all of the pixels in a satellite image into information classes based on land use/cover types (Pax-Lenney *et al.*, 2001). The Iso Cluster Unsupervised Classification tool was then used to perform unsupervised classification in ArcMap 10.8 software. For greater accuracy, the number of classes was tripled to fifteen before grouping them into the five that were considered. These included forest, farmland, built-up, grassland and open water. The researcher was responsible for making Google Earth comparisons and using ground truthing points that represent the ideal classes during the classification process (Tantithamthavorn *et al.*, 2016).

3.4.4 Accuracy assessment and change detection

Before using the results in detecting change, there was a need for accuracy assessment post-classification. This was performed to validate the classification's efficacy by determining how well the resultant LULC agrees with the actual ground features (Muriithi, 2016). The accuracy of topographic maps of the study area for each year was confirmed by KNP map archives, Google Earth's features and field visual interpretation. The accurate environmental land use change for each classified image was then determined by overlaying all the classified images together. Using the Muriithi, (2016) methodology, a detailed cross-matrix derived from change-detection statistics was used to derive information on LULC change trends and quantitative area conversions on a pixel-by-pixel basis.

3.5 Determining the anthropogenic activity index

The degradation levels of the wetlands within and around KNP were determined using a modified index from both the Department of Environment, Land, Water and Planning of Australia (Papas *et al.*, 2022) and New Zealand Land care research (Clarkson & Sorrell, 2018). These assessments used five main criteria of physical appearance including land use, soil disturbance, hydrology, vegetation type and turbidity ranked in a table for each of the wetlands. It was simple and fast since the researcher used his visuals and judgement to fill in the assessment form. On the form, the rating ranged from 1 to 3 with 1 meaning intact or not degraded, 3 as highly degraded and 2 for moderate conditions (Appendix 1).

3.6 Sample collection

Water samples were collected using the eDNA citizen scientist sampler (Smith-Root) for each of the 10 different wetland spots as described in sub-sections 3.6.1 to 3.6.3.

3.6.1 Water sample filtering

In June 2023, samples were collected with the assistance of a field guide and an assistant familiar with the geography of the study area. Ten sampling locations were identified, with five replicas and one control taken within a kilometre radius of each location, yielding a total of 50 samples and 10 controls. Rwenzori water from a Coca-Cola bottling plant was used as the blank control to test for any contaminations during sampling but not for comparison with wetlands. Sampling sites included swamps, streams, and rivers, located either inside or outside the park, where external locations were likely influenced by anthropogenic activities. The specific sites were Mikana Stream, EA Farms Stream, Rwembaita Swamp, Bigodi Swamp, Dura Origin, Dura Kyamugara, Dura Mid, Mpanga BT, Mpanga AT, and Mpanga Sebitoli. Sampling was conducted using the eDNA citizen scientist sampler (Appendix 2) and its specialized filters designed for efficient, rapid collection and long-term preservation of samples without processing. The sampled volumes ranged from 800 ml to 1000 ml, depending on water turbidity, with sampling times ranging from 10 minutes to over an hour. Turbid water caused filter clogging and extended sampling time, whereas clearer water facilitated faster collection. To prevent contamination, sampling proceeded from downstream to upstream. Environmental DNA samples were collected first, followed by the measurement of physicochemical properties and GPS coordinates. To maintain sample integrity, gloves, bleach, and paper towels were used consistently for each sample (Pope *et al.*, 2020). Each sample was then sealed back

into its packet together with silica gel as a preservative. The packets were labelled with brief descriptions of time, volume, location and water properties. The samples were kept sealed in a cool dry place until they were transported to the Makerere University Department of Zoology Laboratory where they were stored at -20 °C (Williams *et al.*, 2016). Samples were exported to the plant ecology laboratory at the University of Zurich Switzerland for processing and sequencing.

3.6.2 Measurement of the physicochemical properties

The Physicochemical properties were measured using a four-in-one electrical metre (AZ86031) (Appendix 2) that recorded dissolved oxygen in milligrams per litre (mg/l), conductivity in micro-Siemens per centimetre ($\mu\text{S}/\text{cm}$), temperature in degrees Celsius ($^{\circ}\text{C}$) and pH (Rahman *et al.*, 2021). Five water samples were collected in a clean container after rinsing it with the same water source for each of the ten wetlands and then the meter dipped into it. This was then allowed to settle for a moment until the readings were stable for recording (Bilewu *et al.*, 2022).

3.7 Determination of bacterial diversity

A systematic procedure of isolating the genetic material, sequencing and analysis was used to determine the bacterial composition found in the 10 wetlands of KNP studied described in sub-sections 3.7.1 to 3.7.5.

3.7.1 Deoxyribonucleic acid extraction

All samples of DNA were extracted and purified using the Qiagen DNeasy[®] PowerWater[®] Sterivex[™] Kit following the protocols at

<https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/microbial-dna/dneasy-powerwater-sterivex-kit>.

3.7.2 Polymerase chain reaction amplification

A two-step PCR approach was used for the amplification of V3 and V4 regions of the 16S gene of bacteria where the first PCR amplification of the desired fragment occurred. Tagged primers were used and three PCR replicates created with different tags for each replicate (National *et al.*, 2019). In the second PCR, the Illumina index and tail were attached. The clean-up pool of all PCR replicate samples was done with Kapa Pure Beads. The Primers used were U431F 5'- CCTACGGGDDGGCWGCA - 3' and U806R 5' - GGACTACHVGGGTMTCTAATC - 3'. For the Amplification procedure, nine microlitres (9 µl) of the mixture was put into each PCR well and one microliter (1 µl) of either a sample or control. The mixture contained ddH₂O (10 µl), Multiplex MasterMix (15 µl) and forward and backward primers (1 µl) each. The number of PCR cycles was 31 following the steps of denaturation (96 °C, 45 seconds), annealing (55 °C, 60 seconds), extension (74 °C, 60 seconds) and hold (10 °C, indefinite). Amplification success was checked using a QiAxcel[®] Screening Cartridge and the three PCR replicates were pooled using the intensity of the QiAxcel[®] band as a guide.

After a bead cleaning step using the Agencourt AMPure XP beads from Beckman Coulter[®] with a ratio of 0.8 and following the manufacturer's protocol was performed. Quantified DNA in each sample using a selective fluorescence dye Qubit[™] BR DNA Assay Kit, read on a Spark[®] Multimode Microplate Reader and pooled all samples at equimolar concentration. Then cleaned the library pool one last time using the AMPure XP beads at a ratio of 0.8 before assessing the quality of the library on an Agilent 4200

TapeStation System and quantified its concentration using the Qubit™ (1.0) fluorometer following the manufacturer's protocol for the dsDNA HS Assay.

3.7.3 Sequencing

Sequencing was done on a NovaSeq SP FlowCell 2x250 base pairs (bp) at the Functional Genomic Centre University of Zurich (FGCZ). The de-multiplexing step was performed using the cutadapt-v2.8 tool to remove both tags and primers allowing one error (13%) on the tags and up to 30% of errors on the primers.

3.7.4 Processing and cleaning

The DADA2 package in R (package version 1.24.0, R version 4.2) was used to identify the amplicon sequence variants (ASV) present in each sample (Callahan *et al.* 2016). The nucleotides were trimmed at 200 bp and 220 bp positions for forward and reverse DNA sequences, respectively. Reads with an expected error higher than 2% of the total nucleotide sites (i.e. 4 nucleotides) were discarded. The error structures of sequences were modelled using the *learnErrors* function in DADA2 with a modified error estimation function. Loess function arguments (setting parameter span to 0.95, the degree to 1 and using log-transformed totals as weights) and enforced monotonicity were then altered. This modification had better performance in predicting the error structure of our sequencing data, which was featured with a banning error rate obtained from the Novaseq sequencing platform as compared to the default setting that was designed for Illumina high-throughput sequencing (Kaushal *et al.*, 2022). After that, the amplicon sequences were de-replicated and ASVs were inferred using DADA2 default parameters. The forward and reverse reads with an overlap >12 nucleotides were merged to construct a sample-by-sequence observation matrix. Nonbacterial sequences and chimeric sequences

were removed in DADA2. Taxonomy was assigned to each ASV by comparison to the SILVA ribosomal RNA gene database (v.138.1) (Quast *et al.* 2013).

High quality 16S rRNA gene of 770, 000 sequences and identified 3975 ASVs were obtained from all samples. The total number of sequences per sample ranged from a few hundred to tens of thousands. To account for this large variation in sequencing depth, rarefied the total number of sequences to 1,000 per sample using the R package ‘vegan’ (v.2.6.2), and excluded sequencing files with lower sequencing depth. Few ASVs were found in PCR negative controls but not in our field sample controls, indicating some potential external contamination during molecular processing. The 16S primers used in the study were very sensitive to eDNA. Contaminated ASVs were removed from all samples. After removing the contaminations and negative controls, the community composition of all ASVs across samples was obtained and ready for statistical analysis.

3.8 Statistical analyses

3.8.1 Analyses for the land use land cover changes

The area pixel changes for each of the five land use and land cover changes was determined as a percentage of the total area studied. This was done for each of the years differently before the comparisons were drawn. This was done using the ArcMap 10.8 software.

3.8.2 Analyses of physicochemical properties and anthropogenic activity index

Two kinds of statistical analysis were carried out on the physicochemical property measurements based on the type of wetland. For wetlands that had two groups of data sets for each of the properties, a student’s t-test with two-tailed distribution and two-

sample unequal variance was carried out to test the significance. Meanwhile, for wetlands that had three groups of data sets, a single-factor analysis of variance was carried out for each of the properties. For the AAI the sum of each all the 5 index scores was calculated for each wetland. All data analyses were performed in the R programme (version 4.2) with an alpha value of 0.05 so as to ensure uniformity in the significance results.

3.8.3 Bacterial diversity analyses

Shannon diversity of bacterial communities was calculated using the *diversity* function in the ‘Vegan’ package at the species level represented by the ASV’s. This was tested whether the Shannon diversity differs between in- and outside forest sites using Student's t-test. To evaluate the effect of deforestation on bacterial Shannon diversity, the river length in or outside the forest from the edge was measured. Shannon diversity in the river as a function of the river length index was then modelled using linear regression.

Non-metric multidimensional scaling (NMDS) was used to visualize the variation in bacterial community composition across rivers, streams and swamps, and between in- and outside the forest habitats. Then, Permutational multivariate analysis of variance (PERMANOVA) was used to statistically test significance (Mbareche *et al.*, 2020). Natural forests were hypothesized to keep and restore bacterial communities in wetland ecosystems. To test this hypothesis, bacterial community dissimilarity among the wetland types for inside and outside forests was calculated. Then to test whether bacterial community dissimilarity decreased or increased from outside to inside the forests, a Wilcoxon signed-rank test was used.

Lastly, the DESeq2 approach was used to identify the bacterial taxa that are differentially abundant between inside and outside forest wetlands. This approach was primarily

designed for the analysis of DNA sequencing data to classify differentially expressed genes between different experimental conditions (Mbareche *et al.*, 2020). Here it was employed to look for bacterial species abundance for forest versus non-forest habitat. Since the bacterial composition in rivers differs from that in swamp and stream, we conducted a DESeq2 model for river and another one for swamp and stream, in which bacterial ASVs with significantly different abundance ($p < 0.01$) between in and outside forests were identified separately for river, then swamp and stream together. All graphs for bacterial diversity were plotted in R version 4.2 (Team, 2022).

3.9 Limitations of the study

Table 3.1. The main limitations/challenges encountered during the study and the solutions provided for each of them.

| Nº | Limitation/challenge | How Limitation/challenge was solved |
|-----------|---|---|
| 1 | Accessing the right wetland points to collect samples | Sampled from accessible wetlands and hired local experts to show the best way and guide through |
| 2 | Threats from wild animals like elephants while collecting samples | Postponed sampling till there was no more threat from the wild animals |
| 3 | Filter clogging due to turbid water | Patience of waiting for hours until the required volume of water sample had been obtained |
| 4 | Sequencing errors and sample contamination | Use of controls and replicas at every stage and processing of sequences before analysis |

CHAPTER FOUR

RESULTS

4.0 Introduction

This chapter gives a detailed account of the findings of this research study in a systematic, simple and easily understandable manner. The findings for each objective pictorially with analysis summarised.

4.1 Land use land cover change in and around Kibale national park

The five parameters studied in LULC changes were farmland, built-up, forest, grassland and open water sources. All the pixels calculated within KNP and the surrounding sub-counties belonged to either one of the above parameters.

4.1.1 Land use land cover changes map

The LULC made were within a span of 20 years with a difference of 10 years between each of the resulting years. These were from 2003-2013 and from 2013 and 2023. The map for each of the study years was put together into one frame for better visual comparisons (Figure 4.1). The forest was represented by dark green, grassland by light green, farmland by brown, built-up by red and water by blue. The year 2003 map in the top left corner of the frame was much darker green (forest) as compared to the bottom right of the year 2023, which looks light green (grassland). The blue seen for water cover were mainly the numerous creator lakes found in the area as confirmed through ground truthing, google earth and KNP map archives. The areas of Rwimi and Hima at the bottom left were mostly red and brown in all the years while the red colour at the location of major towns like Fortportal and Kamwenge kept increasing after every 10 years. It can

be seen that mostly the map shifted from dark green (forest cover) to a light green (grassland) (Figure 4.1).

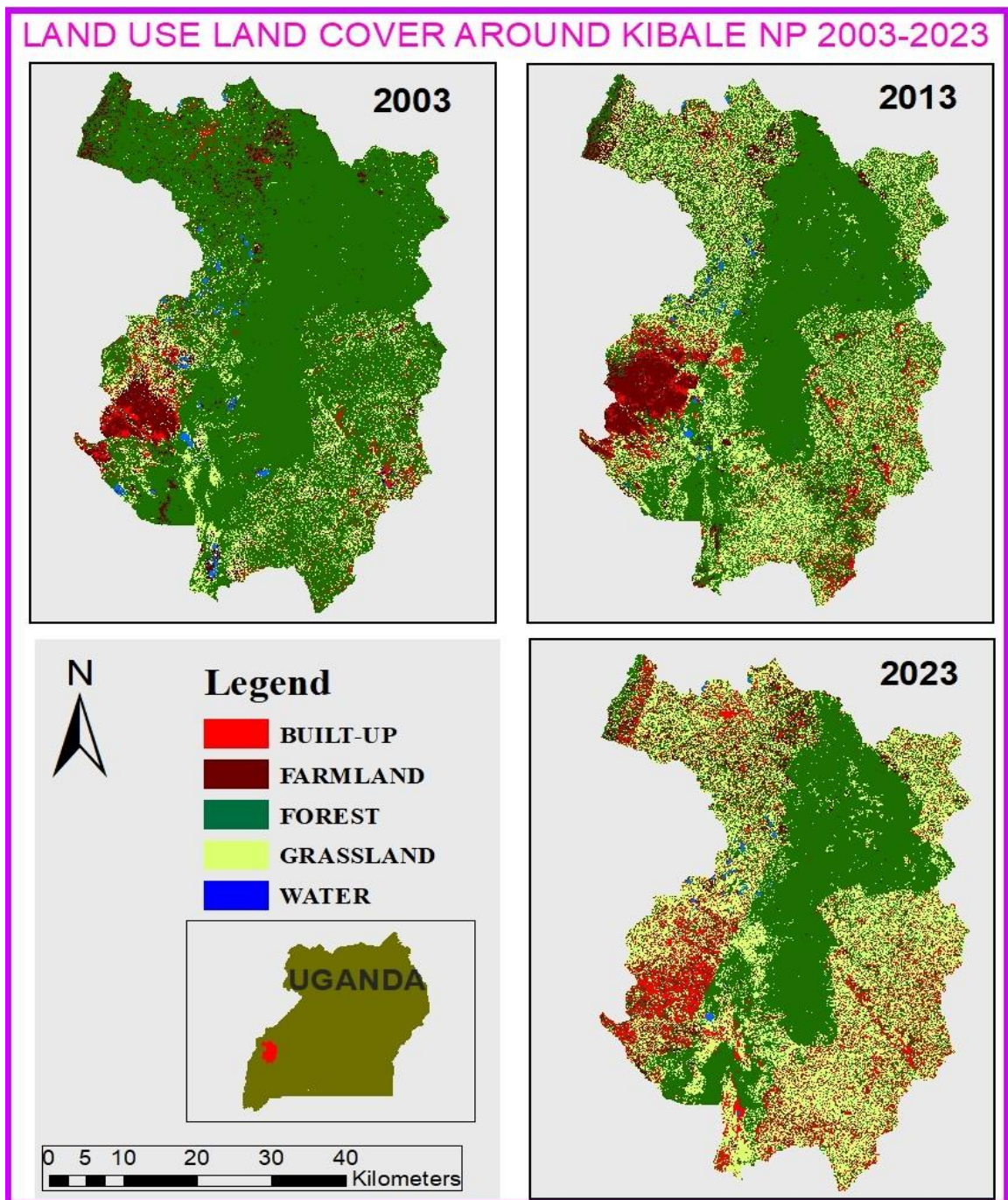


Figure 4.1. Study area depicting the five-land use and land cover changes taking place for three study periods.

4.1.2 Statistical percentage change

The map visuals do agree with the statistical calculations performed on Arc map 10.8. In 2003, forest cover was at 78%, the highest statistical percentage and this gradually dropped from 2013 at 57.7% to 2023 at 42.5% (Figure 4.2). The built-up area increased gently as well as the farmland through the years. Both built-up and farmland increased by an average factor of 2% each between 2003 and 2013 and then by an average factor of 3% from 2013 to 2023. Interestingly, the percentage of grassland more than doubled from 11.7% in 2003 to 27.4% in 2013 and then a smaller increase was seen from 2013 to 2023 at 36.6%. As for the water cover, the 1% in 2003 was halved in 2013 and then decreased further by 0.1% in 2023. Apart from water cover, the changes in other land use and land cover were seen to be significant both from 2003 to 2013 and from 2013 to 2023. Much of the forest cover had decreased while built-up, farmland and grassland increased. Of the increases seen, grassland had the most prominent percentages (Figure 4.2).

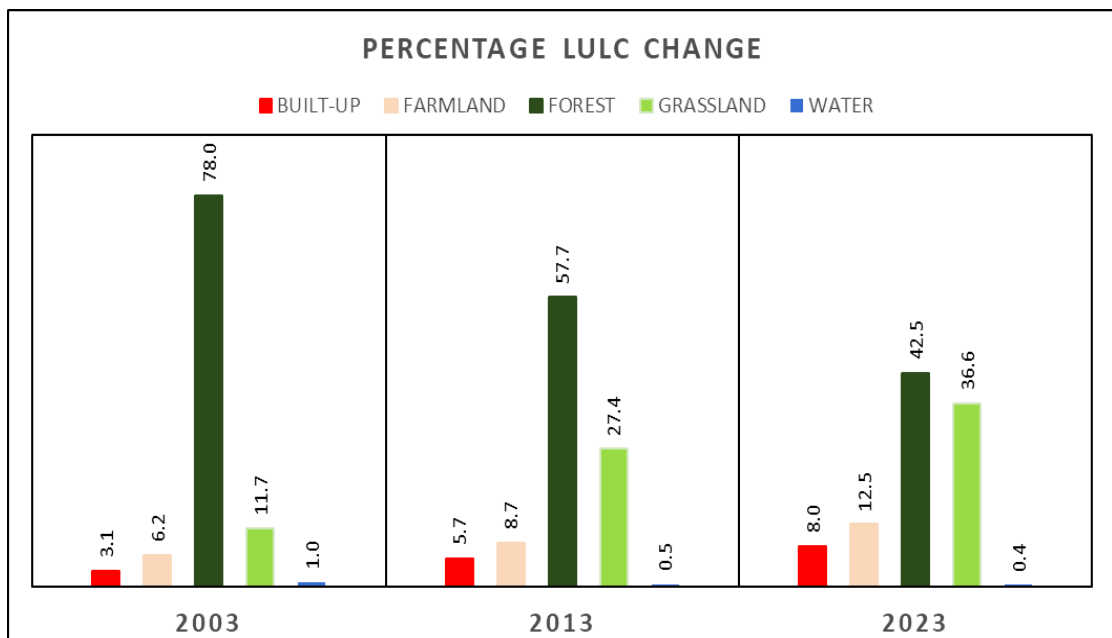


Figure 4.2. The statistical percentage area changes of the five land use land cover classes studied for each particular year.

4.2 Anthropogenic activity index

4.2.1 Anthropogenic activity index scores

Ten wetlands were considered for this study and each was assessed using the same index form (Appendix 1). The wetlands presenting three main types included the Bigodi swamp and Rwembaita swamp for swamps, Mikana stream and EA farm stream for streams, Mpanga BT, Mpanga AT, Mpanga Sebitoli, Dura Origin, Dura Kyamugara and Dura mid for rivers. These names given to the wetlands were as called by the local communities around these areas and other previous researchers who had carried out studies in the area. Five metrics were used to assess the condition of the wetland, which were the land use around the wetland, the level of disturbance of the soil in the area, the hydrology and flow of the water within the wetland, the turbidity of the water and finally the type of vegetation growing within and around the wetland (Appendix 1).

All the metrics in the Bigodi swamp were neutral at two except for land use, which scored a three. As for Mpanga AT River, all metrics were scored at three. These two areas were close to urban areas. The lowest metrics were scored at the Mikana stream which had all low scores except for soil disturbance which was at a moderate score. Considering the wetland types, wetlands outforest tend to score more highly across all the indexes used as compared to their counterparts inside the forest. For example, Bigodi swamp and EA farm stream have more scores of 2 and 3 than Rwembaita swamp and Mikana stream respectively (Table 4.1).

Along the Dura river it was seen that Dura origin and Dura Kyamugara located outforest barely recorded any value of one in the indexes yet Dura mid inforest had all ones except for its turbidity scored at 3. For Mpanga River, Mpanga BT had a one for hydrology and

Mpanga AT has all threes yet both are outforest. Mpanga Sebitoli found inforest had ones then a 2 at soil disturbance and a 3 for turbidity. Among the five indexes used all the wetlands seem to be turbid except for Mikana stream that had a clear water (Table 4.1).

Table 4.1. The ratings of the five metrics used to measure the anthropogenic activity index of each wetland type on a scale of one to three.

| WETLAND | TYPE | STATUS | LAND USE | SOIL | HYDROLOGY | VEGETATION | TURBIDITY |
|-----------------|--------|-----------|----------|------|-----------|------------|-----------|
| Mikana Stream | Stream | Inforest | 1 | 2 | 1 | 1 | 1 |
| EA farm Stream | Stream | Outforest | 3 | 2 | 3 | 3 | 2 |
| Rwembaita Swamp | Swamp | Inforest | 1 | 2 | 1 | 1 | 2 |
| Bigodi Swamp | Swamp | Outforest | 3 | 2 | 2 | 2 | 2 |
| Dura Origin | River | Outforest | 2 | 2 | 1 | 2 | 2 |
| Dura Kyamugara | River | Outforest | 3 | 2 | 2 | 2 | 2 |
| Dura Mid | River | Inforest | 1 | 1 | 1 | 1 | 3 |
| Mpanga BT | River | Outforest | 3 | 2 | 1 | 2 | 2 |
| Mpanga AT | River | Outforest | 3 | 3 | 3 | 3 | 3 |
| Mpanga Sebitoli | River | Inforest | 1 | 2 | 1 | 1 | 3 |

4.2.2 Wetland degradation levels

The sum of the metrics was calculated and scored ranging from 5 to 15 to show the level of degradation for each of the wetland based on the AAI. The values of the sums were interpreted in that a sum of five (5) was no degradation which meant that across all indexes the wetland had scored a one. A ten (10) would mean a neutral point having scored 2 in all the indexes and a fifteen (15) showed total degradation as the wetland would have scored a 3 across all indexes. From the neutral point of 10, a graph of the sum of the indexes for each wetland was then plotted to see which direction the wetland took of either no degradation or total degradation. Wetlands located outforest were seen to take the direction towards total degradation such as EA farm stream, Mpanga AT, Bigodi

swamp and Dura Kyamugara and of these Mpanga AT is the closest to total degradation. Wetlands inside forest generally tend toward no degradation such as Mikana stream, Rwembaita swamp, Dura mid and Mpanga Sebitoli and of these Mikana stream is the closest to being fully intact.

Dura origin despite being outforest is seen heading towards the direction of non-degradation which could be a good sign for the wetland. Mpanga BT registered an exact neutral point meaning that on average all its five indexes scored a two that is to say, land use 2, soil disturbance 2, hydrology 2, vegetation type 2 and turbidity 2 (Figure 4.3). The AAI degradation levels therefore shows that there was a significant difference in the AAI between wetlands of the same type yet surrounded by different activities. Wetlands in the protected areas surrounded by the forest are less degraded than those found outside surrounded by human activities such as tea plantation agriculture and urban settlements.

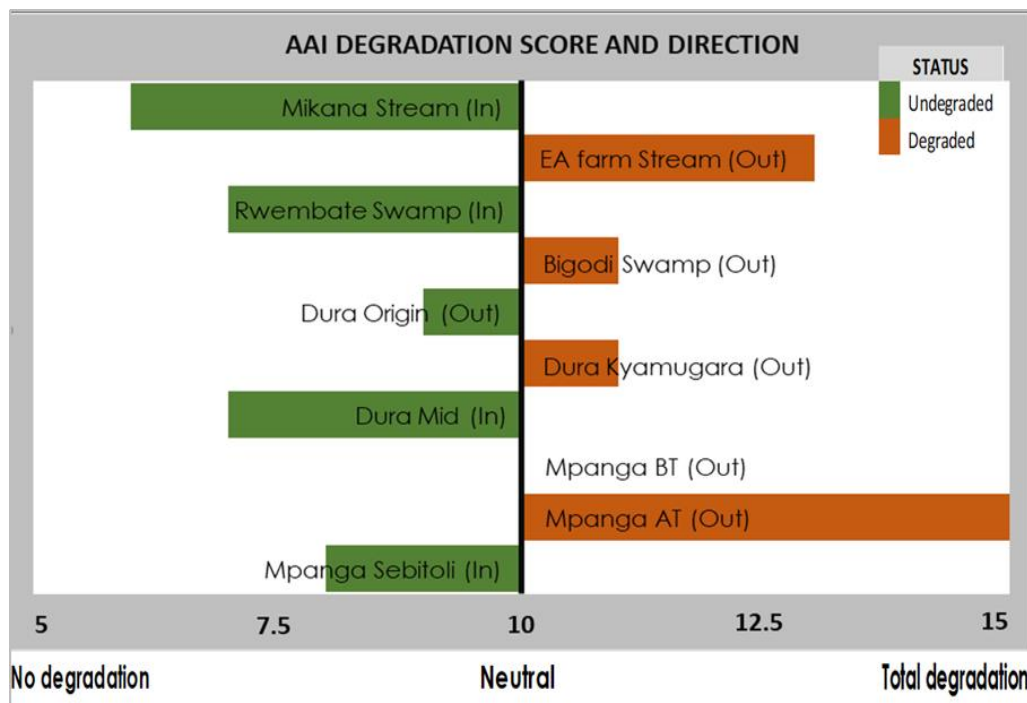


Figure 4.3. Sums of the indexes as each of the wetlands tends towards the condition of either no degradation or total degradation.

4.3 Physicochemical properties

Four physicochemical properties were measured repeatedly five times for each of the wetlands studied i.e., pH, dissolved oxygen, conductivity and temperature. The means of the five recording within a wetland were calculated and represented (Table 4.2). Starting with the streams, there was a significant difference between Mikana stream and EA farm stream in all the in all the four physicochemical properties. Mikana stream had a closure to neutral pH 7.34, a higher dissolved oxygen 7.75 mg/l, a higher conductivity of 55 $\mu\text{S}/\text{cm}$ but a lower temperature of 18.4 $^{\circ}\text{C}$ as compared to its counterpart EA farm stream Outforest. Between the two streams the most significant difference was seen in their dissolved oxygen as EA farm had a very low mean dissolved oxygen value of 2.07 mg/l.

There was no significant difference in the physicochemical properties between the swamps except for conductivity. Both Bigodi swamp and Rwembaita swamp had close to neutral pH (7.0 and 7.5), low dissolved oxygen (0.92 mg/l and 1.78 mg/l) and similar temperatures (21.2 $^{\circ}\text{C}$ and 21.1 $^{\circ}\text{C}$). But their conductivities had a big difference with Bigodi recording a high value of 132 $\mu\text{S}/\text{cm}$ and Rwembaita a low value 57 $\mu\text{S}/\text{cm}$. Along the Dura River, all the physicochemical properties showed a significant difference among its wetlands. The low pH of Dura Kyamugara (5.89) was significantly different from that of Dura origin (6.89) and Dura mid (6.69).

In Dura mid dissolved oxygen and electrical conductivity recorded a significantly higher values (7.31 mg/l and 214 $\mu\text{S}/\text{cm}$ respectively) than Dura origin and Dura Kyamugara which both had similarly low values of dissolved oxygen and electrical conductivity. In the Mpanga river system, all the physicochemical properties were significantly different among the wetlands with electrical conductivity being the most significant. This is due

the fact that Mpanga BT had a very low electrical conductivity of 177 $\mu\text{S}/\text{cm}$ as compared to the high value of Mpanga AT and Mpanga Sebitoli both having 300 $\mu\text{S}/\text{cm}$ and 313 $\mu\text{S}/\text{cm}$ respectively. Temperature values showed low significance difference in comparing wetland types meanwhile dissolved oxygen and electrical conductivity had the most significant differences. Although there were no clear patterns in the variation of the physicochemical among the wetlands, it could be noted that there exists a significant difference between inforest and outforest wetlands of the same type (Table 4.2).

Table 4.2. The means of the four physicochemical after five measurements from each of the wetlands.

| WETLAND NAME | pH | OXYGEN (mg/l) | CONDUCTIVITY ($\mu\text{S}/\text{cm}$) | TEMPERATURE ($^{\circ}\text{C}$) |
|-----------------|------------------------------|------------------------------|---|---------------------------------------|
| Mikana Stream | 7.34 ^a \pm 0.19 | 7.75 ^a \pm 0.24 | 55 ^a \pm 1.2 | 18.4 ^b \pm 1.1 |
| EA farm Stream | 6.50 ^b \pm 0.17 | 2.07 ^b \pm 0.98 | 35 ^b \pm 1.4 | 20.2 ^a \pm 0.90 |
| Rwembaita Swamp | 7.50 ^a \pm 0.43 | 1.78 ^a \pm 2.13 | 57 ^b \pm 3.6 | 21.1 ^a \pm 1.07 |
| Bigodi Swamp | 7.00 ^a \pm 0.05 | 0.92 ^a \pm 0.64 | 132 ^a \pm 2.3 | 21.2 ^a \pm 0.29 |
| Dura Origin | 6.89 ^a \pm 0.55 | 0.98 ^b \pm 0.56 | 81 ^b \pm 15.7 | 19.5 ^b \pm 0.60 |
| Dura Kyamugara | 5.89 ^b \pm 0.12 | 1.39 ^b \pm 1.11 | 80 ^b \pm 11.4 | 22.4 ^a \pm 1.56 |
| Dura Mid | 6.69 ^a \pm 0.31 | 7.31 ^a \pm 0.35 | 214 ^a \pm 1.7 | 21.5 ^a \pm 0.22 |
| Mpanga BT | 7.23 ^b \pm 0.12 | 6.58 ^b \pm 0.03 | 177 ^b \pm 2.2 | 19.8 ^b \pm 0.31 |
| Mpanga AT | 7.31 ^b \pm 0.13 | 6.18 ^c \pm 0.08 | 300 ^a \pm 4.7 | 21.4 ^a \pm 0.48 |
| Mpanga Sebitoli | 7.74 ^a \pm 0.11 | 7.26 ^a \pm 0.25 | 313 ^a \pm 3.2 | 20.5 ^b \pm 0.49 |

4.4 Bacterial diversity

The bacterial diversity was measured in terms of relative abundances, Shannon diversities, Wilcoxon dissimilarities, taxa abundances and non-metric multidimensional scaling.

4.4.1 Relative abundance

Twenty of the most commonly detected bacterial phyla were presented as a ratio of the total ASV found in each wetland (Figure 4.4). The wetlands outside the forest were presented in yellow circles and those in the forest with green circles. The highest ratio of bacterial phylum found in the KNP wetland were the two phyla of *Proteobacteria* (lapis blue) and *Actinobacteriota* (lemon yellow). These two phyla constitute over 75% of the total bacteria found in each of the 10 wetlands. The abundance of *Proteobacteria* was higher in wetlands outside the forest than their respective counterparts inside the forest in streams and rivers. Whereas for the swamps Bigodi swamp outforest had more abundant *Proteobacteria* than Rwembaita swamp Inforest. Oppositely, the abundance of *Actinobacteriota* was higher in wetlands outside the forest than those inside the forest of the same type (Figure 4.4).

Mpanga Sebitoli and Mikana stream had the most abundant *Proteobacteria* consisting of over 65% of the total bacterial population in these two wetlands. As for *Actinobacteriota*, it presented over 50% total abundance in most wetlands except for Mikana stream where it was very low. Other noticeable phyla include *Chloroflexi* (Kelly green) in Dura Origin, *Firmicutes* (Tiffany blue) in Mpanga AT and Bigodi swamp. These are both in town area with high potential of sewage pollution, and *Planctomycetota* (Pthalo blue) in Rwembaita swamp and Dura Origin. EA farm stream and Dura Kyamugara also recorded noticeable

values of Acidobacteria just as the two had the most acidic pH of all the wetlands (Table 4.2). The interchanging abundances of *Actinobacteriota* and *Proteobacteria* shows certain level of competitive interaction between the two dominant phyla (Figure 4.4).

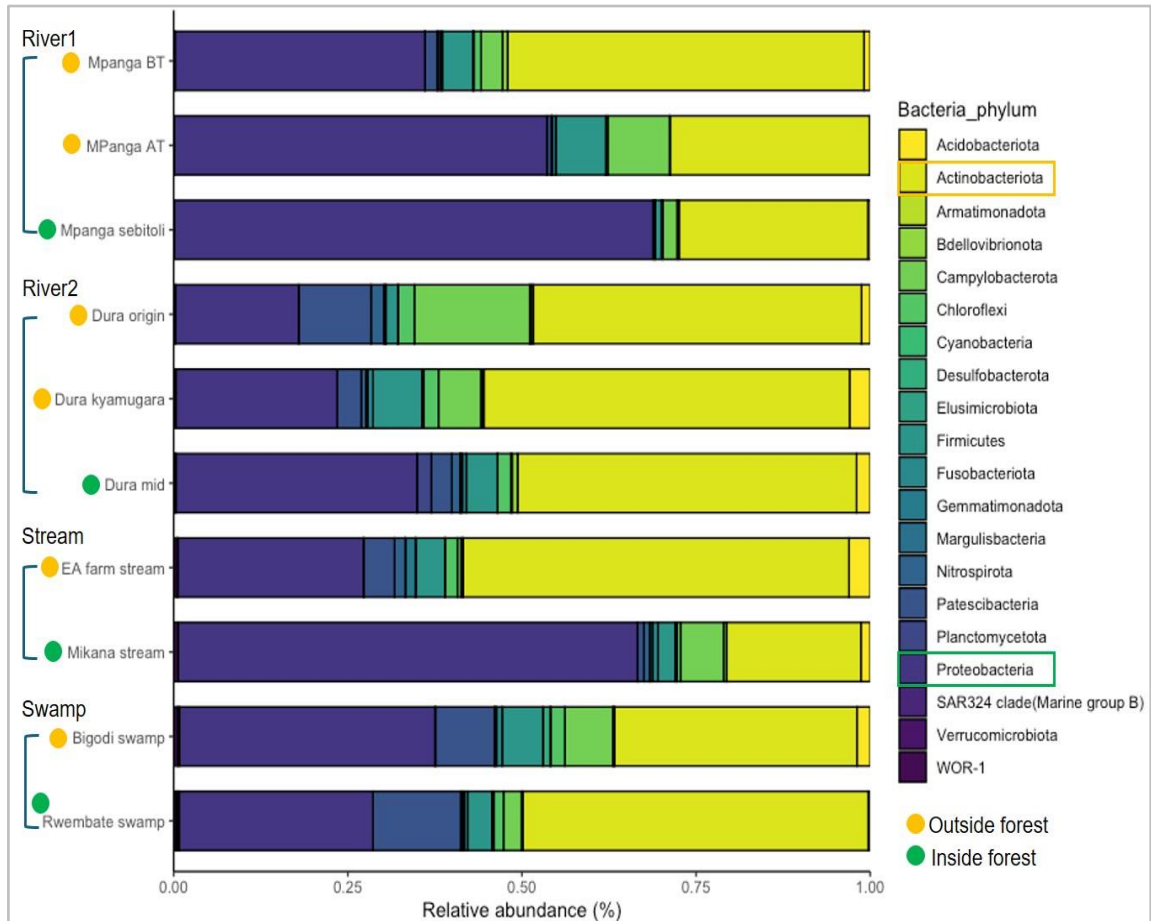


Figure 4.4. The relative abundance of the twenty most common bacterial phylum found in wetlands outforest and inforest.

4.4.2 Shannon diversity

Shannon diversity in this case was used to show bacterial species richness (number of bacterial species) and evenness (uniformity of species abundance) in the wetlands of KNP using the ASV's found. The richness of bacterial species in River Dura increases from Dura origin through Dura Kyamugara to Dura mid as it flowed from outside the forest to inside the forest (Figure 4.5a). The variation in the richness was most significant between

its furthest points of Dura origin and Dura mid with $p=0.01$. Meanwhile, for River Mpanga, the richness decreased as the river flowed from the outside of the forest to the inside. This variation was also the most significant between the two furthest points of Mpanga BT and Mpanga Sebitoli with $p=0.013$ (Figure 4.5b).

The Shannon diversity analysis for all six sites on River Mpanga (3) and River Dura (3) with the distance of the sampling points into or out of the forest is in Figure 4.5c). Variation in the bacterial diversity between the two rivers was highly significant with $p=0.002$. However, only 16% of this result could be attributed to the differences in distance from the forest edge. The furthest point outside from the forest edge was Mpanga BT at a distance of 8.2 kilometres and the furthest inside was Dura mid at 4.7 kilometres.

There was a correlation between bacterial species richness and the distance of the sampling point from the forest edge. Plotting a regression line to understand how distance from the forest edge influences bacterial diversity shows that species richness increases as you move further away from the edge (Figure 4.5d). The R^2 value calculated was very low at 0.16 (Figure 4.5c). The Shannon diversity for the swamps and streams both in and out of the forest was similar with no significant differences seen.

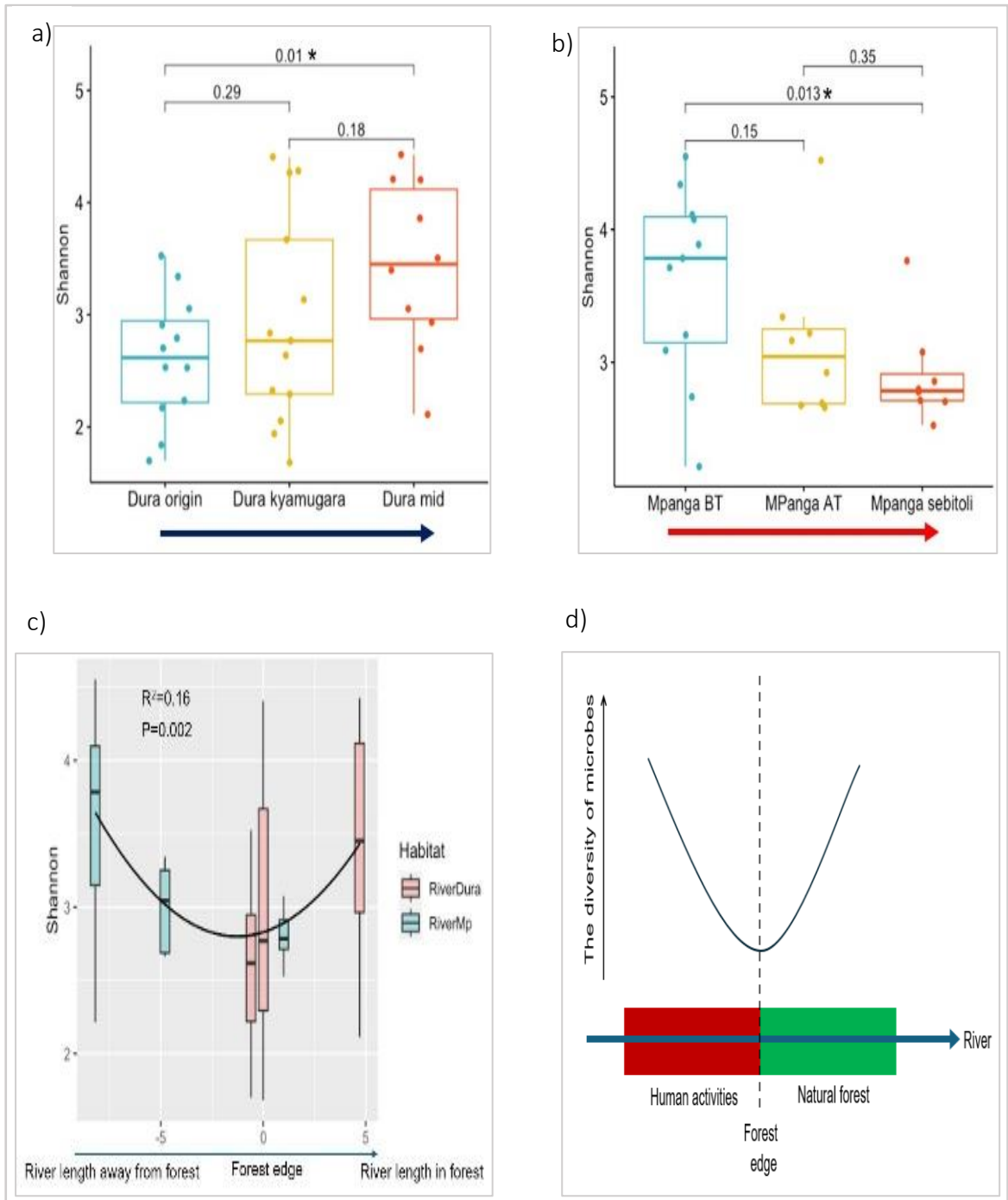


Figure 4.5. Shannon diversity of bacterial species richness and evenness in Dura and Mpanga rivers and the species richness in both rivers with distance from forest edge.

4.4.3 Non-metric multidimensional scaling

The NMDS analysis here was used to visualize the possible similarities and relationships existing between the wetland types. It took into account all the ASVs found in each wetland site as well as the metadata consisting of the physicochemical properties and the AAI scores (Figure 4.6). Considering the wetland types of either River, stream or swamp in Figure 4.6a), the non-metric multidimensional scaling (NMDS) analysis shows that the bacterial community found in the river systems was quite different from those found in streams and swamps.

The stream and swamp samples had a high similarity but varied from the river samples. This variation in species composition among the different wetland types was very significant with $p < 0.001$ in the Permutational Multivariate Analysis of Variance (PERMANOVA) test. A very low R^2 value of 0.06 of this variation could be attributed to the metadata (Figure 4.6a). This means that many other factors not accounted for such as unmeasured physicochemical properties and symbiotic relationships among others could be the cause of this variation. Of the two rivers Mpanga and Dura, the bacterial diversity of river Dura was more closely related to the bacterial diversity of Swamps and Streams than river Mpanga. This was attributed to a slightly higher R^2 value of 0.1 but 90 % is unaccounted for (Figure 4.6b).

On a closure look using NMDS at wetlands with no significance in their Shannon diversity, the swamps Bigodi swamp and Rwembaita swamp showed that they shared a similar bacterial composition while the streams Mikana stream and EA farm stream differed (Figure 4.6c). The green colour was for streams and the red was for swamps with solid and dotted lines representing inside the forest and outside the forest respectively. It

shows the bacterial composition of streams inside the forest had a difference with streams outside the forest while the swamps had similar compositions. There was however, a significant difference between the bacterial composition of swamps and streams at $p < 0.001$ and the highest R^2 recorded of 0.14.

On comparing the river systems only, with green being river Dura and red river Mpanga, dotted lines outside the forest and solid lines inside the forest, the bacterial composition seemed to converge inside the forests (Figure 4.6d). Dura origin and Dura Kyamugara had similar diversity, as do Mpanga BT and Mpanga AT, yet the two sets at different rivers outside forest differ from each other. While their counterparts inside the forest yet still on different rivers had some similarity in their bacterial compositions. The difference in bacterial composition for the two rivers was significant at $p < 0.001$ attributed to 8% of the metadata (Figure 4.6d).

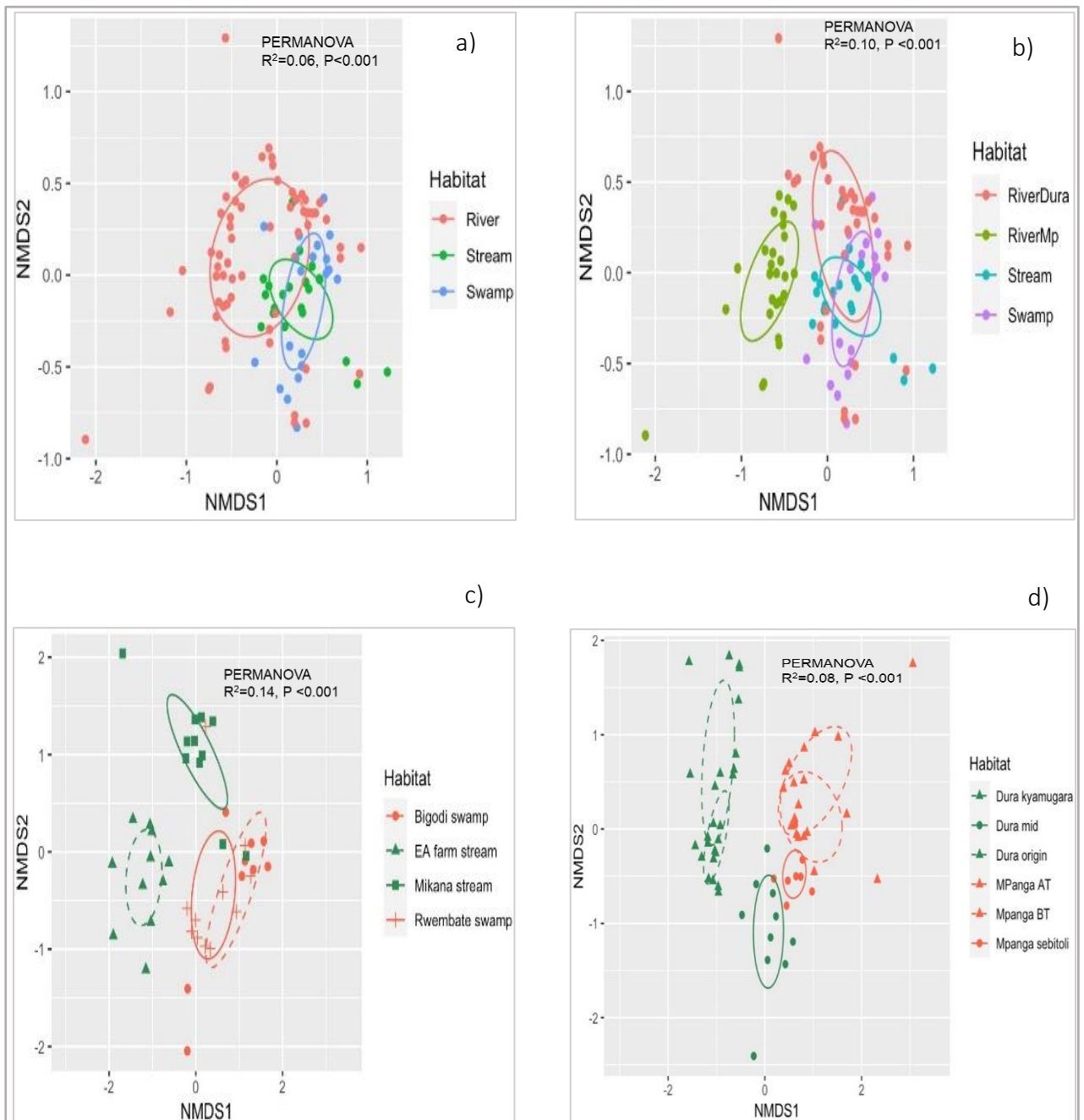


Figure 4.6. The comparison of bacterial composition in the wetland sites using Non-metric multidimensional scaling

4.4.4 Wilcoxon signed rank test

This dissimilarity test was used for the statistical hypothesis that ‘there was no significant difference in the microbial composition of wetland samples outside the forest with those inside the forest of KNP’. A very low p-value ($p < 0.001$) for the Wilcoxon signed-rank test suggests that the null hypothesis was rejected and the alternative accepted.

The lengths of the graphs along the dissimilarity axis can be interpreted in that the longer the graph, the more dissimilar bacterial species within those samples hence in this case more diversity inforest than Outforest. While, the width shows how all the samples within that particular ecosystem type compare to each other. In this case a wider graph would mean that the samples within that ecosystem type differ widely from each other which is the case for outforest wetlands. The overall comparison of the two different ecosystems is that, there was a very high significant difference between the microbial compositions found outside the forest with those inside the forest (Figure 4.7).

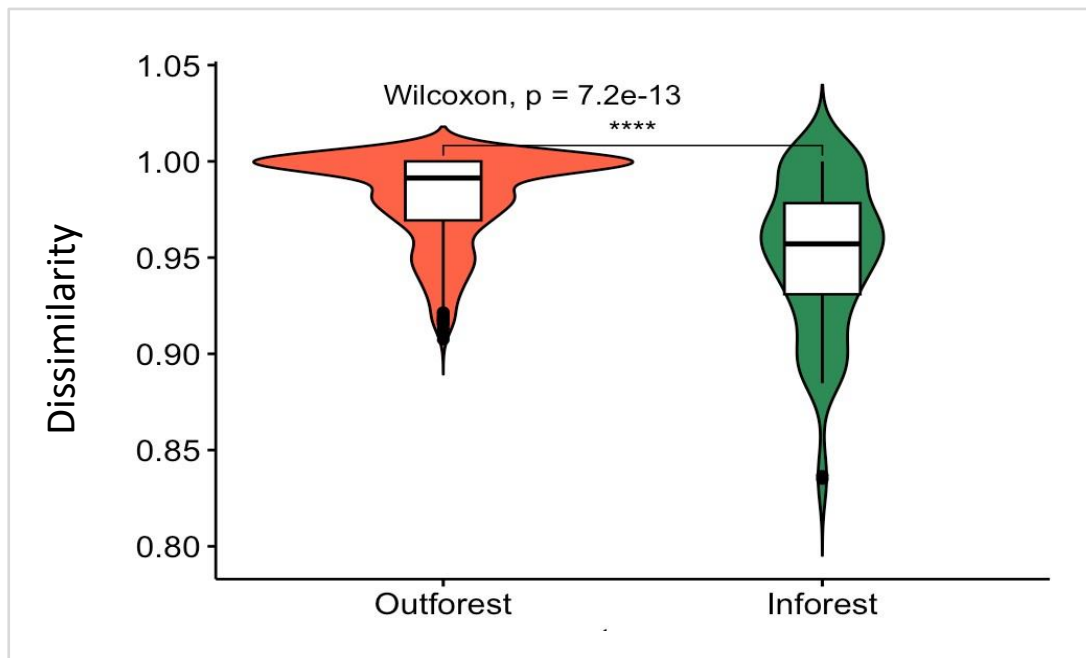


Figure 4.7. A Wilcoxon dissimilarity test and its significance comparing the bacterial diversity of all the wetland samples outside the forest with those inside the forest.

4.4.5 Taxa abundance differences

The DEseq2 method was used to visualize heat maps of abundance of each bacterial species within a particular sample. This was done separately for the river samples and combined for swamps and streams because they showed some similarity in NMDS analysis (Figure 4.6a). The level of redness of the box shows the abundance levels of the different taxa in that particular sample (Figure 4.8). In the river ecosystems, some of the identified abundant bacteria that could be classified across all taxonomic levels of domain – class – order – family – genus - species were bacteria – *Proteobacteria* – *Alphaproteobacteria* – *Rhodobacteraceae* – *Pseudorhodobacter* - *Pseudorhodobacter ferrugineus* inside the forest and bacteria – *Actinobacteriota* – *Actinobacteria* – *Micrococcales* – *Microbacteriaceae* - *Rhodoluna* - *Rhodoluna limnophila* outside the forest. Two other species of interest found in outforest rivers were *Arcobacter cryaerophilus* which is an emerging enteropathogen and potential zoonotic agent transmitted by food and water belonging to the class of *Campylobacteria*. The other is *Thiothrix nivea* which lives primarily in sulphide containing water such as sewage belong to the class of *Gammaproteobacteria*. *Novosphingobium capsulatum* and *Rhodobacter sphaeroide* were abundant in both inside and surprisingly outside river samples.

Generally, the differentially more abundant species inside the forest were those that are good at nutrient cycling like the bacteria – *Actinobacteriota* – *Actinobacteria* – *Mycobacteriales* – *Mycobacteriaceae* – *Lamia* - *Iamia testudinis*. Those abundant outside the forest were either pathogenically harmful species (*Arcobacter cryaerophilus*) or those that are direct indicators of pollution (*Thiothrix nivea*) as seen in Figure 4.8a). For the swamp and stream, the differential abundance of species identified were not very distinctive between inside the forest and outside the forest wetland ecosystems like that

of rivers. However, inside the forest samples were slightly more differentially abundant than those outside the forest. *Rhodoluna limnophila* and *Aurantimicrobium minutum* were some of the identified species abundant in all types of wetlands (Figure 4.8).

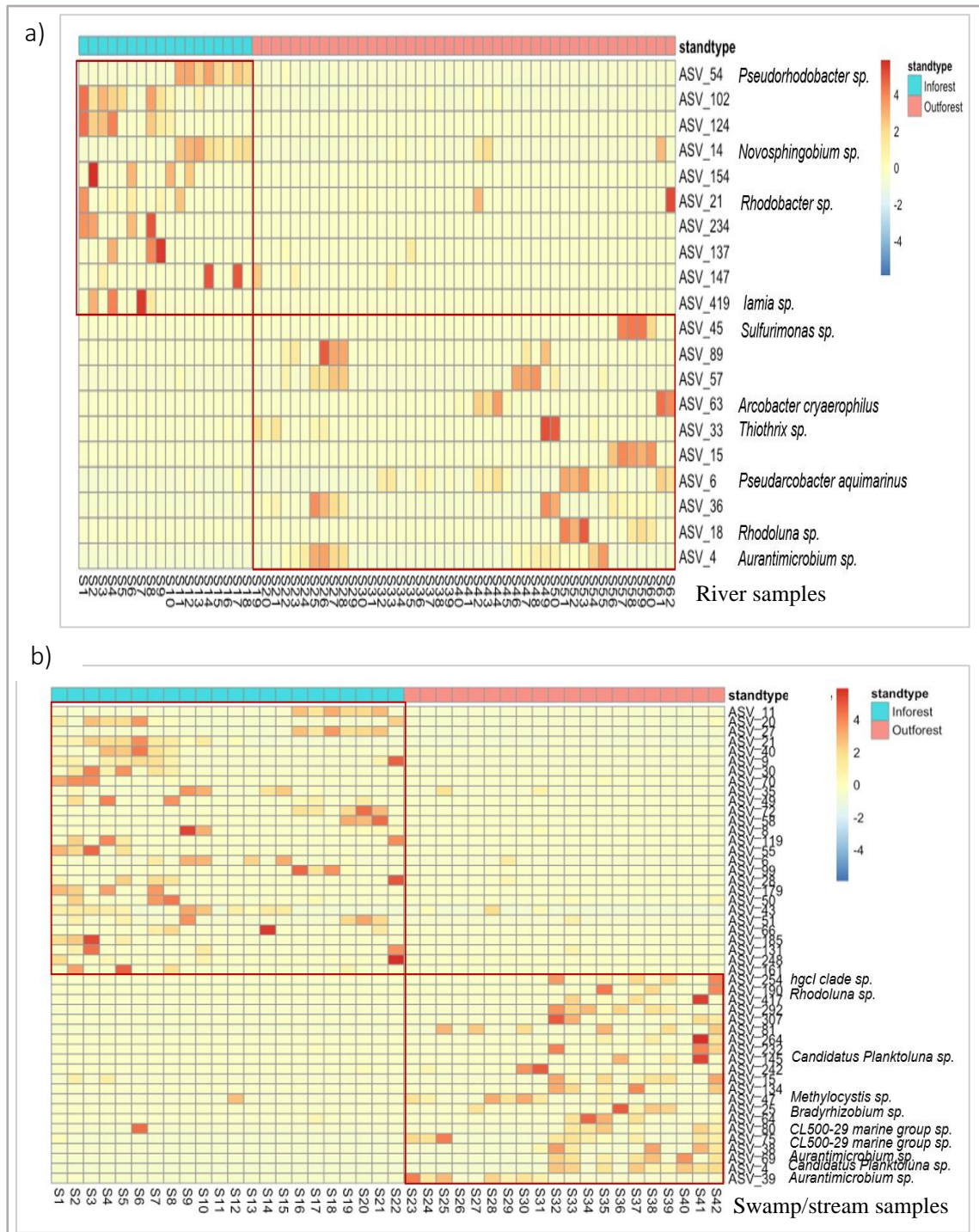


Figure 4.8. Heat map visualization of species abundance across different river and swamp/stream samples.

4.5 Description of the wetlands

4.5.1 Swamps

Two of the wetlands were considered to be swamps due to their characteristics of predominantly hydrophyte vegetation, undrained hydric soil and water-saturated substrate. Bigodi and Rwembaita wetlands, which were outside the forest and inside the forest respectively, met these descriptions.

4.5.1.1 Bigodi swamp

This was the largest of the swamps studied covering an area of about four-square km that lay outside the forested area of KNP. The area was known to harbour a variety of mammals mainly primates like the L'Hoest monkeys and bird species such as Hornbills. These animals attracted tourists managed by KAFRED thereby contributed to the local livelihoods. The wetland vegetation cover was commonly *Cyperus papyrus* and Polita fig trees growing throughout the wetland. Meanwhile, the vegetation surrounding the wetland was not natural but small farms of crops such as maize, beans, rice and cassava with few Eucalyptus tree plantations which are common crops grown around KNP.

On the gentle slopes were human settlements and an urban centre on both sides that showed high anthropogenic activities taking place. All the waste and pollution from the urban centre were washed into the swamp by stormwater hence creating gullies and erosion. The water was clear and flowed steadily undisturbed under the vegetation, with a few openings at the edges for the locals to access water. These access points were mainly for domestic use, cattle drinking and watering of small vegetable gardens. The local

community also harvested wetland products such as reeds and clay soil to make crafts and pottery which they then sold to the tourists.

4.5.1.2 Rwembaita swamp

This was one of the largest swamps found inside the forest. Measuring an area of about 3 km² all surrounded by the KNP forest. The vegetation of the swamp was mainly *Cyperus papyrus* covering most of the swamp with scattered various tree species. The steep slopes were fully covered with thick and green natural trees some of which included *Celtis africana*, *Uvariopsis congensis* and *Diospyros abyssinica*. There were no land use activities recorded for Rwembaita swamp and the only soil disturbances were those made by the elephant tracks as it is a water source for them during dry seasons. The water was turbid and filled with dead vegetation of trees and plants as well as timber broken down from a bridge construction. Both Mikana stream and EA farm stream flowed into the Rwembaita swamp and Rwembaita swamp flowed into the River Mpanga system.

4.5.2 Streams

The wetlands considered as a stream were based on flow and size of the water (<2 meters wide) and the surrounding vegetation. Two wetlands met the description i.e., EA farm stream and Mikana stream found outside and inside the forest respectively.

4.5.2.1 Emmanuel Adegenyira farm stream

The EA farm stream was a very small stream with the least amount of water seen among all the wetlands. The stream started in a sand mine about 3 km away from the forest. It then flowed thinly through a dry land dominated by *Cyperus latifolius* and scattered old eucalyptus trees. There were many land use activities surrounding the steep slopes of this

stream before it entered the forest, these included large tea and eucalyptus tree plantations, grazing lands for cattle and sand mines. Many of the locals use it as a water source for grazing their domestic animals. These activities had caused soil disturbances and poor hydrology therefore degrading the stream.

4.5.2.2 Mikana stream

Mikana stream was entirely located inside the forest surrounded by steep slopes. The natural trees of the Kibale tropical forest shade most parts of the stream. No human activities in the area and moderate soil disturbance were caused by the elephants as they usually graze along the stream. These elephant tracks continue up until the stream enters the Rwembaita swamp. The water was clear and flowed steadily as compared to the EA farm stream with ferns growing along the banks among the dominant trees that happen to shade the water all the way through. Few tourists track can be seen along the stream manually cleared but have overgrown. The soil sediment was mainly black clay with few scattered rocks at the bottom.

4.5.3 Rivers

Six sites were studied as the river ecosystems. Three along river Dura namely Dura origin, Dura Kyamugara and Dura mid. Three along river Mpanga included Mpanga BT, Mpanga AT and Mpanga Sebitoli. Rivers were differentiated from swamps and streams using their larger volumes of water and width of > 4.5 metres of fast-flowing water.

4.5.3.1 Dura origin

River Dura system started within a large swamp of about 5 km² located outside the naturally forested area and passes all the way through KNP and Queen Elizabeth National

Park before draining its water in to Lake George having travelled for over 100 kilometres. *Cyperus papyrus* and *Pennisetum purpureum* (Napier grass) dominated the vegetation of this sampling site. On the gentle slopes were eucalyptus plantations with lumbering, cattle paddocks and small farms growing mainly rice, beans and cassava. The farmland extension into the wetland and the passing of a marram road through the wetland were the main causes of soil disturbance. The water amount was large and clear and its hydrology and flow were intact as it flowed in the middle of the thick papyrus vegetation. This meant it was not easily accessible except at the bridge where the locals got water for domestic use, animal consumption and even washing their motorcycles.

4.5.3.2 Dura Kyamugara

This sampling site was located along the same swamp as the Dura Origin. It was located at the edge of the protected area just as River Dura entered the Kibale National Forest. The swamp was within a narrower valley, the vegetation was mostly common papyrus, and small tree patches. The area had very steep slopes covered with coffee plantations on the east and small farms having crops like cassava, maize, and beans on the west. As a result of the farming activities, the soils on the slopes were disturbed and eroded. The eroded soils washed into the river and this affected its hydrology which caused the turbid water to flow very slowly. Soil erosion into wetlands slows water flow as silting fills the wetlands with soil.

4.5.3.3 Dura mid

Dura mid was the furthest inside the forest sampling point for the whole study. By the time River Dura reaches Dura mid, it would have travelled five kilometres through the forest and been joined by many other tributaries. There were no land use activities and

soil disturbances recorded here as the river was surrounded by natural forest trees growing on the gentle slopes and by the riverbank. The water was turbid and had high flowing volume, and it was fast-flowing on both sandy and rocky surfaces for the sediment. The water was surrounded by tall trees that shade the water from direct sunlight.

4.5.3.4 Mpanga before town

Mpanga BT was part of the River Mpanga system of over 250 kilometres length sampled 8 km away before the river entered Fortportal town. Originating from the foothills of Mountain Rwenzori, river Mpanga flowed through different land uses like farmlands, grazing lands, domestics and other natural vegetation types found in the district of Kabarole. Along the riverbank of the sampling site, the main vegetation was elephant grass. On the flat slopes were cattle grazing areas, small banana plantations and a few eucalyptus plantations. There was minimal soil disturbance resulting from grazing and farming activities than expected which was credited to the dominant elephant grass at the river banks which makes it difficult for humans and animals to access.

4.5.4.5 Mpanga after town

Mpanga AT meaning Mpanga after Fortportal town had the highest AAI in the study by scoring the maximum 3 points in all the metrics used (Table 4.1). Just after the town centre was where the sampling was done. The area showed various human activities which included construction, washing bays, industrial brick making, tree nursery beds, constructions, petrol stations, farming. It also had some direct sewage and waste disposal channels that were connected to the river. The vegetation was found only close to the bank compared to the other sites along River Mpanga and it mainly consisted of short grasses with scattered spots of elephant grass and some cypress trees. Due to the high

population, most of the vegetation had been cleared for other uses like pasture. The soil was highly disturbed by anthropogenic activities that in turn made the water very turbid and brown. Despite all that, the water flowed relatively faster compared to Mpanga BT.

4.5.4.6 Mpanga Sebitoli

About 2 kilometres inside the protected forest area was this sampling site having gentle slopes covered with naturally growing trees and little short vegetation on the banks similar to Dura Mid. After travelling through several tea farms, and town centres such as Fortportal town and many other trading centres, it is joined by various streams that have their own degradation and pollution story to tell along the way. River Mpanga enters the forest as a large, rapid and fast-flowing secondary river spread over the rocky bottom. With no human activities in the area, the only causes of soil disturbance were the elephants, tourist tracks and the tarmac road crossing the river 1 km away. From here the Mpanga River continuous flowing through the forest and the exits down south in Kamwenge before draining its water into Lake George as well.

CHAPTER FIVE

DISCUSSION

5.0 Introduction

A discussion of the results is presented starting with LULC for each period, followed by the AAI, the physicochemical properties and bacterial diversity for each wetland type.

5.1 Land use land cover change

This was visualised using maps and statistically analysed as percentages of total area cover for each year and represented together in form of a graph (Figure 4.2). The map results showed that in 2003, a dark green colour dominated the study map mainly signifying the presence of forest cover even outside of the protected area (Figure 4.1). In 2013, the forest area had reduced, and grassland increased considerably with slight increases in built-up and farmland similar to the findings of Hartter, (2019) in KNP where he observed a decrease in forest cover. This trend then continued for 2023 where the colours light green, brown and red seemed to dominate the map (Figure 4.1). The decrease seen in the forest cover was mainly in the sub-counties around the protected rather than the Kibale forest itself. As from the early two thousand, logging of the forest had been stopped yet the trees outside the forest area were then left to the mercy of the locals who looked up to these trees to meet their timber and wood fuel demands.

The increases in farmland and built-up were more clearly seen in the areas of Rwimi and Hima in the southwest parts of the maps because of huge settlements, open land, cement raw material mining and large prison farms in that area. A splash of red in the north showed Fortportal town and down southeast showed Kamwenge town which were the

main town centres with large human settlements. Many other former small trading centres that have now developed into much built towns such as Kyenjojo, Rutete, Bigodi among many others as well (Rayyanu *et al.*, 2023). There was also a rapid shift in the construction styles from grass thatched houses into iron roofed houses that reflect more on the Landsat images. The water cover remained almost the same throughout the years except for a slightly higher colouring of blue in 2003 due to the image having a higher cloud cover percentage of 8% than in 2013 (3%) and 2023 (1%).

The forest covered 78% of the study area in 2003. This was then reduced to 57.7% in 2013 and further reduced to 42.5% in 2023 (Figure 4.2). However, as the forest was reducing, grassland, built-up and farmland were increasing. This meant that trees were cleared for other anthropogenic land uses (Hartter, 2019). The reduction in forest cover was more severe from 2003 to 2013 by 20.3% than from 2013 to 2023 by 15.2% (Figure 4.2). This can be attributed to increased Eucalyptus tree and Pine tree plantations in the area which had counterbalanced the high deforestation rates as found by Majaliwa *et al.*, (2015) and Kusiima *et al.*, (2022).

The biggest increase in land cover over the years was seen in grassland by 15.7% from 2003 to 2013 and again by 9.2% from 2013 to 2023. Meanwhile, farmland only increased by 2.5% in 2013 from 2003 and then by 3.8% from 2013 to 2023 (Figure 4.2). These changes followed the same trend as Majaliwa *et al.*, (2010), where he found that in 2009 the built-up was at 28% and grassland at 36% with varied figures from the findings of this study. The results also varied from Kusiima *et al.*, (2022) study of 1990–2020 where he found there was a declining trend for grassland, bushland, and tropical high forest by 19.5%, 4.7%, and 2.7% respectively. His subsistence farmland, commercial farmland,

built-up areas studies had an overall change of 19.0%, 5.0%, 3.5% respectively. This variation could be a result of the difference in the classification tools used by each study.

Even though most of the forest was being cleared for farming purposes, the farmlands were used seasonally and when some were left unused for some time, they grew into grassland. This explains the reason as to why the increase in grassland was more than the increase in farmlands (Majaliwa *et al.*, 2015). The increase in built-up was due to developments in the region and the construction of more iron sheet roofed houses (Hartter, 2019). Built-up area had a steady increase of covering 3.1% of the land in 2003 to 5.7% in 2013 and 8% in 2023. According to Kayiranga *et al.*, (2016) in their landscape change studies around Nyungwe-Kibira Park, they attributed such related changes to human activities such as deforestation and land fragmentation. Small scale farmers tend to intensively plough the land throughout the year and use it for crop growing rather than agroforestry hence the increase in farmlands and decrease in forest cover.

Similar to Kusiima *et al.*, (2022) the water cover for the years as expected was constant. Although, for 2003 it was at 1% compared to 2013 at 0.5% and 2023 at 0.4%. This could be so because cloud cover was computed as water cover and 2003 had the most cloud cover at 8%. These variations in cloud cover were considered insignificant to affect changes in the land cover statistics of other land uses. All in all, the null hypothesis that ‘there is no significant change in the LULC of KNP from 2003 to 2023’ was rejected and the alternative was accepted. This was so since significant changes were seen for forest cover, grassland, built-up and farmland as people cut down trees, they used the land for settlement and agriculture.

5.2 Anthropogenic activity index

Mpanga AT was the most degraded wetland having scored the maximum value of three (3) in all the metrics observed (Table 4.1). This could be as a result of its position in the centre of Fortportal town hence receiving all the waste and degradation effects from the urban centre. The findings of Gideon & Bernard, (2018) in Lubigi wetland showed that wetlands positioned in the centre of urban areas tended to be highly degraded. Meanwhile, the Mikana stream was the least degraded where the only sign of degradation seen was soil disturbance because of elephant movements and no human activities as similarly described by Apodaca & Chapman, (2004) on the same wetland. According to Park *et al.*, (2016), Elephants in KNP can shape the ecosystem and impact biodiversity through their movement and browsing.

In general, wetlands outside the forest had a higher degradation level than those inside the forest even when considered for each of the wetland types. According to Beuel *et al.*, (2016), such changes in wetlands are a result of anthropogenic activities. Therefore, people having less access to the protected area compared to the unprotected areas could have been the reason for this variation. Out of the five metrics used, turbidity, land use and soil disturbance were the most affected in most of the wetlands meanwhile vegetation and hydrology were the least affected. These two could only be seen to make an impact in Mpanga AT river and the EA farm stream.

The degradation levels showed more clearly the direction taken by the wetlands towards either degradation or intactness. All the wetlands tending towards fifteen were found outside the protected area of KNP and those tending towards five were inside the forest except for Dura origin and Mpanga BT respectively. Despite being outforest, Dura origin

was surrounded by less human activities like agriculture and settlement which could be the result of its water-logged areas that do not favour these activities. While for Mpanga BT its neutral point of no direction could have resulted from the fact that it was far from the main towns hence not easily accessible for human to impact it. As per these findings in the degradation levels, Mpanga AT and EA farm stream will require much more effort to carry out restorations. On the other hand, Mikana stream, Dura mid and Rwembaita swamp could have their current states maintained with lesser efforts.

This tells us of the importance of protected areas in maintaining the integrity of our wetlands and the role of anthropogenic activities surroundings in destroying that same integrity similar to Gideon & Bernard, (2018). As clearly seen outside wetlands were degraded and forest surrounded wetlands were not. It also ascertains that there is a significant difference in the AAI between inforest and outforest wetlands of KNP. Therefore, rejecting the null hypothesis that ‘there is no significant difference in the AAI for the wetlands of KNP’ and accepting its alternative.

5.3 Physicochemical properties

The physical properties measured for this study include dissolved oxygen, electrical conductivity, temperature and pH. It should be noted that for a healthy fresh water wetland so as for biodiversity to thrive the required values as by the United Nations Environmental Program (UNEP) include as follows; Dissolved oxygen between 5-9 mg/l, Electrical conductivity values of not more than 1000 $\mu\text{s}/\text{cm}$, pH between 6.5-8.5 is preferred and a temperature range of 5-30 °C (UNEP, 2020). Values beyond these ranges could be harmful for most of wetland lifeforms including bacteria (Sui *et al.*, 2021).

5.3.1 The swamps

On average the Bigodi wetland had the lowest dissolved oxygen levels of 0.92 mg/l of all the wetlands studied. This was different from Kyambadde *et al.*, (2004) findings where they found papyrus-covered wetlands had high levels of oxygen. The reason for this could be the slow-flowing water of Bigodi swamp which creates conducive grounds for decomposition of organic matter to take place and deplete its oxygen levels (Lyimo *et al.*, 2021). Meanwhile, the temperature, pH and conductivity were moderate compared to other study areas measuring 21.2 °C, 7.02 and 132 $\mu\text{s}/\text{cm}$ respectively. These results were deemed to be normal for a wetland to have as per the studies of (Chinemerem *et al.*, 2022).

Rwembaita swamp water had the second lowest conductivity recorded at 57 $\mu\text{s}/\text{cm}$ and a low dissolved oxygen of 1.78 mg/l similar to oxygen levels in swampy wetlands caused by their excessive decomposition rates (Mulei *et al.*, 2018). He attributed the low conductivity to the decomposition of dense *Cyperus papyrus* vegetation. Rwembaita swamp also had the second-highest pH of 7.5 which couldn't be a result of rainfalls as suggested by Bilewu *et al.*, (2022) as it had not rained for weeks. Although other factors such as water from EA farm or the soil chemical composition in the area are possible.

The t-test for the physicochemical properties of the swamps (Bigodi and Rwembaita) showed that there was no significant difference between the temperature, pH and dissolved oxygen measurements which all had $p > 0.05$. However, there was a high significant difference in their conductivity with a $p < 0.001$. The cause of this difference in conductivity could directly be linked to the fact that Bigodi swamp located near Bigodi town receives much more ions from dissolved salts and mineral washed from the town. On the other hand, Rwembaita swamp which is surrounded by the forest. Based on these

findings, the null hypothesis ‘there was no significant difference in the physicochemical properties of the swamps’ was accepted for oxygen, pH and temperature while rejected for conductivity. This conclusion was different from the findings of Mulei *et al.*, (2018) on swamp physicochemical properties with different surroundings. It could be that swamps in KNP were more tolerant of changes caused by anthropogenic activities or that the level of pollution in Bigodi wetland has not exceeded its capacity. This enabled it to restore them back to the natural conditions that match with swamps inside the forest.

5.3.2 The streams

The lowest conductivity recorded at 35 $\mu\text{s}/\text{cm}$ was in EA farm stream, which was explained by the lack of ions from dissolved salts and inorganic elements washing in the water which would have increased conductivity according to Rahman *et al.*, (2021). This result is contradicting with the fact that EA farm stream is surrounded by anthropogenic activities and scored on the degraded side of the AAI. Two reasons could explain this, first being that the EA farm stream was shielded from stormwater which could be carrying these ions by a spongy vegetation on the flat terrain. Secondly, the anthropogenic activity around EA farm stream is mainly agriculture which had less polluting ions as compared to town centres. A low dissolved oxygen of 2.07 mg/l was recorded as opposed to high oxygen levels in vegetation-covered wetlands found by Kyambadde *et al.*, (2004). This could as well be due to *Cyperus latifolius* vegetation cover which gives the wetland similar properties to a papyrus swamp like Bigodi swamp with low dissolved oxygen levels. Such vegetation covers reduces the surface area for oxygen to dissolve in wetlands predominantly covered by them (Fugère *et al.*, 2016).

Mikana stream had the lowest temperature of 18.4 °C because the forest cover mostly shaded the water from direct sunlight. Wetland exposed to direct sunlight in the day tend to have higher temperature as found by Maciej Serda *et al.*, (2010). Mikana stream also had the highest dissolved oxygen of 7.75 mg/l was recorded in all the sampling sites. High dissolved oxygen was attributed to two main factors, surrounding vegetation (Kyambadde *et al.*, 2004) and low temperature levels in the water (Lyimo *et al.*, 2021). Its conductivity of 55 µs/cm was among the lowest and opposed to the values of 156 µs/cm average found by Fugère *et al.*, (2016) in forested streams of Kibale. It had a pH of 7.34 slightly above neutral which could be because its pH is not much influenced by the surrounding landscape as suggested in a study by Allan, (2004).

On testing the significance level in the difference between EA farm stream and Mikana stream physicochemical properties using students t-test at alpha 0.05, it was found that temperature ($p=0.032$) was significant. But dissolved oxygen, pH and conductivity were more significant with ($p<0.001$). This meant that compared to other physicochemical properties, electrical conductivity can easily vary between degraded and intact wetland as was similarly seen earlier for the swamps. But this trend is not clear because for the swamps outforest had a higher electrical conductivity value while for the streams it's the opposite. It could be that other factors such the soil type in the wetland is the other determinant of conductivity and not just the surrounding activities (Lyimo *et al.*, 2021).

In conclusion for streams physicochemical properties, the null hypothesis 'there is no significant difference between the physiochemical properties of the streams was rejected for all the four physicochemical properties studied. This is so because in KNP inforest stream differed from outforest streams in their physicochemical properties.

5.3.3 Dura River

The average dissolved oxygen levels recorded at Dura origin were very low at 0.98 mg/l as it flows slowly through thick vegetation with a lot of decomposition going on. This low oxygen was different from other findings of Kyambadde *et al.*, (2004) but related to Bilewu *et al.*, (2022) which he attributed to oxygen being used for aerobic decomposition by bacteria. With a low conductivity of 81 $\mu\text{s}/\text{cm}$ as it had no direct source of dissolved salts, the second lowest temperature of 19.5 °C. The temperature was linked to a cold day of sampling therefore no direct sunlight hitting the water that day which would have raised the temperature as stated by Lyimo *et al.*, (2021).

Dura Kyamugara on the other hand recorded the highest temperature of 22.4 °C and the most acidic pH of 5.89 among all sampling sites. These pH values of Dura Kyamugara were below the recommended UNEP values ranging from 6.5 - 8.5. The reason for this low pH could be explained by sulphur oxides and nitrogen oxides leaching from the heavily fertilized coffee farms on the wetlands sloppy banks as per Peralta *et al.*, (2013) hypothesis. It still had low dissolved oxygen levels and conductivity levels as well, both recorded at 1.39 mg/l and 80 $\mu\text{s}/\text{cm}$ respectively (Table 4.2). These findings were similar to Dura origin and hence attributed to the same explanation of swamp conditions where dissolved oxygen is used for decomposition and less water surface area is exposed for it to dissolve which are both valid explanation according to Beutel *et al.*, (2006). Just as the level of conductivity is determined by dissolved salts, oxygen levels are determined by exposed surface area and bacterial decomposition activities (Chinemerem *et al.*, 2022).

High electrical conductivity and dissolved oxygen values were recorded for Dura mid at 214 $\mu\text{s}/\text{cm}$ and 7.31 mg/l respectively. These two values come as a surprise due to the fact that up rivers of Dura origin and Dura Kyamugara had low values. The high electrical conductivity could be explained by the possibility of its tributaries carrying the ions from other areas. According to Rahman *et al.*, (2021), rivers in urban centres tend to have high electrical conductivity values that can be passed down the river through tributaries. A temperature of 21.5 °C despite it having passed under the tree shades for a long time could be due to the fact that it was a hot day when sampling was done (Bilewu *et al.*, 2022). High dissolved oxygen levels of 7.31 mg/l were attributed to the rapid flow of water and a larger open surface area for oxygen to dissolve in the water of Dura Mid. The science behind this oxygen dissolution was stated by Downing & Truesdale, (1955).

The statistical significance of the physicochemical properties along the three Dura river sampling spots was calculated using a one-way ANOVA at 0.05 alpha. The p-value showing the level of significance varied in the order of pH at $p=0.006$, temperature at $p=0.004$, dissolved oxygen at $p<0.001$ and finally the most significant being conductivity at $p<0.001$. This significance was mainly brought by the high values of Dura mid as compared to the first two rivers which both had low values with no much difference. The null hypothesis 'there is no significant difference in the physicochemical properties along River Dura' was therefore rejected based on the above results and the alternative was accepted as evidenced by the results.

5.3.4 Mpanga river

The dissolved oxygen levels and pH of Mpanga BT were relatively high averaging at 6.58 mg/l and 7.23 respectively. Meanwhile, the conductivity of 177 $\mu\text{s}/\text{cm}$ and temperature

of 19.8 °C were low compared to other sampling sites along River Mpanga (Table 4.2). Mpanga at this point had not reached Fortportal town but already passed through tea plantations and farms which could be the reason as to why its conductivity was higher than other wetlands but lower than subsequent Mpanga sites. Studies of Akhtar *et al.*, (2021) and Sasakova *et al.*, (2018) showed that both chemicals from settlements and agriculture increase conductivity levels similar to that found in Mpanga BT.

The conductivity of Mpanga AT was the second highest at a value of 300 $\mu\text{s}/\text{cm}$ on average. This could be because of inorganic chemical elements from the town activities (Kyambadde *et al.*, 2004). Temperature of 21.4 °C, dissolved oxygen of 6.18 mg/l and pH of 7.31 were above average for the study (Table 4.2). Above-average pH levels could also be attributed to chemical pollution from all the human activities surrounding the river which was true in the case of studies by Akhtar *et al.*, (2021). Oxygen could easily dissolve in this water as the surface area was largely exposed even though it was polluted, larger amounts of oxygen could still be retained as it dissolves in the water faster.

For Mpanga Sebitoli the highest conductivity and pH were recorded here averaging 313 $\mu\text{s}/\text{cm}$ and 7.74 respectively despite it being inside the forest. This increase in conductivity and pH from Mpanga AT were attributed to two factors. The already dissolved ions from Fortportal which have not been bio remediated and many other tributaries joining the river with extra ions from other town centers and farms. Chemicals from agricultural use and urban runoff do add up to give the high conductivity and extreme pH values in water bodies (Walter *et al.*, 2019). High dissolved oxygen levels of 7.26 mg/l were observed which could be due to more water surface being exposed by its rapids splashing on the rocky sediment.

One-way ANOVA at 0.05 alpha level was used to test for the significance difference among each of the physicochemical properties in the three river spots. The results were all significant with $p < 0.001$ for temperature, pH, dissolved oxygen and conductivity. Of these the electrical conductivity significance value was mainly between Mpanga BT and the other two spots as it had a distinct value from theirs. The null hypothesis ‘there is no significant difference in the physicochemical properties along river Mpanga’ was therefore rejected and the alternative hypothesis ‘there is a significant difference in the physicochemical properties along River Mpanga was accepted.

This significance in the physicochemical properties detected between all these wetlands could directly be attributed to the difference in their surrounding ecosystems. Inforest wetlands that tended towards no degradation in the AAI had values closure to the recommended ones. Their counterparts outside forest which had tended towards total degradation had further away values. It can therefore be agreed upon that anthropogenic activities around a wetland can influence its physicochemical properties in a negative way. This is in line with other study findings of Chinemerem *et al.*, (2022) and Amaechi *et al.*, (2022).

5.4 Bacterial diversity

5.4.1 Bacterial diversity for swamp ecosystems

The bacterial composition found mainly in the swamps were the phyla *Proteobacteria* and *Actinobacteriota*. Rwembaita swamp had a higher *Actinobacteriota* than *Proteobacteria* meanwhile in Bigodi swamp the two phyla seem to be relatively in equal amounts. This came as a surprise outside forest wetlands were expected to harbor abundant amounts of the more adapted *Actinobacteriota*. This shift in the findings could

be as a result of high levels of decomposition taking place in Rwembaita swamp creating more favourable conditions for the growth of *Actinobacteriota* than *Proteobacteria* which agree with (Lammel *et al.*, 2018). *Firmicutes* and *Chloroflexi* were the other visible bacterial phyla found in both swamps and these two phyla are commonly found in all swamp related wetlands (Wu *et al.*, 2021). Visible *Acidobacteria* was found in the Bigodi swamp but insignificant in the Rwembaita swamp. This particular bacteria phyla prefers to live in low pH conditions that are acidic as per Eichorst *et al.*, (2018) and since Bigodi had a lower pH then this could be the possible explanation.

All in all, the bacterial composition of these two wetlands was similar as they both had no significant Shannon diversity difference and plotted together in the NMDS analyses. This could be attributed to factors such as them having similar physicochemical properties. The papyrus dominated vegetation of swamps does give them a natural filter from the impacts of anthropogenic activities (Christiansen *et al.*, 2022). Hence then it is possible that the conditions in outside swamps are yet still similar to the swamps inside the forest despite having anthropogenic activities surrounding them. Since electrical conductivity was the only significant difference among swamp physicochemical properties, then it can be concluded that conductivity does not affect bacterial composition in wetlands as it does in mangrove wetland soil reported by Yin & Yan, (2020). Similarly, AAI does not affect the bacterial composition of swamps because despite Bigodi being more degraded than Rwembaita, their bacterial compositions were still similar. But this would then not agree with Wu *et al.*, (2021) who found that degradation does significantly affect the microbial composition of swamps. The variations in both these findings could be due to other factors such as different levels of degradations and the geographical landscapes that differ (Arroyo *et al.*, 2015). There was

no significant variation in bacterial diversity between outforest and inforest swamps in KNP was the final conclusion based on the findings.

5.4.2 Bacterial diversity for stream ecosystems

Both Mikana stream and EA farm stream had high *Proteobacteria* and *Actinobacteriota* phyla as well. EA farm stream has the highest *Actinobacteriota* of all the wetlands covering over 50% of the relative abundance in that stream. Compared to other findings of Feng *et al.*, (2022), this level of *Actinobacteriota* was exceptionally high for such a wetland surrounded by mainly agriculture and no urban centers. This difference could be attributed to a particular type of chemicals used in these farms which does not favour the growth of *Proteobacteria* as it has a thinner cell wall compared to *Actinobacteriota*. EA farm stream had other notable phyla such as *Firmicutes* and *Acidobacteria* which both thrive under degraded conditions as well.

As for the Mikana stream over 70% of the relative abundance was *Proteobacteria* and the other notable phyla was *Chloroflexi* which both prefer undisturbed wetland conditions. *Proteobacteria* and *Chloroflexi* as main the phyla in a wetland was also seen by Feng *et al.*, (2022) but their study covered the urban Bahe river in China. This therefore means that even though *Proteobacteria* and *Chloroflexi* prefer natural conditions they can still thrive in wetlands in urban centers when managed properly. There was no significant difference in the Shannon diversity for the microbial composition of the two streams. However, the NMDS plots show that bacteria composition was different between the two streams. As dissolved oxygen was the most significantly different physicochemical property between these swamps, then a difference in bacterial composition could be

credited to this factor. Other studies like Sun *et al.*, (2015) had previously stated that the level of dissolved oxygen in water does influence its bacterial diversity.

5.4.3 Swamps and streams

The bacterial composition of Mikana stream and EA farm stream showed that the bacterial composition of EA farm stream was more related to those of the swamps than it was to Mikana stream. This could be because EA farm had similar other measured physicochemical properties to the swamps than it had with Mikana such as dissolved oxygen (Table 4.2). For example, Mikana had very high oxygen levels of 7.5 mg/l yet Bigodi swamp, Rwembaita swamp and EA farm stream all had < 2.1 mg/l. Another factor could be the surrounding vegetation made a family of papyrus plants in the three wetlands and not in Mikana stream. The vegetation types within an ecosystem does influence bacterial diversity (Ahn *et al.*, 2007). Mikana was also the least degradation compared to the other three wetlands which could be another factor.

The taxa abundance difference of swamps and streams showed that certain species such *CL500-29 marine group* and *Methylocystis parvus* were abundant in both inforest and outforest samples. This meant that despite there being a significant difference in the bacterial composition of inforest and outforest swamp and streams, certain bacteria are able to thrive in all those conditions. This could be as a result of having adapted to live in any of the two wetlands no matter the surrounding. Such bacteria are usually referred to by scholars as extremophiles (Sims *et al.*, (2013); Abed *et al.*, (2014)).

There were more abundant ASV's found outside the forest swamp and stream than inside the forest swamp and stream (Figure 4.8b). This could be explained by anthropogenic activities creating conditions of natural selection and those species that are selected for

end up thriving in outforest wetlands as suggested by Mansfeldt *et al.*, (2020). Meanwhile inside forest wetlands have conducive environment and no much completion for natural selection to take place hence no much dominant species that would have become more abundant (Feng *et al.*, 2022). It can then be assumed that intact streams favored the flourishing of the most common bacteria phylum in the world like *Proteobacteria* yet at species level none became more dominant.

5.4.4 Bacterial diversity for river ecosystems

The river ecosystems had the most interesting bacterial composition results obtained (Figure 4.4). *Proteobacteria* and *Actinobacteriota* dominated the relative abundance in all the six sites with *Actinobacteriota* having slightly higher levels in all except in Mpanga AT and Mpanga Sebitoli. This is because *Proteobacteria* increased with the flow of the river meaning that *Proteobacteria* became more abundant with the direction of river flow regardless of conditions in the river. This observation agrees with the findings of Atoll *et al.*, (2020) who attributed it to a factor of *Proteobacteria* being washed from the soil along the river banks where it is more dominant.

As for *Actinobacteriota* it reduced in river Mpanga down the river suggesting that the increased degradation conditions in the town influenced it negatively as opposed to the Christiansen *et al.*, (2022) who stated that *Actinobacteriota* dominates in urban catchments. Therefore, in River Mpanga as *Proteobacteria* increased down the river, *Actinobacteria* reduced hence the two alternating. This alternation was seen in all wetland types including swamps and streams which could be evidence of competition between these two phyla. In intact wetland *Proteobacteria* is more competitive while in degraded wetlands *Actinobacteriota* becomes more competitive as its thicker cell wall able to resist

pollution for much longer. Another interesting observation was the high level of *Chloroflexi* in the Dura origin habitat as it gradually reduced downriver to Dura Mid. *Chloroflexi* was commonly found in peat wetlands (Christiansen *et al.*, 2022) which have similar conditions to Dura Origin.

The Shannon diversity decreased in River Mpanga from outside the forest to inside the forest and oppositely for River Dura, Shannon diversity increased from outside the forest to inside the forest. No clear reasonable explanation could be given for the variation other than the fact that each river has its own path and therefore its own Shannon diversity regardless of the direction of the flow. This prompted for test of Shannon diversity relative to the distance from the forest edge. This test showed that Shannon diversity in the rivers increased with the distance further way from the forest edge (Figure 4.5c). This observation could be explained by high anthropogenic activities like built-up and farming affecting bacterial diversity close to the forest edges as compared to away from the forest (Cavicchioli *et al.*, 2019). The intensification of human activities at edges of the protected area could be because of the leakage of human activities that would have happened within the protected area being concentrated at the forest edge as well as people seeking for opportunities that come with tourism activities (Team & Roser, 2023).

According to the NMDS analysis, the bacterial composition of river ecosystems was different from those of swamps and streams (Figure 4.6a) and distinct from each other (Figure 4.6b). River Dura had a relatively similar bacterial composition with the streams and the swamps than it had with River Mpanga. This could be due to the fact that River Dura flows through swampy habitats and was joined by various streams, therefore, had similar characteristics in bacteria as well. However, these observations do not apply to peat swamps as evidenced by the study of Christiansen *et al.*, (2022).

Bacterial composition of rivers inside the forest (Dura mid and Mpanga Sebitoli) were similar compared to the outside river pairs (Dura origin and Dura Kyamugara) and (Mpanga BT and Mpanga AT) which were distinct. This meant that bacterial diversity converged as the river flowed into the forest indicating some restoration of diversity by the forest ecosystem closely relating to the 2023 study by Wu *et al.*, (2023). This also clearly points out the importance of protected areas in conservation.

The abundant ASV's expressed in rivers included *Pseudorhodobacter ferrugineus* and *Lamia testudinis* which were only found inside the forest. These two species are commonly known for their importance in breaking down organic matter and contributing to the trophic status of an ecosystem (Caston *et al.*, 2009). Those only found abundant outforest included indicator species such as *Arcobacter cryaerophilus* which is being widely known as a pathogen for causing gastrointestinal diseases like diarrhoea (Tinta *et al.*, 2022). The other is *Thiothrix nivea* which is used as an evidence of sewage pollution in water bodies (Aislabie *et al.*, 2009). Humans are capable of introducing bacterial species into natural ecosystems hence impacting life in that ecosystem negatively (Islam *et al.*, 2019). There were also species that thrived in both the inforest and the outforest river ecosystems such as *Rhodoluna limnophila* and *Aurantimicrobium minutum* which could be a sign of their ability to adapt to different environments as suggested by Sui *et al.*, (2021).

5.4.5 Wetlands outside the forest vs wetlands inside the forest

Considering the bacterial diversity in all the wetlands, a Wilcoxon dissimilarity graph proved that natural wetlands had both more differentially abundant species as well as similar bacterial composition when compared to degraded wetlands outforest (Figure

4.7). The dissimilarity in bacterial composition between wetlands found inside the forest and those outside agrees with similar studies of bacteria in wetlands under different anthropogenic activities by Yi *et al.*, (2022), Sui *et al.*, (2021) and Islam *et al.*, (2019). This observation could be as a result of inside forest wetlands having conducive conditions for any species to survive. Whereas for degraded wetlands the physicochemical parameters are beyond natural and only few species are able to thrive under such conditions.

Therefore, natural wetlands were better suited for bacteria to thrive and hence perform their functions such as decomposition to maintain the health and trophic status of that particular ecosystem. Meanwhile degraded wetlands create an opportunity for the emergence of pathogenic agents that could lead to disease spread to humans, animals and plants that benefit from such an ecosystem. Since there is a high significant difference in the bacterial composition of these two distinct ecosystems, the null hypothesis of ‘there is no significant difference in bacterial diversity among the wetlands of KNP’ was rejected and the alternative was accepted as suggested by the findings.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.0 Introduction

This chapter presents the conclusions and recommendations about the study objective by objective. It also highlights the study contributions and suggests areas for future research.

6.1 Conclusion

6.1.1 Land use land cover changes

Kibale national park experienced significant anthropogenic expansion which led to forest cover loss and a growth in built-up, grassland, and farmland areas. This is so because increased human activity encroaches and converts forest areas for other uses. This therefore suggests a critical need for improved land-use management practices that can stop the ongoing deforestation.

6.1.2 Anthropogenic activity index of the wetlands

Wetlands outside forest protection area face significant degradation from human activities while those within forest boundaries remain comparatively undisturbed. Limited access to protected areas correlates directly with a reduction in the degradation of wetland. Therefore, in order to preserve wetlands, human access to them should be controlled.

6.1.3 Physicochemical properties of the wetlands

The study found that surrounding land use has a strong influence on wetland physicochemical properties, particularly in unprotected wetlands. Introduction of pollutants and nutrients by anthropogenic activities causes significant changes to these properties beyond the recommended levels. This call for the need of localized sustainable interventions in town and agricultural areas around KNP.

6.1.4 Bacterial diversity of the wetlands

The bacterial diversity of KNP wetlands were significantly different from each other dependent on factors such as physicochemical properties, AAI, wetland type and location all influencing bacterial diversity. However, swamp wetlands resist the effects of degradation on their ecosystem hence they have similar species composition. Bacteria phyla *Actinobacteriota* and *Proteobacteria* abundance alternated between degraded and none degraded wetlands respectively. Pathogenic species such as *Arcobacter cryaerophilus* were identified abundantly in outforest wetlands while useful species such as *Lamia testudinis* abundantly in forest. Inforest wetlands have more biodiversity than outforest wetland thus showing the need to restore wetlands outside of the protected area.

6.2 Recommendations

6.2.1 Bacterial diversity in relation to anthropogenic activities

Bacterial diversity should serve as a critical indicator for assessing wetland ecosystem health, particularly in degraded wetlands outside the forest, where pathogenic species are more abundant. The relationship between bacterial diversity and ecosystem health underscores the need for bacterial diversity assessments to complement other methods.

Local authorities in the surrounding sub-counties should implement measures to reduce deforestation rates by promoting agroforestry, reforestation and restoration initiatives. Reinforcing sustainable land use practices is essential as current projections indicate a significant depletion of tree cover in the next 30 years.

Wetlands out of protected areas require targeted restoration efforts, especially for the River Mpanga system. The Fortportal municipal council should manage River Mpanga more comprehensively by regulating activities along its tributaries, mandating sewage and industrial waste treatment, and promote piped or borehole water. These measures would mitigate the effects of urban runoff and increase safe water access.

Natural wetlands exhibit greater resilience, enhanced biodiversity, and effective pollutant filtration. Therefore, restoring all KNP wetland ecosystems to their natural states is crucial for maintaining biodiversity and preventing health risks from abundant harmful species like *Arcobacter cryaerophilus*. Such restoration would also support tourism.

Expanding community awareness initiatives on the importance of wetland health can drive local support for conservation policies. Community-based conservation efforts, supported by UWA, would promote responsible land use and strengthen protective measures for ecosystems around KNP.

6.2.2 Contributions of the study

The study has contributed to knowledge areas by characterising the bacterial community of wetlands in KNP and Uganda at large. It has also made known the changes in land use and land cover taking place around KNP and the districts of Kabarole, Kamwenge and Kyenjojo. How anthropogenic activities affect the different types of wetlands and their

physicochemical properties have been studied and documented. These knowledge areas can be projected to similar wetland types and conditions in the country and the world over. The authorities such as the local government in the area, UWA and MWE, can adopt these findings and recommendations to improve their activities and policies. Through these the country can be able to achieve sustainable development goals such as clean and safe water, life in water and life on land, which are SDG's 6, 14 and 15 respectively.

6.2.3 Areas for further research

Future assessments should include seasonal monitoring to analyze bacterial composition shifts in response to temporal environmental changes. This approach will provide insights into the ecosystem's adaptability to seasonal variations in human and climate impacts.

Further studies should also investigate the effects of individual anthropogenic activities on dominant bacterial phyla, such as *Actinobacteriota* in degraded wetlands so as to understand how these bacteria respond to specific environmental changes.

Beyond bacterial diversity, future research could expand the eDNA metabarcoding approach to explore other life domains, including Archaea and Eukarya so as to gain a holistic understanding of wetland biodiversity and its ecological implications in KNP.

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APPENDICES

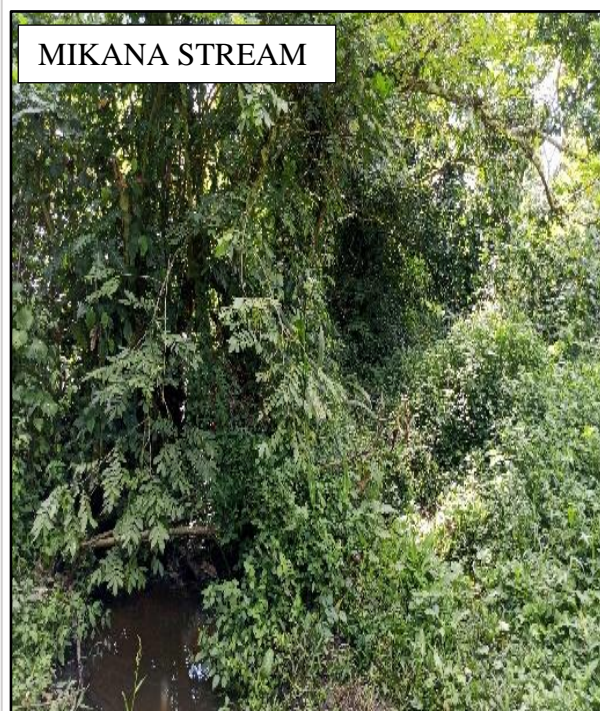
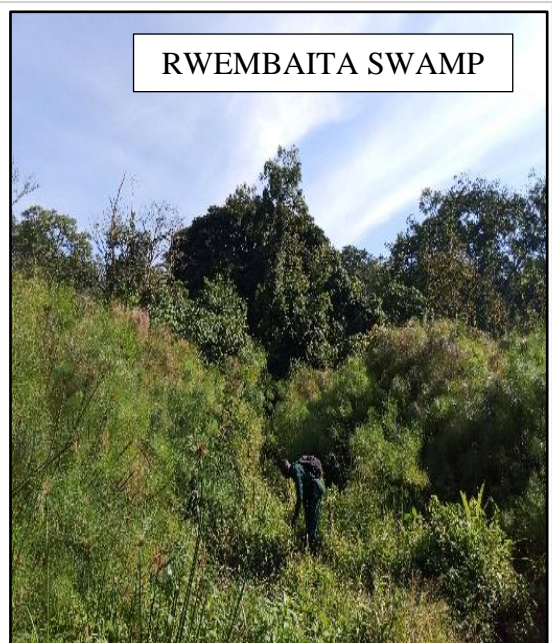
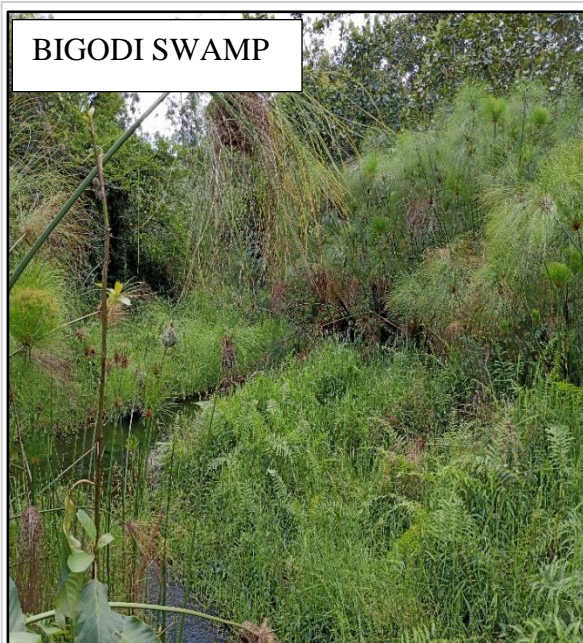
Appendix 1: Anthropogenic activity index form

| Degree/Level | Metric 1: Surrounding land use intensity | Rating |
|---------------------|---|---------------|
| Low | Mainly undisturbed but with little human/animal impact e.g., few animal trails and human footpaths | 1 |
| Moderate | Moderate signs of human and animal impact e.g., livestock activity and agriculture on small scales | 2 |
| High | Much clear evidence of human/animal impact e.g., settlements and farming on a large scale | 3 |
| | Metric 2: Soil disturbance | |
| Low | Small areas of bare soil e.g., mostly vegetation patches | 1 |
| Moderate | Moderate areas of bare soil e.g., small openings in the soil | 2 |
| High | Large areas of disturbance in soil e.g., gullies and rills | 3 |
| | Metric 3: Hydrologic alteration | |
| Low | Small alteration of flow e.g., not affecting the wetland | 1 |
| Moderate | Significant and visible influence currently affecting the wetland | 2 |
| High | High activity and disturbance on flow e.g., ditch inlet, drainage channels, road excavation, cultivation, soil barriers | 3 |
| | Metric 4: Vegetation community quality | |
| Low | High diversity of species and predominantly native species | 1 |
| Moderate | Moderate species diversity with non-native species present | 2 |
| High | Low species diversity with the predominance of non-native species | 3 |
| | Metric 5: Turbidity | |
| Low | Clear and colourless water | 1 |
| Moderate | Moderate colouration of the water | 2 |
| High | Significant and visible turbid colouration of the water | 3 |

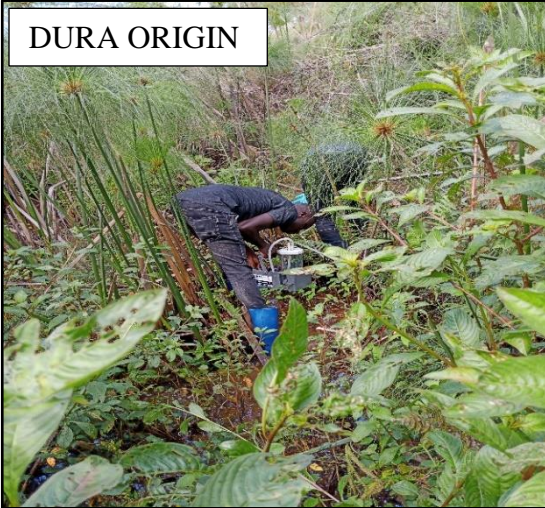
Appendix 2: Main pieces of equipment used



Appendix 3: Field pictures



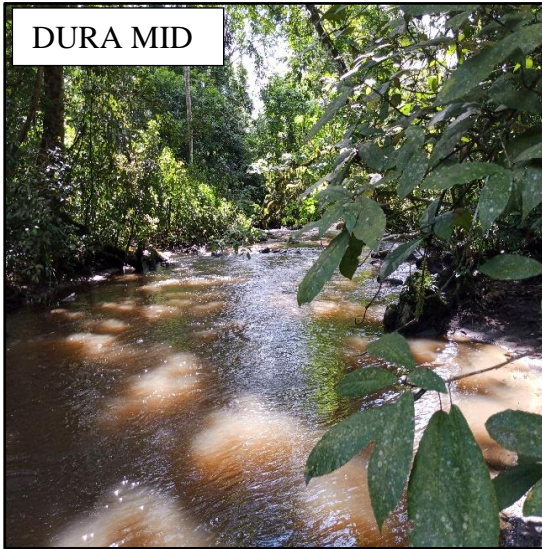
DURA ORIGIN



DURA KYAMUGARA



DURA MID



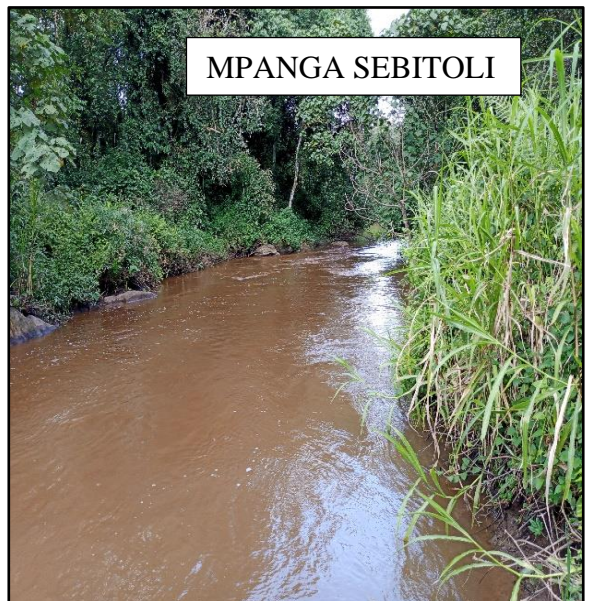
MPANGA BT




MPANGA AT



MPANGA SEBITOLI



Appendix 4: Kyambogo University research permit


KYAMBOGO UNIVERSITY
P. O. BOX 1 KYAMBOGO
Tel: 041 - 4286792 Fax: 256-41-220464
Website :www.kyu.ac.ug, Email: drgt@kyu.ac.ug
Directorate of Research and Graduate Training
Office of the Director

Date:

TO WHOM IT MAY CONCERN

RE: *Murigu Lemi Vincent*

Dear Sir/Madam,

This is to introduce to you the above named student Reg: No *21/UG/2018/14378/PE* pursuing *Master of Science in Conservation and Natural Resource mgmt*, Department of *Biological Science*, Kyambogo University.

She/he intends to carry out research on *Characterization of microbial communities in Kibale National Park - Uganda* in partial fulfillment of the requirements for the award of *Master of Science in Conservation and Natural Resource mgmt*.

The purpose of this letter therefore is to request you to grant him/her permission to carry out his/her study in your institution.

Any assistance rendered to him/her will be highly appreciated.

Yours sincerely,

Bua
Prof. Bosco Bua
AG. DIRECTOR


KYAMBOGO UNIVERSITY

★ 09 MAY 2023 ★

DIRECTOR

DIRECTORATE OF RESEARCH AND GRADUATE TRAINING

Appendix 5: Uganda Wildlife Authority research permit

**UGANDA WILDLIFE AUTHORITY**
OFFICE OF THE EXECUTIVE DIRECTOR
PLOT 7 KIRA ROAD KAMWOKYA
P. O. Box 3530, Kampala, Uganda

Our Ref: COD /96/05 19th May 2023

Sadadi Ojotre
BeZero Carbon
Banner Street, London
UK

RESEARCH APPLICATION APPROVAL

I am in receipt of your research application dated 26th April 2023 seeking permission to undertake a research study in Kibale National Park titled; *"Using Environmental DNA for ecosystem assessment and monitoring"*.

This is to inform you that permission has been granted to you and your co-investigators Mr. Kasekendi Innocent, Mr. Munguleni Vincent, Dr. Nick Atkinson and Prof. Jonathan Davies with effect from 1st June 2023 to 30th September 2023. You are expected to submit to Uganda Wildlife Authority a progress report by September 2023 and a final report of your findings by end of November 2023. In case you are unable to work within these dates, please notify us in writing.


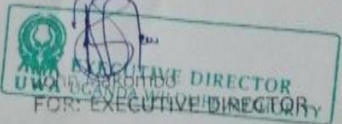
You will be required to pay to UWA a research application fee of UGX 50,000 (fifty thousand shillings), a monthly research fee of UGX 100,000 (one hundred thousand shillings) and a refundable report/ security deposit fee of UGX 200,000 (two hundred thousand shillings). Your co-investigators Kasekendi Innocent and Munguleni Vincent will each be required to pay a research application fee of UGX 20,000 (twenty thousand shillings) while Dr. Nick Atkinson and Prof. Jonathan Davies will each be required to pay a monthly research fee of USD 250 (United States dollars two hundred fifty). Kindly note that monthly research fees are paid by calendar month.

Since your research involves samples collection, you will fill and submit a Material Transfer Agreement to enable you export them for analysis.

You are required by law to seek approval from the Uganda National Council for Science and Technology (UNCST). By copy of this letter, UNCST is duly informed that your research application has been endorsed by UWA and further approval is hereby sought before commencement of research.

Report to the Chief Warden, Kibale Conservation Area (KCA) on arrival for registration, payment of fees and further guidance

Conserving for Generations
Yours sincerely,

Copy: Executive Secretary, UNCST
Chief Warden, KCA

Appendix 6: Uganda National Council for Science and Technology clearance



Uganda National Council for Science and Technology
(Established by Act of Parliament of the Republic of Uganda)

Our Ref: NS608ES

19 July 2023

Sadadi Ojatre
BeZero Carbon LTD
Kampala

Re: Research Approval: Using environmental DNA (eDNA) for ecosystem/biodiversity assessment & monitoring

I am pleased to inform you that on **19/07/2023**, the Uganda National Council for Science and Technology (UNCST) approved the above referenced research project. The Approval of the research project is for the period of **19/07/2023 to 19/07/2024**.

Your research registration number with the UNCST is **NS608ES**. Please, cite this number in all your future correspondences with UNCST in respect of the above research project. As the Principal Investigator of the research project, you are responsible for fulfilling the following requirements of approval:

1. Keeping all co-investigators informed of the status of the research.
2. Submitting all changes, amendments, and addenda to the research protocol or the consent form (where applicable) to the designated Research Ethics Committee (REC) or Lead Agency for re-review and approval **prior** to the activation of the changes. UNCST must be notified of the approved changes within five working days.
3. For clinical trials, all serious adverse events must be reported promptly to the designated local REC for review with copies to the National Drug Authority and a notification to the UNCST.
4. Unanticipated problems involving risks to research participants or other must be reported promptly to the UNCST. New information that becomes available which could change the risk/benefit ratio must be submitted promptly for UNCST notification after review by the REC.
5. Only approved study procedures are to be implemented. The UNCST may conduct impromptu audits of all study records.
6. An annual progress report and approval letter of continuation from the REC must be submitted electronically to UNCST. Failure to do so may result in termination of the research project.

Please note that this approval includes all study related tools submitted as part of the application as shown below:

| No. | Document Title | Language | Version Number | Version Date |
|-----|--------------------------|----------|----------------|--------------|
| 1 | Project Proposal | English | FINAL VERSION | |
| 1 | Approval Letter | English | | |
| 2 | Administrative Clearance | English | | |

Yours sincerely,

Dr. Christopher Ddamulira
For: Executive Secretary

UGANDA NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY