

**INFLUENCE OF HIPPO AND CATTLE DUNG ON THE NUTRIENT LEVELS,
ALGAL DIVERSITY, COMPOSITION, BIOMASS AND ECOSYSTEM
METABOLISM IN AQUATIC ECOSYSTEMS: A MESOCOSM APPROACH**

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DECLARATION

Declaration by the student

This thesis is my original work and has not been presented for the award of any certificate or degree in any other institution or university. No part of this work should be reproduced without prior permission from the author and/or University of Eldoret.

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DEDICATION

This Research Thesis is dedicated to my parents Mr. and Mrs Kiplagat who taught me the value of hardwork and also to my husband Jeremiah for his encouragement.

ABSTRACT

Large mammalian herbivores (LMH) are major agents of the movement of terrestrial organic matter and nutrients into aquatic ecosystems, which can alter the structure and functioning of aquatic ecosystems. Populations of native large mammalian herbivores have been lost or displaced by livestock in many regions of the world, and this may result in changes in the functioning of the aquatic ecosystem due to significant differences in the quality of their dung. A replicated mesocosm experiment was conducted for six weeks (42 days) at the bank of Amalo river to study the influence of large mammalian herbivores (hippo and cattle) dung input on nutrient concentration, algal biomass, composition and diversity, and flume-scale ecosystem metabolism. The treatments included: H100 (no cattle dung, 100% hippo dung), H80 (20% cattle dung, 80% hippo dung), H60 (40% cattle dung, 60% hippo dung), H40 (60% cattle dung, 40% hippo dung), H20 (80% cattle dung, 20% hippo dung), and H0 (100% cattle dung, no hippo dung). These treatments were conducted in triplicate. Nutrients were analyzed by standard colourimetric methods, chlorophyll-a was extracted using methanol and measured spectrophotometrically, total suspended solids (TSS) and ash-free dry mass (AFDM) were determined gravimetrically and algae were counted under a microscope using the counter rafter cell and flume-scale ecosystem metabolism was measured in-situ using miniDOT loggers; where dissolved oxygen and the water temperature was recorded every one minute for the six weeks. Study results showed that cattle and hippo dung inputs have contrasting effects on aquatic ecosystem function; treatment with 100% cattle dung (H0) released higher amounts of limiting nutrients, exhibited higher algal biomass, higher algal abundance and diversity and supported higher rates of gross primary production (GPP) relative to 100% hippo dung (H100). Hippo dung, which has slower mineralization rates and larger particle sizes, increased water clarity relative to cattle dung. Differences in stoichiometry (C: N:P ratio) of major elements and faecal particle sizes between cattle and hippo dung were the likely drivers of decomposition and releasing rates of nutrients, which in turn influenced ecosystem processes differently. The study suggests that large mammalian herbivores (LMH) play a significant role in the movement of organic matter and nutrients from terrestrial to aquatic ecosystems. The loss or displacement of native LMH populations by livestock in various regions of the world can lead to changes in the functioning of aquatic ecosystems due to differences in the quality of their dung.

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LIST OF ABBREVIATIONS AND ACRONYMS

AFDM – Ash fry dry mass
ANOSIM - Analysis of similarities
CA- Correspondence analysis
CCA - Canonical Correspondence Analysis
CPOM - Coarse particulate organic matter
DO- Dissolved oxygen
EC - Electrical conductivity
ER - Ecosystem respiration
FROM - Fine particulate organic matter
GF/F - Glass fiber filters
GLMM - Generalized Linear Mixed Models
GPP - Gross primary productivity
Hippo - Hippopotamus
LVB - Lake Victoria Basin
NEP - Net ecosystem production
NMDS - Non-Metric multidimensional scaling
PCA - Principal Component Analysis
PERMANOVA - Permutational analysis of variance
POM - Particulate organic matter
PPT - Parts per thousand
RCC - River continuum concept
SIMPER - Similarity percentages analysis
SRP – Soluble reactive phosphorous
TP – Total phosphorous
TSS - Total suspended solids
UoE - University of Eldoret
YSI - Yellow Spring Instruments

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

The movement of organic matter from terrestrial to aquatic environments has often been understood to be dominated by litterfall and hydrologic transfers during storms and precipitation events (Wallace *et al.*, 1997; Wantzen *et al.*, 2008; Garzon-Garcia *et al.*, 2017). Even though, it is increasingly recognized that large mammalian herbivores (LMH) can be major agents of the movement of terrestrial organic matter and nutrients into aquatic ecosystems (Bond *et al.*, 2014; Subalusky *et al.*, 2017). Whilst rates vary extensively over broad spatial and temporal scales and most depend on the characteristics of the animal vector and the recipient ecosystem (Vanni, 2002; Subalusky *et al.*, 2015), the amount can be significant, particularly for low-order streams in rangelands and pastoralist areas (Bond *et al.*, 2014; Masese *et al.*, 2018, Stears *et al.*, 2018).

The LMH movements form substantial linkages between ecosystems through the transport of carbon (C) and nutrients across ecosystem boundaries (Vanni, 2002; Subalusky *et al.*, 2015). These animal-mediated resource subsidies can strongly affect nutrient cycling (Kitchell *et al.*, 1999; Vanni, 2002), ecosystem productivity (Marcarelli *et al.*, 2011), and food web structure and stability of the recipient system (Subalusky *et al.*, 2017). The linkage between terrestrial and aquatic ecosystems in many African savannah landscapes occurs because of the vectoring role that large mammalian herbivores play in transferring large amounts of organic matter and nutrients from terrestrial to aquatic ecosystems (Jacobs *et al.*, 2007).

Different ways in which organic matter and nutrients from LMH enter aquatic ecosystems include egestion and excretion during migrations and watering (Hayward, 2012), facilitation of soil erosion (Jacobs *et al* 2002.), and drowning during water crossings (Subalusky *et al.*, 2017). A prominent example is the common hippopotamus (*Hippopotamus amphibius*, hereafter hippo), which migrates daily between savannah grasslands, where it forages, and aquatic ecosystems where it rests and much of its defecation occurs (Subalusky *et al.*, 2015). Resource subsidies from hippos alter primary production and secondary production, most prominently through direct consumption by bacteria, invertebrates, and fish (Dawson *et al.*, 2016; Subalusky *et al.*, 2017).

The expansion of human settlements, crop farming, and conversion of forests and savannah grasslands to pasture for livestock production have contributed to the loss of large populations of wild LMH around the world (Ripple *et al.*, 2015). In most African savannahs, large populations of wild herbivores still dominate the biomass of conservation areas (Young *et al.*, 2013). However, even in these regions, wild LMH is declining concurrently with increases in livestock such as cattle, goats, and sheep (Ogutu *et al.*, 2016). For instance, in the savannah grasslands, hippo populations are however declining, and they are now listed as vulnerable by the International Union for Conservation of Nature (IUCN) (Kanga *et al.*, 2011). Therefore, the continuous decline in hippo populations and subsequent decrease in discharge may influence the ecosystem (Subalusky *et al.*, 2015).

In most areas where livestock has replaced wildlife on the landscape, their impact on aquatic systems is often perceived as negative, with research primarily focusing on issues like habitat degradation, nutrient and organic matter loading, and microbial

contamination (Bond *et al.*, 2014). Ruminants such as cattle and sheep, with their efficient digestive systems, produce smaller faecal particles compared to foregut fermenters like hippos and horses (Fritz *et al.*, 2009). Despite this, foregut fermenters have longer mean retention times, enabling them to extract nutrients more effectively from ingested food than ruminants, as reflected in the C: N:P stoichiometry of their faeces (Vandevenne *et al.*, 2013). The C: N:P stoichiometry of East African buffalo dung, similar in size, digestive physiology, and feeding strategy to cattle, is 153.0:5.3:1.0 (Sitters *et al.*, 2014), while hippo dung from the same region has a higher proportion of carbon to nitrogen, indicating relatively lower dung quality at 222.8:6.3:1.0 (Subalusky *et al.*, 2015). Additionally, cattle have a broader foraging range and consume a wider variety of plant species compared to hippos, resulting in the ingestion of a more diverse array of metabolites and chemicals, such as silicon and phenols (Noirard *et al.*, 2004). This dietary diversity in cattle can have implications for ecosystem processes and dynamics by introducing a greater range of plant-derived compounds into their dung, subsequently influencing nutrient cycling, decomposition rates, and interactions with aquatic organisms in lotic ecosystems (Noirard *et al.*, 2004).

Dung comprising small particles that are relatively high in nutrients, as expected from ruminants such as cattle, is expected to remain suspended in the water column, which could decrease light penetration, thus decreasing both water column and benthic primary production (Garg and Bhatnagar, 1999). Furthermore, the addition of nutrient-rich ruminant dung from cattle to aquatic ecosystems already receiving large inputs of carbon-rich non-ruminant dung from hippos may lead to interactions between the two subsidies in decomposition rates and ecosystem effects (Kominski *et al.*, 2015).

The Mara River and its seasonal tributaries in the Maasai Mara National Reserve (MMNR) in Kenya host more than 4000 hippos (Kanga *et al.*, 2011). There are also over 250 000 cattle in communal lands adjoining the MMNR, where livestock coexist with wildlife (Ogutu *et al.*, 2011). This distribution results in a displacement pattern with hippo areas inside the reserve, mixed hippo and livestock areas outside the reserve, and only livestock grazing areas further away from the reserve (Veldhuis *et al.*, 2019). Previous research by Subalusky *et al* (2017) and Dutton *et al* (2018) showed that the number of inputs by LMH had a substantial impact on the aquatic ecosystem. This study aimed at studying the influence of both hippo and cattle dung inputs on nutrients, algal biomass, composition and diversity, and ecosystem metabolism (a mesocosm approach). A replacement design was used to compare the ecosystem effects of cattle dung and hippo dung inputs both independently and in combination with one another.

1.2. Statement of the Problem and Justification

The expansion of human settlements, crop farming, and conversion of forests and savanna grasslands to pasture for livestock production have contributed to the loss of large populations of wild LMH around the world. In many African savannas, large populations of wild LMH still dominate the biomass of conservation areas, where they significantly influence terrestrial nutrient cycling and primary production. However, even in these regions, native wildlife is declining concurrent with increases in domestic livestock such as cattle, goats, and sheep.

In most areas where livestock has replaced wildlife as the dominant LMH in the landscape, the influence of livestock on aquatic systems has often been seen as negative, with research focusing on habitat degradation, nutrient and organic matter loading, and microbial contamination. However, livestock may take over some of the ecological roles historically filled by native LMH, thereby maintaining the functionally important linkage of riverine ecosystems to their surrounding terrestrial landscapes. The degree to which the ecosystem effects of livestock versus native large mammalian herbivores arising from this functional linkage are similar to one another depends in part on the similarity of the resource subsidies they transfer. Differences in particle size and composition are likely to influence how dung inputs from hippos and cattle influence aquatic ecosystems. Although hippos can increase primary production in some aquatic ecosystems, it can reduce benthic production and lead to hypoxia.

Therefore, the goal of this study was to experiment recirculating stream mesocosms to test the impacts of LMH (hippo and cattle) dung input on nutrient release, algal biomass, composition and diversity, and ecosystem metabolism in aquatic ecosystems; given that

their foraging behaviour and the quality of their dung are different. A replacement design was used to compare the ecosystem effects of cattle dung and hippo dung both independently and in combination with one another. These will help in the development of appropriate management strategies for aquatic ecosystems.

1.3 Study Objectives

1.3.1 Overall Objective

To assess the influence of Hippo and Cattle dung inputs on nutrient concentrations, algal biomass, composition and diversity, and ecosystem metabolism in the mesocosms

1.3.2 Specific Objectives

- i) To determine the nutrient concentrations, at different proportions of hippo and cattle dung, in the mesocosms
- ii) To investigate the algal biomass, composition and diversity at different proportions of hippo and cattle dung in the mesocosms
- iii) To evaluate the correlation of different proportions of hippo and cattle dung on flume-scale ecosystem metabolism in the mesocosms

1.4 Hypotheses

HO₁: There are no significant variations in nutrient concentrations at different proportions of hippo and cattle dung in the mesocosms

HO₂: There are no significant differences in algal biomass, composition and diversity at different proportions of hippo and cattle dung in the mesocosms

HO₃: There are no significant variations in the correlation of different proportions of hippo and cattle dung on flume-scale metabolism in the mesocosms

CHAPTER TWO

LITERATURE REVIEW

2.1 Nutrient sources in lotic ecosystems

Freshwater and terrestrial ecosystems are relatively connected via physical processes and fluxes of energy across the riparian ecotone (Alberts *et al.*, 2017; Schindler & Smits, 2017). The inputs of nutrients and organic matter derived from terrestrial are an important driver of productivity in aquatic ecosystems (Wallace *et al.*, 1999; Burdon & Harding, 2008). Allochthonous organic matter produced in one ecosystem and relocated into another plays potentially important roles in the food web and ecosystem dynamics (Mariash *et al.*, 2018; Allan *et al.*, 2020). Allochthonous resources can have strong and complex effects on the recipient ecosystem such as changing nutrient cycling hence increasing or decreasing ecosystem productivity (Marcarelli *et al.*, 2011).

It is increasingly being recognized that inland waters play critical roles in nutrient cycling at local, regional, and global scales (Battin *et al.*, 2008). Nutrient enrichment from organic inputs and agricultural run-off is placing the world's vulnerable fresh waters in a precarious position (Caraco *et al.*, 1998; Bernal *et al.*, 2009). Nutrient pollution of freshwater ecosystems is pervasive and strongly affects carbon cycling. Excess nutrients stimulate the production of carbon-rich algal biomass but can also stimulate carbon loss through increased organic carbon mineralization that releases carbon dioxide instead of supporting the production of higher trophic levels and other ecosystem functions (Finlay *et al.*, 2013). The organic inputs in the form of excretion and egestion typically occur daily when animals engage in feeding migrations and move into their resting habitats. However, the timing and duration of these movements between habitats can be influenced

by various factors, which in turn affect the timing and duration of the subsidies (Subalusky *et al.*, 2015). Large mammalian herbivores are important vectors for the movement of carbon and nutrients among ecosystems (Carpenter *et al.*, 2005), and these animal inputs can act as subsidies that influence the changes in the recipient ecosystem (Chan *et al.*, 2007; Subalusky and Post, 2019). LMH is exceptionally essential subsidy vectors because they can create hotspots of carbon and nutrient cycling when they aggregate in time and space (McClain *et al.*, 2003; McIntyre *et al.*, 2008), transport carbon and nutrients against naturally-established gradients (Naiman *et al.*, 2009), or supply limiting carbon and nutrients (Vanni, 2002). They input organic matter and nutrients into recipient ecosystems through carcasses and waste excretion/egestion (Vanni, 2002; Subalusky and Post, 2019).

When LMH dies in a recipient ecosystem, the carcass decomposes delivering a complex source of carbon and nutrients (Ciborowski *et al.*, 1997). When animals repose in a recipient ecosystem after feeding elsewhere, they add carbon and nutrients to that ecosystem via the excretion of soluble organic and inorganic nutrients from assimilated resources, and the egestion of particulate carbon and nutrients from consumed resources (Field, 1970). Differences in stoichiometry and bioavailability between these different forms of input from wildlife and livestock can influence their effects on aspects of ecosystem function, such as decomposition, nutrient cycling, and the balance between primary production of autochthonous carbon and microbial respiration of allochthonous carbon (Marcarelli *et al.*, 2011; Tiegs *et al.*, 2011; Sitters *et al.*, 2015).

For hippos, most of their populations feed during the night and deposit subsidies (dung) in aquatic systems during the day (Subalusky *et al.*, 2015). Several studies have

quantified inputs of organic matter (dung) and nutrients by either wild large mammalian herbivores or livestock to disparate aquatic ecosystems (Bond *et al.*, 2014; Subalusky *et al.*, 2015). For African savannas, there is available data for some wild LMH (Hulot *et al.*, 2019) but lacks comparative data for livestock. This study was therefore set to determine the influence of hippo and cattle dung on nutrient release in river ecosystems.

2.2 Factors influencing algal biomass, abundance and diversity in lotic ecosystems

Algae are important primary producers since they are the basis of the whole food chain in open waters (Michaels & Silver, 1988; Christianen *et al.*, 2017). The processes that lead to nutrient stimulation of algal carbon production and terrestrial carbon mineralization are fundamentally different. Algal production increases relatively predictably with the availability of growth-limiting nutrients (Finlay *et al.*, 2013). In contrast, the mineralization process of particulate organic carbon (POC) is a more intricate phenomenon, influenced by the activities of organisms across multiple trophic levels, including microbial decomposers and detritivores animals (Webster *et al.*, 1999).

The maximum production of phytoplankton is obtained when the physicochemical factors are at optimum level (Muhammad *et al.*, 2005; Christianen *et al.*, 2017). The species composition of the algae community is an efficient bioindicator of water quality (Fonge *et al.*, 2012). The three major groups of algae in freshwater ecosystems are Bacillariophyta (diatoms), Chlorophyta (green algae), and Cyanophyta (blue-green algae). Changes in water quality or nutrients affect the algal ecological distribution (Fonge *et al.*, 2012). Ecological factors that regulate algae in rivers include current velocity, substratum, geology, nutrient conditions, grazers, temperature, pollutants, and

light availability (Stevenson, 1997). These factors often interact hence affecting algal growth and survival (Lowe & Pan, 1996; Fonge *et al.*, 2015).

The movement of material and energy across community boundaries plays a crucial role in shaping the ecology of entire landscapes (Baxter *et al.*, 2004). In particular, aquatic species that rely on terrestrial sources of energy and nutrients can have significant impacts on the recipient aquatic habitats. These influences can affect vital ecological processes such as nutrient cycling, food web dynamics, and the overall structure of aquatic communities, particularly when the recipient habitats are smaller in size compared to the sources of subsidies (Hall *et al.*, 2015). This highlights the interconnected nature of terrestrial and aquatic ecosystems and underscores the importance of understanding the cross-boundary interactions for comprehensive landscape-level ecological management.

Direct access to streams by animals can lead to increased nutrient levels in the water (Bond *et al.*, 2014; Stears *et al.*, 2018). Both hippos and cattle, known to defecate and urinate in streams (Subalusky *et al.*, 2015), contribute to elevated nitrogen and phosphorus levels (Bond *et al.*, 2014; Hotchkiss & Hall, 2014). The varying nutrient loads in aquatic ecosystems can have diverse effects on the ecosystem itself. Excessive nitrogen and phosphorus levels can result in direct toxicity issues for aquatic organisms and contribute to eutrophication in rivers (Holt, 2008; Huitu *et al.*, 2014). Notably, high levels of phosphorus specifically favour the growth of benthic diatom communities up to a saturation point beyond which further enrichment does not lead to additional growth or biomass accrual (Hynes, 1941). Conversely, lower concentrations of phosphorus are beneficial for the growth of diatom communities (Hynes, 1941). Moreover, studies have

demonstrated that increased phosphorus concentrations can promote the proliferation of macrophytic algae, including *Phormidium* (cyanobacteria) (Jompa & McCook, 2002).

One significant contributor of nutrient input into streams is dung from hippos and cattle. However, the data on the direct effects of these inputs on algal community structure in lotic ecosystems remain scarce. As a result, the current study was carried out to determine the effect of hippo and cattle dung on algal biomass, composition, and diversity in river ecosystems.

2.3 Importance of allochthonous energy sources in lotic ecosystems

Ecosystem metabolism quantifies the production (P) and respiration (R) of organic matter within a stream reach and provides insights into the relative contributions of external (allochthonous) and internal (autochthonous) carbon sources to the stream's food web (Kemp *et al.*, 1997). Serving as a functional metric of ecosystem activity, ecosystem metabolism integrates the processes that regulate organic matter dynamics and nutrient cycling in streams. Consequently, it is widely utilized as a tool to assess stream health and ecological condition (Kawaguchi *et al.*, 2003; Young *et al.*, 2008; Hall & Hotchkiss, 2017; Masese *et al.*, 2017).

The primary factors that influence stream ecosystem metabolism include light availability, temperature, nutrients, and organic matter supply (Sinsabaugh, 1997; Mulholland *et al.*, 2001), and these factors are influenced by local (riparian zone and geomorphology) and regional (hydrologic and climatic) factors (Bernot *et al.*, 2010; Hall & Hotchkiss, 2017). Kirchman (2000) found that watershed area, discharge, and soluble reactive phosphorus (SRP) concentration predicted primary production across a large spatial scale.

River ecosystem metabolism, which is the combination of gross primary production (GPP; photosynthesis [P]) and ecosystem respiration (ER), is a measure of how much organic C is produced and consumed in rivers (Kemp *et al.*, 1997; Hall & Hotchkiss, 2017). Algae and other aquatic plants are responsible for primary production, whereas ecosystem respiration measures the rates of respiration of all life, including fish, invertebrates, algae, aquatic plants, and microbes.

The ratio of these two variables (GPP/ER or P/R) also provides information on the relative importance of the two key sources of energy that fuel river ecosystems (algae and terrestrial organic matter). If organic carbon production is greater than carbon consumption, then organic matter produced within the system probably is supporting the food web. On the other hand, if carbon consumption is greater than carbon production, then organic matter from upstream or the surrounding catchment probably is maintaining the system (Von Schiller *et al.*, 2017).

Animal dung inputs can have a range of effects on ecosystem function. Common effects include an increase in primary production and a shift towards increased net ecosystem production (NEP) that usually result from elevated concentrations of inorganic nutrients in the system (Naiman *et al.*, 2009). However, when subsidies are in the form of organic nutrients (carcasses) or are high in carbon (faeces), the mineralization of these subsidies could shift the system towards decreased NEP or greater heterotrophy (Marcarelli *et al.*, 2011). The release of inorganic nutrients after mineralization could then be utilized by either the characteristic of the animal vector or recipient system and may also interact with the form of the subsidy to determine how it impacts ecosystem function (Iteba *et al.*, 2021).

Due to their relatively high quality, LMH inputs are often incorporated into the food web even if they occur in relatively low quantities (Marcarelli *et al.*, 2011; Subalusky *et al.*, 2015). However, the pathway through which they are incorporated depends on the input form and its effects on ecosystem function. Animal excretion rich in inorganic nutrients may be readily assimilated by primary producers and incorporated into higher trophic levels via grazing by primary consumers (Brett *et al.*, 2017; Parr *et al.*, 2020).

Widespread declines in many animal species have likely decreased the occurrence of animal subsidies. These declines have contributed further to the loss of species, due to a range of drivers including habitat loss, direct mortality, climate change, and increased barriers to migration (Harris *et al.*, 2009; Subalusky *et al.*, 2014). In a range of species subjected to human exploitation, there has also been a general decline in body size over time, which interacts with declining population numbers to further decrease subsidy quantity (Larsen, Muehlbauer, & Marti, 2016). Other animal species are increasing in response to anthropogenic activities, such as invasive species that have colonized novel habitats with potentially large impacts on ecosystem nutrient cycling (Capps *et al.*, 2012).

As many animal subsidies are decreasing, anthropogenic nutrient loading is increasing. In some systems, anthropogenic nutrients may essentially replace those lost by animal movements (Twining *et al.*, 2013). However, these nutrients are typically available in different forms. Anthropogenic nutrients usually enter ecosystems as human sewage or fertilizer runoff, both of which are high in inorganic nutrients and often connected with large increases in algal growth. Many animal subsidies provide more complex resources with organic nutrients accompanied by a range of other micronutrients, which may be more useful to a wider range of species and may enter the food web at higher levels of

consumers (Brett *et al.*, 2017). In addition to dynamic nutrient levels, many ecosystems are also experiencing higher levels of variability in a range of environmental factors due to climate change and other anthropogenic factors (Lyons *et al.*, 2004; Verboom *et al.*, 2010). This increased variability may alter the way recipient ecosystems interact with animal subsidies. For example, increased rainfall may escalate recipient system productivity and reduce its retention rate, both of which would lessen the effect of an animal subsidy, or rainfall could enhance the decomposition and uptake of a subsidy and increase its effect (Verboom *et al.*, 2010).

In light of these considerations, the current study was carried out to investigate the impact of LMH (hippo and cattle) dung on flume-scale ecosystem metabolism in river ecosystems. The goal was to examine how these inputs influence the production and consumption of organic carbon within the river, thereby examining their role in shaping the dynamics and energy flow of the ecosystem at the flume scale.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The experiment was conducted on the banks of the Amalo River (a tributary of the Mara River).

The Mara River is an international river shared between Kenya and Tanzania. Its basin is about 13,750 km² of which about 65% is located in Kenya and 35% in Tanzania. The river originates from the forested Mau escarpment along the western rim of the Eastern Great Rift Valley in Kenya (at an altitude of 2,900 m above mean sea level), meanders through large-scale agricultural farms, enters the Maasai-Mara and Serengeti National Parks in Kenya and Tanzania, respectively and ends its 395 km journey in Lake Victoria (LVEMP, 2005). In the middle reaches on the Kenyan side, the river and its tributaries host more than 4,000 hippos (Kanga *et al.*, 2011). This region is also home to the Maasai community, traditionally nomadic pastoralists that graze over 220,000 cattle in the Talek region outside the Maasai Mara National Reserve (MMNR) and utilize streams and rivers as watering points and crossings (Marzolf, Mulholland & Steinman, 1998; Ogutu *et al.*, 2011). Livestock is predominantly concentrated in various Talek River sub-catchments outside the MMNR, while hippos are primarily concentrated in tributaries of the Talek within the MMNR (Kanga *et al.*, 2011).

3.2 Dung collection

Dung from large mammalian herbivores wildlife (hippo) and livestock (cattle) were collected in October 2016 from Talek Rivers and Maasai Mara National Reserve respectively. Fresh hippo and cattle dung was collected from hippo paths and Maasai livestock pens, respectively. Dung from 3 cattle pens and 5 different hippo paths was thoroughly homogenized in buckets before use.



Plate 1: Collection of Hippopotamus dung in the field (Source: Author, 2016)

3.3 Experimental Set-Up of Mesocosms

The experiment was conducted from October to December 2016 in artificial experimental streams known as mesocosms. The mesocosms were specifically designed to determine nutrient concentrations at different proportions of hippo and cattle dung and assess their effects on algal biomass, composition, diversity, and flume-scale ecosystem metabolism, including gross primary production and ecosystem respiration. The mesocosms were

constructed using PVC canvas, measuring 4.2 meters in length and 19 centimetres in width, as described in a previous study by Subalusky *et al.* (2015). Water circulation within each mesocosm was facilitated by paddlewheels attached to a shaft, which was powered by a motor. Each shaft managed six streams, and there were three blocks labelled as A, B, and C, indicating a total of 18 streams (Plate 2).

The mesocosms were situated in an open field along the banks of the Amalo river, a tributary of the Mara River. To ensure an even distribution of light, the entire array of mesocosms was covered with a shade cloth. Before filling the mesocosms, they were lined with washed gravel to provide a suitable substrate for algal growth. The mesocosms were then filled with river water obtained from the Amalo River, specifically from an upstream location with minimal input from large mammalian herbivores. This water served as the baseline condition for the experiment, providing a controlled environment free from the direct influence of dung inputs.



Plate 2: (a) Allocation of dung treatments in three blocks driven independently by paddle wheels (b and c) layout and details of mesocosms, (d) hippo dung, and (e) cattle dung. (Source: Author, 2016)

The arrangement of different treatments in the mesocosms followed a randomized block experimental design. The treatments were randomly assigned to the mesocosms, with each treatment replicate distributed across three blocks. To initiate the experiment, 70 L of river water from the Amalo River was added to each mesocosm. There were three replicates for each of the six dung treatments, which ranged from 100% hippo dung (referred to as H100) to 100% cattle dung (referred to as H0). The specific dung treatments were as follows: H100 (100% hippo, 0% cattle), H80 (80% hippo, 20% cattle), H60 (60% hippo, 40% cattle), H40 (40% hippo, 60% cattle), H20 (20% hippo, 80%

cattle), and H0 (0% hippo, 100% cattle) (Plate 2 (a)). The mean velocity and depth across the experimental channels were set at 0.078 ± 0.013 m/s and 7.8 ± 0.7 cm, respectively. Water levels were maintained through natural precipitation and the addition of rainwater every two days.

The water from the Amalo River that was used in the experiment had the following physicochemical characteristics: total suspended materials = 1.11 ± 0.1 mg L⁻¹, temperature = 19.4 ± 0.7 ° C, dissolved organic carbon = 1.62 ± 0.5 mg L⁻¹, nitrate = 1.48 ± 0.4 mg L⁻¹, soluble reactive phosphorus = 0.06 ± 0.06 mg L⁻¹, and low amount of ammonia that was below detection (10µg/). Background nutrient concentrations were lower than treatment level concentrations in all treatments. A total of 120 g (wet weight, 1.7 g L⁻¹) of dung was distributed in each mesocosm only once at the beginning of the experiment. This concentration of dung is lower than field estimates in the Mara River 4 g L⁻¹, (Subalusky *et al.*, 2015), but it provided a sufficient quantity to elicit ecosystem responses without consuming so much dissolved oxygen due to microbial activity that would make ecosystem metabolism difficult to measure. A single batch addition was preferred to continuous additions to study ecosystem responses arising from differences in dung quality due to nutrient release and mineralization rates.

To accelerate biofilm growth, mesocosms were inoculated with 10 ml of periphyton scraped-off rocks from the Amalo River. Each mesocosm was lined with 6 unglazed ceramic tiles that were used for a weekly sampling of biofilms. Each week, one tile from each mesocosm was destructively sampled without replacement, and biofilm was scrubbed off into 50 ml of water and filtered through pre-weighed and pre-combusted

GF/F filters (0.7 μm pore size, 47 mm diameter, Whatman International Ltd., Maid stone, England).

3.4 Water Sampling and Laboratory Analyses

Water samples were collected every week at 10:00 am, starting from day 1, for the analysis of various parameters. These parameters included ammonium (NH_4^+), soluble reactive phosphorus (SRP), total phosphorus (TP), nitrite (NO_2^-), nitrate (NO_3^-), total suspended solids (TSS), ash-free dry mass (AFDM) concentration and composition, and chlorophyll a (Chl-a). To collect water samples for ammonium, nitrate, nitrite, and SRP, on-site filtration was conducted using pre-combusted and pre-washed Whatman GF/F filters (0.7 μm pore size, 47 mm diameter). The filtered water was then stored in acid-washed high-density polyethylene (HDPE) bottles at 4°C until analysis, which took place within 48 hours.

In the laboratory, the analysis of SRP, TP, NO_2^- , NO_3^- , and NH_4^+ in the water samples followed standard colorimetric methods (APHA, 1998). SRP was analyzed using the ascorbic method, NH_4^+ was analyzed using the hypochlorite method, and NO_2^- and NO_3^- were analyzed using the salicylate method (APHA, 2005). For the measurement of TSS, water samples were filtered on-site using pre-combusted (450°C for 4 hours) and pre-weighed Whatman GF/F filters (0.7 μm pore size, 47 mm diameter). The concentration of TSS (g L^{-1}) was determined by drying the filters with adhered sediments in an oven at 60°C and subtracting the filter weight.

To determine the biofilm biomass (AFDM) within TSS, the filters were further subjected to gravimetric analysis. The filters were ashed at 450°C for 4 hours, re-weighed, and the ashed weight was subtracted from the TSS measurement.

The total suspended solids concentration was Calculated as follows:

$$\text{TSS (mg l}^{-1}\text{)} = ((W_c - W_f) \times 1000) V^{-1}$$

Where;

- TSS = Total suspended solids,
- W_f = Weight of pre-combusted filter in grams;
- W_c = Constant weight of filter + residue in grams;
- V = Volume of water sample used in litres

Ash Free dry mass concentration was calculated as follows:

$$\text{AFDM (mg}^{-1}\text{)} = ((W_c - W_a) \times 1000) V^{-1}$$

Where;

- AFDM = Ash-free dry mass;
- W_a = Weight of ashed filter in grams;
- W_c = Constant weight of filter + residue in grams;
- V = Volume of water sample used in liters
- W_a = Weight after ashing

Chl-a was determined by methanol extraction and concentrations were measured spectrophotometrically (APHA, 1998). Five hundred milliliters of water were filtered through a Whatman GF/F filter (0.7 µm pore size, 47 mm diameter) and then the filter

paper was stored frozen until analysis was carried out in the laboratory. The filter paper was torn into 5 or 6 pieces and inserted into a centrifuge tube then 10 ml of methanol was poured into a centrifuge tube to cover the filter paper, shaken, and vortex until the filter paper was broken up. The centrifuge tubes were stoppered with plastic closure and left in the fridge overnight; in the morning they were centrifuged at 3200 rpm for 10 minutes. The supernatant was then poured off into a 1 cm cuvette. The extinction was then measured using a spectrophotometer at both 665nm and 750nm (zero with methanol).

The concentration of chlorophyll-a was calculated as follows:

$$\text{Chl-a (ug/l)} = \frac{(\text{Abs}[665\text{nm}] - \text{Abs}[750\text{nm}] * A * V_m)}{v_f * L}$$

Where;

A = absorbance coefficient of chlorophyll-a in methanol (12.63).

V_m = volume of methanol used for extinction (ml)

V_f = liters of water filtered

L = path length of the cuvette



Plate 3: Analysis of chlorophyll-a in the lab (Source: Author, 2016)

For ecosystem metabolism in each mesocosm, Dissolved Oxygen (DO) and water temperature were recorded every 1 minute for 6 weeks (42 days) using MiniDOT loggers (PME Inc., Vista, CA, USA). GPP and ER from diel changes of oxygen, temperature, and irradiance were estimated by an inverse modeling procedure that included temperature-dependent ER, light-dependent GPP, and reaeration (Fußet *al.*, 2017). A differential equation model was fitted by Hotchkiss and Hall, (2014) to diel DO concentration measured at a single site (Marzolf *et al.*, 1998). The model simulates temporal changes in DO concentration (dDO/dt) as the result of parameterized GPP, ER, and reaeration

$$\frac{dDO}{dt} = (GPP - ER + RF) \times \frac{1}{z}$$

Where GPP adds DO to the water by photosynthesis; ER consumes DO and RF is the gas exchange at the water-air interface. GPP ($\text{g O}_2 \text{ m}^{-2} \text{ min}^{-1}$) was modeled with light saturation (Ratkowsky, 1986; Uehlinger *et al.*, 2000) as:

$$\text{GPP} = \frac{\text{PAR}}{P_1 + P_2 + \text{PAR}}$$

Where PAR (W m^{-2}) is the observed, instantaneous PAR. P_1 ($\text{W min g}^{-1} \text{ O}_2$) is the inverse of the slope of photosynthesis–irradiance curve at a low light intensity and P_2 ($\text{m}^2 \text{ min g}^{-1} \text{ O}_2$) is the inverse maximum photosynthesis rate. Daily GPP (GPP24, $\text{g O}_2 \text{ m}^{-2} \text{ day}^{-1}$) was integrated from P_1 , P_2 , the light record, and the time step Δt between light measurements:

$$\text{GPP24} = \sum_{t=t_0}^{t \text{ end}} \frac{\text{PAR}_t}{P_1 + P_2 + \text{PAR}_t} \times \Delta t$$

Since ER ($\text{g O}_2 \text{ m}^{-2} \text{ min}^{-1}$) is a strongly temperature-dependent process, it was modelled with the van't Hoff–Arrhenius equation (Mathuriau & Chauvet, 2002):

$$R = \frac{\text{ER}_{20}}{(24 \times 60)} \times \theta^{(T-20)}$$

where ER_{20} ($\text{g O}_2 \text{ m}^{-2} \text{ day}^{-1}$) is the daily rate of ER standardized to 20 °C and T (°C) is the observed, time-specific ambient stream temperature, and θ (theta) is the temperature dependence on respiration. To investigate ER *in situ* temperature, ER_{20} was translated to $\text{ER}_{24_{\text{insitu}}}$ ($\text{g O}_2 \text{ m}^{-2} \text{ day}^{-1}$) using recorded *in situ* temperature measurements T (°C) for every time interval Δt :

$$\text{ER}_{24_{\text{insitu}}} = \sum_{t=t_0}^{t \text{ end}} \frac{\text{ER}_{20}}{(24 \times 60)} \times 1.1085^{(T_t-20)} \times \Delta t$$

The reaeration flux RF ($\text{g O}_2 \text{ m}^{-2} \text{ min}^{-1}$) was computed as:

$$\text{RF} = k \times \text{DO}_{\text{deficit}}$$

Where k is the temperature-dependent vertical gas exchange velocity (m min^{-1}) and $\text{DO}_{\text{deficit}}$ (g m^{-3}) is the difference of the observed DO concentration (DO) to DO at 100% saturation (DO_{Sat}):

$$\text{DO}_{\text{deficit}} = \text{DO}_{\text{Sat}} - \text{DO}$$

DO_{Sat} was calculated from observed, time-specific ambient stream temperature and atmospheric pressure (McCook, 2001). The vertical gas exchange velocity k (m min^{-1}) is related to the reaeration coefficient K (min^{-1}) by multiplication with depth (m) (Raymond *et al.*, 2012). The aeration coefficient measured in 6 mesocosms (2 each for each block) was used by degassing water by boiling and then cooling in air-tight containers before carefully filling the mesocosms with minimal bubbling. The slope of the linear increase in DO concentration was used as an estimate of re-aeration. Temperature dependence of gas exchange was calculated (Miller *et al.*, 1999):

$$K_T = K_{20} \times 1.024^{T-20}$$

Where K_T and K_{20} are reaeration coefficients at ambient stream temperature T and 20°C , respectively. For model fitting, the time derivative $d\text{DO}/dt$ of eqn (1) was approximated by differences in incremented Δt across the observed time intervals, and a discretized time series of DO was predicted using observed, time-specific temperature and light conditions, barometric pressure and a chosen parameter set P1, P2, ER24₂₀, and K_{20} :

$$DO_{t+1} = DO_t + (GPP_t - ER_t + RF_t) \times \Delta t \times \frac{1}{z}$$

DO_{t+1} ($\text{g O}_2 \text{ m}^{-3}$) was computed from DO_t and GPP, ER, and RF were computed from temperature and light conditions at the previous time point t . Δt , the time interval between t and $t + 1$, is needed to scale up the minute-specific rates accordingly and is chosen in agreement with the observed time series. Equation (9) was obtained by forwarding differencing or Eulerian integration of Eqn(1) (Soetaert & Herman, 2009). A first observed DO measurement is used as a starting value (DO_{t0}), from which all subsequent DO_t values are computed. To fit P_1 , P_2 , $ER_{24_{20}}$, and K_{20} to empirical data, eqn (9) was used in an inverse modelling approach that repeatedly models a DO time series with updated parameter values and minimizes the sum of squared residuals of the modelled to the observed DO time series. A reaeration coefficient (k) was estimated by filling 6 clean mesocosms (2 for each block) with degassed (boiled and cooled) water and then recording DO and temperature to model reaeration (K_{20}) without GPP and ER. K_{20} was then used as a starting value to reliably model P_1 , P_2 , $ER_{24_{20}}$, and K_{20} .



Plate 4: Light logger (left) and mini DO₂T logger (right) (Source: Author, 2016)

3.4.1 Measuring algal biomass

Algal biomass is quantified as Chl-a, Tss, and Afdm. Chl-a can be measured using a benthotorch or extracted using chemicals. Benthotorch is an in vivo flourometer designed to quantify algal biomass (as chlorophyll-a) and community composition of cyanobacteria, green algae and diatoms in benthic environments. For the experiment, Chl-a was extracted using methanol and then its concentration was measured using a spectrophotometer.

3.5 Data Analysis

Generalized Linear Mixed Models (GLMM) were used to test the effect of dung treatment on response variables Chl-*a*, AFDM, TSS, SRP, NH_4^+ , NO_2^- and NO_3^{2-} . GLMM models included dung treatment (H0, H20, H40, H60, H80, and H100) and time (week 1- week 6) as fixed effects, and block and stream (nested within a block) as random effects.

A three-parameter Sigmoid model was fitted using the Gompertz function (Gompertz, 1825), to daily GPP and ER for each dung treatment to estimate three parameters that measure various aspects of the GPP and ER rates: the upper asymptote, growth rate and time of maximum growth. The Gompertz function was selected over the logistic function as a model for the temporal data structure because of hypothesized asymmetry in GPP and ER in the mesocosms (Paine *et al*, 2012).

Principal Component Analysis (PCA) was used to reduce the dimensionality of the physico chemistry and primary production data in relation to the dung treatments. Two PCs were included to describe water quality physico-chemical variables and primary

production variables separately. PCAs were statistically assessed using PERMANOVA (permutation analysis of variance), based on Bray-Curtis similarity matrices (McArdle & Anderson, 2001).

Community structure was described in terms of taxon richness, abundance and community indices. Species distribution data were summarized and the mean was calculated for each dung treatment category using the number of taxa (S) and the total relative abundances. Several diversity indices were calculated for each dung treatment and means were calculated for each category. Shannon's diversity index (H') was derived as a measure of diversity (Magurran, 2004), and an associated

H'/H' max index (Pielou, 1975) was used as a measure of evenness. The reciprocal form of the Simpson index (1-Ds) (Simpson, 1949) was used as a measure of species richness. Fisher's alpha

(Fisher *et al.*, 1943) was used as an extra measure of algae diversity. Margalef's species richness index was also calculated as an extra measure of algae taxon richness.

A two-way nested analysis of similarities (ANOSIM) was used to compare average rank similarities in algae species composition among dung treatments and time (weeks). These analyses were performed to check if algae changed in composition among dung treatment and with time (weeks). ANOSIM calculates the R-statistic, which is a test statistic that varies between 0 and 1; with higher values indicating bigger differences between factors.

Non-metric multidimensional scaling (NMDS) was used to visualize algae composition in different dung treatments and with time using the mesocosms dataset (Clarke & Gorley,

2006). Dissimilarity matrices based on the Bray–Curtis coefficients (Bray & Curtis, 1957) were derived using the R function “vegdist” (Gardener, 2014). The goodness of fit of the ordination was assessed by the magnitude of the associated stress value, with a value of <0.2 corresponding with a good ordination (Kashian *et al.*, 2007). Permutational multivariate analysis of variance (PERMANOVA) was used as implemented in the “adonis” function of the vegan R package (Oksanen *et al.*, 2018) to test for significant differences in algae species composition among dung treatments and with time in weeks in the mesocosms. Pairwise differences in algae composition among dung treatments were run for all pairs of dung treatments and weeks using the “adonis.pair” function of the EcolUtilsR package (Minshall, 1988), and used Bonferroni correction to set significance levels for p-values. In all the aforementioned tests, statistical significance was determined by 999 permutations.

Similarity percentages analysis (SIMPER) was performed to establish which key algae that were accountable for the variations observed among dung treatments in the mesocosms. The percentage (%) contribution of algae to the overall dissimilarity was quantified among dung treatments. SIMPER is a restrictive pairwise analysis between two-factor levels (Clarke & Warwick, 2001), and in this case, comparisons were done between H0 and H100, H20 and H100, H40 and H100, H60 and H100 and between H80 and H100.

Canonical Correspondence Analysis (CCA) was used to elucidate relationships between the algae and environmental variables. The output was displayed as triplots, in which the plotted points for algal taxa and dung treatment could be related to water quality physicochemical variables that were represented as rays (Ter Braak & Smilauer, 1998).

CHAPTER FOUR

RESULTS

4.1 Characteristics of cattle and hippo dung

Large mammalian herbivores (cattle and hippo) dung used in the experimental mesocosms had different characteristics. The cattle dung had significantly ($p < 0.05$) smaller particle sizes (0.4 mm) as compared to hippo dung (17.8 mm). Cattle dung had a lower C: N: P ratio of 155.2:5.1:1.0 than that of hippo dung which had a C: N: P ratio of 261.4:7.6:1.0 (Table 1). When wet, the smaller particles of cattle dung readily dissolved in water but the larger particles of hippo dung mostly sank to the bottom.

Table 1: Characteristics of hippo dung and cattle dung used in the mesocosms. P = Phosphorous, N = Nitrogen, C = Carbon.

Parameter	Hippo dung	Cattle dung
Particle sizes in mm	17.8	0.4
Nitrogen (% dry matter)	0.98	1.13
P (mg/g)	1.29	2.23
N (mg/g)	9.81	11.32
C: N:P	261.4:7.6:1.0	155.2:5.1:1.0

4.2 Nutrient concentrations at different proportions of hippo and cattle dung in the mesocosms

The mean concentrations of nutrients released by different proportions of hippo and cattle dung varied across the treatments. The mean concentration of soluble reactive phosphorus (SRP) showed significant variation among the treatments ($F_{5,126} = 3.77$, $p = 0.04$) (Figure 1a). Treatment with 100% cattle dung had the highest mean SRP concentration (0.29 ± 0.11 mg/l), while the treatment with 0% cattle dung (100% hippo dung) had the lowest mean concentration (0.14 ± 0.06 mg/l). The mean ammonia concentration also varied significantly among the treatments ($F_{5,126} = 8.32$, $p < 0.001$). Treatment with 100 % cattle dung had the highest mean ammonia concentration (0.07 ± 0.03 mg/l), while 20% cattle dung had the lowest mean concentration (0.01 ± 0.01 mg/l) (Figure 1b). The mean nitrite concentration also exhibited significant variation across the treatments ($F_{5, 126} = 8.32$, $p < 0.001$), with 100% cattle dung having the highest mean concentration (0.058 ± 0.013 mg/l) and 0% cattle dung having the lowest mean concentration (0.019 ± 0.013 mg/l) (Figure 1c). Additionally, the mean nitrate concentration varied significantly across the treatments ($F_{5, 126} = 3.30$, $p = 0.04$). Treatment with 60% cattle dung had the highest mean nitrate concentration (3.5 ± 0.5 mg/l), while 20% cattle dung had the lowest mean concentration (2.1 ± 0.3 mg/l) (Figure 1d).

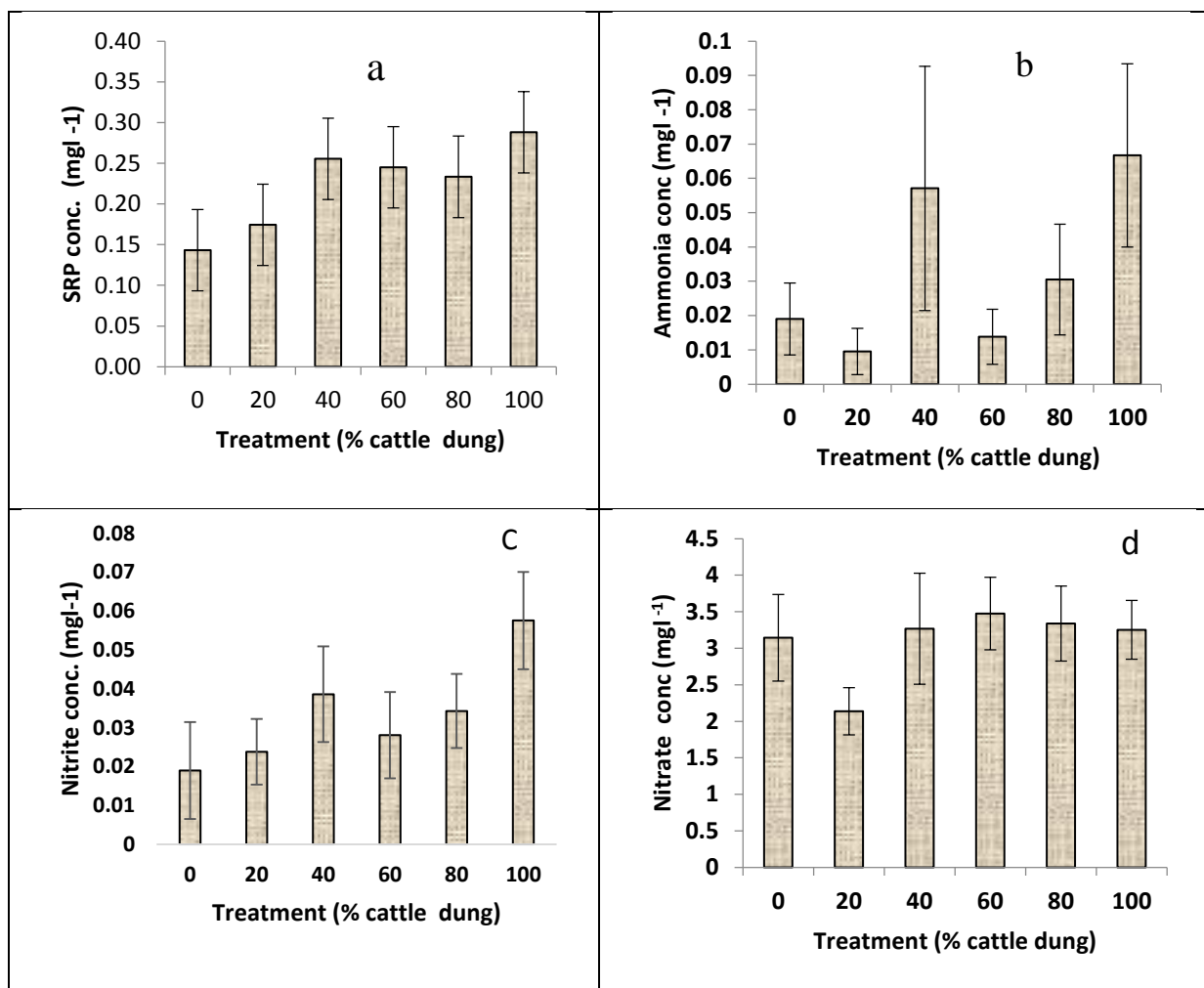


Figure 1: Influence of different proportions of cow dung on the release of various nutrients in experimental mesocosms.

Figure 2 shows that dung treatment had a significant effect on nutrient concentrations. There was a significant increase in soluble reactive phosphorous (SRP), ammonium, nitrite and nitrate with the increasing proportion of cattle dung (Figure 2; Table 2). Also, a significant effect of time on nutrient concentrations was seen reflecting different rates of nutrient release and uptake throughout the experimental period (Table 2). There was more than a 90% reduction in SRP within the first 14 days across all treatments (Figure

2a), while the concentration of ammonia declined by more than 50% during the same period (Figure 2b).

Correspondently, nitrite concentrations significantly declined after the second week, while those of nitrate increased (Figure 2c, d; Table 2). These patterns showed that nitrite was being converted to nitrate as the concentration of nitrite and particularly ammonia decreased, while the concentration of nitrate progressively increased in all treatments.

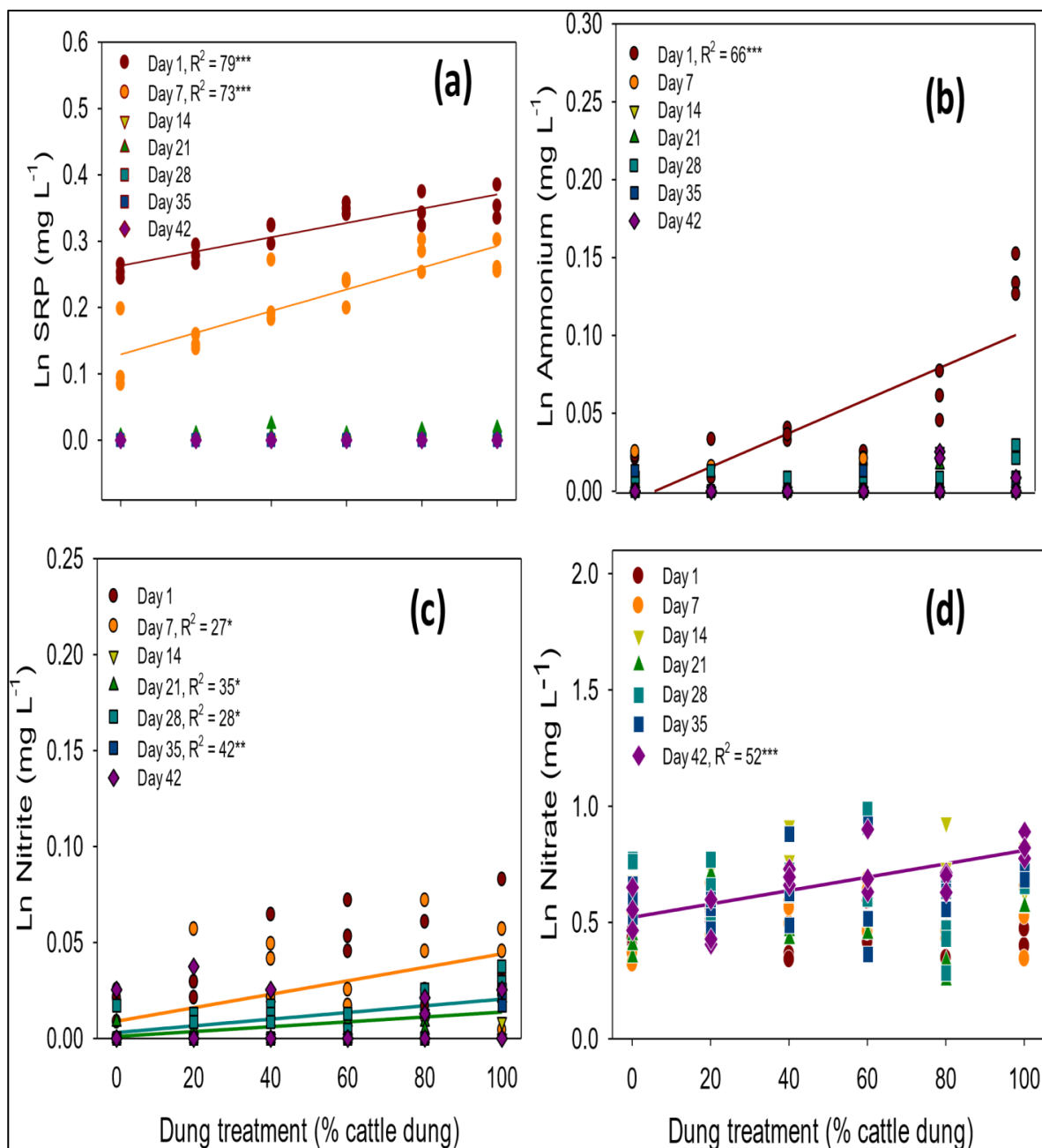


Figure 2: Influence of dung treatment on (a) soluble reactive phosphorus (SRP), (b) ammonium, (c) nitrite, and (d) nitrate concentrations. Asterisks and significant model fits are displayed for linear relationships across low-high proportions of cattle dung for each sampling occasion ($\alpha \leq 0.05$). * $P < 0.05$, ** $P < 0.01$, * $P < 0.001$.**

4.3 Algal biomass, composition and diversity at different proportions of hippo and cattle dung in the mesocosms

4.3.1 Algal biomass

The algal biomass was quantified by analyzing chlorophyll-a (Chl-a) concentration, Ash-Free Dry Mass (AFDM), and Total Suspended Solids (TSS).

Regression analysis showed a linear increase in Chl-a, TSS, and AFDM variables across the dung treatments from a low proportion of cattle dung (H100) to a high proportion of cattle dung (H0) (Figure 3; Table 2). Also, there was a significant effect of time on these parameters (Table 2). The concentrations of Chl-a, AFDM and TSS increased by >100% (Figure 3a-c). The fine cattle dung particles readily dissolved in water and remained in the water column, as exhibited by the higher TSS values (Figure 3c). The increase in TSS also could be attributed to higher phytoplankton biomass in the water column in cattle dung treatments, as indicated by Chl-a concentration

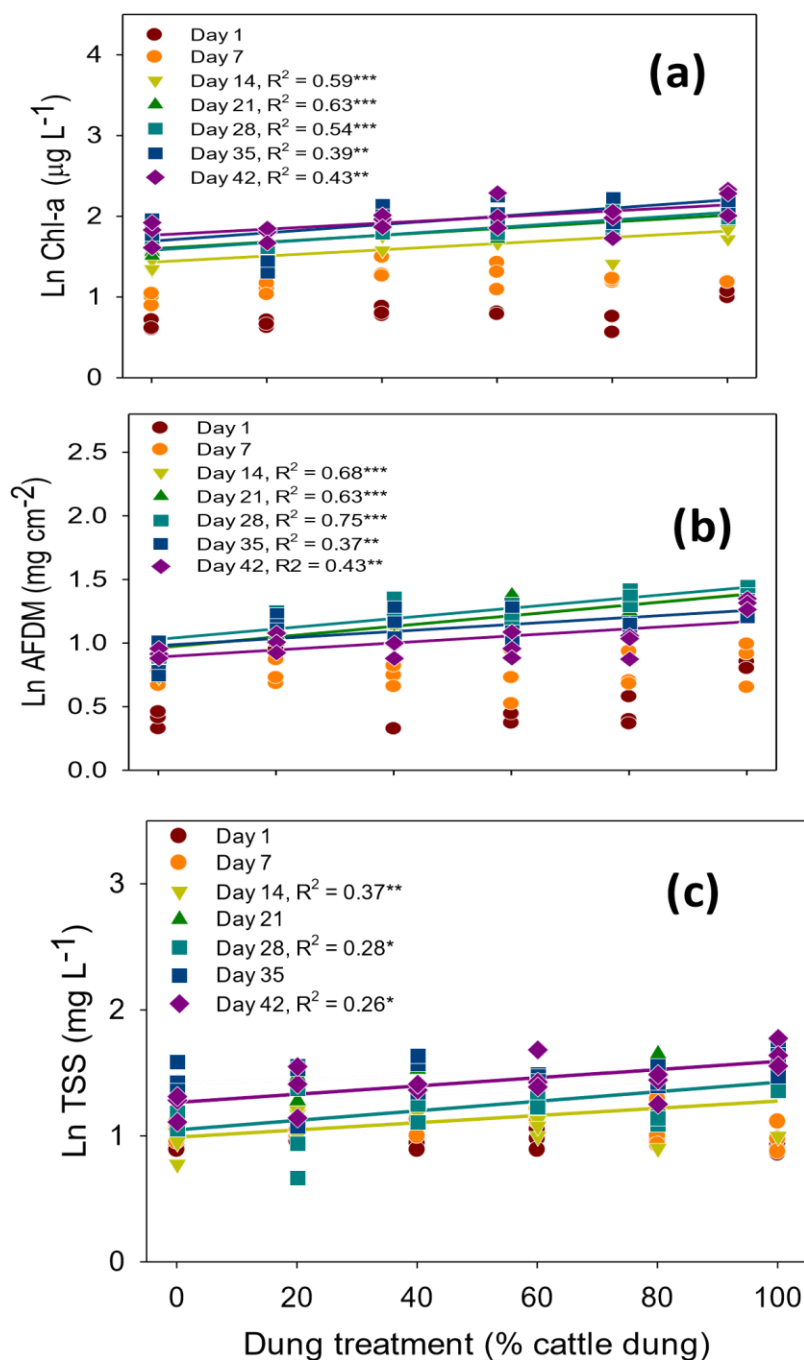


Figure 3: Influence of dung treatment on a) chlorophyll-a (Chl-a), b) ash-free dry mass (AFDM), and c) total suspended solids (TSS) concentrations. Asterisks and model fits are displayed for significant linear relationships ($\alpha \leq 0.05$). * $P < 0.05$, ** $P < 0.01$, * $P < 0.001$.**

Table 2: Results of mixed-effects models for loge (x) transformed (\pm SE) chlorophyll a (chl-a, ugL-1), ash-free dry mass (AFDM, mg cm²) and total suspended solids (TSS, mgL-1), soluble reactive phosphorus (SRP, mg L-1), ammonium (mg L-1), nitrite (mg L). The marginal R² (GLMM[m]; fixed effects only) and the conditional R² (GLMM[c]; fixed and random effects) represent the proportion variance explained by each model. SE = standard error; SD = standard deviation; *P < 0.05, **P < 0.01, *P < 0.001.**

Fixed Effects	SRP	Ammonium	Nitrite	Nitrate	Chl-a	AFDM	TSS
Intercept	0.87(0.08) ***	0.1(0.02) ***	0.14(0.01) ***	0.38(0.05) ***	1.11(0.05) ***	0.79(0.07) ***	1.08(0.04)***
Dung Treatment	-0.004(0.001) **	-0.001(0.0002) *	-0.001(0.0002) ***	-0.002(0.001) *	-0.003(0.001) ***	-0.003(0.001) *	-0.002(0.001) *
Time	-0.19(0.02) ***	-0.02(0.05) ***	-0.02(0.003) ***	0.04(0.01) ***	0.22(0.02) ***	0.12(0.02) ***	0.11(0.01) ***
Dung Treatment * Time	0.001(0.0003) *	0.0002(0.0001) *	0.0002(0.00004) ***	-	-	-	-0.0004(0.0002) *
Random effects	SD	SD	SD	SD	SD	SD	SD
Block (intercept)	0.03	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Stream (intercept)	<0.001	<0.001	<0.001	0.06	0.04	<0.001	0.05
Residuals	0.26	0.06	0.038	0.13	0.24	0.25	0.12
R ² GLMM(m)	0.58	0.20	0.51	0.34	0.74	0.47	0.72
R ² GLMM(c)	0.58	0.20	0.55	0.46	0.75	0.47	0.76

4.3.2 Algae composition

In the experimental mesocosms, a total of 22 algae species were identified, belonging to three classes (Chlorophyta, Bacillariophyta and Cyanophyta). The most abundant class was the Chlorophyta (72.0%) followed by the Cyanophyta (7.4%) with Bacillariophyta (3.9%) being the least in all the weeks (Figure 5a). Similarly, across the treatments, the most abundant division was the Chlorophyta (85.0%) followed by the Cyanophyta (10.8%) with Bacillariophyta still being the least (4.2%) (Figure 5b). Dung treatment had a significant ($p < 0.05$) effect on the abundance of algae. There was a significant increase in Chlorophyta and Bacillariophyta and a decrease in Cyanophyta from a low proportion of cattle dung (H100) to a high proportion of cattle dung (H0) (Figure 5b).

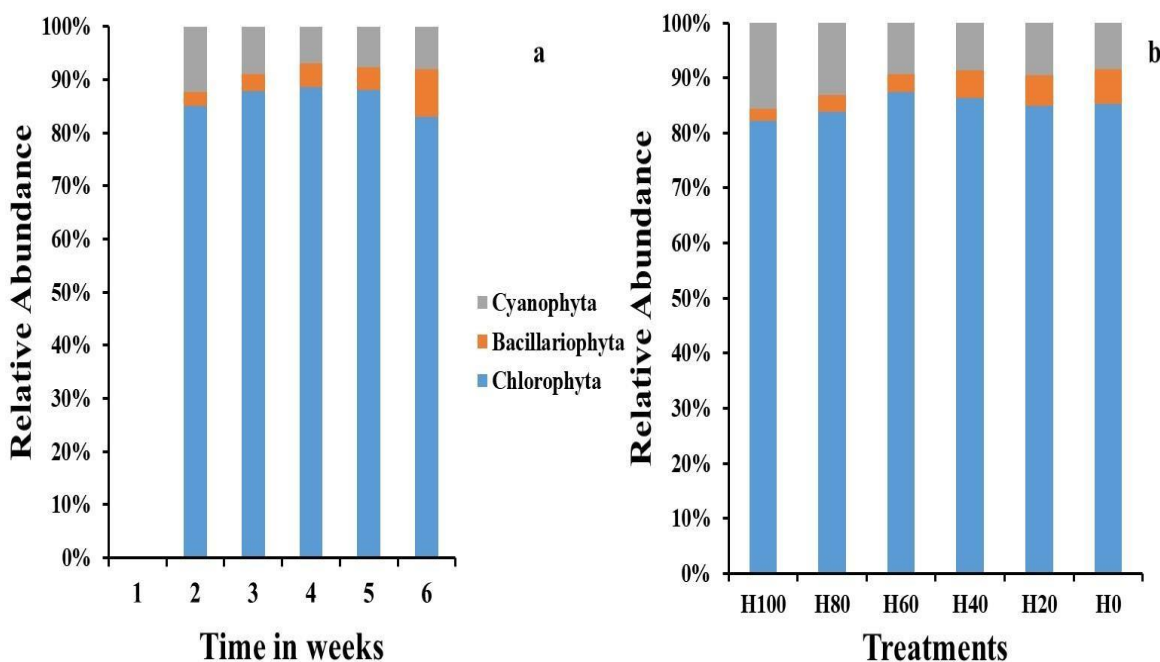


Figure 4: Relative abundances of algae at different weeks (4a) and in different treatments (4b).

Class Cyanophyta had five (5) genera identified (namely; *Oscillatoria* sp., *Anabaena* sp., *Microcystis* sp., *Lyngbya* sp. and *Spirulina* sp.) (Figure 6). All the genera were identified in all the treatments from week 2 (week 1 was not sampled as it was zero days and week 2 was after 7 days when sampling began) to week 5 while in the last week (week 6) *Lyngbya* sp. and *Anabaena* sp. were missing in all the treatments. In week 5, *Spirulina* sp. was missing in the treatments with 80% and 100% cattle dung. The *Microcystis* sp. were the dominant species across the study period mainly in treatments with high cattle dung while *Spirulina* sp. mainly dominated treatments with low cattle dung (H100 - H60) (Figure 5).

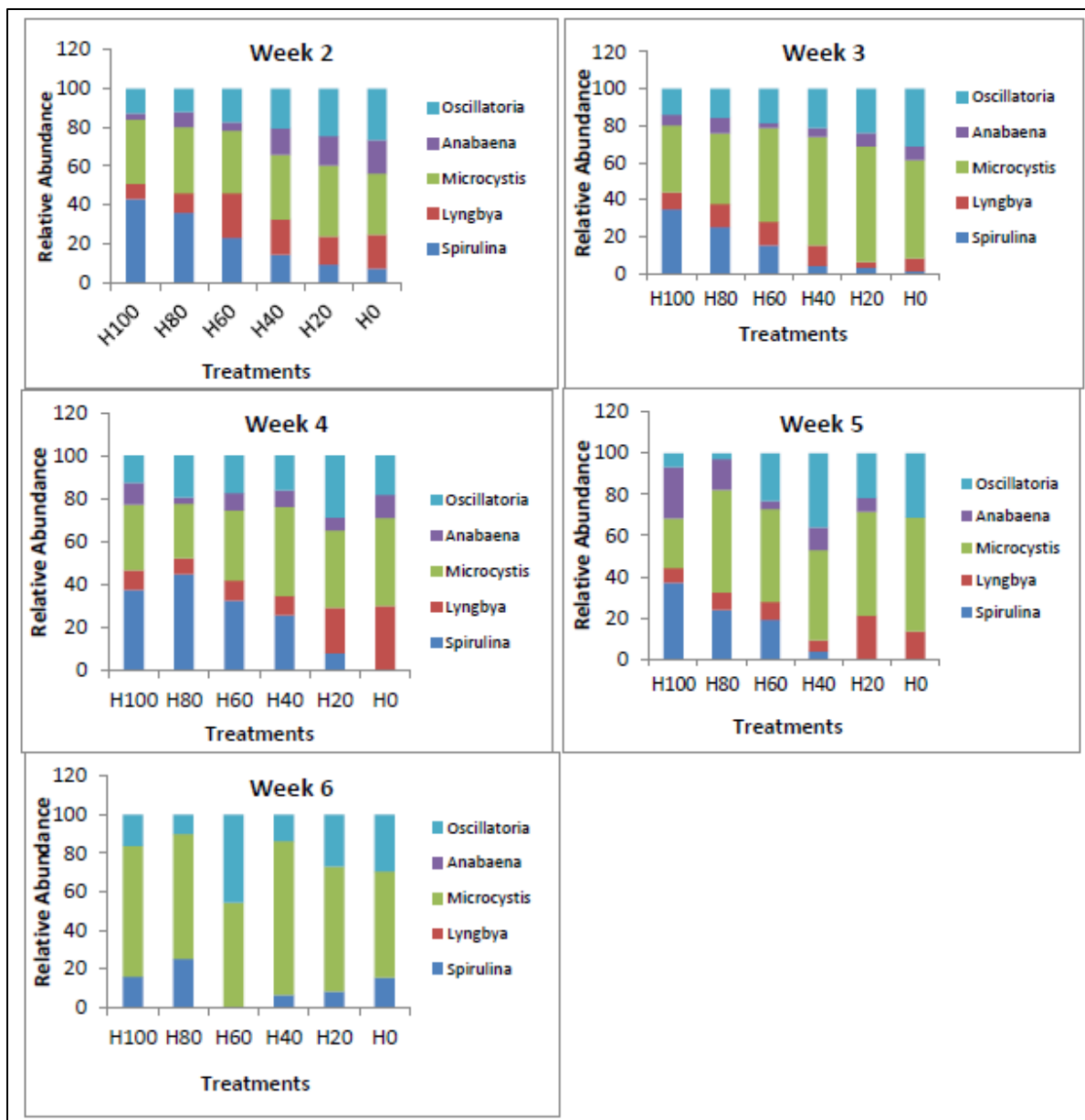


Figure 5: Weekly relative abundance of Cyanophyta in the different dung treatments

Class Bacillariophyta had six genera identified (namely; *Cocconeis* sp. *Achnanthes* sp. *Eunotia* sp., *Navicula* sp, *Gomphenoma* sp. And *Nitzschia* sp.) in all the treatments (Figure 7). *Achnanthes* sp. And *Cocconeis* sp. were abundant in treatments with a high

proportion of cattle dung (H20 and H0) while *Gomphenoma* sp. and *Navicula* sp. dominated treatments with low levels of cattle dung (high hippo dung levels) (Figure 7). During the 1st week of algae colonization (Week 2), the treatment with 100% had no algae colonizing the mesocosms (Figure 7).

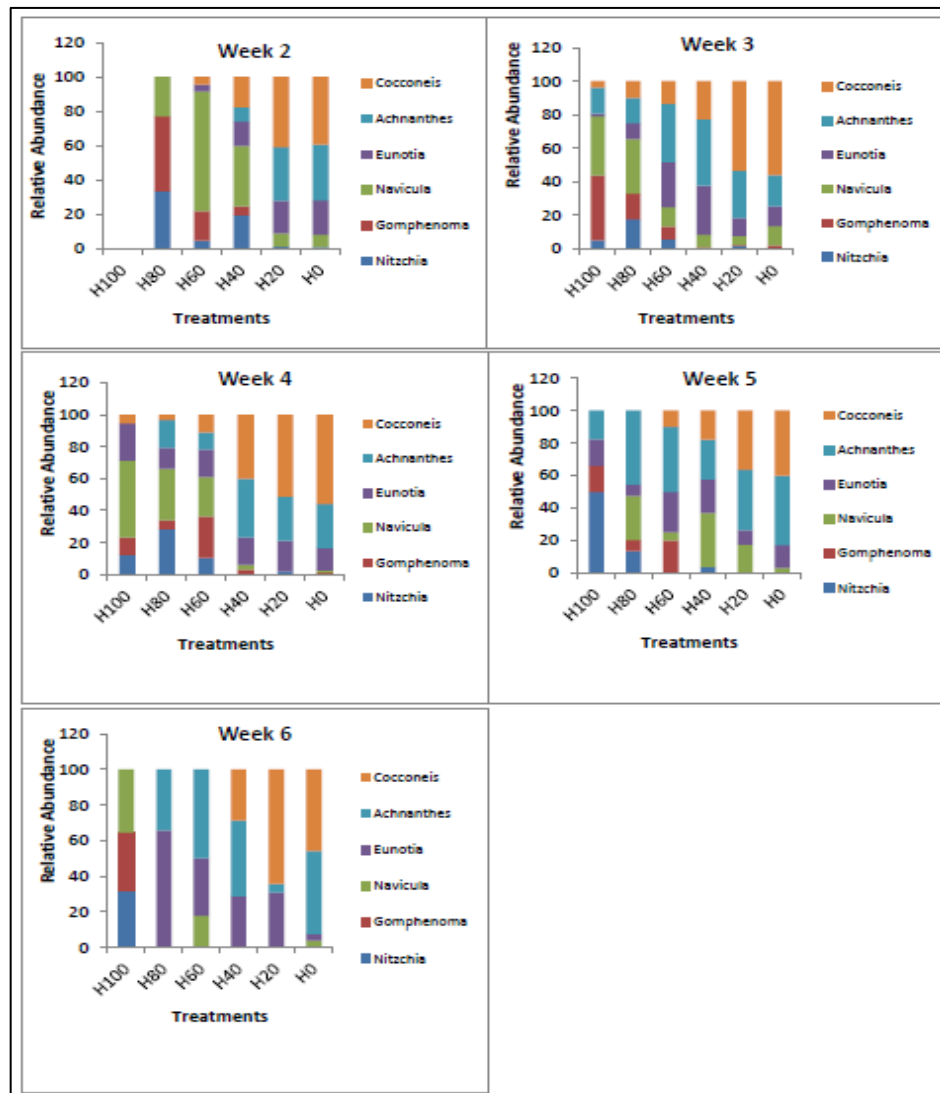


Figure 6: Weekly relative abundance of Bacillariophyta in the different dung treatments

Class Chlorophyta had eleven genera identified (namely; *Zygema* sp., *Oedogonium* sp., *Scenedesmus* sp., *Cladophora* sp., *Spirogyra* sp., *Coelastrum* sp., *Closterium* sp., *Ulothrix* sp., *Pleurococcus* sp., *Pediastrum* sp., and *Mougeotia* sp.) in all the treatments (Figure 8). *Scenedesmus* sp. and *Cladophora* sp. exhibited clear patterns by increasing with an increase in the cattle dung levels (from H100 to H0), while *Oedogonium* sp. exhibited an opposite trend by decreasing with an increase in cattle dung levels across all the weeks (Figure 8). Time had a significant effect on the algal composition. By the 6th week, only three (3) genera (*Scenedesmus* sp., *Ulothrix* sp. and *Cladophora* sp.) were dominating the composition (Figure 8). During the same period, *Oedogonium* sp. was abundant in treatments with high proportions of hippo (H100 and H80) but significantly decreased with an increase in the proportion of cattle dung (Figure 8).

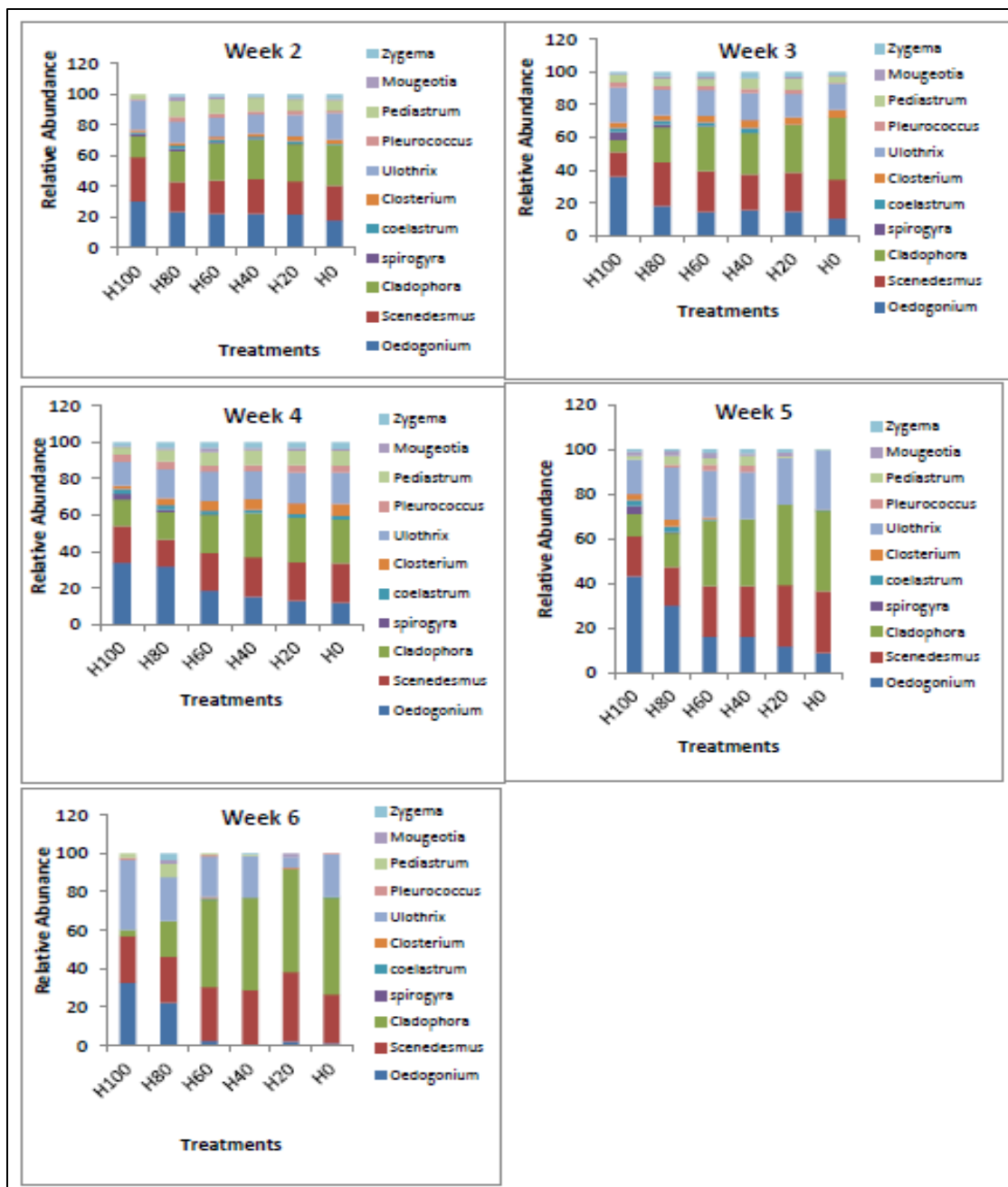


Figure 7: Weekly Relative Abundance of Chlorophyta in the different dung treatments

Analysis of similarity (ANOSIM) indicated significant differences in algae assemblages for untransformed abundance data among dung treatments (R-statistic = 0.69, $p = 0.0001$), and across time in weeks (R-statistic = 0.91, $p = 0.0001$). These findings suggest a stronger effect of “time” across dung treatments as compared to the “dung treatment” effect across time. The non-metric multidimensional scaling (NMDS) based on algae composition showed no differentiation between dung treatments (PERMANOVA $F = 0.21$, $df = 5$, $p = 0.23$) (Figure 9a). The dung treatments NMDS formed with algae genera had two clusters with algae clustering either around the high cattle dung treatments (H0 to H40) cluster or the low cattle dung treatments (H60 to H100) cluster (Figure 9a). However, there were clear differences in algae composition with time in weeks (PERMANOVA $F = 5.10$, $df = 4$, $p = 0.001$) (Figure 8b).

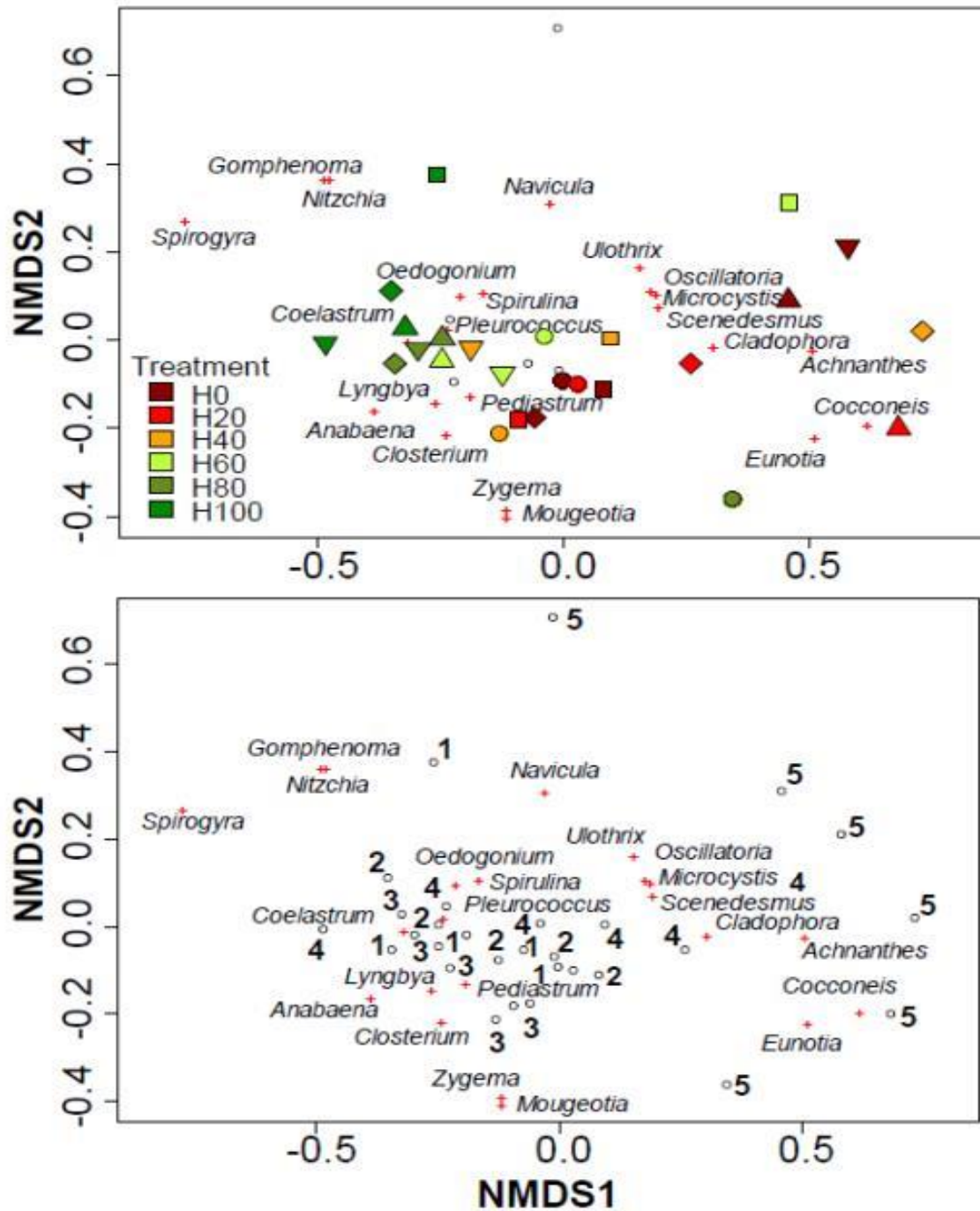


Figure 8: NMDS based on algae species composition in the different mesocosms. The two panels are the same ordination with loadings for (a) species composition and dung treatments, and (b) species composition and time in weeks (1-5). H0 (100% cattle)-H100 (0% cattle) = dung treatments, 1-5 = time in weeks (week 2 to week 6).

Abundance-based SIMPER's pair-wise comparison of H100 vs. H0, H20, H40, H60 and H80 identified *Cladophora* sp., *Scenedesmus* sp., *Oedogonium* sp. and *Ulothrix* sp. as the main taxa contributing the greatest dissimilarity observed between the categories (Table 3). For the comparison between 100% cattle dung and 100% hippo dung, *Cladophora* (24.01%), *Scenedesmus* sp. (17.15%), *Oedogonium* sp. (14.65%), and *Ulothrix* sp. (12.36%) were the main taxa contributing to the % dissimilarity observed with *Cladophora* sp., *Scenedesmus* sp. and *Oedogonium* sp. having a higher mean in the 100% hippo dung treatment while *Ulothrix* sp. had a higher mean in the 100% cattle dung treatment (Table 3). *Cladophora* sp. (21.60%), *Scenedesmus* sp. (16.68%), *Oedogonium* sp. (15.57%), and *Ulothrix* sp. (12.06%) were the main taxa contributing to the observed dissimilarity between the 80% cattle dung and 100% hippo dung. Again *Cladophora* sp., *Scenedesmus* sp., and *Oedogonium* sp. had higher mean in the 100% hippo dung treatment while *Ulothrix* sp. had a higher mean in the 80% cattle dung treatment (Table 3). Still, *Cladophora* sp. (21.36%), *Scenedesmus* sp. (17.39%), *Oedogonium* sp. (16.95%), and *Ulothrix* sp. (11.34%) were the main taxa contributing to the observed dissimilarity between the 60% cattle dung and 100% hippo dung. Similarly, *Cladophora* sp., *Scenedesmus* sp., and *Oedogonium* sp. had a higher mean in the 100% hippo dung treatment while *Ulothrix* sp. had a higher mean in the 60% cattle dung treatment (Table 3).

For the comparison between 40% cattle dung and 100% hippo dung, *Cladophora* sp. (21.31%), *Scenedesmus* sp. (17.99%), *Oedogonium* sp. (17.62%) and *Ulothrix* sp. (11.76%) were the main taxa contributing to the % dissimilarity observed between the 40% cattle dung and 100% hippo dung treatments. *Cladophora* sp. and *Scenedesmus* sp.

had a higher mean in the 100% hippo dung treatment while *Oedogonium sp.* and *Ulothrix sp.* had a higher mean in the 40% cattle dung treatment (Table 3).

Table 3: Abundance-based SIMPER contributors to % dissimilarity in the composition of Algae between H0 vs. H100, H20 vs. H100, H40 vs. H100, H60 vs. H100, and between H80 vs. H100.

H0 vs. H100				
Taxon	Mean		Contrib. %	Cumulative %
Cladophora	2	258	24.01	24.01
Scenedesmus	1	467	17.15	41.17
Oedogonium	666	770	14.65	55.82
Ulothrix	943	405	12.36	68.18
Pediastrum	300	82.2	4.68	72.85
Closterium	234	50.2	3.45	76.3
Microcystis	239	118	3.32	79.62
Spirulina	22.4	134	2.8	82.41
Cocconeis	132	2	2.46	84.88
Oscillatoria	151	44.4	2.02	86.9
H20 vs. H100				
	Mean		Contrib. %	Cumulative %
Cladophora	1	258	21.6	21.6
Scenedesmus	1	467	16.68	38.28
Oedogonium	745	770	15.57	53.85
Ulothrix	764	405	12.06	65.91
Pediastrum	345	82.2	5.43	71.34
Microcystis	265	118	4.02	75.36
Closterium	210	50.2	3.27	78.63
Spirulina	40.8	134	2.84	81.47
Cocconeis	113	2	2.42	83.89
Oscillatoria	154	44.4	2.27	86.16
H40 vs. H100				
	Mean		Contrib. %	Cumulative %
Cladophora	1	258	21.36	21.36
Scenedesmus	1	467	17.39	38.75
Oedogonium	756	770	16.95	55.69
Ulothrix	706	405	11.34	67.03
Pediastrum	329	82.2	5.92	72.95
Closterium	183	50.2	3.37	76.32
Spirulina	47.2	134	2.96	79.28
Microcystis	192	118	2.95	82.24
Pleurococcus	121	57	2.32	84.56
Zygea	123	23.8	2.2	86.76

Table 3: (continued) Abundance-based SIMPER contributors to % dissimilarity in the composition of Algae between H0 vs. H100, H20 vs. H100, H40 vs. H100, H60 vs. H100, and between H80 vs. H100.

	H60 vs. H100		
	Mean		Contrib. %
Cladophora	1	258	21.31
Scenedesmus	1	467	17.99
Oedogonium	784	770	17.62
Ulothrix	686	405	11.76
Pediastrum	281	82.2	5.46
Spirulina	91.2	134	3.11
Closterium	162	50.2	3.1
Microcystis	155	118	2.66
Pleurococcus	132	57	2.57
Zygea	105	23.8	1.97
	H80 vs. H100		
	Mean		Contrib. %
Oedogonium	10	770	21.58
Scenedesmus	8	467	17.07
Cladophora	772	258	15.3
Ulothrix	622	405	12.95
Pediastrum	273	82.2	5.5
Spirulina	168	134	4.08
Microcystis	190	118	3.25
Pleurococcus	124	57	2.74
Closterium	116	50.2	2.45
Coelastrum	99	50.8	2.09

Oedogonium sp. (21.58%), *Scenedesmus* sp. (17.07%), *Cladophora* sp. (15.30%), and *Ulothrix* sp. (12.95%) were the main taxa contributing to the greatest dissimilarity between the 20% cattle dung and 100% hippo dung. *Oedogonium* sp. And *Scenedesmus* sp. had a higher mean in the 100% hippo dung treatment while *Cladophora* sp. and *Ulothrix* sp. had a higher mean in the 20% cattle dung treatment (Table 3).

4.3.3 Algae diversity

A total of 22 species were identified in all treatments except the treatment with 100% cattle dung where 21 species of algae were identified (Table 4). Treatment with 100% hippo dung had a low number of individuals (39,989) while the H0 treatment had the highest number of individuals (93,845) (Table 3). All the indices showed narrow ranges (Table 4). Diversity increased as the proportion of cattle dung increased (Table 4) but dropped with the replacement of hippo dung with cattle dung (Table 3). Shannon diversity index was higher (2.38) in the treatment while the least diversity was recorded in the H0 treatment (2.20) (Table 4). Similarly, the Simpson index ($1/D_s$) had the same trends with higher values in the H80 treatment (0.87) and least in the H0 treatment (0.84) (Table 4).

Pielou's evenness index displayed the same trends as Shannon diversity with a higher value (0.49) in the H80 treatment and the least value in the H0 treatment (0.43) (Table 4). In contrast, Fisher's alpha diversity showed a different trend with a higher value (2.25) in the H100 treatment but still with the least value in the H0 treatment (1.95) (Table 4). Dominance followed the opposite trend of diversity and evenness indices by having the highest values (0.16) at the H0 treatment with the least value being recorded the at H80 treatment (0.13) (Table 4).

Table 4: highest values (0.16) at the H0 treatment with the least value being recorded the at H80 treatment (0.13).

Indices	H100	H80	H60	H40	H20	H0
Taxa_S	22	22	22	22	22	21
Individuals	39989	70156	75796	77380	85999	93845
Dominance_D	0.15	0.13	0.14	0.14	0.14	0.16
Simpson_1-D	0.85	0.87	0.86	0.86	0.86	0.84
Shannon_H	2.26	2.38	2.30	2.30	2.32	2.20
Evenness_e^H/S	0.44	0.49	0.45	0.45	0.46	0.43
Margalef	1.98	1.88	1.87	1.87	1.85	1.75
Equitability_J	0.73	0.77	0.74	0.74	0.75	0.72
Fisher_alpha	2.25	2.11	2.10	2.09	2.07	1.95

4.3.4 Relationship between algae composition, primary production, and water quality

Canonical correspondence analysis (CCA) displayed spatial patterns in algal composition with water quality (Figure 10) and measures of primary production and algal biomass accumulation (e.g., AFDM, Chl-*a*) with water quality (Figure 10). In terms of the relationship between algal composition and Physico-chemical water variables, the first CCA axis (CCA Axis 1) accounted for the greatest variance of 58.3%, while the second CCA Axis (CCA Axis 2) accounted for 18.0%. The two CCA ordinations explained 76.3% of the total association (Figure 10). The CCA ordination for the numerical abundance of algae showed that the nutrients were associated with treatments that had higher levels of cattle dung (60% - 100%) (Figure 10). *Lyngbya* sp., *Oscillatoria* sp., *Anabaena* sp., *Achnanthes* sp., *Cladophora* sp., and *Cocconeis* sp. were associated with higher levels of cattle dung treatments with their levels of the nutrients (Ammonia, SRP, Nitrite, Nitrate, TP, TSS, POM, DOC, and DIN) (Figure 11).

In terms of the relationship between primary production and physico-chemical water variables, the first CCA axis (CCA Axis 1) accounted for the greatest variance of 67.6%, while the second CCA Axis (CCA Axis 2) accounted for 15.5%. The two CCA ordinations explained 83.1% of the total association (Figure 11). Similarly, for the CCA ordination, all the nutrients (SRP, ammonia, nitrates, DIN, TSS, POM, and DOC) were significant at higher cattle dung treatments except TP and nitrites which were significant at lower cattle dung treatments (20% and 40% cattle dung) associated with the Diatoms (Figure 11).

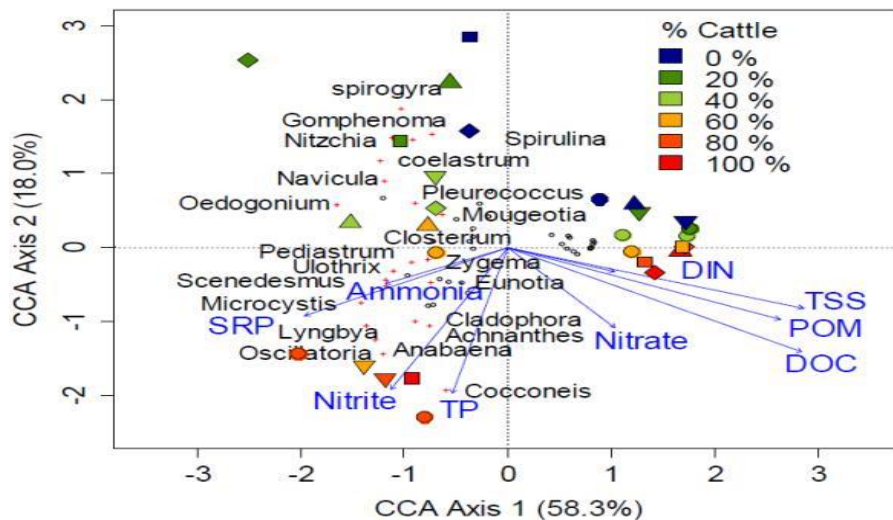


Figure 9: Canonical correspondence analysis (CCA) triplot of algal taxa about Physico-chemical water variables in experimental mesocosms.

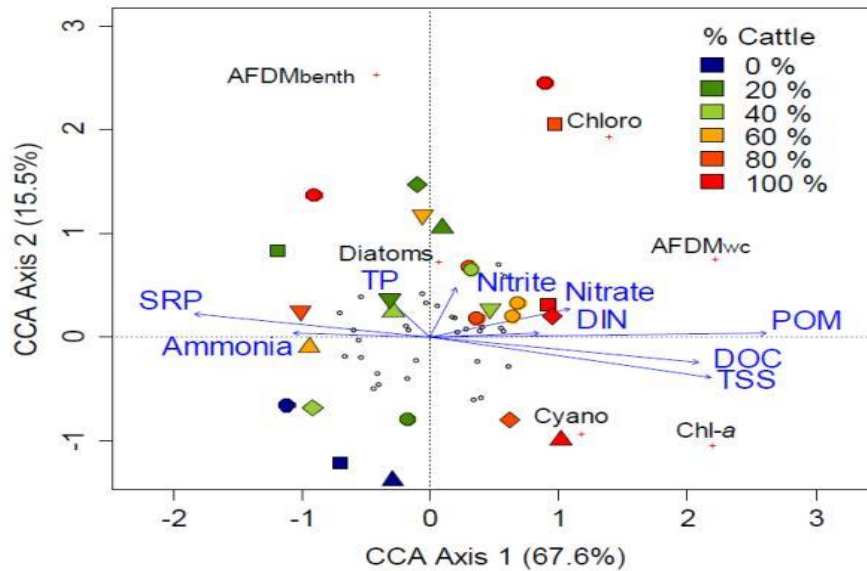


Figure 10: Canonical correspondence analysis (CCA) triplot of measures of primary production about Physico-chemical water variables in the experimental mesocosms.

4.4: Correlation of flume-scale ecosystem metabolism at different proportions of hippo and cattle dung in the mesocosms

Dung treatment had a significant effect on GPP, GPP: ER, and NEP. These parameters showed a linear increase across dung treatments from a low proportion of cattle dung (high proportion of hippo dung) to a high proportion of cattle dung (low proportion of hippo dung) (Figure 12). Differences among dung treatments had strong influences on the temporal trends in GPP and ER

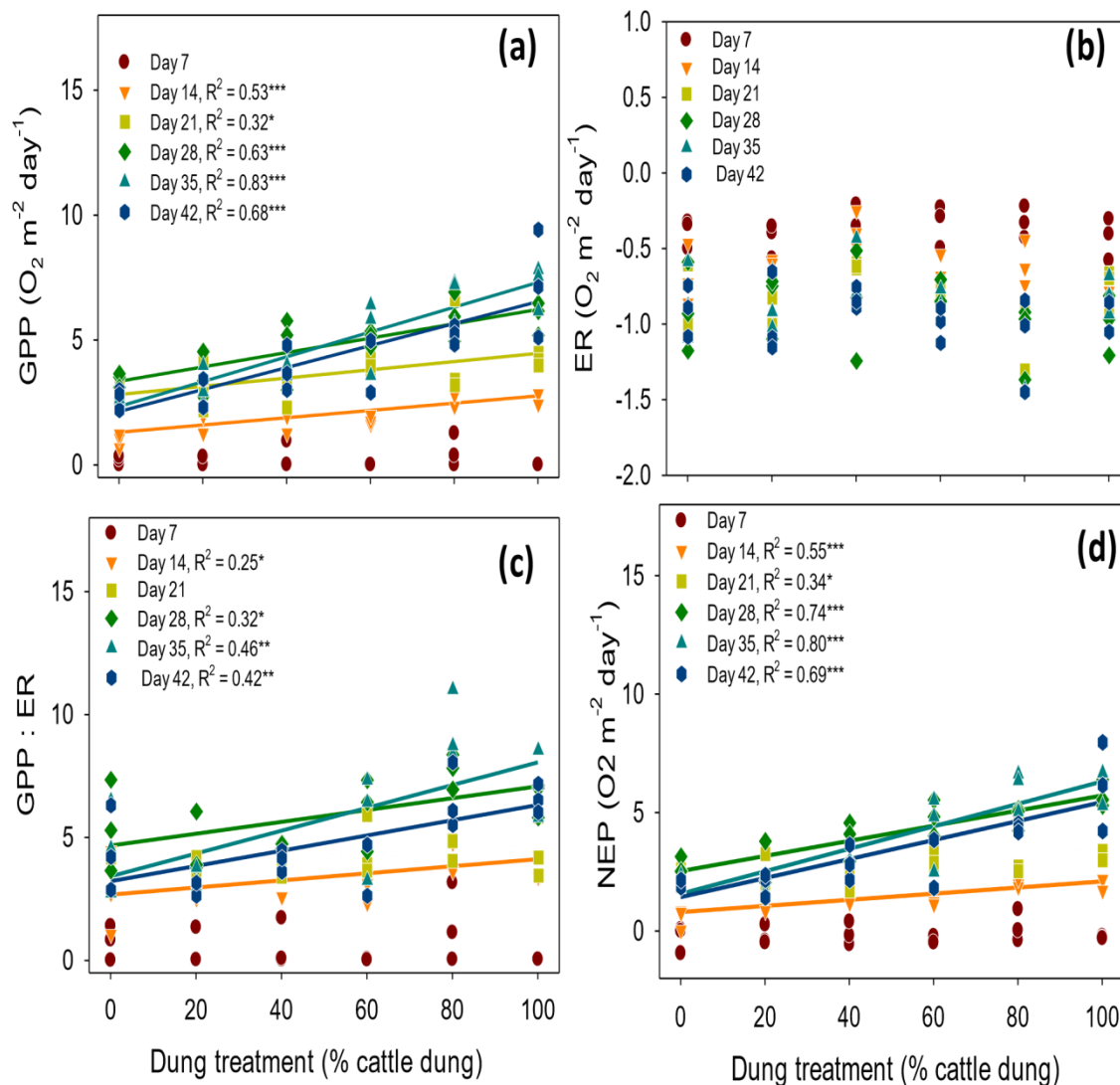


Figure 11: Influence of dung treatment on a) gross primary production (GPP), b) ecosystem respiration (ER) c) GPP: ER and d) net production (NEP). Asterisks and model fits are displayed for significant linear relationships ($\alpha \leq 0.05$). * $P < 0.05$, ** $P < 0.01$, * $P < 0.001$.**

Using sigmoid (Gompertz function) models, the maximum rate (K) of production and respiration was estimated, as the rate of increase in production or respiration, and lag or time (days) to upper asymptote (Figure 13). As the proportion of cattle dung increased,

the experimental streams reached a higher maximum (asymptotic) GPP (Figure 13a) and reached the maximum GPP more quickly (Figure 13b). The relationships between dung treatment and maximum GPP and the rate of increase in GPP were not fully linear (Figure 13a, b).

Replacing a portion of hippo dung with cattle dung (20% and 40% cattle dung) increased the rate of increase in GPP, but not the maximum GPP. For example, GPP reached maximum production faster, but at the same K. The K value increased linearly once cattle dung was added, but from 60% cattle dung, the rates of increase in GPP did not change. However, replacing a bit of hippo dung with cattle dung slightly increased the maximum rate of respiration (Figure 13d) and the rate of increase in respiration (Figure 13e), and reduced the lag (time in days to upper asymptote) (Figure 13f). Similar to GPP, the K value peaked at 60% cattle dung, and captures the interacting effects (mixture effect) of increased respiration rate when the high hippo dung is mixed with the nutrient (P and N) rich cattle dung. Hippo dung treatment (0% cattle dung) differed from cattle dung treatment (100% cattle dung) by supporting a lower maximum rate of respiration, an increased rate of respiration and spending a longer time (lag) to reach the upper asymptote.

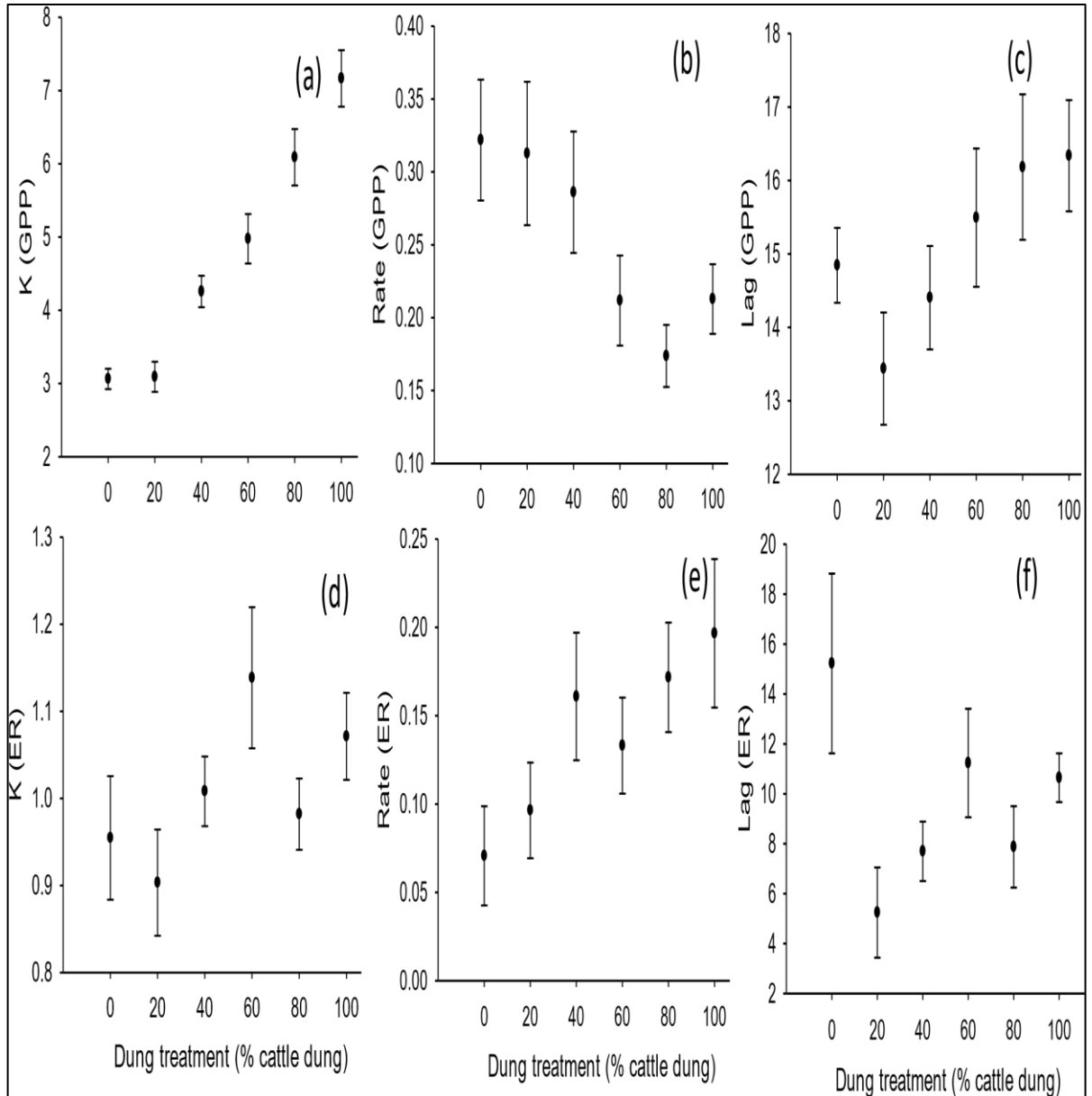


Figure 12: Parameter estimates (mean \pm SD) for sigmoid (Gompertz function) models fitted to gross primary production (a, b, c) and ecosystem respiration (d, e, f). Upper asymptote/ maximum rate of production or respiration (a, d), rate of increase in production or respiration (b, e), and lag or time (days) to upper asymptote (c, f).

4.5. Influence of dung treatment on ecosystem productivity

Primary productivity showed significant differences between dung treatments (PERMANOVA $F = 5.43$, $df = 5$, $p < 0.001$) and time (weeks) (PERMANOVA $F = 11.48$, $df = 5$, $p < 0.001$). However, there were no interactions between dung treatment and time (PERMANOVA $F = 1.02$, $df = 30$, $p = 0.408$) (Figure 12). The principal component 1 (PC 1) of the PCAs explained 42.9% of the total variation, while principal component 2 (PC 2) explained 26.5% of the total association (Figure 12). Primary productivity as measured by water column AFDM (AFDw), Chl-*a*, and algal biomass measured by the biomass of cyanobacteria (Cyanophyceae - Cyano), and green algae (Chlorophyceae - Chloro) increased with increasing levels of cattle dung levels (80% and 100%, low proportion of hippo dung) while Diatoms were significantly higher at low cattle dung levels (20%, high proportion of hippo dung) (Figure 14).

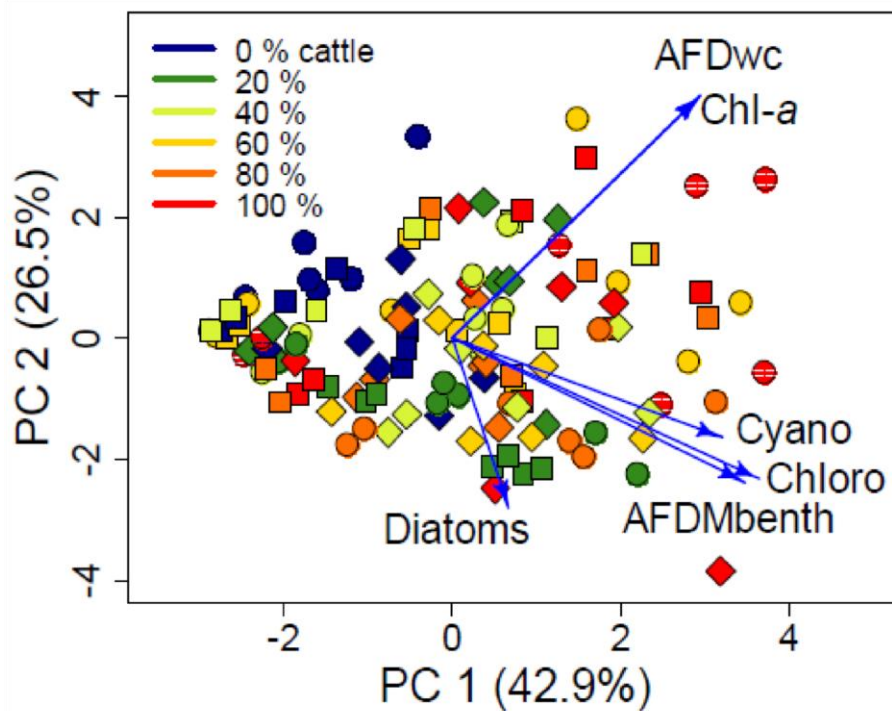


Figure 13: PCA biplot for variables capturing primary productivity or biomass accumulation by primary producers (groups of Chl-*a*, algae, and AFDM) and dung treatment in experimental mesocosms. AFDMbenth = ash-free dry mass of the benthos/ periphyton, AFDwc= ash-free dry mass of the water column, Cyano = Cyanophyceae, Chloro = Chlorophyceae, Chl-*a*= Chlorophyll a

CHAPTER FIVE

DISCUSSION

The findings of the current study demonstrate that substituting hippo dung with cattle dung leads to distinct effects on aquatic ecosystems. Cattle dung exhibits smaller particle sizes and greater concentrations of essential nutrients such as nitrogen (N) and phosphorus (P) per unit mass. These disparities between cattle dung and hippo dung contribute to increased algal biomass, enhanced algal composition and diversity, and elevated ecosystem metabolism at the flume scale in response to cattle dung, in comparison to hippo dung.

5.1 Characteristics of cattle and hippo dung

The experimental mesocosms in this study utilized dung from two large mammalian herbivores: cattle and hippopotamus (hippo). These dung samples exhibited distinct characteristics. Cattle dung had significantly smaller particle sizes, measuring 0.4 mm, while hippo dung had larger particle sizes, measuring 17.8 mm. Furthermore, the C: N: P ratio was also different between the two types of dung. Cattle dung had a lower C: N: P ratio of 155.2:5.1:1.0, whereas hippo dung had a higher C: N: P ratio of 261.4:7.6:1.0. The variation in particle sizes can be attributed to the digestive systems of the respective animals. Ruminants like cattle and sheep possess more efficient digestive systems compared to non-ruminants such as hippos and horses. This difference in digestion leads to the production of smaller faecal particle sizes in ruminants (Thomas and Campling, 1977; Fritz *et al.*, 2009).

When exposed to moisture, the smaller particles of cattle dung readily dissolved in water. On the other hand, the larger particles of hippo dung mostly sank to the bottom, likely due to their size and density. This disparity in behaviour upon wetting further contributes to the contrasting responses of the two dung types in aquatic ecosystems. Generally, these distinct characteristics of cattle dung and hippo dung play a crucial role in shaping the responses of aquatic ecosystems (Fritz et al., 2009). The smaller particle sizes and lower C: N: P ratio of cattle dung make it more easily available and soluble in water. This availability of nutrients promotes algal growth and productivity, leading to higher algal biomass. In contrast, the larger particle sizes and higher C: N: P ratio of hippo dung make it less soluble and accessible in water, potentially limiting its impact on algal growth.

5.2 Nutrient concentrations at different proportions of hippo and cattle dung

The study observed that the release of nutrients, including soluble reactive phosphorus (SRP), ammonium, nitrite, and nitrate, increased with the proportion of cattle dung in the experimental mesocosms. This suggests that cattle dung contributes to a higher release of nutrients into the aquatic ecosystem compared to hippo dung. Furthermore, the analysis revealed a significant effect of time on nutrient concentrations, indicating different rates of nutrient release and uptake throughout the experiment.

Within the initial 14 days of the experiment, there was a substantial reduction of more than 90% in SRP concentrations across all treatments. Similarly, the concentration of ammonia declined by over 50% during the same period. These rapid declines in nutrient concentrations indicate the dynamic nature of nutrient release and uptake in the experimental system. The smaller particle sizes of cattle dung, compared to hippo dung,

appear to facilitate greater decomposition by microbial communities. This increased decomposition leads to a higher release of nutrients in the cattle dung treatments (Mathuriau and Chauvet, 2002). The higher quality of cattle dung, as indicated by its lower C: N: P ratio, allows for easier assimilation of nutrients at shorter time scales. In contrast, non-ruminants like hippos have longer mean retention times, enhancing nutrient extraction from ingested feed. This results in a relatively higher C-to-nutrient ratio in hippo dung, reflecting its lower quality.

The higher nutrient levels observed in cattle dung can also be attributed to the dietary differences between ruminants and non-ruminants. Ruminants, such as cattle, have a broader diet that includes a wider variety of plant species. These plants contain a diverse array of metabolites and chemicals, resulting in a richer nutrient composition in the dung (Noirard *et al.*, 2004). Additionally, cattle often consume supplements and fodder crops with higher protein content (N) compared to hippos, which primarily rely on grass as their food source.

5.3 Algal biomass, composition and diversity at different proportions of hippo and cattle dung

5.3.1 Algal biomass

The findings of the study demonstrate the significant effects of dung treatment on various parameters, including Chl-a (chlorophyll-a), TSS (total suspended solids), and AFDM (ash-free dry mass). There was a linear increase in Chl-a, TSS, and AFDM across dung treatments, ranging from a low proportion of cattle dung (H100) to a high proportion of cattle dung (H0). This suggests that the composition of dung has a direct influence on

these parameters. Furthermore, the concentrations of Chl-a, AFDM, and TSS increased by over 100% over time, indicating the significant influence of time on these parameters.

Cattle dung stimulated higher primary production in the water column, resulting in increased Chl-a, TSS, and AFDM compared to hippo dung. This enhanced primary production can be attributed to the higher concentrations of limiting nutrients such as nitrogen (N) and phosphorus (P) present in cattle dung. Additionally, the smaller particle size of cattle dung allows it to readily dissolve in water, remaining in the water column and contributing to higher TSS values. The increased TSS can also be attributed to the higher phytoplankton biomass in the water column, as indicated by the concentration of Chl-a.

These findings are consistent with a comparative study conducted by Subalusky *et al.* (2017), which also observed that nutrient-rich cattle dung stimulates higher primary production compared to hippo dung. In our current study, we found that the larger particles of hippo dung settled at the bottom of the experimental streams, leading to clearer water. These findings align with the observations made by Subalusky *et al.* (2017), who noted that the large particle size of hippo dung tends to sink to the bottom of pools and streambeds, accumulating during periods of low flows. It has been demonstrated that this accumulation of organic matter can decrease benthic production (Dawson *et al.*, 2016) and have negative consequences such as hypoxia, fish kills, and a significant reduction in both fish and macroinvertebrate species diversity (Masese *et al.*, 2018; Stears *et al.*, 2018).

In contrast, cattle dung tends to dissolve and become distributed by river discharge over a wider area, potentially leading to widespread effects such as algal growth and eutrophication in rivers, lakes, or other receiving water bodies (Iteba *et al.*, 2021). The contrasting behaviour of cattle dung and hippo dung highlights the importance of dung composition and particle size in influencing ecosystem dynamics and the overall health of aquatic environments.

5.3.2 Algal composition

Three classes of algae (Chlorophyta, Bacillariophyta and Cyanophyta) were identified in the study; the most abundant class was the Chlorophyta while Bacillariophyta (diatoms) were the least across the treatments in all the weeks. These results are contrary to the findings of Mbao *et al.* (2013) in the Mara River, where they found Bacillariophytes as the dominant and most abundant class of algae. The high abundance of Chlorophyta in the current study can be attributed to the fact that some members of Chlorophyta are invasive species and thus easily colonize new habitats (Capps *et al.*, 2012). *Cladophora glomerata* was reported to bloom in Lake Erie in the 1960s because of phosphate pollution (Higgins *et al.*, 2008). In the current study, *Cladophora sp.* exhibited clear patterns by increasing with an increase in the cattle dung levels associated with high levels of limiting nutrients.

The spatial variation in the composition of algae at the varying dung levels of cattle and hippos can be attributed to the variation in nutrient levels among these treatments. Water quality variables such as temperature, pH, dissolved oxygen, electrical conductivity, discharge, light availability and nutrient concentrations are considered essential for the growth and development of algae (Hill and Knight, 1988). Nutrient and light availability

(which are influenced by dung input into streams) have been documented to limit benthic algae growth in small streams (Hill and Fanta, 2008).

Treatment with 100% hippo dung had the least number of algae individuals while the treatment with 100% cattle dung had the highest number of individuals. The higher numerical abundance of the algae in the cattle treatment can be attributed to the higher nutrient levels from the cattle dung as compared to hippo dung. Several studies have observed that algal production increases relatively predictably with the availability of growth-limiting nutrients (Hill and Knight, 1988; Naiman *et al.*, 2009; Finlay *et al.*, 2013). *Microcystis sp.*, *Oscillatoria sp.*, *Achnanthes sp.*, *Cocconeis sp.*, *Scenedesmus sp.* and *Cladophora sp.* Species exhibited clear patterns by increasing with an increase in the cattle dung levels (from H100 to H0), while *Oedogonium sp.* exhibited an opposite trend by decreasing with an increase in cattle dung levels across all the weeks. The increase in *Cladophora sp.*, and *Microcystis sp.*, can be attributed to the fact that they are tolerant and are mid- to late-successional species in freshwaters where it is grazer resistant (Dodds and Gudder, 1992). The commonly distributed and abundant *Scenedesmus* and *Cladophora* species at the cattle treatments are indicators of organic pollution and nutrient enrichment (Nakano *et al.*, 1999).

The low algae abundance in the hippo dung treatments can be attributed to the fact that although hippo dung has been reported to fertilize aquatic ecosystems increasing primary and secondary production (Grey and Harper, 2002; Mosepele *et al.*, 2009), other studies have shown that large particles in hippo dung can have detrimental effects on benthic production, especially during the dry season when they settle at the bottom of hippo pools and downstream sections of rivers (Dawson *et al.*, 2016; Dutton *et al.*, 2018).

Similar studies with cattle dung have shown that the weakly digested hippo dung with high C: N: P ratios support heterotrophic microbial activity, while cattle dung with lower stoichiometric ratios can increase primary production faster (Subalusky *et al.*, 2017). Because light is one of the key determinants controlling the production and composition of periphyton or algae in aquatic ecosystems, it is more likely that cattle dung more strongly stimulated the autotrophic component (algae) of periphyton hence the higher abundance of algae recorded.

An increase in time had a significant effect on the algae taxa and by the 6th week only tolerant genera (*Microcystis sp.*, *Oscillatoria sp.*, *Achnanthes sp.*, *Cocconeis sp.*, *Scenedesmus sp.*, *Ulothrix sp.* and *Cladophora sp.*) were dominating the composition. Earlier studies have indicated that the net growth rates in algae can be limited by one or a combination of factors, including nutrients, herbivory, light availability, flow and wave action, and limitations can depend on species, habitat types and region (Naiman *et al.*, 2009; Finlay *et al.*, 2013). Therefore, the reduction in the composition of algal genera with time in this study can be attributed to the high nutrient concentrations and turbidity levels as this inhibited algae development due to nutrient inhibition and light attenuation by suspended particles (higher TSS levels) thus only the tolerant taxa could survive. Time had a significant ($p < 0.05$) effect on both nutrients and TSS as they both increased with an increase in time in weeks.

5.3.3 Algae diversity

The findings of the study demonstrate the influence of dung composition on the diversity and abundance of algae species. The results indicate that the replacement of hippo dung with cattle dung had a profound impact on the overall number of algae species. This

suggests that the specific composition of dung treatments may play a role in shaping algal community composition.

When considering the abundance of individuals, it became evident that cattle dung created a more favourable environment for algal growth. The treatment with 100% hippo dung had a lower number of individuals, while the treatment with the highest proportion of cattle dung (H0) had the highest population density. This suggests that cattle dung provides nutrients or other factors that enhance algal growth and support larger algal populations compared to hippo dung.

Examining the diversity indices provided further insights into the patterns observed. The Shannon-Weiner diversity index, which accounts for both species richness and evenness, was found to be higher (2.38) in the treatment with a relatively high proportion (H80) of cattle dung. This suggests that the presence of a high proportion of cattle dung promoted higher diversity and a more balanced distribution of individuals among species. On the other hand, the use of only cattle dung (H0) resulted in a reduced diversity, potentially due to limitations in nutrient availability or other factors specific to cattle dung.

However, the results regarding dominance deviated from the patterns observed in diversity and evenness indices. The treatment with 100% cattle dung (H0) displayed the highest dominance value, indicating a higher proportion of individuals belonging to a few dominant species. In contrast, the treatment with a relatively high proportion of hippo dung (H80) had the lowest dominance value, suggesting a more equitable distribution of individuals among species.

These findings highlight the intricate relationship between dung composition, algal species diversity, and evenness. The presence of a low concentration of cattle dung and a higher concentration of hippo dung appears to promote higher diversity and more balanced species distribution. The contrasting dominance patterns suggest that the specific composition of dung treatments may influence the relative abundance of dominant algal species.

5.4 Flume-scale ecosystem metabolism at different proportions of hippo and cattle dung

There were notable dung treatment effects on GPP and NEP. Despite GPP generally increasing with increasing proportions of cattle dung, the relationships between maximum production (K) and rate of increase in production and dung treatment were nonlinear (Figure 10a, b). Replacing a bit of hippo dung with cattle dung increased the rate of increase in GPP. The lack of increase in GPP above 60% cattle dung treatment (Figure 10a, b) can be attributed to limitations imposed by the controlled environment (the mesocosms).

The high GPP in cattle dung can be attributed to the fact that light is one of the key determinants controlling the production and composition of periphyton or algae in aquatic ecosystems (Griffiths *et al.*, 2013), it is likely that cattle dung is more strongly stimulated the autotrophic component (algae) of periphyton while hippos stimulated the heterotrophic component (bacteria/fungi), which led to higher GPP per unit biomass of periphyton among cattle dung treatments. The lower GPP rates in hippo dung treated might have been caused by the dung settling to the bottom thus reducing benthic primary

production which displayed a delayed response in GPP. A study by Dutton *et al.* 2018 while working on the Mara River also observed a delay in GPP rates and attributed it to the settling of the hippo dung to the bottom of the stream.

However, it is notable that some proportion of cattle dung could support similar rates of GPP and NEP as a pure cattle dung treatment. Similar mixed effects and interactions have been obtained in decomposition experiments of litter mixtures, whereby 'sharing' of resource facilitates caused the degradation of one litter by the presence of another (Gartner and Cardon, 2004), leading to higher decomposition rates in litter mixtures of different species compared to individual leaf species, in this case, the pure hippo dung treatment.

There were no dung treatment effects on ER, which was intriguing given the significant response of GPP to dung treatment, and suggests different drivers for GPP and ER in the model experiment. ER is particularly sensitive to temperature variation, more so than GPP and given that temperature did not differ among the study treatments, despite wide diurnal variations (mean daily range 14 °C - 26 °C), a lack of differences in ER implies that temperature, and not nutrient concentrations, was the major driver of the ER. There was also a lack of coupling between GPP and ER, which explains the increasing concentration of microbial biomass in cattle dung-dominated treatments over time. Thus, there is a likelihood that increased ER from heterotrophs in the hippo dung treatment was offset by the increased autotrophic respiration in the cattle treatments.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

In this study, we have demonstrated the contrasting effects of large mammalian herbivores, namely cattle and hippos, on aquatic ecosystem function. The differences in stoichiometry (C: N:P ratio) of major elements and faecal particle sizes between cattle dung and hippo dung were identified as key drivers of nutrient decomposition and release rates, which subsequently influenced ecosystem processes in distinct ways. Cattle dung demonstrated higher rates of nutrient release, suggesting its potential as a more efficient nutrient source in the lotic ecosystems.

The varying nutrient release rates and qualities of dung demonstrated notable impacts on the composition and diversity of algae within the study. Specifically, the research highlighted the substantial role of dung quality in shaping the algal community. Notably, cattle dung, characterized by its high-quality nutrient content, fostered higher rates of nutrient release. Consequently, the dominance of green algae was observed in the mesocosms with high levels of cattle dung, underscoring the ecological implications of different dung types on algal dynamics.

Moreover, the research demonstrated that cattle dung input facilitated the transfer of higher amounts of limiting nutrients, major ions, and dissolved organic carbon to the mesocosm ecosystems compared to hippo dung. Consequently, mesocosms with cattle dung input exhibited higher levels of gross primary production and microbial biomass,

indicating the significant role of cattle dung in driving ecosystem-level metabolic processes.

Overall, our results provide valuable insights into the ecological dynamics of aquatic ecosystems, emphasizing the importance of considering the effects of large mammalian herbivores and the implications of their dung on ecosystem processes. Such knowledge contributes to informed conservation and management strategies, particularly in African Savannas where the interactions between terrestrial and aquatic ecosystems are intricate and vulnerable to human-induced changes.

6.2 Recommendations

- 1. Long-Term Monitoring:** Establish long-term monitoring programs in natural streams to track changes in algal biomass, composition, and diversity over time. This will provide valuable data for understanding the effects of dung treatment and other factors on stream health. Long-term monitoring can also help detect any emerging issues or trends, allowing for early intervention and adaptive management strategies.
- 2. Further Research on natural streams:** Conduct additional research in natural streams to validate and expand upon the findings of the experimental study. Investigate the effects of dung treatment under different environmental conditions and stream types to capture the full range of potential responses. Furthermore, explore the interactions between algal communities and other components of stream ecosystems, such as macroinvertebrates and fish, to gain a more comprehensive understanding of the ecological consequences of dung inputs.

3. Further research on other potential sources of nutrients: Our study focused specifically on the effects of cattle and hippo dung on algal dynamics in streams. However, there are other potential sources of nutrients and organic matter inputs to rivers, such as those from goats, sheep, donkeys, and wildlife. It is recommended to conduct further research to quantify the contributions of these additional sources to nutrient loading and algal dynamics. This research should encompass a catchment-scale study, incorporating factors like run-off and agriculture, to gain a comprehensive understanding of nutrient inputs and their effects on aquatic ecosystem processes.

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APPENDICES

Appendix I: List of plates





Plate 1 (a-h): (a) Experimental set up, (b) Fitting stream substrates (c) Collection of water quality parameters, (d) Sample collection, (e) Nutrients analysis (f) Benthotorch, (g) Hippos and (h) Cattle.

(Source: Author, 2016)

Appendix II: Similarity Report

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