ORGANIC MATTER DYNAMICS IN NYLSVLEY WETLAND: DISTRIBUTION AND PROCESSES

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An MSc thesis submitted to the Department of Ecology and Resource Management, School of Environmental Sciences, University of Venda

In fulfillment of the requirements for MSc Environmental Science degree

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February 2021

ABSTRACT

Wetlands play a crucial role in nutrient and biogeochemical cycles and are among the most productive of ecosystems. Physical and biological processes are involved in the distribution and protection of organic matter in wetlands sediments. Productivity among wetlands therefore varies depending on the type of the wetland, climatic condition and vegetation communities. The application of biogeochemical techniques involving stable isotopes of carbon and nitrogen has been successfully used to determine sediment sources in aquatic ecosystems such as wetlands. The study investigated spatial and temporal changes in sources of organic matter in sediments and examined spatial and seasonal changes over two seasons within Nylsvley wetland. Samples of sediments were collected at each wetland site per season in order to determine amount of organic matter and to identify contributors to sediment organic matter. These samples were air dried and impurities removed, then put into the oven and dried at 60 °C to constant weight for 72 hours. Data was analyzed using a three–way ANOVA with Tukey's HSD analysis ed to assess the differences in sediment organic matter (%) among wetland zones, sites and seasons. A Stable Isotope Analysis in R (SIAR) model was used to identify organic matter sources contributing to sediments in wetland zones per season. Results showed distribution was uneven throughout the wetlands; the seasonal zone had the highest sediment organic matter (27%) in the cool-dry season and the permanent zone had the highest (25%) sediment organic matter in the hot-wet season and the temporary zone had the lowest. Several studies have indicated that $\partial^{13}C$ and $\partial^{15}N$ can be used as indicators for environmental change. Carbon and Nitrogen stable isotope may be an alternative means to detect early environmental changes in aquatic ecosystems including wetlands.

The seasonal zone had a high nutrient concentration (Calcium) in both hot-wet and cool-dry season ranging between 1000-1800 mg kg^{-1}. Autochthonous plants were the main source of organic matter in sediments although allochthonous plants also contributed to the sediment organic matter content, autochthonous inputs was still dominant. In sediments the $\partial^{13}C$ mean values were higher than the $\partial^{15}N$ values in all sites and wetland zones. This study showed that sediment organic matter in wetlands is mainly derived from autochthonous sources. This study’s findings help to better understand the distribution of organic matter in wetland ecosystems. This is particularly important as wetlands play a vital role globally by their contribution to provisioning, regulating and cultural services. As such, future studies should continue to assess seasonal variations and identifying other sources of organic matter within the context of distribution and processes in wetland ecosystems not only in South Africa but globally.

Keywords: wetlands, stable isotopes, allochthonous and autochthonous, organic matter

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DECLARATION

I declare that "ORGANIC MATTER DYNAMICS IN NYLSVLEI WETLAND: DISTRIBUTION AND PROCESSES" is my own work. All other sources, used or quoted, have been indicated and acknowledged by means of complete references. This thesis has not been submitted for a degree at another university.

Signature:

Miss Lufuno Makhuvha

21 February 2021

ACKNOWLEDGEMENTS

Firstly, I want to express my deepest gratitude to Dr. Tatenda Dalu my supervisor for everything he has done to make this thesis possible. His guidance and supervision allowed me to develop this thesis in a manner that supported my research interest. I am also grateful to Prof. Ryan-J. Wasserman (Rhodes University) for co-supervising me, being part of this project, and his guidance and dedication to this thesis. Secondly, I want to express my very profound gratitude to my parents Thivhafuni Makhuvha and Stanley M. Makhuvha, my siblings Maele, Rofhiwa and Livhuwani Makhuvha.

I greatly acknowledge the financial support of the University of Venda Niche Grant (SES/18/ERM/10) and NRF Thuthuka Grant (117700) to Dr Tatenda Dalu and Prof Ryan J Wasserman for funding my research studies. I would also like to thank Limpopo Economic Development, Environment and Tourism (LEDET) for granting me permission to collect data at the Nylsvley Nature Reserve. Any opinions, findings, conclusions or recommendations expressed in this material are those of the authors, and the NRF does not accept any liability in this regard.

CHAPTER 1: INTRODUCTION

Background

Wetlands are widely considered to be net sinks of atmospheric carbon dioxide and offer a wide range of other ecosystem services to humankind (Mitra et al. 2005; Mitsch and Gosselink, 2007). Two of these services are climate regulation and nutrient cycling. Large amounts of organic matter are introduced into their soil through various processes, including their pulsing hydrology and autochthonous input (Odum et al. 1995; Cronk and Fennessy 2001; Mitsch and Gosselink 2007). This soil organic matter accumulates in different decomposition stages for long periods of time. When the organic matter is decomposed under anaerobic conditions it produces methane, a carbon-based gas that can be released back to the atmosphere or dissolved into carbon dioxide. When organic matter decomposes aerobically (in the presence of oxygen), living organisms which use oxygen, feed upon the organic matter (Zedler, 2000).

Many wetlands are overlooked since they are neither wholly terrestrial nor aquatic, and not determined solely by either groundwater or surface water (Zedler and Kercher, 2005). Kotze (2010) indicated that wetlands occur in different settings ranging from heads of streams, floodplains along rivers, the wide flat coastal plains or even in endorheic settings, disconnected from rivers/ lakes. In these aquatic ecosystems organic matter sources reach the benthic system through sedimentation events during wet phases, or are directly deposited during dry phases (Graf, 1992). The benthic environment in wetlands is influenced by a variety of organic matter sources including bacteria, benthic microalgae, phytoplankton, terrestrial organic matter and detritus (Canuel et al., 1997; Hu et al., 2006). The relative importance of the diverse food sources within the wetland changes spatially and seasonally are a result of changes in freshwater discharge, together with local differences in habitat characteristics including vegetation type and tidal amplitude (Deegan and Garritt, 1997; Riera and Richard, 1997; Bouillon et al., 2004; Olin et al., 2013). The variability of wetland ecosystems arising from these temporal and spatial fluctuations in physical, chemical and biological factors creates challenges for assessing organic matter dynamics (McLusky and Elliott, 2004).

Physical and biological processes are involved in the distribution and protection of organic matter in wetland sediments including primary productivity, oxygen exposure time, patterns of freshwater discharge and wind (Hedges and Keil, 1995; Arzayus and Canuel, 2005; Goñi et al., 2009; Palomo and Canuel, 2010). In particular, freshwater flow and algal blooms events in the overlying surface water are important factors affecting the type and amount of organic matter that is deposited (Carrie et al., 1998; Zimmerman and Canuel, 2001; Gogou and Stephanou, 2004). In turn, the quality and quantity of organic matter delivery to surface sediments influences the biomass and biodiversity of the benthic fauna (Rowe et al., 1991; Herman et al., 1999). In this context, given the spatial and temporal dynamics of wetland ecosystems, it is important to include spatial and temporal approaches to assess the organic matter composition in sediments. Doing so may enhance our understanding of the functioning of energetic ecosystems such as wetlands.

Wetlands are found in all climatic zones extending from tropics to the tundra (except Antarctica), occupying about five percent of the earth’s land area (Fraser and Keddy, 2005). Wetlands are active natural ecosystems characterized by saturated or standing water surroundings at least part of the year. Water levels change seasonally in most wetlands instead of being stable, this accounts for making wetlands highly productive environments (Richardson et al., 2001). Productivity among wetlands varies depending on the type of the wetland, climatic condition and vegetation communities. Along with productivity, decomposition is a complex process that involves both aerobic and anaerobic processes. The rate of decomposition is a function of climate (temperature and moisture enhanced microbial activity) and quality (composition) of organic matter entering the system (Schlesinger, 1997). However, wetland characteristics lead to the accumulation of organic matter in the soil and sediment serving as carbon (C) sinks and making them one of the most effective ecosystems for storing soil carbon (Schlensinger, 1997).

Stable isotopes have become a popular method for understanding aquatic ecosystems including wetlands because they help scientists in understanding sources links and process information in aquatic environments (Heckey and Hesslein, 1995). These analyses can also be used to a certain degree in terrestrial systems. Certain isotopes can signify distinct primary producers forming the bases of food web and trophic level positioning. Analysis is usually done using a mass spectrometer, detecting small differences between gaseous elements. The three major isotopes used in aquatic ecosystems analysis are $\partial^{13}C$, $\partial^{15}N$ and $\partial^{34}S$ (Minagawa and Wada, 1984). In addition to mass spectrometry and emission spectrometry, techniques such as nuclear magnetic resonance, gas chromatography, mass spectrometry and automated nitrogen and carbon analysis mass spectrometry (ANCA-MS) are playing an increasing role in understanding wetlands (Peterson and Fry, 1987).

In ecological studies, stable isotope analysis (SIA) has been successfully applied to reconstruct diet and migration patterns of organisms, track flows of elemental matter and identify food web structures within and across ecosystems (Fry, 2006). It relies on the existence of chemical elements with two or more stable isotopes that are unevenly distributed among compounds or compartments. Isotopes are analyzed in the gaseous form using an instrument (isotope ratio mass spectrometer, IRMS) which precisely measures isotope ratios of the compound of interest. Laboratory standards and international reference materials (with predetermined isotope values) are needed for ensuring utmost accuracy of isotope analyses. Stable isotopes of carbon and nitrogen in organic matter offers an alternative means to detect early signs of environmental changes in aquatic ecosystems (Michener et al., 2016; Hicks et al., 2012).

Nylsvley Nature Reserve was designated a Ramsar site in July 1998. Ramsar is an international convention that recognizes and urges protection of globally important wetlands. The Nylsvley Nature reserve is one of about twenty sites registered in South Africa and one of 2065+ in the world. The Ramsar defines wetlands as “areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tides does not exceed six meters” (Ramsar Convention, 1971; Article 1.1). Wetlands are scattered over a wide range of biomes, from the tundra to the tropics, and are main components in global biogeochemical cycles. For example, fifteen percent of global terrestrial carbon flux from rivers to coastal environments is estimated to be resulting from wetlands (Hedges et al., 1997). However, the dynamics of dissolved organic matter in wetland influenced coastal rivers is more complex than the simple mixing of upriver-derived dissolved organic matter with saline water, since additional organic matter which is dissolved can be supplied from tidally flooded coastal wetlands, riparian soil or plant residues, exudation from phytoplankton from the flood plains, emergent macrophytes, and sea-grass, microbial mats, and groundwater inputs (Bertilsson and Jones, Jr. 2003; Dittmar et al., 2012; Hedges 1992; Maie et al., 2006; Tzortziou et al., 2008). The Nylsvley wetland is identified as an inland wetland and classified as a seasonal floodplain wetland comprising a seasonal river associated with grassland floodplain. The Nyl River arises outside the reserve and flows through the area and passes through a large dissipative floodplain section that facilitates the development of an extensive wetland during the rainy season when the river arises.

The quality, quantity and nature of organic matter delivery to surface sediments influences the biomass and biodiversity of the benthic fauna, with implications for the entire aquatic food web (Rowe et al., 1991; Herman et al., 1999). Understanding how organic processes and delivery function matter is, therefore, important for management of secondary productivity dynamics, particularly in systems such as the NRR where potential drivers of organic dynamics arise outside of protected areas. It is also important to consider temporal and spatial dynamics approaches to assessing the organic matter composition in sediments, particularly in large wetland systems where edge effects, variable rainfall dynamics and habitat heterogeneity likely facilitate complicated functioning. Given the importance of wetlands, these natural habitats need to be effectively managed, with organic matter dynamic forming an important, yet often overlooked component of system-level understandings (Clymo et al. 1998).

Aim

This study aims to evaluate sediments and diverse sources of organic matter to examine spatial and seasonal changes in the sediment organic matter composition over two seasons: hot-wet and cool-dry season within Nylsvley wetland. The research objectives were to:

- Assess seasonal variation in sediment organic matter across wetland sites
- Identify sources of and contributors to organic matter across different wetland zones using stable isotopes

Hypotheses

- Since organic matter can be influenced by the amount of rainfall and length of the dry season, organic matter dynamics change seasonally; therefore there will be a significant difference in organic matter between the two seasons
- The permanent zone always has water, and the seasonal and temporary zone sometimes has little water depending on the rate of rainfall that has occurred, hence the amount of organic matter is expected be high in the permanent zone during the wet season and dry season since there is always water and they dry out last.
- The temporary zone is mostly dry as there is little to no water making it a less productive zone compared to the permanent zone, hence low stable isotopes signatures will be found in the temporary zone since contributions of sediment organic matter will be less as they are also influenced by temperature and rate of freshwater flow

Justification and significance of the study

As the time period during which a soil area remains waterlogged (hydroperiod) increases, the soil properties of a wetland develop and may increase the storage of organic matter/carbon. (Shaffer and Ernst, 1999). Soil organic matter is one of the most telling indicators of wetland maturity, it is also considered as the primary energy source being derived from litter fall, root turnover, and microbial organisms. (Bruland and Richardson, 2006).This study contributed to knowledge on organic matter dynamics that are found within the Nylsvley Nature Reserve, specifically aiming to determine the sources of organic matter (allochthonous *vs* autochthonous) across low water (cool-dry season) and high water (hot-wet season) periods. Many studies about organic matter within wetlands have been conducted internationally such as the seasonally inundated wetlands in the southwest of United States. Studies such as characterizing organic matter inputs to sediments of small, intermittent prairie: a molecular marker and stable isotope approach conducted in Manhattan USA (Pisani et al., 2016) A study about spatial distribution characteristics of organic matter and total nitrogen of marsh soils in river marginal wetlands was conducted in Erbaifangzi floodplain in the Xianghai Nature reserve in China (Bai et al., 2004). These limesink wetlands support numerous rare plants and they are also an important breeding habitat for rare or threatened amphibians (Sutter and Kral, 1994). In South Africa, only a few of such studies have been conducted in the Nylsvley Nature Reserve (e.g. Greenfield et al., 2007; Dalu et al., 2020). Nylsvley Wetland is a protected area and is limited to human disturbance; this is one of the reasons for choosing this site.

CHAPTER 2: LITERATURE REVIEW

Introduction

Wetlands play a crucial role in nutrient and biogeochemical cycles and are amongst the most productive ecosystems (Sahrawat, 2006; Wu and Liu, 2017). The South African Water Act defines wetlands as: “land which is transitional between terrestrial and aquatic systems where the water table is usually at or near the surface, or the land is periodically covered with shallow water and which in normal circumstances supports or would support vegetation typically adapted to life in saturated soil” (Envass, 2019). Wetlands are known to be important sources of dissolved organic matter (DOM) for rivers and coastal environments (Yamashita et al., 2008). However, the environmental dynamics of dissolved organic matter within wetlands have not been well documented on large spatial scales. (Jaffe et al., 2008). This chapter aims to explore literature on organic matter sources and dynamics in wetlands as well as the nature of wetlands in South Africa.

Although wetlands occupy about 6 % of the earth surface, they contain a large proportion of the carbon stored in terrestrial soils worldwide, at approximately 15 % (Stern et al., 2007; Wang et al., 2016a). Wetland soils are comprised largely of organic matter (Clymo et al., 1998). High rates of organic matter input coupled with slow mineralization rates have made wetland soils important carbon (C) sinks for atmospheric carbon dioxide (CO_2). The low oxygen amounts, acidic peat, and nutrient shortage are the major factors influencing the soil organic matter rate of mineralization (decomposition of the chemical compounds in organic matter) to be slow in wetlands (Bridgham et al., 2003; Yavitt et al., 2004).

There has been a continuous increment in threats to freshwater systems in South Africa and around the globe due to a range of varying anthropogenic factors. South Africa is a water scarce country with inconsistent temporal and spatial rainfall distribution patterns (Baker, 2018). Climate change poses continuous pressure on the country, as water availability may become a development-limiting factor in South Africa. In Southern Africa, for example, water resources are increasingly being polluted due to rapid urbanization, poor catchment management, agriculture and mining practices (Dalu and Froneman, 2016; Dalu et al., 2016). Although wetlands make up such a small proportion of South Africa's surface area, they provide an array of valuable benefits to people. Many of these wetlands comprise natural infrastructure that assists in managing the country's limited water resources (Knoesen et al., 2009). The Nylsvley Ramsar Wetland is an important aquatic system in the dry Limpopo Province (Van der walt, 1997). The wetland is subjected to seasonal flooding of varying intensities. During the dry spells, the wetland dries completely with water available only on the permanent pools in the channel (Haskins and Kruger, 1998). The water depth is influenced by the type of flooding that occurs within the system but rarely exceeds one meter.

Organic matter is found in all soils, with soil organic matter being made up of microbe, plant and animal remains at different stages of decomposition. Litter and roots of plants are the prime sources of soil organic matter in both surface and near–surface horizons (Schoch et al., 2004; Botanical Society of America, 2008; Upton et al., 2011). Living organisms <2 mm are regarded as part of soil organic matter (Alan, 2018). Soil organisms affect soil structure and soil organic matter (SOM) dynamics through processes such as burrowing and root channelling (Puget et al., 1995). The ratio between inputs and outputs of organic matter results in the accumulation of organic matter in wetland soils (Cole et al., 2001; Kayranli et al., 2010). Biogeochemical processes in wetlands are complicated because of the frequent exchange of energy and materials. The organic matter sources that are frequently studied in streams are dead wood and plant litter such as senescent leaves (Karl, 2015). Sediment organic matter is important to soil, however, too much of soil organic matter is problematic (Alan, 2018). When organic matter gets eroded from soils into surface waters, water parameters get affected negatively (Alan, 2018). Since organic matter is typically comprised of phosphorus as one of its building blocks, it makes the organic matter vulnerable to rapid mineralization causing eutrophication (algal blooms) which reduces oxygen concentrations. Furthermore, high organic matter concentrations increase the cost of water purification (Alan, 2018).

The significance of wetlands on various aspects such as biodiversity richness, livelihoods depending on wetlands and its contribution on the purification of water sources is well recognized. The role of these wetlands as carbon sinks locally and globally, however, is still not recognized and no scientific record of carbon deposited stock has been maintained (Bridgham et al., 2006). Floodplain lakes and associated wetlands are generally sinks for carbon, nitrogen, phosphorus, and other elements as they accumulate organic matter (De camps and Decamps 1989; Lugo et al., 1990). In spite of this, the exchange of these elements among landscape boundary components contributes to the formation of a highly dynamic environment, which is the major difficulty in identifying sources and digenetic processes in these systems (Neiff, 1990).

Analysis of naturally occurring stable isotopes have emerged as powerful techniques for addressing research and management related questions in ecology. Over the last decade stable isotopes have been increasingly used in environmental studies. In the laboratory, very small amounts of plants, animal and sediment organic matter samples are oven dried then ground to a fine powder. Stable isotopes are alternative forms of elements with different molecular weights that are found naturally and do not decay radioactively. Stable isotope analysis of elements such as carbon, nitrogen and sulphur is used in ecology to trace food webs (Clynick and Chapman, 2002); they offer an effective natural tracer to investigate the trophic linkages between organisms and wetland resources. Stable isotope ratios are useful as they can be used to trace movement and biological assimilation of nutrient and organic matter in wetlands (Fry and Sherr, 1984; Melville and Connolly, 2003).

A case study in Lesotho showed that wetlands constituted an important aspect of the livelihoods of the rural people. Most of the wetlands across Lesotho are either used for livestock watering, grazing and agriculture, as well as a source of drinking water (Lanas and Turpie, 2009). Hence, one of the major constraints to the sustainable use of wetlands in Lesotho and Africa in general is the lack of information on the diverse benefits that can be obtained from wetlands if properly managed, therefore the need for early and timely identification of systems of ecosystems disturbance is critical (Bino et al., 2015). Stable isotopes of carbon and nitrogen in organic matter offer an alternative means to detect early signs of environmental changes in wetland ecosystems (Michener and Lajtha, 2008). The ratios of ∂^{13}C and ∂^{15}N have been used to provide insight into the sources, sinks and cycling of carbon and nitrogen in aquatic ecosystems as this biota interact with its physical and chemical environments (Gladyshev, 2009).

Sources of soil organic matter

Organic matter is the main characteristic of wetland soils, whether as parent material or as important components of wetland soils. Many physical, biological and chemical properties in wetland soils are influenced by organic matter content and quality. It has a high pH and water holding capacity and provides essential elements for wetland plant growth. The saturation of soils with water generally slows decomposition, which often causes wetlands to accumulate organic matter in the substrate. The peat found in some wetlands is the evidence of this accumulation of organic rich soils in wetlands (Glaser et al., 1981). Not all organic matter that enters or is formed by photosynthesis in wetlands, however, remains within the wetland boundary. Many wetlands export organic carbon to streams and estuaries at a rate greatly higher than that of terrestrial ecosystems (Mulholland and Kuenzler, 1979). In this way, wetlands can make large contributions to the support of organisms that consume non-living organic matter. Soil organic matter in natural wetlands is mainly from the decomposition of animal and plant residues (Bai et al., 2004). Studies (e.g. Aiken and Cotsaris, 1995; Reckhow et al., 2004) have highlighted that allochthonous (i.e. terrestrial plants, soils) and autochthonous (i.e. algae, macrophytes) sources are the major contributors to organic matter (OM) into surface soil. The bulk OM pool is a combination of living organisms such as phytoplankton and detrital matter which includes faecal, plants, humification products, and complex geopolymers (DeLeeuwand and Largeau, 1993; Hedges et al., 2001; Carlson and Hansel, 2014).

Organic soils

Organic soils, known also as peatlands are characterized by high organic matter (more than 12% total carbon) in the upper first meter (Ramesh Reddy et al., 2008). These soils originate from accumulation of partially decomposed organic matter. Here decomposition processes are slower than primary productivity. More precisely the top layer (surface horizon) is generally well decomposed; while below fibrous decomposed material (peat) accumulates. Organic soils have high water-holding capacity and poor drainage (Reddy et al., 2008).

Factors affecting organic matter distribution and accumulation

Generally, the long–term balance between organic matter input and output controls the spatial distribution of soil organic matter (Wang et al., 2016a; Weishampel et al., 2009). Multiple factors affect the accumulation and distribution of soil organic matter in the wetland system making it a complicated process (Zheng et al., 2018). The amount of organic matter in the soil depends primarily on rainfall, air temperature, and the kinds of plants that have been growing in a soil, management practices, soil temperature, and drainage. Soils that are tilled frequently are usually low in organic matter because tilling decreases residue particle size and increase the amount of air in the soil, increasing the rate of organic matter decomposition (Crouse, 2018).

Temperature

Temperature has been highlighted as the main driving factor in animal and plant decomposition (Bot, 2005). The tropics have a high rate of decomposition in due to high average temperature than in temperate regions. Due to slower mineralisation (decomposition), soils in temperate regions will have high amounts of organic matter (Bot, 2005). Organic matter decomposes faster in warm, humid climates and slower in cool, dry climates. The relatively faster rate of decomposition induced by the continuous warmth in the tropics implies that high equilibrium levels of organic matter are difficult to achieve in tropical agro-ecosystems (ibid.).

Soil moisture and water saturation

Air and water are the basic requirements for soil biological activity (Linn and Doran, 1984). An increase in the mean precipitation results in an increase in OM which results in more residue, and thus, more potential food for soil organisms (Bot, 2005). Water saturation occurs during rainy periods causing poor soil aeration (Bot, 2005). Oxygen is a requirement for most soil organisms, and a decrease in the amount of oxygen in the soil causes the mineralization rate to drop and organisms to become inactive or even die (Bot, 2005). This leads to anaerobic transformation, which causes damage to plant roots due to waste products or conditions favourable to pathogens. Slow decomposition rate and continued production of organic matter can result in large amounts of OM contents in soils with prolonged periods of water saturation (Bot, 2005).

Topography

Organic matter at higher elevation decomposes faster under aerobic conditions, and elevation also indirectly affect the production of SOM through vegetation types, microbial processes, and soil physical and chemical properties (Wang et al., 2016b). Organic matter accumulation occurs at low elevations and this accumulation occurs for two reasons: (i) conditions at the bottom of the hills are wetter than at mid or upper slope positions, and (ii) OM is carried to the bottom of the landscape through erosion and runoff (Bot, 2005). Similarly, SOM quantity is high on south–

facing slope (Southern Hemisphere) when compared to the north–facing slopes because of low temperature (Quideau, 2002).

Vegetation and biomass production

The quantity and the quality of organic matter input determine the rate of soil organic matter accumulation (Bot, 2005). Under tropical conditions, decomposition and the increase in the labile nitrogen pool during the growing season is favored by the applications of materials that are readily degradable with low Carbon and Nitrogen ratios, such as green manure and leguminous cover crops (Bot, 2005). On the other hand, applications of plant materials with both large C and N ratios and lignin contents such as cereal straw and grasses generally favour immobilization of nutrients, accumulation of organic matter and formation of humus, with increased potential for improved soil structure development (Bot, 2005).

Soil texture

Soil OM is proportional to clay content in the soil (Rice, 2002). Clay content increases soil organic matter using two mechanisms: (i) by bonding within surface OM particles thereby delaying the process of decomposition and (ii) by increasing chances of aggregate formation due to the presence of high amounts of clay content in soil. Macro–aggregates physically prevent OM molecules from mineralization caused by microbial attack (Rice, 2002). In areas of the same climate condition, the OM content in fine textured (clay) soils is two to four times that of coarse textured (sandy) soils (Prasad and Power, 1997).

Organic matter dynamics

Organic matter of natural waters is divided into two large groups: allochthonous and autochthonous. Allochthonous organic matter is a mixture of organic matter of humic nature and terrigenous origin, the sources of which are products of incomplete decomposition of plant and animal remains (Skopintsev, 1950). Autochthonous organic matter forms in the water bodies as a result of photosynthesis and the destruction of detritus (dead bacteria, phytoplankton). The main distinctive feature of allochthonous organic matter is the presence of the decomposition and condensation products of lignin molecules (Kulish, 2002). Allochthonous organic matter contains far more chromophores which absorb light in the visible region than autochthonous organic matter (Stopintsev, 1950). Autochthonous carbon (C) is considered relatively labile and easily metabolized, in contrast to allochthonous sources which are considered largely intractable (Wetzel, 1992). Allochthonous C inputs can play an important role in whole ecosystem metabolism, with inputs that can equal or exceed internal primary productivity (Cole et al., 2006).

Formation of organic matter

The ultimate source of organic matter for most soils is through the fixation of carbon dioxide from the atmosphere through photosynthetic reactions by plants (Murphy, 2014). Bioavailability of OM is influenced by molecular size class, chemical composition, and how it is transferred through trophic levels and its fate (Amon and Benner, 1996). In certain soils, algae play a crucial role in SOM dynamics (MacEntee et al., 1972; Hunt et al., 1979). Organic matter accumulation is dependent on a suite of biological, physical and chemical requirements and it creates non–

ideal conditions for microbial decomposition (Macías and Camps–Arbestain, 2010; Dungait et al., 2012).

Decomposition of organic matter

Organic matter decomposition is mostly a naturally occurring biological process (Brussaard, 1994). The rate of decomposition is influenced by three factors: soil organisms, physical environment and organic matter quantity (Brussaard, 1994). During the decomposition process, various products are released: energy, water, plant nutrients, carbon dioxide (CO_2) and resynthesized organic carbon compounds (Bot, 2015). Decomposition of dead material and modified OM that occurs successively leads to humification which is the formation of an even more complex organic matter called humus which affects soil properties (Juma, 1998). While humus decomposes at slow rate, it turns the soil darker; increases soil aggregation and stability (i.e. the ability to attract and retain nutrients) and contributes N, P and other nutrients to the soil (Bot, 2015).

The hydrological regime is the driving force in wetland systems, as it directly changes the wetland physicochemical properties, especially oxygen availability, which controls the ratio of organic matter decomposition (Chen et al., 2011; Iacob et al., 2014; Tranvik et al., 2009). Dissolution process has been demonstrated and highlighted to play an important role in the SOM decomposition (Marschner and Kalbitz, 2003). The average time that SOM remains in the soil from its entry to complete decay, which is known as turn over time, ranges from few days to centuries, with most decomposition occurring shortly after deposition but mostly within less than a decade (Arnone et al., 2000; Kenward and Hall, 2000; Tingey et al., 2000; Blazejewski et al., 2005; Lùtzow et al., 2006; Osman, 2013; Strawn et al., 2015).

CHAPTER 3: STUDY AREA

Background

The study was carried out in Nylsvley Nature Reserve (NNR; S24º39’50.0 E 28º39’54.4) in the upper-reaches of the Mogalekwena River, a tributary of the great Limpopo, and stretches over a distance of about 70 km (Figure 3.1). Nylsvley Nature Reserve forms part of the largest floodplain in South Africa (Noble and Hermens, 1978) and covers a total area of 24250 ha (Higgins and Rogers, 1993). The reserve lies at an altitude of 1080-1155 m above sea-level, and receives an average of 648 mm of rain per annum. Summers are warm to hot rising up to 38-39° C (maximum temperature), while winters are mild to warm with temperatures rarely dropping below zero (Scholes and Walker, 1993). Nylsvley is coldest in July with temperatures dropping to an average of 5.7°C at night. The annual wet season ranges from November to April, the summer months, and about 85% of the annual rainfall occurs during these months. The study was carried out during the cool-dry season from the 12th to 15th June 2019 and during hot-wet season from the 20th to 23rd March 2020.

Topography

The landscape of Nylsvley is gentle sloping consisting of three rocky outcrops within the area; The Stemmerskop outcrop is located in the center of the reserve, the second outcrop Maroelakop located in the eastern corner of reserve and the third outcrop is located in the western corner of the reserve (Macfarlane and Teixeira-Leite, 2012).

Geology and soils

The geology of the Nylsvley region is complex, consisting of a variety of igneous, sedimentary and metamorphic rocks of very different ages. Nylsvley has a variety of geological formations. It is mainly characterized by sedimentary rocks of the Waterberg Group including sandstone, conglomerate, shale and siltstone of the Vaal water formation (Harmse, 1977). The most dominant geology is the Cenozoic alluvium which is found in Subtropical Freshwater Wetlands. The topography is a gently sloping 1:1750 m river gradient (Rogers and Higgins, 1993) with a few rocky outcrops.

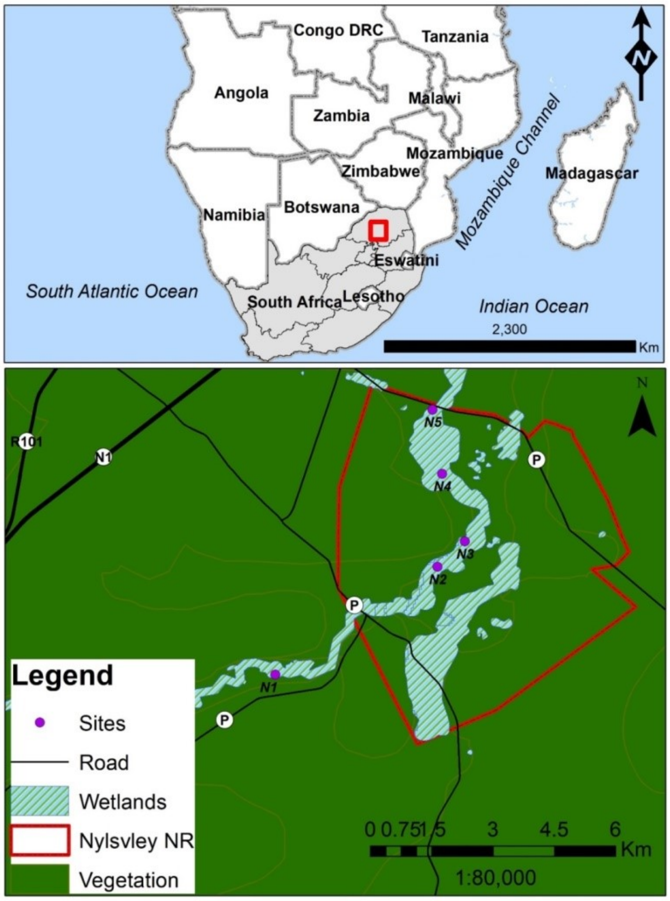

Figure 3.1 Location of the study sites within the Nylsvley Wetland, located in the Limpopo Province.

Nylsvley has a variety of soil forms such as the Springbokvlakte Thornveld soils which are red-yellow pedal and, the vertical soils, with a fluctuating water table. These soils experience swelling and shrinking during the wet and dry periods (Scholes and Walker, 1993). Glenrosa soils are also found in the area, they are shallow and thin. Champagne and Arcadia clayey, waterlogged soils are associated with Subtropical Freshwater Wetlands (Mucina and Rutherford, 2006).

Nylsvley's floodplain

Nylsvley area is naturally exposed to seasonal flooding and fluctuation in water levels. The Nylsvley floodplain Wetland plays an important role in supplying water for wildlife and supporting the biodiversity of the area (Tarboton, 1987). The entire floodplain is 24 250ha and includes hydromorphic grasslands and sodic sites, with the Nyl River serving as the main drainage channel (Higgins et al., 1996). On average, inundation of at least parts of the floodplain occurs during three out of every five years, during the summer season that lasts from October to April (Theron et al., 1992). Flood waters persist occasionally throughout the year until the following wet season. During dry spells the floodplain dries completely and water is available only in permanent pools (refuge areas) in the channel (Tarboton, 1987). The water depth varies according to the type of flooding that occurs but seldom exceeds one meter. The floodplain and catchment represent a diverse and complete ecosystem whose components are dependent on each other to maintain the integrity of the system (Limpopo Economic Development, Environment and Tourism, 2013).

Vegetation

About 800 plant species support the biodiversity that makes Nylsvley famous. Vegetation along the Nylsvley floodplain is differentially distributed along three spatial directions: distance from the channel, elevation above the channel, and distance downstream. This reserve is a mosaic of habitats dominated by *Acacia karroo* (sweet thorn)*, Combretum erythrophyllum* and broad leafed woodland. It also consists of grassveld floodplain plants such as Milkweed, *Panicum shinzii*, Black jack, *Persicaria*, *Phragmites australis, Cyperus fastigiatus* (Bailey, 1990). The reserve comprises Central Bushveld vegetation units of the Savanna Biome and a Freshwater Wetland vegetation unit (alluvium vegetation) of Inland Azonal Vegetation. Central Sandy Bushveld is characterized by sandy plains and catenas supporting tall, deciduous *Terminalia sericea* and *Burkea africana* woodland on deep sandy soils, with *Terminalia sericea* regularly dominant at the lower slopes of sandy catenas (Low and Rebelo, 1996). Alluvium vegetation is a complex and dynamic flora determined by way of the interplay of numerous ecological elements, such as sedimentation-to-erosion rates, sediment load, water chemistry and nutrient load; and the frequency and period of flooding, among others. The flora is made up of an intricate complex of aquatic macrophytes, marginal reed beds and great flooded grasslands, ephemeral herblands and riverine thickets. Dominant tree and shrub species consist of *Acacia erubescens*, *Acacia nilotica* and *Acacia tenuispina*, dominant grasses encompass *Aristida bipartita* and *Bothriochloa insculpta* (Mucina and Rutherford, 2006).

Fauna

The Limpopo Province Nylsvley Nature Reserve (NNR) is managed by Limpopo Economic Development, Environment and Tourism. Limpopo Tourism Agency is responsible for hospitality. Nylsvley provides a suitable habitat to a wide variety of bird, mammal, reptile, fish and insect species. The reserve supports about 1 000 large mammals including breeding herds of the threatened roan and tsessebe. giraffe, zebra, kudu, waterbuck, impala, reed-buck, warthog, porcupine, bush-babies, ostrich, bushpig, leopard and brown hyena all occur on the reserve (Brooke, 1994). It is one of the largest and most important birding areas in South Africa, with a total of 382 bird species recorded within the NNR and 426 bird species recorded in the broader Nyl River floodplain to date. The latter figure accounts for 46% of all bird species found in southern Africa. Nearly 400 species of birds are known to reside or visit occasionally, including 102 water bird species (Tarboton, 1987). Additionally, the species diversity within the NNR constitutes a high level of endemism, with 11 endemic and 18 near-endemic species recorded to date.

A total of 25 different amphibian species have been recorded at NNR, none of which are classified as vulnerable (Tarboton, 1987). Some of the amphibian species found at the reserve are the red toad, bushveld rain frog, banded rubber frog, snoring puddle frog, common platanna, giant bullfrog and the Natal sand frog. There are about twenty-two fish species recorded at the NNR. The importance of fish is often overlooked and their presence is of highest importance for a well-functioning wetland system (Mucina and Rutherford, 2006). This diversity of fish is able to sustain a wide variety of fish-eating birds and mammals and is fundamental to the overall health of the wetland ecosystem. Some of the common species found include sharp tooth catfish, large-scale yellowfish, barb and topminnow species.

CHAPTER 4: ASSESSING VARIATION IN BELOW-GROUND ORGANIC MATTER ALONG THE NYLSVLEY WETLAND, SOUTH AFRICA

Introduction

Soil organic matter has a large number of functions within the biosphere. It is the basis of a favorable soil structure, hydrological properties and biological activity (Stemmer, 1997). It offers sorption sites for plant nutrients and pollutants; it is also an important source of nutrients and acts as a sink for atmospheric carbon dioxide (CO_2). All these functions may be influenced by changes in climate, land use and the overall situation of the environment (Tinker and Ingram, 1994). Organic matter in aquatic systems is either produced internally (autochthonous) or delivered from the terrestrial environment (allochthonous). Terrestrial environment organic matter is increasingly recognized as a strong driver of aquatic productivity. There is strong evidence that significant transformations of both particulate and dissolved terrestrial organic matter fractions occur in wetlands and estuarine waters, as well as along their transport downstream (Coble, 2007; Osburn and Bianchi, 2016). The aim of this chapter was to assess variation in below ground organic matter, how organic matter varies and the SOM percentage within the three wetland zones over the two seasons in Nylsvley Wetland.

Freshwater wetlands are characterized by significant temporal and spatial variations in the pattern and magnitude of water inflows and outflows (Kennedy et al., 2003). These produce distinctive water table fluctuations that reflect hydro-meteorological conditions and changes in the predominant water source (Winter, 1999; Bradley, 2002). Such wetlands can be characterized by a seasonal hydrological cycle in which waters within the wetland will comprise varying quantities of water derived from precipitation, groundwater and surface water sources (Bradley, 1997). Groundwater is likely to be proportionally more important in summer when the water table is depressed by evapotranspiration while in winter the water table recovers as a result of higher precipitation (Bradley, 1997; Zeeb and Hemond, 1998).

Wetlands also vary on a temporal scale based on the climate and season. As such, once a wetland type has been established, it can then be further categorized into either a temporary, seasonal or permanent wetland system depending on the length of time that it remains a feature in the landscape (Department of Environmental Affairs, 2016). Temporary wetlands are saturated for short periods (approximately one to three months per annum) during the rainy season only. Soils are typically characterized by a minimal grey matrix of less than 10 percent of the total soil volume and the occurrence of very few chroma mottles (Mitsch and Gosselink, 2007). Vegetation associated with this type of wetland are predominantly grass species, a mixture of other species that occur in non-wetland areas as well as hydrophytic plants that are largely restricted to wetland areas (Kotze et al., 1996). A seasonal wetland is saturated for most of the growing season (approximately three to six months). Soils are characterized by a grey soil matrix of more than ten percent of the soil volume, a high occurrence of chroma mottles and significant periods of wetness (minimum of three months) (McVicar et al., 1977). Vegetation associated with this type of wetland is predominantly sedges and grasses that are restricted to wetland areas, usually < 1 m tall. In terms of hydrological functions, the seasonal nature of flooding results in aerobic and anaerobic conditions which are more favorable than permanent zones for performing water purification functions (Kotze and Marneweck, 1999). Seasonally, wetlands usually have a lower organic content than permanent wetlands due to frequency of aerobic conditions, which promote the decay of organic matter.

Efficiency of seasonally wet zones with regards to organic matter is therefore lower than in permanent wet zones, but still contributes significantly towards water purification. Lastly, a permanent wetland is saturated all year round. Soils are characterized by a prominent grey matrix, there is few to no high chroma mottles, wet throughout the year and having a sulphuric odour (Kotze et al., 1996). This type of wetland is dominated by highly specialized aquatic plants such as *Phragmites australis* and *Typha capensis* adapted to permanently wet conditions. Of the three identified wetland types, permanent wetlands are the most efficient at water purification, flood reduction and stream flow regulation (Kotze et al., 1996). In river floodplain ecosystems, temporary, seasonal and permanent zones occur based on proximity to the main river channel. These environments offer useful variations for determining differences between temporary, seasonal and permanent wetland zones, which allow for a better understanding of different wetland types. In this study, the below-ground organic matter is assessed across these different zones in the NNR with other regions of the wetland, along the course of the river.

Soil organic matter is fundamental for wetland formation and function. Physical and biological processes are involved in the distribution and preservation of organic matter in wetland sediments including primary productivity, time, patterns of freshwater discharge and winds (Hedges and Keil, 1995). Soil clay contents and wetland plant growth characteristics such as height, density and plant litter greatly affect the spatial distribution characteristics of soil organic matter in floodplain soils. Understanding the distribution of soil organic matter can contribute to knowledge on conservation methods of wetlands (Robinson et al., 2008a). The study explored the distribution of soil nutrient and content organic matter throughout the Nylsvley Wetland. The distribution of nutrients and SOM was investigated in three various zones namely; temporal, seasonal and permanent zone. I hypothesized that (i) the wetland permanent zone would consist of the highest nutrients and SOM concentrations, followed by seasonal and then the temporary zones and; (ii) that upper reach sites would have the lowest nutrient and SOM concentrations as they are narrow than the lower reach sites which have more optimal depositional characteristics. Finally, I hypothesized that (iii) the hot-wet season would produce elevated nutrient and SOM concentrations given the high levels of productivity and allochthonous input associated with this period, including temperatures during the hot-wet season. This also applies to the seasonal zone as there are high levels of productivity due to the intermediary zone, which has a balanced profile.

Materials and methods

Sampling

Sediment samples together with particulate organic matter (POM) macrophytes, microphytobenthos and senescent leaves as sources of organic matter, were collected in winter (cool-dry) and summer (hot-wet). The dominant emergent plant materials were collected by hand and washed using wetland water. Three zones were sampled along the wetland: (1) permanent (2) seasonal and (3) temporary. Surface sediment organic matter (SOM) samples were collected at each site from the upper one cm and frozen until further processing in the laboratory. The growing macrophytes were collected and washed using wetland water. All samples were collected during the day, within the three wetland zones at five sites (Figure 4.1). After collection, all water, sediment and plant samples were stored on ice in a cooler box for transportation to the laboratory for processing after a day or two of collection.

Sediment sampling

The study was designed around the collection of 100 sediment samples across sampling sites, each sampled in two seasons. For each sampling event per site, two transects were used to sample across different zones in the wetland (Figure 4.1). This resulted in four points sampled along the temporary zone (i.e. two each on either side of the permanent zone), four along the seasonal zone (i.e. two each on either side of the permanent zone) and two along the permanent zone per site (i.e. $n = 10$) (Figure 4.1). In the end, a total of only 90 soil samples were collected instead of 100, because of the presence of hippos in site during the hot-wet season, making it impossible to safely sample this site. Soils were carefully retrieved from a 6 cm soil layer using a steel hand auger. The soil samples were placed in labelled polyethylene Zip lock bags. All the soil samples were stored at room temperature and transported to the laboratory within 72 hours for further processing for nutrients. Prior to soil ashing, the soil samples were air dried for 72 hours and then put into the oven and dried at 60 °C for 72 hours.

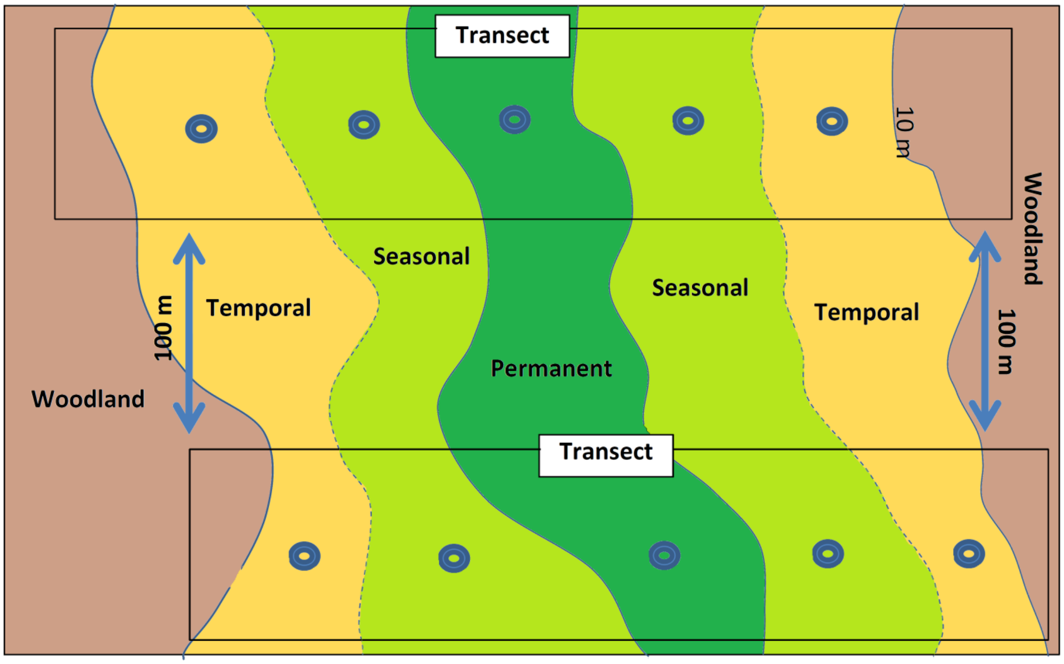

Figure 4.1 Location of the two transects per each sampling site within Nylsvley Wetland. This was conducted at five sites in both the cool-dry June 2019 and the hot-wet March 2020 season, to a total of a 100 samples. Only 90 samples were collected in the end, given that Site 1 in the Hot-wet period could not be sampled.

Nutrient analysis

All dried sediments collected were labeled and sent for analysis at BEMLAB in Cape Town, a South African National Accreditation System (SANAS) certified laboratory for analysis. An inductively coupled plasma- optical emission spectrometer, ICP-OES (Varian, Mulgrave, Australia) was used to determine the nutrient content for cations like K, Ca and Mg. To achieve this, an acid digestion procedure using 1 Nitric acid (HNO_3) to 1 Hydrochloric acid (H_2SO_4) ratio mixture at 80 °C for 30 minutes was carried out. The standard concentrations were then used in the calibration of the instrument and standard blanks. A natural standard of certified reference soil was digested and analyzed in triplicate to estimate the accuracy of the procedure, through

SARM-51 (MINTEK) and (IAEA) recovery tests. The percentage recoveries of the certified values ranged between 87 % and 110 %, respectively. A Bray-2 extract was used for total phosphorus concentrations (Bray and Kurtz, 1945).

Organic matter analysis

In the laboratory, soil organic matter and carbon was measured to estimate the amount of SOM from the dried sediment/soils. This is because it is a reliable and easy method to estimate SOM (Nelson et al, 1996; Baldock et al, 1999). To determine SOM, ~3 g dry mass of soil samples were measured and burnt at 450 °C in a furnace for 16 hours and then weighed again using the loss in ignition (LOI) method and the difference in weight was the organic matter composition (Bruland and Richardson 2004; Cao et al. 2011). Results were presented as percentages (%).

Data analyses

Differences among sites, zones and season were subsequently tested separately using a one-way ANOVA with Tukey post hoc tests. These tests were performed using Statistica version7 (StatSoft, Tulsa, OK).

Results

The potassium (K) concentration was higher in the permanent zone in majority of the sites during the hot-wet season, and it was low in the permanent zone in all sites (N1-N5) during the cool-dry season. The permanent zone had low calcium (Ca) and magnesium (Mg) concentration in all sites; this was during the cool-dry season (Fig 4.2). The permanent, seasonal and temporary zone all had high concentrations of Mg and Ca in all wetland sites in the hot-wet season. The seasonal zone of Mg concentration was the highest in site N2 (793.61 mg kg^{-1}), observed in the cool-dry season. The seasonal zone remained with the highest observed concentrations in all sites, although the permanent zone had recorded increased concentrations in all sites in the hot-wet season. Phosphorus (P) had very low concentration in all wetland zones and sites; and particularly compared to the other three nutrients, phosphorus was very low. The permanent zone had the highest P concentration with 15 mg kg^{-1} (Figure 4.2) and the temporary zone had low P values in all wetland sites with the lowest concentration being 5 mg kg^{-1} observed during the cool-dry season. The phosphorus concentration values increased in all the zones (permanent, seasonal and temporary) in all sites during the hot-wet season. The nutrient concentrations in the permanent zone were higher in the hot-wet season whereas in the cool-dry season the concentrations in permanent zone were lower in all wetland sites.

Based on ANOVA analysis, all other nutrient concentrations across sites were not significantly different with the exception of K (F = 4.99, df = 4, $p = 0.001$). Significant wetland zone difference for P (F = 13.27, df = 4, $p < 0.001$), K (F = 4.82, df = 4, $p = 0.011$), Ca (F = 21.89, df = 4, $p < 0.001$) and Mg (F = 30.28, df = 4, $p < 0.001$) were observed. Significant seasonal differences were observed for K (F = 5.90, df = 4, $p = 0.018$), Ca (F = 27.96, df = 4, $p < 0.001$) and Mg (F = 5.87, df = 4, $p = 0.018$) Using pairwise comparisons, significant ($p < 0.05$) site variations were observed for K, Ca and Mg (Table 4.1) and also significant ($p < 0.05$) wetland zones differences were observed for all nutrients (Table 4.1) with the exception of P (seasonal *vs* temporary, $p = 0.167$) and K (permanent *vs* temporary, $p = 0.423$).

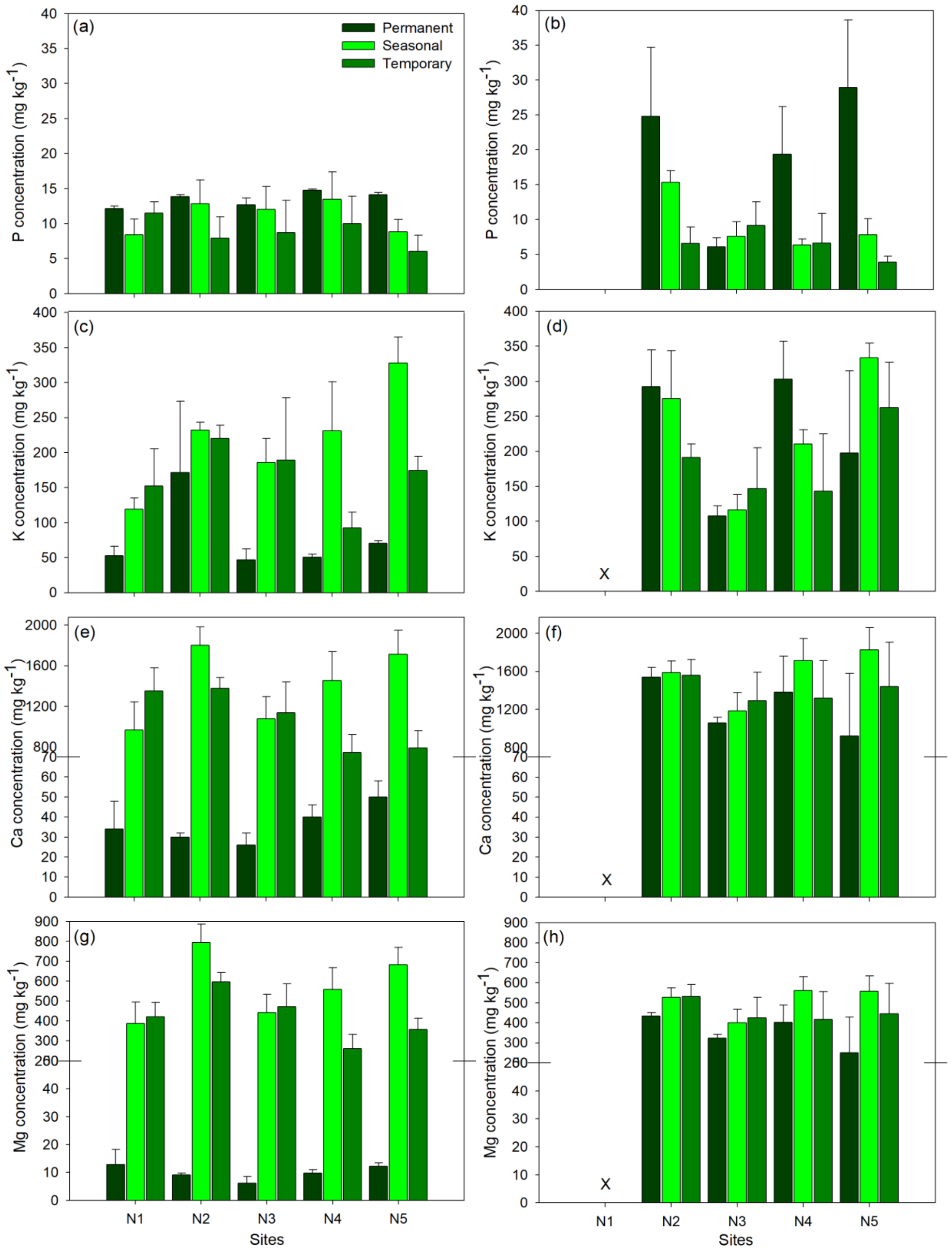

Figure 4.2. Variation in nutrients phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) concentration between two seasons: (**a, c, e, g**) cool-dry, and (**b, d, f, h**) hot-wet seasons in Nylsvley Wetland.

Table 4.2. Tukey's pairwise comparison results of significant ($p < 0.05$) variables of wetland sites and zones

Variable	Pair	*P*
Sites		
K	N1 *vs* N2	0.011
	N2 *vs* N3	0.017
	N3 *vs* N5	0.003
	N5 *vs* N1	0.002
Ca	N1 *vs* N2	0.043
Mg	N1 *vs* N2	0.009
	N2 *vs* N3	0.023
Wetland zones		
P	Permanent *vs* Seasonal	0.002
	Permanent *vs* Temporary	<0.001
K	Permanent *vs* Seasonal	0.004
	Seasonal *vs* Temporary	0.036
Ca	Permanent *vs* Seasonal	<0.001
	Seasonal *vs* Temporary	0.042
	Temporary *vs* Permanent	<0.001
Mg	Permanent *vs* Seasonal	<0.001
	Seasonal *vs* Temporary	0.012
	Temporary *vs* Permanent	<0.001

Sediment organic matter content

During the cool-dry season, the range and mean values of SOM varied throughout three zones of wetland. The mean value was high in the seasonal zone in sites N1 (16.1±5.4 %), N3 (27.1±4.0

%), N4 (20.6±3.5 %) and N5 (18.7±2.7 %) during this season (Fig 4.3). The permanent zone was found to have the lowest SOM amount in site N1 (13.2±2.1 %) in the cool-dry season. During the hot-wet season, the temporary zone had the lowest SOM values in site N4 (16.3±2.9) and N3 (17.8±2.4), high values of sediment organic matter (Fig 4.3) was in site N5 in the seasonal zone with (28.3±2.6).

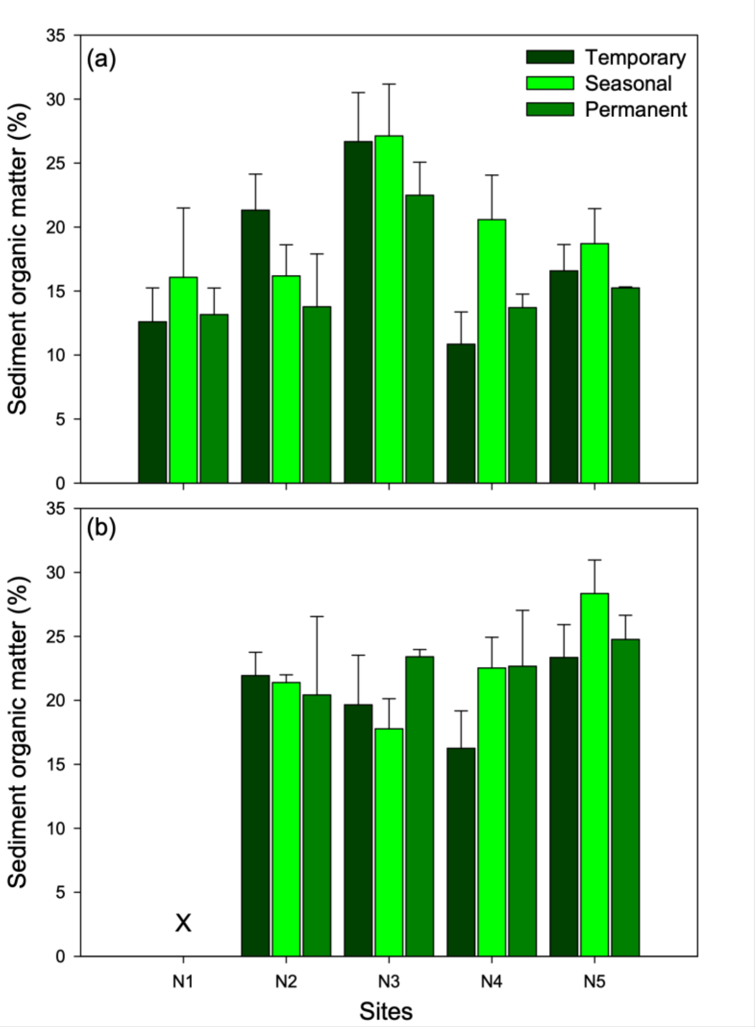

Figure 4.3 Sediment organic matter concentrations (%) in three wetland zones across 5 sites in Nylsvley Wetland for (a) cool-dry and (b) hot-wet seasons.

Discussion

In this study, a quantitative approach was taken to assess the distribution, variation and dynamics of organic matter in Nylsvley wetland. The results indicated that the distribution of organic matter was uneven throughout the wetland. In particular, the wetland zones generally differ in nutrient concentrations as some zones were wet and some were dry with different types of soil and plants found in the wetland in line with the first hypothesis. The only exception to this was resulting from the rainfall during the hot-wet season and the allochthonous input associated with this period, which also affected the nutrient concentrations in different wetland zones to some concentrations being low in the cool-dry season and some high in the hot-wet season. The productivity of organic content was found to be high in the permanent and seasonal zone.

During the hot-wet season, allochthonous inputs likely increased, site N5 had the most sediment organic matter content of above 22 % in the permanent zone (Figure 4.3) Sediment organic matter in the permanent zones of the sites are higher because of the occurrence of rainfall resulting in full wetlands . During the cool-dry season, high amount of nutrients were found in the seasonal zone with 28% in site N3. Site N1 had the lowest sediment organic matter below 15% in all wetland zones. Site N3 had the highest sediment organic matter during the cool-dry season compared to all other sites. The temporary zone had similar, but slightly lower SOM to the seasonal zone (Figure 4.3). The nutrients were different across wetland zones and sites, for example, in some of the temporary zones nutrients were very low and in some high; a result of the type of nutrient concentration and seasonal changes. Significant differences were observed across the five sites, whilst similarities were observed across the three wetland zones (Table 4.1).

Soil nutrient content increased in some vegetation zones while it decreased in others after heavy rainfall. During the hot-wet season, Ca and Mg concentrations were high in all the wetland zones (Figure 4.2). This was due to the occurrence of rainfall. Only P concentration was high in the permanent zone, whilst very low in the seasonal and temporary zones. Phosphorus (P) has a strong attraction to fine clay and it could be expected that during rainfall more sediment is deposited, leading to high concentrations. In the hot-wet season all nutrients (K, P, Ca and Mg) increased with rainfall. High K, Ca, Mg and P in the hot-wet season could be attributed to increased organic matter decomposition rates. During the cool-dry season, the P concentration in the permanent zone was higher than in the seasonal and temporary zones. This was because there was no water/rainfall in this period resulting in drying of the soil and sediments and subsequently less organic matter in the wetland. The permanent zone always has water, even in small amounts at certain times. The amount of P concentration in the permanent zone ranged between 10 and 15 mg kg^{-1} (Figure 4.2). The seasonal zone had the highest concentration in three nutrients i.e. K, Ca and Mg during the cool-dry season, whilst the permanent zone was the lowest in these three nutrients. This was because the area was dry including vegetation and soil, and having less allochthonous inputs in the wetland. The seasonal zone does not dry out completely like the temporary zone; wetlands always have water, though minimal. The soil in the seasonal zone is slightly dry due to wind during the cool-dry season in addition to little to no precipitation in the area. The permanent zone is the one that always has water even in small amounts, resulting in less organic matter in wetlands. During this season birds fly away and some animals migrate to comfortable habitats for survival. These conditions lead to a small output of bacteria, fungi and algae. Magnesium (Mg) concentrations were very low in the permanent zone in all five sites and

Calcium (Ca) concentrations were also low in the permanent (Figure 4.2). This implies that nutrient concentrations in the permanent zone are generally low in the cool-dry season.

Sediment organic matter in natural wetlands is largely a product of the decomposition of animal and plant residues, including mineralization and erosion, which are influenced by many biological and non-biological conditions (Reddy and Patrick, 1975). Abundant biomass, anaerobic conditions, and slow water velocity in wetlands constrain decomposition rates of organic matter in soil and accelerate the accumulation of organic matter (Hu et al., 2015; Wang et al., 2016a). This explains the low amount of SOM in the permanent zone in the cool-dry season (Figure 4.3), which has similar characteristics. The amount of SOM in the seasonal zone is high in both seasons (dry and wet) in sites N3 and N5 (27 % and 28 %, respectively) because it is between temporal and permanent zones. The seasonal zone has moderate conditions (temperature, moisture and anaerobic condition) being an intermediary zone and this quickens the decomposition rate of organic materials. The variation of SOM might be explained by the observation that the capacities for SOM holding in wetland soils and the amount of plant litter inputs were different within the five sites and in three wetland zones, which could be greatly influenced by the distance to riverbed, water table and soil moisture (Vought et al., 1994; Williams et al., 1999; Zhang, 1998).

The SOM concentration is influenced by multiple factors. According to Wang et al (2016), organic matter at higher elevation decomposes much faster under aerobic conditions than that at low elevation. Moreover, elevation also indirectly affects the production of SOM through vegetation types, microbial processes, and soil physical and chemical properties (Wang et al.

2016b). This further explains the variations along slope topography in the distribution of organic matter across the five wetland sites. The permanent zone, which was at lower elevation compared to others, recorded low SOM. Higher elevation in Nylsvley Wetland may cause lager spatial variation of landscape, which may lead to gradient change of SOM among elevation intervals. Slope gradient may fundamentally affect the migration of organic matter by controlling water velocity (Haiou et al., 2016).

Plant litter inputs could have been the major source of organic matter of the soil surface, due to harvesting of *Phragmites australis.* The litter could have been carried to the areas far from riverbed by floodwater, becoming difficult to decompose under inundation or drought conditions (Van Oorschot et al., 2000). The vegetation composition varied greatly along the Nylsvley floodplain. Differences in vegetation composition were observed across five sites. Sites which had great abundance of vegetation were (sites N2, N3, N5) composed of high organic matter. Furthermore, *Phragmites australis* and *Triarrhena* stubbles take longer to return to soil, having more complex structures (lignin and cellulose), which are often harder to degrade than those of *Carex* and *Phalaris* (Wang et al. 2016a). This also plays a role in the variation of soil organic matter in floodplain.

The concentration of dissolved organic matter in soil is higher in summer than in winter and early spring. The higher the temperature, the more favourable the conditions for the development of microorganisms; which increases the microbiological activity of soils. Kalbitz et al. (2000) claims that changes in the dissolved organic matter content in soils are also due to the amount and dynamics of precipitation. Soils in wetter and cooler climates generally contain more organic matter than soils in drier climates. The two seasons (hot-wet and cool-dry) have different climates, air temperatures and water level. During the cool-dry season, wetlands are almost dry and some are completely dry since there is no rain, whilst in the hot-wet season wetlands are full of water because of rainfall events. Consequently, there is less organic matter during the dry season due to less water being available in the wetlands, resulting in fewer animals/living organisms feeding on the wetland.

The distribution across the wetland zones was uneven. The permanent and seasonal zones had the most organic matter. This was because of the freshwater flow rates and the rainfall in the zones. Plants and animals survive well in this zone whether during the cool-dry or hot-wet season, it is the most favorable zone (Brinson et al., 1981). This is because plants grow better with precipitation and a long growing season, adding more organic residues. Very wet soils such as wetlands contain little air and biological activity. As a result of this, decomposition is slow, and the percentage of organic matter is high.

CHAPTER 5: ASSESSING CONTRIBUTIONS OF AUTOCHTHONOUS AND ALLOCHOTHNOUS INPUTS TO SEDIMENT ORGANIC DYNAMICS IN A RAMSAR DECLARED WETLAND USING STABLE ISOTOPE ANALYSIS

Introduction

Wetland ecosystems depend on inputs of both allochthonous and autochthonous organic matter for their energy. Sources of organic material in wetlands also depend on the dimensions of the body of water and the types of terrestrial communities that deposit organic material into it (Tranvik, 1988). In aquatic communities, autochthonous input is provided by the photosynthesis of large plants and algae in shallow waters and by microscopic phytoplankton in the photic zone of the entire water body (Zheng et al., 2003). Allochthonous organic matter is formed outside the ecosystem and is imported through precipitation, dry fall out, groundwater and lateral transport (Zheng et al., 2003). The quantity and quality of the organic matter supply and the relative proportion of allochthonous to autochthonous inputs influence food web structure and determine the potential secondary productivity for wetlands (Algesten, et al., 2004). Some of the organic matter that enters a wetland from the watershed (allochthonous) or is produced within the wetland itself (autochthonous), is ultimately deposited on the wetland bottom and becomes combined permanently into the wetland sediments.

Wetlands thus function as sinks for organic matter and associated macro-elements such as carbon (C), phosphorus (P) and nitrogen (N) (McCallister et al., 2004). As a result, wetland sediments contain an archive of past environmental conditions and biogeochemical processes in and around the water body. These sediments can be used to file ecosystem changes through time (Smeltzer and Swain, 1985). Organic matter accumulation rates in sediments have been studied in conjunction with stable isotopes (∂^{13}C and ∂^{15}N) analyses to infer past environmental changes in riverine and wetland ecosystems (Savage et al., 2004; Hodell and Schelske, 1998).

Wetlands receive external allochthonous input of organic matter from the surrounding terrestrial landscape production in the water column (Darling and Talbot, 2003). Groundwater appears to be the main source of water to some wetlands but in Nylsvley Wetlands water comes from rainfall in the catchment, as the system is a river floodplain wetland type. Most of the allochthonous material arrives in wetlands as dissolved organic carbon (Tranvik, 1988), but this depends on the wetland type. In some wetlands allochthonous material actually arrives as detrital plant material from terrestrial systems.

Stable isotopes occur naturally and changes in their distribution and natural abundance in soils and plants can give important information on the functioning of ecosystems, organic matter dynamics and important processes. Stable isotope analysis relies on the existence of chemical elements with two or more stable isotopes that are unevenly distributed among compounds or compartments. Isotopic values are often influenced by environmental and temporal drivers (Laakmann and Anuel, 2010; Quillfeldt et al., 2015; Oczkowski et al., 2016). In ecological studies, stable isotope analysis (SIA) has been successfully applied to reconstruct diet and migration patterns of organisms, identify food web structures and track flow of elemental within an ecosystem (Fry, 2006).

Stable isotopes of carbon and nitrogen in organic matter offer an alternative means to detect early signs of environmental changes in aquatic ecosystems (Michener et al., 2016; Hicks et al., 2012) .The ratios of $\partial^{13}C/^{12}C$ and $\partial^{15}N/^{14}N$ (defined as $\partial^{13}C$ and $\partial^{15}N$) have been used to provide insight into the sources, sinks and cycling of carbon and nitrogen in aquatic ecosystems, as these elements interact with biotic, physical and chemical components of the environments (Gladyshev, 2009; Hietz, 2005). Past studies of stable isotope signatures in wetland sediment organic matter suggest the following generalizations; that (i) Allochthonous organic matter usually has more negative $\partial^{13}C$ than does autochthonous organic matter (Filley et al., 2001); (ii) Values of $\partial^{13}C$ can also be used to distinguish organic matter deposited during periods of high versus low primary productivity; and (iii) The natural balance between the production and decomposition of sediment organic matter in many aquatic areas has been disturbed by anthropogenic activities (Gao et al., 2012). Many studies have examined the influence of seasonal variations in the environment and anthropogenic activities on sediment organic dynamics, and much useful information has been obtained. (Lesen, 2006) studied the seasonal variations of sediment organic dynamics in San Francisco Bay, and found that the sediment organic carbon and nitrogen were highest in spring and lowest in winter. (Gao et al., 2012) studied the geochemistry of sediment organic matter in China based on stable isotopic signatures and total organic carbon, and found that the inputs of organic matter from anthropogenic activities had a more significant influence on parameter distributions than natural processes.

The temporary zone would be the area with low stable isotope signatures given that the area is very dry, with little to no water available in the area. Organic matter in this zone decomposes rapidly. There is a strong correlation between temperature and productivity, hence it is anticipated that productivity may be less or enhanced through increase in temperatures.

Materials and methods

The study was conducted in Nylsvley wetland over two seasons, the cool- dry (in June 2019). A detailed description of the study area is provided in Chapter 3.

Stable isotope analysis

Sediments and plants were collected in the Nylsvley Nature Reserve. These samples were collected in five sites in transect. Phytoplankton and zooplankton were sampled with a 20 µm and 62 µm plankton respectively. Macroinvertebrates were sampled using a Nylon mesh (1000 µm) with a flat bottom and an aluminum rim that allowed a sampling distance of 1.5 m. A 10 m length sampling area was sampled by submerging the net and sweeping for 5 minutes. This involved walking along the edges of the ponds and dragging the net through the macrophyte vegetation. Plants that were collected were milkweed, *Phragmites australis, Persicaria, Lantana Camara, Digitaria eriantha, Panicum maximum, Gomphocarpus physocarpus*, *Bidens pilosa*, *Cynodon dactylon, Cyperus* spp., *Sporobolus* and *Ludwigia Stolonifera.* Some of these plants are allochthonous and autochthonous plants.

Macroinvertebrates were identified to genus according to Gerber and Gabriel (2002). Fish samples were measured for standard length to the nearest 0.1 mm and fin clip samples were taken from each individual fish.

All samples including sediments, plants and filters were freeze dried in aluminum foil envelopes, and crushed to a fine homogeneous powder using a mortar and pestle. For filtered samples, about 0.5 mg of material was scraped off from the dry filters and placed into tin capsules. For other materials, sub-samples were weighed (25 to 30 mg for sediments, and approximately 1 mg for plants) into tin capsules. All samples were analyzed for stable carbon and nitrogen isotopes. Once all samples were sorted and identified, they were all oven dried at 60 °C for at least 48 hours, then grounded into a homogenous powder and sent for isotopic analysis at the University of Pretoria.

The stable isotope analysis was carried out using a Flash EA 112 Series coupled to a Delta V Plus stable light isotope ratio mass spectromoter via a ConFlo IV system (Thermo Fischer, Bremen, Germany). Standard delta notation (δ) was used to express stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). The isotope ratios were expressed in parts per thousand (‰) differences from a standard reference material as the following:

$$\delta^{13}Cor\delta^{15}N = \left[\left(\frac{Rsample}{Rstandard} - 1\right)\right] \times 1000$$

where R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively (Fry 1991, Hobson and Clark 1992). The C:N was in mass ratios. The standards used were referenced to atmospheric nitrogen for $\delta^{15}N$ (Ehleringer and Rundel 1989) and $\delta^{13}C$ was referenced to Vienna Pee-Dee Belenmite (Craig 1957). The average analytical precision was <0.15‰ for $\delta^{13}C$ and <0.1‰ for $\delta^{15}N$. To correct for lipid effect on $\delta^{13}C$, Post et al. (2007) was used:

$$\delta^{13}C(corr) = \delta^{13}C - 3.32 + 0.99 \times C{:}N$$

Data analysis

Samples were collected at each of the five sites. Sediment mean values of C and N isotopes were compared among the five sites (N1-N5) and wetland zones (temporary, seasonal and permanent) using a Kruskal-Wallis test (SPSS, 2007). Where significance occurred, means were separated by a Posthoc Mann-Whitney U test. The test is justified because the data were not normally distributed after testing with a Kolmogorov Sminorv test.

Bayesian SIAR model Stable Isotope Analysis in R (Parnell et al., 2010) was used to assess the relative contributions to the different organic matter sources to the sediment mixtures. This Bayesian model combines uncertainty and variation in parameters such as trophic enrichment factor. The model was run for each season and each wetland reach in order to assess spatial and seasonal variability in the sources of organic matter in sediments. Assumptions of small carbon and nitrogen fractionation factors (0.5 for both) were based on short-term degradation experiments (Zieman et al., 1984; Wedin et al., 1995, Schweize et al., 1999, Dehairs et al., 2000).

Results

The sediment mean values of ∂^{15}N were very low ranging between 0.01-2.60 ‰. The sediment mean values of ∂^{13}C were much enriched in all wetland zones and across all sites (N1-N5). The most depleted was the temporary zone in site N2 with mean -19.53 ‰. The most enriched with mean of -26.67 ‰ (Figure 5.1) was found in the permanent zone in site N4 using ∂^{13}C. The highest sediment mean was in site N4 in the seasonal and permanent zone with mean 1.9 ‰ and

2.58 ‰, respectively. The temporary zone had the lowest sediment mean with 0.25 ‰ compared to other wetland sites.

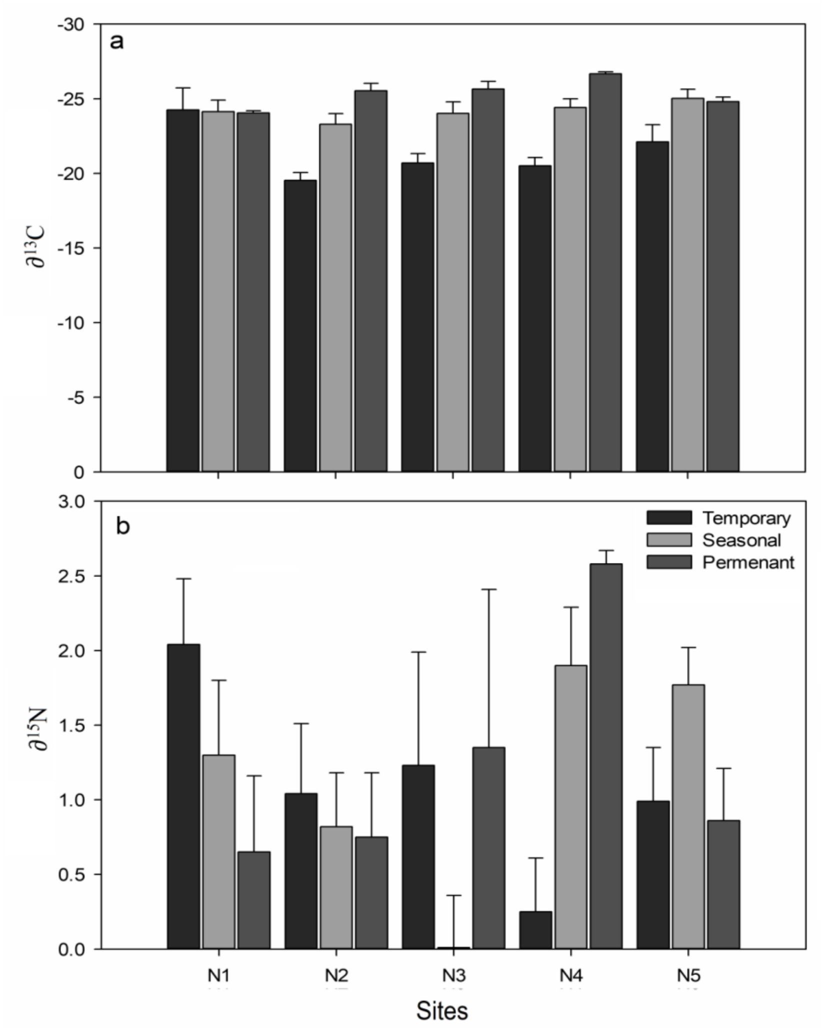

Figure 5.1 Sediment mean values (‰) of (a) Carbon ($\partial^{13}C$) and (b) Nitrogen ($\partial^{15}N$) across study sites (N1-N5).

Sediment N (H = 5.895, df = 4, $p = 0.207$) and C (H = 3.388, df = 4, $p = 0.495$) isotopes were found to be similar across study sites. Whereas, sediment N isotopes (H = 0.263, df = 2, $p = 0.877$) were non-significant across wetland zones, sediment C isotopes (H = 30.977, df = 2, $p < 0.001$) were significantly different across different wetland zones. Using Mann Whitney-U test, significant differences were observed for permanent *vs* seasonal ($p = 0.018$), permanent *vs* temporary ($p < 0.001$) and seasonal *vs* temporary ($p < 0.001$). Table 5.1 shows the mean and standard error of all the taxa that was collected in all wetland sites and in different wetland zones using stable isotope carbon, nitrogen and a combination of both nitrogen and carbon. The mean and se of the taxa varied because of the two stable isotopes used in the sites; carbon (∂^{13}C) and (∂^{15}N) nitrogen, (Table 5.1).

Table 5.1 Mean and standard error (‰) of carbon and nitrogen stable isotopes in all wetland sites for the different taxa.

Taxa	**Site 1** (‰)	**Site 2** (‰)	**Site 3** (‰)	**Site 4** (‰)	**Site 5** (‰)
C/N (∂^{13}C/∂^{15}N)					
Fish	4.63± 0.10		4.77±0.28	4.53±0.08	6.46±0.30
Invertebrates	5.50±0.24		5.39±0.26	5.64±0.29	6.42±0.29
Algae	14.28±1.65		17.05±10.84	14.78±1.03	
Persicaria	12.85±0.44	23.81±1.38	19.81±3.64	15.53±0.90	13.82±1.67
Phragmites australis	23.61±1.46	26.83±2.12		22.46±1.75	19.37±4.91
Digitaria eriantha	29.42±5.54				
Lantana Camara	17.34±0.36			40.52±4.93	
Gomphocarpus physocarpus	16.17±0.46	19.96±0.49	23.38±0.46	22.20±0.34	19.41±0.62
Panicum maximum	27.97±4.24	25.22±3.35	53.61±3.21	72.79±9.80	39.94±5.74
Sporobolus sp.	47.90±8.35				30.60±4.03
Bidens pilosa		65.20±9.97		96.92±2.05	
Cyperus sp.		63.15±1.31	74.73±5.93	64.20±6.32	51.43±2.83
Cynodon dactylon	93.79±6.84		92.96±10.34		

Ludwigia stolonifera			10.67±0.83	20.03±0.50	
Hyparrhenia hirta			112.25±14.85		
Unidentified			68.49±2.58		
Nitrogen (∂^{15}N)					
Fish	8.65±0.14		10.26±0.26	9.97±0.18	0.82±0.11
Invertebrates	6.28±0.26		7.17±0.31	5.76±0.27	4.34±0.26
Algae	2.26±0.49		1.72±0	2.07±0.25	
Persicaria	3.74±0.03	6.32±0.14	5.15±0.44	6.54±0.21	5.74±0.37
Phragmites australis	6.67±0.39	5.15±0.48		6.87±0.07	1.74±1.84
Digitaria eriantha	3.30±0.23				
Lantana Camara	3.58±0.09			0.37±0.25	
Gomphocarpus physocarpus	6.09±0.03	4.33±0.85	2.90±0.17	3.16±0.28	2.99±0.05
Panicum maximum	5.25±0.18	3.08±0.19	2.65±0.54	0.96±0.30	2.28±11.13
Sporobolus sp.	2.78±0.49				4.18±0.35
Bidens pilosa		2.83±0.45			
Cyperus sp.		5.70±0.30	1.27±0.25	0.66±0.14	3.89±0.13
Cynodon dactylon		1.19±0.19	0.52±0.48		
Ludwigia stolonifera			3.08±0.83	7.74±0.15	
Hyparrhenia hirta	1.36±0.59				
Unidentified			0.66±0.20		
Carbon (∂^{13}C)					
Invertebrates	-27.38±0.60		-28.80±0.72	-29.18±0.53	-28.67±1.24
Algae	-22.39±2.72		-1.72±0	-25.29±0.18	
Persicaria	-25.61±0.08	-27.88±0.19	-27.32±0.35	-25.65±0.14	-26.61±0.40
Phragmites australis	-25.30±0.15	-25.62±0.20		-26.16±0.17	-16.19±2.82
Digitaria eriantha	-25.77±0.06				
Lantana camara	-27.14±0.11			-26.04±0.14	
Milkweed	-25.54±0.02	-25.98±0.06	-24.87±0.21	-25.55±0.16	-24.03±0.11
Panicum maximum	-17.39±1.25	-24.65±0.63	-28.14±0.18	-12.48±0.27	-13.04±0.11
Sporobolus sp.	-13.50±0.08				-13.57±7.99
Bidens pilosa		-28.10±0.21		-27.59±0.10	
Cyperus sp.		-12.65±0.21	-12.05±0.10	-12.28±0.14	-11.57±0.18
Cynodon dactylon		-18.11±7.00	-12.51±0.17		
Ludwigia stolonifera			-24.54±0.04	-24.69±0.22	
Hyparrhenia hirta			-12.95±0.18		
Unidentified			-11.94±0.12		

Results showed that the mean and standard error of stable isotope carbon were lower than that of nitrogen but the mean of combined carbon and nitrogen was higher (Table 5.1).

Spatial and temporal variations in sources of organic matter within the Nylsvley Wetland were clearly represented by stable isotope signatures. Higher $\partial^{13}C$ signatures in sediments were found in permanent and seasonal zone (Figure 5.1). The mean value in $\partial^{13}C$ decreased from -26.67 ‰ in site N4 the permanent zone to -19.53 ‰ site N2 in the temporary zone. The permanent zone values are mostly high because the area is rich with water, sediments, and different taxa are found within this zone including animals that come to feed in the wetland. In site N1 using $\partial^{13}C$, the mean values for the temporary, seasonal and permanent zone were equal if not almost equal (Figure 5.1). Sites N2, N3 and N4 mean values varied greatly as the temporary zone was lower than seasonal; and seasonal lower than permanent, resulting in the permanent zone being high in the 3 sites with the mean values being above -25 ‰. In site N1 the temporary zone had the highest mean with 2.04, permanent zone was low with 0.65 whereas in site N4 the temporary zone had the lowest mean of 0.25 and permanent zone very high with mean of 2.58. The seasonal zone in site N3 was completely depleted with a mean of 0.01 ‰.

The SIAR outputs suggested that in the permanent, seasonal and temporary zone, autochthonous was the main organic matter source contributing to sediments in all sites. The most dominant taxa or group was plants. Plants such as *Phragmites australis*, *Persicaria*, *Panicum maximum* were dominant in all five sites. In the permanent zone in site N2, allochthonous organic matter was the main contributor as a lot of plants were allochthonous in sediments. There was the influence of rainfall in the area, with milkweed being the most dominant plant. In seasonal site N3, autochthonous was the most important source of organic material for sediments. Organic matter from animals contributed less to sediments in all wetland zones and sites; this is because not many animals were found in the wetlands (only fish and invertebrates). Animals contributed about 10% in the permanent and temporary zone (Figure 5.2). The other animals only came to the wetlands to feed or drink water. Autochthonous input has higher percentages than allochthonous and animals input, and animals had the lowest percentages. Site N2 was dominated by allochthonous plants which included Milkweed, *Lantana Camara*, *Bidens pilosa*, *Cynodon dactylon* and *Sporobolus*. About 60% in site N3 is dominated by autochthonous plants in the seasonal and permanent zone.

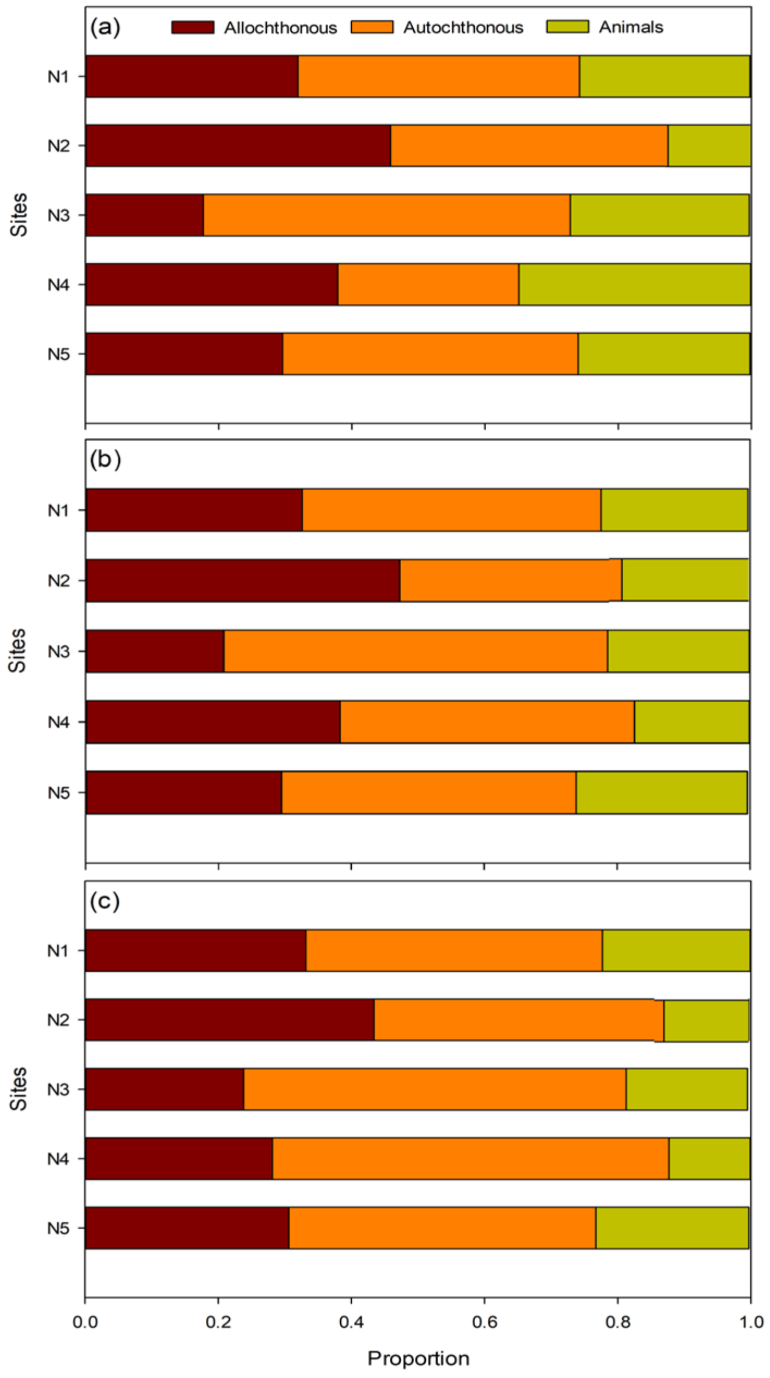

Figure 5.2: SIAR Proportions (%) of allochthonous, autochthonous and animals' inputs across N1-N5 in the (a) permanent, (b) seasonal and (c) temporary zone.

Discussion

Spatial and temporal variations in the sources of organic matter deposited within the Nylsvley wetland were clearly represented by SI signatures. The hypothesis of the temporary zone having low signatures was proved. We found lower $\partial^{13}C$ signatures in sediments in the temporary zone, with the lowest values occurring in all sites (Figure 5.1). It was hypothesized that the lower reach sites would be more autochthonous as they were shallow and slow flowing with increased potential for primary productivity. A possible explanation for this trend was the influence of wetland water in the temporary zone, where carbon sources with low $\partial^{13}C$ signatures terrestrial matter and phytoplankton dominated (ranging from -26.67 to -19.53 ‰). The findings further suggest that $\partial^{15}N$ signature values varied greatly in all wetland sites, in N1 temporary zone was the highest; in site N4 permanent zone was higher than in the first site and in site N5 the seasonal zone was enriched compared to the other two wetland zones. This was because of the many elements necessary to sustain biotic production in wetlands. Nitrogen presents unique challenges due to its chemical versatility. The amount of Nitrogen accumulated in wetland soils depends on the balance between plant production and decomposition, and the balance between allochthonous import and particulate export. Most soils have $\partial^{15}N$ values of 2 ‰ to 5 ‰ (Figure 5.1). On a regional and global scale, soil and plant $\partial^{15}N$ values systematically decrease with increasing mean annual precipitation and decreasing mean annual temperature (Amundson et al., 2003). Nitrogen from human and animal waste is enriched with $\partial^{15}N$ (McClelland et. al., 1997). Enriched $\partial^{15}N$ of various taxa has been used as an indicator for organic matter influence in the freshwater wetland environment (Cole et al., 2004).

Previous studies showed that SOM in wetlands is derived mainly from autochthonous sources including a mixture of fresh and detrital phytoplankton, zooplankton and bacteria (Cannuel and Zimmerman, 2001; Budge et al., 2001). In addition, allochthonous organic matter sources derived from plant materials influenced sediments (Figure 5.2), because biotic and abiotic characteristics such as changes of freshwater discharge and sources of organic matter all influence stable isotope (SI) composition of sediments (Palomo and Canuel, 2010).

The study demonstrated that autochthonous input (plants and animals) was the main source of organic matter in all three wetland zones; permanent, seasonal and temporary (Figure 5.2). However, sources differed in sites because in some sites allochthonous was the main source. Sites N2 and N4 in the permanent zone were dominated by allochthonous plants; plants such as *Cynodon dactylon*, Blackjack and PanSporo. Site N2 was dominated by allochthonous plants in all wetland zones (permanent, seasonal and temporary), therefore allochthonous proportion was high (about 0.5 %). The SIAR proportions indicated that animals also had an input; being a source of organic matter although they did not contribute much compared to autochthonous and allochthonous. The seasonal hydro-period may help explain the relatively high productivity of organic matter in the Nylsvley Wetlands. The sites are characteristically wet during the dormant season and drying when the growing season begins, providing optimal conditions for plant growth (Brinsol et al., 1981).

The values of the combined carbon and nitrogen stable isotope were much enriched; a possible explanation to this is because the carbon values were highly enriched and when combined with the low nitrogen values, the values became more enriched. However, stable isotope nitrogen values were low ranging from 0.52±0.48 to 10.26±0.26 ‰ (Table 5.1). The ∂^{15}N values vary but reach maximum in the permanent zone in site N4 then remain high and stable (-26.67 to -24.05 ‰). The ∂^{15}N values were stable in winter. Stable isotope nitrogen (∂^{15}N) values increased from 0.52 to 9.97 ‰ (Table 5.1), a possible explanation to this was in summer the ∂^{15}N showed strong enrichment in comparison with winter. Approaching the summer season we observed a slow increase in ∂^{15}N, indicating a gradual change in nitrogen chemistry in the atmosphere. Some plants, such as *Hyparrhenia hirta* and *Cyperus* sp*.,* had low values in both ∂^{15}N and ∂^{13}C in wetland sites and in both the hot wet and cool-dry seasons. The ∂^{15}N value of *Hyparrhenia hirta* was 1.36 ‰ and *Cyperus* sp. was between 0.66 -5.70 ‰. These values were low since other plants' values were enriched and above 10 ‰ in this stable isotope. This was also because the plant *Hyparrhenia hirta* plant was not found in many sites, but only in sites N1 and N3 in both seasons.

Previous studies suggested that measurement of ecosystem (plant and soil) stable isotopic compositions (∂^{13}C and ∂^{15}N) can provide insight into soil C and N cycling (Nadelhoffer and Fry, 1994). Management of soil requires techniques that accurately monitor changes in soil organic matter over the short term and long term. Stable isotopes of carbon and nitrogen can help answer fundamental ecological questions, in addition to solving applied problems relating to the source, type and input of organic matter and nutrients in aquatic ecosystems (Fry, 2006; Peterson, 1987). Natural abundance and tracer addition stable isotope studies have revealed much about the processing of organic matter and nitrogen through stream, river, and wetland environments (Peterson and Mulholland et al., 2000). However, there is an increase in nitrogen input and ∂^{15}N isotopic values in wetland areas, due to the decomposition and leaching of soluble nitrous compounds.

CHAPTER 6: GENERAL DISCUSSIONS AND CONCLUSION

Summary and conclusions

Wetlands play a role as sinks for sediments, and wetlands that are connected to adjacent aquatic ecosystems may trap more sediment as compared to wetlands that lack such connectivity (Mitsch and Gosselink, 2000; Fryirs et al., 2007). The purpose of the study was to evaluate spatial and temporal changes and sources of organic matter in sediments within a wetland environment using three different wetland zones over two seasons; hot-wet and the cool-dry season. The first study (Chapter 3) examined the study area of Nylsvley Wetland found in the upper reaches of the Mogalekwena River in Limpopo. The entire Nylsvley's floodplain is 24 250ha (including hydromorphic grasslands and sodic sites) and is naturally endangered to seasonal flooding and fluctuation in water levels. It is a gentle slope consisting of three rocky outcrops and the vegetation is differently distributed, vegetation such as *Phragmites australis, Cyperus fastigiatus.* It is one of the largest and most important birding areas in South Africa, with a total of 382 bird species recorded within the Nylsvley Nature Reserve and 426 bird species recorded in the broader Nyl River floodplain to date.

The second study (Chapter 4) assessed variation in below ground organic matter along the Nylsvley Wetland. This study was conducted in the cool-dry and hot-wet season. The purpose of the study was to investigate the distribution of SOM in the three wetland zones: temporary, seasonal and permanent over the two seasons. Soil samples were measured and burnt in order to determine the sediment organic matter. The results of the study indicated that the distribution pattern was uneven throughout the wetland zones. The permanent zone had the lowest SOM and the seasonal zone had the highest sediment organic matter in the cool-dry season. In the hot-wet season, the permanent had the highest SOM, although in one of the sites (site 1) no samples were taken because of the presence of hippos in the wetland. The hypothesis test of this study was, therefore, positive. The nutrient concentration of K, Ca, P and Mg also had an influence in the contribution of the organic matter in sediments, with Phosphorus being the main source of nutrient in the sediment.

Chapter 5 assessed the contributions of allochthonous and autochthonous inputs to sediment organic dynamics in Nylsvley using stable isotopes analysis. Wetland ecosystems depend on inputs of both allochthonous and autochthonous organic matter for their energy and productivity. The results indicated that high amount of sediment organic matter in wetlands is from autochthonous input, including plants such as *Persicaria, Panicum maximum* and *Phramites australis*, as well as animals. Allochthonous inputs also include matter intentionally or unintentionally introduced by anthropogenic activities near or within the wetland. Stable isotope carbon (∂^{13}C) and nitrogen (∂^{15}N) were used to compare the mean and standard error of different taxa in all wetland sites N1-N5. Results showed that composition of nitrogen and carbon stable isotope means were higher than the individual isotope. The main limitation of the isotopic method is the lack of knowledge in spatio-temporal patterns and dynamics of stable isotopes in aquatic environments. Analyses of naturally-occurring stable isotopes have emerged as powerful techniques for addressing research and management related questions in ecology. Physical and biological processes are involved in the distribution and preservation of organic matter in wetland sediments.

Conclusions

The use of stable isotopes in ecological and environmental studies has increased considerably over the past decade. The distribution of organic matter in Nylsvley Wetland was uneven throughout because the sources and amount of organic matter found in the wetlands differ with season. Higher amounts of soil organic matter were found in the permanent zone, whilst lower amounts were found in the seasonal zone during the hot-wet season. This was because the permanent zone is always moist. High amounts of organic matter are found in areas which are wet than in dry areas. Such is the case also with plants, which are greener, taller and moist after rainfall in the wet season. This study demonstrated that stable isotopes are generally sensitive environmental indicators and can be used to assess responses of aquatic plants to nutrient stress, enrichment and water management. One of the advantages of stable isotopes is that they occur naturally, and changes in their distribution and natural abundance in soils and plants can give important information on the functioning of ecosystems (including wetlands), organic matter dynamics and other important processes.

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