DOCTOR OF PHILOSOPHY

An investigation of the process of microbial decomposition and the ‘enzymic latch’ mechanism in coastal wetland ecosystems

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Award date:
2018

Awarding institution:
Bangor University

An investigation of the process of microbial decomposition and the ‘enzymic latch’ mechanism in coastal wetland ecosystems

A thesis submitted to Bangor University by

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In candidature for the degree of Philosophiae Doctor

July 2018

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Summary

Coastal wetlands are one of the world's most valuable habitat types, due to the numerous ecosystem services they provide, including protection from storm surges and coastal erosion, supporting biodiversity and carbon sequestration. The soil property of waterlogging leads to slow rates of decomposition of organic matter, leading to net carbon sequestration, making these ecosystems valuable tools against climate change. The carbon storage capacity of coastal wetlands can be lost due to anthropogenic disturbances and natural causes, such as changes in land use and global warming. Accordingly, more than 50% of salt marshes and 35% of mangrove swamps have so far been lost around the world, resulting in significant losses of soil organic carbon. This study investigated the biogeochemical processes in the soils of some coastal wetland types: a mangrove swamp in Florida, USA; a salt marsh ecosystem in the UK; and a salt marsh site encroached by mangroves, also in Florida. At each site, indices of soil organic matter decomposition were examined, particularly those relating to the 'enzymic latch' mechanism. The enzymic latch mechanism was stronger where the soil water content is high, for example the red mangrove soils and the mid salt marsh soils, which are frequently flooded, in line with the hypothesis. Analysis of decomposing mangrove leaf litter falls around these three species revealed much greater contributions of phenolics from the white and black mangrove species, whereas the red mangrove plants contributed comparably more nutrients. In the salt marsh, the mid tidal zone had the lowest rate of decomposition, despite the low zone being located closest to the sea, disproving the hypothesis. The mangrove encroached areas had more biological activity compared to the salt marsh area, suggesting that the colonisation of salt marshes with mangrove vegetation will have short-term negative impacts on below-ground carbon sequestration in coastal wetlands.

Acknowledgements

I sincerely wish to express my thanks and gratitude to my lead supervisor Professor Chris Freeman for the invaluable support, encouragement and good guidance he has given me throughout my research period. In spite of tight schedules, you are always there for me to ensure that I am on track, I am sincerely grateful to him for giving me this opportunity.

I would like to thank most sincerely my co-supervisor, Dr Christian Dun, for the help and excellent guidance. You are indeed more than a friend. Special appreciations also to Dr Timothy Jones, my mentor, brother and friend. Your guidance and patience in the laboratory did not just produce excellent results, but exposed me to variety of instrumental analysis and new laboratory techniques. I assure you that the skills learned has better repositioned me to face grater tasks ahead. I remain highly grateful to Dr Rachel Gough, for your precious time and going through my write up and effecting the necessary corrections. In addition, I remain grateful to the entire Bangor Wetland Group, you have been such an amazing group to work with.

Special thanks also goes to the Management of Plateau State Polytechnic Barkin ladi and TETFUND Nigeria for funding which made this research work a reality.

This endeavour was made possible by the support I enjoyed from my dear wife Alheri (Larep Jipal) and the kids, Tongret, Nissi, Nanret, Elyon and Nankling. Thanks also to Nanyet, Nanwal and a host of others for your good will. Special thanks too to my siblings Abalis, Iliya, Ndah Sihitshai and many others for your constant prayers and encouragements.

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Lists of abbreviations

CH_4 Methane

CO_2 Carbon dioxide

DIC Dissolved Inorganic Carbon

dicq 2,3-dihydroindole-5,6-quinone-2-carboxylate

DOC Dissolved Organic Carbon

DOM Dissolved Organic Matter

FAO Food and agricultural organisation

FID Flame ionisation detector

GAE Garlic acid equivalent

GHG Greenhouse gas

DW Dry weight

ECD Electron capture detector

IC Inorganic Carbon

IPCC Intergovernmental Panel on Climate Change

L-DOPA L-dihydroxyphenylalanine

MUF Methylumbelliferone

NOAA National oceanic and atmospheric administration

NPP Net primary productivity

Nmol nano mole

NO_2 Nitrous oxide

REDD Reducing emissions from forest and forest Deforestation

PES Payment for Ecosystem Services

POX Phenol oxidases

POM Particulate Organic Matter

SOC Particulate Organic Carbon

SOM Soil Organic Matter

WHO World Health Organisation

Chapter 1

General introduction

1.1 Wetland ecosystems

Wetlands are considered to be one of the world's most productive and carbon-rich ecosystems on Earth, a vital biome that provides numerous important ecosystem services (Shange *et al*., 2013; Mitsch & Gosselink, 2015; Adame & Fry, 2016). Wetlands are biologically complex ecosystems due to the physical, chemical and biological processes that occur in such environments. They form a critical feature of the global landscape due to the unique role they play in regulating global biogeochemical cycles (Mitsch & Gosselink, 2015). Many definitions of wetlands exist, but the International Ramsar Convention, 1971, defined a wetland as:

> 'Wide range of soil types saturated with water, comprising of salt marshes, lakes, floodplains, mangroves, (peat) swamps, rivers, flooded forests, rice-fields, and coral reefs. It also includes areas of marine water up to a depth of six meters at low tide (Uluocha & Okeke, 2004).

The above definition, therefore, integrates all of the variable aspects of wetlands, with the dominant characteristic of a persistently high water-table and the effect this has on biogeochemical processes.

Wetlands are found in all climatic conditions ranging from the tropics to the tundra (Reddy & DeLaune, 2008; Mitsch & Gosselink, 2015). Mitsch and Gosselink (2015) noted that the presence of water lasting throughout the growing season with distinctive soil conditions and vegetation types well adapted to withstand saturated soils are a significant feature of wetlands. Mitsch *et al.* (2010) further identified three main features that constitute wetland ecosystems:

I. The hydrological regime usually characterized by water level, flow regime, and the frequency and duration of inundation over time.
II. The physicochemical nature of the soil that includes sediments reduced or oxidized soil conditions, as well as the chemical reactions in the soil.
III. The biotic environment, which is comprised of plants, animals, and microbial organisms tolerant of anoxic conditions.

Arguably, wetlands has one of the highest carbon densities among all the terrestrial ecosystems (Stern *et al*., 2007; Adame & Fry, 2016). Several sources state that the stability and functioning of wetlands are highly dependent on the quality and quantity of soil organic matter and stored carbon (Kirwan & Blum, 2011; DeLaune & White, 2012; Stagg *et al*, 2017).

Waterloging creates anoxic conditions in wetland soils. The anoxic conditions place a constraint on microbial activities accounting for the slow rate of decomposition of organic matter, leading to significant carbon storage in such soils (Gorham, 1991; Freeman & Nevison1999). The anoxic conditions also encourage the activities of methanogenic microbes which produce methane (CH_4) as a metabolic by-product, to the extent that wetlands account for 15-40% of global methane emissions (IPCC 2007). CH_4 is a greenhouse gas that has 25 times higher potential for contributing to global warming than carbon dioxide (Whiting & Chanton, 2001; Bridgham *et al.* 2006; Zheng *et al.*, 2013; Suratman, 2017).

Consequently, the Ramsar Scientific and Technical Review Panel reported that, although wetlands occupy only a small portion of the Earth's surface (approximately nine percent), they hold an estimated 35% of the World's terrestrial carbon stock in soil sediments (Amthor *et al.*, 1998; Whiting & Chanton 2001; Schlesinger & Lichter 2001; Akoko *et al*., 2013). Wetlands are known to sequester carbon dioxide (CO_2) from the atmosphere into the soil. Additionally, once wetlands are threatened by either natural or anthropogenic activities, they could release potentially large amounts of carbon dioxide, methane and nitrous oxide which are the primary greenhouse gases (GHGs) linked to climate change (Liikanen *et al*., 2006; Malmer *et al*., 2005; Mander *et al*., 2008; Mer & Roger, 2001; Whiting & Chanton, 2001). Some studies revealed that wetlands serve as a global store for atmospheric carbon, and recommend further research to bring out a clearer picture of the pathways and various forms of carbon that constitute the carbon budget of wetlands (Loulergue *et al*., 2008; Adame & Fry, 2016).

The release of these GHGs from wetland ecosystems is attributed to the increased rates of decomposition of organic matter, which take place at varying rates depending on a number of environmental conditions prevailing at any given time. These conditions include hydrological dynamics of the soil, oxygen availability, pH, temperature, leaf litter, and the number of decomposing microbes (Limpens *et al*., 2008; Kayranli *et al.*, 2010). Approximately 15% of materials in the form of organic matter, because of decomposition by microbial activities, that are exported into the sea originate from wetlands (Stern *et al*., 2007; Adame & Fry, 2016).

The unique biogeochemical characteristics of wetlands encourage the storage of carbon from sediments and organic matter as they gradually accrete in the soil. This critical role of wetlands to the ecosystem notwithstanding, they are threatened by anthropogenic activities as well as

natural causes (Olalekan *et al*., 2014). Some climate change models predict a warm and drier climate across the globe and the response of wetlands represents a positive feedback in the process of global warming which could increase oxygen availability in such ecosystem with a negative consequence for carbon mobilisation (Updegraff *et al*.,2001). As a result, this could be reflected within a drawdown of the water table, influencing the biogeochemical processes in the soil resulting in an increase in the rate of organic matter decomposition by microbes and increasing GHGs emissions (Updegraff *et al*., 2001). This situation could convert wetlands from natural carbon sinks into net carbon sources (Freeman & Lock, 1992). Similarly, Freeman *et al*. (1996) reported a series of laboratory and field studies suggesting that drier climatic conditions could significantly alter many wetland biogeochemical properties by impacting on its water quality and trace gas emissions. The studies further reveal that the activities of soil microbes dramatically promote such adverse changes in wetlands and, surprisingly, only minimal work has been done to investigate such impacts on wetland soils.

Deforestation and ecosystem degradation are identified as the most significant cause of CO_2 emissions to the atmosphere after fossil fuel combustion (Adame *et al*., 2015). The highest global deforestation rates are in wetland ecosystems, accounting for the loss of about one-third of the world's mangroves in the last five decades with salt marshes suffering similar fate since the eighteenth century (Mcleod *et al*., 2011). Also of concern is the report of Hansen & Nestlerode (2014) that about half of the coastal wetlands in the United States alone are found around the Gulf of Mexico but, unfortunately, these vital ecosystems are reducing annually at an alarming rate of approximately 25,000 hectares as a result of anthropogenic and natural stresses. The loss of these vital ecosystems and further degradation compromise their ecosystem services. Recent efforts aimed at curtailing vast losses of carbon from ecosystem services resulted in proposing programmes such as Reducing Emissions from Forest Deforestation and Forest Degradation (REDD+) and many similar other forms such as Payment of Ecosystem Services (PES) which has global acceptance. These programmes were aimed at mitigating climate change in developing countries by enhancing reduction in deforestation and conservation of water resources through some form of financial inducements (Alongi, 2011; Everard *et al*., 2018).

Accordingly, the Ramsar Convention, which is the international inter-governmental treaty signed in Iran in 1971, provides the framework for natural action and international cooperation for the conservation and wise use of wetlands. The reports have so far identified 1,723 wetland sites of international importance that are under anthropogenic and natural threats (Reddy &

DeLaune, 2008). To this end, the 1971 Ramsar Convention classified wetlands into two major components:

1. Coastal wetlands comprised mainly of mangrove swamps and salt marshes, which are tropical and sub-tropical ecosystems serving as interphases between the coast and the sea.

2. Non-coastal wetlands comprising of freshwater wetlands, which are situated within interior landscapes such as floodplains, rivers and streams, wet prairies and hardwood swamps, marshy areas around lakes and ponds, and peat lands serving as a link to estuaries that support coastal marshes. Figure 1.1 presents a depiction of major wetlands of the World.

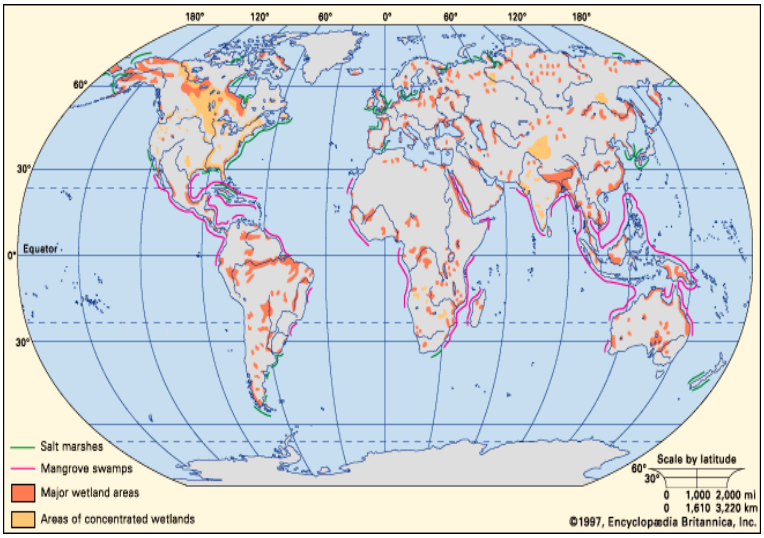

Figure 1.1. Major Wetland Areas of the World showing global distribution of Salt Marshes and Mangrove Swamps. Adapted from Encyclopaedia Britannica, Inc. (1997). Retrieved from https://www.britannica.com/science/mangrove-forest.

1.2. Major wetland types

Global wetlands inventory are seen to include swamps and marshes, lakes and rivers, wet grasslands and peatlands, oases, estuaries, deltas and tidal flats, near-shore marine areas, mangroves and coral reefs, and other human-made sites such as fish ponds, rice paddies, reservoirs, and salt pans (Ramsar, 2004; Anthonj, 2017). Wetlands have different characteristics with one feature similar to all, which is the water table is close to the soil surface or inundated for an extended period or covered with shallow water for the most part of the year. The main features of a wetland are determined by taking into account the salinity of the water,

type of soil and the biodiversity. Classification of wetlands can be difficult because of the high variability of conditions in such habitats (Mitsch & Gosselink, 2015). Figure 1.2 shows comparisons in annual carbon sequestration rates of different coastal wetland types and tropical forest ecosystem soil sediments with the tropical forest having the lowest annual carbon sequestration.

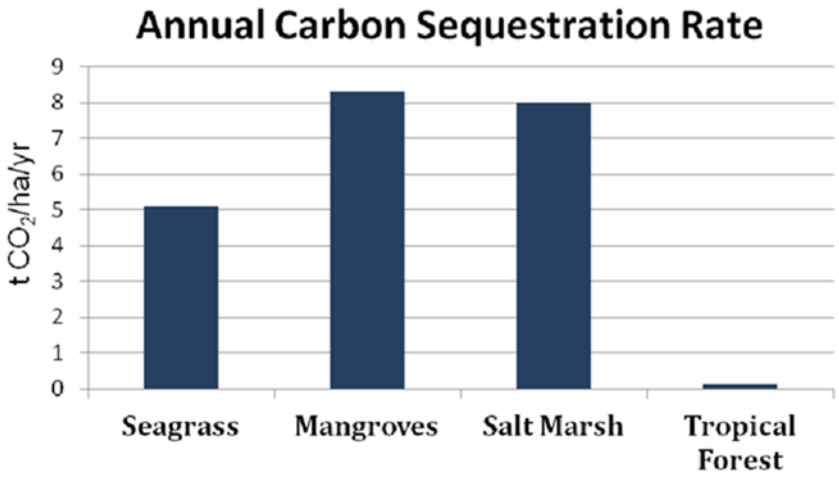

Figure 1.2. Comparison of annual carbon sequestration rates of coastal wetlands compared to tropical forests (Kempka 2015). Retrieved from: https://climatetrust.org/blue-carbon-rising/

1.2.1. Coastal wetlands

Wetland types found in coastal watersheds include mangrove swamps, salt marshes, bottomland hardwood swamps, fresh marshes, and shrub (Mitsch & Gosselink, 2015). Coastal wetlands provide numerous ecosystem services to many coastal communities such as protection against natural disasters including erosion and heavy windstorm. They also improve water quality, support fisheries and other biodiversity.

1.2.2. Mangrove swamps

Mangrove swamps are the leading naturally occurring coastal wetland type found in tropical and subtropical regions, providing beneficial services to humanity and maintaining coastal ecological equilibrium (Srikanth *et al.*, 2016; Liu *et al.*, 2017). Mangrove ecosystems are among the most biologically active and productive ecosystems, providing many essential ecosystem services. These ecosystem services include protecting coastal communities against natural disasters, nutrient cycling, fisheries, water filtration for adjacent coastal communities,

medicines as well as building and construction materials and serving as sinks for greenhouse gases (Giri *et al*., 2011; Feller *et al*., 2017; Doughty *et al*., 2017).

Mangroves are found distributed in the sub-tropical and tropical shores between the sea and the land between 30°N and 30°S latitude (Giri e*t al*., 2011). Though mangroves occupy a small portion of the world's coastal areas (0.5%), they are vital natural ecosystems for carbon sequestration and mitigate the effect of climate change (Alongi, 2014).

Mangrove plants across the world mostly consist of about nine orders, and over 70 species, notable among them are the red (*Rhizophora mangle),* black (*Avicennia germinans*) and white (*Laguncularia racemosa*) mangroves. The World Food and Agricultural Organisation (FAO) stated that Indonesia, Australia, Brazil, and Nigeria account for about 43% of the world mangrove forests covering an estimated total land area of 160,000 km^2 (FAO, 2003). Murdiyarso *et al*. (2015) reported that the mangrove swamps in the Indopacific region alone contain on average 1,023 Mg C ha^{-1} buried in the soil and play a significant role in mitigating the effect of climate change. Mangrove plants have developed some unique physiological features that enable them to survive in anaerobic, waterlogged soil conditions with low oxygen. Such anoxic conditions encourage a high rate of long-term storage of carbon which gives mangrove swamps their global importance as one of the world's most efficient carbon sinks with greater capacity to mitigate the effect of global warming (Murdiyarso *et al*., 2015).

Similarly, mangrove swamps have a substantial impact on the global carbon budget of tropical and coastal areas due to a high percentage (49-98%) of organic matter in their sediments because of higher productivity and low rate of decomposition of organic matter (Saraswati e*t al*., 2016). Therefore, mangrove swamps continue to draw attention because of their ability to sequester carbon. These are carbon stocks stored in the biomass and sediments of tidal vegetated ecosystems such as mangroves, salt marshes, the beds of seagrasses which have potential for mitigating the effect of climate change (Alongi, 2015; Woodroffe *et al*., 2016; Sousa *et al*., 2017). The accumulation of such massive amounts of organic carbon in mangrove ecosystem is attributed partly to high leaf litter fall and detritus. In fact, leaf litter fall in healthy mangrove swamps is estimated to be in the range of 2-3g dry wt m^{-2} day^{-1} (Saraswati *et al.*, 2016).

Analysis of carbon exchange from mangrove environments revealed an average carbon export of 202 g C m^{-2} y^{-1}, representing 50% of average mangrove litter fall. Rainfall and high temperature account for 77% (Dittmar *et al.*, 2006) of the variation in global litter fall. Export of dissolved organic carbon (DOC) from mangrove ecosystems is 26.6 g C m^{-2} y^{-1}, with tidal amplitude being the main driver of export (Adame & Lovelock, 2011; Breithaupt *et al.*, 2012; Dittmar *et al*., 2006; Kristensen, 2008; Mcleod *et al*., 2011).

Mangroves are one of the most threatened tropical ecosystems as a result of large-scale anthropogenic activities as well as natural causes, which includes population expansion, industrialization, agricultural practices, pollution, road construction, over-harvesting, and grazing (Doughty *et al*., 2017). Each of these factors influence mangrove swamps negatively and cause a northward migration of mangrove plants as well as large-scale die-offs of such vegatation as observed recently in Australia and the Gulf of Mexico (Duke *et al*., 2017; Lovelock *et al*., 2017).

1.2.3. Salt Marshes

Salt marshes are a particular type of coastal wetland, which are highly productive and widely distributed in estuarine systems around the world. They also occur in deltas, the upper coastal intertidal zones, between seas and land. These are often flooded by salty or brackish water dominated by the natural terrestrial halophytic ecosystem. Salt marshes play an essential function in the environment serving as excellent sinks for GHGs, niche habitats for certain organisms as well as providing coastal protection from storms through wave attenuation (Adam, 1993; Poirier *et al*., 2017; Martin *et al*., 2017; Orson *et al.,* 2018).

Coastal salt marshes are species-poor, with grasses, shrubs and herbs that are tolerant to such salty environment as the dominant flora with sometimes a few algae. Occasionally, species of small mammals, birds, fishes, spiders, insects and some marine invertebrates constitute part of the biodiversity. Salt marshes could also serve as nurseries and feeding grounds for a variety of water fowl such as ducks, herons, sharp-tail sparrows among others while, some benthic communities such as mollusks, *Polychaeta* and *Oligochaeta* species can colonize such environments (Doody, 2007). Salt marshes are frequently inundated, the height of which can vary from a few centimeters in enclosed areas such as littoral zones of the Baltic Sea to several meters in the Atlantic coast. A meandering network of tidal channels controls the drainage of

this seawater. Through these channels sediments, detritus, dissolved nutrients, plankton and small fishes are flushed in and out of the salt marshes (Genovese & Witman, 2004).

Coastal salt marshes play a vital role in counterbalancing atmospheric carbon dioxide and serve as an excellent blue carbon sink sequestering about 210 g $C/m^2/yr^1$, (Bai *et al*., 2016). The high productivity levels in coastal salt marshes make it a focal point in the management of carbon sequestration owing to their long-term storage ability. Coastal salt marshes, unlike most upland soils have the capacity to continuously bury a vast amount of carbon in the soil throughout the lifespan of available vegetation in addition to tidal inundation, hence they are referred to as an essential and efficient carbon sink as well as blue carbon ecosystems (Hansen & Nestlerode, 2014; Yuan *et al*., 2015). Zonation of salt marshes occurs based on the topography and the types of plant that dominate the area. Based on this, salt marshes are often classified as low, medium and high marshes which is dependent on the rate of annual tidal submergences that occur (Adam, 1993).

The process of decomposition of plant litter materials in salt marsh soils could give rise to accumulation of large deposits of organic matter as well as deposition of heavy metals. Industrial pollutants from urban industrial waste from river flows have been reported in salt marshes situated close to polluted areas (Duarte *et al*., 2010). Consequently, when heavy metals find their way into salt marshes, they tend to be absorbed alongside other soil nutrients, which finally settle in the arial shoots and the root system. In this case, the heavy metals become part of the root biomass and upon decomposition the root litter could add up to the soil mineral sediment components. Previous studies (Duarte *et al*., 2010) revealed that varying amounts of some heavy metals (Zn, Cu, Cd and Co) were recovered from analysis of some parts of salt marsh vegetation (*Sarcocornia fruticosa, perennis, Halimione portulacoides, and Spartina maritima*) with highest concentrations from the root exudates in the Targus–Portugal estuary salt marsh situated close to an industrial area. The outcome of the investigation thus suggests that salt marsh could also serve as a sink for heavy metals supporting previous studies. Vegetation density could play vital role in retaining materials from tidal flow and inundation by slowing down the velocity of water, implying that pollutants containing heavy metals could be trapped alongside other nutrients which eventually will find their way into the sediment pathway (Wienner *et al.,* 2006; Drake *et al.,* 2015; Whiting, 2016).

Invasive plant species that colonise salt marshes in response to warming could alter carbon cycling processes, as they are linked with greater CH_4 emission and uptake of CO_2 than vegetation native to the coastal salt marsh environment (Mueller *et al*., 2016). For example, *Phragmites australis* has a higher biomass and gas transport capability than most salt marsh vegetation types, therefore could increase the soil-atmospheric exchange of GHGs (Colmer, 2003).

Despite the importance of coastal salt marshes as a carbon sink and a vital natural medium for mitigating the effect of global warming, they are also under serious threat like other coastal wetlands types due to anthropogenic activities as well as natural causes. Recent models have, unfortunately, predicted that the rise in global atmospheric temperatures could negatively impact coastal salt marshes releasing centuries of stocked soil carbon into the atmosphere as a result of anthropogenic activities such as land reclamation and industrial activities among others (Wu *et al*., 2017). Current threats to coastal salt marshes could include changes to hydrology regimes, development of coastal communities, pollution arising from waste disposals, invasion from non-native woody plant species including mangroves and ever-rising global temperatures (Martin *et al*., 2017). It is therefore pertinent to have a clear understanding of the impact of these environmental variations on carbon capturing ability of such natural ecosystems in order to predict future threat these natural carbon sinks might have on the global climate.

1.3. Carbon sequestration and cycling in coastal wetland ecosystems

As a result of large-scale anthropogenic activities and natural causes, atmospheric concentrations of carbon dioxide (CO_2), a greenhouse gas, continue to rise from the recorded pre-industrial levels of 280 ppm to an alarming level of 410 ppm (Forster *et al*., 2007, NOAA, 2018), the highest values recorded so far in over 500,000 years (Jansen *et al*., 2007). The continued rise in atmospheric CO_2 has resulted in an increase in sea volumes with implications for the global climate and ecosystem carbon cycling dynamics (Denman *et al*., 2007). To this end, wetlands remain a useful tool to capture and sequester the atmospheric CO_2 as a long-term mitigation strategy (Forster *et al*., 2007; Adame & Fry, 2016). Since wetlands are highly efficient sequesters of organic matter, their role in the global carbon cycle is crucial (Mcleod *et al*., 2011).

Although information regarding coastal carbon cycling and sequestration is on the increase, there appears to be a significant underestimation and uncertainties in the component stocks and fluxes such as the 'missing carbon' in mangrove swamps (Mitsch *et al*., 2013; Barr, *et al*., 2014). This is why wetlands globally are estimated to have the ability to store over a third of the world's terrestrial carbon (Macreadie *et al*., 2013). Therefore, carbon sequestration is referred to as the process of locking carbon dioxide away from the atmosphere into the soil sediments (Whiting & Chanton, 2001; IPCC, 2014).

These wetlands form a transition between the terrestrial and the marine habitat, characterized by a unique soil type, hydrological regime and vegetation types (Mitsch *et al.*, 2013; Zhang *et al*., 2017). A coastal wetland is a unique ecosystem type where sediment accretion into its soil and the ability to sequester carbon is highly dependent on the efficient build-up of organic matter mostly from vegetation (Macreadie *et al*., 2013). Therefore, these types of ecosystems are by nature referred to as the World most efficient carbon sinks owing to the massive amount of sediments and organic matter deposited in such soils making them an essential component of the World carbon cycle (Macreadie *et al*., 2013; Barr *et al.*, 2014).

All wetland types have the capacity to capture and store carbon through the photosynthetic process (Foster *et al*., 2012). Due to the complex biogeochemical processes that take place in wetlands, plants in such ecosystems generally grow at a much faster rate than the rate at which break down of organic materials occurs, thereby encouraging a net annual build-up of carbon sinks. The waterlogged conditions in wetland soils restrict oxygen penetration into the sediments thereby creating anaerobic conditions, which slows down the rate of material breakdown resulting in accumulation and storage of large quantity of carbon in the soil (Foster *et al*., 2012). Wetlands could therefore become sources or sinks of carbon, and can equally switch between being sinks of carbon to become net sources depending on some environmental changes attributed to either anthropogenic or natural happening. Mcleod *et al*. (2011) reported that an estimated total global accumulation of organic carbon of coastal wetlands ranges from 57 to 87 Tg/yr^{-1}. Furthermore, Mitsch *et al*., (2013) and Zhang *et al*., (2017) reported that the carbon sink ability of coastal wetlands had become an interesting area of global carbon cycle investigations.

The outcome of investigations carried out in 158 saltmarshes revealed that an average of 242.2 g/m^2 of carbon were found in the soils of such coastal wetlands (Lovelock *et al*., 2014). In addition, the spatiotemporal accumulation of soil organic carbon (SOC) found in most coastal

wetlands is attributed to vegetation cover, which largely contributes to the above ground organic biomass arising from leaf litter, and other parts of the plants. Exudates from the root system and rhizomes also contribute some fraction of below ground biomass. Accordingly, it is estimated that over 50% of the biomass found in coastal wetlands are buried below ground as a consequence of tidal deposition thereby influencing the total soil organic carbon (SOC) (Duarte *et al*., 2013).

Donato *et al*. (2011), Mcleod *et al*. (2011) and Adame & Fry, (2016) noted that mangrove and marsh ecosystems have the capacity to sequester three times as much carbon than terrestrial ecosystems. They also stated that mangroves in the Caribbean and Indo-pacific could sequester up to 1,023 Mg C ha^{-1} yr^{-1}. Foster *et al*. (2012) stated that the outcome from recent research advocates that healthy, intact coastal wetland ecosystems such as mangrove forests, tidal marshes, and seagrass meadows are inherently good at sequestering vast amounts of atmospheric carbon dioxide and could store it in the soils for hundreds to thousands of years. Such systems could serve as an excellent mitigation tool in managing the effect of global warming. Figure 1.3 below further describes the process of carbon sequestration and cycling in the wetland ecosystem by the vegetation capturing atmospheric CO_2 during photosynthetic process; eventual dropping of carbon rich leaf litter and other vegetation parts are submerged and buried in oxygen-limited soils resulting in carbon burial.

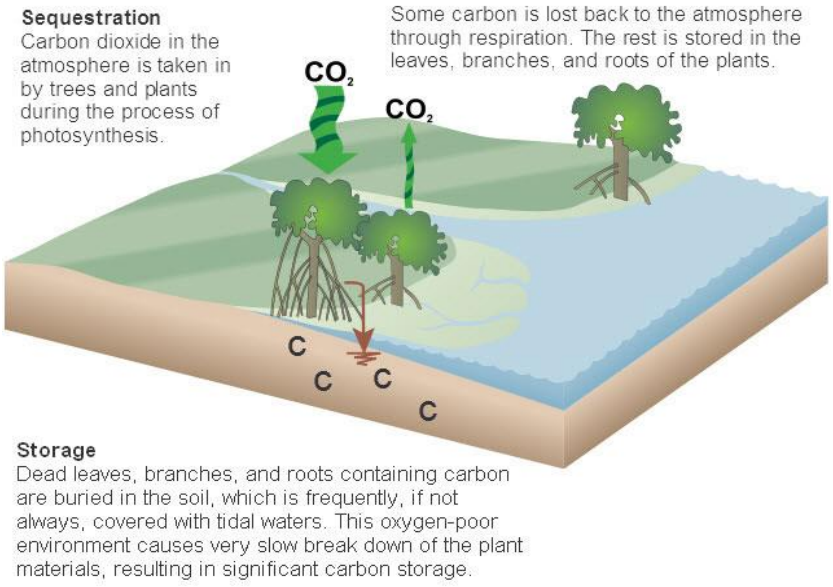

Figure 1.3: Carbon Cycling in a Coastal Wetland Ecosystem. Adapted from NOAA Habitat Conservation Retrieved from http://www.habitat.noaa.gov/coastalcarbonsequestration.html.

Plants through photosynthetic process absorb atmospheric carbon. Carbon is processed and stored in the plant's organs such as leaves, branches, and roots during the process of respiration and some is returned to the atmosphere. As leaf litter falls and is covered by tidal water, leaching occurs. Biochemical process and microbial action converts the complex organic compounds into inorganic minerals, which becomes bioavailable for plant uptake (Zhao *et al*., 2015).

1.4. Decomposition of organic matter in coastal wetlands

Soil organic matter decomposition could be explained as the process whereby plant and animal waste are degraded or broken down through leaching and the activities of decomposing microbes, which are the central mechanism for carbon loss in wetlands environment (Stagg *et al*., 2017). Kayranli *et al.* (2010) stated that the process of decomposition in wetland soils could be complex as it involves both aerobic and anaerobic processes using various electron acceptors. The absence of oxygen could be the primary determinant for plant detritus turnover in such environments. Keiluweit e*t al*. (2015) and Stagg *et al*. (2017) stated that the rate of soil organic matter decomposition in wetland ecosystem is influenced by external environmental factors such as temperature, oxygen and nutrient availability. They also identified salinity and nutrient availability as well as the composition of microbial diversity and the quality of material waste to be influencing the process of decomposition. In the wetland ecosystem, especially mangrove swamps, aerobic decomposition of organic matter occurs on detritus materials deposited on soil sediment surface. Decomposition under anaerobic conditions occurs under the soil sediments as oxygen rarely penetrates greater than 2mm below the surface of the sediment except in the borrows created by macroinvertebrates and vegetation roots (Kristensen *et al*., 2008; Holguin *et al*., 2001). It then implies that an increase in temperature, oxygen and nutrient availability could trigger microbial activities thereby resulting in increased rates of decomposition of organic materials.

Litter fall from plants forms part of the net primary productivity (NPP) in wetland ecosystems as this depends upon decomposition and eventually serves as a significant source of soil nutrients (Zhao *et al.,* 2015). The process of decomposition of litter materials, unlike soil organic matter, starts with a physical process of leaching followed by biochemical breakdown involving soil microbial and faunal actions were complex compounds are converted into simple inorganic materials, which becomes bioavailable. It is estimated that >90% of nitrogen and

phosphorus and up to 60% of mineral nutrients assimilated by wetland plants are from decomposed plant litter materials. Accordingly, the breakdown of plant litter materials significantly impacts nutrient cycling in wetland ecosystem (Zhao e*t al*., 2015). Soil enzymes are the biological catalysts of decomposition of organic matter, and extracellular hydrolase enzymes, which have the potential to break down complex organic matter, serve as the primary decomposers of such organic matter into nutrients. The anoxic conditions in coastal wetland soils thus ensure that the hydrolase enzymes have a low rate of activities resulting in low decomposition rates (Freeman *et al*., 2001).

1.5. Climate change impact on coastal wetlands

Extreme global climatic conditions are greatly impacting on ecosystem structure and functions as well as influencing the distribution of species (Osland *et al*., 2017). Natural occurrences such as ever-increasing drought, heat waves, storms such as hurricanes, flooding, and freezing are all changes due to extreme climatic conditions which could have significant implications on the structure and functioning of the ecological habitats (Allen *et al*., 2010; Zhang *et al*., 2017).

Coastal wetland ecosystems provide essential services such as water quality improvement, flood protection, groundwater recharge, carbon sequestration, ecotourism and support to biodiversity (Hammack & Brown, 2016). Hydrological dynamics in wetlands regulate the biogeochemical process and GHGs emission such as CH_4, CO_2 and N_2O, and therefore, have an impact on global climate. A drawdown of the water table (less 10 cm) could, therefore, have a negative consequence on the structure and ecosystem services of such wetlands (Paschalis *et al*., 2017). A number of studies have investigated the potential effects of such changes on coastal wetlands, and it was generally predicted that coastal wetlands would become drier if the world gets warmer (Mitsch *et al*., 2010) due to more significant surface water evaporation, which will increase aeration of the soil. Once climatic conditions change, wetland ecosystems, particularly in the tropics, are the first to feel such impacts. The aforementioned conditions could imply that most coastal wetlands could dry out, implying that centuries of buried carbon stocks would be vulnerable to oxidation (Mitsch *et al*., 2010).

Simpson *et al.* (2017) reported that changes in the global climatic conditions have altered the distribution and functioning of vegetation in coastal wetlands. For instance, rising winter temperatures in the south-eastern United States of America have caused a poleward shift of

mangrove vegetation literally taking over pure salt marsh ecosystems which has an entirely different type of vegetation well adapted to that soil. It is worrisome that the salt marsh ecosystem which currently has the highest average carbon storage per land area among unmanaged terrestrial habitats could lose a significant portion of stored carbon due to invasion of alien species particularly mangroves because of climate change-induced expansion (Cavanaugh *et al*., 2014). Similarly, the open boundary that had existed between salt marshes and mangrove swamps in North America is fast depreciating in response to changes in environmental conditions. Mangrove dieback in some areas has increased due to prolong freezes, likewise expansion in mangrove habitats has been observed in response to extended warmer period (Rodriguez *et al.,* 2016; Osland *et al*., 2017). As a result, an appreciable level of alteration in the distribution of Florida USA mangroves swamps has been recorded (Rodriguez *et al*., 2016). Furthermore, the northernmost mangrove limit in Florida has expanded more than twice its original size in area since 1984 in response to rising temperatures. The expansion of mangroves into salt marshes will considerably alter carbon storage in coastal ecosystems (Simpson *et al.*, 2017). The implications of changes in ecosystem structure and function could have consequences on both regional and global carbon pools as these two ecosystems have demonstrated great ability to sequester and store huge amounts of carbon (Mcleod *et al*., 2011; Duarte *et al*., 2013).

Forster *et al.* (2007) observed that elevated global temperatures might result in an increase in the decomposition rates and subsequent emissions of GHGs, particularly in tropical wetlands. Their assessment is based on a comparative work carried out on the decomposition rates of organic matter in tropical wetlands in Nigeria in comparison with boreal peatlands in Canada. The outcome of the investigations revealed that decomposition rates were ten times faster in the tropical region than in colder climate environments. This implies that the biogeochemistry of Nigerian wetlands could be greatly impacted in response to draining because of warming alternations in rainfall patterns. These changes could lower the water table, thereby stimulating significant releases of GHGs and leading to nutrient depletion (Freeman e*t al*., 1993; Martikainen *et al.*, 1993).

Page *et al.* (2002) and Moore *et al*. (2013) reported an increase in drainage and clearance of tropical wetland swamps and forests for agricultural purposes. This action poses a serious threat to such ecosystems as they could fast be losing their status as net global carbon sinks and become net sources of GHGs, principally CO_2, which is the major greenhouse gas associated

with global warming (Hooijer *et al*., 2010). Global warming mitigation is increasingly becoming an interesting subject matter as the effects of climate change are becoming apparent around the World (Kayranli *et al*., 2010). Despite the vital ecosystem services offered by coastal wetlands, anthropogenic and biogeophysical activities that include population pressure, industrialization, uncontrolled tilling for agriculture, overgrazing, logging, marine and coastal erosion, and construction of dams remain a significant threat. In the context of climate change, it is therefore necessary to take every measure in addition to the Paris deal to prepare for the ecological implications of changes in the frequency and intensity of climate extremes (IPCC, 2014). Due to global warming, coastal wetlands are increasingly becoming vulnerable in response to increases in atmospheric temperatures. This condition could encourage the activities of soil enzymes notably hydrolases, the key indicators of organic matter decomposition in wetland soils as suggested by Freeman *et al*. (2001).

1.6. Enzymic latch mechanism

The main cause of slow rate of organic matter decomposition in the wetland soils is due to the activity of hydrolases enzyme (Freeman *et al*., 2001; Dunn *et al*., 2014). These enzymes have their origin in heterotrophic soil organisms and can cleave large and complex high molecular weight organic matter macromolecules, reducing them in the process for assimilation by microbes (Dunn, 2013).

Anaerobic conditions in wetland soils place a constraint on the activities of soil microbes resulting in slow rates of decomposition of organic matter (Freeman *et al*., 2001). Dunn *et al.* (2014) stated that the activities of extracellular soil enzymes influence the rate of decomposition of organic matter in wetland soils. Therefore, measurement of their activities in the soil could give an indication of the biogeochemical process, and nutrient and carbon cycling rates in such ecosystems. Furthermore, analysis of the activities of these extracellular soil enzymes gives clear understanding to researchers on how environmental factors could influence the microbial ecology of such ecosystems.

Most free or soluble soil enzymes could be traced to soil microbes where they are found integrated with soil colloids, attached to clay minerals and outer surfaces of dead and viable cells, from where they are synthesized and secreted, though, some plants and animals within the same environment contribute to the soil enzyme budget (Burns, 1982; Dunn *et al*., 2014).

From a number of investigations, Freeman *et al*. (2001) submitted that Phenol oxidase could control the concentration of phenolic compounds in peat matrix, which has consistently proved to suppress the activities of hydrolases, the leading suite of enzymes implicated with the breakdown of organic matter (Freeman *et al*., 2001). Similarly, outcome from further findings of Freeman *et al*. (2001; 2004), Fenner and Freeman (2011), and Freeman *et al*. (2012) revealed that the global northern peatlands are seen to be an unbalanced ecosystems, this is because the rate of primary production is higher than that of decomposition resulting in storage of 20-30% of the global soil carbon. This considerable amount of stored carbon could be linked to inhibition of a single extracellular soil enzyme, phenol oxidase, under low oxygen availability (Freeman et al., 2001). This then results in accretion of phenolic materials, which places a constraint on the activity of hydrolase enzymes in such peats thereby limiting the mineralization of organic matter. This condition is referred to as the 'enzymic latch' mechanism as proposed by Freeman *et al*. (2001; 2004). Saraswati *et al*. (2016) investigated the effectiveness of the enzymic latch in northern Florida red mangrove soils and discovered it is active. Figure 1.4 below illustrates how the enzymic latch operates in a wetland ecosystem.

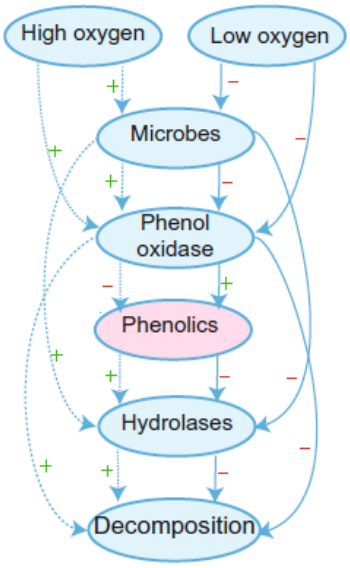

Figure 1.4: Schematic diagram showing the role of oxygen in controlling decomposition of wetland soils. Solid arrows show effects of increased oxygen and dashed arrows show effects of low oxygen in the 'enzymic latch' mechanism. '+' sign indicate stimulating (positive) effects and '-' sign indicate suppressing (negative) effects. Processes on the right-hand side occur in the 'enzymic latch' and the process on the left-hand side shows when the latch opens up. Adapted from Saraswati *et al*., (2016).

The activity and production of these important extracellular hydrolase enzymes in the soil can be supressed by some conditions which are both biotic and abiotic, this then encourages the accumulation of a huge quantity of high molecular weight organic matter in wetland soils and some group of polyphenols or phenolic compounds as well (Freeman *et al*., 2001).

Phenolic compounds are secondary metabolites of diverse organic compounds, characterized by the presence of benzene ring of aromatic compounds with hydroxyl groups or their derivatives such as esters and glucosidase. They include many classes of structurally diverse natural products arising from the shikimate-phenylpropanoid to flavonoids pathways, which have some antimicrobial properties. Virtually all plants contain various phenolic compounds depending on the type of plant (Bhattacharya, *et al.*, 2010). Polyphenols impact soil nutrients and affect the composition and actions of decomposer microbes and nutrient cycling in soils. Feralic acid, garlic acid or flavonoids can either stimulate or inhibit spore germination and hyphal growth of saprophytic fungi (Lattanzio *et al*., 2006). A variety of factors affects the activity of enzymes in soils, some of which are highlighted below.

1.7. Extracellular soil enzymes

Nutrient cycling in soils involves biochemical, chemical as well as physicochemical reactions with the biochemical process being mediated by soil microbes. Biochemical reactions are catalyzed by enzymes, which are proteins with catalytic properties owing to their power of specific activities (Reece *et al*., 2011). The presence of heavy metals in the soils may have an impact on the biochemical processes by affecting both microbial population and enzyme activities. This usually happens when the heavy metals obstruct enzyme activities by surrounding catalytically active groups, thereby creating denaturing effects on the conformation of proteins, or competing with the metal ions involved in the formation of enzyme-substrate complexes (Gianfreda *et al*., 1996). Soil enzyme activities could be used as an indicator to measure the activities of soil microbes which could involve its biochemical functioning including the formation of organic matter, rates of decomposition and nutrient cycling (Dick *et al.*, 1992).

1.7.1. Phenol oxidases

Phenol oxidase is a natural microbial soil enzyme and occasionally produced from exudates of certain plant roots which have the ability to degrade phenolic compounds from simple aromatics to complex polyphenols (Dunn *et al*., 2014). This group of enzymes could be

severely suppressed in wetlands due to the anaerobic soil conditions as it has an absolute affinity for oxygen (Freeman *et al.*, 2001). Studies have revealed high concentrations of phenolic compounds in coastal wetland soils such as mangroves and salt marshes among others. This then implies that phenol oxidase plays a central role in the rate of organic matter decompositions. Therefore its lack of activity in such predominantly anaerobic soils explains the low rates of enzyme activities and organic matter decomposition in wetland soils. Consequently, phenolic compound could be said to place a 'latch' or a constraint on the activities of hydrolase enzymes, preventing the release of large terrestrial stored carbon but instead facilitate its storage (Freeman *et al*., 2001; Marx *et al*., 2001).

1.7.2. Hydrolases

The activities of extracellular hydrolysing enzymes in soil control the rate of substrates degradation to become bioavailable for uptake by both plants and microorganisms, making them the key mediators of breakdown of soil organic matter, cycling of nutrients and mineralisation (Marx *et al*., 2001; Dunn *et al*., 2014). Therefore, the activities of hydrolase enzymes indicates a direct expression of biological activity of soil microbes and they are linked to the specific process of organic matter turnover and nutrient cycling in wetland ecosystems. Accordingly, suits of hydrolase enzymes, which include β-D-glucosidases, arylsulphatases, β-D-xylosidase, N-acetyl-β-D-glucosaminidase and phosphatases, have been associated with decomposition of substrates of varying composition and complexity (Jordan & Wagschal 2010; Deng & Popova, 2011; Dunn *et al*., 2014).

N-acetyl-β-D- glucosaminidase is widely distributed in nature and is involved in the breakdown of chitin $(C_8H_{13}O_5N)_n$, which is a significant fraction of humus bound nitrogen in wetland soils. The decomposition of chitin is therefore considered a vital process of nitrogen cycling (Kang *et al*., 2005). The enzyme arylsulphatases are from fungi and soil bacteria, though plants and animals can produce it too (Klose *et al.*, 2011). Arylsulphatases are involved in cycling of sulphur in the soil and catalyses the removal of sulphate ion from plant derived esters (Press *et al.*, 1985; DeForest, 2009). The phosphatase enzyme removes phosphate group from organic molecules. The dominant phosphatase enzyme in most soil is phosphomonoesterase (Turner e*t al*., 2002).

1.8. Factors affecting soil enzyme activities

Enzyme activities in the soil are controlled mainly by climatic and physicochemical factors such as temperature, pH, redox potential, inhibitors (e.g. phenolic) and activators (e.g., $Mg^{2+,}$ Ca^{2+}) and nutrient supply (Eed, 2012; Leake & Read, 1990). To a large extent, enzyme production in the soil depends on microbial activity which is regulated by temperature, water content, nutrient availability, and plant type (Insam, 1990). The rate at which decomposition of soil organic matter occurs is primarily facilitated by extracellular enzyme activities. Organic matter in wetlands is primarily comprised of high molecular weight polymeric compounds. These compounds are not assimilated directly by microbes, they must first be broken down into low weight molecular compounds by the hydrolyzing action of extracellular enzymes before assimilation by microbes (Sinsabaugh *et al*.,1991).

In wetlands, β-glucosidase, N-acetyl-β-D-glucosaminidase, and phosphatase which are synthesized and released by bacteria, fungi, and plant roots, play vital roles in accessing soil C, N & P cycles (Kang *et al*., 2005). A large proportion of soil enzymes are stabilized by colloidal organic matter or clays through an ionic bond or salt bridges. The binding of enzymes to clays and organic matter in the soil affects their activities by increasing their half-life even though the enzyme substrate affinity could decrease (increase in K_m and / or decreases in the maximum reaction rate (V_{max}). This could alter the velocity of the reaction in line with Michaelis-Menten equation: $V_1=V_{max}$ (S)/k_{max}+(S) (V is the velocity of reaction, and S is substrate concentration) (Tabatabai, 1971; Keating & Quinn, 1998).

1.8.1. Temperature

Enzyme activities in the soil are activated by an increase in temperature with the Q_{10} value of most enzymes ranging between 1.5 and 2.5 (Wallenstein *et al*., 2012). An increase in temperature has a corresponding increase in enzyme activity because substrates collide with active sites on the enzyme regularly as the molecule move rapidly (Reece *et al.*, 2011). An experiment to study the effect of temperatures at 9°C, 37°C, and 41°C on enzyme activity (Eed, 2012) revealed that activity at 37ºC was 3 times the enzyme activity at 9°C while enzyme activity at 41°C was about 1.5 times the activity at 37°C. Elevated temperatures could also indirectly affect enzyme activities due to bacterial proliferation and directly by modifying the enzyme kinetics. An increase in the global atmospheric temperature could have the same effect on coastal wetland biogeochemistry processes.

1.8.2. Drought

Water table drawdown in wetland soils increases oxygen availability thereby increasing enzyme activities by setting them free from inhibitors, e.g. the concentration of phenolic compounds, a potential inhibitor of hydrolysing enzymes in northern peatlands or any coastal or tropical wetland, may decline due to more activity of phenoloxidase thereby weakening the enzymic latch mechanism (Freeman *et al*., 2001).

Jing-Shuang (2005) reported that drought might alter enzyme activities and the rate of decomposition in wetland environment. Climatic changes may directly or indirectly affect microbial soil community and enzyme activities and the entire biogeochemical process in wetland ecosystem (Henry, 2013). Prolong drought could result in nutrient limitation due to substrate diffusion. Recent studies revealed that reduction in water level in both constructed and natural wetlands affected extracellular enzyme activities as well as decomposition rates, subsequently resulting in greenhouse gas emission and a shift in plant species composition (Venterink *et al.*, 2002; Wright & Reddy, 2001). Changes in soil water content equally affected nitrification and denitrification which controls N cycling in wetlands. Low water table could also result in increased concentrations of inhibitors such as Fe^{2+} (Hefting *et al*., 2004).

1.8.3. pH

Soil pH may also affect enzyme activity by changing the interaction between immobilized enzymes and the soil matrix, as each enzyme has an optimum pH (Frankenberger & Johanson, 1982). Enzymes can be altered by the concentration of hydrogen ions because they are made up of both acidic and basic amino acids. Variation in soil pH can have an impact on the activities of enzymes. pH is a factor, which, in part, is driving the effect of the biogeochemical processes in wetland soils (Dunn, 2013).

1.8.4. Nutrient availability

Various soil microbial communities control the rate of their enzyme production based on nutrient availability. The amount of nutrient in any wetland soils could, therefore, have an influence on the activities of enzymes. Altering environmental conditions, such as a rise in temperature and/or drop in water table could increase oxygen availability resulting in massive microbial proliferation and high rate of decomposition with high GHG emissions.

1.8.5. Redox potential

The decrease in redox potential influences enzyme activity by changing the microbial population or reducing metal ions which can inhibit soil enzyme activities (Pulford & Tabatabai, 1988). Changes in redox potential could have an impact on oxygen availability, water vapour content, pH, as well as temperature (de Mars & Wassen, 1999; De Groot & Van Wijck, 1993), e.g., redox potentials, increases with a decrease in pH (Wright & Reddy, 2001).

1.9. Objectives of the thesis

The primary objective of this PhD is to investigate the process of decomposition and the effect of the 'enzymic latch' mechanism in coastal wetland soils by determining the biogeochemical processes in mangrove and salt marsh ecosystems. This follows the initial novel work on the enzymic latch mechanism by Freeman *et al*. (2001) on peat samples from northern peatlands. This study analyses mangrove and salt marsh soils by determining the biogeochemical processes and GHGs fluxes and the extent to which the enzymic latch mechanism is active in these types of ecosystems.

The objectives of this study were:

1. To determine variation in organic matter and key constraints on the enzymic latch mechanism in the two types of coastal wetland soils: mangrove swamps and salt marshes
2. To determine the effect of mangrove encroachment into salt marsh habitats on organic matter and the enzymic latch mechanism
3. To assess the influence of vegetation litter in determining organic matter cycling in mangrove ecosystem

1.10. References

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Chapter 2

Carbon cycling and sequestration in coastal salt marshes

2.1 Abstract

Coastal salt marsh ecosystems are a dynamic wetland type, offering numerous ecosystem services. One of the most important is their ability to sequester globally significant amounts of organic carbon. This study examined the processes involved in carbon sequestration along the elevation gradient in an internationally recognised salt marsh habitat in north Wales, UK. Soil samples were collected from the high, mid and low tidal zones of the salt marsh habitat. The results showed that soil samples from the mid zone of the salt marsh is the most efficient for carbon sequestration. It had the highest soil water content and, as a result, the lowest rates of decomposition and greatest content of soil organic matter (SOM). This may be due to the presence of vegetation in the mid zone of the salt marsh and the effect that the roots have on trapping moisture in the soil. Although the low zone is closer to the sea, the sparse vegetation and absence of roots may be the main reason why the soils are less able to hold onto water and therefore effectively accumulate organic matter.

2.2 Introduction

Salt marshes are regarded as one of the World's most valuable sinks of blue carbon, which is carbon stored in the biomass and deep sediments of vegetated coastal ecosystems such as tidal marshes, mangroves, and seagrass beds. It is estimated that salt marshes can bury carbon at a rate 55 times faster than tropical rainforests (Macreadie *et al.*, 2013). The annual rate of carbon sequestration in salt marshes on a global scale has been estimated at up to 87.2 ± 9.6 Tg C yr^{1}, exceeding that of tropical rainforests (53 ± 9.6 Tg C yr^{-1}), despite the former occupying only 0.5-2% of the geographical range of the latter (Mcleod *et al.*, 2011; Macreadie *et al*., 2013). Macreadie *et al*. (2013) and Orson *et al*. (2018) stated that salt marshes, if left undisturbed, have the capacity to bury carbon in soil sediments for millennia, whereas the carbon stored in rainforests, which is principally as above-ground vegetation, lasts only for a few decades (Keller *et al*., 2012).

Recent studies have provided improved estimates of the rate of carbon sequestration in salt marshes. Bridgham *et al*. (2006) state that salt marshes accumulate carbon at an average rate of 220 g C m^{-2} y^{-1}, whilst Sousa *et al*. (2017) estimate a very similar rate of 245 ± 26 g C m^{-2} y^{-1}. Arriola & Cable (2017) provide a range of 49.5-109.5 g C m^{-2} y^{-1}, stating that variability is due to differences in vegetation type and density, inundation range, sediment sources and geomorphology.

The high rate of carbon burial in salt marshes is attributed to a high rate of primary production exceeding that of decomposition, which results in a build-up of organic matter in the soil (Persico *et al*., 2017). Accretion of organic matter in salt marshes is mainly due to the slow and incomplete decomposition of plant biomass in the anoxic soils and sediment (Chmura *et al*., 2003; Mudd *et al*., 2009). Temperature, microbial community, redox potential, vegetation type, and hydrology regime all influence the rate of decomposition of organic matter in salt marsh ecosystems, similar to other wetlands types. Vegetation type and density are key influences in organic matter accretion because they slows down water velocities and facilitates the physical binding of organic matter in the soil profile (Drake *et al*., 2015; Whiting, 2016), as it could also prevent erosional losses. Decomposition rates in salt marsh ecosystem are a key control of the build-up of organic matter and net carbon sequestration will raise the height of the land surface. In the event of a rise in sea level, salt marshes will play a vital role in protecting coastal ecosystems from the effect of climate change (Morris *et al*., 2002).

Despite their role as regulators of global climate and providers of protection from sea level rise, the extent of coastal salt marshes is declining at an alarming rate. It is estimated that 25% has been lost since the 18th century, due primarily to anthropogenic land use changes (Beach & Kennish, 2001; Coverdale *et al*., 2013; Macreadie *et al*., 2013; Persico *et al*., 2017). Current rates of salt marsh losses are estimated at 0.7–7% per year (Hopkinson *et al*., 2012; Arriola & Cable, 2017). The loss of salt marsh habitats has serious implications for global carbon storage, with anthropogenic changes often converting these systems from net sinks to net sources (Macreadie *et al*., 2013; Henry, 2014; Pavlov *et al*., 2014). This has raised some concerns that huge quantities of ancient buried carbon could be lost to the atmosphere, which could accentuate climate change (Macreadie e*t al*., 2013).

Based on elevation and the frequency of tidal inundation, salt marshes are zoned into low marsh areas, which are frequently flooded, middle marshes, and high marsh areas that are less frequently inundated (Rabenhorst, 2001), as seen in Figure 2.1.

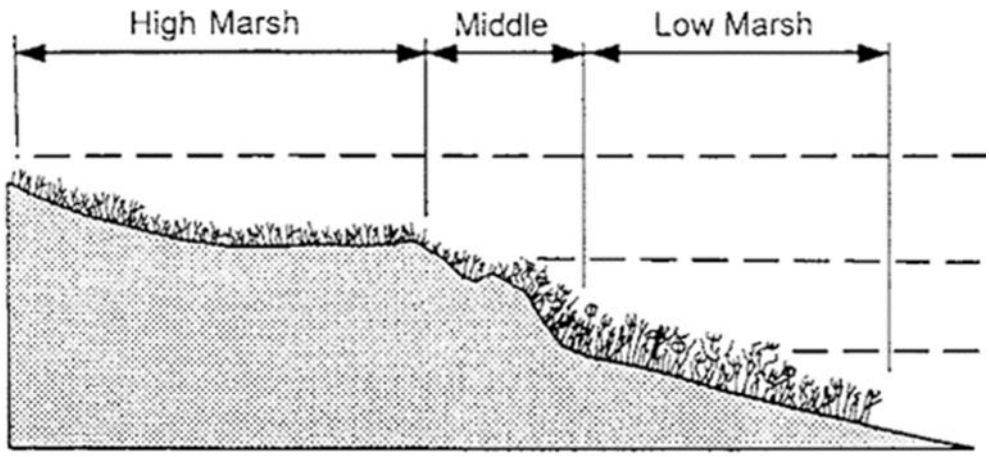

Figure 2.1. Classification of a typical coastal salt marsh into low, middle, and high marsh zonse base on proximity to water edge (Rabenhorst, 2001)

The decomposition of plant litter materials in salt marsh vegetation occurs in three stages, as described by Valiela *et al*. (1985). The first phase is characterized by loss in weight of about 5-40% of soluble materials due to leaching. The second phase is quite slow due to the anaerobic conditions in the soil and could last close to a year. This step involves microbial and biochemical actions with degradation of organic matter, as well as subsequent leaching of hydrolyzed substances, resulting in the losses of about 40-70% of the original materials. The

third and final stage involves the slow decomposition of the remaining refractory materials, which is about 10% of the original material and could last about a year. Sequestered organic matter in a salt marsh system is largely determined by primary productivity in the mid and high zones (Bouchard & Lefeuvre, 2000; Henry, 2014).

In recent years, increasing anthropogenic GHG emissions have raised global atmospheric temperatures, causing significant changes in ecosystem services. In response to warmer climatic conditions, some tropical and sub-tropical vegetation, especially mangroves, are shifting northwards displacing coastal salt marshes. Alterations in such marshes could alter the structure and compromise ecosystem services of such environments, with implications for carbon cycling in one of the World's most important carbon sinks (Yando *et al*., 2016; Martin *et al*., 2017). Climatic changes such as a rise in sea level, subsidence intensity, and frequency of storm surges could have implications for stored carbon in coastal salt marshes and adjacent coastal forest or communities. Once threatened by sea rise level at the land-sea interface, coastal salt marshes could be pushed further landward, encroaching or literally taking over coastal land forests and could even displace adjacent coastal communities beside destroying potential carbon storage sites and may increase the release of organic matter into the ocean (Morris *et al*, 2002; Arriola & Cable, 2017). Anthropogenic alterations due to land use changes, such as land reclamation for agriculture, livestock grazing and drought in coastal salt marshes could compromise the ability of such ecosystems to store carbon by smothering or killing the vegetation. Drought can stimulate microbial activities, enhance soil respiration and export centuries of stored carbon. The loss of carbon in coastal salt marshes has been primarily linked to changes in land use, which have resulted in carbon losses in the last half century (Wantzen *et al*., 2012).

A salt marsh exists along an elevation gradient, and will be influenced by contrasting levels of inundation and consequently soil oxygen concentrations. Therefore, it would be relevant to investigate whether different zones of a salt marsh are more or less effective at sequestering carbon. This study examined the soil organic matter contents and decomposition rates in the high, mid and low zones of a salt marsh in north Wales, UK. The hypothesis is that the low zone of the salt marsh will have a higher capacity to sequester carbon due to more frequent flooding by seawater and therefore more anoxic soils that have low rates of organic matter cycling.

2.3. Materials and methods

2.3.1. Study site and sample collection

Soil samples were collected from Cefni salt marsh (Figure 2.2), within the Malltraeth estuary, on the island of Anglesey, north Wales, United Kingdom (SH400664). The region is protected for its sand dune and salt marsh habitat under Site of Special Scientific Interest and Special Area of Conservation designations. The vegetation community is identified as Atlantic Salt Meadow, dominated by *Salicornia* species. This habitat is characterised into three zones of high, mid and low base on proximity to the sae and gradient. The low zone is the closest to the water edge and poorly vegetated with a longer period of inundation due to tidal action, followed by the mid zone which a thicker vegetation cover. The high zone is situated further inland at a higher gradient and less frequently subjected to tidal action with a thicker vegetation.

Figure 2.2. Sampling location within Maltreat estuary, north Wales, UK

Sampling was undertaken at the high, mid and low marsh zones based on the difference in vegetation communities and cover between the three sites during the low tide period on 6th July 2016. A grab sample of soil (approx. 200g) was collected randomly within each zones at at a distance of about 3 meters apart were vegetation characteristic were similar from a depth of 10-12 cm using a trowel at five randomly chosen locations within each of the three zones. Large items such as stones, shells and vegetation parts were removed before the sample was

placed in a labelled plastic bag and sealed. GPS coordinates and soil temperature at each sampling point was recorded (mean temperature values - low: 16.3°C, mid: 15.0°C, high: 15.2°C). All samples were stored in the cold room at 4°C before use within two weeks.

2.3.2. Laboratory analysis

Before any biological analyses were undertaken, all soil samples and required reagents were incubated at field temperature for 24 hours in order to conduct the analyses at field temperature, which helps to replicated field conditions (see Fenner *et al*., 2005 and Kang & Freeman, 2009). Soil samples were homogenized by hand thoroughly for about two minutes to ensure uniformity, and to remove any unwanted debris. All laboratory chemicals, biological reagents and enzyme substrates used in this experimental investigation were certified analytical grade acquired from Sigma-Aldrich Company limited (Poole, Dorset, UK). Plastic consumables, such as Eppendorf micro-centrifuge tubes, pipette tips, 50 ml falcon tubes and 96 well microplates were supplied by Fisher Scientific (Loughborough, UK).

2.3.2.1. Soil water and organic matter contents

Soil water and organic matter weights were determined following the method of Frogbrook *et al.* (2009). Approximately 5g of soil were placed in pre-weighed crucibles, weighed again and placed in an oven (Memmert- Schwabach, Germany) at 105°C for 24 hours to ensure thorough evaporation of soil water content. After weighing again, the crucibles were placed in a muffle furnace (Carbolite, Sheffield, UK) for 120 minutes at 550°C and weighed again thereafter. The weights were used to calculate the water and organic contents as a percentage of the original sample on excel spreadsheet.

2.3.2.2. Water extraction and water chemistry

A water extract was prepared by adding 5g soil to a 50ml falcon tube, followed by 40ml deionised water. The tubes were placed on a digital rotary shaker (KIKA®-Werke, Gmbh & CO.KG, Germany) at 300 rpm for 24 hours. pH and electrical conductivity were determined on an aliquot of the water extract using SevenEasy and FiveGo (Mettler-Toledo, Leicester, UK) bench-top meters respectively. The pH meter was calibrated with pH 4 and 7 buffers, and the conductivity meter with 84 µS cm^{-1}, 1,413 µS cm^{-1} and 12.88 µS cm^{-1} solutions. Following determination of pH and conductivity, the samples were centrifuged at 5000 rpm using the Sorall ST-16R centrifuge (Thermo Scientific, UK) and filtered through 0.45 µm filters (Cole Palmer, St. Neots, UK).

2.3.2.3. Measurement of the activity of phenol oxidases

2.3.2.3.1 Principles of the assay

Determination of the activity of phenol oxidase uses L-DOPA (phenolic amino acid L-3,4-dihydroxyphenylalanine) as the model substrate (Pind *et al*., 1994). L-DOPA and phenol oxidases form an enzyme-substrate complex resulting in the formation of a red pigment compound called dopachrome (2-carboxy-2, 3-dihydroindole-5, 6-quinone) (Figure 2.3), which has a molar extinction coefficient of 3,700 M^{-1} cm^{-1} at 475nm (Pomerantz & Murthy, 1974). The absorbance of dopachrome can be measured on a spectrophotometer with the presence of dopachrome being proportional to enzyme activity.

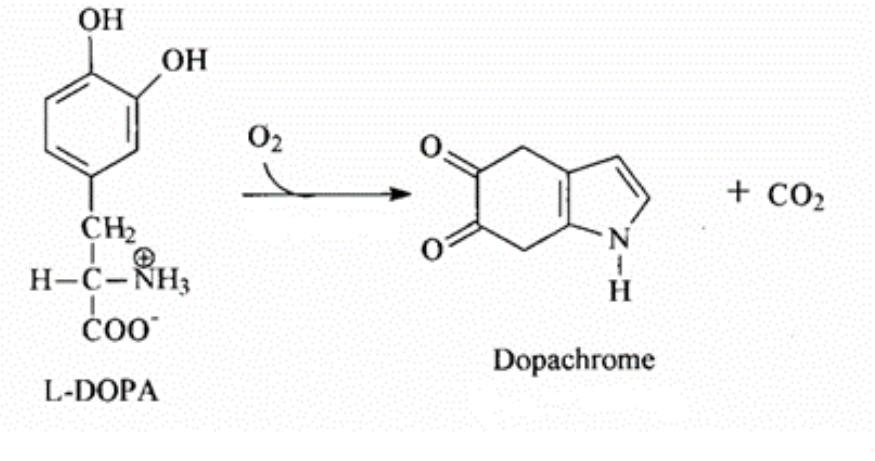

Figure 2.3: The oxidation of L-DOPA (L-3,4-dihydroxyphenylalanine) to dopachrome (2- carboxy-2,3-dihydroindole-5,6-quinone) by phenol oxidases during the enzymic assay.

2.3.2.3.2. Assay procedure

The activity of phenol oxidase was determined using the methods outlined in Dunn *et al*. (2014). A 10mM solution of substrate was prepared by weighing 0.986g L-DOPA, transferring to a dry 500ml capacity glass bottle, adding 500 ml deionized water and dissolving with the aid of a magnetic stirrer. Once the reagent and soil samples were at field temperature, two separate 1 g (±0.05) sub-samples of soil were placed in separate stomacher bags (Seward, Worthing, UK). Nine ml deionized water (maintained at a field temperature with soil samples) was added to each bag and the bags homogenized in a laboratory paddle blender (Stomacher® circulator, Seward, Worthing, UK) for 30 seconds. Ten ml deionized water was pipetted into one of the bags (‘blank’) and 10 ml L-DOPA solution was pipetted into the other bag (‘substrate’). Both bags were further homogenized in the laboratory paddle blender for 30

seconds and then incubated at field temperature for ten minutes to ensure linear oxidation of L-DOPA. After the allotted time, homogenate from each bag was transferred into three 1.5 ml Safe-Lock Eppendorf microcentrifuge tubes and centrifuged at 10,000 rpm for 5 minutes. 300 µL of the supernatant was then transferred from each tube into a well of a clear 96-well Sterilin microplate (Triple Red, Buckinghamshire, UK). The absorbance of the red pigment (dopachrome) was measured on a SpectraMax M2e spectrophotometer (Molecular Devices Ltd., Wokingham, UK) at 475nm.

2.3.2.3.3.. Calculating the activity of phenol oxidase

In order to calculate the activity of phenol oxidase in the soil sample, the average value of the blank sample was subtracted from the average value of the substrate sample. This then gives an average absorbance value (A); this was then used in the following formula based on the Beer-Lambert Law regarding the relationship between absorbance and transmission of ultraviolet and visible light:

$$\boldsymbol{c = A / \varepsilon b}$$

where c = concentration of phenol oxidase (mols)

A = average absorbance

ε = molar absorption coefficient for phenol oxidases (i.e. 3,700 M^{-1} cm^{-1})

b = path length (when using 300µL in a 96 well Sterilin® microplate this is 0.865cm)

Expressing the activity of phenol oxidase in the correct units, the following equation was used:

Phenol oxidase activity (nmol dicq g^{-1} min^{-1}) = ((c x (V_{total}/V_{soil}) x 10 x 1,000) / w) / t

where c = concentration of phenoloxidase (mols)

V_{total} = total volume of solutions in Stomacher bag (i.e. 19,000µL)

V_{soil} = volume in Stomacher bag occupied by soil homogenate (i.e. 10,000µL)

10 = dilution of peat used in original homogenate (i.e. 1 in 10)

1,000 = conversion of activity from µM to µmol

w = dry weight of 1 g of wet peat used in the assay (g)

t = length of reaction time (min) e.g. 10 minutes

The activity of phenol oxidase is expressed as nmol of product formed (dopachrome or 2-carboxy-2, 3-dihydroindole-5, 6-quinone, referred to in the equation as diqc) per minute (min^{-1}) per gram (g^{-1}) of the dry weight of soil - nmol dicq g^{-1} min^{1}.

2.3.2.4. Measurement of the activity of hydrolases

2.3.2.4.1. Principles of the assay

Following an enzymic reaction in the soil, the hydrolase enzymes break down organic matter into smaller components, becoming bioavailable for microbes and respired as CO_2 in the process of mineralization. The presence of phenolic compounds in wetland soils directly inhibits the activities of hydrolase enzymes. Consequently, the activity of the enzyme phenol oxidase is fundamental in indirectly regulating the activities of hydrolases enzymes (Freeman *et al*., 2001; Dunn *et al*., 2014).

To quantify hydrolase enzyme activities, assays utilize fluorogenic 4-methylumbelliferone (MUF) (Figure 2.4) as the enzyme substrates (Freeman *et al*., 1995; Dunn *et al*., 2014). The hydrolase enzyme is able to break the bond between the MUF molecule and the target compound, causing the MUF molecule to fluoresce, which could be determined spectroscopically. The fluorescence can then be measured and is proportional to the activity of the enzyme (Dunn e*t al*., 2014).

Successful determination of hydrolases enzyme activities has some challenges, such as:

- interferences caused by the highly colored phenolic compounds present in a high concentrations in wetland soils (Freeman *et al*.,1995).

- quenching, which reduces the intensity of fluorescence in the detection of MUF in soil samples.

- pH and temperature effects on fluorescence (Dunn, *et al*., 2014).

Freeman *et al.,* (1995) reported quenching of 21-61% for wetland soils. Therefore, in this method, a calibration curve for each soil is required for quantification of MUF in any given sample to minimise this effect. The concentrations of MUF calculations are based upon a calibration curve and enzyme activities are expressed as milli moles (mmol) or micromoles (µmol) of MUF released per gram (g^{-1}) of soil, per hour (h^{-1}).

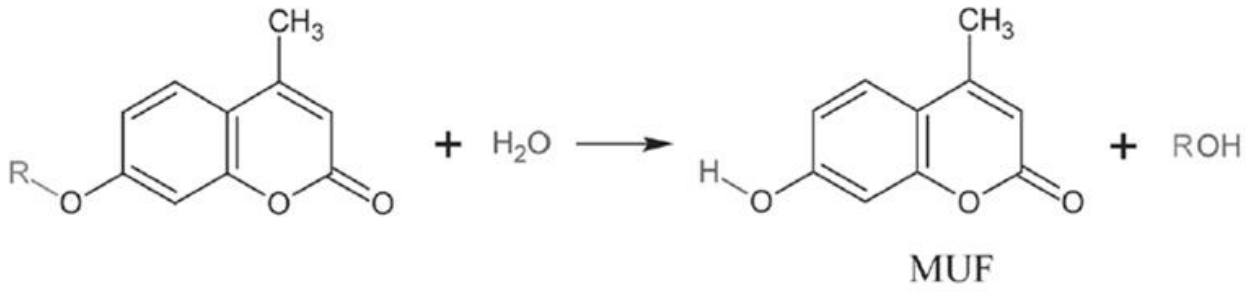

Figure 2.4: Structure of 4-methylumbelliferone.

2.3.2.4.2. Procedure for hydrolases soil enzyme assay.

Five hydrolase enzymes were tested; β-D-glucosidase, arylsulphatase, β-D-xylosidase, N-acetyl-β-D-glucosaminidase and phosphatase. The solutions of the enzyme substrates were prepared by dissolving the required quantity to make a 1L solution of 400 µM 4-MUF (200 µM for phosphatase), as described in Table 2.1. These concentrations are used to ensure optimal enzymic activity for wetland soils (Freeman *et al*., 1995). Following addition of the compound to a 1L flask, 20 ml 2-Ethoxyethanol (Cellosolve) solvent was added to the flask to allow the compound to dissolve. The flask was made up to a final volume of 1000 ml with deionized water and stirred on the magnetic stirrer where necessary.

For the calibration curve, a 1,000 µM MUF stock solution was prepared by dissolving 0.0881g MUF-free acid (4-methylumbelliferone sodium salt, Sigma Aldrich Ltd, Dorset, UK) in a 500 ml bottle. Ten ml of cellosolve solvent (2-Ethoxyethanol) added, then deionized water to a final volume of 500mL and placed on the magnetic stirrer as required (Dunn e*t al*., 2014).

Table 2.1.The MUF-labelled substrates needed for measuring the specified enzyme's activity

Substrate	**Enzyme**	**Enzyme commission number**	**Grams needed for 1L 400µM (200µM for Phosphatase)**
4-MUF β-D-glucopyranoside	β-glucosidase	3.2.1.21	0.1353
4-MUF Sulfate potassium salt	Arylsulphatase	3.1.6.1	0.1177
4-MUF β-D-xylopyranoside	β-D-xylosidase	3.2.1.37	0.1233
4-MUF N-acetyl-β-D-glucosaminide	N-acetyl-β-D-glucosaminidase	3.2.1.52	0.1518
4-MUF phosphate	Phosphatase	3.1.3.2	0.0512

After thorough homogenizing of the soil by hand, six 1g (±0.05) subsamples were placed into separate stomacher bags (5 for each enzyme and 1 for the calibration standards). To each of the 5 enzyme bags, 7 ml of the relevant substrate (maintained at field temperature) was added to the relevant stomacher bag and homogenised in a laboratory paddle blender for 30 seconds. All of the bags were incubated at field conditions for 60 minutes except phosphatase, which was for 45 minutes. Following incubation, one 1.5 ml microcentrifuge tube per bag was filled with sample and centrifuged in a micro centrifuge (Eppendorf 5415C Centrifuge, Stevenage, UK) at 10,000 rpm for five minutes. Following centrifugation, 250µl of supernatant was added to the wells of a black flat-bottomed 96-well Sterilin microplate (Sterilin, Cambridge, UK). To each of these wells, 50 µl of deionized water was added to ensure a comparable dilution to the standard solutions in the calibration curve.

To the sub-samples in the remaining stomacher bags, which are for the calibration curve, 7ml of deionized water was added, homogenized, transferred to centrifuge vials (2 per sample) and centrifuged, as previously described (no need for incubation). After centrifugation, 250µl of supernatant was transferred into 8 separate wells for each sample (approx. 4 x 250µl from each of the 2 vials). A calibration curve of MUF was created by adding 50µl of standard solution to

these 8 wells, following the creation of a dilution series from the 1000 µm stock MUF solution (0, 5, 10, 20, 40, 60, 80, 100 µm).

The microplate was analysed on a SpectraMax M2e spectrophotometer (Molecular Devices Ltd., Wokingham, UK) at 330 excitation, 450 nm emission to determine the fluorescence of the MUF (Dunn *et al*., 2014).

2.3.2.4.3. Calculation of hydrolases enzyme activity

MUF concentrations in the reaction mixtures were calculated against the calibration curve created for each sample. The enzyme activity was then calculated as follows:

$$\textbf{Enzyme activity (µmol g}^{-1}\textbf{ h}^{-1}\textbf{) = F / M}_{dry}\textbf{ / t}_{assay}\textbf{ / 8}$$

Where: F = fluorescence value

M_{dry} = dry weight of 1 g of wet soil

t_{assay} = time of assay, e.g. 60 minutes or 45 for phosphatase.

8 = converts the original units from µM to µmol, due to the original dilution (1:8) of the soil to substrate mixture in the Stomacher bags.

2.3.2.5. Measurement of soil total phenolic concentration

2.3.2.5.1. Principles of the assay

Phenolic compounds are those that contain a phenol group. Phenol itself is the simplest phenolic compound (Figure 2.5), while those such as lignin (Figure 2.6) and tannin (Figure 2.7) are much more complex, containing several phenol groups.

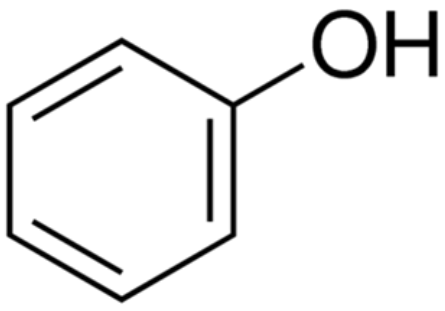

Figure 2.5: Phenol compound

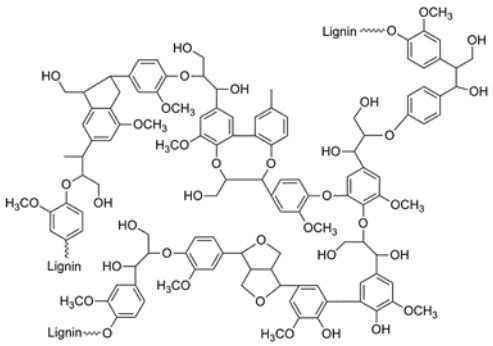

Figure 2.6: Structure of Lignin

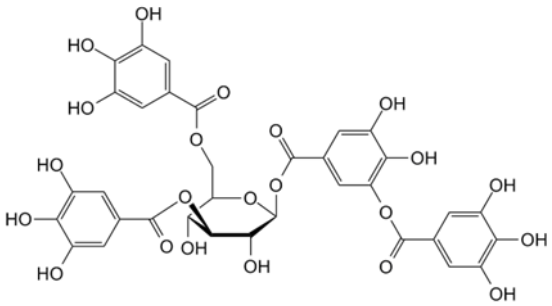

Figure 2.7: Structure of Tannin

Laboratory analyses tend to use colorimetric procedures, involving UV/visible absorbance analysis, because they are easy and reliable methods (Yu & Dahlgren, 2000). The Folin-Ciocalteau reagent procedure is one of the most popular (Bueno *et al*., 2012; Blainski *et al*., 2013) and works effectively for wetland soil samples (Freeman *et al*., 2001; Fenner *et al*., 2005; Dunn *et al*., 2014). It quantifies the total concentration of phenolic hydroxyl groups in a sample. Polyphenols react with Folin-Ciocalteu reagent to form a blue chromophore constituted by a phosphotungsticphosphomolybdenum complex (Blainski *et al*., 2013). The maximum absorption of the chromophores is contingent upon the concentration of phenolic compounds. However, this reagent rapidly decomposes in alkaline solutions, which necessitates the use of excess reagent to obtain a complete reaction. This excess can result in precipitates and high turbidity, making spectrophotometric analysis difficult. To resolve this problem, Folin and Ciocalteu's reagent is comprised of lithium salts which minimises the turbidity (Blainski *et al*., 2013). Subsequently, 'the reaction generally provides accurate and specific data for several groups of phenolic compounds, because many compounds change colour differently due to differences in unit mass and reaction kinetics' (Blainski *et al*., 2013).

Phenolic compounds place a constraint on the activity of hydrolase enzymes in wetland soils (Freeman *et al*., 2001; Dunn *et al*., 2014). However, they can be decomposed to less complex compounds by a specific group of extracellular enzymes, phenol oxidases (Freeman *et al*., 2001). The anaerobic conditions in wetland soils leads to low activity of phenol oxidase, encouraging the accumulation of phenolic compounds and the suppression of hydrolase enzymes.

2.3.2.5.2. Assay procedure

The phenolic assay was carried out based on methods described by Box (1983) and Dunn *et al*. (2014). Firstly, two solutions must be prepared; phenol stock solution and sodium carbonate buffer. To prepare the 1000 ppm phenol stock standard solution, 0.25 g of phenol compound

was weighed on an analytical balance, transferred to a clean 250 ml volumetric flask, and filled to the mark with deionised water. The calibration solutions were prepared by diluting the 1000 ppm stock solutions according to table 2.2, to create a range of calibration standards from 0-30 ppm in 100 ml volumetric flasks. After adding the relevant volume of stock solution, the flasks were filled to the 100 ml mark with deionized water and stored at 4°C until required.

Table 2.2. Stock solution of phenol standards for calibration curve

Phenol concentration (ppm)	**1000 ppm phenol stock (mL)**
0.25	0.025
0.50	0.050
0.75	0.075
1.0	0.1
2.0	0.2
3.0	0.3
4.0	0.4
5.0	0.5
6.0	0.6
8.0	0.8
10.0	1.0
15.0	1.5
20.0	2.0
30.0	3.0

To prepare the sodium carbonate solution, 200g of Na_2CO_3 (Sigma-Aldrich UK Ltd) was added to a 1litre flask and filled with deionized water. The solution was placed on the magnetic stirrer for 1 hour to ensure that all of the compound dissolved completely. The

solution was then filtered using a 47 mm GF/F (Phenomenex, Macclesfield, UK) filter on a vacuum pump.

The concentration of water extractable phenolics was determined on the water extract samples. The standards 0, 0.25, 0.5, 0.75, 1, 2, 3 ppm were chosen because the samples were slightly coloured. For each calibration standard and sample, 1 ml was pipetted into separate labeled 1.5 ml eppendorf microcentrifuge tubes. To each of these, 50 µl of Folin-Ciocalteau Phenol Reagent (Sigma-Aldrich UK Ltd) and 0.15 ml of Na_2CO_3 solution was added to all tubes and they were mixed by turning the vials upside down in a rack a number of times. The samples were left to incubate for 1 hour 15 minutes at room temperature, during this time a gradual colour change to blue was observed, indicating the presence of phenolics. After the incubation period, three replicates of 300 µl of each standard and sample was transferred into wells of a clear 96 well microplate. The plate was analysed for absorbance at 750 nm on a SpectraMax M2e Spectrophotometer (Molecular Devices Ltd., Wokingham, UK). The instrument averages the three replicates of each standard and sample and calculates the concentrations of the samples from the calibration curve.

2.3.2.6. Microbial soil respiration

2.3.2.6.1. Principles of gas chromatography

Biogeochemical cycles in wetland soils include compounds in gaseous phases and these gases are generally referred to as trace gases. The majority of trace gases cycle in low concentrations, often being rapidly taken in by plants and the soil or involved in other chemical conversions. The cycling of the relatively inert gases CO_2, CH_4 and N_2O however, are more significant and, coupled with their importance as greenhouse gases, make them the subject of most soil respiration studies. The gas generally produced by wetlands in the largest quantities is CO_2, mainly due to the microbial respiration continuously breaking down organic matter (Gammelgaard *et al*., 1992; Vasander & Kettunen, 2006). In the predominantly anoxic soils in wetlands, aerobic metabolism is less prevalent, and therefore CO_2 emissions are lower. Instead, anaerobic metabolism by *Archaea* (methanogenic microorganisms) favour CH_4 production through the utilisation of a limited set of substrates, hydrogen and acetate being the most important (Segers, 1998). Some of this CH_4 can be oxidised to CO_2 in the uppermost layer of the soil profile by methanotrophic microorganisms (Lai, 2009; Freeman *et al*., 2002). N_2O production is linked to the microbial soil processes of nitrification and denitrification (Freeman *et al*., 1993).

Gas chromatography is a technique for separating compounds in gaseous or liquid phases. There is a mobile phase, in this case a carrier gas (generally helium, hydrogen or nitrogen) that carries the components of the mixture over a stationary phase (a column usually wound into spiral coil) where the compounds are separated according to the degree to which they interact with the stationary phase. Those compounds that interact the most will move through to the detector fastest. This instrument is fitted with two detectors; a flame ionisation detector (FID) and electron capture detector (ECD). The FID consists of a hydrogen flame burning in air. As a substance leaves the column, it burns in the flame producing ions, which can be detected by measuring the electrical conductivity of the flame. The conductivity is proportional to the concentration of the sample. With the ECD, as the sample is carried through the column, the electrons are temporarily excited to a higher energy level. When this energy is reduced, the electrons are captured by the ECD detector, which is reflected as a chromatography peak.
Analysis of CO_2 by gas chromatography can be problematic due to the inert nature of the CO_2 molecule. To overcome this, catalytic converters can be fitted to convert CO_2 to something more easily detected. In this case, a methaniser has been fitted to convert CO_2 to methane. This allows for the detection of CO_2, converted to CH_4, using the same FID.

2.3.2.6.2. Procedure

Soil respiration was performed by placing 10 g of homogenized soil into a 50 ml falcon tube fitted with rubber septa in the lids and incubated at field temperature for a period of 60 minutes. After the allotted 60 minute period of incubation, gases were collected from the tubes using a 10 cm^3 syringe fitted with a short bevel hypodermic needle (Sigma Aldrich Ltd, Dorset, UK) and transferred into labelled (Time 2; T2), pre-evacuated 10 ml Exetainers (Labco Ltd, Lampeter, UK) fitted with screw caps with pierceable rubber septa. An air sample from above the centrifuge tubes containing the samples at the start of the experiment was collected into five exetainers labeled as Time 1 (T1).

Gas samples were analyzed on a Varian model 450 gas chromatograph (GC) instrument, fitted with a flame ionization detector (FID) and a catalytic converter (methaniser) to measure CO_2 and CH_4 concentrations, and an electron capture detector (ECD) for N_2O. Oxygen-free nitrogen is used as the carrier gas. CH_4, CO_2, and N_2O (retention times 1.08, 1.87 and 2.25 minutes respectively) were quantified by comparison of peak area with that of the three standards of

known concentrations (Table 2.3), prepared by Scientific and Technical Gases Ltd (Newcastle under Lyme, Staffordshire, UK), used in the preparation of a standard curve.

Table 2.3.Concentrations (ppm) of the commonly used standard gases

	CO_2	**CH_4**	**N_2O**
Standard A	**250**	**2**	**0.5**
Standard B	**500**	**25**	**1**
Standard C	**1,000**	**50**	**2**

The measured concentrations were used to calculate a flux, based on the difference between the T1 and T2 samples and corrected for the 10 g weight of soil and 60-minute incubation time. Fluxes of the greenhouse gases are expressed in $\mu g^1\ g^{-1}\ s^{-1}$. As a general commentary, all analytical processes are clearly described in detail in Chapter 2.

2.3.3. Statistical analyses

Significant differences in the measured parameters between the three zones were tested for using one-way ANOVA. Relationships between the measured variables were tested using Pearson's correlation. All statistical tests were performed using IBM SPSS v22 (IBM Corporation, New York, USA).

2.4. Results

The mid zonation area had the highest soil water (56.23%) and soil organic matter (9.98%) content, significantly greater than the low and high zones (Figure 2.8; $p<0.05$). The soil water content of the low and high zone were 25.23% and 24.08%, and the SOM contents 1.52% and 2.31% respectively. These differences are not significant for either parameter ($p>0.05$).

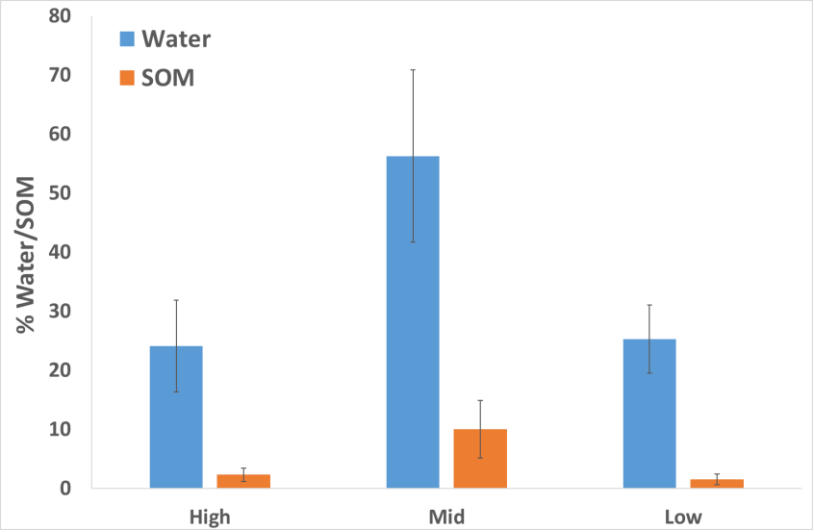

Figure 2.8: Bar chart showing mean soil water and organic contents in each salt marsh zone (n=5, error bars ± SD)

The high and mid zones of the salt marsh had a near neutral pH; 7.34 and 7.02 respectively, and this difference is not significant (Figure 2.9, $p>0.05$). The low zone had a significantly higher mean pH of 7.95 compared to both ($p< 0.05$).

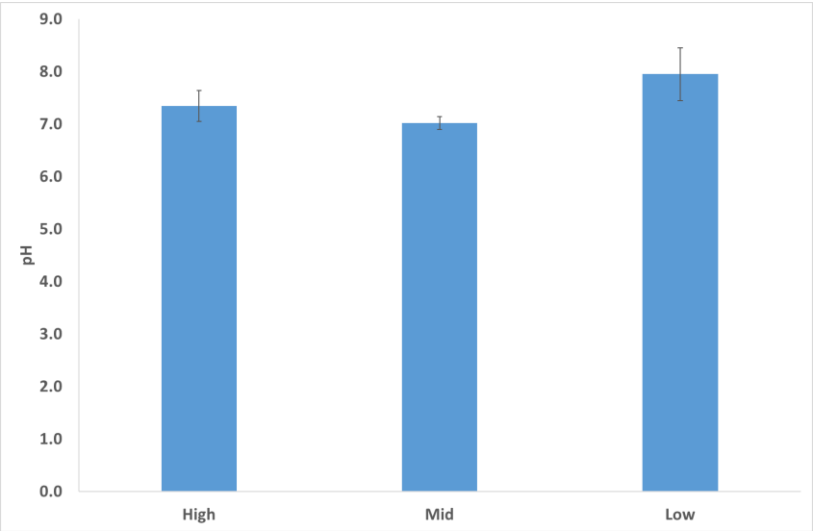

Figure 2.9: Bar chart showing mean soil pH at the three salt marsh zones (n=5, error bars ± SD)

The phenol oxidase activity (Fgure 2.10) was greatest in the low zone, with an average activity of 20. 96-µmol dicq g^{-1} h^{-1}. The activity in the high and mid zones was significantly lower, at 6.44 and 3.50 µmol dicq g^{-1} h^{-1} respectively ($p<0.05$). The high and mid zones activities are not statistically significant ($p<0.05$).

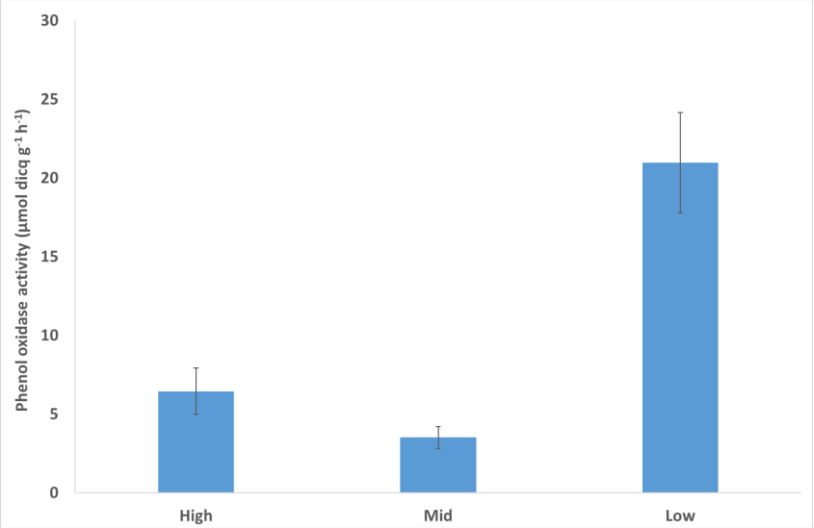

Figure 2.10: Bar chart of mean phenol oxidase activity at the three salt marsh zones (n=5, error bars ± SD)

The phenolic concentration of the soil was found to be highest in the mid zone, with an average concentration of 53.25 μg^{-1} g^{-1} (Figure 2.11). This is significantly higher than both the high and low zones ($p<0.05$). The concentrations in the high and low zones were 15.66 μg^{-1} g^{-1} and 4.18 μg^{-1} g^{-1} respectively; these values are not significantly different.

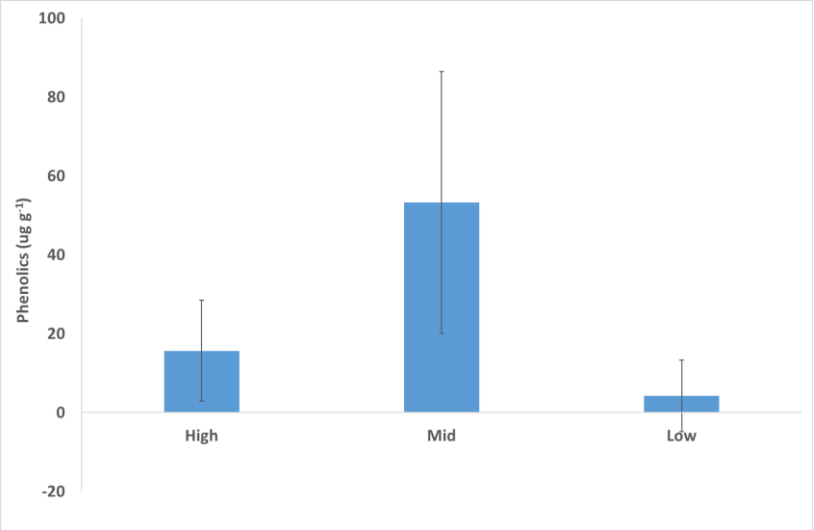

Figure 2.11: Bar chart of mean phenolic concentration at the three salt marsh zones (n=5, error bars ± SD)

Figure 2.12 shows the activity of the five hydrolase enzymes; β-D-glucosidase, arylsulphatase, β-D-xylosidase, N-acetyl-β-D-glucosaminidase, and phosphatase. A similar trend is observed for all enzymes; similar values for the high and low zones and much lower activities in the mid zone. The difference between the high and low zones is insignificant for all enzymes ($p>0.05$) but the difference between the high and mid zones is significant for all enzymes ($p<0.05$). The difference between the mid and low zones is significant for β-D-glucosidase, β-D-xylosidase and phosphatase ($p<0.05$) but not arylsulphatase and N-acetyl-β-D-glucosaminidase ($p>0.05$).

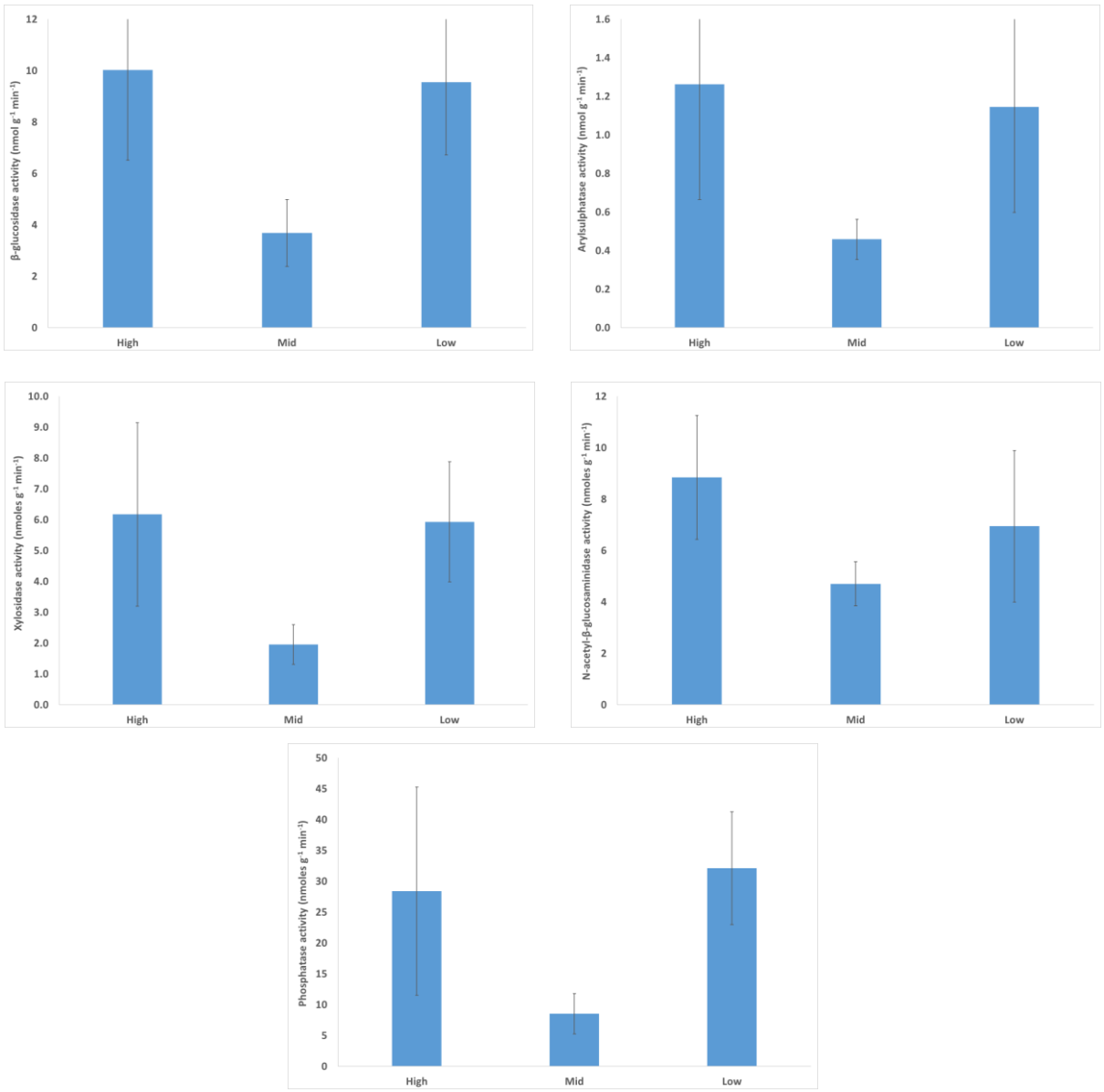

Figure 2.12: Bar chart of mean hydrolase enzyme activities at the three salt marsh zones (n=5, error bars ± SD)

Figure 2.13 shows the flux of carbon dioxide for the three salt marsh zones. The CO_2 flux from the mid zone (6.79 ug g^{-1} h^{-1}) was significantly greater than the negative flux recorded for the high zone (-2.29 ug g^{-1} h^{-1}; $p<0.05$). The flux from the low zone (1.30 ug g^{-1} h^{-1}) was not significantly different to either ($p>0.05$).

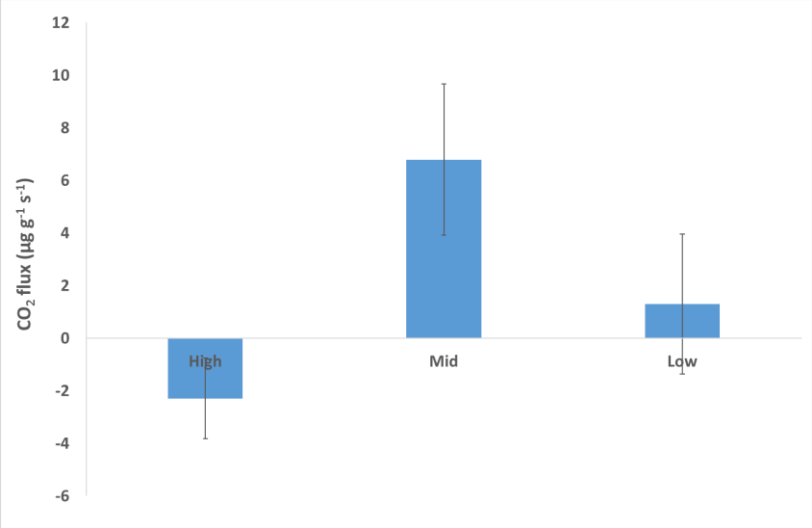

Figure 2.13: Bar chart of mean carbon dioxide flux at the three salt marsh zones (n=5, error bars ± SD).

There are a number of significant correlations between some of the measured parameters. There is a very strong positive relationship between the percentage contents of water and organic matter across the three sites ($r=0.959$, $p<0.001$); on closer inspection this relationship is non-linear (Figure 2.14). The soil water content correlated negatively with all six measured enzymes, but only significantly for three; β-D-glucosidase ($r=-0.785$, $p<0.001$), β-D-xylosidase ($r=-0.750$, $p<0.001$), and Phosphatase ($r=-0.745$, $p<0.001$). The negative relationships between soil water content and the activities of arylsulphatase, N-acetyl-β-D-glucosaminidase and phenol oxidase were all significant at the <0.05 level, but not <0.01. Phenol oxidase activity correlated positively with soil pH ($r=0.922$, $p<0.001$), but the same relationship was not observed for the hydrolase enzymes ($p>0.05$). The CO_2 flux correlated positively with the water content of the soil ($r=0.726$, $p<0.01$).

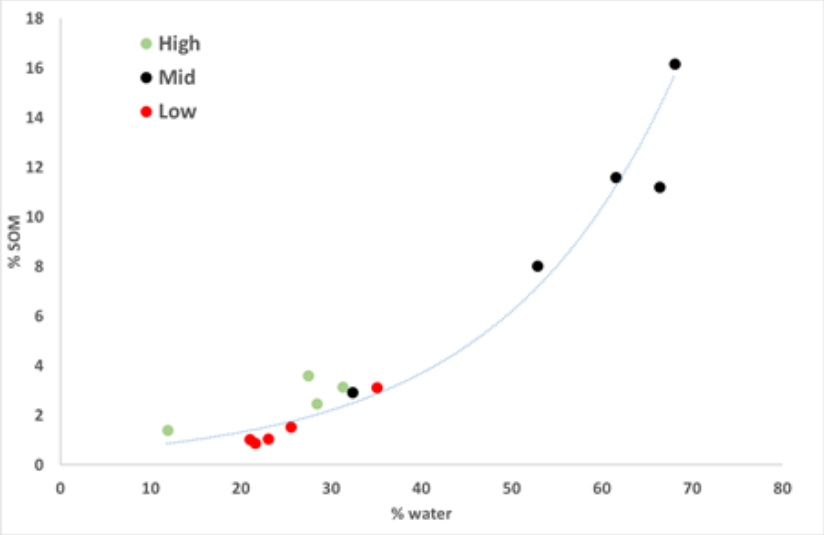

Figure 2.14: Scatterplot showing the relationship between % water and % soil organic matter across the 3 salt marsh zones. The trendline shows an exponential relationship

2.5. Discussion

Coastal salt marsh ecosystems play an important role in global carbon sequestration, serving as a significant blue carbon pool with great potential to mitigate the extent of climate change (Mcleod *et al*., 2011; Siikamäki *et al.*, 2013). Salt marsh ecosystems could equally serve as a source of greenhouse gas emissions if the processes that are responsible for sequestration are compromised in any way due to anthropogenic disturbances (Pavlov *et al*., 2014).

In this study, soils from the mid zone of Cefni salt marsh stood out as having the lowest rate of biological activity, as it was characterised by the lowest enzyme activities, highest soil phenolic content, and highest percentages of soil water and SOM. It is speculated that the water content that have effect on the oxygen levels/redox in the soil sediments could be the key drivers of the biogeochemical differences observed between the three zones. With the greater water content of the mid zone, the result of the interplay between proximity to the sea, which supplies water, and a dense vegetation cover, which retains water appear to be the key controls on organic matter contents.

Previous studies have shown that vegetation slows down water velocity and trapped sediments in between plants, as litter materials from dying plants increase inputs, thereby encouraging build-up of organic matter and sediments in such ecosystems (Drake *et al.*, 2015; Whiting, 2016). Within the context of this experiment, the lack of vegetation in the low salt marsh zone increased oxygen availability thereby stimulating microbial activities. The mid zone is more vegetated than the low zone and has the advantage of roots entrapping sediments and its being closer to the sea promote greater water retention in this area. Consequently, soils of the mid zone of the Cefni Maltreat salt marsh could be better in carbon storage than those from the low and high zone areas. Another reason for the higher contents of the soil organic matter in the mid zone could be due to higher rate of productivity which exceed that of decomposition which has been linked to vegetation cover as well (Persico *et al*., 2017).

The high soil water content of the mid zone will create an anoxic soil environment. This is apparent from the low activity of the key enzyme phenol oxidase in this zone. Previous studies have demonstrated how phenol oxidase is a regulator of decomposition processes in wetland soils

(Pind e*t al*., 1994; Freeman *et al*., 2004; Saraswati *et al*., 2016) and it appears to come into play also in this salt marsh. The mid zone had the highest concentration of soil phenolics, which is a direct result of the low phenol oxidase activity. The greater activity of phenol oxidase in low and high zones has led to a reduced build-up of phenolics, as the enzyme is able to break down these compounds in the presence of oxygen. The contrasting availabilities of phenolic compounds is directly influencing the hydrolase enzymes, with reduced activities in the mid zone and greater activities in the low and high zones due to reduced phenolic constraints (Freeman *et al*., 2001). This trend was observed for all five hydrolase enzymes. In addition to influencing enzyme activities, the higher soil water availability at the mid zone could be mobilising free ions such as Fe^{2+}, which could be impairing microbial activities further, providing an additional explanation for the reduced rates of decomposition and greater accretion of SOM (Kang & Freeman, 2005).

Higher rates of decomposition and lower SOM contents were recorded at the low zone compared to the mid, which disproves the hypothesis that carbon sequestration would be most efficient at the closest point to the sea. This could be based on the fact that the low zone of Cefni Maltreat salt marsh has a lower vegetation density, reducing organic inputs to the soil and the ability to trap water. Therefore, it can be assumed that soil oxygen concentrations would be higher, reducing constraints on decomposition, although this was not directly measured.

Higher rates of decomposition and lower SOM contents were also recorded at the high zone. Although the above- and below-ground biomass is greatest here, being located furthest from the sea this zone is only occasionally inundated with water, so the soil environment will be mostly oxic. This is the most likely explanation for why enzyme activities here were greater than in the mid zone. Arriola & Cable (2017) discuss how vegetation density and inundation extent are key determinants of sediment accumulation in salt marshes. Despite the likely high inputs of organic matter at this zone, due to the extensive above-ground biomass, the site is simply too dry to be a significant zone of carbon sequestration.

Despite the clear observation that the mid zone is the most effective for carbon sequestration, this site also had the highest CO_2 emissions, an observation that does not fit in with the rest of the data. In fact, a positive correlation between CO_2 flux and soil water content was observed. It is most unlikely that soil water content in the mid zone could be responsible for the high CO_2 flux as there was a high positive correlation with soil water. Soil water content has been

previously reported to correlate with CO_2 and CH_4 emission in wetlands (Furukawa *et al*., 2005). Furthermore, Xu & Qi (2001); Reichstein *et al.* (2002); Xu *et al*. (2004) and Curiel Yust *et al.* (2007) noted that water availability can influence the rate of organic matter decomposition and CO_2 fluxes in soils. Orchard & Cook , (1983) after measuring the activity of microbes in soil by varying the water content reported that changes in availability of soil moisture influenced microbial activity as decreasing the water content from -6.01 to -0.02 MPa caused a 10% reduction in microbial activity. Rewetting the soil greater than 5 MPa afterwards, caused a large and rapid upsurge of microbial activity up to 40-fold for a short period. They also reported a log-linear relation between water potential and microbial activity.

2.6. Conclusion

Coastal salt marshes occupy a small fraction of about 1% of global wetlands, yet they are significant carbon pools. However, relatively little attention has been given to the role of the enzymic latch mechanism in salt marsh ecosystems and how this might vary within the different zones. These findings revealed that:

- The mid-zone of Cefni salt marsh had the lowest rate of decomposition of organic matter and is where the enzymic latch mechanism is most prevalent.
- The interplay between proximity to the sea and vegetation cover appears to be the driving factor responsible for the biogeochemical differences observed in soils between the sites.
- On a general scale, the Cefni salt marshes are likely to be a sink for GHGs.

The Cefni salt marsh, typical of many other temperate salt marshes, face both anthropogenic and natural threats and need to be well protected due to the many ecosystem services they offer. Protecting and conserving these ecosystems from such threats will be a necessary strategy to maintain their globally significant stores of organic carbon and prevent them becoming net sources of greenhouse gases. The data obtained suggests that the carbon sequestration dynamics of salt marshes are complex and driven by the interplay between water content and biomass.

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Chapter 3

Carbon sequestration and the enzymic latch mechanism in red, black and white mangrove soils

Carbon sequestration and the enzymic latch mechanism in red, black and white mangrove soils

3.1. Abstract

Mangrove swamps are an important habitat types providing several ecosystem services, such as protection of coastlines from erosion and extreme weather conditions, including hurricanes, water filtration, nutrient cycling and carbon sequestration among others. Although the carbon storage ability of mangroves is well documented, few studies have addressed the effect of mangrove species on carbon sequestration. Soil samples collected from red (*Rhizophora mangle),* black *(Avicennia germinans)* and white (*Laguncularia racemose)* mangroves in Florida, USA, showed that the red mangrove soil is the most efficient for carbon sequestration. It had the lowest phenol oxidase activity, highest phenolic concentration and lowest hydrolase enzyme activity and, as a result, the highest concentration of soil organic matter (SOM). It is believed that the high soil water content of the red mangrove, due to its close proximity to the sea, is a key driver of these observations. The 'enzymic latch' mechanism appears to be prevalent in the red mangrove soil in particular, allowing these ecosystems to be efficient at carbon storage and therefore a vital natural ecosystem mitigating the effect of climate change.

Key words: Mangroves, Carbon, Sequestration, Enzymic latch, Enzymes, Decomposition

3.2. Introduction

Mangroves swamps are highly productive and carbon-rich inter-tidal wetland ecosystems (Giri *et al.*, 2011; Alongi, 2014) found in many countries between latitudes 30° North and South of the equator (Alongi, 2000; Donato *et al.*, 2011). Globally, mangroves occupy a total coastal area of 157-160,000 km^2 (Donato *et al.*, 2011), with 43% of the world's mangroves being found along the coasts of Indonesia, Australia, Brazil and Nigeria (Alongi, 2011).

Mangroves offer the key ecosystem services of coastal protection from storms, water filtration, wood production, support for biodiversity among many others (Everards *et al.*, 2014) . They also play a key role in carbon sequestration and on a global scale are estimated to capture somewhere in the region of 18.4 Tg C yr^{-1} (Donato *et al.*, 2011; Alongi, 2011) to 23.2 Tg C yr^{-1} (Giri *et al.*, 2011). They are efficient natural ecosystems for mitigating the extent of climate change (Mcleod *et al.*, 2011; Siikamaki, 2012). Mangrove swamps store 45-98% of organic carbon below ground (Saraswati *et al.*, 2016) and can accumulate up to 5 metres of peat (Bridgham *et al.*, 2006; Wiener *et al.*, 2006). Murdiyarso (2011) and Donato *et al.* (2011) reported that mangrove swamps have sequestered an average of 1023 Mg ha^{-1} of carbon. These sequestered carbon stocks can be attributed to the dense canopies of trees in the swamps, with high rates of leaf litter input (estimated to be in a range of 2 to 16 Mg ha^{-1} yr^{-1}) (Twilley *et al.*, 1992) into waterlogged, anaerobic soils where organic matter is only partly decomposed and net sequestration results. Total carbon storage in mangrove forests soils is high compared to other tropical forest types. Donato *et al.* (2011) estimate that mangroves contain an average of three to four times the quantity of carbon (above + belowground) typically found in boreal, temperate and upland tropical forests per unit area.

Mangroves provide a unique ecological environment for diverse microbial communities, which are fundamental to the functioning of these habitats. They are particularly important in controlling the biogeochemical environment of mangrove soils. For example, sulphate-reducing bacteria are critical to the cycling of nitrogen. Mangroves are also home to a group of fungi called "manglicolous fungi" which play a particularly important role in the decomposition of mangrove leaf litter (Kathiresan & Bingham, 2001).

The rate of decomposition in wetland soils are slower due to the anoxic conditions than under aerobic conditions, accounting for accumulation of high organic matter in such environments.

In soils, extracellular hydrolytic enzymes are considered to be the main mediators of organic matter decomposition and nutrient cycling (Marx *et al.*, 2001). In wetlands, the anoxic conditions supresses enzyme activities, leading to a build-up of inhibitory phenolics, which reduces the rate of organic matter decomposition and causes a build-up of organic material as peat. This process is regulated by oxygen constraints on a single enzyme, phenol oxidase resulting in phenolic build up and as a result placing a constraint on hydrolases enzyme, a process known as the 'enzymic latch' mechanism (Freeman *et al.*, 2001). Mangrove wetlands are a globally significant store of carbon, due to limited availability of oxygen to soil microbes which accounts for slow rate of decomposition of organic matter trapped within such soils (Keller, 2011). Saraswati *et al.* (2016) investigated the influence of the enzymic latch mechanism in red mangrove soil from Florida, USA, and found the mechanism is present.

Human development, particularly for agriculture and urban expansion, has devastated mangroves, such that approximately one-third of the world's mangrove forest has been lost in the last 50 years (Alongi, 2002; Giri *et al.*, 2008). Loss of mangrove vegetation can have the undesired impact of switching the systems from a net sink to a net source of greenhouse gases (GHGs) (Alongi, 2002). Moreover, carbon losses from mangrove ecosystems due to anthropogenic activities or natural causes could have consequences on the global climate, which may include a rise in atmospheric temperatures and an increase in coastal storms, both of which could have important consequences for mangrove ecosystems (Walters *et al.*, 2008). Furthermore, changes in land use could overturn sediment carbon, exposing it to atmospheric oxygen resulting in microbial proliferation and increased rate of decomposition with subsequent release of stored GHGs into the atmosphere. Pendleton *et al.* (2012) reported a 50% loss of sediment carbon within 8 years after clearing mangrove swamps in Panama. Likewise, Lovelock *et al.* (2011) measured large short term CO_2 fluxes of 29 Mg CO_2 ha^{-1} yr^{-1} from the surface of cleared mangrove swamps. Other losses of carbon could be due to tidal action (erosion), tree respiration and re-mineralization (Kridiborworn *et al.*, 2012; Sweetman *et al.*, 2010). Rising sea-level will also impact on mangroves (Gilman *et al.*, 2008), with longer periods of inundation being a threat but changes in elevation could cause a landward retreat and an increased opportunity for seedlings and colonisation (Lu *et al.*, 2013). Without protection it is expected that mangroves will be lost at a similar or even greater rate than tropical forest ecosystems (Valiela *et al.*, 2001; FAO, 2007). (Duke *et al.* (2007) stated that 30-40% of coastal wetlands and 100% of mangrove forests could be lost in the next 100 years if the current rate of losses continue.

Bubier (1995) revealed that soil bacteria, fungi, actinomycetes as well as other soil fauna are responsible for organic matter decomposition in aerated soils, while anaerobic bacteria are responsible for the breakdown of organic matter in waterlogged soils under anoxic condition.

There remains much to be understood about the carbon sequestration dynamics of different mangrove forest species. This study built on that of Saraswati *et al.* (2016) by determining the rate of decomposition of organic matter and the impact of the 'enzymic latch' mechanism in the soils of red, black and white mangroves in Florida, USA. It is therefore, hypothesise that white mangrove soil has the highest rate of decomposition, whereas soils from the red mangrove has the lowest, due to their proximity to the sea and resultant extent of inundation.

3.3. Materials and methods

3.3.1. Study sites

Sampling was performed in southern Florida, USA, near Barefoot Beach County Preserve, Bonita Springs, in an area containing all three of mangrove tree species that are dominant in this region; red (*Rhizophora mangle)*, black *(Avicennia germinans)* and white (*Laguncularia racemose)* base on proximity to the water edge. The red mangrove is situated closest to the sea, frequently flooded by tidal action, and closely followed by the black mangrove. The white mangrove is located inland at a higher gradient than the red and black mangroves and as a result less frequently flooded. The three different areas had a dense vegetation dominated by species peculiar to its area. The mean annual temperature range of the site is 18-29°C, and rainfall 1318 mm (Min *et al.*, 2015). The vegetation and environmental factors influencing mangrove ecosystem formation in Florida have been extensively studied (e.g. Ball, 1980). Soils were randomly sampled from the following locations in five replicates at a distance of about three meters apart:

- Red: Latitude: 26.29553, Longitude: -81.83155
- Black: Latitude: 26.29600, Longitude: -81.83200
- White: Latitude: 26.29465, Longitude: -81.83157

Figure 3.1: Sampling locations in Red, Black and White mangroves in Florida, USA

3.3.2. Sample collection

At each site, about 500g of soil from a depth of 10-12 cm was collected in five random locations using a trowel and the samples placed in plastic bags and sealed. Average soil temperatures were 18°C in the red, 19°C in the black and 22°C in the white mangrove soils. Samples were sent back to the UK in cooler boxes with ice packs and maintained at 4°C. All analyses were conducted within 2 weeks of sample collection.

3.3.3. Laboratory analyses

3.3.3.1. Water extraction

Soil samples were homogenised by hand and unwanted debris removed. A 5 gram sub-sample was transferred to a 50 ml falcon tube, followed by 40 ml deionised water. The tubes were then placed on a shaker (KIKA®-Werke, Gmbh & CO.KG, Germany) at 300 rpm for 24 hours. The samples were then removed from the shaker and pH and conductivity determined on an aliquot of the sample using SevenEasy and FiveGo (Mettler-Toledo, Leicester, UK) bench-top meters. Following this, the remaining samples were centrifuged at 5000 rpm using a Sorall ST-16R centrifuge (Thermo Scientific, UK) and 20 ml of sample filtered through 0.45 µm cellulose nitrate filters (Cole Palmer, St. Neots, UK).

3.3.3.2. Soil water and organic contents

Soil water and organic weights were determined following the method of Frogbrook *et al.* (2009). Approximately 10g of soil were placed in pre-weighed crucibles, weighed again and placed in an oven (Memmert- Schwabach, Germany) at 105°C for 24 hours to ensure thorough evaporation of water content from the soil. After weighing again, the crucibles containing the soil were placed in a muffle furnace (Carbolite, Sheffield, UK) for 120 minutes at 550°C and weighed again thereafter. The weights were used to calculate the water and organic contents as a percentage of the original sample.

3.3.3.3. Phenol Oxidase enzyme assay

A 10 mM solution of the substrate phenolic amino acid L-3,4-dihydroxyphenylalanine (L-DOPA) (Sigma Aldrich Ltd, UK) was prepared by dissolving 0.986 g of powder in 500 ml deionised water. The solution and soil samples were placed in an incubator set to field temperature 24 hours before undertaking analysis. Two 1 g homogenised soil sub-samples were placed into two separate stomacher bags, one assigned as blank and the other as substrate. Nine ml deionised water was added to each bag and the sample homogenised using a stomacher (Seward, Worthing, UK). Following this, 10 ml of the L-DOPA solution was added to the

substrate bag and 10 ml of deionised water was added to the blank bag. Both were homogenised again and then placed in the incubator for 10 minutes. After the period of incubation, three 1.5 ml microcentrifuge tubes were filled with solution from each bag and centrifuged at 10000 rpm for 5 minutes. 300 µL of the supernatant was then pipetted from each microcentrifuge tube into separate wells of a clear 96 well microplate and the absorbance at 475nm read using a SpectrMax M2e (Molecular Devices, Wokingham, UK) plate reader. The activity of the enzyme was calculated by subtracting the average blank absorbance value from the average substrate absorbance value and correcting for the dry weight of soil, to give an activity expressed as nmol of product formed (dopachrome or 2-carboxy-2,3-dihydroindole-5,6-quinone,) per minute (min^{-1}) per gram (g^{-1}) of soil (dry weight), as detailed in Dunn *et al.* (2014).

3.3.3.4. Hydrolase enzyme assay

Methylumbelliferone-based enzyme substrate solutions were created for all 5 enzymes (400µM for β-glucosidase, β-xylosidase, sulphatase and chitinase; 200µM for phosphatase) were prepared according to Dunn *et al.* (2014). The solutions and soil samples were placed in an incubator set to field temperature 24 hours before undertaking analysis. For each sample and each enzyme, 1 g of soil was placed in labelled stomacher bags and 7 ml of the relevant substrate added. The bag was then homogenised and incubated for 1 hour (45 minutes for phosphatase) at field temperature. Following incubation, soil slurries were dispensed into 1.5 ml microcentrifuge tubes and centrifuged at 10,000 rpm for 5 minutes. 250 µl supernatant was extracted from each and added to separate wells of a black 96 well microplate, to which 50µl of deionised water had been previously added. A similar procedure was adopted for the standard solutions, but instead using deionised water rather than enzyme substrate in the stomacher bags and 50 µl of varying concentrations of MUF-free acid solution in the microplate wells. The concentration of the samples and standards was then measured using the SpectraMax M2e plate reader and converted into a value of activity following Dunn *et al.* (2014).

3.3.3.5. Gas fluxes

Soil respiration was performed by placing 10g of homogenized soil into a 50 ml falcon tube fitted with rubber septa in the lids and incubated at field temperature for a period of 60 minutes. After the allotted 60 minute period of incubation, gases were collected from the tubes using a 10 cm^3 syringe fitted with a short bevel hypodermic needle (Sigma Aldrich Ltd, Dorset, UK) and transferred into labelled (Time 2; T2), pre-evacuated 10 ml Exetainers (Labco Ltd, Lampeter, UK) fitted with screw caps with rubber septa. An air sample from above the

centrifuge tubes containing the samples at the start of the experiment was collected into five exetainers labeled as Time 1 (T1).

Gas samples were analyzed on a Varian model 450 gas chromatograph (GC) instrument, fitted with a flame ionization detector (FID) and a catalytic converter (methaniser) to measure CO_2 and CH_4 concentrations, and an electron capture detector (ECD) for N_2O. Oxygen-free nitrogen is used as the carrier gas. CH_4, CO_2, and N_2O (retention times 1.08, 1.87 and 2.25 minutes respectively) were quantified by comparison of peak area with that of the three standards of known concentrations, prepared by Scientific and Technical Gases Ltd (Newcastle under Lyme, Staffordshire, UK), used in the preparation of a standard curve.

3.3.4. Statistical analysis

Data were analysed by one-way ANOVA to test for the effect of one factor on the measured parameters; site (three levels, white, black, red). Relationships between the enzyme activities and physico-chemical factors across the three mangrove zones were determined by correlation analysis. SPSS v22 (IBM Corporation, New York, USA) was used for all analyses. A p value of <0.05 was used to denote significance for the ANOVA analysis, but <0.01 for the correlation analysis.

3.4. Results

The red mangrove soil had a greater water content (84.2%) than soils in the black mangrove stands (73.1%), but this difference was not significant ($p>0.05$; figure 3.2). Soil beneath white mangroves had a much lower soil water content (44.1%), which is significantly different to the red and black ($p<0.05$). This trend was mirrored for the SOM (Figure 3.2), with the red mangrove (57.9%) having the highest SOM, followed by the black mangrove (36.5%) and the white (9.9%), however, only the red and white mangroves sites were significantly different ($p<0.05$).

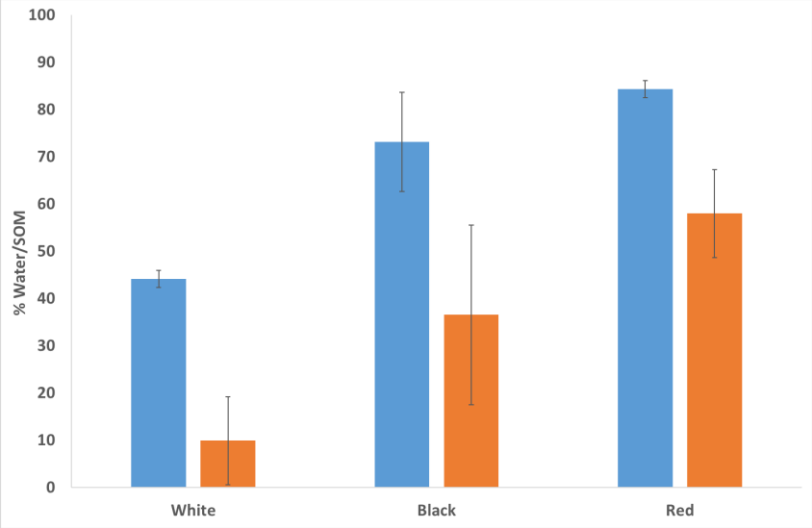

Figure 3.2: Bar chart showing mean soil water and organic contents in each mangrove zone (n=5, error bars ± SD)

The mean soil pH differed substantially between the three zones (Figure 3.3), with ANOVA analysis demonstrating significant differences for all comparisons ($p<0.05$). The white was the most alkaline (mean pH 8.09), followed by black (7.40) and red (6.32).

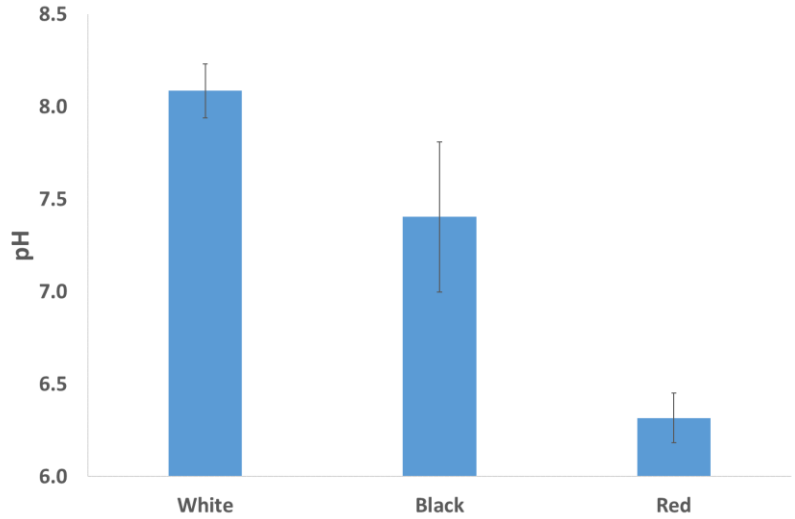

Figure 3.3: Bar chart showing mean soil pH in each mangrove zone (n=5, error bars ± SD)

The white mangrove soil had a more than threefold greater phenol oxidase activity (853.74 nmol dicq g^{-1} h^{-1}) in comparison to the red mangrove soil (206.15 nmol dicq g^{-1} h^{-1}), which was a significant difference ($p<0.05$). The black mangrove had more than twice the activity (439.48 nmol dicq g^{-1} h^{-1}) compared to the red soil, but this was not statistically significant (Figure 3.4).

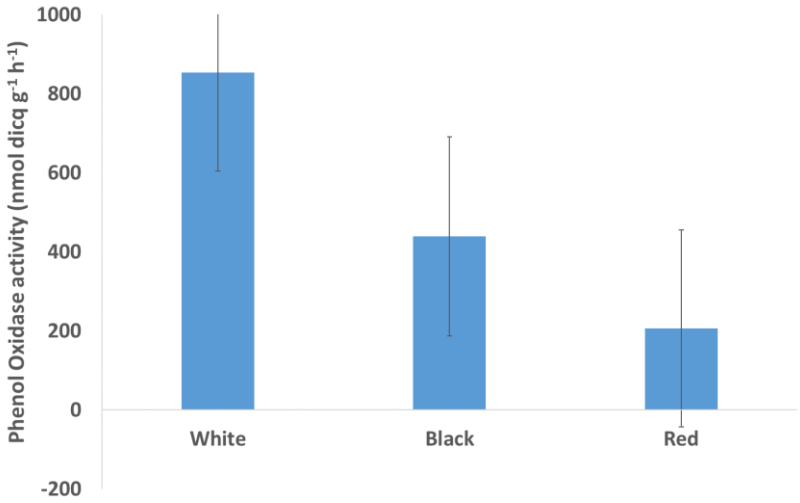

Figure 3.4: Bar chart showing mean activity of phenol oxidase in each mangrove zone (n=5, error bars ± SD)

The concentration of soil phenolics (Figure 3.5) was similar in the black (282.06 µg g^{-1}) and red (262.33 µg g^{-1}) mangrove soils but for the white was significantly lower than both (45.03 µg g^{-1}) ($p<0.05$).

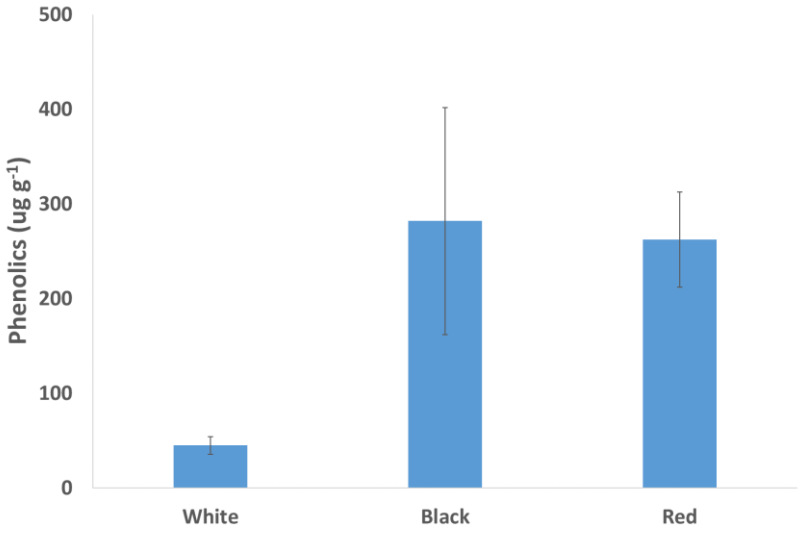

Figure 3.5. Bar chart showing mean concentration of soil phenolics in each mangrove zone (n=5, error bars ± SD)

Similarly, the activity of β-glucosidase (Figure 3.6) does not differ much between the black (4.41 nmol g^{-1} min^{-1}) and red (3.04 nmol g^{-1} min^{-1}) mangrove soils but there was a significantly higher rate of activity in the white (9.42 nmol g^{-1} min^{-1}) ($p<0.05$). The other four hydrolase enzymes showed similar trends, with the white mangrove soil always having the highest activity and the lowest activities usually being in the red mangrove.

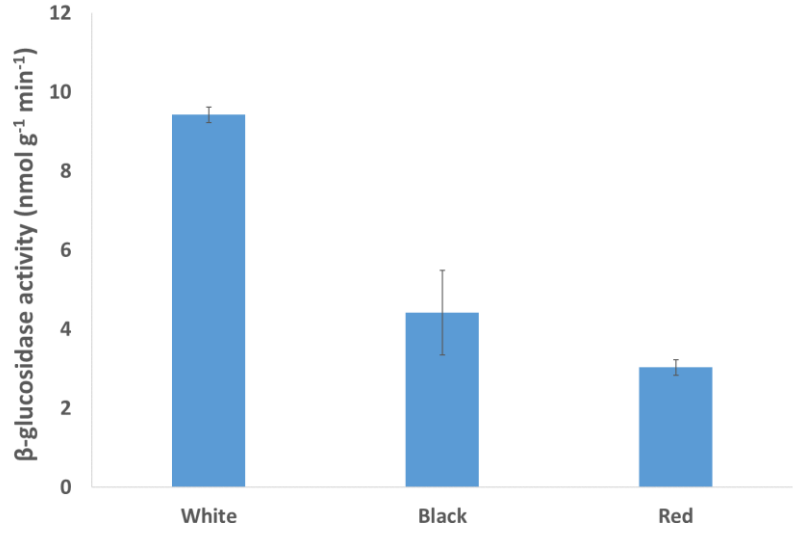

Figure 3.6. Bar chart showing mean activity of the enzyme β-glucosidase in each mangrove zone (n=5, error bars ± SD)

The flux of CO_2 (Figure 3.7) does not show the same pattern as the previous parameters, with the highest flux measured from the red mangrove soils (9.33 µg g^{-1} s^{-1}) and the lowest from the black (4.39 µg g^{-1} s^{-1}), but these differences are not statistically significant ($p>0.05$).

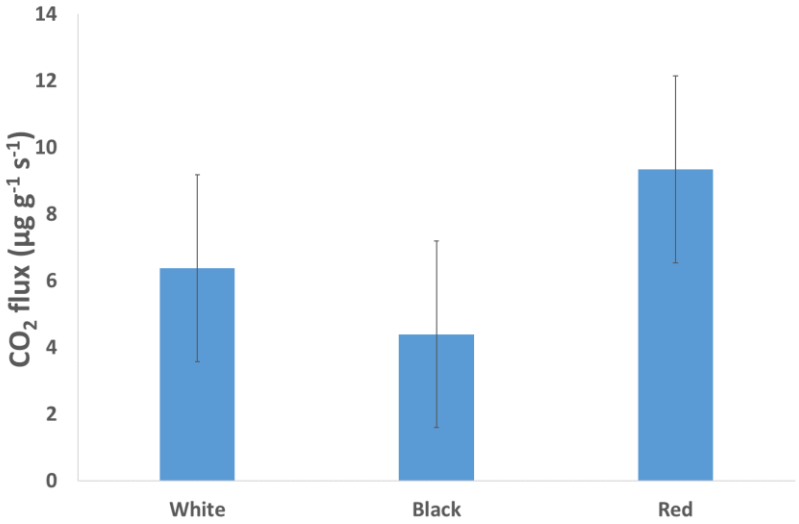

Figure 3.7. Bar chart showing mean CO_2 flux in each mangrove zone (n=5, error bars ± SD)

Correlation analysis revealed negative relationships between the concentration of phenolics and the activity of all six enzymes. The relationships were significant for β-glucosidase ($r=-0.674$, $p<0.01$), xylosidase ($r=-0.819$, $p<0.001$), phosphatase ($r=-0.882$, $p<0.001$) and phenol oxidase ($r=-0.770$, $p<0.01$) but not sulphatase ($r=-0.524$, $p=0.045$) or chitinase ($r=-0.448$, $p=0.094$). Furthermore, soil pH correlated positively with all enzymes ($r=0.688$-0.833, $p<0.01$) excluding sulphatase ($p>0.01$).

There was a strong positive relationship between the percentage contents of water and organic matter ($r=0.953$, $p<0.001$); on closer inspection this relationship is non-linear (Figure 3.8). No measured parameter correlated significantly with the CO_2 flux.

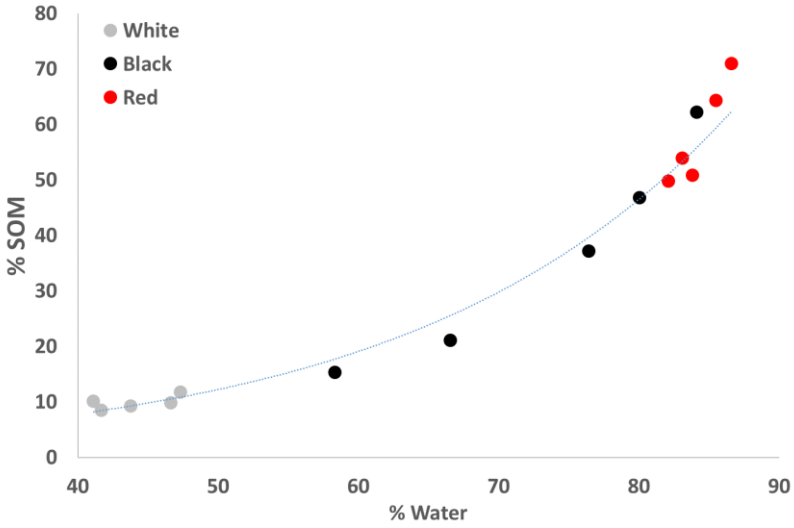

Figure 3.8. Scatterplot showing the relationship between % water and % soil organic matter across the 3 mangrove zones. The trendline shows an exponential relationship

3.5. Discussion

A clear trend in the measured parameters was observed along the mangrove species gradient, with the white mangrove soil having high enzyme activity and low soil organic matter, the red mangrove soil having low enzyme activity and high soil organic matter and the black mangrove soil occupying a mid-point in between.

The mean height of the water table and resulting inundation is likely to be the key driver of these observations, with the red mangrove being closest to the sea and therefore having much more saturated soils. The black mangrove soils show some similarity by having higher organic matter and soil water contents with low hydrolase activity than the white mangrove soils, suggesting low rate of decomposition. The white mangrove soil will be less frequently subject to flooding because of its location further away from the water's edge at a higher gradient. Soil water content influences the extent of oxygenation in soils and therefore exerts a strong influence on decomposition processes, with low soil oxygen availability implying low rates of decomposition (Freeman *et al.*, 2012) encouraging net sequestration of organic matter (Donato *et al.*, 2011, Saraswati *et al.*, 2016). Friesen *et al.* (2018) investigated the rate of decomposition as a factor of carbon accretion in mangroves and identified tidal inundation, vegetation types, faunal community and microbial processes as factors that could influenced organic matter accretion in mangrove ecosystems.

Measurement of the activities of extracellular enzymes was a key component of this investigation, as it allows for the examination of the 'enzymic latch' mechanism in the ecosystem. The mechanism by which wetland soils are able to sequester globally significant amounts of organic matter in soil sediments (Freeman *et al.*, 2001).

The white mangrove soils exhibited some higher level of activities in all parameters measured except gas fluxes suggesting that the enzymic latch mechanism could not be as strong as it is in the red and black mangrove soils. Usually, the activity of phenol oxidase is perceived to be inversely correlated with phenolic compound concentrations, and this was the case in this study; the greater the activity of phenol oxidase the more phenolic compounds are broken down into smaller compounds (Pind *et al.*, 1994, Freeman *et al.*, 2001). This is why the highest phenolic content was found in the red mangrove soil compared to the white mangrove soil. Freeman *et al.* (2012) demonstrated that an abundance of inhibitory phenolics strengthens the

enzymic latch mechanism in peatlands, because phenolics are known to possess anti enzyme properties. The data obtained fits the mechanism that phenolic compounds are inhibitory to hydrolase enzymes (Freeman *et al.*, 2001). The lowest activity of β-glucosidases was observed in the red mangrove soils, followed by soils under the black mangrove stand while soils analysed from the white mangrove sites had higher enzymes activity. There was also a strong and significant negative correlation between phenolics and β-glucosidase activity (and for other hydrolase enzymes).

A strong variation in pH, by almost two fold units, was also observed along the mangrove gradient, with the white mangrove soil having the highest followed by the black mangrove soil and then soils from the red mangrove areas. The impact of pH on decomposition processes is complex because of the range of pH optima that different microorganisms and enzymes can have (Lynch, 1995; Turner, 2010). Soil pH correlated negatively with all but one of the assayed enzymes, suggesting that it may be an additional factor as to why the red mangrove soil had the lowest rate of decomposition.

Soil samples were analysed for carbon dioxide (CO_2) emissions, which as the end-point of decomposition provide additional information alongside rates of enzyme activities. The CO_2 flux data did not conform to the pattern shown by the rest of the measured parameters. In fact, there were no significant differences recorded between the three sites. This suggests a number of things; either that soil decomposition processes are more complex than can be interpreted by only measuring enzyme activities, or there may have been some experimental error during the analysis. The large error bars suggest the latter may have been a contributing effect. Given the importance of CO_2 emissions in effecting climate change (Sweetman *et al.*, 2010; Kridiborworn *et al.*, 2012) , it would be worth investigating the CO_2 emissions from mangrove soils under these three species in more detail. Kristensen (2008) evaluated CO_2 flux in pristine and anthropogenically-impacted mangrove forests in Tanzania, demonstrating that the pristine forest is a sink of greenhouse gases and the anthropogenically-impacted forest has reduced capacity to absorb CO_2 (although is still a net sink of carbon).

3.6. Conclusion

The outcome from this investigation was the discovery that the red mangrove soil had a higher capacity for water retention and build-up of organic matter than that beneath the black and white mangrove species, making it the most effective carbon sink out of the three dominant mangrove species found in much of the southern USA. The reasons for this appear to be due to the 'enzymic latch' mechanism and related to the greater soil water content of the red mangrove soil, which is the key driver of the biogeochemical differences observed. The mechanism is present in the black mangrove soils, but to a lesser extent this species, alongside the red, is a vital natural ecosystem for carbon sequestration and important in helping the fight against climate change (Jones, 2013).

This investigation agrees with the hypothesis that the white mangrove soil has the highest enzyme activities while soils from the red has the lowest base on proximity to water edge. The key conclusions are:

- Waterlogging is a key driver of the biogeochemical observations between the three mangrove species.
- The enzymic latch mechanism is prevalent in the red mangrove soils, due to the low phenol oxidase activity, high phenolic compound concentrations, low hydrolase enzyme activities and high content of organic matter.

The importance of mangrove ecosystems as a globally significant store of carbon cannot be understated. Mangrove swamp conservation and restoration will greatly improve the ecosystem services derived from mangroves, and will allow for improved sequestration of carbon (Marois & Mitsch, 2015). This study suggests that the red mangrove should be prioritised if the main goal of restoration is carbon sequestration.

Acknowledgements

The assistance and good guidance of Bob Wasno of the Florida Gulf Coast University, USA was very instrumental in identifying the sites for sample collection and is highly appreciated.

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Chapter 4

An investigation of decomposition process in coastal wetland soils (mangrove and salt marsh transect)

4.1. Abstract

As global temperatures continue to rise, mangrove ecosystems are seen to be moving poleward, displacing salt marsh ecosystems. Mangroves and salt marshes are both highly valued habitats, providing numerous ecosystem services such as carbon sequestration, nutrient cycling and protection of coastal communities from natural disasters among others. Soil samples were analysed from a black mangrove – high zone salt marsh transect in north-eastern Florida, USA, to assess the impacts of mangrove encroachment on soil decomposition processes. The main findings from the analysis were that the soils from the mangrove takeover area had a lower water content (30.9-37.5%) compared to those from the salt marsh area (49.3-81.1%). Similarly, the soil organic matter (SOM) content was 4.8-7.1% and 9.4-34.6% in the mangrove and salt marsh areas respectively. The soils from the mangrove part of the transect had a greater activity of phenol oxidase, lower soil phenolics concentration and greater activity of hydrolases and CO_2 emissions compared to the salt marsh. These data indicate that mangrove takeover of salt marsh habitat by mangroves could have a negative impact on carbon storage in coastal environments, at least during the transitional period.

Keywords: mangrove, salt marsh, carbon, decomposition, sequestration, transect

4.2. Introduction

Mangrove swamps and tidal salt marshes are coastal ecosystems that form a critical interface between terrestrial and marine habitats (Jones *et al.*, 2014; Yando *et al.*, 2016). They perform a number of key ecosystem services such as improvement of water quality, protection of coastal communities from natural disasters, food production, serving as nurseries for aquatic life and carbon sequestration (Alongi, 2008;Yando *et al*., 2016). Salt marshes are predominantly found along temperate and arctic coasts dominated by perennial and succulent vegetation types that are freeze-tolerant, while mangroves which are not freeze-tolerant are woody plants (Stevens *et al.*, 2006; Barbier *et al*., 2011; Osland *et al.*, 2017).

Salt marshes are regarded as one of the worlds' most important carbon sinks with an estimated carbon burial rate 55 times faster than that of the tropical rain forest (Mcleod *et al.*, 2011). Globally, salt marshes occupy a small fraction of 1-2% (Bouillon *et al.*, 2002) of the total coastal areas but can sequester 87.2 ± 9.6 Tg C yr^{-1} carbon annually, exceeding that of tropical rainforests (53 ± 9.6 Tg C yr^{-1}). Bridgham *et al.* (2006) estimated 4.6 Tg C yr^{-1} as the carbon sequestration rate of global salt marshes, whereas Duarte *et al.* (2004) noted that global tidal salt marshes could sequester about 60.4 Tg C yr^{-1}. In addition, salt marshes have the ability to hold carbon in the soil for millennia *(*Macreadie *et al.*, 2012) as against the rainforest's decades. Similarly, mangroves occupy a total global coastal area of 157,000-160,000 km^2 (Laffoley & Grimsditch, 2009) with a global annual carbon burial rate of approximately 18.4 Tg C yr^{-1}. Simpson *et al*, (2017) reported that mangrove ecosystem occupies a small fraction (0.7%) of global tropical forest area, but have the capacity to store three times soil carbon per hectare than tropical rain forest, thus mangrove swamp is also a significant global carbon pool. Therefore, these coastal wetland ecosystems collectively play a vital role in global carbon sequestration (Jones, 2013).

The mean global temperature increase of 0.75°C in the last three decades (Solomon *et al.*, 2007; Saintilan *et al*., 2014; Asbridge, 2015), has altered the structure and distribution of ecological communities (Walther *et al.*, 2002; Parmesan & Gary, 2003). Changes in climatic conditions are expected to cause more biotic alterations, which could lead to tropicalisation of certain temperate habitats due to poleward expansion of plant communities. One of the most significant observations has been the expansion of mangrove swamps into salt marsh habitats (Rogers *et al.*, 2005; Osland *et al.*, 2013; 2017).

Lately, the rate at which mangroves are taking over salt marsh habitats in both hemispheres has received substantial attention because of the implications of such takeover on salt marsh ecosystem functions (Saintilan *et al.*, 2014; Alongi, 2015; Ward *et al.*, 2016). Expansions in the range of mangroves are in response to climatic changes, fire, hurricanes, rainfall (Raabe *et al.*, 2012; Smith *et al.*, 2013), rise in global temperatures, increased in nutrients availability due to tidal influence and other disturbances (Eslami-Andargoili *et al*., 2009; Doyle *et al*., 2010; Simpson *et al*., 2017). As the planet continues to warm, mangrove habitats will continue to expand into saltmarsh areas (Armitage, *et al.*, 2015; Johnston & Caretti, 2017). Natural expansion of mangroves into saltmarsh habitats could create less favourable environmental conditions for the survival of salt marsh species due to changes in edaphic conditions (Osland, *et al.*, 2013; 2016). Mangrove vegetation is likely to outcompete salt marsh vegetation in terms of nutrient uptake as the latter cannot survive under mangrove canopies (Stevens *et al.*, 2006). Typical salt marsh vegetation such as *Spartina* spp cannot withstand mangrove-dominated habitat due to high salinities and fast sediment accretion and could easily be replaced by mangrove plants, as observed in Paranagua Bay, Brazil (Lana *et al*., 1991). Prolonged freezing events could also reverse the transition, at least in the short-term, allowing salt marsh vegetation to recolonise areas where mangroves have started to take over (Patterson, 1993).

Based on the current knowledge of ecosystem structure and function, the shifting of mangrove swamps into salt marshes could have implications for some of its ecosystem services. Since coastal salt marshes play an important role in counter-balancing atmospheric carbon dioxide and serving as an important and highly efficient blue carbon ecosystem, displacing such habitats could have some initial consequences for the blue carbon sink (Yuan *et al.*, 2015). There are concerns that such encroachment could increase decomposition rates of the soil organic matter and lead to these ecosystems becoming net sources of GHGs, at least during the transitional phase (Osland *et al.*, 2013; Cavanaugh *et al.*, 2014; Saintilan *et al.*, 2014). The potential release of centuries of sequestered soil carbon could have serious implications for the atmospheric carbon pool and may necessitate a change in coastal management strategies (Solomo *et al.*, 2007; Osland *et al.*, 2013; Cavanaugh *et al.*, 2014; Saintilan *et al.*, 2014; Osland *et al.*, 2017).

Salt marsh vegetation loss as a result of mangrove encroachment could also reduce the overall structure of soil organic matter, due to changes in plant leaf litter and biomass inputs, which

contributes to soil carbon capture during plant senescence and photosynthetic processes (Bull *et al.*, 1999). Consequently, the loss of buried organic matter and ancient sedimentary carbon as a result of erosion is likely to increase during the transitional period as juvenile *Avicennia germinans* (black mangrove) might not be sufficiently physically developed to withstand high environmental stress (Turner, 1993; Couwenberg *et al.*, 2010).

In contrast, Kelleway *et al.* (2016) stated that quantitative evidence suggests that mangrove encroachment into salt marsh habitats could eventually increase carbon storage and wood production among other important ecosystem services. Similarly, (Doughty *et a*l., 2016) reported that newly established mangrove sites in Florida contained twice the amount of above ground carbon (biomass) than salt marsh habitat. Loomis & Craft (2010) and Adam (2002) stated that complete transition of salt marsh vegetation to mangrove swamps could take 4 to 6 years. However, Osland *et al.* (2012) and Kelleway *et al.* (2017) suggested that it could take decades for the full impact of mangrove expansion on the soil sediment properties of salt marsh to be realised. They further noted that the ability to support anaerobic pathways of microbial metabolism vital in stabilising the habitat and organic matter accretion in the soil could be greatly compromised within such transitional periods. Accordingly, theoretically, mangrove encroachment into salt marshes could have both positive and negative impacts on key ecosystem services. Consequently, mangrove encroachment could in part increase other ecosystem services including serving as nurseries for fish and shellfish, storm protection among others.

In spite of several studies on mangroves and salt marshes as separate habitats, only a few studies have examined the mangrove-salt marsh ecotone. Most of these studies focused on documenting the spatial and temporal changes of the black mangrove-salt marsh transitions and associated variation in physical factors (Patterson & Mendelssohn, 1991; Perry & Mendelssohn, 2009; Stevens *et al.*, 2006; Comeaux *et al.*, 2012). Documented evidence has shown a two decadal progressive mangrove to salt marsh takeover attributed to responses to changes in climatic conditions along the coast of the Gulf of Mexico in Florida, USA (Everitt *et al.*, 2010, Bianchi *et al.*, 2013; Osland *et al.*, 2013; Cavanaugh *et al.*, 2015; Rodriguez *et al.*, 2016; Peterson & Bell 2016; Osland *et al.*, 2017). Furthermore, asserted that the entrapment of black mangrove propagules by salt marsh plants could have facilitated the landward encroachment of black mangrove in Florida coast.

This study was aimed at investigating the implication of mangrove encroachment into saltmarsh habitat on soil carbon sequestration. To this end, it is hypothesise that mangrove encroachment into salt marsh habitat could increase the rate of decomposition in the takeover areas, which could have implications for soil carbon storage. The hypothesis was tested by conducting laboratory-based biogeochemical analyses by examining decomposition indices such as soil enzyme activity, soil phenolic contents and microbial soil respiration.

4.3. Materials and Methods

4.3.1 Study site

Soil samples were collected across a salt marsh–black mangrove transition zone close to the Matanzas River, near Marineland, northern Florida, USA (Figure 4.1). In total, 33 soil samples were collected at an interval of two metres, from the black mangrove forest edge towards the sea (N 29° 41.353', W 081° 13.689' to N 29° 41.307' W81° 13.707'). Soils samples were collected at 2 meters interval along the transect starting from the encroached areas comprised of emerging juvenile black mangrove vegetation (*Avicennia germinans*) with infrequent presence of *Salicorna* spp transitioning to a true salt marsh high zone dominated by three species of grasses *(Salicornia europaea, Batis maritima* and *Spartina patens).* Soils sampled at the encroached areas dominated with the black mangrove were less water logged while those at the salt marsh areas were more water logged inward towards the sea at each transect point.

Figure 4.1: Study site in northern Florida, USA, showing the locations of the 33 sampling points along the black mangrove to salt marsh gradient.

4.3.2. Sample collection and preparation

Soil samples were collected starting from an area dominated by emerging juvenile black mangrove plants and a high zonation salt marsh transect area dominated by *Salicornia* and *Batis* spp vegetation. Soil samples were collected at a steady interval of 2 meters transect between each point beginning from the edge of the black mangrove encroached area, while maintaining a straight path right into the high tidal zone salt marsh dominated areas towards the water edge. Altogether, 33 transects were identified and approximately 500 g of soil was

collected from a depth of about 12 cm at each transect intervals using trowel. Large roots and stones were removed from the soil sample before it was placed in a labelled plastic bag. The GPS coordinates and soil temperature were also recorded at each sampling point. The samples were transported back to the UK in a cooler box and stored at 4°C until analysis.

4.3.3. Laboratory analyses

All laboratory analyses were conducted within two weeks from the time of collection to minimise any changes in the samples due to storage effects. In the laboratory, the samples were brought back to field temperature (18-25°C) for 24 hours before undertaking any analyses and, on the day of analysis, all samples were homogenised by hand for a few minutes and any more unwanted materials such as roots, stones and shell were removed.

4.3.3.1. Hydrolase enzyme assay

Five hydrolase enzymes were assayed; β-D-glucosidases, arylsulphatases, β-D-xylosidase, N-acetyl-β-D-glucosaminidases and phosphatase, following procedures outlined in Dunn *et al.* (2014). Solutions of enzyme substrates were prepared by dissolving the required quantity to make a 1 litre solution of 400 µM 4-MUF (200 µM for phosphatase) and together with soil sub samples incubated at field temperatures 24 hours before analysis.

Six 1g (±0.05) subsamples were placed into separate appropriately labelled stomacher bags (5 for each enzyme and 1 for the calibration standards). To each of the 5 enzyme bags, 7 ml of the relevant substrate was added to the relevant stomacher bag with the aid of a 10ml pipette, homogenised and incubated at field temperature for 60 minutes except phosphatase, which was for 45 minutes. After incubation, one 1.5 ml microcentrifuge tube per bag was filled with the soil slurry and centrifuged in a microcentrifuge (Eppendorf 5415C Centrifuge, Stevenage, UK) at 10,000 rpm for five minutes. Following centrifugation, 250µl of supernatant was extracted into wells of a black flat-bottomed 96-well Sterilin microplate (Sterilin, Cambridge, UK). To each of these wells, 50 µl of deionized water was added to ensure a comparable dilution to the standard solutions in the calibration curve.

To the subsamples in the remaining stomacher bags, which are for the calibration curve, 7ml of deionized water was added, homogenized, transferred to centrifuge vials (2 per sample) and centrifuged, as previously described (no need for incubation). After centrifugation, 250µl of supernatant was transferred into 8 separate wells for each sample (approx. 4 x 250µl from each of the 2 vials). A calibration curve of MUF was created by adding 50µl of standard solution to

these 8 wells, following the creation of a dilution series from the 1000 µm stock MUF solution (0, 5, 10, 20, 40, 60, 80, 100 µM). The microplate was analysed on a SpectraMax M2e spectrophotometer (Molecular Devices Ltd., Wokingham, UK) at 330 excitation, 450 nm emission to determine the fluorescence of the MUF (Dunn *et al.*, 2014).

4.3.3.2 Phenol Oxidase enzyme assay

The activity of phenol oxidase was determined using the methods outlined in Dunn *et al.* (2014). A 10mM solution of L-DOPA substrate was prepared by weighing 0.986g L-DOPA, transferring to a dry 500 ml capacity glass bottle and dissolving in 500 ml deionized water. All reagents and soil samples were incubated at field temperature for 24 hours before analysis. Two separate 1 g (±0.05) sub-samples of soil were placed in separate stomacher bags (Seward, Worthing, UK). Nine ml deionized water was added to each bag and the bags homogenized in a laboratory paddle blender (Stomacher® circulator, Seward, Worthing, UK) for 30 seconds. Ten ml deionized water was pipetted into one of the bags ('blank') and 10 ml L-DOPA solution was pipetted into the other bag ('substrate'). Both bags were further homogenized in the laboratory paddle blender for 30 seconds and incubated at field temperature for ten minutes to ensure linear oxidation of L-DOPA. After the allotted time, homogenate from each bag was transferred into three 1.5 ml Safe-Lock Eppendorf micro centrifuge tubes and centrifuged at 10,000 rpm for 5 minutes. 300 µL of the supernatant was then transferred from each tube into a well of a clear 96-well Sterelin microplate (Triple Red, Buckinghamshire, UK). The absorbance of the red pigment (dopachrome) was measured in a SpectraMax M2e spectrophotometer (Molecular Devices Ltd., Wokingham, UK) at 475nm (Dunn *et al.*, 2014).

4.3.3.3. Percentage water and soil organic matter (SOM)

Soil water and organic weights were determined following the method of Frogbrook *et al.* (2009). Approximately 10g of soil were placed in pre-weighed crucibles, weighed again and placed in an oven (Memmert, Schwabach, Germany) at 105°C for 24 hours to ensure thorough evaporation of water content. After weighing again, the crucibles were placed in a muffle furnace (Carbolite, Sheffield, UK) for 120 minutes at 550°C and weighed again thereafter. The weights were used to calculate the water and organic contents as a percentage of the original sample using an Excel spreadsheet.

4.3.3.4. Measurement of soil phenolic concentration

The soil phenolic assay was carried out based on methods described by Box (1983) and Dunn *et al.* (2014). Briefly, phenol stock solution and sodium carbonate buffer were prepared. To prepare the 1000ppm phenol stock standard solution, 0.25g of phenol compound was weighed on an analytical balance, transferred to a clean 250ml volumetric flask, and filled to the mark with deionised water.

The calibration solutions were prepared by diluting the 1000ppm stock solutions accordingly to create a range of calibration standards from 0-30ppm in 100 ml volumetric flasks. After adding the relevant volume of stock solution, the flasks were filled to the 100ml mark with deionized water and stored at 4^0C until required.

To prepare the sodium carbonate solution, 200g of Na_2CO_3 (Sigma-Aldrich UK Ltd) was added to a 1L flask and filled with deionized water. The solution was placed on the magnetic stirrer for 1 hour to ensure that all of the compound dissolved completely. The solution was then filtered using a 47mm GF/F (Phenomenex, Macclesfield, UK) filter on a vacuum pump.

The concentration of water-extractable phenolics was determined on the water extract samples. The standards 0, 0.25, 0.5, 0.75, 1, 2, 3ppm were chosen because the samples were slightly coloured. For each calibration standard and sample, 1ml was pipetted into separate labeled 1.5ml eppendorf microcentrifuge tubes. To each of these, 50µl of Folin-Ciocalteau Phenol Reagent (Sigma-Aldrich UK Ltd) and 0.15ml of Na_2CO_3 solution was added to all tubes and they were mixed by turning the vials upside down in a rack a number of times. The samples were left to incubate for 1 hour 15 minutes at room temperature, during which time a gradual colour change to blue was observed, indicating the presence of phenolics.

After the incubation period, three replicates of 300µl of each standard and sample was transferred into wells of a clear 96 well microplate. The plate was analysed for absorbance at 750nm on a SpectraMax M2e Spectrophotometer (Molecular Devices Ltd., Wokingham, UK). The instrument averages the three replicates of each standard and sample and calculates the concentrations of the samples from the calibration curve.

4.3.3.5. Microbial soil respiration

Soil respiration was performed by placing 10g of homogenized soil into a 50 ml falcon tube fitted with rubber septa in the lids and incubated at field temperature for a period of 60 minutes. After this time, the gases were collected from the tubes using a 10 cm^3 syringe fitted with a short bevel hypodermic needle (Sigma Aldrich Ltd, Dorset, UK) and transferred into labelled

(Time 2; T2), pre-evacuated 10 ml exetainers (Labco Ltd, Lampeter, UK) fitted with screw caps with pierceable rubber septa. An air sample from above the centrifuge tubes containing the samples at the start of the experiment was collected into five exetainers labeled as Time 1 (T1).

Gas samples were analyzed on a Varian model 450 gas chromatograph (GC) instrument, fitted with a flame ionization detector (FID) and a catalytic converter (methaniser) to measure CO_2 and CH_4 concentrations, and an electron capture detector (ECD) for N_2O. Oxygen-free nitrogen is used as the carrier gas. CH_4, CO_2, and N_2O were quantified by comparison of peak area with that of the three standards of known concentrations, prepared by Scientific and Technical Gases Ltd (Newcastle under Lyme, Staffordshire, UK), used in the preparation of a standard curve. The measured concentrations were used to calculate a flux, based on the difference between the T1 and T2 samples and corrected for the 5 g weight of soil and 60-minute incubation time. Fluxes of the greenhouse gases are expressed in $\mu g^1\ s^{-1}$.

4.3.4 Statistical analysis

Correlation analysis was used to test for relationships between the measured parameters using IBM SPSS v22 (IBM Corporation, New York, USA).

4.4. Results

The data revealed lower contents of both water and organic matter in the soils at the mangrove end of the gradient, with gradually increasing contents into the transition to salt marsh, reaching a plateau midway along the gradient (Figure 4.2). Soil water contents were in the range of 30.9-37.5% in the mangrove area and 49.3-81.1% in the salt marsh section. Similarly, the SOM content was 4.8-7.1% and 9.4-34.6% in the mangrove and salt marsh areas respectively. There is a very strong correlation between the water and SOM contents along the gradient ($r=0.976$, $p<0.001$), although further inspection reveals that this is a non-linear relationship (Figure 4.2).

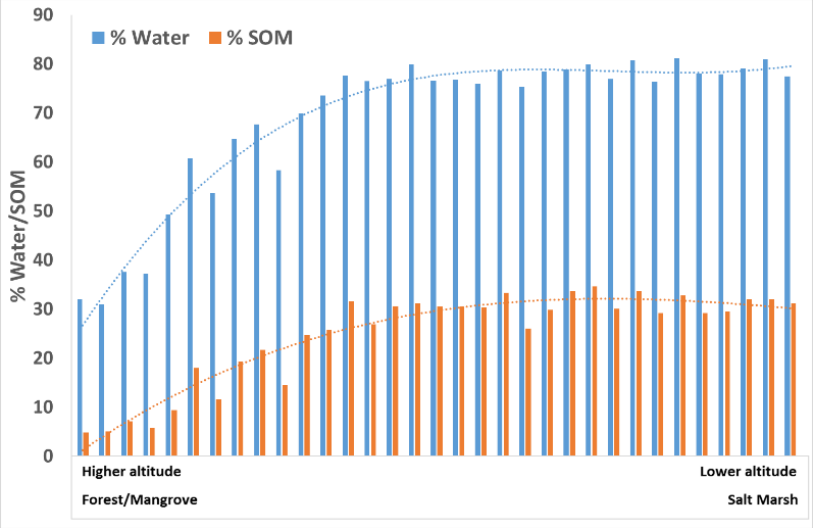

Figure 4.2: Bar chart showing percentage soil water and SOM content along the black mangrove-high zone salt marsh gradient. Both trendline shows a third order polynomial relationship

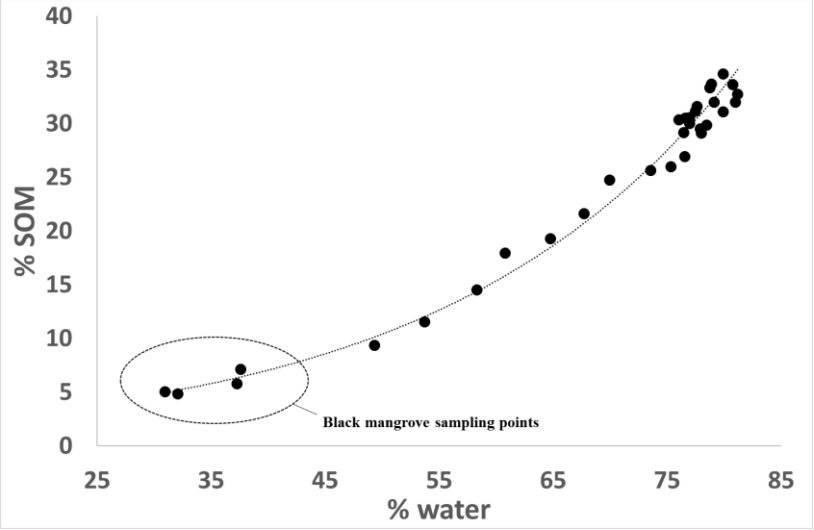

Figure 4.3: Scatterplot showing the relationship between percentage soil water and SOM content along the black mangrove-high zone salt marsh gradient. The trendline shows an exponential relationship

Analysis of the abiotic factors, pH and conductivity, reveals a clear trend along the gradient (Figures 4.4 and 4.5). The mangrove soils have a much more neutral pH, ranging from 7.11-6.20. The pH decreases into the salt marsh, with values between 6.10-4.34. The conductivity was much lower in the mangrove, ranging from 37.3-58.1 µS g^{-1}, compared to the salt marsh, where the conductivity varied from 104.4-387.7 µS g^{-1}. The pH and conductivity correlated very strongly (r=-0.874, p<0.001). Soil phenolics showed a small increasing trend along the gradient, although there is a lot of variability between the individual samples. Concentrations were generally lower in the mangrove area, ranging from 10.5-23.0 µg g^{-1}. In the salt marsh part of the transect the phenolics varied from 20.3-70.5 µg g^{-1}.

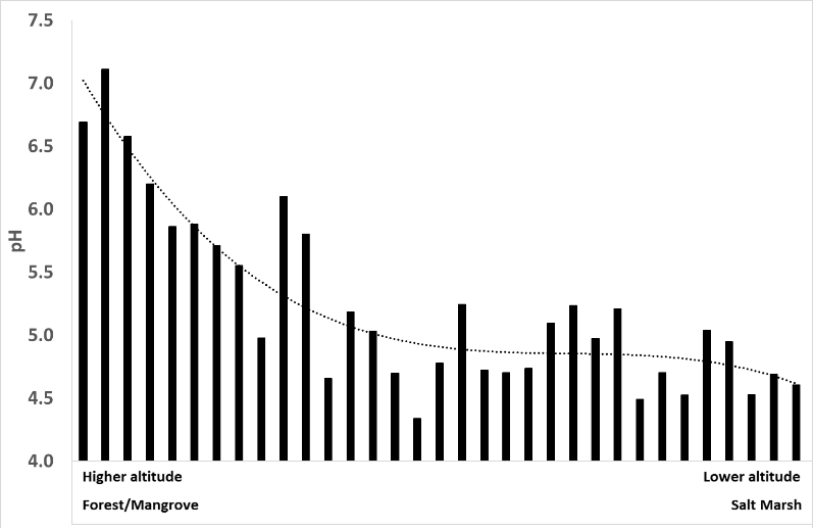

Figure 4.4: Bar chart showing soil pH along the forest/mangrove/salt marsh gradient. The trendline shows a third order polynomial relationship

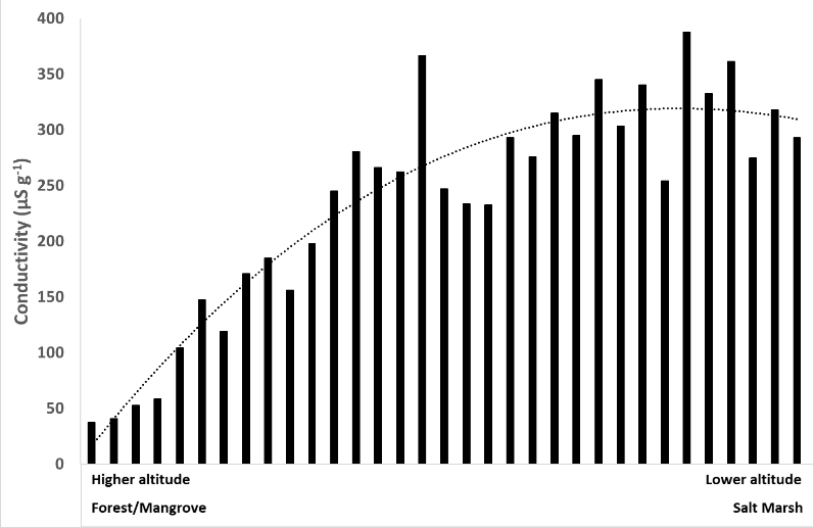

Figure 4.5. Bar chart showing soil conductivity along the forest/mangrove/salt marsh gradient. The trendline shows a third order polynomial relationship

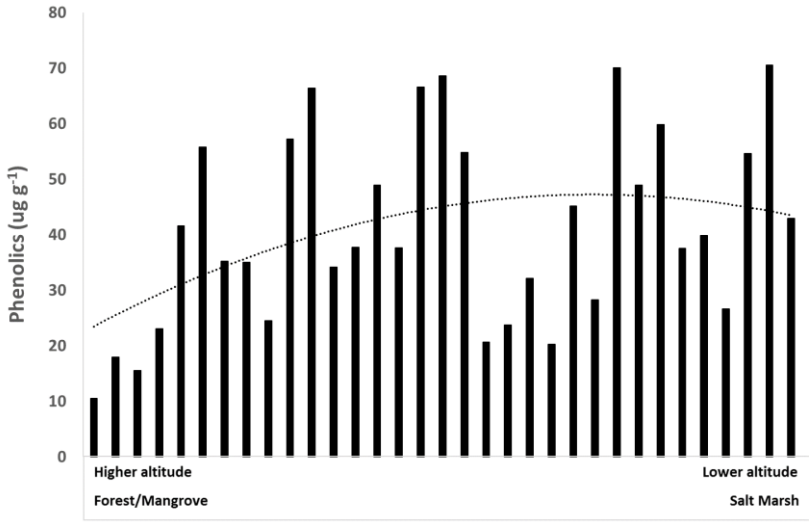

Figure 4.6: Bar chart showing soil phenolics concentrations along the forest/mangrove/salt marsh gradient. The trendline shows a third order polynomial relationship

For the biological parameters, most of the empirical evidence suggests higher rates of decomposition in the mangrove part of the transect. The activity of phenol oxidase was higher in the mangrove encroached areas (378.9-1522.2 nmol dicq g^{-1} h^{-1}) compared to the salt marsh sites (69.4-849.1 nmol dicq g^{-1} h^{-1}), as seen in Figure 4.7. None of the other measured parameters correlated significantly with phenol oxidase activity, including phenolics ($p>0.05$).

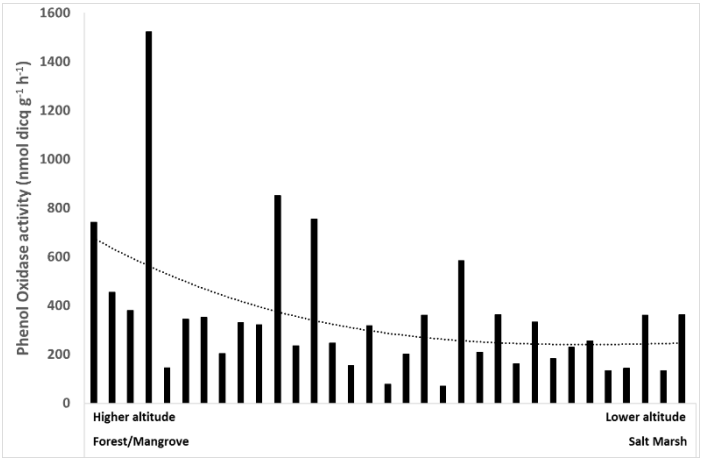

Figure 4.7: Bar chart showing phenol oxidase activity along the forest/mangrove/salt marsh gradient. The trendline shows a third order polynomial relationship

The activity of three of the five hydrolase enzymes; β-glucosidases, β-xylosidases and Phosphatase showed a declining trend in activity from the mangrove to the salt marsh parts of the gradient. The activity of β-glucosidase ranged from 4.04-7.01 in the mangrove area and 0.71-4.44 nmol g^{-1} min^{-1} in the salt marsh area. For chitinase the activity showed a very similar trend, with activities recorded at 3.18-4.86 nmol dicq g^{-1} h^{-1} in the mangrove area and 0.93-3.83 nmol dicq g^{-1} h^{-1} in the salt marsh part. There is a strong correlation between the activities of both enzymes ($r=0.951$, $p<0.001$). The trend was a little different for β-xylosidase; activities ranged from 1.56-1.99 nmol dicq g^{-1} h^{-1} in the mangrove soils, but there was much greater variability in the salt marsh part of the transect, ranging from 0.33-3.11 nmol dicq g^{-1} h^{-1}, with the greater activities at the end closest to the mangroves. There is a strong correlation between the activity of β-xylosidase and both β-glucosidase ($r=0.791$, $p<0.001$) and chitinase ($r=0.793$, $p<0.001$). The activity of all three enzymes correlated very strongly and positively with pH ($r=0.657$-0.890, $p<0.001$), very strongly and negatively with soil water content ($r=-0.590$-0.868, $p<0.001$), soil organic matter ($r=-0.632$-0.845, $p<0.001$) and conductivity ($r=-0.668$-0.818, $p<0.001$), but there is no correlation with phenolics ($p>0.01$).

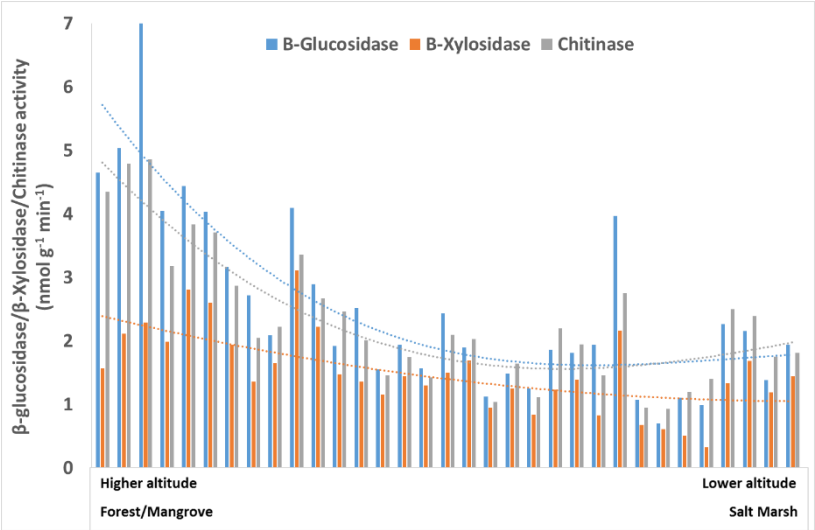

Figure 4.8: Bar chart showing β-glucosidase, β-xylosidase and chitinase activities along the forest/mangrove/salt marsh gradient. The upper two trend lines shows a third order polynomial relationship while the lower trend line shows second order polynomial relationship

The activities of arylsulphatase and phosphatase did not show a clear trend along the transect and did not correlate with any other soil parameters, although the activities for both enzymes were generally lower in the mangrove soils.

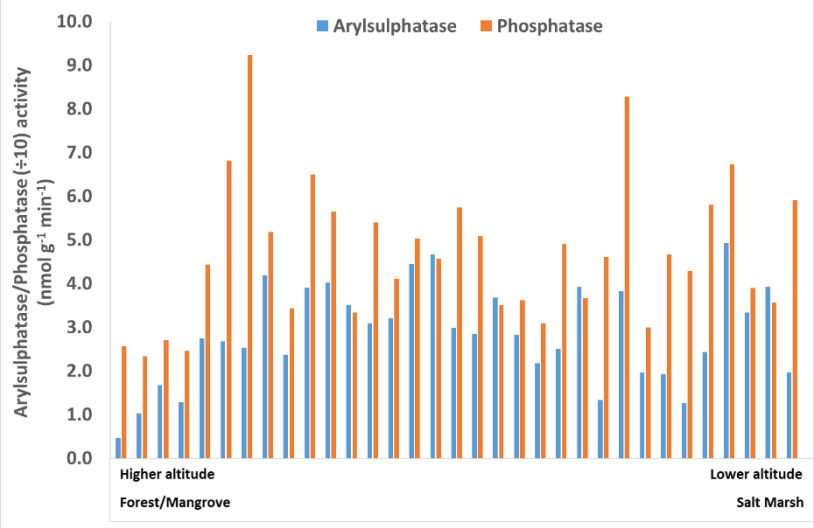

Figure 4.9: Bar chart showing arylsulphatase and phosphatase activities along the forest/mangrove/salt marsh gradient

A gradual decline in CO_2 emissions was observed along the transect, although the high degree of variability from sample to sample point means that there is not much confidence in this trend. Emissions ranged from 6.16-14.05 µg g^{-1} h^{-1} in the mangrove area, compared to0.02-27.57 µg g^{-1} h^{-1} in the salt marsh area. None of the measured parameters correlated significantly with the CO_2 flux ($p>0.05$).

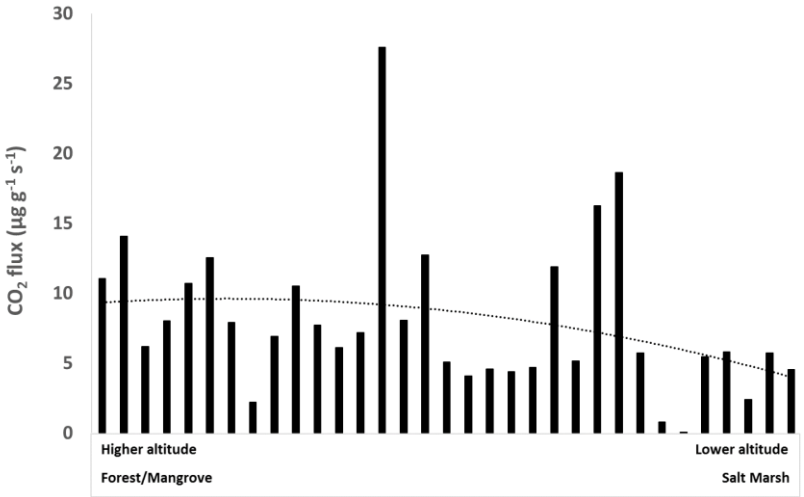

Figure 4.10: Bar chart showing CO_2 emissions along the forest/mangrove/salt marsh gradient. The trendline shows second order polynomial relationship

4.5. Discussion

The rate of decomposition of organic matter in wetland soils is to a large extent dependent on edaphic factors such as temperature, oxygen and water availability. To this end, various biological processes such as growth of vegetation and microbial activities in such ecosystems is affected by such factors.

This investigation seem to suggest that water logging and vegetation cover could have some influence on the differences in soil enzyme activities and accumulation of phenolics, which are key controls on decomposition and carbon storage in wetland environments. The low water content in the soil around the mangrove encroached area, compared to the higher water content in soils from the salt marsh dominated areas, could be attributed to differences in the range of tidal exposures as well as vegetation cover.

These findings from this work implies that degradation of phenolic inhibitors could leave the enzymic latch 'opened' and a reduction in hydrolases enzyme activity and aiding decomposition and mineralisation of accumulated organic matter in the soil. This then implies that the organic matter is decomposed at a higher rate at mangroves take over areas while the reverse is the case in the salt marsh zones, which appeared to be more waterlogged. Once the enzymic latch mechanism is opened, decomposition is enhanced and the soil loses its carbon storage ability, instead it becomes net source of carbon into the atmosphere, which will equate to a negative impact on climate change (Yabuki *et al.*, 2009a; Kitaya *et al*., 2002; Joaquin 2014)

This findings agree with similar previous studies that as mangrove plants encroach into salt marsh vegetation, the soil conditions are altered, decreasing soil carbon and nitrogen due to breakdown of organic matter (Osland *et al*., 2017; Yando *et al*., 2016; Cavanaugh *et al*., 2014; Saintilan *et al*., 2014).

The results obtained suggest a higher trend of biological activities in the soils sampled from the mangrove takeover area, which was less waterlogged and dominated by emerging black mangrove vegetation while soils from the salt mash zone, which was water logged and dominated by *Spartinia* and *Batis* spp plants had lower biological activities. It is hypothised that the higher rate of decomposition in the black mangrove take-over area is likely to be due to the presence of the black mangrove trees' pneumatophores which is likely to increase oxygen levels. These specialised roots oxygenate the substrate and therefore 'open' the enzymic latch allowing phenol oxidases to break down the inhibitory phenolic compounds at those sites

encouraging the activities of hydrolases enzyme to break down accumulated organic matter and release sequestered carbon in the process (Yabuki *et al.*, 2009a; Freeman *et al*., 2011).

Other factors that might come into play include the role of gradient and altered soil moisture content, the latter being because of increased water uptake by the mangrove compared to salt marsh vegetation. This will affect microbial activity and rhizodeposition from the mangrove roots. The waterlogged condition of the salt marsh zones has created an anoxic environment in the soil which will place a constraint on the activities of hydrolases enzymes and increase the concentration of inhibitors such as Fe^{2+} (Pulford & Tabatabai, 1988; Freeman *et al*., 1996).

It has been reported that mangrove encroachment into salt marsh vegetation is accompanied by initial depletion of the salt marsh type vegetation as the latter cannot withstand the loss of nutrients due to mangrove plants (Stevens *et al*., 2006; Osland *et al*., 2017). As a result of die-off of salt marsh vegetation, the soil surface is exposed to the atmosphere, encouraging oxygenation, evaporation and triggering microbial activities (Saintilan & Williams, 1999; 2000; Joaquin, 2014). Soils along the salt marsh zones with less vegetation cover had rather higher rates of biological activities, which supports the earlier argument that vegetation cover could in part impact the biogeochemical processes in wetland soils rather than elevation alone.

Freeman *et al.* (2012) stated that oxygen and nutrient availability, temperature and pH are vital factors that drive enzyme activities in the wetland environment. Low water content in the soil affects the activities of soil enzymes by promoting microbial proliferation and modifying the enzyme kinetics (Kang & Freeman, 1999). This could be driving the stimulated decomposition in the mangrove take over sites.

Carbon dioxide flux is higher at the take-over area, but the effect is not as strong as for the carbon cycling enzymes. Additional work, involving the measurement of net ecosystem exchange (NEE), is needed to determine whether the black mangrove-encroached area could be a net source of GHGs, in comparison to the likely net sink of the salt marsh area. In this investigation, increasing soil water content and higher amount of soil organic matter is credited to increasing waterlogging along the transect towards the salt marsh zones.

Guo *et al.* (2013) investigated biotic interactions between black mangrove and salt marsh vegetation along the Texas coast across longitudinal and elevational gradients and different life stages of black mangroves and discovered that salt marsh vegetation facilitated black mangrove

seedlings at high latitude but inhibit it at a low latitude. Similarly, Yando *et al*., (2016) investigated the effects of woody plant encroachment on plant-soil interaction and ecosystem carbon pools in salt marsh-mangrove ecotones and submitted that mangrove displacement of salt marsh are highly dependent on the properties of the displaced salt marsh.

Empirical evidence has suggest that mangrove takeover of salt marsh usually takes 4 years, before the mangrove develops fully into a swamp (Kangas and Lugo, 1990; Turner, 1993; Couwenberg *et al*., 2010). Similar studies reveal that the saltmarsh grass *Spartina patens* failed to survive high salinity and sediment accretion caused by mangrove take-over in Paranagua Bay, Brazil (Lana *et al*., 1991).

Some scholars have suggested that mangrove encroachment into salt marshes could enhance carbon storage and increase elevation in response to rising sea levels (Kelleway *et al*., 2017). There are other points of view stating that the full impact of takeover could last from years to decades particularly in terms of net benefits for carbon storage (Osland *et al*., 2012; Kelleway *et al*., 2017). Changes as result of a long transition period could have serious implications for centuries of stored soil carbon and could create a great deal of uncertainties over other vital ecosystem services offered by salt marsh habitats.

Another concern of mangrove takeover of salt marshes is that the ecological protective role of the salt marsh would have been compromised within the succession period. The majority of the findings thus agree with the hypothesis that mangrove take-over of salt marshes will compromise the traditional carbon storage ability of saltmarshes within the transition period. This has the potential to provide negative feedback for the global atmosphere, though some findings do suggest a more beneficial influence mangrove takeover has on the salt marsh habitat. This finding therefore indicate that the expansion of black mangroves has an initial impact on the accumulated belowground salt marsh carbon stock and also provide a foundation for better understanding for both above- and below-ground effects of mangrove takeover of salt marsh ecosystem.

4.6. Conclusion

Outcome from this investigation seems to provide some vital insights into the potential impacts of mangrove encroachment into saltmarsh type vegetation. It is, however, worth noting that the full range of ecological consequences of full mangrove takeover of salt marsh ecosystems has not been fully explored. Moreover, it is on record that the two types of ecosystems in this study provides numerous ecosystem services and any form of alterations in these habitats could have implications for coastal communities and the global climate (Barbier *et al.*, 2011; Osland *et al*., 2017). It is therefore speculated that mangrove take over implies that microbial activities in the soil could be stimulated in response to increased atmospheric oxygen ingress into the soil profile, with increased temperature and nutrient availability. This will trigger microbial activities with increased decomposition rates along encroached areas thereby compromising vital ecosystem services of this important blue carbon sink within the transition period that could last several decades to realise the full potential of carbon storage, which is a key role of salt marsh ecosystems (Adam, 2002; Loomis & Craft, 2010; Osland *et al*., 2012; Kelleway *et al*., 2017).

The 'enzymic latch' mechanism is being weakened by the mangrove encroachment into salt marsh habitat, which is increasing decomposition of organic matter and the release of CO_2 to the atmosphere. The reduction in biological activities as witnessed in the salt marsh areas indicates that the invasion or replacement of salt marsh type shrubs by a woody tropical and sub-tropical mangrove type vegetation with entirely different physiology could have serious transient implications for the saltmarsh ecosystem. Morever, saltmarshes are regarded as one of the most dominant carbon sinks in the world, capturing and storing up to 87.2 ± 9.6 Tg C yr^{-1} (Mcleod *et al*., 2011), so their replacement by mangroves can potentially release centuries of stored carbon into the atmosphere. To this end, a drastic reduction in anthropogenic activities responsible for increasing global warming, is necessary if mangrove encroachment into salt marshes habitat is to be curtailed.

Acknowledgements

Much appreciation to Bob Wasno of the Florida Golf Coast University, USA and Dr Todd Osborne, Whitney Laboratory for Marine Bioscience, University of Florida for their guidance in the field and Wetland science conservation MSc students 2016/17 set of Bangor University,

UK for their kind assistance in collecting soil samples. Dasat G.S is grateful to TETfund Nigeria for funding to undertake the research study in Bangor University Wales, UK.

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Chapter 5

Effects of tree species on leaf litter decomposition and carbon cycling in a mangrove ecosystem

5.1. Abstract

Carbon sequestration and nutrient dynamics in mangrove soils depend on the turnover of organic materials. Leaf litter accounts for 32% of the total net primary productivity (NPP) in mangroves and contributes significantly to the total soil phenolic budget in mangrove ecosystems. Analysis of the phenolic and nutrient contents of partially decomposing leaf litter materials obtained from the three main mangrove species found in Florida, USA, reveals that the decomposing leaves of white mangrove (*Laguncularia racemose*) and black mangrove (*Avicennia germinans*) contain much higher concentrations of phenolics than those from the red mangrove (*Rhizophora mangle*), whereas decomposing red mangrove leaves contribute much greater loads of key nutrients. The contrasting inputs of phenolics and nutrients from the leaf litter of the three different species will have important consequences for soil carbon cycling as one of the key ecosystem services that mangrove ecosystems offer.

5.2. Introduction

Mangroves are one of the world's most productive ecosystems, rich in organic matter and nutrients, supporting a wide range of biodiversity ranging from fungi, bacteria, algae and vertebrates, as well as invertebrate fauna (Reef *et al.*, 2010; Shafique *et al.*, 2015). Mangrove swamps are a reservoir and source of organic matter and nutrients to coastal environments due to high levels of detritus inputs that, once decomposed, enrich the soil and supply water with particulate organic matter (POM) and dissolved organic matter (DOM). Therefore, mangrove detritus are a vital source of energy and organic matter for tropical estuarine and coastal ecosystems (Lima & Colpo, 2014; Nordhaus *et al.*, 2017).

Leaf litter materials are an integral component of net primary productivity (NPP) as well as a source of soil nutrients in mangrove swamps (Zhao e*t al*., 2015; Shafique *et al*., 2015). Nordhaus *et al.* (2017) noted that leaf litter production accounts for about 32% of the total NPP in mangroves. It is estimated that the NPP for mangrove systems is in the range of 2 to 50 Mg C ha^{-1} $year^{-1}$ (Alongi, 2009), matching that of some of the most productive old growth terrestrial tropical forests (Clark *et al*., 2001). Freshly fallen leaf litter is either submerged and buried under mangrove forest soil sediments or washed away by tidal action to adjacent waters.

In the mangrove ecosystem, litter fall comprises predominantly leaves, which are an important source of food for decomposers in such anoxic environments (Jennerjahn & Ittekkot, 2002, Mfilinge *et al.*, 2005; Numbere & Camilo, 2017). Leaf litter fall from mangrove vegetation serves as a key source of energy within the food web, which has an impact on commercial coastal fisheries. The contribution of litter fall to the carbon budget in red mangrove ecosystems alone is estimated at 89 g dry weight m^{-2} y^{-1} as reported by Bremer, (1995). The mangrove swamps of southern Florida, USA and Tabasco, Mexico have an estimated leaf litter fall of 4 to 8 tons ha^{-1} $year^{-1}$ which eventually gets broken down by microbial action and integrated into the soil sediments as organic matter (Contreras *et al*., 2017).

The decomposition of leaf litter from mangrove vegetation is an important part of nutrient cycling involving various biochemical and physical processes. On contact with water, leaching takes place and the leaf is subsequently broken down by microbial and faunal action. The biochemical process involves converting complex organic compounds into simple bioavailable inorganic nutrients for plant and microbial uptake. Consequently, the breakdown of organic

substances from leaf litter and recycling of detritus can increase nutrient levels in the soil and make them more available for plant growth and development (Contreras *et al*., 2017).

Leaf litter decomposition in wetland habitats has attracted the attention of many researchers as it is vital for several key wetland functions including sequestering carbon, improvement of water quality, phenolic enrichment as well as dissolved organic carbon (DOC) production (Mitsch and Gosselink, 1993; Freeman *et al.*, 1993; Freeman *et al*., 2001a). Several studies have attributed the abundance of bacteria and fungi to leaf litter decomposition (Steinke, 2000; Mumby *et al.*, 2004; Rajendran & Kathiresan, 2007; Kathiresan & Bingham, 2001; Barroso Matos *et al.*, 2012). Litter production has been measured in mangroves in Kenya, Bermuda and Australia to be 0.011 t ha^{-1} y^{-1}, 9.4 t ha^{-1} y^{-1}, and 23.69 t ha^{-1} y^{-1} respectively (Kathiresan & Bingham, 2001). Therefore, it is important to assess litter decomposition as an important tool for determining ecosystem functions in mangroves.

The cycling of nutrients and trace elements in mangrove swamps has received less attention compared to other forest types in the tropical and sub-tropical regions of the world (Alongi *et al*., 2005). Mangroves, like any other plant in the environment, have the capacity of losing some stored nutrient elements due to environmental as well as physiological related stresses. This is as a result of their robust physiological and biogeochemical mechanisms that efficiently manage such material losses as a result of tidal or atmospheric exchange while surviving in a typically anoxic environment (Alongi *et al*., 2005). Decomposed organic matter from plant leaf litter can greatly influence mineral cycling in mangrove wetlands. It is estimated that >90% of nitrogen and phosphorous and up to 60% of mineral nutrient intake by wetland plants are from plant litter, this then serves as a critical link in nutrient cycling in wetland ecosystem (Zhao *et al*., 2015).

In the process of decomposition of leaf litter materials, a large amount of organic substances such as dissolved organic carbon (DOC) are released into the soils (Reef *et al*., 2010). Nitrogen, amino acids and hexosamines are building blocks of proteins and constitute the bulk of organic matter. They also make up a large proportion of mangrove leaves and sediments. Moreover Alongi e*t al*. (2005) found that the main nutrient pool of mangrove forests is buried underground and not in above-ground biomass. Studies on mangrove leaf litter decomposition in the tropics revealed that fungi to a large extent are consequential decomposers of submerged leaves in mangrove swamps and they play critical roles in biochemical cycling, increasing soil

nutrients in the process (Mackey & Smail, 1996; Gulis & Suberkropp, 2003; Romero *et al*., 2005).

The microbial breakdown of plant litter materials in mangrove swamps is largely dependent on the microbial biomass, abundance and structure of the decomposing organisms, quality of organic matter and environmental condition of the soil (Clark *et al*., 2001; Chanda *et al*., 2016). Bacteria and fungi in particular are the key agents of decomposition of mangrove litter materials and thereby control the conversion and cycling of nutrients (Kathiresan & Bingham, 2001). Decomposer organisms depend on leaf detritus as an important base for food webs, which could either be utilised within the ecosystem or exported.

Kathiresan *et al*. (2011) noted that fungi are associated with decomposition of leaf materials in the mangrove environment and, as leaf material falls, it is colonised and broken-down by fungi. Three stages are identified in the process of decomposition: leaching of soluble compounds; microbial oxidation of refectory compounds such as cellulose and lignin; and physical and biological fragmentation (Bremer, 1995). The rapid conversion of leaf litter to organic matter by microbial enzyme activity in the mangrove swamp floor greatly contributes to the soil sediment carbon budget in this habitat alongside other inputs from estuarine or marine sources (Jennerjahn & Ittekkot, 2002; Bouillon *et al*., 2002).

Fungi are the first microbes to colonise mangrove leaves once submerged. *Phylloplane* fungi do not attack live mangrove leaves, their action starts as soon as the leaves are submerged. This is followed by cellulase-producing fungi in the first 21 days of submergence, then the action of xylanase producers between 28-60 days. Pectinase, amylase and protease are dominant throughout the decomposition period (Kathiresan & Bingham, 2001). Recycling of nutrients continues with the degradation of leaves where a number of substances are removed and contribute to the dissolved organic matter budget. Carbohydrates are removed quickly in the early part of decomposition. However, tannins, which have some antimicrobial properties leach slowly and its abundance in the soil depletes microbial populations (Kathiresan & Bingham, 2001).

Interestingly, decomposition of mangrove leaf litter materials varies with species type and depends on the structure and chemical components of the leaf. For example, *Avicennia* spp. leaves decomposed faster than others decompose because the leaf is thinner and more easily

submerged and have a lower tannin content than others. *Rhizophora* spp. floats for several days hence takes longer to be submerged and decomposed (Kathiresan & Bingham, 2001). Mackey & Smail (1996) investigated decomposition in *Avicennia* spp. leaves and reported a significantly faster rate of decomposition in the lower tidal zones where there are longer submersion periods.

Phenolics are chemical compounds comprised of one or more aromatic ring, bearing one or more hydroxyl functional groups. They occur universally in high concentrations in higher and woody plant materials, soils, microorganisms and more recently have been found in industrial waste effluents. Usually, phenolics could get into the soil either through leachates or as particulate matter as plant materials gets decomposed by microbial action (Hättenschwiler & Vitousek, 2000). Once integrated into the soil, phenolics can regulate biological activities such as decomposition, mineralisation and cycling of organic materials (Freeman *et al.*, 2001b; Rovira &Vallejo, 2002; Toberman *et al*., 2010). Several studies have identified polyphenols from plants as regulators of soil processes which are also thought to limit decomposition and nutrient recycling in wetland soils (Hättenschwiler & Vitousek, 2000). Also, polyphenols from plants can control the carbon pool and the form of nutrients available for plants and microbes (Hättenschwiler & Vitousek, 2000).

The decomposition of leaf litter material is reported to significantly contribute to the carbon budget in mangrove ecosystems and eventually increase its carbon sequestration potentials. To this end, Twilley *et al.* (1992) reported an approximate annual total of 38 mol m^{-2} $year^{-1}$ of captured carbon in the mangrove ecosystem. Recent work by Freeman *et al*. (2012) demonstrated that genetic modification of *Sphagnum* could result in more synthesis of phenolics into such wetlands and suggested that the addition of phenolic materials in a typical wetland environment has potential to be used as a geoengineering tool for carbon sequestration in terrestrial environments.

The impact of phenolic compounds on the rate of breakdown of organic matter has been investigated (Min *et al*., 2015) directly and indirectly by employing litter bag and microbial biomass methods, respectively. Toberman *et al*. (2010) and Min *et al*. (2015) measured the activities of phenol oxidase and hydrolases and the rate of decomposition of leaf litter and concluded that decomposition of soil organic matter was impaired due to the inhibitory action of phenolics on decomposing hydrolases enzymes. This point of view supports the ‘enzymic

latch' mechanism (Freeman *et al.*, 2001a). Opelt *et al*. (2007) and Mellegård *et al*. (2009) demonstrated that phenolic acids released from *Sphagnum* spp. suppressed bacterial and fungal growth and the activities of β-glucosidase, phosphatase, sulphatase, chitinase, and xylosidase by 21, 15, 32, 18, and 14% respectively (Freeman *et al*., 2004). Furthermore, a reduction in the production of CO_2 was encountered in peat samples containing phenolic compounds under anaerobic conditions (Freeman *et al*., 2004) as the rate of litter decomposition was shown to be inversely proportional to the phenolic content in leaf litter (Min *et al*., 2015).

The role of mangrove leaf litter as a source of phenolics has received relatively less attention than plants such as *Sphagnum* mosses. Therefore, this study aimed to determine phenolic compound inputs from mangrove leaf litter materials into the mangrove soils by analysing the phenolic content of leaf litter materials obtained around red, black and white mangrove swamps in northern Florida, USA. It is therefore hypothesise that leaf litter materials from red mangrove contribute more phenolics into the soil because it is thinner and easily submerged and decomposed as against the black and white mangrove the leaves of which are wider with longer floating periods.

5.3. Materials and methods

5.3.1. Sampling site and sample collection

The leaf litter samples for this investigation were collected in southern Florida, USA, near Barefoot Beach County Preserve, Bonita Springs, in an area containing all three of the mangrove tree species that are dominant in this region; red (*Rhizophora mangle)*, black *(Avicennia germinans)* and white (*Laguncularia racemose)*. More details about the sampling location can be found in Chapter 3 were soils were sampled in the same locations. Sampling was carried out at low tide period. At each site, about 100g of partially decomposed litter leaves were collected devoid of stones, shells and sand at five different sites about 3 meters apart under each of the three different mangrove stands. This was placed into labelled plastic bags and sealed.

5.3.2. Laboratory analyses

Samples were analysed for phenolic content and percentage water and solid organic matter, using procedures outlined in Zheng & Wang (2001), Cai *et al*. (2004) and Dunn *et al*. (2014). Briefly, samples were air-dried in the dark at room temperature for 2 weeks to allow for evaporation of moisture. After obtaining a constant weight for three days, the litter were ground into fine powder using a dry mill grinder (Kenwood Ltd., UK) and passed through a sieve (24 mesh) and packed into sealed plastic bottles until required.

5.3.2.1. Percentage organic matter

To determine the percentage organic matter in the leaf litter, pre-weighed porcelain crucibles were half-filled with leaf litter powder, weighed again and placed in a muffle furnace (Sanyo Ltd., UK) at 550˚C for 1 hour. Following this, the crucibles were weighed again and the organic matter determined as the loss on ignition.

5.3.2.2. Water extraction

To a 50ml falcon tube, 5 gram of powdered leaf litter was extracted with 40 ml ultrapure water at 80˚C for 20 minutes in a water bath shaker (Shaking Bath 5B-16, Techne Ltd., UK). After cooling, the pH and electrical conductivity were determined using SevenEasy and FiveGo (Mettler Toledo, Leicester, UK) pH and conductivity meters and the extract centrifuged at 5,000 rpm for 30 minutes. Following this, the samples were filtered using a 0.45 µm nylon membrane under vacuum. The filtrate was stored at 4˚C until analysis.

5.3.2.3. Determination of phenolic compound concentrations

The concentration of water extractable phenolics from the powdered sample was determined using the Folin-Ciocalteu colorimetric method described by Box (1983), Zheng & Wang (2001) and Cai *et al*., (2004) with some modifications. Briefly, 1 ml of the filtered extract was pipetted into a 1.5 ml self-locked Eppendorf tube (Fisher Scientific Ltd., Loughborough, UK), then oxidized with 50 µl Folin-Ciocalteu reagent (Sigma Aldrich Ltd., UK). The reaction was neutralized with 0.15 ml saturated sodium carbonate (200 g/l). A standard curve was prepared by adding 0-10 mg L^{-1} phenol standard solutions, all preparations mixed and allowed to react for 1 hour 15 minutes at room temperature. Following incubation, for each standard and sample, 300 µl was pipetted into wells of clear flat 96-well microplate. The absorbance at 750 nm of the resulting blue colour was measured with a SpectraMax M2e spectrophotometer (Molecular devices Ltd, Wokingham, UK) and the phenolic concentration of the samples quantified against the standard curve.

5.4. Statistical considerations

Data were analysed by one-way ANOVA to test for the effect of one factor on the measured parameters; site (three levels, white, black, red). Relationships between the enzyme activities and physico-chemical factors across the three mangrove zones were determined by correlation analysis. SPSS v22 (IBM Corporation, New York, USA) was used for all analyses. A p value of <0.05 was used to denote significance for the ANOVA analysis, but <0.01 for the correlation analysis.

5.5. Results

Analysis of the pH (Figure 5.1) of the leaf extracts revealed similar mean values for the white (6.88) and red (7.03) species, but a significantly lower mean value for the black (6.06; $p<0.05$).

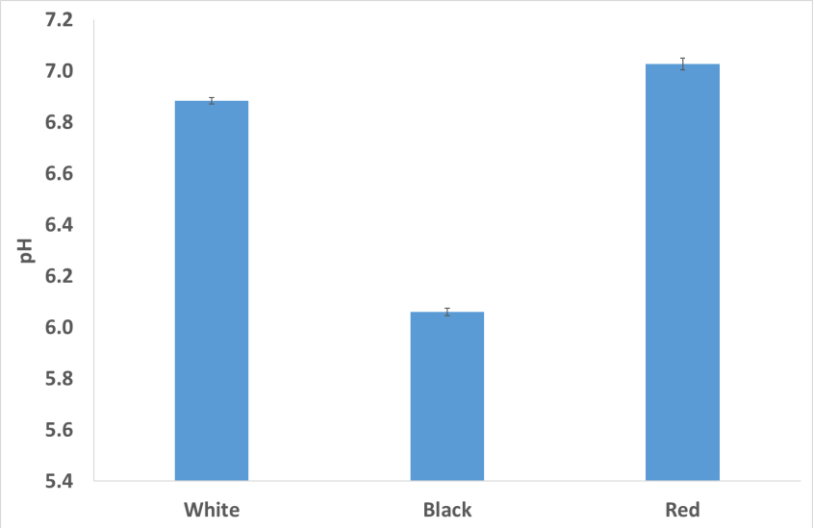

Figure 5.1. Bar chart showing mean pH of the white, black and red mangrove leaf litter extract (n=5, error bars ± SD).

For the phenolic compound concentrations (Figure 5.2), the red mangrove litter had the lowest concentration, with a mean value of 737.51 µg g^{-1}. The black mangrove leaf litter extract had a significantly higher value at 6,480.82 µg g^{-1}, with the white mangrove having significantly higher again at 8,015.56 µg g^{-1} (both $p<0.05$).

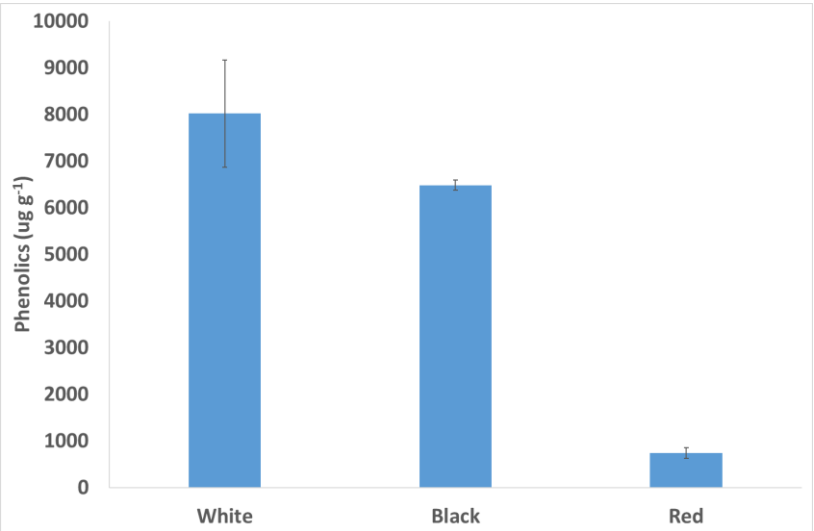

Figure 5.2. Bar showing mean phenolics concentrations of the white, black and red mangrove leaf litter extract (n=5, error bars ± SD).

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The mean concentration of nitrate (Figure 5.3) in the red mangrove leaf litter was 5.89 µg g^{-1}, which is significantly higher than the negligible amounts in the black and white mangrove leaf extract.

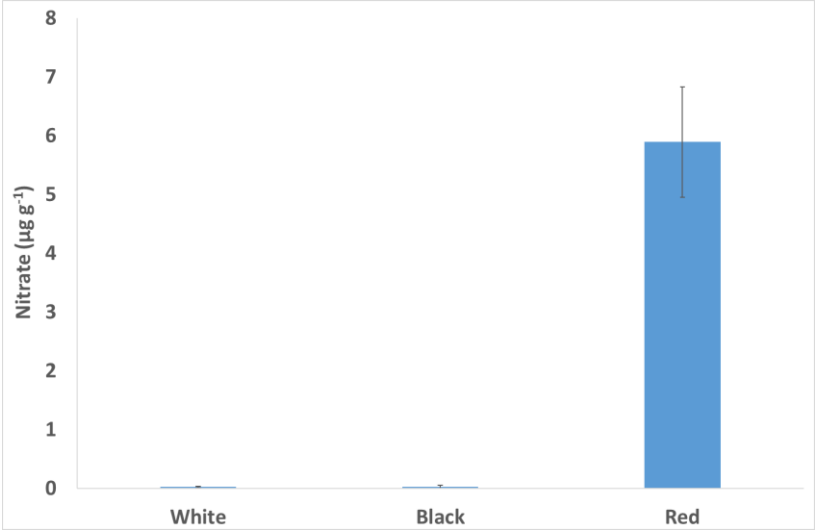

Figure 5.3. Bar chart showing mean nitrate concentrations of the white, black and red mangrove leaf litter extract (n=5, error bars ± SD).

A very similar pattern was observed with ammonium (Figure 5.4), with high concentrations in the red mangrove leaf litter (27.34 µg g^{-1}), compared to very low concentrations for the black (0.12 µg g^{-1}) and white (0.13 µg g^{-1}).

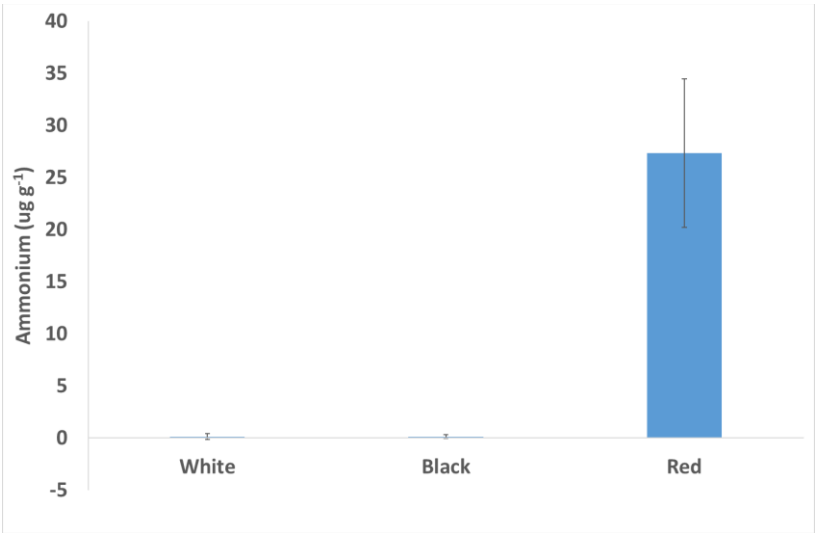

Figure 5.4. Bar chart showing mean ammonium concentrations (n=5) of the white, black and red mangrove leaf litter extract (n=5, error bars ± SD).

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In terms of phosphate (Figure 5.5), the leaf litter material from the red mangrove had the highest content (51.28 µg g^{-1}), which was significantly higher than the black (16.74 µg g^{-1}) and the white (2.05 µg g^{-1}).

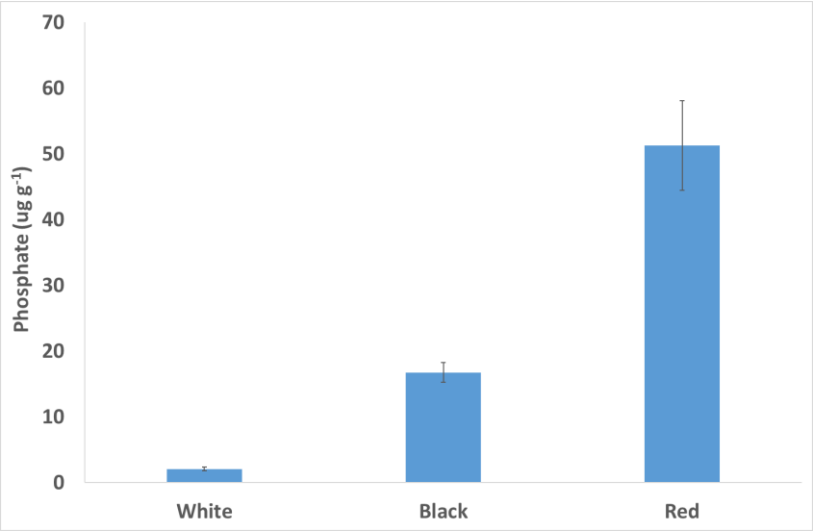

Figure 5.5. Bar chart showing mean phosphate concentrations of the white, black and red mangrove leaf litter extract(n=5, error bars ± SD).

5.6. Discussion

Mangrove ecosystems have large inputs of litter arising from falling leaves, branches and other debris (Kathiresan & Bingham, 2001). Microbial breakdown of such litter materials largely contributes to the overall soil organic matter budget and the recycling of nutrients in the mangrove ecosystems. Therefore, plant leaf litter forms one of the major sources of phenolic compound and nutrient inputs into the mangrove soil sediments (Twilley *et al.*, 1992). The decomposition of leaf litter materials into the mangrove soil sediments increase nutrient availability through the process of mineralisation and contributes to the nutrient demand of decomposing microbes and plant growth and development (Lima & Coplo, 2014).

This investigation has demonstrated that the partially decomposing white mangrove leaves analysed have the highest concentrations of phenolic compounds, closely followed by the black mangrove. The red mangrove leaf litter had much lower phenolics than both, in fact just less than 10% of the value of the white mangrove. This disproves the hypothesis that litter materials from red mangroves have the highest concentration of phenolic compounds.

Red mangrove swamps in particular serve as a breeding ground for aquatic invertebrates such as fishes and crabs and the activities of such macro-invertebrates produce much detritus from the leaves. It is therefore speculated that proximity to the water's edge with frequent flooding from tidal action and turbulence from receding water could accelerate mass loss of undecomposed materials in the red mangrove zone. This could technically lead to more rapid breakdown of phenolic compounds from the red mangrove leaf litter. Ashton *et al.*, (1999) reported that the activities of crabs and other macro invertebrates contributes to fast break down of leaf litter enhancing faster decomposition rates in subtidal regions than intertidal regions.

The leaf litter materials from the red mangrove appears to be richer in some mineral nutrients such as nitrogen, phosphate and ammonium than those from the black and white. This may be related to the faster decomposition of red mangrove leaf litter, as speculated previously regarding the observations for phenolic compounds. Faster degradation of leaf litter would be expected to release nutrients such as nitrate, phosphate and ammonium and the greater input of these to the near shore coastal environment would be expected to fuel microbial growth, stimulate primary productivity and enhance food webs (Lima & Colpo, 2014).

White mangrove stands are rarely flooded, which promotes build-up of leaf litter. Therefore, upon decomposition of the litter materials, more phenolics could be introduced to the soil sediments. However, the phenolic inputs from leaf litter represent only a fraction of total phenolic compounds in the soil phenolic pool because empirical evidence from soil samples analysed from the same sites (Chapter 3) reveals that the red and black mangrove soils had a much higher concentration of soil phenolic compounds than the white mangrove. The activities of macro invertebrates, flooding and leaf litter chemistry of the different species are all contributing factors in accounting for the soil phenolic contents across the three zones. Similarly, Numbere & Camilo (2016) investigated mangrove leaf litter decomposition under mangrove forest stands with different levels of pollution in Nigeria's Niger Delta rivers. They reported denser accumulation of leaf litter materials around the white mangrove stand compared to the red and black species, which they attributed to proximity from the sea.

This results also indicate that the leaf litter materials from the black mangrove stands contained more phenolics than those from the red mangrove. The black mangrove is located in between the red and white mangrove swamps, at a higher elevation than the red and is therefore less frequently flooded. The presence of pneumatophores (Ball, 1980), which serve as complementary roots in black mangroves, trap leaf litter materials. It is further speculated that this action could encourage high built-up of litter materials and aid fast burial by sediments and eventual break down by microbial action giving rise to more phenolic in such soils. The presence of invertebrate organisms such as crabs and shrimps, which depend on the leaf falls as a source of detritus, are key to the breakdown of these materials.

The leaves of *Avecinnia* spp (black mangrove) have less tannins compared to others and are easily submerged due to small surface area hence faster rate of decomposition. Several studies reported variation in decomposition rates of mangrove leaves (Lima & Colpo, 2014). Kathiresan & Bingham (2001) reported that tidal regime, leaf structure and chemical composition of leaves are determinant factors of decomposition of mangrove leaf litter, and therefore decomposition varies with species type. Mackey & Smail (1996) investigated decomposition in *Avicennia* leaves and reported significantly faster rate of decomposition in lower tidal zones with longer submersion periods.

Phenolic compounds in wetland soils encourage the build-up of organic matter by supressing the activity of hydrolases, the main enzyme implicated with organic matter decomposition in

line with the enzymic latch mechanism as proposed by Freeman *et al*. (2001a). The effects of phenolics on the decomposition of soil organic matter in wetland ecosystems has been investigated directly using the litterbag method and indirectly in a laboratory set up using microbial biomass, extracellular enzyme activity, and heterotrophic respiration. The concentration of phenolic compounds was a determinant factor on the rate of decomposition of such materials.

Similarly, Dunn (2013) suggests that addition of supplementary phenolics could further enhance carbon storage capability of such ecosystems. Therefore, overharvesting of mangrove plants for commercial purposes and other anthropogenic activities implies reduced phenolic inputs from leaf litter falls in mangrove soil sediments. This could further compromise mangroves carbon sequestration and storage potential with negative consequence for the global climate.

5.7. Conclusion

Findings from this analysis reveal that the three species of mangrove leaf litter contribute varying amounts of phenolic compounds to the soil sediments. Phenolic content was highest in litter materials from the white mangrove stand, closely followed by the black, but with much lower concentrations from the red mangrove litter. The differences are associated with the activity and abundance of detritivores, leaf structure and chemical composition, which affects palatability. The red mangrove leaf litter also releases much more concentration of the key nutrients nitrate, phosphate and ammonium compared to the white and black species. The contrasting inputs of phenolics and nutrients will have important implications for carbon sequestration and primary productivity in mangrove ecosystems.

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Chapter 6

Final discussion

6.1. Overview

Coastal wetlands are dynamic ecosystems providing numerous ecosystem services, such as providing protection from storms and supporting biodiversity. On a global scale, coastal wetlands are very efficient carbon sinks, mitigating the effect of climate change through carbon burial in the form of soil organic matter and often-high productivity of aboveground biomass. It is estimated that 2,500 Pg carbon (C) is stored in the Earth's soils, of which about 30% is stored in wetland soils (Mitsch *et al*., 2013) (Chapter 1).

Mangrove and tidal salt marsh wetland ecosystems hold a large quantity of carbon as soil biomass. The estimates of global carbon stored in mangrove swamp soils range between 1.22 - 4.98 Pg C with an annual burial rate of 52 Tg C yr^{-1} (Quintana-Alcantara, 2004). Tidal salt marsh ecosystems have an estimated global stored carbon ranging from 49.5-109.5 g C m^{-2} y^{-1} with an annual carbon burial rate estimated to range between 4.6 - 8.7 Tg C yr^{-1} (Arriola & Cable, 2017). Despite their global importance, coastal wetlands are increasingly becoming threatened by anthropogenic disturbances and natural events. Climate change is causing the northward expansion of mangrove swamps into salt marsh habitat in both hemispheres in recent decades. This process could convert these all-important carbon sinks into net sources of greenhouse gases (Chapter 4).

Ultimately, these investigations were aimed at determining the carbon sequestration and storage capabilities of mangrove and saltmarsh ecosystems and whether the 'enzymic latch' mechanism is a key control on decomposition processes, like it is in temperate peatlands. The outcome of this investigation revealed that the biogeochemical processes in mangrove swamps and salt marsh soils are largely dependent on hydrological regime as well as vegetation density, which play crucial roles in determining soil water and oxygen contents. In contrasting zones of both mangrove and salt marsh, high water content supresses the activity of phenol oxidase, encouraging the accumulation of phenolic compounds, which inhibit the activity of hydrolase enzymes. Therefore the 'enzymic latch' mechanism is closed, as is the case in northern hemisphere peatlands (Freeman *et al.*, 2001a), leading to sequestration of organic carbon.

6.2. Activities of extracellular enzyme in coastal wetland soils

The activity of extracellular enzymes in wetland soils influences the process of decomposition and accumulation of organic matter, and therefore the rate of carbon sequestration, of such ecosystems. The activities of extracellular enzymes is generally thought to be an indication of the level of biological activity, which determines the processes of nutrient cycling and organic matter turnover in wetland soil sediments. Microbes in soil are unable to directly assimilate organic matter, but rather absorb simple and dissolved bioavailable nutrients for their growth and development. Therefore, microbes in soil produce extracellular enzymes to make readily usable dissolved compounds. The enzyme phenol oxidase is usually extremely low in water-logged wetland soils, which encourages the build-up of phenolic compounds, which are enzyme inhibitors (Dunn *et al.,* 2014). Phenolic build-up places a 'latch' on the activities of hydrolases; the main suite of enzymes responsible for decomposition. This creates the perfect conditions for wetland soils to build up organic material and sequester carbon in such soils (Merino *et a*l 2016).

In general, results of this investigation revealed that most soils analysed from water-saturated tidal zones (mid zone of Cefni salt marsh (Chapter 2), red and black Florida mangrove soils (Chapter 3) and the salt marsh zone in Chapter 4 were characterised by low activities of phenol oxidase, high soil phenolics and low hydrolase enzyme activities. As a result, they had a higher content of organic matter. Soil samples from less frequently flooded areas (high salt mash zone, white mangrove soil and black mangrove soils, part of the mangrove /salt marsh area) had lower soil water contents, higher biological activities and lower content of soil organic matter.

6.3. Gas fluxes

The three main greenhouse gases (GHGs); carbon dioxide, methane and nitrous oxide have been the main focus of climate change monitoring by the IPCC since 1990, and any disturbance of wetland ecosystems has serious implications for GHG fluxes from the natural environment (IPCC, 2013). Mangrove swamps and salt marsh habitats are important blue carbon sinks as previously stated (Chapter 1, section 1.1.2), yet persistent deforestation, land use conversion and other anthropogenic as well as natural causes are increasingly turning them from sinks into net sources. Moreover Chen *et al.* (2010) reported that vegetation and soil characteristics could influence GHG fluxes in mangrove soils.

Determination of gas fluxes from the mangrove and salt marsh ecosystems shed light on the contribution of such ecosystems in mitigating or exacerbating the effect of global warming.

The results of this investigation suggest that the black and red mangrove soils have a much lower carbon dioxide flux than the white mangrove soils (Chapter 3). The low- and mid-zonation areas in the salt marsh soils also had lower fluxes than the high zonation site (Chapter 2, section 2.4). The analysis of soils from the mangrove/salt marsh transect revealed that the mangrove encroached areas had higher CO_2 fluxes than the salt marsh dominated area (Chapter 4, section 4.3).

There is an increasing demand to identify the major sources of GHG emissions from natural ecosystems to help develop effective climate change legislation. It is therefore imperative that research is carried out to ascertain the level of emission of GHGs, particularly in tropical wetlands in developing countries, which are continuously drained for agricultural purposes, overharvesting of vegetation for wood fuel and grazing.

6.4. Relationship between organic matter and water contents

Accumulation of soil organic matter and water content in mangroves and saltmarshes depends on hydrological regime and vegetation cover. Sources of organic matter in mangroves and salt marshes are largely from plant litter materials such as leaves, branches, propagules, sea grasses and roots of dead plants. Proximity to water edge and tidal inundation significantly influences the accumulation of the soil organic matter and the water contents of soils sampled from both ecosystem types (Chapters 2, 3 and 4). Other sources include the activities of macro fauna and phytoplankton in the water column (Friesen *et al.*, 2018). Frequent flooding and longer period of inundation displaces air in the soil and increase compaction, which also increases the ability of such soils closer to the sea to retain water. The red and black mangrove soils as well as the low and mid zonation areas of the salt marsh are situated closer to the water edge and experience frequent flooding as compared to the white mangrove and high salt marsh zone, which are situated at a higher elevation. This accounts for the higher percentages of SOM and water in soils from those areas. Positive and highly significant relationships were observed between water and organic matter contents of the soils sampled for Chapters 2, 3 and 4. Combining these data together results in a highly significant relationship across the three sites, incorporating both salt marsh and mangroves from the UK and USA. Figure 6.1 shows the relationship, with a Pearson correlation coefficient of 0.872 ($p<0.001$).

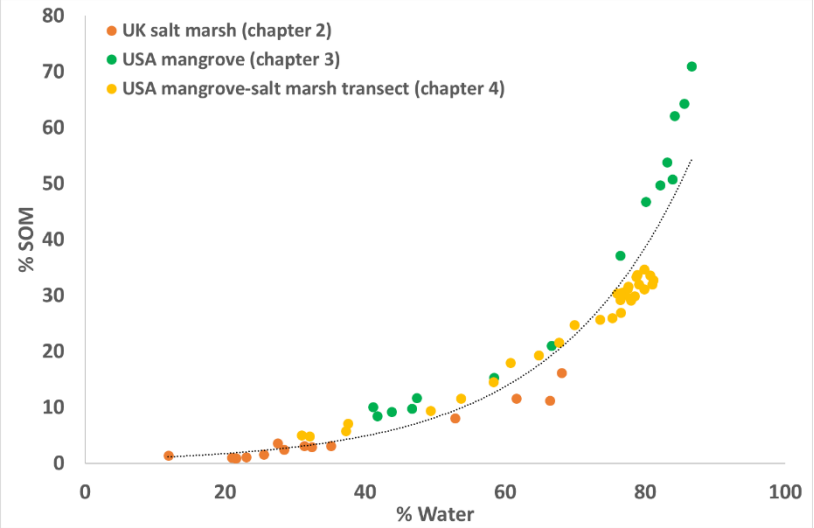

Figure 6.1: Relationship between soil water and organic matter contents from different coastal wetland types. The trendline shows an exponential relationship

The relationship between the two variables is actually non-linear. A 30% increase in water content at the lower end of the scale (20-50% water) results in only an approximate 10% increase in SOM content. However, a 30% increase in water content at the higher end of the scale (60-90% water) increases the SOM content by as much as 60%. The mangrove ecosystem studied in Chapter 3 has a generally higher SOM content than the salt marsh sampled in Chapter 2. This may be because mangroves have thicker, woody vegetation with more robust physiology compared to the salt marsh perennial shrubs, and as a result, higher net primary productivity. It may also be because the sites that were sampled at the salt marsh have a generally lower water content than the mangrove sites, which will be due to the proximity of the sites to the sea and the regularity with which they experience inundation by seawater. Therefore, the salt marsh soils will be relatively more aerated, resulting in more favourable conditions for decomposition of organic matter. Interestingly, the observation of greater SOM in the mangrove soils (Chapter 3) is in direct contrast to the lower SOM content of the mangrove encroached salt marsh sampled in chapter 4. However, this is further evidence of the transient effects that mangrove vegetation can have when it colonises salt marsh habitat. The growth of mangroves is initially expected to deplete soil organic matter, due to enhanced oxygenation of the soil, but over time, it is expected that these sites will go back to being a net carbon sink. This will happen once the mangrove vegetation is fully established, with high rates of NPP and high inputs of litter to the soil (Osland *et al.*, 2013; Cavanaugh *et al.*, 2014; Saintilan *et al.*, 2014).

6.5. Effect of pH

Recent studies acknowledge that low pH is responsible for supressing enzyme activities in wetlands soils (Dunn, 2013). Enzymes comprise both acidic and basic groups of amino acid side chains hence they are amphoteric globular proteins whose activity or structure could alter the concentrations of hydrogen ions. For this reason, the binding sites of enzymes must be at an optimum level or state of ionisation for effective functioning. Variation in soil pH can have an impact on the activities of both ionising and deionising reactive groups. The low pH in wetland soils modifies the binding sites of enzymes, which suggest differences in pH of free enzymes from that of clay bound enzymes (Dunn, 2013). For these reasons, pH can be seen as a major driver of most of the biogeochemical processes in both mangrove and salt marsh soils (Chapters, 2, 3 and 4). Dunn (2013) reported that adding hydrochloric acid to northern hemisphere peat samples supressed decomposition for 24 hours. Similarly, many investigations on the effect of pH on phenol oxidase has demonstrated supressed phenol oxidase activity at low pH (Williams *et al*., 2000).

6.6. Phenolic inputs from leaf litter materials

Vegetation densities play vital roles in controlling the biogeochemical processes in wetland soils. Root exudates promote microbial diversity as well as soil enzymes by providing vital nutrients, gases and minerals, which influence the rate of mineralisation. Leaf litter materials form an integral part of net primary productivity (NPP) as well as being a source of soil nutrients in mangrove swamps (Zhao *et al.*, 2015; Shafique *et al*., 2015), accounting for about 32% of total NPP in mangrove ecosystems (Nordhaus *et al*., 2017). Moreover, leaf litter fall from mangrove vegetation forms one of the major sources of phenolic compounds and carbon inputs into the mangrove soil sediment biomass (Twilley *et al.*, 1992). Freeman *et al*. (2001b) suggest that degradation of oxopolysaccharide from *Sphagnum* and phenolic compounds are responsible for the preservation of 'bog bodies' in peatlands by supressing microbial activities in the soil, furthermore, phenolic compounds produced from *Sphagnum* mosses supressed the activities of microbes and fungi resulting in low rates of decomposition of organic matter (Bragazza *et al.*, 2006).

Analysing leaf litter materials under red, black and white mangrove stands provided an insight into the contribution of the different species of vegetation to the overall soil phenolic budget in the ecosystem (Chapter5). This results indicated that leaf litter materials contribute significantly to the overall soil phenolics pool with the highest coming from the white and

black species, and much less from the red mangrove leaves (Chapter 5, section 5.10). Phenolic compounds supress the activity of the hydrolase enzymes responsible for the decomposition of organic matter and its presence in wetland soils strengthens the 'enzymic latch' mechanism.

Outcome from the investigation along a mangrove salt marsh transect in northern Florida, USA indicated that transitional areas dominated by the few emerging black mangrove vegetation had reduced quantity of soil phenolics and greater enzyme activities and microbial soil respiration, suggesting that vegetation has an important influence on the soil biogeochemistry (Chapter 4). Soil samples from poorly vegetated areas showed low phenolics and other measured parameters (Figure 4.3), suggesting that vegetation cover may be a key regulator of biogeochemical processes in areas threatened by mangrove encroachment at the time of this investigation.

Similarly, Dunn (2013) enriched peat samples by adding lignin and observed suppression in hydrolases activity implying that the presence of phenolic compounds in the peat inhibits decomposition in those wetlands.

Recent reports indicate that overharvesting of mangrove vegetation for commercial purposes has led to deforestation and estimates suggest that 90–970 Tg C y^{-1} of carbon is returned to the atmosphere from mangrove clearance, raising ecological and socioeconomic concerns (Alongi, 2014). Consequently, a decrease in vegetation canopy in mangrove swamps will compromise phenolic inputs from leaves and expose the soil to atmospheric oxygen encouraging microbial proliferation with eventual release of GHGs. Furthermore, changes in land use will have a negative impact on mangrove swamps as observed in a massive die back of mangrove swamps covering several kilometres near Marco Island, Florida, USA (Rookery Bay, 2017) and the worst in recorded history in Australia's Gulf of Carpentaria, covering about 1000 km of coastline (Harris *et al*., 2017). Human development, particularly for agriculture and urban expansion, has devastated mangroves, such that approximately one third of the world's mangrove forest has been lost in the last 50 years (Alongi, 2002; Giri *et al*., 2008). Mangrove swamp restoration and conservation will greatly improve mangrove ecosystem services and storage of globally significant amounts of carbon (Marois & Mitsch, 2015), which will be aided by vegetation growth and inputs of phenolic compounds from mangrove leaf litter.

6.7. Implication of mangrove encroachment into salt marsh habitats on ecosystem services

A poleward shift of tropical woody plants (mangroves) into temperate shrubs (salt marsh) has been observed in recent years (Osland *et al.*, 2013; 2017). Such encroachments could cause structural changes in some ecosystem services (Kelleway *et al.*, 2016). There has been some quantitative evidence suggesting a decrease in carbon storage capacities in the mangrove takeover area (Kelleway *et al*., 2016), but there appears to be no direct assessment of the impact of encroachment on the key biogeochemical processes in the takeover areas, which is a motivation for this novel investigation. On a global level, mangrove encroachment of salt marsh ecosystems are in response to a range of changing abiotic factors such as rise in atmospheric temperatures, sea level and atmospheric CO_2 (Saintilan *et al.*, 2014).

Analysis of soil samples from a mangrove/salt marsh transect where black mangrove vegetation is taking over salt marsh habitat in northern Florida USA gave some insight into the implications of such take over on the biogeochemical process in salt marsh ecosystems. The hypothesis that such displacement could initially stimulate decomposition during the transition to full mangrove swamp holds some ground. Enzyme activities were discovered to be mostly higher at the mangrove end of the salt marsh transect. It is therefore speculated that this could be due to reduced waterlogging and the below ground effects of the mangrove vegetation. Most biological indices of decomposition appeared to maintain a downward trend from the mangrove take-over areas, which were drier than the waterlogged salt marsh zone. It is evident from the results obtained that the rate of decomposition of organic matter is higher in the mangrove encroached take over area. The pneumatophores of the emerging juvenile black mangrove (*Avicennia geminans*) increase oxygenation of the soil, which are likely to also be responsible for increasing the rate of decomposition.

The results suggest that the ‘enzymic latch’ mechanism is weak at the take over area but strong in the salt marsh-dominated habitat. Therefore, it is not out of place to speculate that mangrove encroachment into salt marsh habitat is likely to initially affect some ecosystem services during the transition period while others could improve. Literature suggests that full mangrove take-over of salt marsh habitat could take up to 4 years with others suggesting decades before full realisation (Osland *et al*., 2013; Kelleway *et al*., 2016, 2017) and that salt marsh store up to a

century of carbon as soil sediments. Such stored carbon could be released into the atmosphere within the transition period thereby increasing the flux of GHGs.

Based on the current understanding of the structure and function of coastal wetland ecosystems, it is not out of place to speculate that mangrove encroaching into salt marsh is likely to increase some ecosystem services such as nutrient accretion, erosion control and storm protection for coastal communities. However, the process could also pose a serious threat to the stability of blue carbon stores within the takeover period. It could also result in decline in the abundance of fauna requiring open and low vegetation structure as well as a decline in ecotourism and recreational activities such as bird watching and fishing. Furthermore, such ecosystem changes could pose significant uncertainties on some countries where such habitats are located. Kelleway *et al*. (2017) reported that the annual global ecosystem services of these coastal wetlands are worth an estimated US $24.8 trillion. It is therefore theorised that salt mash ecosystem is efficient in carbon sequestration and other ecosystem services before its integration with black mangrove thereby compromising such ecosystem services within the transition period. However, overtime with full takeover, resulting in a black mangrove habitat such ecosystem services are enhanced as presented in Figure 6.2 below.

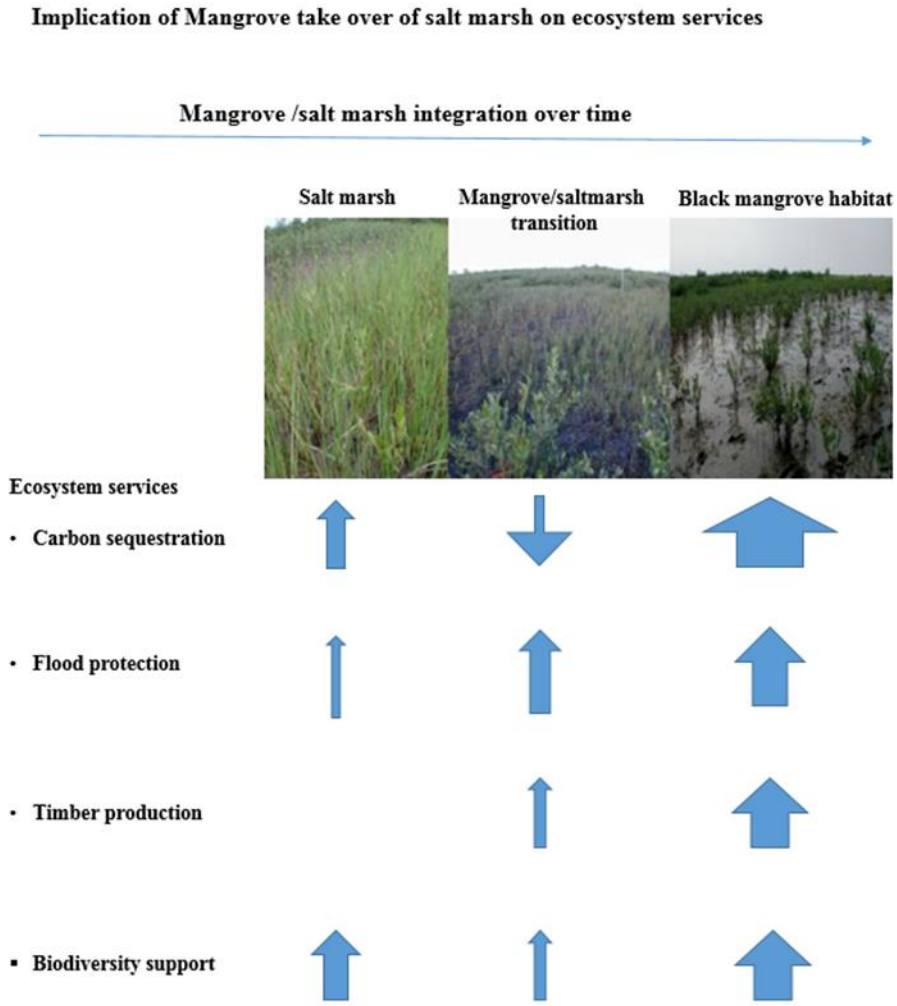

Figure 6.2. A conceptual schematic model indicating ecosystem services of salt marsh and how integration with black mangrove vegetation could compromise such services before enhancement after full takeover over time. Up and down arrows indicating increase or reduction in specific ecosystem services before encroachment, during transition period and after colonisation.

6.8. The implication of mangrove encroachment on biogeochemical processes

The rates of organic matter decomposition and nutrient cycling in mangrove and salt marsh habitats are thought to vary according to structural complexity and litter fall. For example, recycling of carbon takes longer in mangrove habitats than in salt marsh ecosystem due to variation in vegetation types, as the former comprises woody plants while the latter comprises herbaceous plants. For this reason, mangrove encroachment into salt marsh could have initial significant impacts on carbon and nutrient storage (Kelleway *et al.*, 2017). Furthermore, woody mangrove vegetation and herbaceous salt marsh vegetation vary in terms of both above and

below ground biomass structure as well as carbon sequestration and cycling capabilities, therefore mangrove encroachment into salt marsh could deplete typical salt marsh plants such as *Spartinia*, *Batis* spp etcetera but in the long run will result in an enhanced woody vegetation density. Figure 6.3 below presents a conceptual diagram of how mangrove encroachment could alter ecosystem structure and consequences for fauna habitat quality and wave reduction capacity. The low vegetation structure of salt marsh provides security for roosting and feeding birds as they could easily notice any ambush predators due to longer line of sight. Insectivorous bats prefer to feed over open salt marsh or at the marsh–forest edge, avoiding the acoustic clutter of closed forest. The higher biomass and structural rigidity of mangroves may also increase storm wave attenuation (Kelleway *et al*., 2017).

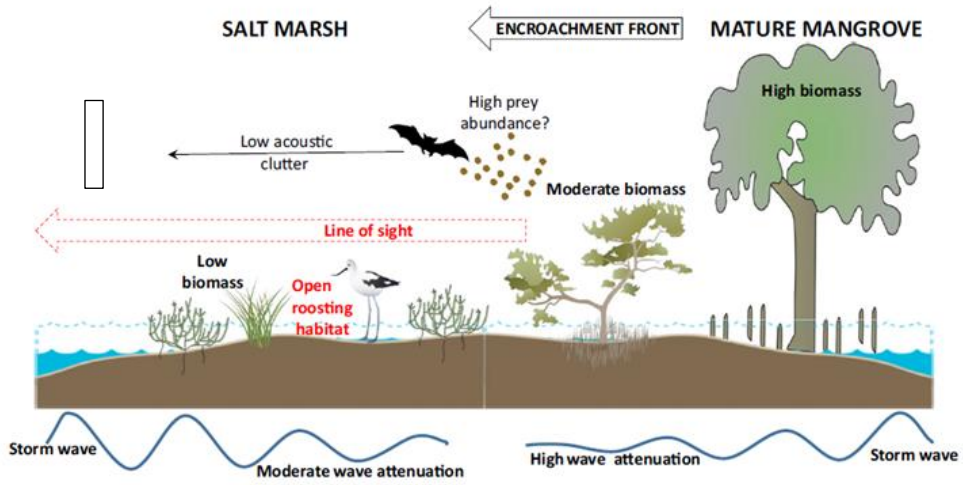

Figure 6.3: Conceptual diagram of the ways in which mangrove encroachment can alter ecosystem structure and ramifications for fauna habitat quality and wave attenuation capacity (from Kelleway *et al.*, 2017).

6.9. The enzymic latch mechanism and coastal wetland ecosystems

Wetland ecosystems plays an important role in the global carbon cycle because they have the highest deposit of soil carbon in storage among all terrestrial ecosystems. Whilst occupying only about 1% of the Earth's surface, the soil carbon storage value of these ecosystems is nearly 15% (~450 Pg) of total global terrestrial soil carbon storage (Jin *et al.*, 2013).

For several decades, the process of carbon storage in wetland ecosystems appeared to have been understood. However, the discovery by Freeman *et al*. (2001a) that an 'enzymic latch' mechanism is the cause of carbon accumulation in northern peatlands brings a clearer understanding on the process of carbon cycling in such ecosystems changes this earlier perception.

The ‘enzymic latch’ mechanism works through low oxygen availability in wetland soils suppressing the activity of the extracellular enzyme phenol oxidase. This allows for the build-up of phenolics (an organic compound found in abundance in wetland soils because of breakdown of plant materials). Phenol oxidase activity is generally low in wetlands and this encourages phenolic build-up. Phenolics inhibit the activity of hydrolases, which are the main enzymes responsible for decomposition thereby creating an ideal condition for carbon storage by massive accumulation of organic matter in the soil. However, in the presence of oxygen the reverse happens, as the activity of phenol oxidase increases breaking inhibitory phenolics thereby encouraging the activities of hydrolases triggering massive decomposition of organic matter because of microbial proliferation. Thus breaking the enzymic latch mechanism. The diagram below further illustrates the enzymic latch mechanism based on the presence/absence of oxygen as a constraint.

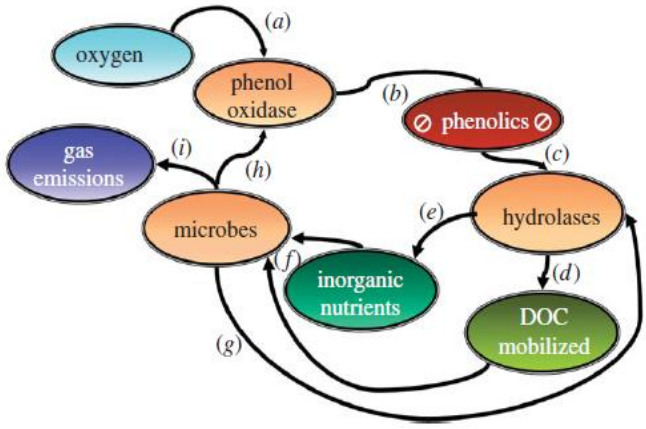

Figure 6.4: A conceptual illustration of the role of phenol oxidase in regulating wetland carbon storage through the ‘enzymic latch’ mechanism. Source: (Freeman, *et al.*, 2012).

a- O_2 triggers the enzyme phenol oxidase resulting in reduced amount of inhibitory Phenolics,

b- The resultant reduction of inhibiting Phenolics encourage kindling of hydrolase enzyme in the soil,

c- The activities of the hydrolase enzyme results in-

d- Increase break down of dissolve organic carbon (DOC) and

e- release of inorganic nutrients hitherto captured in the soil.

f- as a result of the activities in events d and c above, more substances and nutrients are produce which promote microbial activity by secreting more hydrolase enzyme.

g- more phenol oxidase and

h- Increased emission of CH_4, CO_2 and N_2O.

Results obtained from the mangrove soils indicate that the enzymic latch mechanism is much present in the red and black mangrove soils as well as in the low and mid zonation area of the salt marsh soils. This is evident by the lower phenol oxidase activity, higher phenolics and lower hydrolase enzymes activity compared to those in the soils of the white mangrove and high salt marsh zones (Chapters 2, 3 and 4 results section). By keeping the enzymic latch mechanism closed, these parts of the mangrove and salt marsh zonation are excellent carbon sinks and natural ecosystems in the fight against climate change.

6.10. Geoengineering approaches to mitigate climate change.

Anthropogenic activities have increased atmospheric CO_2 concentrations by an estimated 8 Pg yr^{-1}, which has been linked to rising global temperatures. It is projected by the IPCC that the atmospheric CO_2 concentration could reach 535–983 ppm by the year 2100 and this could mean that the global atmospheric temperature could increase by 1.1–6.4^{0}C (Freeman *et al.*, 2012; IPCC, 2013). Concerns have been raised that such increases could have negative global implications which may include drought, increased in sea volumes, loss of habitats, etc. (Zhang *et al.*, 2015). Accordingly, a geoengineering approach aimed at sequestering and storing carbon in wetland ecosystems has been suggested. Geoengineering involves large-scale manipulation of the environment in order to offset rising global temperatures (Freeman *et al*., 2012; Zhang *et al*., 2015).

Freeman *et al.* (2012) suggested wetlands could be used for geoengineering through the addition of phenolic supplements to peat substrates to capture and sequester more carbon, or through the genetic modification of some wetland plants such as *Sphagnum*, which is responsible for phenolic synthesis. The work of Alshehri (2018) demonstrated that the addition of lignin solutions and wood chips from different vegetation types can reduce decomposition and strengthen the enzymic latch in peatland ecosystems. The advantage of such geoengineering tools is that they are cost-effective and could strengthen the enzymic latch mechanism in wetland soils.

In terms of coastal wetlands, It is therefore suggested that;

- discouraging anthropogenic activities that could increase deforestation and pollution, encouraging massive afforestation and other initiatives such as the REDD+ and PES initiatives in coastal communities (REDD+ and PES are international initiative aimed at attaching economic incentives for countries to enhance ecosystem services and protection of water

quantity and quality for downstream uses sustainable management of forests, and enhancement of forest carbon stocks) (Piffer Salles *et al*., 2017; Everard *et al*., 2018).

- More research is needed in developing a geoengineering strategy for mangrove and salt marsh ecosystems.

6.11. Final conclusions

The main conclusions of the project are listed below:

- The 'enzymic latch' mechanism that was initially discovered in northern peatlands (Freeman *et al.,* 2001a) is also present in mangrove and salt marsh ecosystems. This is the main reason for the low rate of microbial decomposition of organic matter in soils from certain parts of these ecosystems.
- Soil water content, pH and vegetation cover are key drivers affecting the rate of organic matter accretion in wetland ecosystems.
- Extent of waterlogging, tidal action and gradient are key controls on biogeochemical cycling along mangrove and salt marsh gradients.
- There is a strong non-linear relationship between soil water and soil organic matter in coastal wetland ecosystems.
- Red mangrove and mid-zone salt marsh soils are the most efficient parts of these ecosystems for capturing carbon and mitigating the effect of climate change.
- Phenolic compounds in soil are an effective material in supressing decomposition in wetland soils
- The three commonly occurring mangrove species contribute different amounts of phenolics and nutrients to the soil.
- Mangrove takeover of salt marsh habitats is likely to have transient negative implications for blue carbon stores.

Periodical review of existing policies with financial backing and commitment by relevant authorities is hereby encouraged for conservation and protection of coastal wetlands. Efforts aimed at the protection and conservation of natural carbon pools could significantly contribute in curtailing the emission of greenhouse gases and further preserve the environment. Furthermore, to ensure sustainability of the wetland, continuous monitoring and assessment of key ecosystem variables could be adapted as part of the management system. Governments should create deliberate coastal wetland conservation policies, which could promote funded research projects covering various aspects of wetlands by non-governmental organisations, which could greatly help in maintaining and sustaining these crucial wetlands.

6.12. Future work

Although this study has shown that the mangrove and salt marsh wetlands are important sites of carbon sequestration, additional research is needed to ascertain the status of wetlands in the developing World (Africa, Asia and South America) and their role in the global climate as either carbon sinks or net sources. The 5th assessment report of the IPCC (2014) identified anthropogenic activities as the main causes of global warming since the mid-20th century. Consequently, there is a serious gap in implementation of mitigation strategies to curtail anthropogenic activities that exacerbate the situation between the developed world and the developing countries; of particular example in the developing world is the Lake Chad basin in sub-Sahara African region among several others with similar challenge. The Lake Chad basin has been described as 'one long climate catastrophe' and is in part responsible for the current crisis in the West African region, which includes Nigeria, Chad, Niger and Cameroon, and has left over 10.7 million people in need of urgent humanitarian assistance (Carius, 2017). To address this crisis, resources must be channelled into undertaking research work. Similar approach of integrating ecosystem–based with engineering water resources management for water security as in the case of Banas catchment, Rajasthan, India (Everard *et al*., 2018). This could serve as a long-term solution in conservation, restoration and wise use of water resources across the Chad basin and other wetlands that are under anthropogenic threat across the region.

Most wetlands are drained for agricultural purposes in several developing countries to improve food security particularly in Nigeria. Nigeria's extent of mangroves is the third largest in the world and is currently under serious threat by the activities of oil companies. Research should be undertaken in such mangroves to ascertain the biogeochemical properties and the impact of the enzymic latch mechanism in such tropical wetland types, alongside major Ramsar recognised wetlands such as the Pandam wetland, Baturiya wetland, Lower Kaduna-Middle Niger floodplain in Nigeria and Tambi wetland National park in the Gambia and many others.

If the fight against rising global temperature is to be successful on a global scale, significant research must be conducted in Africa's wetlands as Mitsch and Gosselink (2015) noted that 58% of total global CH_4 emissions originates from tropical wetlands and rice paddies.

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