

**The Density, Permanganate Oxidisability, and Stability of
Soil Organic Carbon in Wetlands of the South African
Highveld**

by

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DECLARATION

I hereby certify that this project write-up is my own work, except where duly acknowledged. I also certify that no plagiarism was committed in writing this.

A handwritten signature in black ink, appearing to read 'Brendon Fourie', enclosed within a hand-drawn oval. A long, thin horizontal line extends from the right side of the oval.

Signed _____

Brendon Fourie

Abstract

Wetland soils are highly effective ecosystems for long term storage of carbon. Prolonged periods of soil saturation stimulate high rates of productivity by appropriately adapted hydrophytic vegetation and cause a depletion of oxygen within the soil that significantly reduces the rate of mineralisation of organic material, the process that results in the release of CO₂ back into the atmosphere. As a result, the rate of production exceeds the rate of mineralisation of organic material and leads to a net accumulation of soil organic carbon over time.

This study aims to serve as a baseline estimate of carbon storage in wetlands of South Africa. This study was not intended to be on an extensive spatial scale but rather an intensive focus on carbon storage as well as spatial distribution, variation and factors that influence carbon storage.

Three aspects of soil organic carbon were assessed for sections of four selected wetlands. Firstly, the soil organic carbon density was estimated in tons per hectare as well as the differences in soil organic carbon density of different wetland hydrological zones. Secondly, the nature and stability of soil organic carbon were assessed based on the proportion of soil organic carbon that was readily oxidised by potassium permanganate (permanganate oxidisable carbon). Lastly, correlations were assessed between soil organic carbon and the elements N, P, K, Ca, Mg, Mn, Fe, Cr, S, and Al to assess the relationships between soil concentrations of these elements and the density of soil organic carbon.

Four wetlands were selected including three mineral and one organic wetland containing peat. The mean SOC densities of the three mineral wetlands at a soil thickness of 20 cm were 49.6 t ha⁻¹ (tons per hectare), 54.5 t ha⁻¹ and 413 t ha⁻¹. The mean SOC density of the organic wetland was 127.0 t ha⁻¹.

There were significant differences in soil organic carbon density between different wetland hydrological zones, the highest densities found in the permanently saturated zones. A negative correlation between wetland disturbance in general and POXC content was found. Total soil N, P, and S concentration showed the highest

correlation between SOC and soil POXC concentration. The soil concentrations of Fe, Mn and S did not seem to have any appreciable relationship with the oxidisability of SOC or SOC density.

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1 Chapter one: Background

In recent years natural carbon sequestration processes as a mitigation measure to curb increasing atmospheric carbon have become a major topic within the climate change arena. Worldwide wetlands store a disproportionately large amount of soil carbon despite only occupying approximately 5% of terrestrial land area. It has been estimated that different kinds of wetlands contain 350-535 Gt of carbon, corresponding to 20-25% of the world's total soil organic carbon (Adhikari et al., 2009). Among all terrestrial ecosystems, wetlands have the highest carbon density (Kayranli et al., 2010).

Wetlands can be both sources and sinks of atmospheric carbon, depending on their age, operation, disturbance and environmental boundary conditions such as location and climate. When carbon input into wetlands exceeds output, accumulation of organic carbon occurs within the soil (Kayranli et al., 2010).

A major concern regarding wetlands and climate change is that wetlands emit the greenhouse gases carbon dioxide (CO₂) and methane (CH₄). Methane is particularly potent greenhouse gas that has approximately 25 times the greenhouse effect of CO₂ in the atmosphere (Mitsch et al., 2012). Mitsch et al., (2012) have shown through dynamic modeling of carbon flux that methane emissions of created or restored wetlands become unimportant within 300 years compared to carbon sequestration, and that worldwide wetlands may be net carbon sinks of approximately 830 Tg/year with an average of 118 g C m⁻² year⁻¹.

Wetland ecosystems provide a diversity of services that are vital for human well-being. Provisioning services such as food (notably fish) and fibre are essential to many communities and are directly linked to the carbon cycle within these systems. Supporting and regulating services such as nutrient cycling is critical to sustaining vital ecosystem functions that deliver many benefits. Fresh water both directly and indirectly is a particularly important ecosystem service. In addition to life sustaining services, wetlands also have significant aesthetic, educational, cultural and spiritual importance to people and provide invaluable opportunities for recreational activities and tourism (MEA 2005). Wetland soil organic matter (SOM) has other beneficial

environmental effects. SOM contains essential nutrients and elements and enriches soil microbiota, which in turn contribute to nutrient cycling in the soil, holding nutrients in the root-zone layers of the soil for longer, slowing the rate of passage of pollutants to the groundwater table. Pollutants may become bound to SOM, purifying water through the attenuation of pollutants (Victoria et al., 2012). Despite the huge ecological and economical value of wetlands they remain some of the most threatened ecosystems in the world (Zedler & Kercher, 2005).

Wetlands are often broadly divided into peatlands (organic soils) and freshwater mineral soil wetlands, based on the soil organic carbon content and the presence of peat (Bridgham et al., 2006). In peatlands carbon is sequestered predominantly through organic matter production and an accumulation rate higher than organic matter decomposition under anaerobic conditions (Grover et al., 2012). In mineral soil wetlands, carbon sequestration occurs through sediment deposition from upstream catchments and in situ biomass production, at rates higher than decomposition of organic matter (Bridgham et al., 2006). A common definition for peat is a pure organic layer at least 20 cm in thickness (Mitra et al., 2005). Under South African classification a peat horizon contains more than 20% dry mass organic carbon in environments associated with water inundation or that are at least saturation for extended periods (Soil Classification Working Group, 2018). One of the requirements for the formation of peat is that the wetland must be saturated on a virtually permanent basis to prevent oxidative decomposition of SOM (Reddy & DeLaune, 2008).

Only around 10% of wetlands in South Africa are considered peatlands, with the remainder of wetlands being mineral soil dominated wetlands (van Vuuren, 2010) which do not have an O horizon.

Wetland carbon stocks have mainly been reported for peatlands (Ausseil et al., 2015; Mitra et al., 2005). Loisel et al., (2014) Reported the latest estimate of northern peatland carbon stock at 436 Gt C.

Information on mineral wetland carbon stock is scarce (Ausseil et al., 2015). Bridgham et al., (2006) proposed the first carbon balance for both mineral and

organic soil wetlands in North America, by compiling information on area and carbon pools. Zhang et al., (2008) reported carbon densities of around 171 – 274 t ha⁻¹ in the marshes of China. Limited studies in Argentina and Australia have reported considerable variability in mineral wetland carbon density ranging from 12 – 557 t - ha⁻¹ (Page & Dalal, 2011).

The accurate estimation of carbon stock of wetlands over a large geographical area requires extensive datasets. The variables required to calculate soil carbon stock of an area of any magnitude include the carbon density (t ha⁻¹) and the total surface area (ha). The product of carbon density multiplied by the area gives the total mass of carbon (t) of that area (Ausseil et al., 2015). Calculation of surface area of a wetland requires a comprehensive field delineation to establish the boundaries of that wetland following the wetland delineation guidelines of the Department of Water Affairs and Forestry (DWAF) (Rountree et al., 2008). The delineation of wetlands in South Africa is an on-going process that will require some time given the total number of wetlands, their expanse, effort and expenses that go into their delineation and generating data. The National Freshwater Ecosystem Priority Areas (NFEPA) project achieved a significant step in mapping and prioritising freshwater ecosystems within South Africa. However, the national scale of the project means that finer-scale applications are constrained by data accuracy issues. In an area such as the Mpumalanga Highveld, where highly accurate data is needed to support regulatory decision-making, further refinement of the wetland data is required (Mbona et al., 2015). The complete delineation and more extensive sampling of the wetlands selected for this study were beyond the time limit and budget of the project. Therefore, it was considered more practical to focus on and estimate carbon density rather than complete stock of each wetland as a baseline assessment.

POXC has been found by several workers to be useful and practical as an indicator of organic carbon sensitive to changes caused by land use practices (Bonilla et al., 2014; Tirol-Padre & Ladha 2004; Nan et al., 2017; Culman et al., 2012). POXC may be effective in tracking management practices that promote SOC sequestration, making it a particularly useful indicator for soil quality research (Culman et al., 2012). Disturbances such as acid mine drainage (AMD) and agricultural practices may have

a significant effect on the SOC of the wetland soils reflected in POXC, possibly nutrient imbalances as well and can be used as an indicator in this regard.

The capacity of a wetland to sequester carbon is a function of the carbon cycle and is dependent on the differences between the rate of primary productivity by which atmospheric CO₂ is fixed and the rate of mineralisation of SOM releasing the carbon back into the atmosphere predominantly as CO₂ (Essington, 2003; Reddy & DeLaune, 2008). This study also seeks to investigate the factors that may influence these differences in primary productivity and mineralisation of SOC

Nitrogen (N) and phosphorus (P) are two of the most important macro nutrients that limit the productivity of terrestrial, wetland, and aquatic ecosystems (Reddy & DeLaune, 2008). Soil N and P content have also been shown to have an important impact on the soil microbial community responsible for the mineralisation of SOM in wetlands (Sundareshwar et al., 2003). The elements K, Ca, and Mg are also essential plant nutrients, the concentrations of these elements in soil may be limiting factors that affect the rate of primary production.

In saturated anoxic soils MnO₂, FeOOH, and SO₄²⁻ serve as alternative terminal electron acceptors to appropriately adapted microorganisms for the oxidation of SOM (Essington, 2003). Acid mine drainage (AMD) is a serious environmental problem in South Africa and contains high concentrations Fe and Mn species and SO₄²⁻ that are eventually transferred into many ecosystems including wetlands. If Fe³⁺, Mn⁴⁺ and SO₄²⁻ can be transferred to the wetland and settle out within the soil they could potentially be used by iron/manganese reducing and sulphate reducing bacteria respectively to mineralise SOM in highly reduced conditions where O₂ and NO₃⁻ are in limited supply. Aluminium is liberated from clay minerals in acidic soils and is toxic in plants (Reddy & DeLaune 2008). Aluminium toxicity might be relevant if soil pH is decreased by AMD.

To the authors knowledge there is neither published literature on the carbon densities of the wetlands of South Africa nor any literature on the impact of human induced disturbance on carbon density. The lack of knowledge about wetland carbon sequestration as an important ecosystem service in South Africa in an age of ever-

increasing atmospheric CO₂ levels and the intensive pressures that the coal mining industry poses on the wetlands of the Highveld are the primary motivation for this study.

The primary aim of this study is to provide the first estimates of wetland soil organic carbon density of South Africa for selected wetlands on the Highveld. The secondary aim is to assess factors that are potential drivers of soil organic carbon (SOC) density. The objectives are:

- Generate the first estimates of soil organic carbon density of selected wetlands on the South African Highveld as well as the differences in SOC density of different wetland hydrological zones
- Assess POXC as a potential inexpensive indicator for tracking changes in SOC carbon stability caused by land use practices
- Assess total soil N, P, K, Ca, Mg, Mn, Fe, Cr, S, and Al concentrations as potential drivers of SOC density

The hypotheses tested for are:

1. There are significant differences in SOC density between different wetland hydrological zones, with SOC being positively correlated to the period of saturation
2. Disturbance in wetlands will have a negative effect on the stability of SOC reflected in lower concentrations of POXC compared to undisturbed wetlands
3. Total soil N, P, K, Ca, Mg, Mn, Fe, Cr, S, and Al soil concentrations are significantly correlated with SOC density in wetland soils. As essential plant nutrients the correlation was expected to be positive for the elements N, P, K, Ca, Mg, and S. Excessive concentrations of Fe, Mn, S and Al will be negatively correlated with SOC

The four selected wetlands included three channelled valley bottom wetlands (refer to chapter 2.4 in literature review for wetland hydrogeomorphic type) with mineral soil horizons and one seepage wetland connected to a channelled valley bottom wetland with an organic (O) horizon and a river.

The layout for this dissertation is as follows: chapter two is a literature review and relating to processes of carbon accumulation in wetlands. Chapter three deals with the sampling programme methodology for chapters four and five. SOC estimates and the analysis of POXC are included in chapter four. Chapter five investigates selected soil elements that may influence the SOC density of wetlands. Chapter six is a synthesis that includes a summary, conclusions and recommendations.

Abbreviations: AMD, acid mine drainage; POXC, permanganate oxidisable carbon; SOC, soil organic carbon; SOM, soil organic matter.

2 Chapter two: Literature review

2.1 Introduction

The enrichment of the atmosphere with greenhouse gasses (GHGs) by humans and the cumulative radiative forcing of all GHGs have led to an increase in the average global surface temperature of 0.6 °C since the late 19th century, with the current warming rate of 0.17 °C/decade. A global warming rate of 0.1 °C/decade is considered critical as ecosystems are unable to adjust rapidly enough to environmental changes beyond this rate (Lal, 2004).

The COP21 or 21st Conference of the Parties to the United Nations Framework Convention on Climate Change in Paris (November 30 to December 11, 2015) produced the Paris Climate Agreement. This is a global agreement that strives to reduce the impacts of climate change with the goal of limiting global warming to less than 2 °C compared to pre-industrial levels and to limit the overall increase to a maximum of 1.5 °C (Minasny et al., 2017). To achieve the goal of keeping global warming less than 2 °C, we need to limit our annual GHG emissions with an estimate of 9.8 Gt C at a 64% probability (Meinshausen et al., 2009).

The South African government has been proactive in respect to carbon stocks and associated GHG emissions in natural and semi-natural ecosystems with 39 policies. These mostly aim at limiting the spread of alien invasive plants, controlling vegetation fire and restoring wetland and woodland ecosystems (Minasny et al., 2017).

On earth there are five carbon reservoirs that are in dynamic equilibrium. These reservoirs are; the atmosphere, hydrosphere, geosphere, pedosphere (soil) and the biosphere. The oceanic pool is the largest, followed by the geologic, pedologic, biotic and the atmospheric pool (Lal, 2004; Reddy & DeLaune, 2008). The pedologic C pool consists of two principal components; SOC and soil inorganic carbon (SIC). The SOC pool is more prominent in wet regions and the SIC pool in arid regions. The total soil C pool (SOC + SIC) is four times greater than the biotic pool and around three times that of the atmospheric pool (Lal, 2004), (Figure 2.1). Of interest is the

atmosphere carbon reservoir as an increase in global mean atmospheric temperature is predicated on the greenhouse effect caused by rising concentrations of greenhouse gasses. The major forms of carbon present in the atmosphere are CO₂, CH₄, CO and volatile organic compounds. The combustion of fossil fuels and biomass convert and shift carbon from the lithosphere and biosphere reservoirs respectively into the atmospheric carbon reservoir (Reddy & DeLaune, 2008).

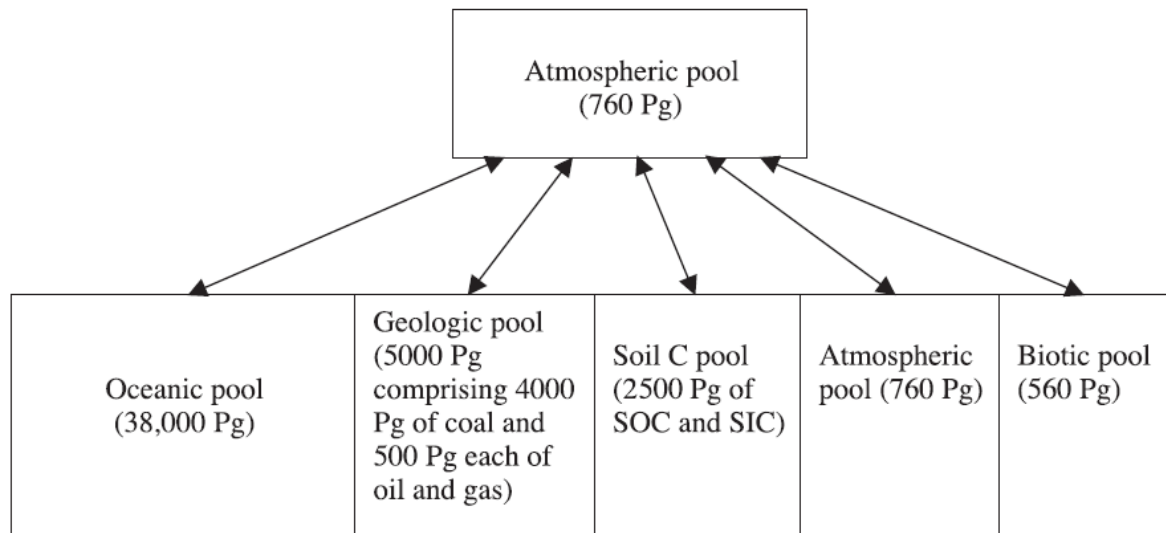


Figure 2.1: Principal carbon pool distribution between the main components of the global ecosystem (Lal, 2004)

The term “soil carbon sequestration” implies removal of atmospheric carbon as CO₂ by photosynthetic organisms and the storage of fixed C as SOC. The goal is to increase SOC density in the soil, improve depth distribution of SOC and stabilise SOC by encapsulating it within stable micro-aggregates so that C is protected from rapid decomposition by microbial processes or as recalcitrant C with long turnover time (Lal, 2004). Historically poor land use practices and soil mismanagement have caused a depletion of SOC with CO₂ emissions into the atmosphere consequently. There is however strong evidence that this process can be reversed to some extent and enhancing the SOC pool could significantly offset fossil fuel emissions (Kauppi et al., 2001).

Land use changes are the second largest anthropogenically induced cause of greenhouse gas emissions globally (12-20%), predominantly due to deforestation in

the tropics and sub-tropics. The conversion of primary tropical forest into agricultural land results in a SOC stock loss of 25 % and 30% for perennial crops and forest conversion into grassland results in a loss of 12%. Losses of SOC are partly reversible if agricultural land is afforested, under cropland fallow, or cropland conversion into grassland, resulting in SOC increases of 29%, 32%, and 26% respectively (Don et al., 2010). In general, disturbance of wetlands can accelerate SOC decomposition rates by increasing soil aeration, increasing soil temperature, and increasing soil substrate availability to microorganisms (Richardson & Vepraskas 2001).

Solving the global warming problem is a complex issue that requires combining as many approaches as possible. Soil carbon sequestration is a practical and viable means of reducing the impact of GHG emissions. It is by no means the 'silver bullet' solution to climate change by itself but its effects will have a substantial impact on reducing the rate of global warming and will buy us time over the next few decades while other effective sequestration and low carbon impact technologies are being developed (Minasny et al., 2017). Worldwide wetlands may be net carbon sinks of approximately 830 Tg/year with an average of 118 g C m⁻² year⁻¹ (Mitsch et al., 2012). Furthermore, increasing SOC density of soils will have the additional benefit of improving soil structure and conditions (Lal, 2016).

2.2 Soil organic matter and soil organic carbon stocks

Soil organic matter (SOM) consists of the total of all substances in soils containing elements in organic form, including organic C, organic P, and organic N, and may be defined as all plant and animal residues in various stages of decomposition. Soils inherit organic matter from living organisms that eventually die, leaving their remains in the soil. Living organisms in soil are not included in the definition. Soil organic carbon (SOC) is a component of soil SOM (Richardson & Vepraskas, 2001). In general, it is assumed that approximately 58% of SOM consists of SOC, however, this ratio varies and is dependent on the specific characteristics of a given soil and its SOM (Schumacher, 2002).

The subdivision of SOM is a contentious topic (Lehmann & Kleber, 2015). SOM is often divided into humic and non-humic substances. Humic substances are considered the most stable and resistant to decomposition portions that make up the bulk of SOM. These are amorphous, dark-coloured, partly aromatic, chemically complex substances that no longer exhibit specific chemical and physical characteristics normally associated with well-defined organic compounds. Non-humic substances include those with still-recognisable chemical characteristics, such as amino acids, carbohydrates, fatty acids, pigments, proteins, resins, and waxes. These compounds are generally more easily degraded than their humic counterparts and are often referred to as labile SOM (Richardson & Vepraskas, 2001).

Fresh input of dead plant and animal matter consists of labile and refractory components. Labile fractions are associated with non-humic substances that are readily degraded. Refractory (non-labile) substances (e.g. lignin) are more resistant to degradation and are associated with the formation of humic substances (Richardson & Vepraskas, 2001).

Current models, such as those above, used to describe the fate of SOM input into soil assume that organic matter is somehow transformed into chemically unrecognisable fractions that are older and more resistant than other fractions of SOM (humification or secondary synthesis), or that labile fractions of SOM are preferentially decomposed by soil microorganisms over more refractory substances (selective preservation). There are however no specifically proposed pathways and evidence to support the theory of humification. Recent and robust evidence has also shown that under stable conditions appropriately adapted decomposer organisms are able to decompose 'refractory substances' at rates much higher than previously anticipated (Lehmann & Kleber, 2015). Under unstable and/or disturbed conditions, however, as is often found in unprotected areas, the interaction between labile, non-labile carbon, and the soil microbial community may be significantly altered (Zou et al., 2005).

Lehmann and Kleber (2015) proposed an alternative concept for the decomposition of SOM, the Soil Continuum Model (SCM). Quoted; "*In the SCM concept, organic matter exists as a continuum of organic fragments that are continuously processed*

by the decomposer community towards smaller molecular size. The breakdown of large molecules leads to a decrease in the size of primary plant material with concurrent increases in polar and ionizable groups, and thus to increased solubility in water. At the same time, the opportunity for protection against further decomposition increases through greater reactivity towards mineral surfaces and incorporation into aggregates. Modern analytical tools for the characterization of biomolecules in microbial cells and soils now suggest a direct and rapid contribution of microbial cell walls to soil organic matter protected by interaction with minerals. Adsorption may be followed by desorption, exchange reactions with competing organic compounds, and biotic or abiotic degradation. An obvious consequence of microbial involvement in the decomposition process is the direct deposition of microbial cells, cell debris, exopolysaccharides, and root exudates on mineral surfaces”.

Organic carbon and inorganic carbon are the two major forms present in the soil-water-plant components of wetland ecosystems. The carbon cycle in wetland systems can be depicted as storage of carbon in major reservoirs that function as either a sink or a source of carbon, and flux between reservoirs. The reservoirs of organic carbon in a wetland can be grouped as follows (Reddy & DeLaune, 2008):

- Plant biomass (living, standing stock)
- Particulate organic carbon (detritus, soil and water)
- Dissolved organic carbon (detritus, soil and water)
- Microbial biomass carbon (detritus, soil and water)
- Gaseous end products (atmosphere, detritus, soil and water)

2.2.1 Plant biomass

Vegetation consisting of plants (including macrophytic and algal species) is responsible for primary production and the transformation of inorganic CO₂ into organic carbon. Net primary productivity of wetlands is higher than many other terrestrial ecosystems and is approximately the same order of magnitude as that of tropical rain forests. Despite covering only approximately 6% of the earth's land area, their relative contribution to the earth's biogeochemical cycle far exceeds their

surface area. It has been estimated that wetlands collectively contain 68% of terrestrial soil carbon reserves and represent a highly significant land-based carbon sink. The primary productivity in wetlands is highly variable and is influenced by type of vegetation composition, nutrient status and geographic location. The ratio of below ground to above ground biomass can range from 0.2 to 3.9, depending on the species and the geographic location (Reddy & DeLaune, 2008).

2.2.2 Particulate organic matter

As plants senesce, a portion of the aboveground biomass is returned to the detrital pool, where it undergoes decomposition. The heterotrophic microorganisms that decompose the organic matter convert organic carbon back to inorganic carbon through respiration. Organic carbon is stored in both living (vegetation, algae and microbial biomass) and non-living (plant detritus and native soil organic matter) reservoirs. This is the largest reservoir of organic carbon in a wetland system constitutes approximately 95% of the total carbon (Reddy & DeLaune, 2008).

2.2.3 Dissolved organic matter

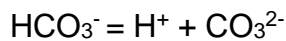
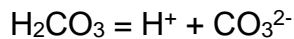
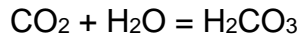
Dissolved organic matter (DOM) is defined operationally as material that passes through a 0.45 µm filter. DOM represents approximately <1% of total soil organic matter, whereas in surface waters dissolved organic carbon accounts for approximately 90% of total organic carbon (Reddy & DeLaune, 2008).

2.2.4 Microbial biomass

The decomposition of organic matter is the primary role of heterotrophic microflora in soils. Microbial biomass accounts for only approximately 3–5% of the total organic carbon in wetland; yet most of the net ecosystem production passes through the microbial loop during decomposition. Microbial decomposers derive their energy and carbon for growth from SOM and contribute to the recycling of carbon and energy within and outside wetland ecosystems (Reddy & DeLaune, 2008).

2.2.5 Gaseous end products of carbon

Under aerobic conditions CO₂ is the gaseous end-product of decomposition, whereas both CO₂ and CH₄ are the end products of anaerobic conditions. The CO₂ formed during decomposition readily dissolves in water and is partitioned H₂CO₃, HCO₃⁻ and CO₃²⁻ inorganic fractions as follows:



The ratio between dissolved and gaseous inorganic carbon depends on the pH of the water column and soil pore water. The pH of soils in wetlands is usually buffered between 6 and 7, depending on the reduction of available terminal electron acceptors as microorganisms decompose organic matter. Water pH values may fluctuate between day and night as a result of photosynthesis of algae and submerged macrophytes (Reddy & DeLaune, 2008).

2.2.6 Labile Soil Organic carbon

Labile soil carbon is considered the fraction of carbon with the most rapid turnover rates and its oxidation/mineralisation drives the flux of CO₂ between soils and the atmosphere (Zou et al., 2005). Labile carbon is most sensitive to disturbance and management practices (Coleman et al., 1996; Harrison et al., 1993). Soil organic matter with high labile carbon content is often referred to as high quality (Dalal et al., 2005).

The drivers of SOM mineralisation in soils in most soil C-models are often considered to be environmental conditions (e.g. temperature and moisture) and organic matter quality (e.g. labile carbon and C-N ratio) exclusively, but the important role of biotic factors such as the microbial community structure and their activity as decomposers are now increasingly recognised (Garcia-Pausas & Paterson, 2011; Strickland et al., 2009; Blagodatsky et al., 2010). The most significant biotic influence in SOM mineralisation is the well-recognised but poorly understood

process of SOM priming, whereby inputs of labile C stimulate turnover of SOM. Conceptually it is often thought that SOM is a chemically recalcitrant substrate for microorganisms that requires more investment of cellular resources (e.g. enzymes) to break down than is recovered from its metabolism. In environments with recalcitrant SOM and low nutrient status soils, inputs of easily metabolised labile C sources may provide the energy required to metabolise more recalcitrant SOM and mobilise nutrients limiting microbial activity and growth (Fontaine et al., 2003; Garcia-Pausas & Paterson, 2011; Paterson et al., 2009). The most abundant source of labile carbon inputs in natural soil environments is through rhizodeposition of low molecular weight organic substances by growing plant roots (Garcia-Pausas & Paterson, 2011). Live roots release a substantial amount of C into the soil; estimated to account for up to 20% of photo assimilate (Hütsch et al., 2002), and has been shown to prime mineralisation of SOM to variable extents (Cheng & Kuzyakov, 2005).

Garcia-Pausas and Paterson (2011) reported that application of labile C (glucose) to soil resulted in increased mineralisation of SOM (priming) and that the magnitude of priming was reduced in fumigated soils where microbial biomass carbon was less and the microbial community structure was altered. In this experiment 16 out of 32 soil microcosms were fumigated with chloroform to kill off a large proportion of the microbial community and significantly alter community structure before incubation. This suggests a link between microbial community composition and SOM-mineralisation (Garcia-Pausas & Paterson, 2011).

Considering the above findings in the context of this study, human disturbances may significantly alter soil microbial community structure and activity and may result in differences in POXC.

The SOC concentrations in mineral surface soils are usually quite low and typically do not exceed 6% (60g kg⁻¹ soil), typically less than 1 % for terrestrial soils in South Africa. Worldwide, Histosols, which are organic soils and are dominated by SOC, contain on average 419 g C Kg⁻¹ soil and up to 100% SOM. Andisols and Spodosols have greater median concentrations of organic carbon (38.3 g C Kg⁻¹, and 27.9 g Kg⁻¹ respectively) than Alfisols, Udisols, and Aridisols (3.8 g C Kg⁻¹, 4.6 g C Kg⁻¹, and 5.4

g C Kg⁻¹ respectively), mainly due to differences in climatic conditions (temperature, precipitation and humidity) and vegetation. Wetlands that are permanently or near-permanently saturated with water tend to have predominantly organic soils (Histosols) (Richardson & Vepraskas, 2001).

Despite the relatively low concentrations of SOC in mineral soils, this pool of carbon is an important component of the global carbon budget and cycle. It is estimated that world soils contain 1576 Pg of SOC to a depth of 1 m, while soils contain an estimated 1738 Pg of inorganic carbon, mainly as carbonates. The soil orders that primarily contribute to the global SOC budget are Histosols (390 Pg) and Inceptisols (352 Pg) (Table 2.1). Although these numbers are relatively large, their true significance lies in the fact that the estimated size of the global SOC pool is more than twice the estimated size of the atmospheric carbon pool (750 Pg), and approximately three times the organic carbon estimated to occur in vegetation biomass (550 Pg). The ocean and geologic carbon reservoirs contain the greatest quantities of carbon (38,000 and 65.5×10^6 Pg carbon), but the latter is not in dynamic equilibrium with the ocean and terrestrial reservoirs (Essington, 2003).

2.3 Factors and processes that determine the accumulation and storage of soil organic carbon

Organic carbon accumulates in soil when the production of organic materials is high or when the conditions are not optimal for the decomposition of organic materials. When conditions are optimal for the decomposition of organic matter microbial activity is so high that the accumulation of organic matter is relatively insignificant. Wetlands that are saturated throughout the year tend to have a higher accumulation of organic matter than wetlands that are periodically saturated. Permanently saturated soils are in a continuous anaerobic state, producing conditions that are not optimal for microbial activity and therefore the decomposition of SOC occurs at a slower rate than soils that are periodically aerobic (Adhikari et al., 2009).

The rate and degree to which organic carbon accumulates in wetland soils is dependent on the balance between two fundamental processes; 1) the net primary

productivity of wetland vegetation and 2) heterotrophic metabolism (decomposition) of soil microorganisms. The growth and activity of vegetation and decomposers is affected by factors including; topography and geological position (parent material) of the wetland; the hydrological regime; temperature and soil moisture (climate); species composition; pH and soil morphology. Thus, the combination of all these factors makes carbon dynamics in wetlands a complicated process (Richardson & Vepraskas, 2001; Reddy & DeLaune, 2008).

2.3.1 Geology and topography

The composition of parent material has a significant effect on the chemical (elemental content and pH) and physical characteristics (texture; structure; sand, silt, and clay mineralogy; bulk density; and hydraulic conductivity) of the soils from which it is derived. The elemental content of parent material determines soil content and ratios of elements essential to plant growth such as; Ca, Mg, Mn, K, Na, Fe, Ni, and Co. Soils formed with lower concentrations of exchangeable base cations (Ca, Mg, Na, and K) are more prone to acidification by leaching (Buol et al., 2011).

Topography (relief) of a landscape is the physical configuration of the land surface with respect to relative elevation and slope, and frequently directs and controls the flow of both surface and subsurface water to and from a wetland. Attributes of relief include elevation above sea level, slope and natural soil drainage condition, aspect, and slope configurations of convexity and concavity. A cross section overview of landscape positions is illustrated in figure 2.2; the summit of a landscape is characterized by a crest with a rather gentle slope, followed by a narrow shoulder area with a steep slope. From the side slope through the footslope, toeslope, and flood plain the angle of slope progressively decreases.

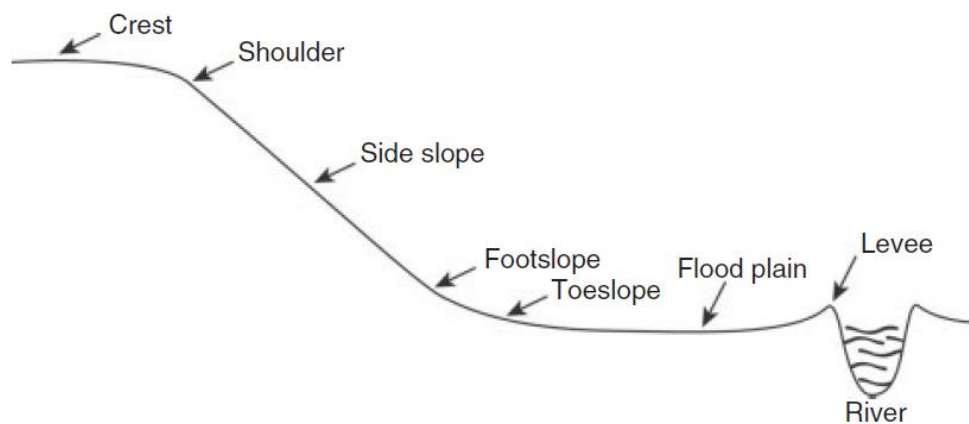


Figure 2.2: Cross section diagram of landscape positions

The position within a landscape and its relief are influential factors in determining soil hydrology, water table position, and hydrodynamics (direction and rate of surface and subsurface water flow). Water at the crest of the landscape has the highest gravitational potential energy relative to the lowest point in the landscape. As water flows laterally from the crest through the shoulder position this potential energy converts to kinetic energy and accelerates down the slope, progressively losing energy as the slope decreases. The erosive potential of water is proportional to the kinetic energy (flow velocity) of the water. Sediments and nutrients are transported by water runoff and deposition occurs as the angle of relief decreases and the water flow velocity subsequently decreases. The largest sediment fraction is coarse sand-sized particles and is deposited first, followed by silt, then clay where flow velocity is at its lowest (Buol et al., 2011 : Richardson & Vepraskas 2001).

Organic matter accumulates in soils primarily due to limited O₂ supply caused by waterlogging. Wetlands tend to form in the lowest positions of the landscape where the water table is closest to the soil surface and where low slope angles result in slow water movement. In addition, the hydrodynamics of water movement through wetlands has an impact on the sedimentation rates and recharge of soil O₂. Water moving at low velocity allows for sedimentation to occur and a slower recharge of O₂

from the surrounding landscape. Sedimentation occurs simultaneously with organic matter accumulation and affects storage of SOC by burying it in the soil profile (Richardson & Vepraskas, 2001).

A requirement for the formation of peat in peatlands is permanently saturated (anoxic) soils (Grover et al., 2012). Mineral soil wetlands can be further divided into permanent wetlands that are at least partially saturated throughout the year and temporary wetlands that experience seasonal or occasional flooding during wet seasons. Within permanent mineral wetlands there are different hydrological zones that experience different degrees of saturation throughout the year often following the topographical gradient with permanently saturated zones situated in the lowest depression where the water table is higher than the soil surface followed by seasonal and temporary zones further upslope (Figure 2.5). Wetlands may have all three of these zones, two or only one (Collins, 2005).

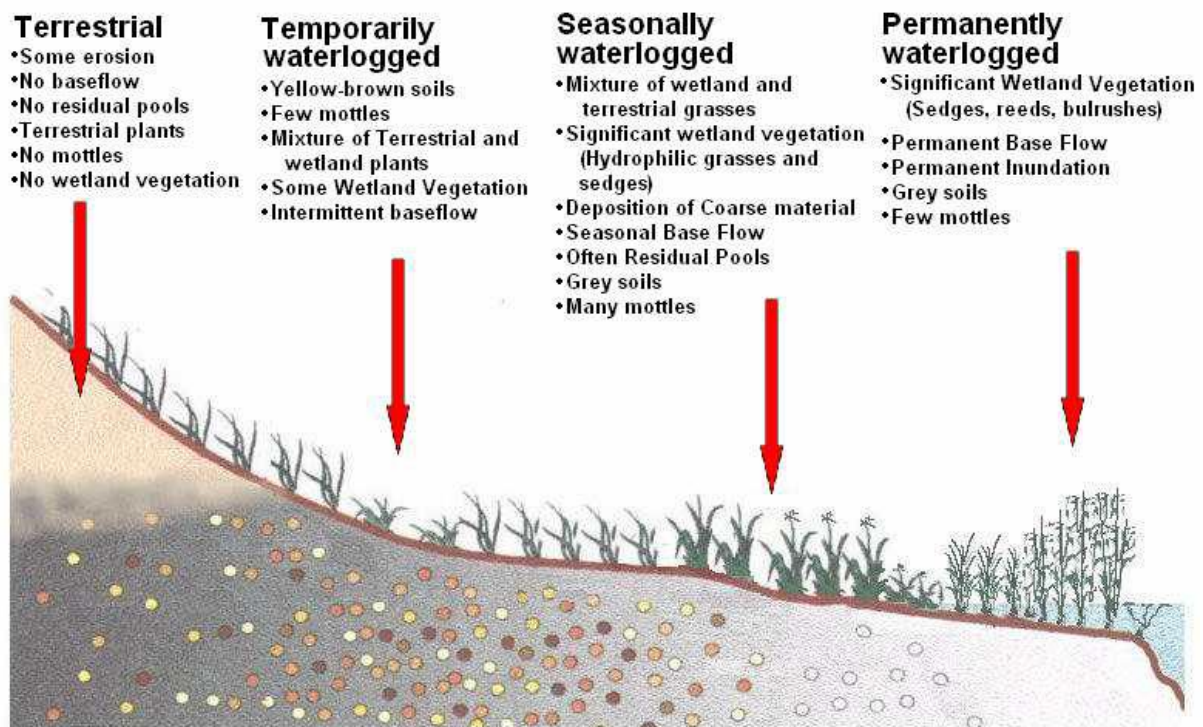


Figure 2.3: Cross section through a valley bottom wetland, indicating how the soil wetness and vegetation indicator change as one moves along a gradient of decreasing wetness, from the permanent wet hydrological zone (higher relative SOC concentration) to the temporarily wet hydrological zone (lower relative SOC concentration) and eventually into non-wetland (lowest relative SOC concentration), (Collins, 2005)

2.3.2 Climate and hydrological regimes

As a factor in soil genesis, climate is responsible for two of the most significant components affecting the processes of soil formation: water and temperature (energy). Precipitation and water play an important role in erosion and deposition of soil material. Vegetative biomass usually increases as precipitation increases if soils are of adequate nutrient status. Heat energy directly drives the rate at which chemical and physical soil processes occur (Buol et al., 2011).

There is a strong correlation between climate and soil carbon stock as organic carbon content decreases with increasing temperatures. Enzymatic reaction rates and therefore decomposition rates double with every 10°C increase in temperature and therefore carbon output is expected to be higher relative to input in warmer

climates compared to cooler climates. Findings however contradict this theory as some tropical wetlands can store 80% or more carbon than some temperate wetlands, indicating that a combination of complicating factors is at play (Adhikari et al., 2009).

2.3.3 Species composition of wetland vegetation

Wetland ecosystems can be grouped according to their water source, either freshwater or estuarine (saltwater) ecosystems. Freshwater wetlands are predominantly dominated by either herbaceous emergent vegetation (marshes), woody vegetation (swamp forests), or peatmosses (*Sphagnum* spp.) and acid tolerant plants (bogs). Estuarine wetlands are dominated either by herbaceous vegetation or mangroves (Richardson & Vepraskas, 2001). The chemical composition of organic matter deposited by different types of vegetation is species dependent (Buol et al., 2011). The chemical composition of different plant types contributes to the rate of decomposition of organic matter. Properties such C:N ratio, lignin content and tannin content determine the rate at which microorganism can break down organic matter. Plants that produce highly fibrous organic matter such as Sphagnum moss are more difficult to decompose and subsequently have longer turnover rates (Richardson & Vepraskas, 2001).

Soils inherit SOM from living organisms that eventually die, leaving their remains in the soil. Living plant roots are usually not considered as part of SOM, but both fresh and decomposing residues of plants and animals (microorganisms) are included, as well as tissue of living and dead microbes (microorganisms) (Richardson and Vepraskas, 2001).

2.3.4 Redox chemistry, pH and nutrient availability

Oxygen availability is the main driver of wetland SOM mineralisation (Richardson and Vepraskas, 2001).

Nitrogen (N) and Phosphorus (P) are two of the most important macro nutrients that limit the productivity of terrestrial, wetland, and aquatic ecosystems (Reddy & DeLaune, 2008). Primary production in coastal wetlands is conventionally thought to be limited by N (Sundareshwar et al., 2003). Tropical montane forests are often characterised by slow decomposition of organic matter and poor litter quality relative to tropical lowland forests. Cool moist conditions reduce the rate of litter decomposition and mineralisation of N, thereby restricting the release of plant available N (Dalling et al., 2016). Sundareshwar et al., (2003) found that in a pristine salt marsh primary productivity was primarily limited by N availability but the bacterial community was limited by P. Therefore in coastal wetlands and possibly other ecosystems, individual trophic groups may respond differently to N and P loading.

Treseder (2008) performed a meta-analysis on Nitrogen additions and microbial biomass and reported for the microbial community as a whole, biomass declined by an average of 15% under N fertilisation and even more substantially under larger N loads for longer durations of fertilisation. Moreover, soil CO₂ emissions responded in concert with biomass. The rate SOC mineralisation was therefore reduced. Zak et al., (2008) found a decade of experimental NO₃⁻ deposition significantly increased amounts of organic matter (+12%) and N (+9%) in forest floor and mineral soils, despite no increase in detritus production. Rejmanková and Sirová (2006) found that in oligotrophic, P-limited herbaceous wetlands of northern Belize P-enriched plots resulted in an improved development of the microbial decomposer community, which in turn leads to higher production of hydrolytic extracellular enzymes, resulting in faster decomposition of SOC.

From the above references it seems that higher N and P loads may stimulate increased primary productivity whereas high N loads have a negative effect on microbial biomass while high P loads have a positive effect on microbial decomposition. It is therefore reasonable to speculate that there might be optimal ratios of soil N and P that may enhance primary productivity whilst limiting SOM decomposition by microorganisms, thereby maximising a wetland's potential for net carbon sequestration.

Microbial metabolic activity and therefore mineralisation of organic matter is mediated by redox couples in soil. Organic matter is a source of electrons and in aerobic soils O_2 is the main electron acceptor where it is reduced to H_2O . Soil saturation leads to a decline in O_2 availability as soils become more reduced. Appropriately adapted microorganism then have to utilise alternative redox couples with other electron acceptors that are available. This is known as the redox sequence and alternative electron acceptors are used in the following sequence; $O_2 > NO_3^- > MnO_2 > Fe(OH)_3 > FeOOH > SO_4^{2-} > CO_2$ with each successive couple yielding less metabolic energy return (Essington, 2003).

The rationale for including Mn, Fe, and S levels in this study is as an indirect measure of the impact of AMD from coal mines that directly affect two of the wetlands in this study (Plant and Zaalklap) and how Mn and Fe oxides and SO_4^{2-} may influence SOC density. AMD contains high concentrations of SO_4^{2-} as well as other elements such as iron, aluminium and manganese as well as low concentrations of heavy metals, derived from the the oxidation of sulfide-bearing rocks such as pyrite (FeS_2) when exposed to water and air during mining operations (Akcil & Koldas, 2006). As illustrated by the redox sequence above, the addition of these pollutants to wetlands through AMD may alter the redox status and microbial community composition of these affected wetlands. An influx of predominantly SO_4^- , Fe^{3+} oxides and Mn oxides from AMD can be expected to lead to an increase in soil oxidation and therefore a reduction in the labile SOC fraction and an accelerated rate of SOC mineralisation.

Generally the pH values of acid soils increase after flooding and those of alkaline soils decrease. Soils with high carbon concentrations and reduced iron tend to reach a pH of around 6.5 within a few weeks after flooding. Increase in pH of acid soils low in carbon or reactive iron occurs much more slowly, and these soils rarely reach a pH of 6.5. Acid sulphate soils with lower SOM content may never reach a pH of greater than 5 even after several months of flooding (Reddy & DeLaune, 2008).

2.4 Relative carbon storage capacity of different hydrogeomorphic wetland types

The concept of hydrogeomorphic wetland type

To aid in identifying the water transfer mechanisms of different wetland types, the concept of hydrogeomorphic (HGM) wetland classification was developed by Brinson (1993). HGM units are distinguished primarily based on Ollis et al. (2013):

1. Landform, which defines the shape and localised setting of the aquatic ecosystem.
2. Hydrological characteristics, which describe the nature of water movement into, through and out of the aquatic ecosystem.
3. Hydrodynamics, which describe the direction and strength of flow through the aquatic ecosystem.

Seven primary HGM wetland types are recognised for inland systems (Ollis, 2013)

2.4.1 Rivers

Linear fluvial, eroded landforms which carry channelized water flow either permanently, seasonally or ephemerally. A river flows through a channel that is confined to valley (gorge) or an incised macro-channel. Both the active channel and riparian zone adjacent to it make up the river (Rountree et al., 2008).

2.4.2 Channelled Valley Bottoms

Depositional valley bottoms are linear fluvial with little to no meandering. Water flow is either permanent or seasonal. The valley floor is a depositional environment with the channel flowing through fluvially-deposited sediment. These systems are more prevalent in upper catchment areas (Rountree et al., 2008)

2.4.3 Unchanneled Valley bottoms

Water flow is linear fluvial with net depositional valley bottom surfaces that do not have a channel. The valley floor is a depositional environment with sediment that is deposited fluvially or colluvially. These systems are more prevalent in upper catchment areas, or at tributary junctions where the sediment from the tributary smothers the main drainage line (Rountree et al., 2008).

2.4.4 Seepage wetlands

These are the most common wetlands in South Africa and are located on the mid- and footslopes of hillsides; either as isolated systems or connected to downslope valley bottom wetlands or as fringes of depression pans. Seepages occur where springs decant into the soil profile where it is close enough to the water table or where through-flow in the soil profile is forced close to the soil surface as a result of impervious rock layers (Rountree et al., 2008).

2.4.5 Depression Pans

These depressions are usually small and circular or oval in shape and occur on the crest positions of the landscape. Although commonly endorheic (inward draining), many pans are hydrologically connected to adjacent valley bottoms through sub-surface diffuse flow paths (Rountree et al., 2008).

2.4.6 Wetland Flats

These wetlands occur in areas of flat topography and weakly developed drainage patterns. The drainage of rainfall is limited by the low topographical relief and accumulates in the landscape. During the wet season the water table may rise to or above the soil surface, producing extensive area of inundation. These wetlands may be permanent or seasonal in nature (Rountree et al., 2008).

Although little information is available specifically on the effectiveness of different HGM wetland types in storing carbon, inferences can be made based on differences

in factors that influence these different wetland types. Some of these factors include hydrology, topography and nutrient status.

Closed systems, such as wetlands that are fed predominantly through precipitation (e.g. bogs, pocosins and some seasonal or ephemeral wetlands), are oligotrophic (low nutrient levels) and typically have low primary productivity. Higher inputs of surface and ground water tend to increase primary productivity in fens and marshes. Wetlands that receive pulses of nutrients, such as river floodplains, littoral zones in lakes, and tidal marshes are typically very productive (Reddy & DeLaune, 2008).

The carbon sequestration capacity of a wetland is directly dependent on the balance between net primary productivity and decomposition. Both processes are regulated by hydroperiod, nutrient availability, and abiotic stressors; salinity, concentrations of H_2S , and acidity, as well as other factors such as; solar radiation and air/soil temperature. The availability of O_2 is regulated by hydroperiod and limits aerobic respiration. The availability of essential nutrients like nitrogen (N), phosphorus (P) and Calcium (Ca) can accelerate or limit primary productivity and decomposition, while stressors such as salinity, H_2S , and acidity can inhibit both processes. The enrichment of nutrients appears to stimulate primary productivity to a greater extent than decomposition and therefore carbon accumulation tends to be higher (Richardson & Vepraskas, 2001).

Primary productivity is expected to be higher in wetlands that are recharged predominantly by rivers, streams, surface water and ground water (channelled valley bottoms, unchannelled valley bottoms, depressions, and seeps) as there are higher influxes of sediments, clay minerals and nutrients, especially NO_3^- , P, as well as K and Ca, than wetlands that are recharged predominantly through precipitation and fluctuating water tables (floodplains and wetland flats) (Richardson & Vepraskas, 2001). The rate of hydrological flow will also have an impact on decomposition rates as anaerobic conditions are only maintained in stagnant or slow-moving water (Collins, 2005).

2.5 Carbon sequestration in wetlands

A major concern regarding wetlands and climate change is the fact that wetlands emit the greenhouse gases carbon dioxide (CO₂) and methane (CH₄). Methane is particularly potent greenhouse gas that has approximately 25 times the effect of CO₂ in the atmosphere (Acreman, 2012; Mitsch et al., 2012). Mitsch et al., (2012) have shown through dynamic modeling of carbon flux that methane emissions of created or restored wetlands become unimportant within 300 years compared to carbon sequestration, and that worldwide wetlands may be net carbon sinks of approximately 830 Tg/year with an average of 118 g-C m⁻² year⁻¹.

Among all terrestrial ecosystems wetlands have the highest carbon density (Kayranli et al., 2010). Of the various wetland types, peatlands have been recognised as the most significant carbon storage ecosystem as these wetlands account for almost 50% of the terrestrial carbon storage despite occupying only 3% of the world's terrestrial area (Adhikari et al., 2009). Peatlands in South Africa are uncommon, accounting for approximately 10% of all wetlands in the region. These wetlands are however crucial ecosystems and many of them are threatened (van Vuuren, 2010).

In addition, wetland soil organic matter (SOM) has other beneficial environmental effects. SOM contains essential nutrients and elements and enriches soil microbiota, which in turn contribute to nutrient cycling in the soil, holding nutrients in the root-zone layers of the soil for longer, slowing the rate of passage of pollutants to the groundwater table. Pollutants may become bound to SOM, purifying water through the attenuation of pollutants (Victoria et al., 2012).

SOM is intimately associated with soil minerals and is important for the maintenance of soil structure and aggregate stability. Organic matter improves the water holding capacity of the soil and has an impact on the thermal properties of the soil. SOM plays an important role in buffering soil solution pH, increasing the cation exchange capacity of the soil, enhances the dissolution of soil minerals, complexes and chelates metal cations (including toxic heavy metals), and retains non-ionic organic compounds (altering their efficacy or potential toxicity) (Essington, 2003). The ability of wetlands to filter and provide fresh water is highly dependent on these processes (Richardson & Vepraskas, 2001).

Soil organic carbon (SOC) is a component of soil organic matter (SOM). Soils inherit SOM from living organisms that eventually die, leaving their remains in the soil. Living plant roots are usually not considered as part of SOM, but both fresh and decomposing residues of plants and animals (microorganisms) are included, as well as tissue of living and dead microbes (microorganisms) (Richardson & Vepraskas, 2001).

From a climate change mitigation perspective, the conservation of wetlands is important for two reasons; 1) it is estimated that collectively the wetlands of the world contain around 350-535 Gt of carbon (Gt = billion tonnes). The poor management and destruction of wetlands will result in the oxidation of this carbon into CO₂ which will then ultimately be released into the atmosphere, further accelerating global warming. 2) The protection, proper management of healthy wetlands, and restoration of disturbed wetlands will ensure that these wetlands sequester atmospheric carbon consistently in the long term, reducing atmospheric CO₂ concentrations (Mitra et al., 2005). Furthermore, appropriate conservation and management strategies will have a positive impact on biodiversity which in turn has a positive feedback on the SOC pool. All other factors being equal, higher biodiversity in ecosystems results in improved sequestration of SOC and biota than systems with reduced biodiversity (Lal, 2004).

Carbon sequestration as an ecosystem service

In the context of studying carbon sequestration of wetlands as an ecosystem service, the following general key questions should be asked (Adhikari et al., 2009):

- Can wetlands function as an effective carbon sink to mitigate changes in atmospheric carbon?
- What are the effects of carbon storage on the function of wetland ecosystems including water quality and biodiversity?
- Is the capacity of wetlands to sequester carbon economically viable and environmentally sound?

Carbon is present in the atmosphere as CO₂, CH₄ (methane), CO (carbon monoxide) and volatile organic compounds. From 1850 to 2000 atmospheric CO₂ concentrations have risen from 270 ppm (parts per million) to 360 ppm (Figure 2.2), during the same period CH₄ concentration rose from 0.7 ppm to 1.7 ppm. Much of the increase in CO₂ is anthropogenic and is attributed to combustion of fossil fuels since the industrial revolution. A variety of sources including natural wetlands, rice paddies, livestock, livestock waste, termites and biomass burning have been attributed to the increase of CH₄ concentrations in the atmosphere. The total amount of CO₂ in the atmosphere is estimated at about 735 Pg (1 Petagram = 10¹⁵ g) compared to 3 Pg for CH₄ and 0.2 Pg for CO (Reddy & DeLaune, 2008).

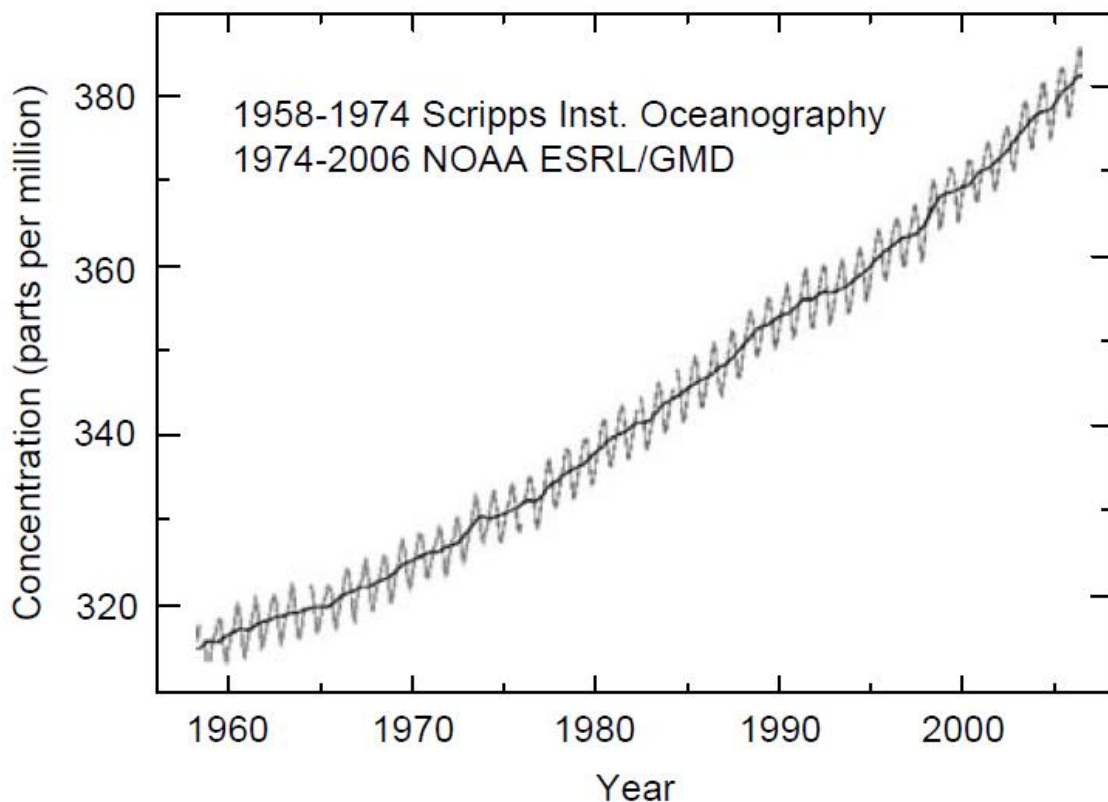


Figure 2.4: Increase of atmospheric CO₂ from 1960 to 2000 (ppm) (Reddy & DeLaune, 2008)

Carbon sequestration is the process by which atmospheric carbon is captured and stored over a long term. It is a function of the rate of carbon that is accumulated via

fixation of atmospheric carbon through the process of photosynthesis vs. the opposing process of decomposition and mineralisation of organic matter that in turn releases carbon back into the atmosphere. Different ecosystems have varying capacities to sequester atmospheric carbon. Ideally the most effective ecosystem for carbon sequestration would be one with the highest possible rate of primary production (photosynthesis) and the slowest possible rate of decomposition (Reddy & DeLaune, 2008).

Terrestrial ecosystems store most of their carbon in two main fractions; vegetation and as soil organic matter. The relative proportion of these fractions is dependent on the conditions of the ecosystem. For example, tropical forests have the highest ratio of carbon stored in vegetation compared to soil (Figure 2.7). Wetlands store most of their carbon in soil (Figure 2.7) (Mitra et al., 2005).

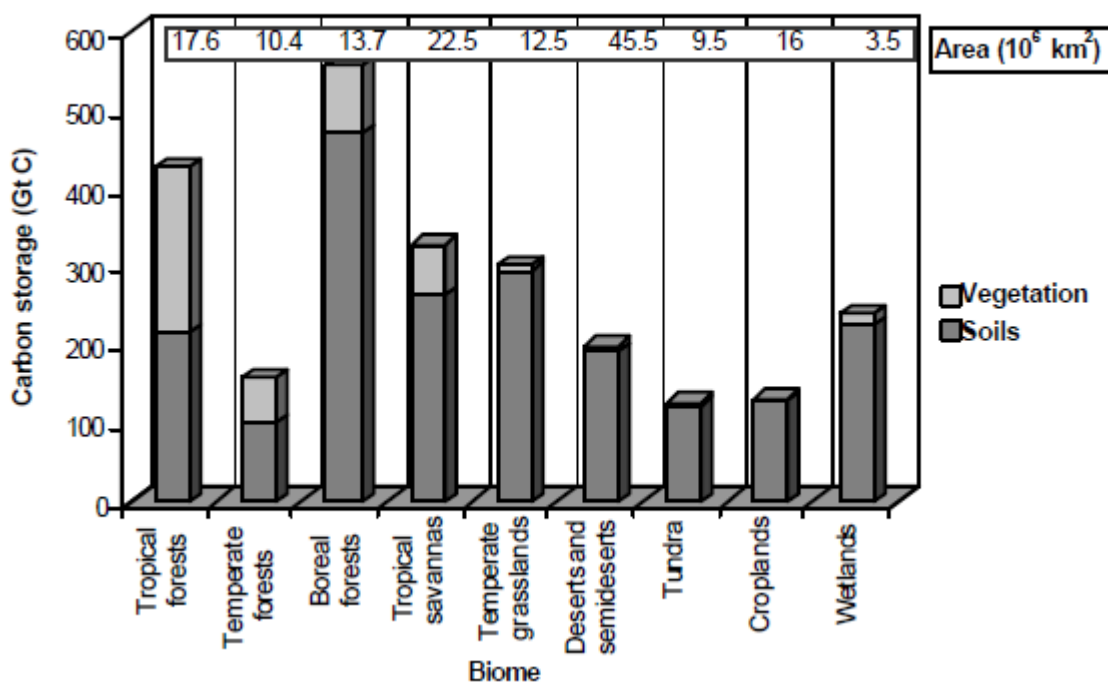


Figure 2.5: Organic carbon storage and area of different global biomes (Mitra et al., 2005)

3 Chapter three: Sampling programme

3.1 Site selection

The first location was a relatively small patch of a channelled valley bottom wetland situated in the Rietvlei Nature Reserve in Gauteng. This wetland and its catchment are situated in a protected area and relatively isolated from human settlement (Figures 3.1 and 3.2).

The second and third locations are near the Highveld Steel processing plant in Mpumalanga, following a stream that extends from the plant into the extensive Zaalklap wetland (Figures 3.3 and 3.4). There is also a large store of coal on the property of this plant. The wetland nearest to the plant is directly adjacent to the plant, approximately 130 m away from the steel plant slag dump and is fed by a stream that is polluted and obstructed by the slag and coal store (Figure 3.3). Further downstream is the third wetland site and forms part of the larger Zaalklap wetland (Figure 3.4). This wetland has undergone extensive rehabilitation since 2013 thanks to the efforts of the Working for Wetlands group.

The fourth site is a seepage wetland situated approximately 450 m upstream of a sand mine (Figure 3.5). This wetland is an organic wetland and contains peat.

These four wetland sites hereafter will be referred to as Rietvlei, Plant, Zaalklap and Organic respectively (Figures 3.1 to 3.6).

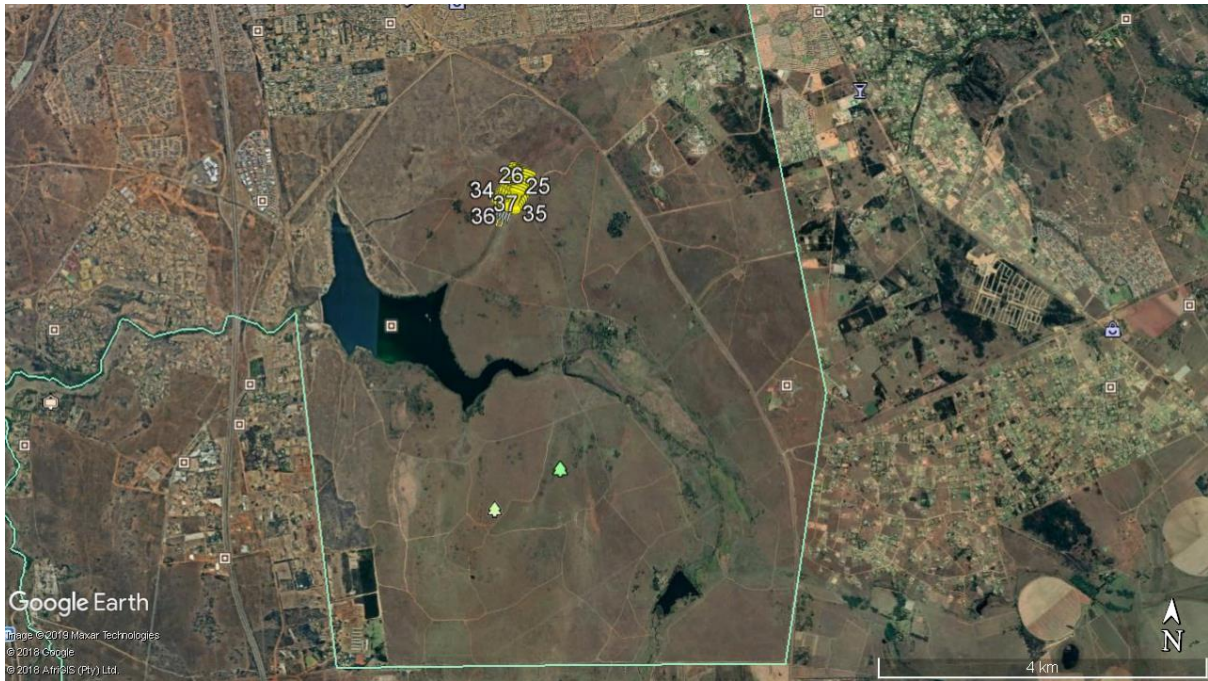


Figure 3.1: Rietvlei Nature Reserve



Figure 3.2: Rietvlei nature reserve wetland sampling grid (300 x 178 m)



Figure 3.3: Steel Plant with adjacent wetland and respective sampling grid (151 x 149 m)

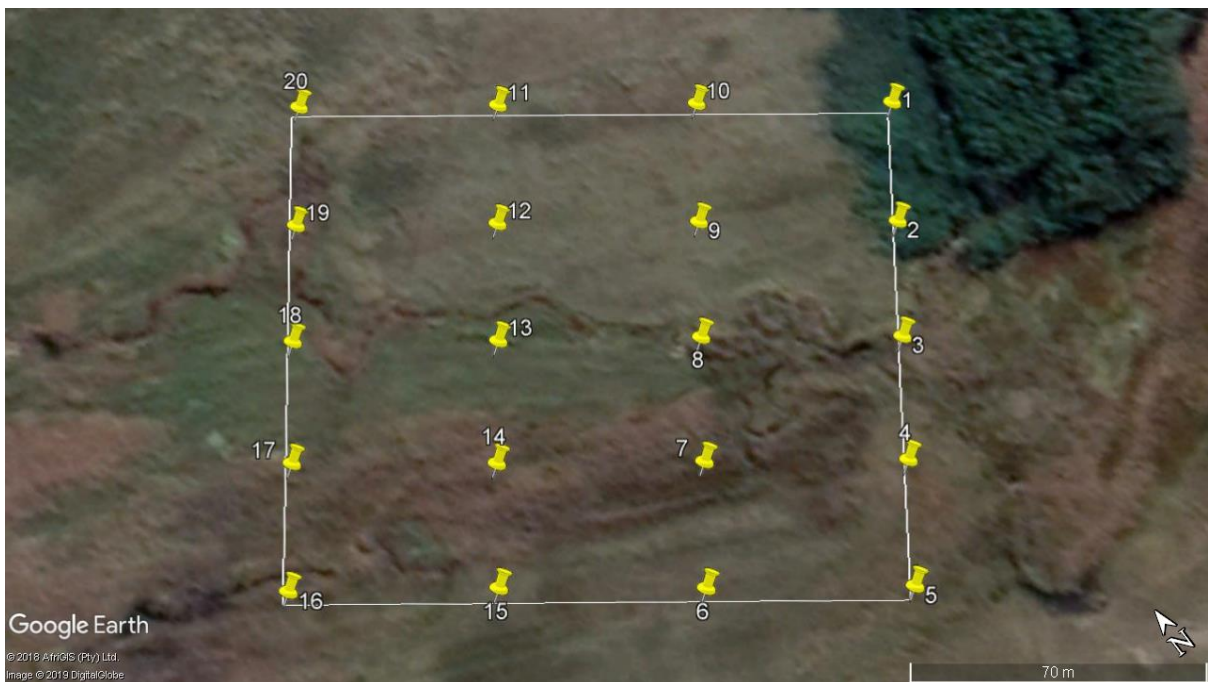


Figure 3.4: Zaalklap wetland section sampling grid (150 x 119 m)



Figure 3.5: Organic wetland section sampling grid (119 x 110 m), with sand mine visible (top right)

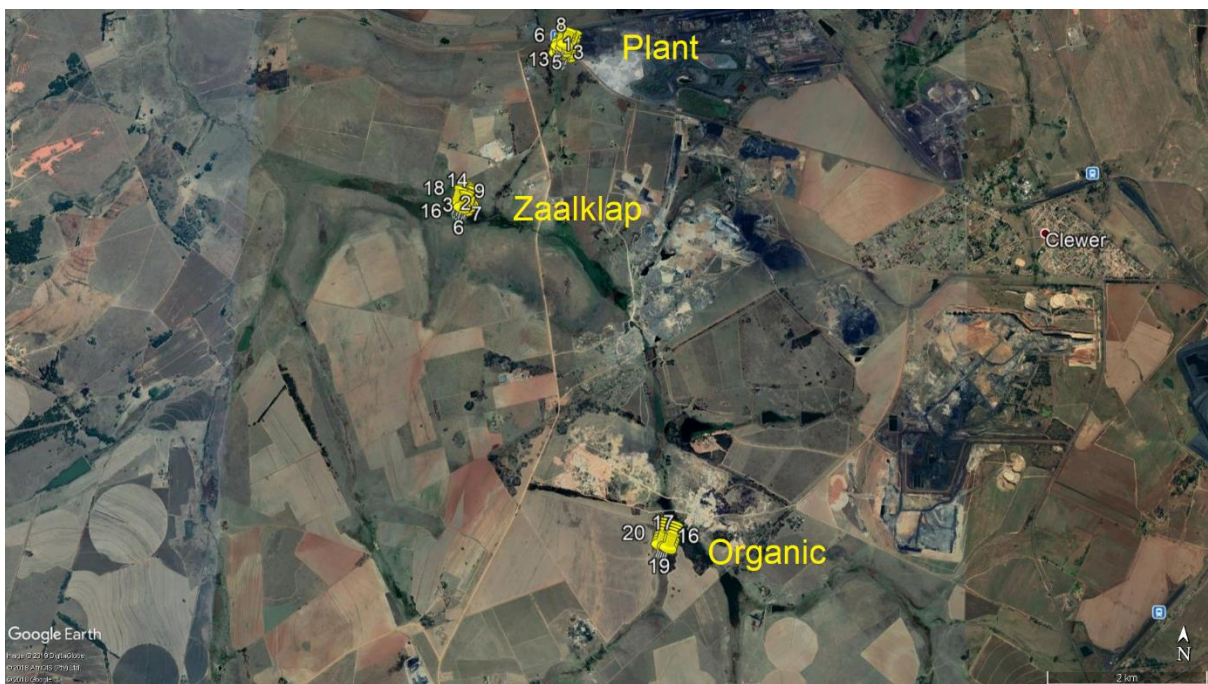


Figure 3.6: Proximity of the Plant, Organic and Zaalklap wetland sites

3.2 Sampling

For Rietvlei a grid consisting of 40 sampling points was created in Google Earth (Figure 3.2). For the Rietvlei wetland, extensive soil classification was performed in the field during sampling (Table A1, Appendix A). This study was part of a larger research project and the same areas were also used in another M.Sc. study which focused on CO₂ emissions of wetland soils (van Rooyen, 2018). The soil classification data set of the wetlands was shared for the larger research project.

For the other three sites grids of 20 sampling points each were created. The grid sampling points of all the sites positions were saved as GPS coordinates in Google Earth. These coordinates were uploaded onto a handheld GPS device for locating points in the field. Samples were collected in airtight containers and stored at 4° C.

The following is an example of the sampling methodology that is conventionally followed by researchers relating to carbon stocks in wetlands;

Adame et al., (2013) researched carbon stocks in wetlands with soft organic soils and used peat augers to extract semi-cylindrical undisturbed cores. Cores were divided into arbitrarily selected intervals (0-15 cm, 15-30 cm, 30-50 cm and >100 cm where possible) that represent layers within the soil profile. These intervals can easily be cut and removed from the peat auger after which they are homogenised before carbon and nitrogen analysis was carried out (Leco CNS-2000 Macro Analyser). The SOC density for each layer was calculated from the SOC concentration, bulk density and thickness of the layer. SOC density for the entire profile (0 cm to 50 cm, or more) was determined by adding the carbon concentration values of each individual depth layer. These undisturbed cores were also used to calculate soil dry bulk density. Soil organic carbon concentration tends to decline in relation to soil depth, which is why it more useful to work in depth intervals as opposed to considering the soil profile as a whole.

A similar method to Adame et al., (2013) was used. It was not possible to use a peat auger for these wetlands because the soil was too hard in most places, a normal soil auger had to be used. For the Rietvlei wetland samples were collected in depth intervals of 15 cm, from the soil surface (0 cm depth) to a depth of 30 cm. For the

remaining three wetlands (Zaalklap, Plant and Organic) samples were collected in depth intervals of 10 cm, from the soil surface (0 cm depth) to a depth of 20 cm. All samples were then stored in a cold room before analyses.

Sampling proved challenging for Rietvlei and Zaalklap. The terrestrial soils of Rietvlei were too hard to auger through at the time of sampling and so only surface samples could be collected for those points. Some of the points in Zaalkap were nearly impossible to reach due to dense vegetation cover and deep water. Only sample points for which samples of all depths (0 cm to 10 cm and 10 cm to 20 cm) could be collected were included in statistical analysis.

4 Chapter Four: Soil Organic Carbon

4.1 Introduction

The primary aim of this chapter was to achieve the first estimates of SOC density (t ha^{-1}) for sections of selected wetlands on the South African Highveld. These baseline estimates can be used as comparative references to which soil carbon density data from other countries can be compared. The total SOC concentrations were determined for all wetland samples as well as permanganate oxidisable carbon (POXC) fractions for selected transects for each wetland site.

The secondary aim of this chapter was to determine the permanganate oxidisable carbon (POXC) and to assess its viability as an indicator of wetland disturbance. Several workers have used the POXC as an index for labile carbon, such as the method proposed by Blair et al. (1995) suggesting that a potassium permanganate solution (KMnO_4) oxidises the most reactive organic carbon. Labile soil carbon is the fraction of carbon with the most rapid turnover rates and its oxidation/mineralisation drives the flux of CO_2 between soils and the atmosphere (Zou et al., 2005). Labile carbon has been found to be the organic carbon fraction most sensitive to disturbance and management practices (Coleman et al., 1996; Harrison et al., 1993). These fractions include POC and MBC and are important indicators of changes in soil ecosystems brought about by management practices (Culman et al., 2012).

The nature of the fractions of organic carbon oxidised by the abovementioned method, however, had not been well elucidated (Tirol-Padre & Ladha, 2004; Culman et al., 2012). Work by Tirol-Padre and Ladha (2004) showed that in soil, KMnO_4 more rapidly oxidised less readily available organic compounds than the water-soluble carbohydrates, indicating that it did not discriminate the nonlabile from labile carbon. Culman et al. (2012) found that POXC is significantly related to POC, MBC, and SOC and more closely related to smaller sized ($53 - 250 \mu\text{m}$) than larger POC fractions ($250-2000 \mu\text{m}$), and more closely related to heavier ($>1.7 \text{ g cm}^{-3}$) than lighter POC fractions, indicating that it reflects a relatively processed pool of labile carbon.

Despite the fact that POXC is not an exclusive reflection of soil labile carbon it has been found by several workers to be useful and practical as an indicator of organic carbon sensitive to changes caused by land use practices (Bonilla et al., 2014; Tirol-Padre & Ladha, 2004; Nan et al., 2017; Culman et al., 2012). POXC may be effective in tracking management practices that promote SOC sequestration, making it a particularly useful indicator for soil quality research (Culman et al., 2012). Mining activities that produce AMD runoff and agricultural practices that produce fertiliser and pesticide runoff may have a significant effect on the SOC of the wetland soils reflected in POXC, and can potentially be used as an indicator in this regard (refer to chapter 2.2.6 in literature review for more on this topic).

4.2 Methodology

4.2.1 Total Soil Organic Carbon

Total soil organic carbon concentrations were analysed following the modified Walkley-Black method described by Schumacher (2002). Samples were prepared by mixing to achieve homogenisation before being oven dried at 45° C for approximately 48 hours after which they were ground with pestle and mortar and sieved (2 mm) to exclude gravel and large pieces of solid plant material.

The Walkley-Black method has historically served as the standard reference method of soil organic carbon analysis as it is a relatively simple and inexpensive procedure. A limitation of the standard method however is that the oxidative digestion of organic carbon by a Potassium dichromate solution ($K_2Cr_2O_2$) is incomplete and generally ranges between 60 and 86% with a mean recovery of 76%. Therefore, a correction factor of 1.33 is commonly applied to the results to adjust the organic carbon recovery (Schumacher, 2002).

The use of a correction factor is an additional source of error. To overcome this, a modified method was incorporated which involves extensive heating during the oxidative digestion step to a temperature of 150° C for 30 minutes. This additional

step results in a virtually complete oxidative digestion of organic carbon and negates the use of a correction factor (Schumacher, 2002).

As for the method by Adame et al., (2013), (chapter 3), the SOC concentration for each depth interval layer was measured by analysis from homogenised samples, 0 cm to 15 cm and 15 cm to 30 cm for Rietvlei and 0 cm to 10 cm and 10 cm to 20 cm for Zaalklap, Plant and Organic wetlands.

4.2.2 Inorganic carbon

Total soil carbon stock mainly consists of organic and inorganic carbon, with inorganic carbon as predominantly carbonates. Soils with a pH value of lower than 7.4 indicate the absence of carbonates, predominantly dolomite and calcite (Schumacher, 2002). Soil pH (pH H₂O method) was measured for various randomly selected points for each location. For pH values <7.4% it was assumed that soil carbonate concentrations are negligible.

4.2.3 Dry Bulk Density

Bulk density is a measure of the mass of dry soil per unit volume and is a parameter required for determining soil carbon density (Ausseil et al., 2015). Attempts were made to extract soil cores to determine the volume and mass of soil samples. However, the saturated and fibrous plant matter dominated nature of the soil made it virtually impossible to extract cores with minimal disturbance. As a result, the measurements made from the disturbed samples were of very low confidence (results not shown). An alternative approach was therefore followed and the development of it is treated in the results and discussion.

4.2.4 Soil Organic Carbon Density (CD)

Soil carbon density (CD) is the measure of mass of carbon per unit volume of soil expressed in unit area (ton C ha^{-1}). Equation 3 was used to determine CD for each site (Ausseil et al., 2015):

$$\mathbf{CD = CC \times BD \times T \times 10^4} \qquad \mathbf{\text{equation 1}}$$

Where **CC** = carbon content ($\text{gram C } 100\text{g}^{-1}$ soil), **BD** = bulk density (t m^{-3}), **T** = soil thickness (m), and **10⁴** = m^2 conversion factor for area (hectares)

Carbon Density was calculated using equation 1.

To calculate the organic carbon density for the Rietvlei wetland to a soil depth of 30 cm the carbon density was first calculated for the two intervals from 0 cm depth to 15 cm and from 15 cm to a depth of 30 cm. To get the value of carbon density in total from the soil surface (0 cm) to 30 cm the two intervals of 15 cm each were added together. The same process was followed for the remaining three wetlands at intervals of 10 cm to a total depth of 20 cm.

4.2.5 Permanganate oxidisable carbon

Following the method of Blair et al. (1995) POXC was estimated by oxidising soil with Mn^{+7} in the form of a KMnO_4 solution. Surface soil samples from one transect of each wetland were analysed ($n=5$). The samples were prepared by pulverising the soil in a ball mill at 210 RPM for 35 minutes, resulting in a very fine powder.

Soil samples containing 15 mg of C were weighed into 35 ml centrifuge tubes and 25 ml of 333 mM KMnO_4 were added to each tube. The mass of each sample required to contain 15 mg of C was calculated from Walkley-Black C concentration data (calculations in Appendix, Table A1). Blank samples, containing no soil were also included. The centrifuge tubes were tightly sealed and mechanically shaken for one hour. The tubes were then centrifuged for 5 minutes at 2000 rpm and the supernatants diluted 1:250 with deionised water. The absorbance of the diluted samples and standards were read using a spectrophotometer at 565 nm. The

concentration range for the standard series was chosen as 250, 300, 320 and 333 mM KMnO_4 (regression chart in Appendix, Figure A1).

The change in the concentration of KMnO_4 is used to estimate the amount of carbon oxidized, assuming 1 mM MnO_4 is consumed (MnVII) in the oxidation of 0.75 mM, or 9 mg, of carbon. The results are expressed as mg C g^{-1} soil. The two fractions are POXC = the C oxidized by 333 mM KMnO_4 and non-POXC = the C not oxidized by 333 mM KMnO_4 .

4.2.6 Statistical Analysis and calculations

To assess the repeatability of the modified Walkley-Black analysis, three replicates were carried out for every fifth sample to estimate the variability of the analytical method. There were too many samples for it to be practical to perform replicates for each sample ($n = 225$). The coefficient of variation (CV) was determined for each sample for which three replicates were performed. To obtain an estimate of the variation of a larger population resampling statistic was used. A set of CV data was generated, and bootstrap analysis was performed to approximate the mean CV (10 000 random replications with replacement) in R studio to achieve a high confidence approximation of the coefficient of variation of the Walkley-Black method.

4.3 Results and Discussion

4.3.1 Dry Bulk Density

As previously mentioned, direct measurements of bulk density were of very low confidence and an alternative indirect approach was adopted. Work by Ruehlmann and Körschens (2009) has shown that SOC concentration is significantly correlated with soil bulk density.

A model was produced for the calculation of bulk density from SOC data for wetlands by performing a meta-analysis from available published literature data of various wetland types ranging from low carbon mineral soils to high carbon organic soils,

assuming SOC concentration is the main bulk density affecting factor. From appendix A (Table A2) the correlation between SOC concentration ($\text{g } 100 \text{ g}^{-1}$ soil) and bulk density (kg m^{-3}) was determined. The model of best fit was produced as follows (Figure 4.1):

$$\rho_b = 1.1861 - 0.263 \ln(\text{SOC}) \quad (R^2 = 0.677) \quad \text{equation 2}$$

Where ρ_b = Bulk Density (kg m^{-3}) and SOC = Soil Organic Carbon ($\text{g C } 100^{-1} \text{ g soil}$)

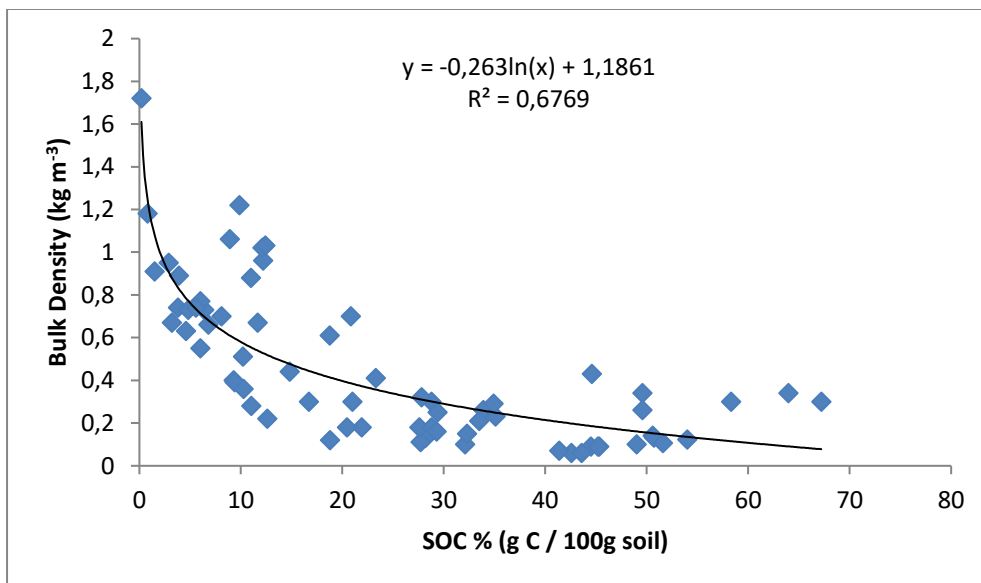


Figure 4.1: Correlation between bulk density and SOC concentration. Data from Appendix (table A2).

The validity of this model was tested by comparing measured bulk density values from literature to estimated bulk density values from equation 2. To avoid bias the dataset used to test the accuracy of the models was not included in the meta-analysis (Data used from Bernal 2012, table A3 in appendix). The mean accuracy (measured value/estimated value x 100) of this model (figure 4.2) is $88 \pm 26\%$ ($R^2 = 0.675$), ($n=128$). Equation 2 was used for bulk density calculations.

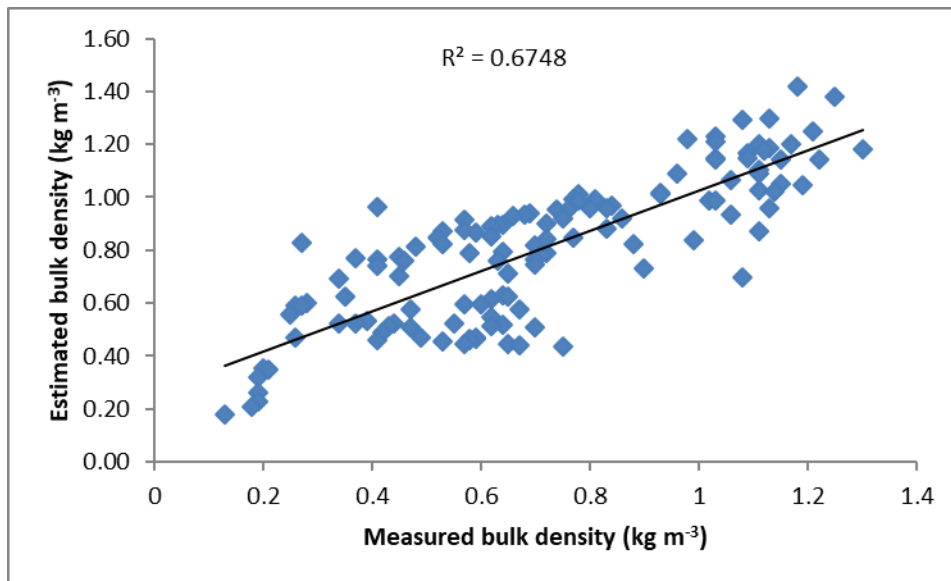


Figure 4.2: Correlation between measured bulk density and bulk density calculated from equation 2 (n=128). Data from Bernal (2012), (appendix, table A3)

As can be expected bulk density increased in relation to soil depth, based on estimates and physical examination in the field. The general negative correlation between SOC concentration and bulk density was also observed, because organic matter has a lower density than mineral soil particles. Bulk density values are indicated in Table 4.1, chapter 4.3.2.1.

4.3.2 Soil organic carbon density

The coefficient of variation for SOC concentration of the Walkley-Black method determined from three replicates for every fifth sample was 8.23 %. First the SOC densities are estimated for the entire wetland grid area, after which wetland grids are delineated into their constituent hydrological zones to indicate the differences in SOC density of these zones.

4.3.2.1 Total wetland area

To a depth of 30 cm Rietvlei has an estimated carbon density of $74.5 \pm 14.8 \text{ t ha}^{-1}$ (median = 48.7 t ha^{-1}) and $49.6 \pm 9.87 \text{ t ha}^{-1}$ to a depth of 20 cm (normalised approximation achieved to a depth of 20 cm by multiplying the SOC density value to a depth of 30 cm by 2/3). This large difference between the mean and the median shows appreciable skewing of the data distribution, which can most likely be attributed to the variability in SOC density of the different hydrological zones. Zaalklap had the lowest carbon density at $41.3 \pm 15.5 \text{ t ha}^{-1}$ (median = 42.9 t ha^{-1}) (to 20 cm). The Plant wetland had the second highest carbon density to a depth of 20 cm at $54.5 \pm 14.8 \text{ t ha}^{-1}$ (median = 56.5 t ha^{-1}) with the Organic wetland having the highest density, more than twice that of any of the other wetlands to a depth of 20 cm at $127 \pm 45.5 \text{ t ha}^{-1}$ (median = 141 t ha^{-1}). Table 4.1 summarises SOC concentrations for depth intervals, estimated bulk densities and carbon densities of the four wetlands.

The Organic wetland consists of a hillslope seep connected to a channelled valley bottom wetland that experiences permanent saturation throughout the year. Carbon concentration increased in relation to the gradient of the hillslope seep to a maximum at the lowest point within the landscape. A G horizon was encountered in the hillslope seep up to the point where it merges with the valley bottom wetland. The permanently saturated zone of the valley bottom wetland consisted of an organic (O) horizon extending to an unknown depth greater than the maximum of 1.2 m that could be reached with the soil auger used. The combination of the high carbon concentrations and unknown depth of the O horizon in the organic wetland indicate that the carbon density would be much greater at full profile depth than the value estimated to a depth of 20 cm ($127 \pm 45.5 \text{ t ha}^{-1}$). For example;

Assuming an average SOC content of 25% ($\text{g C } 100\text{g}^{-1} \text{ soil}$) and a bulk density of 0.34 kg m^{-3} , the permanently saturated zone of the organic wetland may store $>1000 \text{ t ha}^{-1}$ carbon to a depth of 1.2 m.

Table 4.1: Mean soil organic carbon concentration (SOC), dry bulk density, and SOC density. Standard deviation indicated in brackets. Bulk density values are calculated from equation 2. CV is coefficient of variation.

Soil depth intervals (cm)	SOC (%)	Bulk density	SOC density	SOC density CV
	(g C 100g ⁻¹ soil)	(t m ⁻³)	(t ha ⁻¹)	(%)
Rietvlei (n=23)				
0-15	4.01 (1.16)	0.83 (0.07)	48.5 (9.31)	19.2
15-30	1.65 (0.58)	1.07 (0.09)	25.7 (6.74)	26.2
0-30			74.5 (14.8)	19.9
0-20 *			49.6 (9.87)	19.9
Plant (n=20)				
0-10	4.17 (1.45)	0.83 (0.11)	33.1 (8.21)	24.8
10-20	2.27 (1.05)	1.00 (0.14)	21.4 (7.36)	34.4
0-20			54.5 (14.8)	27.2
Zaalklap (n=14)				
0-10	2.90 (1.56)	0.96 (0.19)	25.0 (10.5)	42.0
10-20	1.56 (0.68)	1.11 (0.17)	16.3 (5.82)	35.7
0-20			41.3 (15.5)	37.5
Organic (n=20)				
0-10	19.2 (11.7)	0.47 (0.20)	68.8 (19.2)	27.9
10-20	15.9 (12.6)	0.58 (0.29)	58.4 (27.1)	46.4
0-20			127 (45.5)	35.8

* = Normalised approximation

4.3.2.2 Separation of Hydrological zones

As mentioned in chapter two, wetlands in general are made up of different hydrological zones (although all zones are not necessarily present in all wetlands) based on the degree and duration of saturation namely; permanent, seasonal, and temporary, followed by the terrestrial zone that marks the boundary of the wetland. These zones are however not necessarily defined as abrupt transitions but more as a continuum from one zone to the next. To simplify matters, in this section wetlands are only delineated into the permanent and seasonal zones. Temporary zones can be difficult to distinguish from the terrestrial soil boundary and experience more sporadic periods of saturation than the seasonal and permanent zones. Furthermore, most of the sample points fall within the permanent and seasonal zones.

In this section only the Rietvlei and Organic wetlands are delineated into permanent and seasonal zones. The Zaalklap wetland is excluded due to insufficient sample size, as it was impossible to collect all samples during the sampling process, due to physical barriers. The Plant wetland is excluded since most of the sample grid falls within the permanent zone and the few points that fall within the seasonal zone are of insufficient sample size to be representative of this zone.

The purpose of this section is to look for any potential differences in SOC density between different hydrological zones as opposed to determining SOC density for the entire sample grid area as in Table 4.1, where no differentiation was made between the different hydrological zones.

Delineation was achieved by identifying different soil forms through soil classification performed in the field during sampling as well as historical satellite imagery to assist as indicators for hydrological zone boundaries (wetter zones tend to have denser vegetation than surrounding terrestrial zones and are visible in satellite images).

According to the South African soil classification system there is one soil form that is always associated with organic wetlands namely, the Champagne soil form, and three soil forms that are always associated with mineral soil wetlands namely, Katspruit (Orthic A over G horizon), Willowbrook (Melanic A over G horizon), and Rensburg (Vertic A over G horizon), (Rountree et al., 2008; Soil Classification Working Group, 2018).

For the Rietvlei wetland the Katspruit soil form (Orthic A over gleyed G horizon) is assumed to be the permanent hydrological zone and the seasonal is Westleigh soil form (Orthic A over soft plinthic B horizon, approximately 5% mottles), (Figure 4.3). The Westleigh soil form is associated with seasonal wetlands (Rountree et al., 2008). For the organic wetland the permanent zone is defined as a peat horizon/organic O horizon (peat depth > 1.2 m) and the seasonal zone is a Katspruit soil form (Orthic A over gleyed G horizon), (Figure 4.4).



Figure 4.3: Delineated hydrological zones of Rietvlei wetland. The permanently wet zone is Katspruit soil from and the seasonally wet zone is Westleigh soil form.



Figure 4.4: Delineated hydrological zones of the Organic wetland. Permanently wet zone is Champagne soil form and seasonally wet zone is Katspruit soil form.

Table 4.2 shows the SOC concentration, bulk density (calculated from Equation 2), and SOC density for permanent and seasonal zones of the Rietvlei and Organic wetlands. For the Rietvlei wetland the permanently wet zone has a higher SOC density than the seasonally wet zone, 81.7 ± 14.4 (median = 77.1 t ha^{-1}) and $69.8 \pm 13.1 \text{ t ha}^{-1}$ (median = 70.0 t ha^{-1}) respectively to a depth of 30 cm, and 54.5 ± 9.60 and $46.5 \pm 8.73 \text{ t ha}^{-1}$ respectively to a depth of 20 cm (SOC density to a depth of 20 cm is approximated by multiplying the SOC density value to a depth of 30 cm by 2/3). The Organic wetland's permanently wet zone similarly has a higher SOC density than the seasonally wet zone, 169 ± 11.3 (median = 174 t ha^{-1}) compared to $108 \pm 27.3 \text{ t ha}^{-1}$ (median = 109 t ha^{-1}) respectively to a soil depth of 20 cm. For both wetlands there are significant differences ($p < 0.05$, t-test) in means between the permanently and seasonally wet zones.

Table 4.2: SOC concentration (%), bulk density, SOC density, and SOC density coefficient of variation (CV) for the permanent and seasonal hydrological zones of the Rietvlei and Organic wetlands. Standard deviation indicated in brackets.

Soil depth intervals (cm)	SOC % (g C 100 g ⁻¹ soil)	Bulk density (t m ⁻³)	SOC density (t ha ⁻¹)	SOC density CV (%)
Rietvlei				
Permanent zone (n = 9)				
0-15	4.61 (1.06)	0.79 (0.06)	53.8 (17.9)	33.3
15-30	1.84 (0.62)	1.04 (0.32)	27.9 (7.02)	25.2
0-30			81.7 (14.4)	17.6
0-20 *			54.5 (9.60)	17.6
Seasonal zone (n = 14)				
0-15	3.62 (1.05)	0.86 (0.07)	45.3 (8.55)	18.9
15-30	1.53 (0.52)	1.09 (0.08)	24.3 (6.14)	25.3
0-30			69.8 (13.1)	18.8
0-20 *			46.5 (8.73)	18.8
Organic				
Permanent zone (n = 9)				
0-10	27.0 (11.6)	0.30 (0.08)	84.6 (5.32)	6.29
10-20	28.4 (7.72)	0.29 (0.08)	83.9 (5.99)	7.14
0-20			169 (11.3)	6.69
Seasonal zone (n = 8)				
0-10	12.7 (4.16)	0.47 (0.09)	64.0 (10.8)	16.9
10-20	6.32 (4.58)	0.65 (0.18)	44.0 (17.5)	39.8
0-20			108 (27.3)	25.3

* = Normalised approximation

Comparing the coefficients of variation for SOC density to a depth of 30 cm from Table 4.1 to Table 4.2 the values are similar for Rietvlei at 19.9 % in table 4.1 compared to 17.6 % and 18.8 % for Table 4.2 (permanently and seasonally wet zones respectively). The separation of the Rietvlei wetland into permanent and seasonal zones did not significantly change the SOC density coefficients of variation (Table 4.1 compared to Table 4.2), this would suggest that the soil profile for this wetland is relatively heterogenous and shows spatial variation in SOC density. Considering the topography of this wetland it is a channelled valley bottom wetland with a stream flowing through it in the centre, i.e. where the soil surface is at or above the water table. Extending from the stream perpendicularly there is a slope on either side meaning that the soil surface gradually rises further above the water table

moving through the permanent-seasonal-temporary-terrestrial hydrological zone continuum as opposed to there being well defined individual zones with distinct boundaries. Therefore, SOC density gradually decreases moving away from the permanent zone.

For the Organic wetland the SOC density coefficients of variation to a depth of 20 cm are 35.8 % in Table 4.1 compared to 6.69 % and 25.3 % in Table 4.2 (permanently and seasonally wet zones respectively). Considering the topography of this wetland it is a hillslope seep wetland that merges with a level channelled valley bottom peatland. The SOC coefficient of variation of the seasonal zone is larger compared to the permanent zone. This likely indicates that there is a gradual increase in SOC density from the crest of the hillslope seep down to where it merges with the permanent zone, where the water table is slightly above the peat surface. The relatively low SOC density coefficient of variation of the permanent zone means that this zone is more homogenous in terms of SOC density suggesting that waterlogging increases long term stability of SOC.

4.3.3 Comparing SOC densities to literature

According to the National Terrestrial Carbon Sink Assessment (2015) for South Africa the savannah and grassland biomes have an estimated mean SOC density of $54.2 \pm 30.8 \text{ t ha}^{-1}$ and $101 \pm 44.3 \text{ t ha}^{-1}$ respectively to a depth of one meter and account for a total land area of 358473 km^2 and 224377 km^2 respectively (total surface area of South Africa = 1218414 km^2). According to this assessment these two biomes contribute most to the total terrestrial SOC stock in South Africa. The lowest SOC estimate for this study is for the Zaalklap wetland at $41.3 \pm 15.5 \text{ t ha}^{-1}$ to a depth of 20 cm. If this value were to be scaled up to a depth of one meter the SOC of Zaalklap may potentially be $>70 \text{ t ha}^{-1}$ (depending on the SOC density of the G horizon), approximately 30% more than the mean SOC of the savannah biome. The total land area of wetlands in South Africa is not yet known and therefore the contribution of wetlands to total SOC stock in South Africa remains to be determined.

Table 4.3 summarises a review by Ausseil et al. (2015) of wetland SOC densities from different countries around the world. From this table it is apparent that there is variability in the soil depths to which carbon densities are measured in different countries. Many of the mineral wetland carbon densities are calculated to a depth of 30 cm probably in accordance with IPCC recommendations.

Table 4.3: Comparison of carbon density in freshwater wetlands around the world. Review adapted from Ausseil et al., (2015). These authors defined organic soils as a SOC concentration of above 18 % or a bulk density of less than 0.4 t m⁻³.

Region/Country	Carbon density (t C ha ⁻¹)		Depth (m)	Reference
	Organic	Mineral		
New Zealand	1348		Full peat	Ausseil et al., (2015)
New Zealand	102	121	0.3	Ausseil et al., (2015)
Australia (NSW)		45 – 240	0.3	Webb (2002)
USA and Mexico	1500	162	1	Bridgham et al., (2006)
Ohio (USA)		90 – 147	0.24	Bernal and Mitsch (2008)
Costa Rica		68 – 99	0.54 – 0.6	Bernal and Mitsch (2008)
Mexico		95 – 238	0.3 – 1	Adame et al., (2013)
Argentina		51 – 76	1	Ceballos et al., (2012)
China	828	171 – 274	1.5 – 2	Zhang et al., (2008)
Global-temperate climate		87 – 88	0.3	IPCC (2006); Batjes (2011)
Global	353	128 – 135	0.3	Batjes (2011)

4.3.3.1 Upscaling SOC density to a soil depth of 30 cm

Many of the wetland SOC densities in Table 4.3 were estimated to a depth of 30 cm. In this study the SOC density for the Rietvlei wetland was estimated to a depth of 30 cm, whereas SOC density was estimated to a depth of 20 cm for the remaining three wetlands. To compare SOC densities with those of Table 4.3, the SOC density estimations for the Zaalklap, Plant and Organic wetlands were scaled-up from a depth of 20 cm to a depth of 30 cm. To achieve this the SOC density values to a depth of 20 cm were multiplied with a factor of 3/2. Even though this method is not strictly speaking accurate due to the non-linear fashion in which SOC concentration decreases with soil depth it will still provide some estimation to compare to data in Table 4.3.

From Table 4.1 the average estimated SOC density of the Rietvlei wetland is 74.5 t ha⁻¹. The average SOC densities for the Plant, Zaalklap, and Organic wetlands scaled to a depth of 30 cm are 81.8, 62.0, and 191 t ha⁻¹. The mean SOC density to a soil depth of 30 cm for the three mineral soil wetlands (Rietvlei, Plant and Zaalklap) is estimated at 72.8 t ha⁻¹, which is close to the IPCC (2006) global mean estimate for temperate climate mineral wetlands at 87 – 88 t ha⁻¹ (table 4.3). The Organic wetland with an estimated mean SOC density of 191 t ha⁻¹ to a depth of 30 cm is more than that of the mean reported for organic wetlands in New Zealand at 102 t ha⁻¹ (Table 4.3) but less than that of the global mean of 353 t ha⁻¹ (Batjes 2011, Table 4.3) and 375 t ha⁻¹ (Buringh 1984, Table 4.3) for organic wetlands.

4.3.4 G horizons

Most (>90%) of the wetlands in South Africa are mineral wetlands (van Vuuren 2010), the majority of which are characterised by an A horizon or an E horizon overlying a G horizon (Soil Classification Working Group, 2018). The mean SOC concentration for the G horizon (40 cm) for Rietvlei is 0.71% ±0.45 which is relatively low compared to that of the overlying horizon. However, G horizons are often subjected to illuviation and accumulation of colloidal particles with a high clay content (Soil Classification Working Group, 2018) resulting in dense horizons with a high

bulk density. Considering that bulk density is a parameter required for the calculation of SOC density (Ausseil et al., 2015) the relatively low total SOC concentration may be offset by this horizon's high bulk density and thickness. In mineral wetlands G horizons may store substantial amounts of carbon. For example;

Assuming a G horizon with an average carbon concentration of 0.5%, and a bulk density of 1.37 g cm^{-3} it may store $>20 \text{ t ha}^{-1}$ organic carbon at a thickness of 30 cm and $>27 \text{ t ha}^{-1}$ at a thickness of 40 cm.

Furthermore, adsorption of organic matter to soil minerals and occlusion within soil aggregates have been universally demonstrated to shield SOC from decomposition. Sand, silt and clay have been shown to have affinities for different types of SOC depending on the size and mineralogy of the soil minerals. SOC turnover time and age consistently increases with soil depth due in part to the larger protective potential of SOC by minerals and the unfavourable conditions for microbial decomposition in subsoils. The content of SOC associated with soil minerals has been found to be highest for clay with an average of 3.68%, followed by silt and sand at 3.27% and 1.93% respectively. The stronger association between clay minerals and SOC compared to silt and sand may be attributed to clay's larger surface area, cation exchange capacities (especially 2:1 minerals such as smectite), and the presence of Fe/Al oxides that provide additional sites for adsorption (Han et al., 2015). Therefore, G horizons with high clay content may provide stable environments for mineral-SOC associations and long term storage of carbon.

4.3.5 Permanganate oxidisable carbon

Rietvlei and the Organic wetland had the highest average percentage of POXC (POXC/SOC x 100) at $42.4\% \pm 8.43$ and $44.3\% \pm 7.42$ respectively ($n = 5$ for each wetland). The Plant wetland had the lowest percentage of POXC ($29.1\% \pm 2.43$) followed by Zaalklap ($38.0\% \pm 3.28$). Figure 4.5 illustrates the relative contributions of POXC and non-POXC to total SOC as percentages.

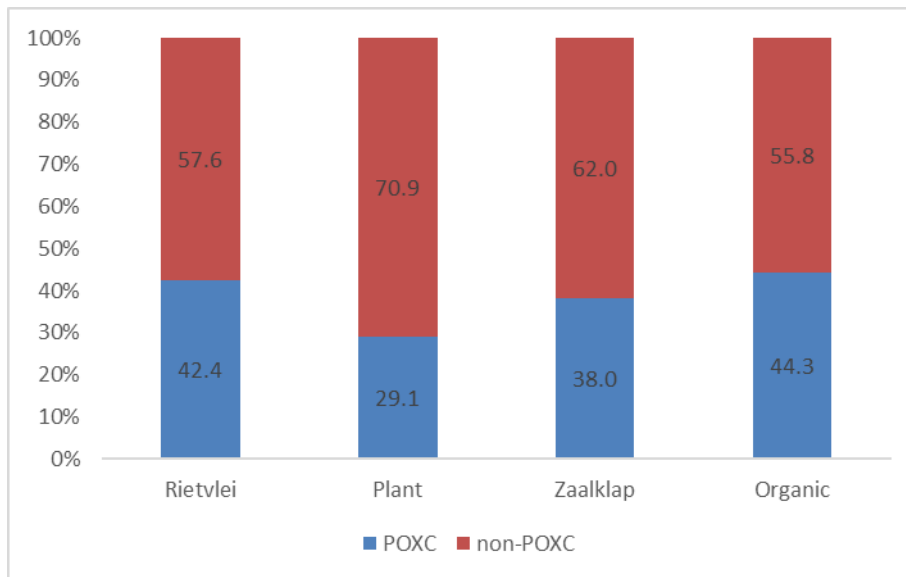


Figure 4.5: Percentages of POXC relative to non-POXC for each wetland

These results may be evidence that land use practices and disturbance are correlated with POXC, supporting literature from other researchers (Coleman et al., 1996; Harrison et al., 1993; Dalal et al., 2005). The Plant wetland is subjected to AMD and has the lowest POXC relative to the other wetlands. Zaalklap is a recently rehabilitated wetland that has historically been subjected to agricultural disturbance and the same AMD as the Plant wetland just further downstream; it has the second lowest POXC. The Rietvlei and Organic wetlands have higher POXC percentages and are not subjected to AMD.

To test for significant differences in POXC concentration data from the Plant and Zaalklap were grouped together, these two wetlands are known to be disturbed by surrounding mines and the Highveld Steel processing plant. Data from the Rietvlei and Organic wetlands were grouped together, these two wetlands were least disturbed. The difference between the means ($n = 10$ for both groups) of these two groups is significant ($p < 0.05$, t - test). From this result it seems like there is a negative correlation between wetland disturbance and POXC concentration.

The use of POXC as an indicator for changes in SOC caused by land use practices is a relatively new concept first introduced by Loginow et al. (1987) and from available literature seems to have only been applied in agricultural related studies. It

is therefore unclear how land use practices and disturbances may change POXC concentrations over time in wetland soils.

4.4 Summary

It was not physically possible to extract intact and undisturbed soil samples for bulk density measurements from the mineral wetlands. Soil cores for organic wetlands such as peatlands are easier to extract with the use of a peat auger, however soils in most mineral wetlands are too hard for this type of auger.

The dry bulk density of these wetland soils could not be directly measured with enough accuracy following commonly used methodology. Instead bulk density had to be estimated using a model produced from a meta-analysis of SOC and bulk density data. This reduces the confidence of SOC density estimations.

There were significant differences in SOC density in the different hydrological zones. Permanently wet hydrological zones on average have a higher SOC than Seasonally wet zones.

Three of the wetlands in this study are mineral soil wetlands that all have an estimated mean carbon density of $>40 \text{ t ha}^{-1}$ to a depth of 20 cm. The SOC density (t ha^{-1}) of these wetlands may potentially be significantly greater than that of the savannah and grassland biomes, the two biomes with the highest contribution to total terrestrial SOC stock for South Africa.

Most mineral wetlands in South Africa have sub soils with G horizons. These horizons have relatively low concentrations of SOC compared to their overlying topsoil horizons but may be stable environments for the long-term storage of a significant concentration of SOC carbon due to their high clay content, density, depth, and thickness.

The POXC method is a relatively easy and inexpensive method that is increasingly used as an indicator for changes in SOC caused by land use practices within the field of agricultural research. From the results in this chapter it appears this method may also be useful in for a similar purpose in wetland soil research. Results

indicated that disturbance may have altered the nature of SOC indicated by lower concentrations of POXC in affected wetlands.

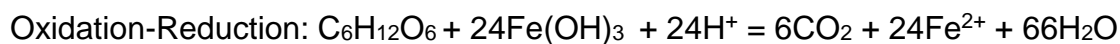
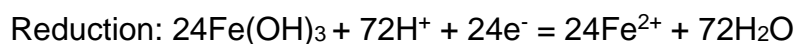
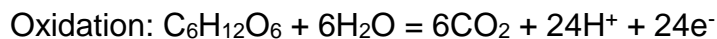
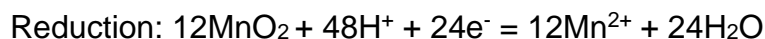
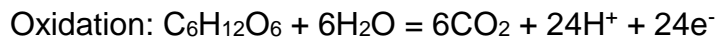
5 Chapter five: Correlations between SOC and POXC and total soil concentrations of N, P, K, Ca, Mg, Mn, Fe, S, and Al.

5.1 Introduction

It is useful to understand what factors significantly impact on the rate at which atmospheric carbon is sequestered as well as those that effect the ratio between carbon input and output. The aim of this chapter is as an initial, non-intensive probe into which common soil elements, whether naturally occurring or introduced by humans, may be correlated with SOC storage. Total soil concentrations of the elements N, P, K, Ca, Mg, S, Mn, Fe, Cr, and Al were measured to investigate if there are relative enrichments or depletions of these elements in these wetlands and if deductions can be made from these results as possible drivers with respect to SOC accumulation and the nature of SOM as reflected by POXC. These results may be useful in guiding decision making for more intensive future research into factors that need to be considered for efficient carbon sequestration in wetlands in the context of climate change mitigation strategies.

Nitrogen (N) and Phosphorus (P) are two of the most important macro nutrients that limit the productivity of terrestrial, wetland, and aquatic ecosystems. The elements K, Ca, Mg, Mn, Fe, Cr are essential nutrients required by plants (Reddy & DeLaune, 2008). Soil N and P concentrations have also been shown to have an important impact on the soil microbial community responsible for the mineralisation of SOC in wetlands (Sundareshwar et al., 2003). In saturated anoxic soils Mn^{4+} , Fe^{3+} , and SO_4^{2-} serve as alternative terminal electron acceptors to appropriately adapted microorganisms for the oxidation of SOC (Essington, 2003). Acid mine drainage (AMD) is a serious environmental problem in South Africa, it contains high concentrations of Mn^{4+} , Fe^{3+} , and SO_4^{2-} that are eventually transferred into many ecosystems including wetlands (Akcil & Koldas, 2006). The enrichment of soils with oxidised Mn, Fe and S species may ultimately accelerate the rate of SOM mineralisation in wetlands. Two of the four wetlands included in this study are subjected to AMD.

Reduction of Fe(III) and Mn(IV) can occur enzymatically through bacterial metabolism or non-enzymatically in saturated soil environments. In reduced soil horizons where O₂ is limited Mn(IV) and Fe(III) oxides dominate as electron acceptors for several facultative and obligate anaerobic bacteria during anaerobic oxidation of organic matter which contributes more to the oxidation of organic matter than non-enzymatic oxidation. The oxidation of glucose by Mn(IV) and Fe(III) is as follows (Reddy & DeLaune, 2008);



On an equal molar basis, the reduction of Mn(IV) and Fe(III) amounts to 70% and 20% of the energy yield in the form of ATP generated compared to Aerobic (O₂) metabolism respectively. The reduction of manganese is likely the preferred electron acceptor for anaerobic bacteria (Reddy & DeLaune, 2008). Furthermore, the POXC method uses Mn⁷⁺ as an oxidiser and should therefore indicate that a fraction of SOC is indeed sensitive to oxidation by Mn⁴⁺ in the natural environment. In these wetlands soils where O₂ supply is limited the enrichment of the soil with Mn(IV) and Fe(III) may lead to an accelerated oxidation of SOC by anaerobic bacteria.

5.2 Methodology

5.2.1 ICP analysis

The same soil samples were used as for POXC analysis, one surface soil transect per wetland. Pulverised and homogenised soil samples were prepared following the EPA 3051a method; Microwave assisted acid digestion of sediments, sludges, soils,

and oils. Sample masses for digestion were 0.3 g to which 20 ml 65% HNO₃ was added to each sample. Samples were microwave digested at peak temperature (175 ± 5° C) for 5 minutes. During this 5-minute period there is a stepping up period during which the samples are heated to the required temperature for digestion followed by cooling period. Deionised water was added to 30 ml after which the solutions were filtered.

The extracts were then analysed with a Spectro Genesis ICP-AES to measure total soil concentrations (mg kg⁻¹ soil) of P, K, S, Fe, Cr, Mn, Mg, Ca, and Al.

5.2.2 Nitrogen analysis

The same soil samples used as for POXC analysis and P, K, Mg, Mn, Fe, Cr, S, and Al analysis, a single transect for each wetland. Total nitrogen analysis was performed following the Leco dumas method, using a LECO CN 628 Dumas analyser.

5.2.3 Statistical analysis

Data from the points (n=20) of transects from all four sites were pooled together. Stepwise regression analysis was performed to look for significant correlations between soil element concentrations and SOC as well as POXC. Correlation coefficients (R²), regression line equations as well as significant p-values are given. The Organic wetland has a much higher carbon density than the other three mineral wetlands. Therefore, regression analysis was performed on all four wetlands as well as for the three mineral wetlands (Rietvlei, Mine and Zaalklap) excluding the Organic wetland.

5.3 Results and Discussion

Table 5.1 summarises the mean total soil concentrations of SOC, POXC, N, P, K, Ca, S, Mg, Mn, Fe, Cr, and Al, as well as SOC:POXC, C:N, C:S, N:P ratios, and pH for surface soil transects from each wetland.

The mineral wetlands (Rietvlei, Plant and Zaalklap) all have similar total N soil concentrations. The total soil N concentration for the organic wetlands soil is approximately an order of magnitude greater than the other wetlands, as is the SOC concentration. There is a correlation between N and SOC concentration because most of the N in soil is in organic forms with less than 5% present in inorganic forms (Reddy & DeLaune, 2008).

The Rietvlei, Plant and Organic wetlands have very similar C:N ratios at 17.0 ± 2.85 , 17.8 ± 1.73 , and 16.0 ± 3.48 mg kg⁻¹ soil respectively. The Zaalklap wetland in comparison has a lower C:N ratio (11.5 ± 5.83 mg kg⁻¹ soil) likely due to disturbance.

The normal range for total P concentration in wetland soils is between 30 – 500 mg kg⁻¹ (Reddy & DeLaune, 2008). The total P concentration for the Rietvlei and Zaalklap wetlands fall within this range (253 ± 69.4 and 158 ± 92.3 mg kg⁻¹ soil respectively). The Plant and Organic wetlands have total soil P concentrations above this range (755 ± 429 and 764 ± 250 mg kg⁻¹ soil respectively).

Of all four wetlands the Plant wetland has the highest relative concentrations of Mn and Fe in its soil. The Zaalklap wetland has the second highest concentration of Mn relative to the other wetlands. Excess Mn may be liberated by the steel plant and transported in runoff from the plant to these two wetlands or as translocation of colloidal sediment from higher lying areas. The normal range for soil Mn concentration varies between 20 – 6000 mg kg⁻¹ soil (Reddy & DeLaune, 2008).

Table 5.1: Summary of means of indicated variables for selected transects for each wetland site. Standard deviation indicated in brackets.

	Rietvlei	Plant	Zaalklap n=5	Organic
SOC (%)	5.22 (1.56)	5.82 (1.26)	1.65 (1.01)	27.6 (12.0)
POXC (%)	2.33 (1.04)	1.68 (0.35)	0.62 (0.39)	11.4 (4.72)
N g 100g ⁻¹ soil	0.33 (0.14)	0.33 (0.07)	0.13 (0.05)	1.59 (0.60)
P	253 (69.4)	755 (429)	158 (92.3)	764 (250)
K	503 (180)	911 (347)	443 (110)	439 (155)
Ca	3962 (2245)	57848 (45109)	2212 (157)	2269 (1111)
S	440 (193)	1150 (708)	722 (241)	2621 (903)
Mg mg kg ⁻¹ soil	2023 (1386)	3826 (1274)	1089 (207)	596 (298)
Mn	588 (333)	2616 (1836)	940 (1110)	324 (278)
Fe	26547 (5127)	35728 (7488)	21891 (15092)	15643 (7320)
Cr	136 (22.6)	213 (55.1)	65.7 (25.5)	78.7 (16.1)
Al	12633 (4056)	12582 (3142)	9127 (2667)	7548 (1534)
SOC:POXC	0.42 (0.08)	0.29 (0.02)	1:0.38 (0.03)	1:0.44 (0.07)
C:N	17.0 (2.85)	17.8 (1.73)	11.5 (5.83)	16.0 (3.48)
C:S	127 (18.7)	70.6 (34.8)	22.8 (13.3)	96.3 (26.3)
N:P	12.7 (3.76)	5.28 (1.90)	11.2 (5.36)	20.0 (3.21)
pH (H₂O)	6.0 - 7.0	6.07 (0.51)	6.20 (0.47)	6.74 (0.22)

The Plant and Zaalklap wetlands have the lowest C:S ratios. The C:S ratio is a useful indicator as most soil S is organic and therefore contained within SOM and increases in proportion to SOC concentration in undisturbed environments (Reddy & DeLaune, 2008) These results are possibly an indicator of the enrichment of S by AMD in these two wetlands.

The soil of the Plant wetland has a considerably higher concentration of Ca relative to the other wetlands. The enrichment of Ca may be a result of dissolved Ca²⁺ from the use of limestone (Calcite, CaCO₃) during the processing of steel yielding water that is saturated with gypsum. The saline water in turn reports to waterways and eventually into wetlands. White precipitation of gypsum was clearly visible on the soil surface in the Zaalklap wetland. Water seeping through steel slag is often alkaline and Ca bearing. It is therefore possible that alkaline runoff from the steel slag in the vicinity reacts with and neutralises AMD that may be present in water from surrounding sources.

The Organic wetland has the lowest concentration of K, Mg, Mn, Fe, and Al within its topsoil compared to the other three mineral wetlands. There are at least two possible reasons for this; 1) The mean SOC concentration for this transect of the wetland (n=5) is 27.6 % (g C 100 g⁻¹ soil), more than three times greater than all the mineral wetlands. If the SOM consists of 58% SOC then the concentration of SOM is approximately 47.6 % which means that the mineral fraction makes up only 52.4 % of the soil. The high concentration of SOM in this soil in effect dilutes the concentrations of the abovementioned metals. The densities of these metals are higher in soil minerals compared to plant biomass (Reddy & DeLaune 2008), 2) The soil of this wetland might be derived from parent material such as quartzite that contained lower concentrations of these metals.

The lower concentrations of Mn and Fe in the topsoil of the Organic wetland compared to the mineral wetlands might also be related to the permanently saturated conditions within this wetland, reducing insoluble Fe and Mn oxides to Fe²⁺(aq) and Mn²⁺(aq) cations respectively which are mobile and free to leach from the topsoil horizon into the deeper subsoil horizons (Reddy & DeLaune, 2008).

5.3.1 Regression analysis for SOC and POXC

Table 5.2 shows the correlations between SOC and the elements from table 5.1 for all four wetlands combined as well as for the three mineral wetlands excluding the Organic wetland.

Regression analysis was performed first including data from the Organic wetland to determine correlations between SOC and the element in table 5.2. Significant correlations ($\alpha < 0.05$) were found for N, P, S, N:P ratio, and C:S ratio. Excluding the Organic wetland data significant correlations ($\alpha < 0.05$) were found for N, P, K, Ca, S, Mg, Al, Cr, and C:N ratio.

Table 5.2: Regression analysis showing correlations between selected elements and SOC. All element concentrations are in mg kg⁻¹ soil.

Soil element	R ²	Regression line equation	R ²	Regression line equation
	Including organic wetland		Excluding organic wetland	
N	0.99	y = 18.0x + 6737	0.89	y = 15.9x + 207
C:N	0.10	y = 0.85x - 3.19	0.28	y = 0.25x + 0.39
P	0.38	y = 194x + 6971	0.43	y = 40.2x + 26668
N:P	0.45	y = 1.23x - 5.13	0.07	y = -0.12x + 5.39
S	0.84	y = 106x - 29899	0.22	y = 19.6x + 27170
C:S	0.15	y = 0.10x + 2.02	0.14	y = 0.02x + 2.96
Mg	0.07	y = -20.2x + 138605	0.50	y = 10.1x + 18951
K	<0.01	y = -9.61x + 106159	0.49	y = 50.1x + 11261
Al	0.06	y = -8.03x + 184731	0.39	y = 3.76x - 735
Ca	0.01	y = -0.44x + 107859	0.30	y = 0.33x - 35144
Mn	0.02	y = -11.5x + 111984	0.32	y = 8.57x + 31966
Fe	0.08	y = -2.84x + 171482	0.16	y = 0.77x + 20612
Cr	0.03	y = -314x + 139335	0.51	y = 227x + 10886

Table 5.3 shows the correlations between POXC and the elements from table 5.1 for all four wetlands combined as well as for the three mineral wetlands excluding the organic wetland.

Table 5.3: Regression analysis showing correlations between selected elements and POXC. All element concentrations are in mg kg⁻¹ soil.

Soil element	R ²	Regression line equation	R ²	Regression line equation
	Including organic wetland		Excluding organic wetland	
N	0.98	y = 7.43x - 4281	0.86	y = 6.78x - 2424
C:N	0.08	y = 0.31x - 0.79	0.11	y = 0.07x + 0.49
P	0.32	y = 74.1x + 4306	0.11	y = 8.83x + 12039
N:P	0.51	y = 0.55x - 2.71	0.01	y = 0.02x + 1.36
S	0.79	y = 42.8x - 12662	0.03	y = 3.30x + 12926
C:S	0.16	y = 0.04x + 0.61	0.22	y = 0.01x + 0.87
Al	0.06	y = -3.18x + 73336	0.36	y = 1.58x - 2578
Mg	0.09	y = -9.39x + 57743	0.24	y = 3.05x + 8409
K	<0.01	y = -9.67x + 45615	0.16	y = 12.6x + 7685
Ca	0.03	y = -0.27x + 44487	0.04	y = 0.05x + 14366
Mn	0.03	y = -6.24x + 46206	0.07	y = 1.76x + 13353
Fe	0.09	y = -1.22x + 70469	0.02	y = 0.12x + 12004
Cr	0.05	y = -168x + 60795	0.13	y = 49.0 + 8703

Regression analysis was performed first including data from the Organic wetland to determine correlations between POXC and the element in table 5.3. Significant correlations ($\alpha < 0.05$) were found for N, P, S, and N:P ratio. Excluding the Organic wetland data significant correlations ($\alpha < 0.05$) were found for N and Al.

The SOC concentration in soil is controlled by the balance between two major opposing processes, the rate at which SOC is produced (input) by plants versus the rate at which SOC is decomposed (output). The input of SOC is limited primarily by the rate of primary productivity and therefore the rate at which organic matter is produced. The output of SOC is limited primarily by the rate of decomposition and mineralisation of SOC that returns C to the atmosphere as CO₂ and CH₄ gas (Essington, 2003). The single most significant factor that limits the rate of decomposition in wetland soils is the deficit of available oxygen caused by saturation (Reddy & DeLaune, 2008). The Organic wetland experiences a greater degree of saturation than the mineral wetlands and has the highest concentration of SOC relative to the mineral wetlands.

The concentration of the elements N, P, and S were significantly and positively correlated with SOC including and excluding the data from the Organic wetland, meaning that higher concentrations of these elements within the soil coincided with higher concentrations of SOC. This shows that the balance between primary production and decomposition is shifted to a greater extent to primary production and the input of SOC relative to its decomposition.

Nitrogen after C is the largest constituent of plant biomass. It is an essential nutrient for all plants and algae. It is often the the most important limiting element for primary productivity in wetlands and other ecosystems. More than 95 % of soil N is in organic form and is therefore complexed with organic matter (Reddy & DeLaune, 2008). The results from tables 5.2 and 5.3 show that N by far had the highest correlation between SOC and POXC respectively and indicate the importance of N for SOC accumulation.

There was a positive correlation between SOC (excluding Organic wetland data) and the C:N ratio. Soil bacteria require C and N to grow which they acquire from plant

detritus. The C:N ratio (mass) of microbes is typically around 10. Microbes take up nitrogen by two processes; first, organic nitrogen is converted to inorganic ammonium (NH_4^+) during the process of ammonification. Secondly, NH_4^+ is immobilised/assimilated into the microbial biomass. Organic substrates with a higher C:N ratio (more C relative to N) favour the immobilisation/assimilation of N (storage within microbial cells) whereas a lower C:N ratio favours ammonification (release) of N from organic substrates and soil. Ammonification occurs during the decomposition of organic substrates which also results in the loss of SOC as CO_2 whereas N is conserved within the microbial biomass. As the the two opposing processes of ammonification and immobilisation proceed a critical C:N ratio of approximately 10 (the same as for microbial cell C:N ratio) in plant detritus is reached where most of the labile SOC has been decomposed resulting in more stable recalcitrant SOC (Reddy & DeLaune, 2008). All of the C:N ratios in table 5.1 are above 10, this might suggest that the higher C:N (higher concentration of C relative to N) ratios mean that there is a larger concentration of labile SOC that contributes to the total SOC.

The primary source of P in wetlands is from upland areas transported to wetlands where it is accumulated in both organic and inorganic forms. Under natural conditions the most common forms of P are $\text{H}_2\text{PO}_4^{2-}$ and HPO_4^- . From tables 5.2 and 5.3 positive correlations were found between P and SOC as well as POXC meaning that in general higher P concentrations resulted in higher SOC and POXC concentrations. One possible reason for these results is that inorganic P is largely unavailable for plant uptake due to strong adsorption to amorphous and crystalline forms of Fe, Al, Ca, Mg and other elements thus limiting primary productivity. A second perhaps more likely reason is that higher concentrations of soil organic P is stored within SOM and does not limit primary productivity. The P requirement for organisms is typically much lower than for N (typical molar requirement of N:P ratio is 16:1) and therefore wetland ecosystems are usually not limited by P. Wetlands have a finite capacity to store P in either organic or inorganic forms (Reddy & DeLaune, 2008). Therefore, the the total P concentration in the wetland soils might be higher due to a larger store of organic P in SOM compared to inorganic P. The method used for analysis however cannot distinguish between inorganic and organic P.

There was a positive and significant correlation between S and SOC (including and excluding Organic wetland data) as well as for POXC (excluding Organic wetland data). This positive correlation shows that the balance between primary productivity and decomposition/mineralisation of SOM might be shifted in favour of primary productivity. Sulphur is an essential element required by all known organisms. Plants and microorganisms assimilate S in inorganic forms mainly as SO_4^{2-} . Therefore it can be assumed that the oxidation of SOC by SO_4^{2-} reducing bacteria did not have any significant effect on the decomposition of SOC relative to the accumulation of SOC. Furthermore, there was no correlation between Mn and Fe and SOC, suggesting that the oxidation of SOC with Mn^{4+} and Fe^{3+} as alternative electron acceptors during the anaerobic oxidation of SOC had no significant effect on the mineralisation of SOC relative to the accumulation of SOC. The use of Mn^{4+} and Fe^{3+} as alternative electron acceptors in anaerobic conditions is favoured over SO_4^{2-} (Essington, 2003), so if Mn and Fe had no effect on SOC than it is unlikely that SO_4^{2-} reduction will have any significant effect on the oxidation of SOC. These soil samples were collected at the soil surface. The anaerobic oxidation of organic matter by SO_4^{2-} reducing bacteria is more likely to occur deeper in the soil profile in highly reduced conditions where all available Mn^{4+} and Fe^{3+} have been reduced to Mn^{2+} and Fe^{2+} .

Regression analysis for the mineral wetlands (excluding the organic wetland data) also showed significant positive correlations between N, P, and S, as well as for K, Ca, Mg, Al, Cr, and C:N ratio compared to SOC. The soils of the mineral wetlands are saturated to a lesser degree than the Organic wetland and are therefore not as reduced as the Organic wetland. For these wetlands the balance between input of SOC and the decomposition thereof are likely shifted more in favour of decomposition compared to the Organic wetland. It can therefore be assumed that any factor that limits the rate of primary productivity will result in a lower input and accumulation of SOC compared to the Organic wetland. The elements K, Ca, Mg, and Cr are essential plant nutrients (Reddy & DeLaune, 2008). Higher concentrations of these elements coincided with higher SOC concentrations. This likely indicates that deficiencies of these elements limit primary productivity and therefore the input of SOC in the mineral wetlands.

The positive correlation between Al and SOC and POXC might be caused by the presence of Al oxides and clay minerals containing Al. Some forms of SOC are adsorbed to Al and Fe oxides and to clay minerals resulting in a shielding effect that reduces the availability of SOC to mineralisation (Reddy & DeLaune, 2008).

Mineral soil wetlands tend to have a less negative redox potential than organic wetlands and therefore the oxidation of SOC is more dependent on the reduction of O_2 and NO_3^- than on the reduction of Mn^{4+} , Fe^{3+} , and SO_4^{2-} (Reddy and DeLaune 2008). Therefore, the accelerated oxidation of SOC caused by excessive enrichment of Mn^{4+} , Fe^{3+} , and SO_4^{2-} is probably less likely to occur in mineral wetlands and probably more likely in permanently waterlogged, highly reduced organic wetlands. Or is more likely to occur deeper in the soil profile where conditions are more reduced.

5.4 Summary

With the Organic wetland data included significant correlations were found between SOC and N, P, S, N:P ratio, and C:S ratio. Excluding the Organic wetland data significant correlations were found between SOC and N, P, K, Ca, S, Mg, Al, Cr, and C:N ratio.

With the Organic wetland data included significant correlations were found between POXC and for N, P, S, and N:P ratio. Excluding the organic wetland data significant correlations were found between POXC and N and Al.

Overall N, P, and S were most consistently correlated with SOC and POXC.

Mn, Fe, and S from AMD did not seem to have any effect on the decomposition of SOC.

6 Chapter 6: Synthesis

6.1 Summary

The primary aim of this study was to estimate carbon density of four wetlands, one organic and three mineral wetlands. Mineral wetlands have a much lower carbon density than organic wetlands. There is a significant correlation between wetland hydrological zone and SOC density, permanent zones on average have a higher SOC density than seasonal zones. The carbon content of the G horizon for one of the mineral wetlands was also assessed. This horizon is very prevalent because most wetlands in South Africa are mineral wetlands with an average SOC concentration of less than 10% to a depth of 20 cm. These horizons may be stable long-term stores of significant quantities of SOC.

Secondly, POXC was measured for these wetlands. Many of South Africa's wetlands are under threat by human encroachment. From literature on agricultural soils, POXC has been shown to be a useful indicator in tracking changes in SOC caused by land use practices. Results from this study indicate that the two wetlands subjected to the most pollution had lower relative POXC concentrations compared to the wetlands not subjected to AMD. POXC may therefore be a useful indicator in monitoring the changes in SOC caused by disturbances.

From chapter five N had the highest correlation (positive) with SOC and POXC potentially indicating that N is the most significant element limiting primary productivity (input of SOC) within these wetlands. There were also positive correlations between SOC and P, K, Ca, Mg, and Cr indicating that these elements could potentially be limiting nutrients associated with primary productivity and the input of SOC. The positive correlation between S and SOC as well as POXC suggests that S has a greater effect on primary productivity than decomposition of SOC or at least has no significant effect on the decomposition of SOC within the topsoil of the soil profile where the soil is not reduced enough for SO_4^{2-} to be used as an alternative electron acceptor by microbes for the anaerobic decomposition of SOC. The positive correlation between SOC and Al is potentially associated with clay minerals and Al mineral complexes within the soil that have a shielding effect on

the decomposition of SOC. The elements Mn and Fe showed no significant correlation between SOC and POXC suggesting that these elements had no effect on either primary productivity nor decomposition of SOC and POXC.

6.2 Conclusions and recommendations

The three mineral wetlands in this study all have a SOC density of more than 40 t ha⁻¹ to a depth of 20 cm, and the organic wetland has an estimated carbon density of 127 t ha⁻¹ to a depth of 20 cm. The mean SOC density for the three mineral wetlands in this study are in the same range as those reported for the global average SOC density for temperate climate mineral wetlands.

There is a significant correlation between wetland hydrological zone and the SOC density. On average permanent hydrological zones have higher SOC densities than seasonal zones, suggesting that there is a strong correlation between the degree of saturation in wetland soil and SOC density. Long periods of saturation in wetlands cause reduced conditions resulting in anaerobic oxidation of SOC that occurs at a slower rate than aerobic oxidation. The measurements of C stocks in wetlands with different hydrological zones should take into consideration the differences in SOC density between these zones. From literature the separation of carbon densities of different wetland hydrological zones seems to be lacking. The reason for this might be that most estimations of carbon densities and stocks are done for organic wetlands where the soils are more homogenous in terms of saturation compared to mineral wetlands that experience greater fluctuations in water table and where different hydrological zones are more distinct. It might therefore be imperative for future wetland carbon density and stock research to shift focus more on the differences of SOC density between different hydrological zones, especially for mineral wetlands.

G horizons are potentially significant long-term and stable environments for the storage of SOC and should be included in SOC density and stock calculations. At a thickness of 30 cm G horizons may store >20 t SOC ha⁻¹.

The permanganate oxidizable carbon method is a relatively easy and inexpensive means of tracking changes in SOC that has been proven effective for agricultural soil management practices. Results from this study seem to support the use of this method for assessing organic carbon in wetland soils. Two of the wetlands in this study were directly impacted by AMD and possibly agriculture. These two wetlands had a significantly lower percentage of POXC than the two wetlands not effected. However, if AMD were the cause of the lower POXC concentrations in the plant and Zaalklap wetlands relative to the wetlands not affected by AMD then a correlation between POXC and Fe and Mn would be expected to be significant. This is however not the case, suggesting that the lower relative concentrations of POXC in the plant and Zaalklap wetlands are caused by other disturbance factors. For example, it has been confirmed that mining activities in this region have caused a lowering in the general water table. The refinement of this method for use in wetlands soils might be a valuable topic for future research.

The elements N, P, and S seemed to be most correlated with SOC and POXC. The correlation between N and SOC and between N and POXC was the highest. In these wetlands total soil N concentration was likely the most important factor that limits primary productivity and SOC density. The elements Mn, Fe, and S did not seem to influence SOC mineralisation.

Given the necessity of bulk density measurements in the calculations of SOC density it is important that further research be conducted in the methods for the accurate measurements of bulk density in the mineral wetlands of South Africa. Bulk density could not be directly measured in this study. Refined methods for accurate and direct measurement of these soil types, mineral wetland soils, would be a valuable topic for future research.

7 References

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Appendix A

Table A 1 Soil classification of each sample point for the Rietvlei Wetland

Sample point	Description with Munsell colour indicated	Soil form
1	<ul style="list-style-type: none"> • B horizon: 10YR 3/4 • No mottles 	Clovelly
2	<ul style="list-style-type: none"> • High clay • A horizon :5Y 2.5/1, 5% mottles • B horizon >10% mottles 	Westleigh
3	<ul style="list-style-type: none"> • Orthic A horizon, 5Y 2.5/1. 5 % mottles • G horizon: gley1 2.5/N 	Katspruit
4	<ul style="list-style-type: none"> • Orthic A: 10YR 3/4 • Soft plinthic B >10% mottles 	Westleigh
5	<ul style="list-style-type: none"> • Orthic A: 10YR 2/2, 1% mottles • Yellow brown apedal B, 10YR 3/4, 2-5% mottles 	Avalon

	• Soft plinthic horizon >10% mottles	
6	• Orthic A, 1% mottles • Yellow brown apedal B 10YR 3/4	Avalon
7	• Orthic A: 10YR 3/4 • Soft plinthic B >10% mottles	Westleigh
8	• Orthic A horizon, 5Y 2.5/1. 5 % mottles • G horizon: gley1 2.5/N	Katspruit
9	• Orthic A: 10YR 3/4 • Soft plinthic B >10% mottles	Westleigh
10	• Orthic A • Yellow brown apedal B, 10YR 4/4 • No mottles	Clovelly
11	• Orthic A, 1% mottles • Yellow brown apedal B 10YR 3/4	Avalon
12	• Orthic A, 1% mottles • Yellow brown apedal B 10YR 3/4	Avalon
13	• Orthic A horizon, 5Y 2.5/1. 5 % mottles • G horizon: gley1 2.5/N	Katspruit
14	• Orthic A: 10YR 3/4 • Soft plinthic B >10% mottles	Westleigh
15	• Orthic A, 1% mottles • Yellow brown apedal B 10YR 3/4	Avalon
16	• Orthic A, 3% mottles • Yellow brown apedal B, 10YR 3/4, 2-5% mottles • Soft plinthic >10% mottles	Avalon
17	• Orthic A, 5Y 2.5/1 • G horizon: gley 1 2.5/N	Katspruit
18	• Orthic A, no mottles • G horizon: gley1 2.5/N	Katspruit
19	• Orthic A • Soft plinthic B >10% mottles	Westleigh
20	• Orthic A, 1% mottles • Yellow brown apedal B 10YR 3/4	Avalon

21	<ul style="list-style-type: none"> • Orthic A • Yellow brown apedal B, 10YR 4/4 • No mottles 	Clovelly
22	<ul style="list-style-type: none"> • Orthic A, 1% mottles • Yellow brown apedal B 10YR 3/4 	Avalon
23	<ul style="list-style-type: none"> • Orthic A horizon, 5Y 2.5/1. 5 % mottles • G horizon: gley1 2.5/N 	Katspruit
24	<ul style="list-style-type: none"> • Orthic A: 10YR 3/4 • Soft plinthic B >10% mottles 	Westleigh
25	<ul style="list-style-type: none"> • Orthic A, 1% mottles • Yellow brown apedal B 10YR 3/4 	Avalon
26	<ul style="list-style-type: none"> • Orthic A • Yellow brown apedal B, 10YR 4/4 • No mottles 	Clovelly
27	<ul style="list-style-type: none"> • Orthic A, 1% mottles • Yellow brown apedal B 10YR 3/4 	Avalon
28	<ul style="list-style-type: none"> • Orthic A: 10YR 3/4 • Soft plinthic B >10% mottles 	Westleigh
29	<ul style="list-style-type: none"> • Orthic A: 10YR 3/4 • Soft plinthic B >10% mottles 	Westleigh
30	<ul style="list-style-type: none"> • Orthic A, 1% mottles • Yellow brown apedal B 10YR 3/4 	Avalon
31	<ul style="list-style-type: none"> • Orthic A • Yellow brown apedal B, 10YR 4/4 • No mottles 	Clovelly
32	<ul style="list-style-type: none"> • Orthic A: 10YR 3/4 • Soft plinthic B >10% mottles 	Westleigh
33	<ul style="list-style-type: none"> • Orthic A horizon, 10YR 2/1. 5 % mottles • G horizon: gley1 2.5/N 	Katspruit
34	<ul style="list-style-type: none"> • Orthic A, 1% mottles • Yellow brown apedal B 10YR 3/4 	Avalon
35	<ul style="list-style-type: none"> • Orthic A • Yellow brown apedal B, 10YR 4/4 	Clovelly

	• No mottles	
36	• Orthic A, no mottles • Yellow brown apedal B, no mottles, 10YR 4/4	Clovelly
37	• Orthic A • Soft plinthic B >10% mottles	Westleigh
38	• Stream	Katspruit
39	• Orthic A, 10YR 2/1 • Soft plinthic >10% mottles	Westleigh
40	• Orthic A • Yellow brown apedal B, 10YR 4/4 • No mottles	Clovelly

Appendix B – Data analysis relating to chapter 4

Table B1: Calculations for sample soil mass required for labile carbon method by Blair, et al. (1995)

wetland	sample number	CC (gC/100g soil)	CC (gC/g soil)	CC (mgC/g soil)	15 mg C (g soil)
Rietvlei	6	3.3897	0.0339	33.8974	0.4425
	7	6.8434	0.0684	68.4345	0.2192
	8	7.0353	0.0704	70.3532	0.2132
	9	5.3085	0.0531	53.0847	0.2826
	10	3.5177	0.0352	35.1766	0.4264
plant	6	3.6284	0.0363	36.2837	0.4134
	7	5.2879	0.0529	52.8786	0.2837
	8	6.7422	0.0674	67.4223	0.2225
	9	6.2470	0.0625	62.4696	0.2401
	10	7.1804	0.0718	71.8037	0.2089
Zaalklap	6	2.4682	0.0247	24.6818	0.6077
	7	0.5622	0.0056	5.6220	2.6681
	8	0.4172	0.0042	4.1719	3.5955
	9	2.9680	0.0297	29.6797	0.5054
	10	1.8289	0.0183	18.2891	0.8202
organic	11	3.6336	0.0363	36.3360	0.4128
	12	33.9634	0.3396	339.6341	0.0442
	13	34.6420	0.3464	346.4199	0.0433
	14	34.2452	0.3425	342.4517	0.0438
	15	31.3760	0.3138	313.7596	0.0478

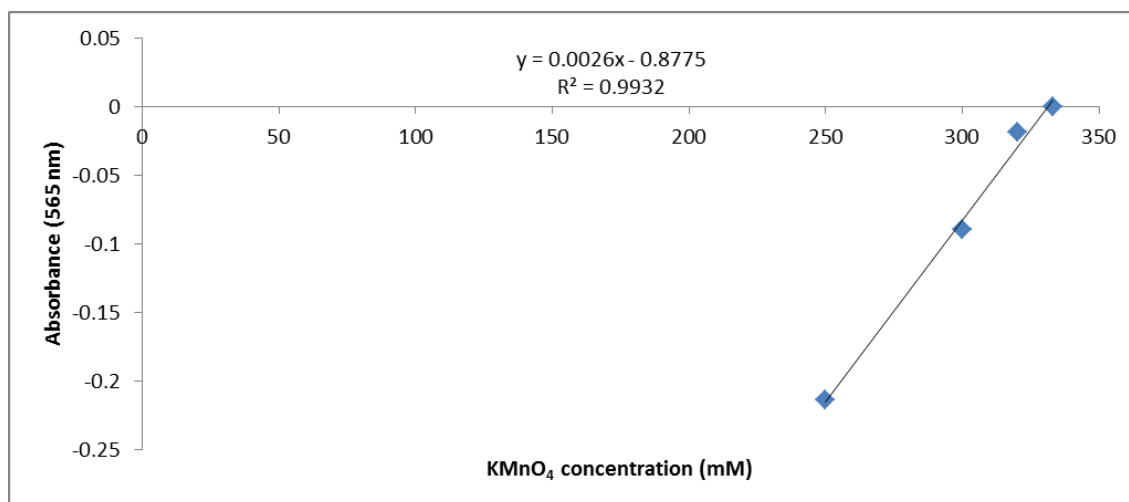


Figure B1: Correlation coefficient and equation from standard series for POXC method by Blair, et al. (1995)

Table A2: Meta-analysis data set for bulk density model

Reference	Country	Wetland type	SOC %	<i>n</i>	<i>sd</i>	BD (g cm ⁻³)	<i>n</i>	<i>sd</i>
Adame et al (2013)	Mexico	Mangroves	28.8	6	2.6	0.30	6	0.06
Adame et al (2013)	Mexico	Mangroves	29.4	6	2.4	0.25	6	0.03

Adame et al (2013)	Mexico	Mangroves	33.9	6	1.7	0.26	6	0.03
Adame et al (2013)	Mexico	Mangroves	33.5	6	1.5	0.21	6	0.03
Adame et al (2013)	Mexico	Mangroves	35.1	6	0.8	0.23	6	0.03
Adame et al (2013)	Mexico	Mangroves	32.3	6	1.0	0.15	6	0.01
Adame et al (2013)	Mexico	Mangroves	27.7	6	2.2	0.11	6	0.01
Adame et al (2013)	Mexico	Mangroves	12.6	6	7.8	0.22	6	0.08
Adame et al (2013)	Mexico	Mangroves	3.2	6	0.4	0.67	6	0.01
Adame et al (2013)	Mexico	Mangroves	27.8	6	5.2	0.32	6	0.08
Adame et al (2013)	Mexico	Mangroves	23.3	6	6.4	0.41	6	0.11
Adame et al (2013)	Mexico	Mangroves	14.8	6	6.9	0.44	6	0.08
Adame et al (2013)	Mexico	Mangroves	1.5	6	0.1	0.91	6	0.11
Adame et al (2013)	Mexico	Mangroves	27.6	6	6.8	0.18	6	0.01
Adame et al (2013)	Mexico	Mangroves	21.9	6	5.7	0.18	6	0.17
Adame et al (2013)	Mexico	Mangroves	21.0	6	8.3	0.30	6	0.20
Adame et al (2013)	Mexico	Mangroves	8.1	6	3.8	0.70	6	0.01
Adame et al (2013)	Mexico	Mangroves	9.3	6	2.2	0.40	6	0.05
Adame et al (2013)	Mexico	Mangroves	6.0	6	1.6	0.55	6	0.06
Adame et al (2013)	Mexico	Mangroves	2.9	6	0.1	0.95	6	0.02
Adame et al (2013)	Mexico	Mangroves	3.8	6	0.5	0.74	6	0.04
Adame et al (2013)	Mexico	Mangroves	28.8	6	4.6	0.18	6	0.02
Adame et al (2013)	Mexico	Mangroves	6.0	6	1.6	0.77	6	0.09
Adame et al (2013)	Mexico	Mangroves	3.9	6	0.5	0.89	6	0.03
Adame et al (2013)	Mexico	Mangroves	4.8	6	1.0	0.73	6	0.04
Adame et al (2013)	Mexico	Mangroves	29.3	6	1.2	0.16	6	0.02
Adame et al (2013)	Mexico	Mangroves	16.7	6	6.2	0.30	6	0.04
Adame et al (2013)	Mexico	Mangroves	4.6	6	0.2	0.63	6	0.02
Adame et al (2013)	Mexico	Mangroves	9.4	6	3.2	0.39	6	0.07
Ausseil et al (2015)	New Zealand	Bog	49.0	26	4.5	0.10	26	0.05
Ausseil et al (2015)	New Zealand	Fen	32.1	35	14.3	0.10	24	0.07
Ausseil et al (2015)	New Zealand	Swamp	28.2	42	15.6	0.13	40	0.07
Ausseil et al (2015)	New Zealand	Marsh	18.8	1	NA	0.12	2	0.06
Ausseil et al (2015)	New Zealand	Fen	10.2	1	NA	0.51	1	NA
Ausseil et al (2015)	New Zealand	Swamp	6.4	8	4.0	0.73	8	0.22
Ausseil et al (2015)	New Zealand	Marsh	8.9	1	NA	1.06	2	0.09
Ausseil et al (2015)	New Zealand	Pakihi	11.0	2	6.0	0.88	1	NA
Ausseil et al (2015)	New Zealand	Ephemeral	0.2	3	0.2	1.72	3	0.23
Cui et al (2014)	China	Meadow	12.2	3	1.1	0.96	3	0.04
Cui et al (2014)	China	Meadow	12.1	3	0.2	1.02	3	0.04

Cui et al (2014)	China	Meadow	12.4	3	0.3	1.03	3	0.02
Cui et al (2014)	China	Meadow	9.9	3	0.8	1.22	3	0.02
Cui et al (2014)	China	Wet meadow	20.8	3	5.3	0.70	3	0.06
Cui et al (2014)	China	Wet meadow	18.8	3	1.5	0.61	3	0.03
Cui et al (2014)	China	Wet meadow	44.6	3	3.3	0.43	3	0.02
Cui et al (2014)	China	Marsh	49.6	3	8.7	0.34	3	0.02
Cui et al (2014)	China	Marsh	64.0	3	0.7	0.34	3	0.04
Cui et al (2014)	China	Marsh	49.6	3	2.1	0.26	3	0.01
Cui et al (2014)	China	Marsh	58.3	3	5.0	0.30	3	0.02
Cui et al (2014)	China	Marsh	67.2	3	1.0	0.30	3	0.02
Korschens (2009)	Alaska	Peat land-Forest	34.9	27	17.4	0.29	27	0.39
Korschens (2009)	USA	Peat land-Forest	50.6	46	7.2	0.14	46	0.05
Korschens (2009)	USA	Relict deltaic marsh	20.5	8	7.9	0.18	8	0.09
Korschens (2009)	USA	Constructed salt marsh	0.8	5	0.4	1.18	5	0.19
Korschens (2009)	USA	Freshwater created marsh	5.6	13	0.8	0.74	13	0.17
Korschens (2009)	USA	Freshwater marsh	11.0	10	4.3	0.28	10	0.08
Korschens (2009)	USA	Salt marsh	6.8	10	4.7	0.66	10	0.30
Korschens (2009)	USA	Salt marsh	10.3	10	5.7	0.36	10	0.14
Korschens (2009)	USA	Sawgrass marsh	42.6	10	2.6	0.06	10	0.01
Korschens (2009)	USA	Sawgrass marsh	44.5	10	3.4	0.09	10	0.01
Korschens (2009)	USA	Sawgrass marsh	43.6	8	1.7	0.06	8	0.01
Korschens (2009)	USA	Sawgrass marsh	45.3	8	2.9	0.09	8	0.01
Korschens (2009)	USA	Sawgrass marsh	41.4	8	1.8	0.07	8	0.01
Korschens (2009)	USA	Sawgrass marsh	45.2	8	1.6	0.09	8	0.01
Korschens (2009)	USA	Sedge meadow	11.7	12	9.1	0.67	12	0.22
Warren et al (2012)	Indonesia	Peat	50.7	433	2.2	0.13	433	0.04
Warren et al (2012)	Indonesia	Peat	54.0	96	3.3	0.12	96	0.05
Warren et al (2012)	Indonesia	Peat	51.6	68	3.6	0.11	68	0.03

Table B3: Bulk density and SOC data for natural and created wetlands from Bernal (2012)

Bulk Density (kg m⁻³) SOC (g C 100g⁻¹ soil)

1.18 0.41
1.25 0.48

1.13	0.66
1.08	0.67
1.21	0.78
1.03	0.85
0.98	0.88
1.03	0.91
1.11	0.94
1.17	0.94
1.13	1.00
1.3	1.01
1.12	1.03
1.09	1.08
1.03	1.15
1.09	1.16
1.03	1.17
1.22	1.17
1.15	1.18
1.11	1.37
1.11	1.44
0.96	1.45
1.06	1.58
1.15	1.67
1.19	1.69
1.11	1.82
1.14	1.87
0.93	1.90
0.93	1.93
0.78	1.94
0.81	2.08
0.77	2.09
1.02	2.11
1.03	2.12
0.78	2.13
0.84	2.30
0.41	2.32
0.8	2.36
1.13	2.37
0.83	2.39
0.76	2.41
0.74	2.44
0.69	2.54
1.06	2.61
0.68	2.62
0.66	2.67
0.75	2.74
0.86	2.75

0.57	2.78
0.72	2.98
0.64	3.00
0.63	3.01
0.62	3.09
0.83	3.16
0.57	3.25
1.11	3.33
0.53	3.33
0.59	3.37
0.62	3.58
0.77	3.61
0.52	3.62
0.72	3.71
0.99	3.74
0.27	3.93
0.53	3.96
0.88	3.96
0.7	4.02
0.48	4.13
0.64	4.42
0.58	4.51
0.72	4.54
0.71	4.62
0.45	4.79
0.37	4.89
0.41	4.94
0.7	4.99
0.63	5.02
0.46	5.05
0.7	5.30
0.41	5.43
0.9	5.62
0.65	6.06
0.45	6.24
1.08	6.41
0.34	6.47
0.64	8.23
0.65	8.50
0.35	8.51
0.62	8.84
0.28	9.35
0.6	9.39
0.57	9.45
0.26	9.53
0.27	9.56

0.47	10.13
0.67	10.24
0.25	10.89
0.62	11.35
0.39	12.09
0.55	12.36
0.34	12.43
0.44	12.48
0.37	12.48
0.64	12.77
0.62	12.86
0.43	12.96
0.7	13.03
0.47	13.24
0.42	14.04
0.59	15.11
0.26	15.12
0.49	15.13
0.59	15.44
0.58	15.59
0.58	15.73
0.41	15.83
0.53	16.08
0.57	16.73
0.65	16.78
0.67	17.00
0.75	17.41
0.2	23.56
0.21	24.11
0.19	27.15
0.19	33.49
0.19	38.06
0.18	41.12
0.13	46.34