

RESPONSE OF SOIL CARBON FRACTIONS TO LAND USE SYSTEMS UNDER ARID TO SEMI-ARID CLIMATES IN SOUTH AFRICA

by

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DECLARATION

I declare that the thesis hereby submitted by me for the Philosophiae Doctoral degree at the University of the Free State is my own independent work and has not previously been submitted by me at another university/faculty. I furthermore cede copyright of this thesis in favour of the University of the Free State.

Signed:

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Palo Francis Loke

Date:

26-01-2018

SUMMARY

RESPONSE OF SOIL CARBON FRACTIONS TO LAND USE SYSTEMS UNDER ARID TO SEMI-ARID CLIMATES IN SOUTH AFRICA

Unprecedented pressure on food-producing ecosystems as a result of increasing population has resulted in substantial losses of soil carbon (C). Carbon loss as a basic and major precursor of soil degradation, is more prevalent in the semi-arid to arid environments, which cover more than half of South Africa's total land surface. Low inputs due to disposal of crop residues by burning in cultivated soils or by overgrazing in rangelands together with climatic conditions interact to influence the quantity and quality of soil C and food production. Management systems with the underlying goal to maintain high levels of soil C and reverse soil degradation in the dryland ecosystems are available and have been tested under different agro-ecological settings locally and abroad. However, there is still limited information on the relationship between soil C quality and quantity, especially in the semi-arid and arid regions. This study was therefore carried out to characterize soil C fractions and evaluate their response to different land use systems under semi-arid to arid climates in South Africa.

Soil samples used in this study were selected from previous collections under three different studies by other researchers. **Study one:** Applied treatments included two methods of straw management (unburned and burned), three methods of tillage (no-tillage, mouldboard ploughing and stubble mulch) and three methods of weed control (chemical and mechanical) in a long-term wheat trial in the Eastern Free State near Bethlehem. Selected samples were collected in different treatment combinations at the 0-50 mm soil layer. **Study two:** Selected soil samples were collected in the primary grasslands, croplands and secondary perennial pastures at the 0-200 mm soil layer in three agro-ecosystems: Harrismith, Tweespruit and Kroonstad. **Study three:** Three farms with two rangeland conditions (poor and good) were selected in two ecosystems: Thaba

Nchu (grassland) and Kuruman (savanna), and samples were taken at the 0-50 mm soil layer in poor and good rangeland conditions. All soil samples were analyzed for soil organic (SOC) and inorganic (SIC) carbon, permanganate oxidizable carbon (POXC), cold (CWEC) and hot (HWEC) water extractable carbon, extractable humic substances (C_{EX}), humic acids (C_{HA}) and fulvic acids (C_{FA}). Cross polarization magic angle spinning (CPMAS) ^{13}C nuclear magnetic resonance spectroscopy (NMR) was used for structural characterization of SOC. Humification (HI) and polymerization (PI) indices as well as alkyl C/O-alkyl C ratio were calculated as indicators of the extent of SOC decomposition.

Results demonstrated that adoption of proper crop residue management (unburned straw in this case) in no-tillage treatments can reverse the current trend of soil degradation in arable landscapes as revealed by an increase in different soil C fractions and SOC quality, compared to treatment combinations that involved mouldboard ploughing. Although indicators of the extent of decomposition were generally similar under the applied treatment combinations, accumulated SOC in no-tilled treatments was less humified, and thus suggested high lability and susceptibility to losses if the soil can be brought under intensive cultivation again.

Our results further indicated that conversion of primary grasslands into arable cropping had a negative impact on the quantity and quality of soil C. However, the magnitude of loss for most C fractions generally followed the order: Harrismith > Tweespruit > Kroonstad, which was unexpected because the increase in mean annual rainfall (MAR) and clay content occurred in the opposite direction. O-alkyl C, a measure of lability of SOC, remained almost the same in Harrismith despite prolonged soil cultivation. This could be explained by higher mean annual rainfall and clay content. Reversion of cultivated soils to secondary perennial pastures demonstrated capability to restore historic C fractions and improve SOC quality to comparable levels to primary grasslands. However, there were no clear trends regarding the magnitude of gain across the three agro-ecosystems. Besides soil management and climatic or clay content,

losses or accumulations of the measured C fractions were modulated by saturation deficits and vegetation quality.

Rangeland conditions in the grassland and savanna ecosystems did not influence soil C fractions, due to rotational grazing, which allowed regeneration of overgrazed areas. However, vegetation regeneration in the savanna was delayed due to hot-dry conditions, hence lower soil cover. Significant changes in the measured C fractions arose when the two ecosystems were compared, and our results indicated that the hot-dry sandy savanna ecosystem was more vulnerable to degradation as revealed by 2-4 times lower C fractions, compared to the cool-moist clayey grassland ecosystem. The CPMAS ^{13}C NMR spectroscopic results highlighted that SOC composition was affected by decomposition in the grassland ecosystem. The four SOC functional groups did not display clear trends in the savanna ecosystem, probably due to heterogeneous (grass-shrub-trees) vegetation composition.

Based on the results of this study it is evident that maintenance of a good permanent soil cover can restore lost C fractions and counteract soil degradation processes in the drought prone ecosystems. This means that for arable cropping, adoption of no-tillage with proper residue management could be an option. No-tillage or secondary pasture management can also be used to reclaim degraded cultivated soils. In the rangelands, permanent soil cover can be maintained or improved by rotational grazing depending with not only availability of rangeland resources, but also with prevailing climatic and soil conditions. Where possible reseeding of grass species can be implemented to avoid desertification and erosion losses of C fractions.

Keywords: Arable cropping, Carbon, CPMAS ^{13}C NMR spectroscopy, Dryland ecosystems, Rangeland condition, Secondary pasture management, Soil degradation.

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To my wife

Mamoeketsi Loke

CHAPTER 1

INTRODUCTION

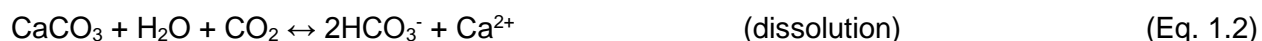
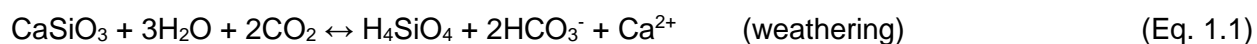
1.1 Motivation

This study was inspired by the request made in 2005 by the Minister of Agriculture to develop a soil protection strategy for South Africa after establishing that close to three million hectares (ha) of moderate to high potential agricultural land was at risk of severe degradation (Pretorius, 2009). Among the degradation processes identified, a decline in soil organic carbon (SOC) was tipped to be the most serious and basic cause (Barnard & Du Preez, 2004; Du Preez *et al.*, 2011a, b). Quantifying the impact and extent of soil degradation on dryland ecosystems and C storage was proposed and included as one of the priorities in the strategic plan of the department (Pretorius, 2009). A baseline SOC study within and between soil forms across different environments was then recommended (Du Preez *et al.*, 2011a). Though the focus was mainly on SOC, soil inorganic C (SIC) seems to have gained more attention lately.

Based on the origin of these two C forms, it is obvious that not every soil consists of SIC; however, SIC can exceed SOC particularly in arid to semi-arid soils formed from calcareous parent material (Batjes, 1996). Soil organic C is a complex heterogeneous mixture of organic materials that reside in the soil. Such organic substances include plant and animal remains in various stages of decomposition, a wide range of soil organisms, and a dark-coloured humus consisting of humic and non-humic substances (Du Preez *et al.*, 2011a). Soil inorganic C refers to a total amount of carbonate minerals present in the soil, of which calcite (CaCO_3) is the dominant component (Sanderman, 2012). Both SOC and SIC are dynamic pools that need to be safeguarded at all costs due to their inherent relations with other properties that comprise the minimum dataset to assess soil degradation (Bronick & Lal, 2005; Lal *et al.*, 2015). In summary, these C forms govern

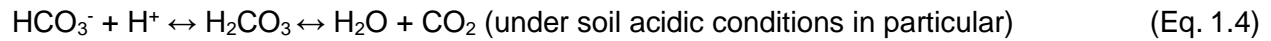
the physical, chemical and biological processes that occur in the soil, including nutrient cycling, cation exchange capacity, buffer capacity, nutrient and water retention, structural stability, aeration, biodiversity and chelation of toxic substances to mention but a few.

Emerging evidence suggests that SOC and SIC can act as a source or sink of atmospheric C (Wu *et al.*, 2009; Wang *et al.*, 2010; Shi *et al.*, 2012). This phenomenon is not fully understood as it depends on a suite of factors such as soil forming factors, land use, management and their interaction. The type of C present in the soil is also critically important in this regard. Conceptually, SOC can be subdivided into three kinetic pools, and depending on their biological stability, turnover or mean residence times are referred to as labile/active, slow and passive. The turnover rates or mean residence times of these pools range from < 10 years (labile) to millinia (passive), with the slow pool being intermediate (10-100 years) (Von Lützow *et al.*, 2006). Soil inorganic C can be characterized into lithogenic (derived from parent material) and pedogenic (product of pedogenic processes) carbonate. Pedogenic carbonate can further be divided into pedo-lithogenic and pedo-atmogenic carbonate (Shi *et al.*, 2012; Ahmad *et al.*, 2015). The difference between the two is that calcium (Ca) in the pedo-lithogenic carbonates is derived from carbonate minerals, whereas that in the latter originates from non-carbonate minerals (Sanderman, 2012; Ahmad *et al.*, 2015). Carbon exchange between SIC and the atmosphere occurs through a series of reactions such as weathering of soil minerals and dissolution or formation of carbonates as indicated by the three chemical equations (Eq. 1.1-1.3) below (Sanderman, 2012). Changes in SIC are attributed to pedogenic carbonate (Wu *et al.*, 2009). Therefore, formation of passive SOC and pedogenic SIC can lead to mitigation of climate change and improvement of soil resilience and food production owing to their long residence times (Lal *et al.*, 2015).



Soil C has been affected by a complexity of factors that include poor agricultural practices. Intensive soil cultivation and overgrazing have been acknowledged to be among the major precursors to soil degradation and escalating concentrations of C in the atmosphere. Cultivation of virgin soils disrupts aggregates and subjects protected organic C to biological degradation (Chen *et al.*, 2009; Kösters *et al.*, 2013). Usually, readily available or labile compounds such as carbohydrates and proteins are affected first, leading to great losses of O-alkyl C (Baldock *et al.*, 1992; 1997). As decomposition proceeds, more recalcitrant fractions of organic matter accumulate with concomitant increase in signal intensities of aromatic, alkyl and carbonyl C (Baldock *et al.*, 1992, 1997; Kögel-Knabner, 1997; 2002; Mathers *et al.*, 2003; Helfrich *et al.*, 2006). However, recent studies have shown that continuous cultivation of arable lands also depletes soils of lignin (Lobe *et al.*, 2002) and humic substances (Guimarães *et al.*, 2013; Kotzé *et al.*, 2016), which are known to be very resistant to microbial decomposition (Kögel-Knabner, 2002; Von Lützow *et al.*, 2006).

Soil organic C decomposition also affect SIC, especially when the released CO₂ is consumed to precipitate or dissolve SIC (Sanderman, 2012). Rapid SOC oxidation along with removal of base cations and nitrate ions by plants and/or leaching and frequent applications of ammonium forming or containing fertilizers, which are commonly observed in arable lands, accelerate soil acidification and thus SIC dissolution (Eq. 1.1) (Wu *et al.*, 2009; Sanderman, 2012; Shi *et al.*, 2012). Liming of acid-affected soils also dissolves SIC (Eq. 1.1). However, the formed bicarbonate should be completely leached out of the soil system otherwise these processes would be regarded as sources of atmospheric C (Eq. 1.4) (Sanderman, 2012; Shi *et al.*, 2012). Overgrazing also leaves soils prone to erosion, acidification and desertification following low soil C inputs, less vegetative cover and animal trampling, which destroys plant stands and topsoil structure (Yong-Zhong *et al.*, 2005; Kotzé, 2015). Overgrazing, and hence grassland deterioration, often results in a random emergence of woody and less palatable plant species (Kotzé, 2015).



Conservation programs have been examined and implemented in many parts of the world due to their effectiveness in suppressing erosion, desertification and improving soil C (Conant *et al.*, 2001; Yong-Zhong *et al.*, 2005; Conant, 2010; Yuan *et al.*, 2012). Similar projects were introduced in South Africa, and some postgraduate students were placed to assess their sustainability under local conditions: (1) In 1999, Kotzé (2004) completed a masters study on a wheat trial set up in 1979 in the Eastern Free State titled “*Influence of long-term wheat residue management on some fertility indicators of an Avalon soil at Bethlehem*” with the aim of establishing the best management that could restore and possibly improve fertility of prolonged cultivated soils. (2) During the past century some commercial farmers in Vryburg, Kroonstad, Koppies, Tweespruit and Harrismith converted their rangelands to arable dryland cropping of maize, wheat and sunflower. Du Toit (1992) completed a masters study titled “*Effect of cultivation on the organic carbon and total nitrogen in selected dryland soils*” to investigate patterns of SOM loss in the upper 200 mm soil layer along cultivation chronosequences in these five ecotopes (agro-ecosystems). Over three decades ago the South African government overturned the farmers’ decision and compensated them to revert degraded arable lands to perennial pastures in central South Africa (Harrismith, Tweespruit and Kroonstad). Birru (2002) completed a masters study titled “*Organic matter restoration by conversion of cultivated land to perennial pasture on three agro-ecosystems in the Free State*” to study organic matter restoration in the upper 200 mm soil layer. (3) As part of the collaboration between University of the Free State and University of Bonn a project termed “Vulnerability and resilience of soils under different rangeland use” was initiated, and Kotzé (2015) completed a doctoral study titled “*Response of soil properties to rangeland use in grassland and savanna biomes of South Africa*” to assess soil degradation induced by rangeland management.

Some soil samples collected for these three mentioned projects were selected to study C fractions schematically presented in Figure 1.1. These previous research works focused more on measurements of total organic C. In fact, there is limited information and understanding of the relationship between SOC quantity and quality characterization and we are not aware of any study conducted along these lines, particularly in the South African context. Globally, very few studies have demonstrated the utility of the combined use of chemical and spectroscopic analytical procedures to characterize SOC into labile and recalcitrant fractions under semi-arid to arid conditions. Understanding different C pools is crucial and of course a prerequisite for effective management of soil C in terrestrial ecosystems as they shed light on the nature or type of C present in the soil. Changes in total C induced by land use or soil management practices may be negligible and undetectable compared to functional pools, and can provide inadequate information. In addition, most local researchers have dedicated their work more on soil C loss than restoration, which forms the main focus of this study.

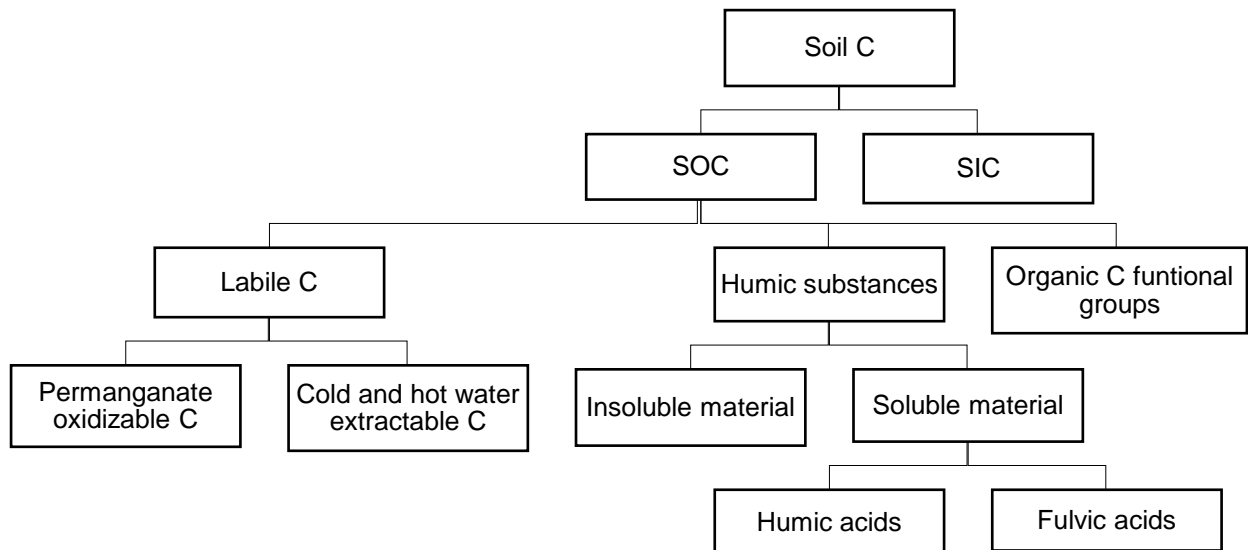


Figure 1.1 Soil C fractions analyzed from the selected samples.

1.2 Aims

The interactions between human activities, low rainfall, high temperatures and low phytomass production have escalated soil degradation in the semi-arid to arid cropped and grazed terrestrial environments, and many countries including South Africa have fallen prey to this chronic phenomenon due to lack of sound soil protection strategies and policies. Establishment or development of safe and sustainable management practices that can reverse soil degradation and improve ecosystem functioning has remained a great challenge in South Africa due to financial and time constraints. Therefore, this study attempts to provide coordinated data for the development of a sound protection strategy for cropland and rangeland soils using both quantitative and qualitative analytical procedures to characterize soil C fractions and spectroscopic changes in SOC as indicators of soil degradation or restoration of the soil's productive capacity. Soil C quantity is as important as quality, as such knowledge of the amount and type of C present in the soil system is critical for soil C management and assessment of atmospheric C sequestration potential of the applied land uses or management systems.

1.3 Objectives

This study focused on specific objectives indicated in the respective chapters. Description of experimental sites and methods used for data collection and analysis are also given in the respective chapters. This study was divided into five chapters and organized as outlined below:

- Chapter 1 presents the thesis introduction, which consists of motivation, aims and hypothesis of the study. This chapter provides a general overview of soil degradation and identifies declining soil C as the major cause. A brief outline on how soil degradation can be addressed is also given in this chapter.
- Chapter 2 compares wheat residue management systems (unburned and burned) combined with tillage (no-tillage, stubble mulch tillage and mouldboard ploughing) and

weed control methods (chemical and mechanical) in order to determine the best treatment combination that can restore and if possible improve soil C in the 0-50 mm soil layer. This chapter is a follow-up to the study conducted by Kotzé (2004) and is prepared for publication as an original research article in Soil Research under the title “*Long-term effects of wheat production management practices on some carbon fractions of a semi-arid Plinthustalf*”.

- Chapter 3 assesses the spatial effect of prolonged soil cultivation and reversion of degraded arable lands to secondary perennial pastures on soil C and related fractions in the upper 200 mm soil layer. Adjacent primary grasslands are used as reference. This chapter is a follow-up to studies conducted by Du Toit (1992) and Birru (2002), and is prepared for publication as an original research article in Agriculture, Ecosystems and Environment under the title “*Dynamics of soil carbon concentrations and quality induced by agricultural land use in central South Africa*”.
- Chapter 4 assesses rangeland degradation along the grazing gradients in commercial livestock farming using different soil C fractions in the 0-50 mm soil layer. This chapter is a follow-up to the study conducted by Kotzé (2015) and is prepared for publication as an original research article in the Journal of Arid Environments under the title “*Changes in soil carbon fractions induced by grazing regimes in commercial grassland and savanna rangelands of South Africa*”.
- Chapter 5 titled “*Synthesis and general conclusions*” synthesizes and integrates the key findings and provides limitations of the study as well as recommendations for future research.

1.4 Hypothesis

Human demands on world soils have affected soil C, which is mainly concentrated in the top soil horizon. Development of sound soil protection strategies should therefore be a priority of

every country if we want to protect the topsoil against losses, maintain or improve soil C levels, mitigate climate change and create healthy hunger-free nations. Therefore, the hypothesis of this study is that replacement of mouldboard ploughing with conservation tillage combined with proper crop residue management, reversion of degraded arable lands to secondary perennial pastures and maintenance of good rangeland condition are capable of restoring historic C and keeping it in the soil.

CHAPTER 2

Long-term effects of wheat production management practices on some carbon fractions of a semi-arid Plinthustalfs

Abstract

Soil cultivation and disposal of crop residues by burning are still common practices in South Africa despite their detrimental effects on soil C and environmental quality. This study evaluated effects of wheat production management practices on different C fractions and SOC molecular composition of a semi-arid Plinthustalfs in a long-term trial established in the Eastern Free State near Bethlehem. The applied treatments included two methods of straw management (unburned and burned), three methods of tillage (no-tillage, mouldboard ploughing and stubble mulch) and two methods of weed control (chemical and mechanical weeding). Selected samples collected from 0-50 mm depth of soils subjected to specific treatment combinations given in the material and methods section were analyzed for soil organic C (SOC), soil inorganic C (SIC), permanganate oxidizable C (POXC), cold water extractable C (CWEC), hot water extractable C (HWEC), extractable humic substances (C_{EX}), humic acids (C_{HA}), fulvic acids (C_{FA}) and organic C functional groups. Humification (HI) and polymerization (PI) indices as well as alkyl C/O-alkyl C ratios were also calculated. The combination of unburned straw, no-tillage and chemical weeding resulted in a significantly higher SOC, SIC, POXC, CWEC, C_{EX} and C_{FA} , but lower HI and PI compared to the interaction between unburned or burned straw and either chemical or mechanical weeding in the ploughed treatments. The HI and PI values were not always significant between the treatment combinations. The ^{13}C nuclear magnetic resonance (NMR) spectra indicated that the analyzed soils contained all the major organic C functional groups, which differed with treatment combinations. Alkyl C and carbonyl C were highest in the unburned, ploughed, mechanically weeded plots and lowest in the burned, ploughed, mechanically weeded plots.

Unburned straw combined with chemical weeding in no-tilled treatments increased O-alkyl C, but resulted in the lowest aromatic C and alkyl C/O-alkyl C ratio compared to the rest of the treatment combinations. In general, the combination of unburned straw, no-tillage and chemical weeding showed potential to restore soil C fractions despite higher accumulation of less humified SOC. Regardless, levels of the measured C fractions were lower, which could be due to long-term production of wheat under monoculture. Therefore crop rotation that involves leguminous plants and deep-rooted high biomass producing crops and elimination of the fallow period are recommended for evaluation in this trial.

Key words: Carbon fractions, Functional groups, Semi-arid regions, Soil management

2.1 Introduction

Declining soil fertility and increasing atmospheric carbon (C) could be a signal to escalating pressure on arable lands and lack of sustainable management practices to rehabilitate prolonged cultivated soils. Continuous cultivation following increased food demand for the growing population has resulted in the breakdown of traditional farming practices such as recycling of crop residues, fallowing, crop-livestock farming and agroforestry that were capable of restoring C and the soils' productive capacity (McNeill & Winiwarter, 2004). Soil management systems like incorporation of crop residues by tillage accelerate decomposition and mineralization of crop residues, causing great emissions of greenhouse gases into the atmosphere. Tillage also disrupts soil aggregates and subjects protected C to biological oxidation and erosion (Chen *et al.*, 2009). Consequently, all the processes governed by soil C deteriorate and pose a long-term threat to soil quality and food security.

Disposal of crop residues is another method used to manage crop residues after harvesting. In many countries, including South Africa (Kotzè & Du Preez, 2007; Loke *et al.*, 2012), these organic materials are still regarded as trash and are disposed of by burning to ease tillage operations and

destroy the habitat for pests and diseases (Singh & Rengel, 2007). From a soil fertility perspective, residue burning could be regarded as the quickest method to release nutrients tied up in crop residues and a cheap technique to improve the pH of acid-affected soils, but has been banned in many European countries due to environmental and health concerns (Singh & Rengel, 2007). The use of chemical inputs to improve soil fertility and crop production has also been a challenge in most developing countries due to high costs, dwindling purchasing power and limited credit facilities to farmers (Bakht *et al.*, 2009). Previous studies also show that crop production can still decline even in well-fertilized soils if their C content is low (Lobe *et al.*, 2001; Kotzé & Du Preez, 2007). Therefore, restoration of soil C is essential to regenerate degraded soils and improve ecosystem functioning.

One basic mechanism to restore or improve soil C in arable lands is to increase C inputs and reduce its losses (Janzen, 2005; Chen *et al.*, 2009). These attributes are associated with conservation tillage, which has been touted to be a perfect alternative for mouldboard ploughing. In conservation tillage systems, at least 30% of crop residues are retained at or near the soil surface after harvesting to provide cover against erosion and improve water infiltration (Singh & Rengel, 2007). Surface retained residues and minimum soil disturbance also reduce C losses through decomposition or erosion and improve nutrient use efficiency (Singh & Rengel, 2007). However, to assess the sustainability of conservation tillage systems on soil C storage, it is crucial to measure different C pools as they differ in terms of their response timeframes to land use and management systems as well as the roles they play in the soil (Gregorich *et al.*, 1994; Janzen, 2005).

Soil C exists in organic and inorganic forms, and is estimated to be the largest C pool in terrestrial ecosystems (Batjes, 1996; Shi *et al.*, 2012), storing 1550 Pg of SOC and 950 Pg of soil inorganic C (SIC) (Batjes, 1996). Although both SOC and SIC have been shown to play a critical role in global C cycling, SIC has received little attention (Wang *et al.*, 2010; Lal *et al.*, 2015). Many

researchers disregard SIC citing its negligible contribution towards C sequestration (Batjes, 1996). New research developments however, have revealed that SIC is a dominant pool in the arid to semi-arid regions (Wu *et al.*, 2009; Shi *et al.*, 2012), which occupy at least one-third of the earth's land surface (Batjes, 1996; Shi *et al.*, 2012). In fact, alkaline/saline soils in China were able to absorb 62-622 g C m⁻² yr⁻¹ (Xie *et al.*, 2009), showing that SIC dynamics are increasingly becoming important to understanding C cycling (Mikhailova & Post, 2006).

Based on its recalcitrance to microbial decomposition, SOC could be classified into labile, slow and passive pools. Labile C serves as energy source for the decomposer community and is a sensitive indicator to changes in land use and soil management, whereas slow to passive C pools are more relevant to long-term soil structural formation and stability as well as C sequestration (Von Lützow *et al.*, 2006). Soil inorganic C exists as carbonates either derived from parent material (primary or lithogenic) or formed through soil processes (secondary or pedogenic) (Wu *et al.*, 2009; Shi *et al.*, 2012; Jin *et al.*, 2014; Lal *et al.*, 2015). The latter can further be subdivided into pedo-lithogenic carbonate due to calcium (Ca²⁺) and/or magnesium (Mg²⁺) inherited from parent material and pedo-atmogenic carbonate due to Ca²⁺ or Mg²⁺ originating from external sources (Sanderman, 2012; Ahmad *et al.*, 2015). Inorganic C is also important for soil structural stability and nutrient cycling (Bronick & Lal, 2005). Since SOC is a reservoir of Ca²⁺ and Mg²⁺ and a source of soil carbon dioxide (CO₂), its presence in sufficient levels can stimulate dissolution and precipitation of SIC when it decays (Bronick & Lal, 2005). Produced CO₂ during SOC decomposition can be incorporated to form SIC, and thus C sequestration (Sanderman, 2012).

Influence of conservation tillage systems on total organic C has been extensively researched; however, little is known about their effects on quantity and quality of soil C for the Bethlehem region. In fact, studies involving the combine use of chemical and spectroscopic characterization methods on soil C are scarce, especially under semi-arid conditions. For example, Wiltshire and Du Preez (1993), Kotzé (2004), Kotzé and Du Preez (2007) and Loke *et al.* (2012) investigated

the effect of residue management under conservation (no- and mulch tillage) and conventional tillage systems on SOC content of a semi-arid Plinthustalfs in a wheat trial located at Bethlehem, South Africa and found that surface retained residues in no-tilled treatments increased SOC in the upper 0-50 mm layer compared to incorporated burned or unburned wheat straw in the ploughed plots.

In these studies (Wiltshire & Du Preez, 1993; Kotzé, 2004; Kotzé & Du Preez, 2007; Loke *et al.*, 2012), soil C compartments such as labile C, humic substances, organic C functional groups or SIC, which are important to deepen our understanding of C dynamics were not considered. In addition, adoption rates of conservation tillage systems in South Africa are steadily growing from 300 000 ha in 2005 to 368 000 ha of land currently under no-tillage (Derpsch & Friedrich, 2009; Friedrich *et al.*, 2012), presumably due to associated benefits and availability of implements (e.g. planter) adapted to conservation tillage systems. However, Friedrich *et al.* (2012) is of the opinion that there is still room to expand and spread conservation agriculture in this country, but limited research and practical results as well as poor information dissemination to farmers have negatively affected its adoption rates.

The objective of this study was to evaluate the long-term impact of two wheat residue management (unburned and burned) under three tillage (mouldboard ploughing, stubble mulch tillage and no-tillage) systems and two weed control methods (chemical and mechanical) on soil C pools of a semi-arid Plinthustalfs in the surface layer (0-50 mm). We hypothesized that surface retained wheat residues in conservation tillage (no- and mulch tillage) systems will restore soil C pools. To test this hypotheses, the selected soil samples were analyzed for soil carbon (C), soil organic C (SOC), soil inorganic C (SIC), labile organic C fractions (permanganate oxidizable (POXC), cold water extractable (CWEC) and hot water extractable (HWEC) C), humic substances and organic C functional groups.

2.2 Material and methods

2.2.1 Site description

A trial was established in 1979 at the Agricultural Research Council (ARC) Small Grain Institute (28°9'S, 28°17'E; 1,680 m above sea level) near Bethlehem in the Eastern Free State of South Africa to study the effects of some wheat management practices on soil fertility and crop productivity. Prior to acquisition of the site by the Institute, it was conventionally tilled for at least 20 years, but other management details are unknown. The mean annual rainfall in this area is 695 mm and the mean annual class-A pan evaporation is 1883 mm, resulting in a mean annual aridity index of 0.37. Most of the rain (79%) falls from October to March, with mean daily temperatures ranging from 6.7°C in July to 20.1 °C in January.

According to the Land Type Survey Staff (2001), the trial is on land type Ca6n, which covers a substantial 420 000 ha. This land type is defined as a plinthic catena, which in upland positions has margalitic and/or duplex soils derived from Beaufort mudstone, shale, sandstone and grit with dolerite sills in places. According to the USDA system, the soil would fall under the Great Group Plinthustalfs (Soil Survey Staff, 1998). This Plinthustalf consists of three diagnostic horizons: an orthic Ap (0-30 mm), yellow-brown apedal B1 (300-650 mm) and soft plinthic B2 (> 650 mm), containing 18, 23 and 36% clay, respectively. The parent material comprises a mixed deposit of aeolian and colluvial origin on shale that increases with depth from 750 to 900 mm.

2.2.2 Experimental design

The trial was laid out on a terrain unit 3 with a 2-3% north-facing slope using a randomized complete block design with three blocks serving as replicates. Each block comprised 36 field treatments: two straw management treatments (unburned and burned), three tillage systems (no-tillage, stubble mulch tillage and ploughing), two weed control methods (chemical and mechanical) and three levels of nitrogen (N) fertilization in a factorial arrangement. Nitrogen levels

were 20, 30 and 40 kg N ha⁻¹ and soil samples were taken only from plots that received the intermediate N level (30 kg N ha⁻¹). This intermediate N rate reflects common farmers' practice in this region. Plots are 6 m x 30 m with 10 m borders and are cropped annually with winter wheat (*Triticum aestivum* L.) without any rotation or replacement with a summer crop. However, in 1990 and 1991 oats (*Avena sativa* L.) was used as a substitute crop, as a way to reduce soil-borne diseases (Take-all, *Gaeumannomyces graminis*) that occurred in some treatments. A fallow period of five months is maintained in this trial to restore soil water between harvesting and seeding, during which most of the rainfall events are expected.

Immediately after harvesting in December, wheat straw in the relevant no-tilled, stubble mulched and ploughed treatments is burned or left unburned. In the ploughed treatments, just after burning, a two-way offset disc is used to incorporate either the unburned wheat straw or the wheat straw ashes to 150 mm depth, followed by mouldboard ploughing to a depth of 250 mm in February, when the soil is sufficiently moist and easy to work. The stubble mulch treatments are not disked, but cut at 100-150 mm using a v-blade and then ripped with a 50 mm width chisel plough at 300 mm spacing to the same depth and at the same time as the ploughed treatments. The no-tilled plots are not ploughed.

Weeding is done once in the relevant treatments when needed the first time, generally in March. This is done either by a mechanical cultivator (rodweeder or v-blade depending on the soil water level) or by spraying non-selective herbicides. Initially only glyphosate (Roundup, Monsanto Co.; 369 g a.i. l⁻¹) was used, but later on it was alternated with Paraquat at 200 g a.i. l⁻¹ to reduce chances of herbicide resistance developing.

All of the treatment plots were slightly disturbed with a combined seeder-fertilizer drill used for sowing the wheat seed together with the premixed fertilizer. A 3:2:0 NPK (25%) + 0.75 Zn fertilizer blend was applied at a rate that results in N, P (phosphorus) and Zn (Zinc) applications of 20, 13 and 1 kg ha⁻¹, respectively. Limestone ammonium nitrate (LAN, 28% N) was thoroughly mixed

with this fertilizer blend and applied to supplement the N levels to 30 and 40 kg ha⁻¹ in the relevant treatments. From the start of the experiment, the wheat cultivar Betta was planted.

2.2.3 Soil sampling and selection

To allow for maximum soil settling after the last cultivation, sampling was done after the rainy season just before planting in June. Soil samples were collected in June 1999, at the intervals of 0-50, 50-100, 100-150, 150-250, 250-350 and 350-450 mm. However, only samples collected from 0-50 mm depth of soils subjected to the following treatment combinations were selected for this study as the effects of the applied treatment combinations were concentrated in the upper 50 mm layer. The applied treatment combinations include; unburned x no-tillage x chemical weeding (UB-NT-CW); burned x mouldboard plough x chemical weeding (B-MP-CW); unburned x mouldboard plough x chemical weeding (UB-MP-CW); unburned x stubble mulch x chemical weeding (UB-SM-CW); burned x mouldboard plough x mechanical weeding (B-MP-MW); and unburned x mouldboard plough x mechanical weeding (UB-MP-MW). Three auger cores (70 mm diameter) were taken from the centre-line of each treatment plot and mixed thoroughly. Samples were dried at room temperature, sieved through a 2 mm sieve and stored for analysis.

Only concentration values of SOM indices will be dealt with since bulk density was measured neither in the previous studies nor the current study; therefore, calculations of the actual contents were impossible. In addition, we believe that the latter would show similar trends as with the concentrations since at the time of sampling bulk density should almost be similar. For these South African soils (also sampled in June/July), a difference in bulk density of less than 4 % between ploughed and native land was reported in two different studies (Lobe *et al.*, 2001; 2002).

2.2.4 Laboratory analysis

Analyses were carried out to determine soil C, SOC, SIC, POXC, CWEC, HWEC, C_{EX}, C_{HA}, C_{FA} and organic C functional groups.

2.2.4.1 Soil carbon

Soil C was analyzed by dry combustion (Nelson & Sommers, 1982) with a TruSpec Leco CN analyzer. Approximately 0.43 g sieved air-dried encapsulated soil samples were placed in a loading head, and one by one dropped into a 950°C hot furnace and flushed with oxygen for rapid and complete combustion. Combustion gases were then passed through a secondary furnace (850°C) for further oxidation before collected in a collection vessel where oxygen was injected and mixed with combustion gases. These gases were purged through a CO₂ infrared detector, which measures C as CO₂.

2.2.4.2 Soil organic and inorganic carbon

Soil organic C was measured with a modified Mebius procedure (Nelson & Sommers, 1982). A 0.5 g of sieved air-dried soil was weighed in a 150 ml glass beaker and reacted with 10 ml of 0.5 N potassium dichromate (K₂Cr₂O₇) and 15 ml of concentrated sulphuric acid (H₂SO₄). Samples were placed on a preheated sand bath at a temperature of 130 °C for 10 minutes. Samples were then removed from the sand bath and 35 ml of deionized water was added to each. Excess K₂Cr₂O₇ was titrated with 0.2 N ferro-ammonia sulphate [Fe(NH₄)₂(SO₄)₂·6H₂O] until the end point is reached, which was detected by a millivoltmeter with a platinum electrode. Soil inorganic C was calculated as the difference between soil C and SOC.

2.2.4.3 Permanganate oxidizable carbon

Permanganate oxidizable C was analyzed according to Culman *et al.* (2012). Briefly, 2.5 g of sieved air-dried soil was weighed into 50 ml falcon tubes, wherein 18 ml deionized water and 2 ml 0.02 M potassium permanganate (KMnO₄) solution were added. The tubes were shaken for 2 minutes at 240 oscillations per minute on an oscillating shaker and then centrifuged for 5 minutes at 906 x g for the soil to settle down. Immediately thereafter, 0.5 ml of the supernatants were

transferred to new 50 ml falcon tubes and diluted with 49.5 ml deionized water before reading the sample absorbance on a spectrophotometer at 550 nm wavelength to obtain POXC.

2.2.4.4 Cold and hot water extractable carbon

Cold and hot water extractable C were determined according to Ghani *et al.* (2003). In short, 3 g air-dried soil samples were transferred to 50 ml falcon tubes and extracted with 30 ml of deionized water. Samples were shaken for 30 minutes on an end-over-end shaker and centrifuged for 20 minutes at 1233 x g. The supernatants were filtered through a 0.45 µm cellulose membrane filter into separate vials for C analysis. A 30 ml aliquot of deionized water was again added to the sediments in the same tubes and shaken on a vortex shaker to suspend the sediments. The capped tubes were then left in a hot-water bath at 80°C for 16 hours. Samples were again shaken on a vortex shaker and centrifuged for 20 minutes at 1233 x g. The supernatants were filtered through a 0.45 µm cellulose membrane filter. Carbon in both extracts was determined according to the modified Mebius procedure, and 5 ml of the extracts was used instead of soil. Carbon obtained from the first extraction was referred to as CWEC, while that from the second extraction was classified as HWEC.

2.2.4.5 Humic substances

The sequential procedure of Schnitzer (1982) was slightly modified to extract and fractionate humic substances. A 5 g of sieved air-dried soil was weighed out in falcon tubes and reacted with 30 ml extraction solution [0.1 N sodium hydroxide (NaOH) and 0.1 M sodium pyrophosphate decahydrate ($\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$)]. The contents were shaken on an oscillating shaker for an hour and centrifuged at 906 x g for 15 minutes. Insoluble material contained in the supernatant was isolated from soluble alkaline material (extractable humic substances). Soluble alkaline material was then precipitated with 0.05 N sulfuric acid (H_2SO_4) to fractionate humic and fulvic acids. Carbon concentrations of extractable humic substances (C_{EX}) and humic acids (C_{HA}) were

determined with the Mebius procedure (Nelson & Sommers, 1982). Carbon in fulvic acids (C_{FA}) was calculated as the difference between C_{EX} and C_{HA} . Humification index ($HI = C_{HA}/SOC$) and polymerization index ($PI = C_{HA}/C_{FA}$) were calculated according to Abril *et al.* (2013).

2.2.4.6 Nuclear magnetic resonance spectroscopy

Bulk soil samples were pre-treated with hydrofluoric acid (HF) to remove magnetic materials, concentrate organic C and increase the signal-to-noise ratio of the resultant NMR spectra as recommended by Skjemstad *et al.* (2001) and Mathers *et al.* (2002). Briefly, 5 g of ground sieved air-dried soil was weighed into 50 ml falcon tubes and 45 ml of 2% HF was added. The tubes were shaken on end-over-end shaker for 8 (4 x 1 hour, 3 x 16 hour and 1 x 64 hour) successive times. Samples were then centrifuged after every extraction at 671 x g for 20 minutes and the supernatant was filtered through a 5 mm Millipore Durapore membrane filter to recover the light fraction. After the final extraction, residues together with the light fraction trapped on the membrane filter were washed 5 times with deionized water, oven dried at 75 °C and ground to powder using a mortar and pestle for NMR analysis.

Whole HF-treated soil samples were packed in cylindrical zirconia rotors before analysis. The nuclear magnetic resonance (NMR) analysis was done on a 400 MHz Bruker AVANCE III spectrometer equipped with a 4 mm VTN multinuclear double resonance magic angle spinning probe, operating at room temperature. The ^{13}C NMR spectra were recorded at 100.6 MHz, using the cross polarization magic angle spinning (CPMAS) technique. A rotating speed of 14 000 Hz was used with a contact time of 1 minute, a recycle delay of 1 second and an acquisition time of 12.8 minutes. All the spectra were recorded with 86 016 scans. The following ranges were integrated: 0–50 ppm (alkyl-C), 50–110 ppm (O-alkyl C), 110–160 ppm (aromatic C), and 160–180 ppm (carbonyl C).

2.2.5 Statistical analysis

The experiment was of completely randomized block design in a factorial arrangement. However, because some of the treatments that involved no-tillage and stubble mulch tillage were not representative (e.g. residue was burned and mechanical weed control method was applied) of conservation agriculture, such treatments were excluded during sample selection. Statistical analyses were performed with SPSS version 24 software package (SPSS Inc.). A one-way analyses of variance (ANOVA) was used and means were compared with Tukey's honestly significant difference post-hoc test (HSD_T). All data were tested for normality and homogeneity using Shapiro-Wilk and Levene's test, respectively before ANOVA were carried out. Statistical analyses were performed at 95% confidence level. Pearson's correlation coefficients were calculated to assess relationships among the measured variables. For ^{13}C NMR spectra, three replicate soil samples per treatment combination were mixed thoroughly and subjected to NMR spectroscopy as composite samples due to high costs and time needed to obtain spectra, as such no statistical analyses were performed.

2.3 Results

2.3.1 Soil organic and inorganic carbon

Applied treatment combinations had significant effects on SOC and SIC (Figure 2.1). The SOC and SIC concentrations were higher ($P < 0.05$) in the plots that received a combination of unburned straw, no-tillage and chemical weeding compared to treatment combinations that involved mouldboard ploughing regardless of the method of straw management and weed control. A significant ($P < 0.05$) increase in SIC was also detected in the unburned-no-tilled-chemically weeded plots relative to unburned-stubble mulched-chemically weeded treatments. The SOC and SIC values, respectively ranged from 9.68 and 0.30 g kg^{-1} in the unburned-ploughed chemically weeded plots to 13.86 and 3.99 g kg^{-1} in the unburned-no-tilled-chemically weeded plots.

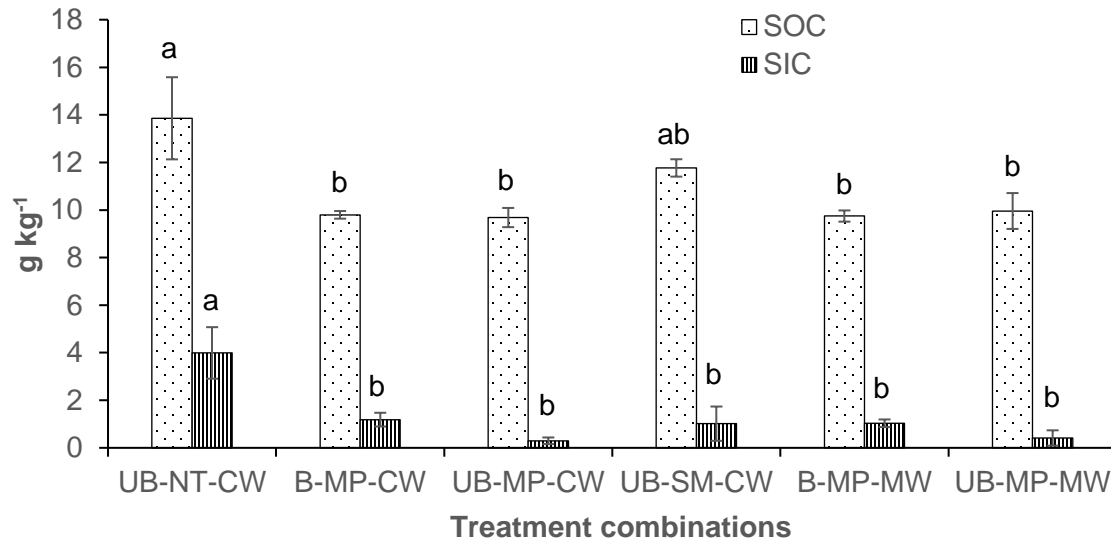


Figure 2.1 Dynamics of soil organic (SOC) and inorganic (SIC) carbon as influenced by long-term wheat production management practices in the upper 0-50 mm layer. Significant differences ($P < 0.05$) are indicated by different letters for each fraction. Vertical bars with horizontal caps indicate standard deviation. UB-NT-CW, unburned x no-tillage x chemical weeding; B-MP-CW, burned x mouldboard plough x chemical weeding; UB-MP-CW, unburned x mouldboard plough x chemical weeding; UB-SM-CW, unburned x stubble mulch x chemical weeding; B-MP-MW, burned x mouldboard plough x mechanical weeding; UB-MP-MW, unburned x mouldboard plough x mechanical weeding.

2.3.2 Labile carbon fractions

Not all the three labile C fractions (e.g. HWEC) were significantly ($P < 0.05$) affected by the applied treatment combinations (Figure 2.2). The CWEC concentrations were only affected by the burned straw-mouldboard plough-chemical weeding combination, which reduced ($P < 0.05$) CWEC levels (616.49 vs 1081.18 mg kg⁻¹) relative to unburned-no-tillage-chemical weeding interaction. Treatment combinations that involve mouldboard plough whether wheat straw was burned or left unburned and weeds controlled by herbicides or mechanically resulted in lower ($P < 0.05$) levels of POXC compared to no-tillage combined with unburned wheat straw and chemical weeding. If

accepted that the combination of no-tillage served as reference, combinations that involved mouldboard ploughing resulted in a loss of 27% POXC on average.

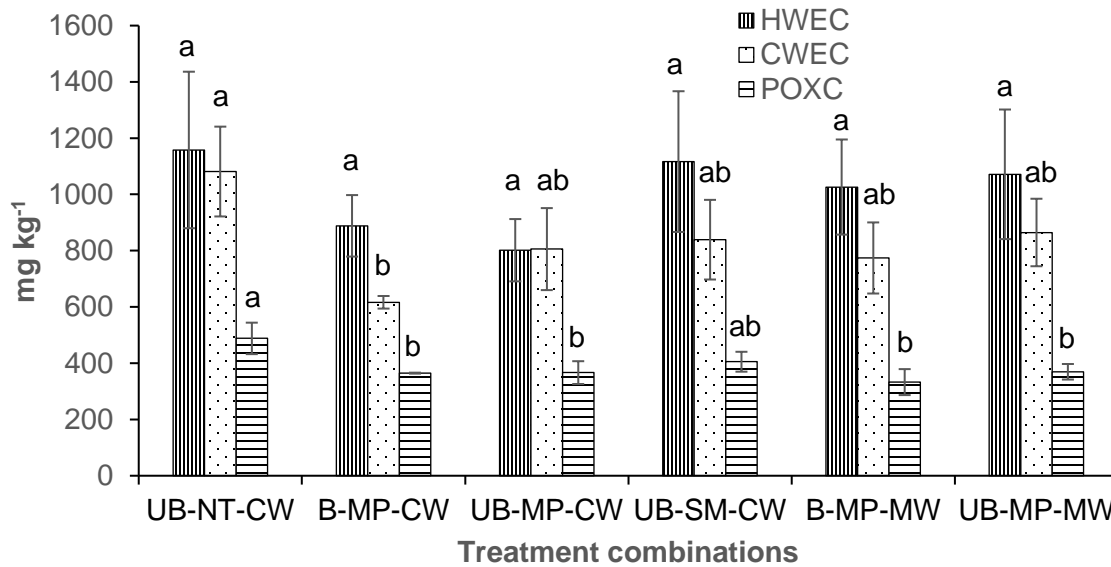


Figure 2.2 Effects of wheat production management practices on labile carbon (C) fractions in the upper 0-50 mm soil layer. Significant differences ($P < 0.05$) are indicated by different letters for each fraction. Vertical bars with horizontal caps indicate standard deviation. HWEC, hot water extractable carbon; CWEC, cold water extractable carbon; POXC, permanganate oxidizable carbon; UB-NT-CW, unburned x no-tillage x chemical weeding; B-MP-CW, burned x mouldboard plough x chemical weeding; UB-MP-CW, unburned x mouldboard plough x chemical weeding; UB-SM-CW, unburned x stubble mulch x chemical weeding; B-MP-MW, burned x mouldboard plough x mechanical weeding; UB-MP-MW, unburned x mouldboard plough x mechanical weeding.

2.3.3 Humic substances

Among the three humic fractions, only C_{EX} and C_{FA} concentrations showed significant changes as a result of long-term wheat production management practices (Figure 2.3). The C_{EX} and C_{FA} concentrations increased significantly ($P < 0.05$) as tillage intensity decreased, with the highest

values recorded in the unburned chemically weeded no-tilled treatments. However, the C_{EX} values did not differ significantly between no-tilled, ploughed and stubble mulched plots where wheat straw was left unburned and weeds were treated with herbicides. Similarly, unburned wheat straw and chemical weeding also did not change C_{FA} significantly in the no-tilled compared to stubble mulched plots. The C_{EX} and C_{FA} concentrations varied from 8.41 and 5.83 $g\ kg^{-1}$, respectively in the burned ploughed chemically weeded plots to 10.86 and 8.78 $g\ kg^{-1}$, respectively in the unburned no-tilled herbicide treated plots.

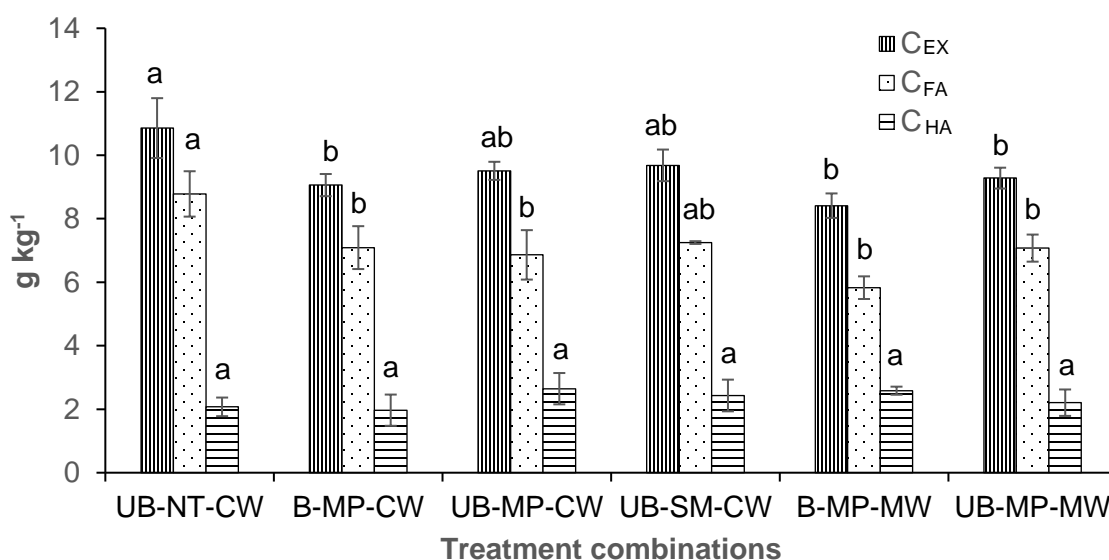


Figure 2.3 Interactive effects of wheat straw management, tillage and weed control methods on soil humic substances; C_{EX} , extractable humic substances, C_{HA} , humic acids; C_{FA} , fulvic acids. Significant differences ($P < 0.05$) are indicated by different letters for each fraction. Vertical bars with horizontal caps indicate standard deviation. UB-NT-CW, unburned x no-tillage x chemical weeding; B-MP-CW, burned x mouldboard plough x chemical weeding; UB-MP-CW, unburned x mouldboard plough x chemical weeding; UB-SM-CW, unburned x stubble mulch x chemical weeding; B-MP-MW, burned x mouldboard plough x mechanical weeding; UB-MP-MW, unburned x mouldboard plough x mechanical weeding.

2.3.4 Structural composition of soil organic carbon

Figure 2.4 shows a solid-state CPMAS ^{13}C NMR spectra of a soil cropped annually with winter wheat and interpretations were done according to Baldock *et al.* (1992; 1997) and Kögel-Knabner (1997, 2002). In the 0-50 ppm, resonances at 28-33 ppm are indicative of the presence of aliphatic C in long chain polymethylene structures. Distinctive shoulders appearing around 25 ppm in some spectra (UB-MP-MW, B-MP-MW and B-MP-CW) arise from short chain methyl C. In the 50-110 ppm chemical shift region, signals appearing in the vicinity of 50-61 ppm originate from methoxyl C structures of lignin, but can also be assigned to amine C of proteins. Dominating signals at 61-75 ppm are often ascribed to oxygenated C of carbohydrate structures. Peaks resonating at 103-105 ppm (di-O-alkyl C) region are characteristic of anomeric C of polysaccharides. Broad bands in the 125-130 ppm chemical shift confirm the presence of C- and hydrogen- (H) substituted aromatic C predominantly of lignin origin, while peaks displayed at 169-173 ppm are typical of carboxylic, ester and amide C groups.

Distributions of ^{13}C over chemical shift regions in the spectra are presented in Table 2.1. The highest alkyl C was recorded in the ploughed plots subjected to unburned straw and mechanical weeding (23%), followed in a descending order by the burned mechanically weeded ploughed plots (22%), unburned chemically weeded stubble mulched plots (22%), unburned chemically weeded ploughed plots (20%), unburned chemically weeded no-tilled plots (18%) and burned chemically weeded ploughed plots (17%). Oxygenated carbohydrates were higher in no-tilled (55%) and stubble mulched (50%) plots due to unburned straw and chemical weeding compared to the rest of the treatment combinations. Aromatic C ranged from 21% in unburned chemically weeded no-tilled to 31 % in the burned mechanically weeded ploughed plots, while carbonyl C varied from 4% in the burned mechanically weeded ploughed to 8% in unburned mechanically weeded ploughed plots.

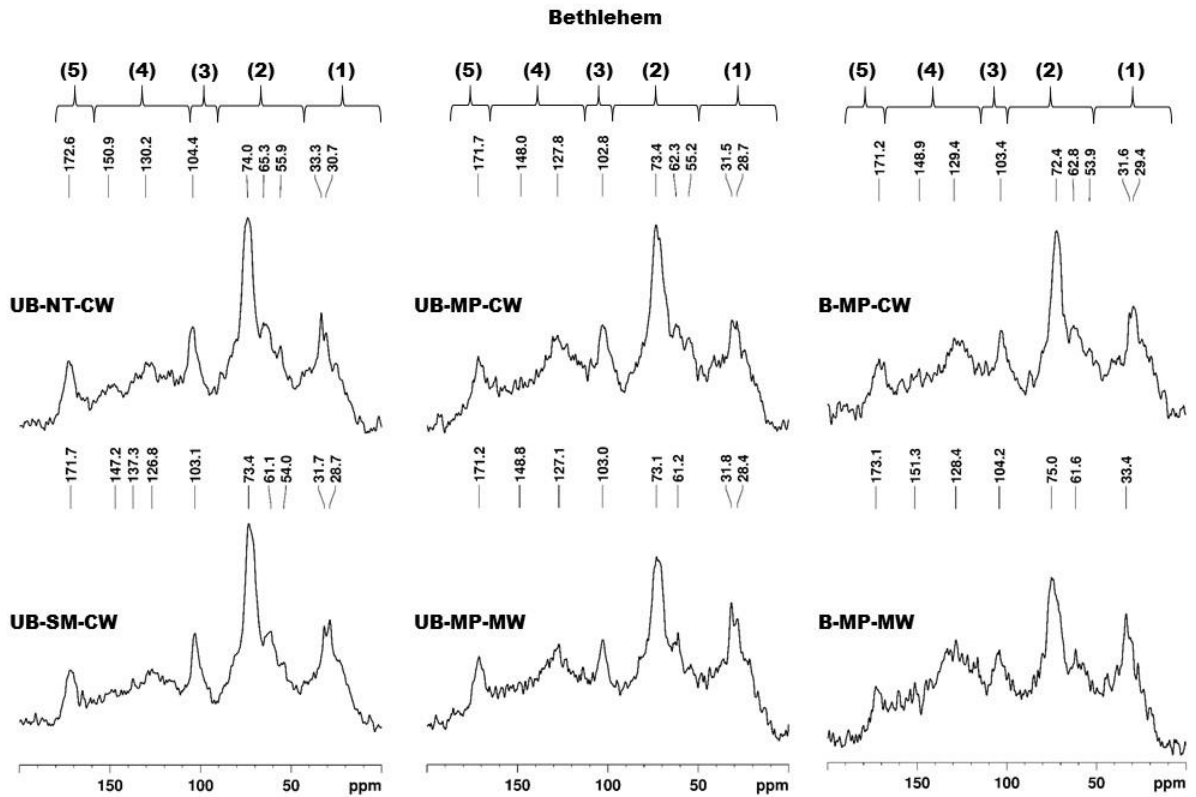


Figure 2.4 Solid state CPMAS ^{13}C NMR spectra of surface soil (0-50 mm) cropped annually with winter wheat. UB-NT-CW, unburned x no-tillage x chemical weeding; UB-MP-CW, unburned x mouldboard plough x chemical weeding; B-MP-CW, burned x mouldboard plough x chemical weeding; UB-SM-CW, unburned x stubble mulch x chemical weeding; UB-MP-MW, unburned x mouldboard plough x mechanical weeding; B-MP-MW, burned x mouldboard plough x mechanical weeding. (1), alkyl C; (2) O-alkyl C; (3), di O-alkyl C; (4), aromatic C; (5), carbonyl C.

Table 2.1 Relative concentrations (%) of alkyl C, O-alkyl C, aromatic C and carbonyl C as affected by wheat production management practices in the 0-50 mm layer

Functional groups	Treatment combinations					
	UB-NT-CW	B-MP-CW	UB-MP-CW	UB-SM-CW	B-MP-MW	UB-MP-MW
Alkyl C	18	22	20	22	17	23
O-alkyl C	55	45	49	50	48	43
Aromatic C	21	26	24	22	31	26
Carbonyl C	6	7	7	6	4	8

UB-NT-CW, unburned x no-tillage x chemical weeding; B-MP-CW, burned x mouldboard plough x chemical weeding; UB-MP-CW, unburned x mouldboard plough x chemical weeding; UB-SM-CW, unburned x stubble mulch x chemical weeding; B-MP-MW, burned x mouldboard plough x mechanical weeding; UB-MP-MW, unburned x mouldboard plough x mechanical weeding.

2.3.5 Extent of decomposition

The HI and PI values did not vary much between the applied treatment combinations except in no-tillage combinations where both HI and PI values were significantly ($P < 0.05$) lower compared to those recorded in the unburned ploughed chemically weeded treatments and burned ploughed mechanically weeded plots, respectively (Table 2.2). The HI values ranged from 0.15 in no-tilled treatments to 0.28 in the unburned ploughed plots where mechanical weeding was used as a mode of weed control. The PI values were in the range of 0.24-0.44, whereby no-tillage combination conceded the lowest values. Although alkyl C/O-alkyl C ratios were not statistically tested, they exhibited more or less the same pattern as the HI and PI. The alkyl C/O-alkyl C varied slightly between treatment combinations, from 0.34 to 0.52. The lowest values were observed in no-tilled plots that received unburned straw and herbicides and the highest in the ploughed plots subjected to unburned wheat straw and mechanical weeding.

Table 2.2 Response of indices of soil organic carbon decomposition to different wheat production management systems

Treatment combinations	Degree of decomposition		
	HI	PI	Alkyl C/O-alkyl C
UB-NT-CW	0.15±0.01a	0.24±0.02a	0.34
B-MP-CW	0.20±0.04ab	0.28±0.09ab	0.49
UB-MP-CW	0.28±0.06b	0.39±0.11ab	0.41
UB-SM-CW	0.21±0.05ab	0.34±0.07ab	0.44
B-MP-MW	0.27±0.01ab	0.44±0.03b	0.36
UB-MP-MW	0.22±0.04ab	0.31±0.07ab	0.52

Significant differences ($P < 0.05$) are indicated by different letters for each ratio. HI, humification index; PI, polymerization index; UB-NT-CW, unburned x no-tillage x chemical weeding; B-MP-CW, burned x mouldboard plough x chemical weeding; UB-MP-CW, unburned x mouldboard plough x chemical weeding; UB-SM-CW, unburned x stubble mulch x chemical weeding; B-MP-MW, burned x mouldboard plough x mechanical weeding; UB-MP-MW, unburned x mouldboard plough x mechanical weeding.

2.4 Discussion

2.4.1 Soil organic and inorganic carbon

Surface accumulation of wheat straw was of much significance in no-tillage treatments as it slows down the rate of residue decomposition and mineralization processes compared to when crop residues are incorporated in the soil (Bakht *et al.*, 2009; Chen *et al.*, 2009). Therefore, higher SOC in the unburned no-tilled chemically weeded plots could be attributed to a constant placement of crop residues at or near the soil surface after harvesting with limited soil disturbance as opposed to mouldboard ploughed plots where the burned or unburned wheat straw was incorporated in the soil. In the tropical central region of Brazil, Carvalho *et al.* (2009) also found that incorporation of crop residues by tillage accelerated residue decomposition compared to no-

tillage, and cited intimate contact of crop residues with the soil mineral phase in the ploughed plots as the main cause.

Soil and crop residue mixing favours biological activity and diversity, consequently increases decomposition rates and SOC losses. Many studies, including the current results substantiated this statement (Carvalho *et al.*, 2009; Clayton, 2012; Moussadek *et al.*, 2014). Contrarily, results obtained in the arid region of Cardoba province, Argentina showed that the effects of tillage management are site-specific (Vázquez *et al.*, 2016). In their study, SOC in the cultivated soils was found to resemble that in the virgin soils. Such unusual SOC responses in the cultivated soils were associated with crop irrigation and fertilization, which improved the phytomass returned to the soil and masked the losses. Fertilizer applications also probably regulated microbial activity and slowed down SOC decomposition rates (Gregorich *et al.*, 1994).

Plant biomass was not sampled in this study; however, grain yield and the harvest index, which was assumed to be 50% for wheat under rain fed conditions (Dr Ceronio, personal communication) were used to estimate the amount of plant residues returned to the soil after harvesting (Biomass = Yield/0.5). If these calculations are accepted, on average, 4.00-4.36 t ha⁻¹ plant biomass has been returned to the ploughed treatments since the inception of this trial in 1979 compared to no-tilled plots where 3.64 t ha⁻¹ wheat straw was recycled (Table 2.3). This suggests that long-term cultivation of arid to semi-arid soils under dryland crop production has detrimental effects on SOC and inherent fertility regardless of the residue quantity incorporated in the soil (Lobe *et al.*, 2001; Kotzé & Du Preez, 2007; Chen *et al.*, 2009; Loke *et al.*, 2012).

Rapid SOC decomposition increases production of soil CO₂ and accelerates dissolution of carbonates, restricting SIC re-precipitation and accrual, which could have been the case in this study (Wu *et al.*, 2009; Shi *et al.*, 2012). This is confirmed by the results obtained in the eastern part of Northern China where intensive soil cultivation resulted in a substantial loss (around 51%)

Table 2.3 Quantities of plant biomass ($t\ ha^{-1}$) returned to the soil after harvesting since the establishment of the wheat trial in near Bethlehem

Year	Treatment combinations					
	UB-NT-CW	B-MP-CW	UB-MP-CW	UB-SM-CW	B-MP-MW	UB-MP-MW
1979	2.89	3.66	3.33	3.05	3.12	2.92
1981	3.93	4.29	3.57	4.23	4.58	4.82
1982	3.64	2.27	2.77	2.52	3.26	2.49
1983	2.13	2.39	4.00	4.16	2.80	3.36
1984	5.55	5.61	5.42	5.67	6.00	5.54
1985	1.55	2.10	2.12	1.95	2.91	2.50
1986	6.12	7.88	7.02	6.90	7.67	6.83
1987	3.38	5.53	4.12	4.45	5.35	4.83
1988	3.71	5.85	4.87	4.83	5.50	5.09
1991	3.39	3.70	4.49	3.10	3.30	3.65
1993	8.45	9.96	9.04	8.97	10.37	8.48
1994	0.41	0.53	0.73	0.53	0.53	0.73
1995	3.42	5.37	4.52	3.61	2.56	2.91
1996	5.09	5.32	6.40	5.44	5.88	5.70
1997	3.64	4.10	3.87	3.65	3.56	3.62
1998	0.85	1.10	0.95	1.47	1.11	1.11
1999	3.79	4.39	3.38	3.53	4.15	3.55
Mean	3.64	4.36	4.15	4.00	4.27	4.01

UB-NT-CW, unburned x no-tillage x chemical weeding; B-MP-CW, burned x mouldboard plough x chemical weeding; UB-MP-CW, unburned x mouldboard plough x chemical weeding; UB-SM-CW, unburned x stubble mulch x chemical weeding; B-MP-MW, burned x mouldboard plough x mechanical weeding; UB-MP-MW, unburned x mouldboard plough x mechanical weeding.

of SIC compared to native grasslands (Wu *et al.*, 2009). Exposure of calciferous layers by tillage to erosive forces was also attributed to such losses. Lobe *et al.* (2001; 2002) also reported incidences of wind eroded silt-associated C in the cultivated soils of Harrismith, not far from our experimental site. As such, another possible reason for lower SOC and SIC in the ploughed plots could be displacement of bare C-rich surface soil by erosion (Janzen, 2006). On the contrary, results of this study are inconsistent with those obtained from Russian Chernozem (Mikhailova &

Post, 2006). Higher SIC in the cultivated soils were presumed to be due to fertilizer and organic manure applications and released base cations (Ca^{2+} and Mg^{2+}) during SOC decomposition that resulted in SIC formation and accumulation (Mikhailova & Post, 2006; Wu *et al.*, 2009; Shi *et al.*, 2012; Lal *et al.*, 2015).

In our case, the pH and base cations (Ca^{2+} and Mg^{2+}) values were almost the same across the sampled treatment combinations (Table 2.4), and therefore did not contribute to changes in SIC. However, strong positive relationships between SIC and POXC ($r = 0.70^{**}$, $P < 0.01$), C_{EX} ($r = 0.63^{**}$, $P < 0.01$) and C_{FA} ($r = 0.72^{**}$, $P < 0.01$) indicate that these SOC fractions stimulated SIC re-precipitation and accumulation in no-tillage combinations, while a negative correlation with PI ($r = -0.52^*$, $P < 0.05$) (Table 2.5) implies that increased decomposition in the ploughed and to some extent stubble mulched plots constrained SIC accrual (Bronick & Lal, 2005; Lal *et al.*, 2015).

Table 2.4 Chemical soil properties of the selected samples (Kotzé, 2004)

Treatment combinations	Chemical properties		
	pH (H_2O)	Calcium (mg kg^{-1})	Magnesium (mg kg^{-1})
UB-NT-CW	5.37	660	106
B-MP-CW	5.39	694	79
UB-MP-CW	5.56	709	87
UB-SM-CW	5.12	590	84
B-MP-MW	5.55	661	83
UB-MP-MW	5.29	615	88

UB-NT-CW, unburned x no-tillage x chemical weeding; B-MP-CW, burned x mouldboard plough x chemical weeding; UB-MP-CW, unburned x mouldboard plough x chemical weeding; UB-SM-CW, unburned x stubble mulch x chemical weeding; B-MP-MW, burned x mouldboard plough x mechanical weeding; UB-MP-MW, unburned x mouldboard plough x mechanical weeding.

Table 2.5 Pearson's correlation coefficients (r) estimated among different soil C fractions

	SOC	SIC	C _{EX}	C _{HA}	C _{FA}	POXC	CWEC	HWEC
SOC	-							
SIC	ns	-						
C _{EX}	0.63**	0.65**	-					
C _{HA}	ns	ns	ns	-				
C _{FA}	ns	0.72**	0.90**	-0.50*	-			
POXC	0.59*	0.70**	0.84**	ns	0.87**	-		
CWEC	ns	0.51*	0.50*	ns	ns	0.68**	-	
HWEC	0.71**	ns	0.48*	ns	ns	0.48*	ns	-
HI	ns	ns	ns	0.82**	-0.75**	-0.63**	ns	ns
PI	ns	-0.52*	-0.49*	0.90**	-0.81**	-0.63**	ns	ns

** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed); ns, non-significant. SOC, soil organic carbon; SIC, soil inorganic carbon, C_{EX}, extractable humic substances; C_{HA}, humic acids; C_{FA}, fulvic acids; POXC, permanganate oxidizable carbon; CWEC, cold water extractable carbon; HWEC, hot water extractable carbon; HI, humification index; PI, polymerization index.

2.4.2 Labile carbon fractions

Although the applied treatment combinations induced changes in SOC, this property is often slow to change due to a myriad of C compounds is made of (Janzen, 2005). Vázquez *et al.* (2016) also indicated that soil quality depends mostly on the quantities of individual fractions other than on total SOC. Some of these C fractions are sensitive enough to monitor short- and medium-term SOC changes as a result of soil management (Gregorich *et al.*, 1994; Janzen, 2005; Chen *et al.*, 2009; Vázquez *et al.*, 2016). Results of this study revealed that POXC and CWEC were sensitive

labile fractions that distinguished changes in SOC as a result of the applied treatment combinations.

The quantities of these fractions observed in this study are lower, but consistent with those reported in other studies conducted under similar climatic conditions (Sequeira & Alley, 2011; Vázquez *et al.*, 2016). The lower quantities are probably due to site-specific conditions. For example, in this trial, wheat has been produced under monoculture, while in other areas irrigation is included as part of management, fallow periods are replaced with cover crops and crop rotation is practiced where high biomass producing crops are rotated with legumes, which recharge the labile pool upon litter or plant fall (Sequeira & Alley, 2011; Vázquez *et al.*, 2016).

According to Chen *et al.* (2009), no-tillage not only restricted intimate interactions between crop residues and mineral particles, but also preserved labile C fractions through soil aggregation and therefore limited their accessibility to the decomposer community, which could be the case in the present study. Additionally, POXC and CWEC as reservoirs of plant nutrients and energy source for soil organisms were perhaps preserved biologically through microbial immobilization in no-tilled treatment combinations (Baldock *et al.*, 1992; 1997). However, this phenomenon could be an area of interest within soil science researcher as it has not been explored extensively. Higher POXC and CWEC in the treatment combinations that included no-tillage was due to exclusion of excessive soil mixing by tillage, and therefore suggest that SOC therein was less humified compared to that in the cultivated treatment combinations. The positive correlation between SOC and POXC ($r = 0.59^*$, $P < 0.05$) and CWEC ($r = 0.71^{**}$, $P < 0.01$) indicated linear increase in these labile fractions as SOC increased (Table 2.5). Chen *et al.* (2009) also reported strong positive correlations between SOC and labile C fractions, including POXC and CWEC, and indicated that such correlations suggested the lability nature of SOC due to regular additions of crop residues in no-tillage treatments as opposed to their removal in the ploughed plots. However, our results revealed that even if equal amounts of residues are added in no-tilled and ploughed plots (Table

2.3), regular cultivation will always provide ideal conditions for their rapid decomposition and SOC loss until an equilibrium state is reached.

2.4.3 Humic substances

Equilibrium state of SOC is often reflected by lower concentrations of labile C fractions and higher recalcitrant fractions. Partial burning of crop residues also remove most of the fragile and flammable plant parts consisting of labile organic compounds, leaving behind plant parts that are more resistant to microbial decomposition. However, an increase in C_{EX} and C_{FA} in no-tilled plots where wheat straw was left unburned and weeds treated with herbicides, particularly when compared to the ploughed plots where straw was burned and weeds controlled mechanically is an indication that exclusion of cultivation stimulated C_{EX} and C_{FA} formation and SOC was less humified (Guimarães *et al.*, 2013; Seddaiu *et al.*, 2013). The positive correlations (Table 3) between labile C fractions (POXC, CWEC and HWEC) and C_{EX} and C_{FA} also demonstrated that these fractions are more or less the same with regard to their chemical composition (Sequeira & Alley, 2011). The distribution of ^{13}C in the chemical shift intervals of the NMR spectra reported by Spaccini *et al.* (2006) revealed that C_{FA} contained higher carbohydrates relative to C_{HA} . Moral *et al.* (2005) also reported strong positive relationships between C_{FA} and carbohydrates, indicative that C_{FA} is highly labile and readily available energy source for soil organisms compared to C_{HA} (Guimarães *et al.*, 2013). The negative correlations between C_{EX} and PI ($r = -0.49^*$, $P < 0.05$) as well as between C_{FA} and HI ($r = -0.82^{**}$, $P < 0.01$) and PI ($r = -0.90^{**}$, $P < 0.01$) are in support of this argument, and further demonstrated that increased humification and polymerization processes deprived cultivated treatments of C_{EX} and C_{FA} (Table 2.5).

According to these results, C_{EX} and C_{FA} are enriched with labile compounds and therefore imply that oxidative losses of humic substances begin with the two before C_{HA} is affected (Vázquez *et al.*, 2016). Thus prolonged soil cultivation destabilized the balance between humification and mineralization of SOC by accelerating mineralization process, resulting in lower C_{EX} and C_{FA} (Sun

et al., 2012; Guimarães *et al.*, 2013; Kotzé *et al.*, 2016). However in the three semi-arid agro-ecosystems located in central South Africa, Kotzé *et al.* (2016) found that prolonged cultivation deprived soils of C_{EX} , C_{FA} and C_{HA} , except in Kroonstad where C_{FA} in the cultivated soils resembled that in the adjacent native grasslands. Such behaviour was attributed to warm and dry soil conditions in Kroonstad that certainly restricted microbial activity and the breakdown of C_{FA} . In other words, decomposition of humic substances does not only depend on how long the soil has been cultivated, but also on local conditions such as climate, soil texture and vegetation.

In general, residue incorporation by tillage not only subjected freshly added crop remnants to rapid microbial decomposition, but also affected stable C (C_{EX} and C_{FA}) fractions. With that said, this study revealed that mean residence times or accumulation of recalcitrant C fractions are not exclusively dependent on their selective preservation, but also on microbial availability and soil management (Kögel-Knabner, 2002; Kotzé *et al.*, 2016). Thus, contrary to mouldboard ploughing, higher C_{EX} and C_{FA} in no-tillage treatments could be due to slower decomposition rates of wheat straw and high labile C fractions (Guimarães *et al.*, 2013; Sun *et al.*, 2012). Turnover rates of C_{EX} and C_{FA} are more rapid than those of C_{HA} , as such frequent supply of organic materials to the soil and slow mineralization constantly recharge labile C of humic substances (C_{EX} and C_{FA}) (Moral *et al.*, 2005; Kotzé *et al.*, 2016; Vázquez *et al.*, 2016).

2.4.4 Structural composition of soil organic carbon

Concentrations of the four SOC functional groups followed the order: O-alkyl C > aromatic C > alkyl C > carbonyl C across the sampled treatment combinations (Figure 2.4 and Table 2.1). Oxygenated C and anomeric C1 of cellulose and hemicellulose were the major contributors to O-alkyl C, which decreased from 55 to 43% as tillage intensity increased. This indicates that minimum soil disturbance accompanied by surface retention of crop residues in no-tillage and stubble mulch tillage recovered labile C fractions unlike in the ploughed plots where unburned or burned straw was incorporated in the soil, thereby stimulating rapid crop residue decomposition

and loss of O-alkyl C. Dieckow *et al.* (2009) also reported higher O-alkyl C in no-tilled plots compared to the ploughed treatments, and ascribed the observed response to the interaction of O-alkyl C with oxide minerals and/or occlusion by soil aggregates in no-tilled treatments.

Although it is difficult to separate effects of crop residue management from tillage effects, unburned straw combined with chemical weeding seemed to be the most influential combination as it also increased O-alkyl C by 1 to 6% in the ploughed plots compared to unburned straw combined with mechanical weeding or burned straw combined with either chemical or mechanical weeding. This is not surprising because burning removed most combustible plant parts, which are rich in labile and easily digestible organic compounds like carbohydrates and amino acids (Kavdir *et al.*, 2005), while mechanical weeding accelerated the fate of already decomposing residues due to mouldboard ploughing. Burning might have also reduced soil microbes and their source of energy, and therefore limited re-synthesis of O-alkyl C (Baldock *et al.*, 1992; 1997).

Many reports have it that during the initial phase of decomposition, O-alkyl C is the first SOC component to decrease, as it is dominated by easily oxidizable organic compounds (Mathers *et al.*, 2003; Helfrich *et al.*, 2006; Spaccini *et al.*, 2006; Carvalho *et al.*, 2009). A decrease in O-alkyl C due to SOC decomposition is often accompanied by an increase in alkyl, aromatic and carbonyl C (Baldock *et al.*, 1992; Mathers & Xu, 2003). However, results of this study exhibited a slightly different pattern in some treatments as the lowest alkyl and carbonyl C (17 and 4%, respectively) were observed in the burned mechanically weeded ploughed plots (Table 2.1). This probably had something to do with differences in fire intensity or the used weed control method, because in the other burned but chemically weeded ploughed plots, the lowest alkyl and carbonyl C accounted for 22 and 7%, respectively. Higher aromatic C in the burned treatments and lower alkyl and carbonyl C is an indication that SOC was dominated by black C (Kavdir *et al.*, 2005), while in the plots where wheat straw was left unburned could imply that SOC therein was dominated lignin units, which are also resistant to microbial degradation (Kögel-Knabner, 1997; 2002).

Despite that these results showed that the degree of decomposition was lowest in unburned chemically weeded no-tilled plots and highest in the unburned mechanically weeded ploughed plots, this suggests that SOC in the latter was more humified and richer in recalcitrant aliphatic C derived from lipids, fatty acids and waxes and lower in carbohydrates (Baldock *et al.*, 1992; Helfrich *et al.*, 2006; Hilscher *et al.*, 2009). Thus, soil organisms utilized carbohydrates resulting in higher polymethylene C due to its selective preservation and/or *in situ* synthesis (Baldock *et al.*, 1992; Kögel-Knabner, 2002).

2.4.5 Extent of decomposition

The HI, PI and alkyl C/O-alkyl C values reflected the degree of SOC decomposition and polymerization (Baldock *et al.*, 1992; Guimaraes *et al.*, 2013). Thus, lower levels of HI, PI and alkyl C/O-alkyl C in no-tilled treatment combinations is indicative of mobile and less humified SOC compared to the ploughed plots (Mathers *et al.*, 2003; Helfrich *et al.*, 2006; Sun *et al.*, 2012; Guimarães *et al.*, 2013). In other words, constant supply and surface placement of wheat straw in no-tillage constrained rapid decomposition and humification processes, resulting in higher concentrations of labile C fractions including C_{EX} and C_{FA} (Sun *et al.*, 2012; Vázquez *et al.*, 2016). Thus, levels of recalcitrant C fractions (C_{HA}) in the cultivated landscapes are relative, implying that they increase as the extent of humification-polymerization processes increase. This statement holds truth according to the positive correlations detected between C_{HA} and HI ($r = 0.82^{**}$) and PI ($r = 0.90^{**}$), which also indicate that C_{HA} is a marker of SOC stabilization (Table 2.5). Despite that, all the HI, PI and alkyl C/O-alkyl C values were less than one across the sampled treatment combinations (Table 2.2), and that implies lower aromaticity of C_{HA} , higher labile C fractions and good quality SOC that can improve soil biological fertility and nutrient cycling for plant nutrition (Guimarães *et al.*, 2013).

2.5 Conclusion

Semi-arid soils have low soil C due to dry and warm climates. Incorporation of wheat straw, burned or left unburned coupled with chemical or mechanical weed control methods did not bring any significant changes in SOC, SIC, labile C and humic substances in the ploughed plots. However, this study revealed that replacement of mouldboard ploughing with conservation tillage can improve atmospheric C sequestration in this region of South Africa. Results of this study further indicated that long-term use of conservation tillage systems, particularly no-tillage under semi-arid conditions, has a potential to restore historic C and keep it in the soil. Thus, although no-tillage treatment combinations resulted in an accrual of less humified SOC, which is considered to be a source rather than a sink for atmospheric C, lack of soil mixing improves macro-aggregates within which micro-aggregates are formed to occlude SIC, decomposable and recalcitrant C fractions (Baer *et al.*, 2015). Occlusion of soil C fractions within aggregates restricts microbial accessibility, while at the same time stabilizing even labile C fractions. However, further research is needed, as the location and stabilization of C in the soil are still poorly understood.

Chapter 3

Dynamics of soil carbon concentrations and quality induced by agricultural land use in central South Africa

Abstract

Prolonged soil cultivation has been identified as a major cause of land degradation and a threat to soil quality, particularly in drought-prone ecological regions. This study evaluated effects of land use change on different soil carbon (C) fractions and organic C quality across three semi-arid agro-ecosystems in central South Africa. Selected soil samples were collected from primary grasslands, croplands and secondary pastures at the 0-200 mm layer in each agro-ecosystem and analyzed for soil C, organic C (SOC), inorganic C (SIC), permanganate oxidizable C (POXC), cold and hot water extractable C (C_{WEC} and C_{HWC}), C in extractable humic substances (C_{EX}), humic acids (C_{HA}) and fulvic acids (C_{FA}). The SOC structure was characterized with cross polarization magic angle spinning (CPMAS) ¹³C nuclear magnetic resonance (NMR) spectroscopy. All analyses were done on bulk soil samples. Conversion of grasslands into arable cropping resulted in losses of SOC, SIC, POXC, C_{EX}, C_{HA} and C_{FA}, with the magnitude of loss following the order: Harrismith > Tweespruit > Kroonstad. The use of CPMAS ¹³C NMR spectroscopy revealed a decrease in O-alkyl C and increase in aromatic, alkyl and occasionally carbonyl C following cultivation of grassland soils. Virtually unaltered O-alkyl C in Harrismith suggest immobilization and storage of labile C by soil microbes. Reversion of cultivated soils into perennial pastures restored these C fractions, indicative of surface accumulation of plant biomass. In fact, restored soils had higher O-alkyl C in Harrismith and Kroonstad that resembled virgin soils. Surprisingly, cultivated soils in the Tweespruit agro-ecosystem consisted of less humified SOC compare to the restored or virgin soils as revealed by lower humification (HI) and polymerization (PI) indices. Lower HI and PI values in the restored soils of Kroonstad also indicated younger

SOC relative to their counterparts in the other two regions. Besides vegetational, soil textural and climatic differences across the three agro ecosystems, losses or gains of the measured C fractions and SOC qualitative changes were also influenced by initial C concentrations before conversion to arable cropping or secondary perennial pastures. Regardless, secondary pasture management generally proved its capability to reverse soil degradation in these drought-threatened areas.

Keywords: CPMAS ^{13}C NMR spectroscopy, Carbon fractions, Semi-arid agro-ecosystems, Soil restoration

3.1 Introduction

Grasslands, which were at some point deemed infertile and unsuitable for crop production, are continuously converted into arable cropping in an attempt to meet food demand for the growing population (Herrick *et al.*, 2012). Prolonged cultivation of these lands results in soil degradation and threatens environmental quality and agricultural sustainability through reduced vegetative cover and nutrient retention and increased breakdown of soil aggregates (Lobe *et al.*, 2001; 2002; Janzen, 2006; Von Lützow *et al.*, 2006). Ultimately, these soils become a source rather than a sink for atmospheric carbon dioxide (CO_2). Restoration of these physical and chemical properties and regulation of CO_2 emissions into the atmosphere are possible with land use and management systems that preserve soil carbon (C) (Preger *et al.*, 2010; Lauer *et al.*, 2011; Kösters *et al.*, 2013; Kotzé *et al.*, 2016).

Soil C is an ecosystem regulator that exists in two discrete compartments: soil organic (SOC) and inorganic (SIC) C. However, majority of studies that measured C sequestration and soil quality changes under arid and semi-arid environments focused mainly on SOC and neglected SIC, despite its vital role in terrestrial C cycling (Batjes, 1996; Wang *et al.*, 2010; Shi *et al.*, 2012). Understanding the dynamics of SOC and SIC is crucial as they are both involved in C transfers

between terrestrial biomes and the atmosphere (Wu *et al.*, 2009; Shi *et al.*, 2012). Moreover, in arid to semi-arid regions, which cover approximately 40% of the earth's total land surface, SIC concentrations sometimes exceed those of SOC (Batjes, 1996; Wu *et al.*, 2009; Shi *et al.*, 2012). Limited plant biomass production as a result of high temperatures and low-erratic rainfall events is poised to be the major precursor of low SOC in semi-arid regions (Abril *et al.*, 2013; Vázquez *et al.*, 2016).

The quantity and quality of primary biomass and other organic inputs are among other factors that influence SOC; however, it is the quality of these organic materials that defines SOC - a complex heterogeneous mixture of non-humic and humic substances residing in the soil. Non-humic substances are composed of a wide range of labile compounds with low molecular weight and rapid turnover rates, whereas humic substances are highly transformed stable compounds with high molecular weight and very slow turnover rates (Gregorich *et al.*, 1994; Von Lützow *et al.*, 2006). Non-humic substances serve as a temporary reservoir for plant nutrients and an energy source for microbial communities, hence are often used as early indicators of soil quality changes as a result of land use and/or soil management (Von Lützow *et al.*, 2006). Humic substances are responsible for remediation of toxic materials, soil structural stability and indicators of atmospheric C sequestration, owing to their recalcitrant nature to microbial degradation and strong associations with the soil mineral component (Von Lützow *et al.*, 2006; Abril *et al.*, 2013; Kotzé *et al.*, 2016; Vázquez *et al.*, 2016).

Soil inorganic C on the other hand, refers to carbonate minerals, which can be divided into lithogenic (primary) carbonates - derived from parent material and pedogenic (secondary) carbonates - formed during soil processes (Batjes, 1996; Mikhailova & Post, 2006; Wang *et al.*, 2010; Sanderman, 2012; Shi *et al.*, 2012; Ahmad *et al.*, 2015). Pedogenic carbonate can be referred to as pedo-lithogenic due to calcium (Ca^{2+}) or magnesium (Mg^{2+}) inherited from parent material or pedo-atmogenic due to Ca^{2+} and/or Mg^{2+} originating from non-carbonate sources

(Sanderman, 2012; Lal *et al.*, 2015). As with SOC, SIC also plays a critical role in nutrient cycling and soil aggregation (Bronick & Lal, 2005), and its formation could result in C sequestration on condition that CO₂ and Ca²⁺ or Mg²⁺ are derived from soil respiration and non-carbonate minerals, respectively (Sanderman, 2012).

Losses of soil C, mainly SOC, due to cultivation are more prevalent in arid and semi-arid regions (Abril *et al.*, 2013; Lal *et al.*, 2015), and have severely affected arable lands resulting in many being abandoned due to inflated reclamation costs or deliberately reverted to perennial pastures to regain their original productivity (Bach *et al.*, 2010; Lauer *et al.*, 2011; Kösters *et al.*, 2013; Baer *et al.*, 2015). The latter has been practiced for over three decades in central region of South Africa in response to the government's call to curb soil degradation (Birru, 2002). The government compensated commercial farmers in Harrismith, Tweespruit and Kroonstad agro-ecosystems of the central region to pilot this initiative. Selection of these three agro-ecosystems was based on their vulnerability to degradation due to a combination of intensive cultivation, semi-arid climates and sandier soil conditions, which then led to the central region losing its status as the 'food basket' for South Africa.

These three sites have previously provided an opportunity to study and understand patterns and rates of SOC loss due to prolonged cultivation (Lobe *et al.*, 2001; 2002; Kotzé *et al.*, 2016) and accrual as a result of reversion of degraded soils to perennial pastures (Preger *et al.*, 2010; Lauer *et al.*, 2011; Kösters *et al.*, 2013). Conservation Reserve Programs of this nature have long been established in many parts of the world. For instance, Baer *et al.* (2015) compared SOC recovery rates following secondary pasture management in the US Tallgrass Prairie and South African Highveld, and found that differences in SOC content at the onset of restoration strongly affect recovery rates. Other local and international studies also indicate that indeed SOC loss is a reversible process under secondary pasture management, but full recovery that resembles primary grasslands is nearly impossible, which therefore calls for long-term secondary pasture

management (Bach *et al.*, 2010; Preger *et al.*, 2010). Moreover, the amassed literature devoted little attention on SIC (Wu *et al.*, 2009; Shi *et al.*, 2012) and the integrated use of quantitative and qualitative procedures to appraise the ability of secondary pasture management to recover SOC fractions under semi-arid environments (Batjes, 1996; Von Lützow *et al.*, 2006; Abril *et al.*, 2013; Vázquez *et al.*, 2016).

Quite a number of analytical tools are available for SOC characterization, ranging from classical extraction procedures to non-destructive spectroscopic methods. Chemical extraction methods are cheaper and do not necessarily require highly skilled labour, but are destructive, tedious and less efficient compared to solid-state spectroscopic techniques, such as ^{13}C nuclear magnetic resonance (NMR) spectroscopy (Baldock *et al.*, 1992; 1997; Kögel-Knabner, 1997; 2002). Despite the limitations, chemical extraction and solid-state ^{13}C NMR spectroscopic procedures can be used to complement each other and better our understanding regarding the fate of organic materials entering the soil as well as the quantity and quality of the resultant SOC (Baldock *et al.*, 1992; Dieckow *et al.*, 2009).

Soil inorganic C is gradually gaining attention, but to our knowledge, no specific procedures are available that can differentiate between lithogenic and pedogenic carbonates or pedo-lithogenic and pedo-atmogenic carbonates unless isotopic C signatures are used (Wu *et al.*, 2009; Shi *et al.*, 2012). Fractionation of SIC with an isotopic C analyzer is also expensive and requires highly trained personnel, hence many studies reported on total inorganic C with little information specifically directed towards the fractions (Shi *et al.*, 2012; Ahmad *et al.*, 2015). Although still under debate, correlations of SOC and SIC sometimes could be used to speculate on SIC fractions (lithogenic and pedogenic carbonates) affected by the applied land use systems (Bronick & Lal, 2005; Wu *et al.*, 2009; Shi *et al.*, 2012). In other words, the presence of SOC indirectly influences SIC or vice versa (Bronick & Lal, 2005).

The aim of this study was therefore to investigate the influence of land use change in the three semi-arid agro-ecosystems on the quantity and quality of SOC and the response of SIC to the applied management systems in the upper 200 mm soil layer. Although the selected sites belong to the semi-arid region, they slightly differ in terms of site-specific conditions such as vegetation, temperature, rainfall and clay content. Slight differences in these local climatic and soil conditions under the current management could significantly influence soil C fractions (Preger *et al.*, 2010). This is particularly important as the loss or recovery of soil C is not only a function of land use, but a collective influence of land use, management and environmental conditions (Preger *et al.*, 2010; Abril *et al.*, 2013; Kotzé *et al.*, 2016). As such, the three agro-ecosystems were also compared to determine the effect of site-specific conditions on the measured soil C fractions and SOC functional groups.

We hypothesized that secondary pasture management can restore historic C to resemble that in the adjacent primary grasslands. It was further hypothesized that the local climatic and soil conditions also mediated recovery of the measured C fractions. If the observations support these hypotheses, this study will add to a database needed to develop a sound soil protection strategy and policy not only for South Africa, but also for other countries with frequent droughts of varying intensity.

3.2 Material and methods

3.2.1 Site description

The chosen three agro-ecosystems include Harrismith, Kroonstad and Tweespruit (Figure 3.1), which vary with altitudes between 1400 and 1800 m above sea level, with Harrismith at the highest and Kroonstad at the lowest location (Table 3.1). Mean annual rainfall (MAR) ranged from 624 mm at Harrismith to 544 mm at Tweespruit of which most rain falls in summer from October to March. The mean annual temperature (MAT) was 13.8 °C at Harrismith, 16.6 °C at Kroonstad

and 14.8 °C at Tweespruit. All three agro-ecosystems had some primary grassland used for livestock farming, mainly cattle. Harrismith is in the Cool Moist Highveld Grassland; Kroonstad in the Dry Sandy Highveld Grassland and Tweespruit in the Cool Dry Highveld Grassland (Bredenkamp *et al.*, 1996). Aridity index (MAR/mean annual evaporation) for each agro-ecosystem is also indicated in Table 3.1. The botanical composition of the natural grassland was dominated by *Cymbopogon plurinodis*, *Themeda triandra*, *Setaria sphacelata*, *Elionurus muticus* and *Eragrostis curvula* at Harrismith, *Eragrostis lehmanniana*, *Eragrostis obtusa*, *Panicum coloratum*, *Stipagrostis uniplumis* and *Pentzia globosa* at Kroonstad, and *Themeda triandra* at Tweespruit. Stocking density was respectively 0.4, 0.5 and 0.6 large stock units per hectare at Harrismith, Kroonstad and Tweespruit with rotational grazing periods of 1 to 3 months in a year (Lobe *et al.*, 2001).

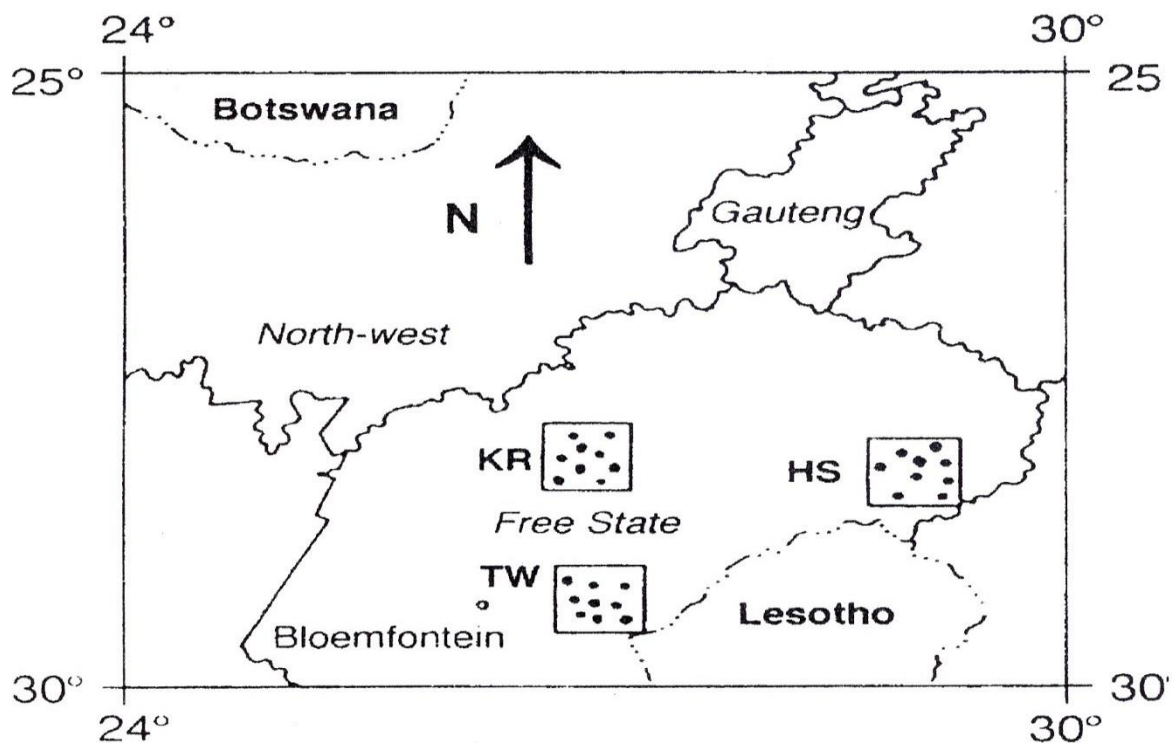


Figure 3.1 Map showing three selected agro-ecosystems; HS, Harrismith; TW, Tweespruit; KR, Kroonstad, in central South Africa.

Table 3.1 Site-specific conditions across the three agro-ecosystems; Harrismith (HS), Tweespruit (TW) and Kroonstad (KR)

Site	Altitude (m)	Climate			Soil form	⁴ Virgin		⁴ Cultivated			⁵ Restored		
		¹ MAR (mm)	² AI	³ MAT (°C)		Clay (%)	pH(H ₂ O)	Clay (%)	pH(H ₂ O)	Years	Clay (%)	pH(H ₂ O)	Years
HS	1800	624	0.36	13.8	Avalon	12.7	5.8	13.1	5.6	20	13.5	5.7	25
TW	1600	544	0.27	14.8	Westleigh	11.6	6.2	12.1	5.9	85	12.6	6.1	25
KR	1400	566	0.28	16.6	Avalon	11.1	6.0	10.6	5.6	20	11.5	5.8	18

¹Mean annual rainfall; ²Aridity index (rainfall/evaporation); ³Mean annual temperature from ARC-ISCW (2011); ⁴Samples from Du Toit *et al.* (1994); ⁵Samples from Birru (2002).

During the past century, some of the primary grasslands that were never cultivated before in the three agro-ecosystems were converted to croplands resulting in different ages under cultivation. This arable land had been ploughed to a depth of 200–300 mm and cultivated with a rotation of wheat, maize and occasionally sunflower. The primary tillage operation was done with mouldboard plough, and when needed mechanical cultivator (tiller) was used to control weeds. At planting, inorganic fertilizer (nitrogen (N), phosphorus (P), potassium (K) and zinc (Zn) in a single or mixture depending on soil analysis and target crop yield) was applied annually (maize: 50-70 kg N, 10-25 kg P, 0-10 kg K ha⁻¹; wheat: 10-40 kg N, 10-25 kg P, 0-15 kg K ha⁻¹; sunflower: 20-50 kg N, 10-20 kg P, 2-6 kg K ha⁻¹) to the dryland crops. Limestone ammonium nitrate (LAN) was also applied, but only on maize after planting to supplement N levels. No organic fertilizer was applied, except for the contribution of dung and urine from animals grazing crop residues during a fallow period. Average grain yields varied from 2.2 to 3.75 t ha⁻¹ for maize, 1.2 to 2.75 t ha⁻¹ for wheat and 1.0 to 1.25 t ha⁻¹ for sunflower. Soils were usually kept in a fallow period for up to six months in the dry season so that the soils retained their stored water (Du Toit *et al.*, 1994; Lobe *et al.*, 2001).

In the past three decades, some croplands of all the three agro-ecosystems were converted to secondary perennial pasture resulting in different ages under conversion. The pasture comprised mostly of *Eragrostis curvula* that was used either for grazing or haying. Inorganic fertilization was mainly restricted to the application of N (LAN) at annual rates of 28–84 kg ha⁻¹ at Harrismith, 0–75 kg ha⁻¹ at Kroonstad and 0–46 kg ha⁻¹ at Tweespruit (Birru, 2002). Soils of these agro-ecosystems were mainly of the Avalon and Westleigh forms according to the South African Soil Classification System (Soil Classification Working Group, 1991), which can be described also as Plinthosols (FAO, 1998) or Plinthustalfs (Soil Survey Staff, 1998). At Harrismith and Kroonstad, the Avalon soil form dominated. This soil has a characteristically brown to dark brown sandy loam orthic A horizon, followed by a yellowish brown to dark brown sandy clay loam apedal B horizon

and then a gray mottled red sandy clay soft plinthic B horizon. At Tweespruit, the Westleigh soil form dominated, which characteristically has a very dark brown sandy loam to sandy clay loam orthic A horizon overlying a dark yellowish brown mottled sandy clay plinthic B horizon.

3.2.2 Soil sampling and selection

Soil samples used in this study were selected from the collections of Du Toit *et al.* (1994) and Birru (2002), which were stored, air-dried and sieved (< 2 mm) in paper bags in a cool, dry and dark room free from contaminants in the basement to minimize soil reactions. In each agro-ecosystem, Du Toit *et al.* (1994) sampled primary grasslands (virgin soils) used for commercial livestock farming and sites under arable cropping (cultivated soils), while Birru (2002) sampled former arable lands reverted to secondary perennial pastures (restored soils). Sampling of the virgin and cultivated soils was done after the rainy season in March 1991. At about the same time in 2000, restored soils were also sampled. In both occasions, no vegetation sampling was done, as such the chemical composition of the inputs could not be determined in the current study.

The approach followed by Du Toit *et al.* (1994) and Birru (2002) in the selection of sites and sampling of soils varied somewhat since their objectives differed. Du Toit *et al.* (1994) sampled six sites at Harrismith and five at both Kroonstad and Tweespruit agro-ecosystems. At each site six plots were sampled from a virgin and cultivated land, chosen to be in close proximity (less than 50 m apart). Twenty sub-samples were collected from each sampling plot (10 m x 10 m plot size) with a 70 mm diameter soil auger to a 200 mm depth to obtain a composite sample. These soil samples represented cultivation periods of 0–59 years at Harrismith, 0–56 years at Kroonstad and 0–85 years at Tweespruit.

Restored sites older than five years, which had previously been under continuous cultivation for more than 20 years, were also sampled. Birru (2002) sampled eight sites at both Harrismith and Tweespruit and twelve at Kroonstad agro-ecosystem at the depth intervals of 0–50, 50–100 and

100–200 mm, with a 70 mm diameter soil auger. Each replicate sample was a composite of six sub-samples taken in a 2 m radius. These soil samples represented secondary perennial pasture periods of 5–25 years at Harrismith, 4–20 years at Kroonstad and 5–25 years at Tweespruit agro-ecosystems.

However, for this study, three replicate samples from each treatment in each agro-ecosystem were selected, and for comparison purposes with virgin soils half of each replicate sample from 0-50, 50-100 and 100-200 mm layers of restored soils were thoroughly mixed to represent 0-200 mm soil depth. Furthermore, samples from cultivated lands for over 20 years and restored lands for more than 15 years were selected for this study. The 20 years of soil cultivation and 15 years of secondary pasture management or beyond are believed to allow substantial organic matter depletion and restoration (Du Toit *et al.*, 1994; Lobe *et al.*, 2001; Birru, 2002). Records of data on these samples are available for referrals, for example: pH and clay content (Table 1); organic C and total N (Du Toit *et al.*, 1994; Birru, 2002); total, inorganic and organic S (Du Toit & Du Preez, 1995); and total, inorganic and organic P (Van Zyl & Du Preez, 1997). Regardless of the agro-ecosystem or land use, bulk densities of the sampled soils ranged from 1.38 g cm⁻³ in the cultivated soils to 1.43 g cm⁻³ in the virgin soils (Lobe *et al.*, 2001), while intermediate values were detected in the restored soils (Preger *et al.*, 2010). Corrections of C concentration values with bulk density resulted in negligible changes in the actual contents (stocks), hence results of this study were presented in concentrations.

3.2.3 Laboratory analysis

Analyses were carried out to determine soil C, SOC, SIC, permanganate oxidizable C (POXC), cold (CWEC) and hot (HWEC) water extractable C, humic substances (extractable humic substances (C_{EX}), humic (C_{HA}) and fulvic (C_{FA}) acids) and organic C functional groups.

3.2.3.1 Soil carbon

Soil C was analyzed by dry combustion (Nelson & Sommers, 1982) with a TruSpec Leco CN analyzer. Approximately 0.43 g sieved air-dried encapsulated soil samples were placed in a loading head, and one by one dropped into a 950 °C hot furnace and flushed with oxygen for rapid and complete combustion. Combustion gases were then passed through a secondary furnace (850 °C) for further oxidation before collected in a collection vessel where oxygen was injected and mixed with combustion gases. These gases were purged through a CO₂ infrared detector, which measures C as CO₂.

3.2.3.2 Soil organic and inorganic carbon

Soil organic C was measured with a modified Mebius procedure (Nelson & Sommers, 1982). A 0.5 g of sieved air-dried soil was weighed in a 150 ml glass beaker and reacted with 10 ml of 0.5 N potassium dichromate (K₂Cr₂O₇) and 15 ml of concentrated sulphuric acid (H₂SO₄). Samples were placed on a preheated sand bath at a temperature of 130 °C for 10 minutes. Samples were then removed from the sand bath and 35 ml of deionized water was added to each. Excess K₂Cr₂O₇ was titrated with 0.2 N ferro-ammonium sulphate [(NH₄)₂Fe(SO₄)₂·6H₂O] until the end point is reached, which was detected by millivoltmeter with a platinum electrode. Soil inorganic C was calculated as the difference between soil C and SOC.

3.2.3.3 Permanganate oxidizable carbon

Permanganate oxidizable C was analyzed according to Culman *et al.* (2012). Briefly, 2.5 g of sieved air-dried soil was weighed into 50 ml falcon tubes, wherein 18 ml deionized water and 2 ml 0.02 M potassium permanganate (KMnO₄) solution were added. The tubes were shaken for 15 minutes at 240 oscillations per minute on an oscillating shaker and then centrifuged for 5 minutes at 906 x g for the soil to settle down. Immediately thereafter, 0.5 ml of the supernatants

were transferred to new 50 ml falcon tubes and diluted with 49.5 ml deionized water before reading the sample absorbance on a spectrophotometer at 550 nm wavelength to obtain POXC.

3.2.3.4 Cold and hot water extractable carbon

Cold and hot water extractable C were determined according to Ghani *et al.* (2003). In short, 3 g air-dried soil samples were transferred to 50 ml falcon tubes and extracted with 30 ml of deionized water. Samples were shaken for 30 minutes on an end-over-end shaker and centrifuged for 20 minutes at 1233 x g. The supernatants were filtered through a 0.45 µm cellulose membrane filter into separate vials for C analysis. A 30 ml aliquot of deionized water was again added to the sediments in the same tubes and shaken on a vortex shaker to suspend the sediments. The capped tubes were then left in a hot-water bath at 80 °C for 16 hours. Samples were again shaken on a vortex shaker and centrifuged for 20 minutes at 1233 x g. The supernatants were then filtered through a 0.45 µm cellulose membrane filter. Carbon in both extracts was determined according to the modified Mebius procedure, and 5 ml of the extracts was used instead of soil. Carbon obtained from the first extraction was referred to as CWEC, while that from the second extraction was classified as HWEC.

3.2.3.5 Humic substances

The sequential procedure of Schnitzer (1982) was slightly modified to extract and fractionate humic substances. A 5 g of sieved air-dried soil was weighed out in falcon tubes and reacted with 30 ml extraction solution [0.1 N sodium hydroxide (NaOH) and 0.1 M sodium pyrophosphate decahydrate ($\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$)]. The contents were shaken on an oscillating shaker for an hour and centrifuged at 906 x g for 15 minutes. Insoluble material contained in the supernatant was isolated from soluble alkaline material (extractable humic substances). Soluble alkaline material was then precipitated with 0.05 N H_2SO_4 acid to fractionate humic and fulvic acids. Carbon concentrations of extractable humic substances (C_{EX}) and humic acids (C_{HA}) were determined with the Mebius procedure (Nelson & Sommers, 1982). Carbon in fulvic acids (C_{FA}) was calculated

as the difference between C_{EX} and C_{HA} . Humification index ($HI = C_{HA}/SOC$) and polymerization index ($PI = C_{HA}/C_{FA}$) were calculated according to Abril *et al.* (2013).

3.2.3.6 Nuclear magnetic resonance spectroscopy

Bulk soil samples were pre-treated with hydrofluoric acid (HF) to remove magnetic materials, concentrate organic C and increase the signal-to-noise ratio of the resultant NMR spectra as recommended by Skjemstad *et al.* (2001) and Mathers *et al.* (2002). Briefly, 5 g of ground sieved air-dried soil was weighed into 50 ml falcon tubes and 45 ml of 2% HF was added. The tubes were shaken on end-over-end shaker for 8 (4 x 1 hour, 3 x 16 hour and 1 x 64 hour) successive times. Samples were then centrifuged after every extraction at 671 x g for 20 minutes and the supernatant was filtered through a 5 mm Millipore Durapore membrane filter to recover the light fraction. After the final extraction, residues together with the light fraction trapped on the membrane filter were washed 5 times with deionized water, oven dried at 75 °C and ground to powder using a mortar and pestle for NMR analysis.

Whole HF-treated soil samples were packed in cylindrical zirconia rotors before analysis. The NMR analysis was done on a 400 MHz Bruker AVANCE III spectrometer equipped with a 4 mm VTN multinuclear double resonance magic angle spinning probe, operating at room temperature. The ^{13}C NMR spectra were recorded at 100.6 MHz, using the cross polarization magic angle spinning (CPMAS) technique. A rotating speed of 14 000 Hz was used with a contact time of 1 minute, a recycle delay of 1 second and an acquisition time of 12.8 minutes. All the spectra were recorded with 86 016 scans and baseline corrected. The following ranges were integrated: 0–50 ppm (alkyl-C), 50–110 ppm (O-alkyl C), 110–160 ppm (aromatic C), and 160–180 ppm (carbonyl C).

3.2.4 Statistical analysis

Statistical analyses were performed with SPSS version 24 software package (SPSS Inc.). A two-way analysis of variance (ANOVA) was carried out to determine the influence of land use, agro-ecosystem and their interaction on soil C fractions. Means were compared with Tukey's honestly significant difference (HSD_T) post hoc tests at 95% confidence level. All data were tested for normality and homogeneity using Shapiro-Wilk and Levene's test, respectively before carrying out ANOVA. For ^{13}C NMR spectra, three replicate soil samples per treatment were mixed thoroughly and subjected to NMR spectroscopy as composite samples due to high costs and time needed to obtain spectra, as such no statistical analyses were performed.

3.3 Results

The dilution effect of cultivation to a soil depth of 200-300 mm could result in underestimated values of SOC, SIC, labile C fractions, humic substances and organic C functional groups, especially when compared to those recorded in the virgin soils. This is derived from the fact that preliminary analysis of SOC by Lobe *et al.* (2001) from samples collected at the depth intervals of 0-100 and 100-200 mm did not reveal any significant ($P < 0.05$) changes when the cultivated and virgin soils were compared. Significant ($P < 0.05$) differences between C values obtained in the cultivated and grassland soils arose when the 0-200 mm soil depth was considered, which declared the dilution effect in the former. However, the wide differences in the measured C fractions between virgin, cultivated and restored soils indicate that dilution was not the principal factor that resulted in lower concentrations of these C fractions in the cultivated soils.

3.3.1 Soil organic and inorganic carbon

Prolonged soil cultivation resulted in a significantly ($P < 0.05$) lower SOC and SIC compared to adjacent native grasslands and secondary pastures (Figure 3.2). In Harrismith and Tweespruit however, SIC concentrations in the cultivated soils did not differ significantly ($P < 0.05$) with those

recorded in the restored soils (Figure 3.2b). The SOC contents decreased from 26.23 ± 1.60 to 10.64 ± 0.32 g kg⁻¹ in Harrismith, from 23.22 ± 0.73 to 9.99 ± 0.47 g kg⁻¹ in Tweespruit and from 9.04 ± 1.12 to 4.82 ± 0.44 g kg⁻¹ in Kroonstad, which indicated that 59, 57 and 47% of SOC, respectively, was lost due to cultivation (Figure 3.2a). The SIC contents were in the range of 6.08 ± 3.35 - 8.94 ± 3.11 g kg⁻¹ in the virgin soils and 0.90 ± 0.56 - 1.96 ± 0.55 g kg⁻¹ in the cultivated soils, and the highest losses due to cultivation were recorded in Tweespruit (90%) followed in a descending order by Kroonstad (82%) and Harrismith (71%).

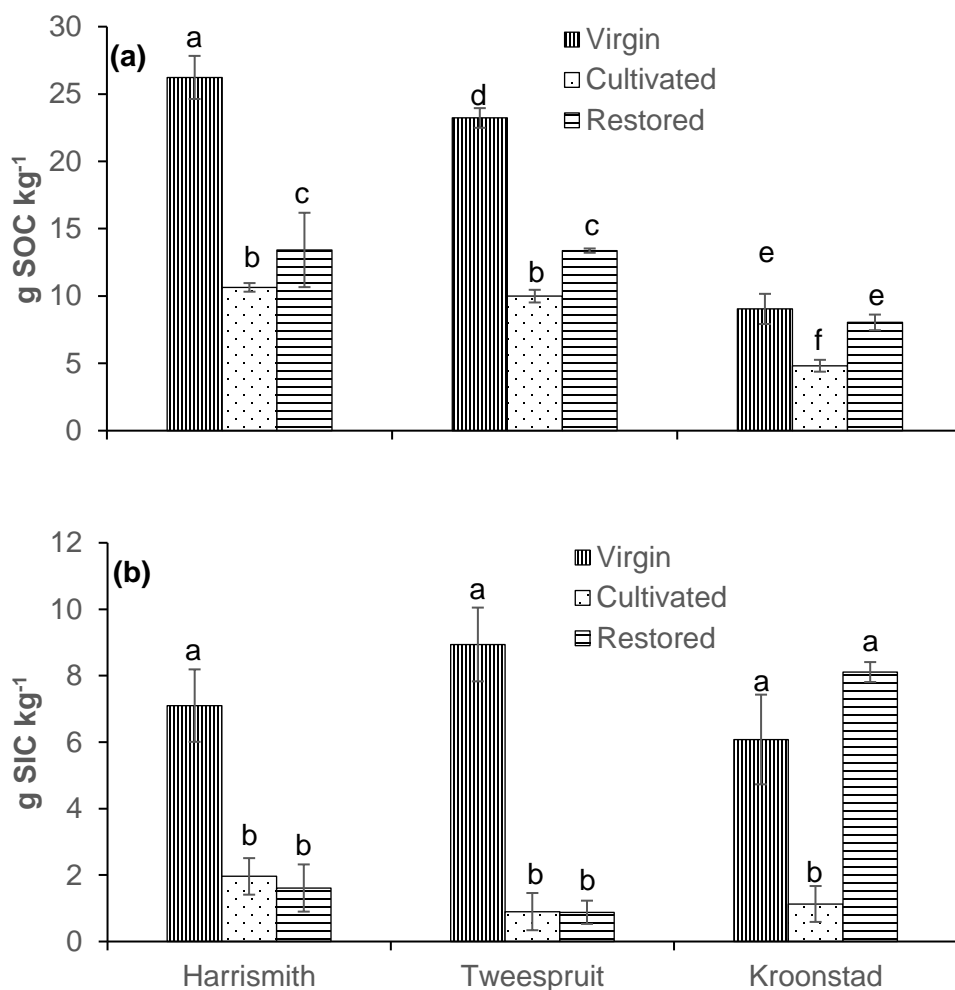


Figure 3.2 Effects of virgin, cultivated and restored land use on concentration of soil organic [SOC (a)] and inorganic [SIC (b)] carbon in the three agro-ecosystems. Significant differences ($P < 0.05$) are indicated by different letters. Vertical bars with horizontal caps indicate standard deviation.

Secondary pasture management recovered 12-36% of the lost SOC as a result of cultivation, with the highest recovery observed in Kroonstad and lowest in Harrismith agro-ecosystem (Fig. 2a). The SIC concentration in the restored soils of the Harrismith and Tweespruit agro-ecosystems did not differ significantly ($P < 0.05$) with those recorded in the cultivated soils (Fig. 2b). In Kroonstad on the other hand, secondary pasture management significantly ($P < 0.05$) increased SIC (from 1.13 ± 0.54 to 8.11 ± 0.30 g kg⁻¹), exceeding SIC concentrations obtained in the primary grasslands (6.08 ± 3.35 g kg⁻¹).

3.3.2 Labile carbon fractions

Changes in land use significantly ($P < 0.05$) affected POXC and CWEC, but not HWEC (Figure 3.3). The POXC concentration was higher in the virgin soils and lower in the cultivated soils (Figure 3.3a). A decline in POXC due to cultivation followed the order: Harrismith (from 893.36 ± 76.74 to 427.36 ± 32.87 mg kg⁻¹) > Tweespruit (from 707.16 ± 39.93 to 436.05 ± 5.01 mg kg⁻¹) > Kroonstad (from 405.17 ± 17.69 to 294.22 ± 2.89 mg kg⁻¹). Restored POXC concentration was significant ($P < 0.05$) only in the Harrismith agro-ecosystem, where at least 18% of the 52% C lost was reinstated. Reversion of degraded arable lands to secondary perennial pasture was the only management that influenced ($P < 0.05$) CWEC in the Kroonstad agro-ecosystem (Figure 3.3b). Application of secondary pasture management restored antecedent CWEC, exceeding that detected in the virgin soils by 129%.

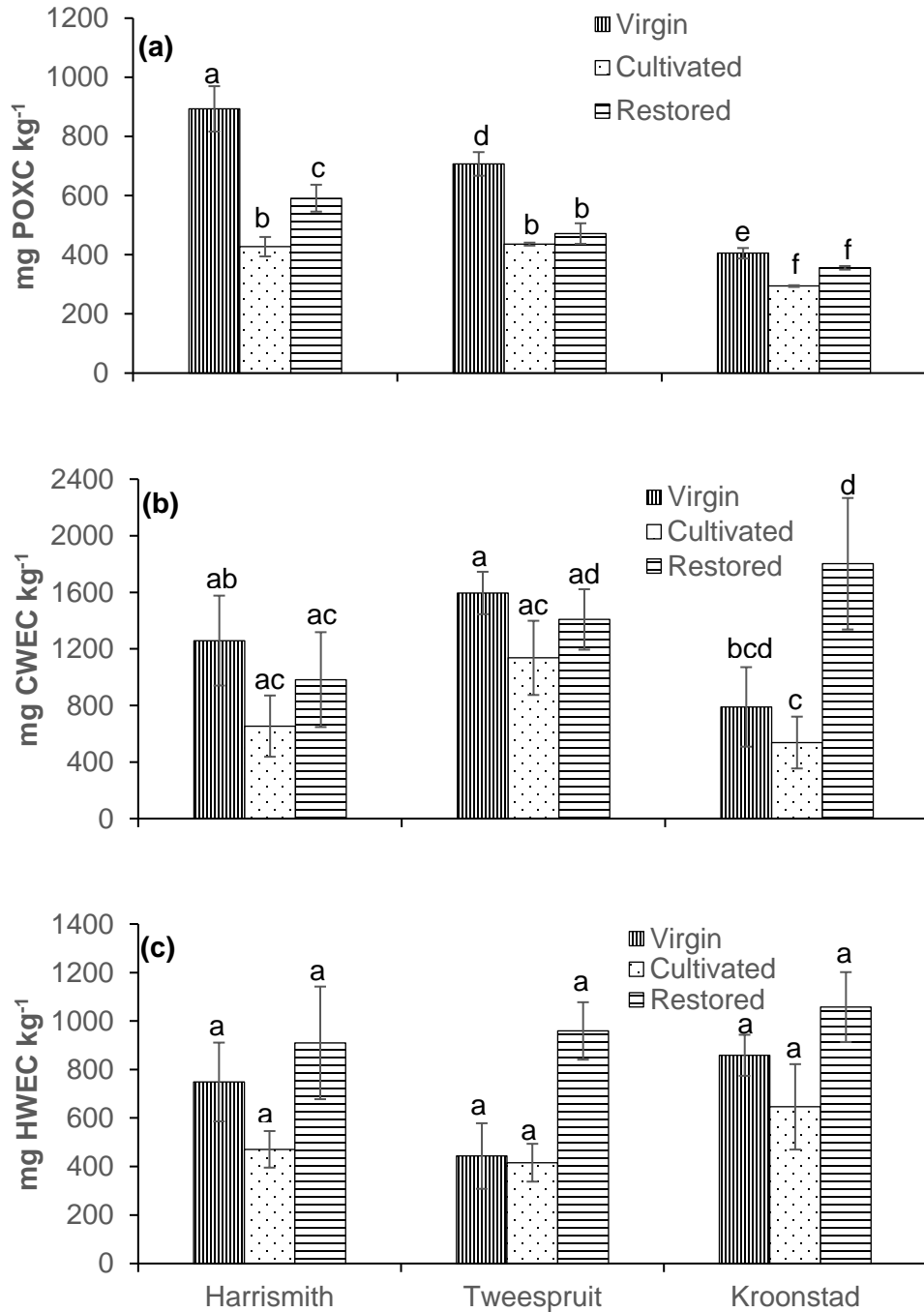


Figure 3.3 Effects of virgin, cultivated and restored land use on concentrations of labile carbon fractions: permanganate oxidizable carbon [POXC (a)], cold water extractable carbon [CWEC (b)], and hot water extractable carbon [HWEC (c)] in the three agro-ecosystems. Significant differences ($P < 0.05$) are indicated by different letters. Vertical bars with horizontal caps indicate standard deviation.

3.3.3 Humic substances

Humic substances also showed significant ($P < 0.05$) changes as a result of the applied land use systems across the three agro-ecosystems, all following the order Harrismith > Tweespruit > Kroonstad (Figure 3.4). Despite their recalcitrant nature, long-term cultivation resulted in a significant loss of C_{EX} (Figure 3.4a), C_{HA} (Figure 3.4b) and C_{FA} (Figure 3.4c) in ranges of 58-68% in Harrismith, 45-76% in Tweespruit and 37-54% in Kroonstad. The highest loss was detected in C_{HA} where the concentration decreased from $6.05 \pm 0.28 \text{ g kg}^{-1}$ in the virgin soils to $1.47 \pm 0.23 \text{ g kg}^{-1}$ in the cultivated soils of the Tweespruit agro-ecosystem. The lowest loss was observed in C_{EX} , declining from $6.67 \pm 0.50 \text{ g kg}^{-1}$ in the virgin soils to $4.18 \pm 0.18 \text{ g kg}^{-1}$ in the cultivated soils of the Kroonstad agro-ecosystem.

On the other hand, reversion of arable lands into secondary perennial pastures significantly ($P < 0.05$) restored humic substances (Figure 3.4), except for C_{HA} in the Kroonstad and C_{FA} in the Tweespruit agro-ecosystem, where both cultivated and restored soils had almost the same concentrations of these humic C fractions. On average, secondary pasture management replenished the lost C_{EX} , C_{HA} and C_{FA} by 16% in Harrismith. The C_{EX} and C_{HA} concentrations increased by 10 and 31%, respectively in Tweespruit, while C_{EX} and C_{FA} contents improved by 16 and 3%, respectively in Kroonstad as a result of the applied secondary pasture management.

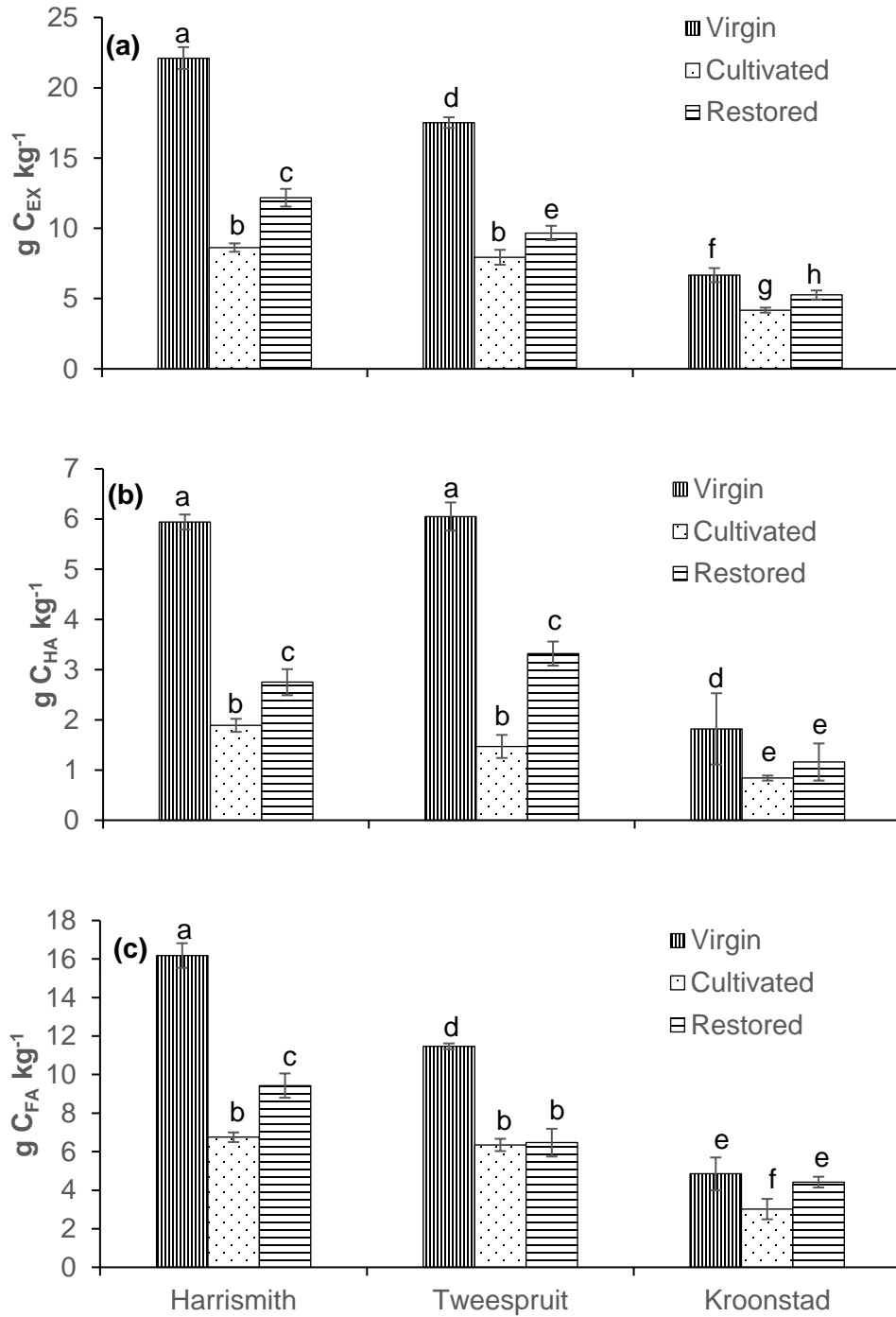


Figure 3.4 Effects of virgin, cultivated and restored land use on concentrations of humic substances: extractable humic substances [C_{EX} (a)], humic acids [C_{HA} (b)] and fulvic acids [C_{FA} (c)] in the three agro-ecosystems. Significant differences ($P < 0.05$) are indicated by different letters. Vertical bars with horizontal caps indicate standard deviation.

3.3.4 Structural composition of soil organic carbon

The CPMAS ^{13}C NMR spectra for the sampled virgin, cultivated and restored soils from Harrismith, Tweespruit and Kroonstad agro-ecosystems are presented in Figure 3.5, and interpretations were done according to Baldock *et al.* (1992; 1997) and Kögel-Knabner (1997, 2002). All the spectra generally show the major four chemical shifts regions based on chemical types indicated in the material and methods section. The 0-50 ppm chemical shift was dominated by peaks within the range of 30-32 ppm arising from long-chain polymethylene structures, while shoulders that appear in the vicinity of 28-30 ppm in the cultivated and restored soils signify the presence of branched structures of these aliphatic compounds. Two distinct peaks in the 50-110 ppm region resonating within 71-73 and 103 ppm represent C2-C5 and anomeric C1 (mainly hemicellulose) carbons of polysaccharides, respectively. Shoulders at 55 and 58-64 ppm are attributed to lignin-derived methoxyl C or C-N bonds of proteins and C6 of carbohydrates, respectively. Broad bands in the 110-160 ppm chemical shift with peaks at 126-130 ppm are assigned to C- or H-(hydrogen) substituted aromatic C, while signals at 147-150 ppm indicate O-substituted aromatic C, both derived from lignin. Tannins in the restored soils of Tweespruit are observed at 144 ppm. Resonances within the vicinity of 170/171 ppm are caused by C in the carboxyl, ester and amide groups.

Relative contributions of organic C functional groups are given in Table 3.2. In Harrismith, the relative intensities of the four functional groups did not vary widely between the cultivated and virgin soils. In fact, the difference was 1% for alkyl, O-alkyl and carbonyl C and 4% for aromatic C. Slightly wider variations were observed in Tweespruit and Kroonstad, where cultivation of grasslands resulted in a lower O-alkyl C with concomitant increase in alkyl, aromatic and carbonyl C. Differences were in the ranges of 1% for alkyl C to 8% for O-alkyl C in the Tweespruit agro-ecosystem, and 2% for aromatic C to 11% for O-alkyl C in the Kroonstad agro-ecosystem. In contrast, virgin, cultivated and restored soils in Tweespruit had equal proportions of carbonyl C.

Reversion of cultivated soils into secondary perennial pastures restored the antecedent O-alkyl C in all the sampled agro-ecosystems. However, in Harrismith and Kroonstad the signal intensities of O-alkyl C surpassed those detected in the primary grasslands by 3 and 5%, respectively. Similarly, alkyl C under secondary pasture management in the Tweespruit and Kroonstad rose to represent primary grasslands. Proportions of aromatic and carbonyl C in the restored soils remained lower than those in the cultivated soils.

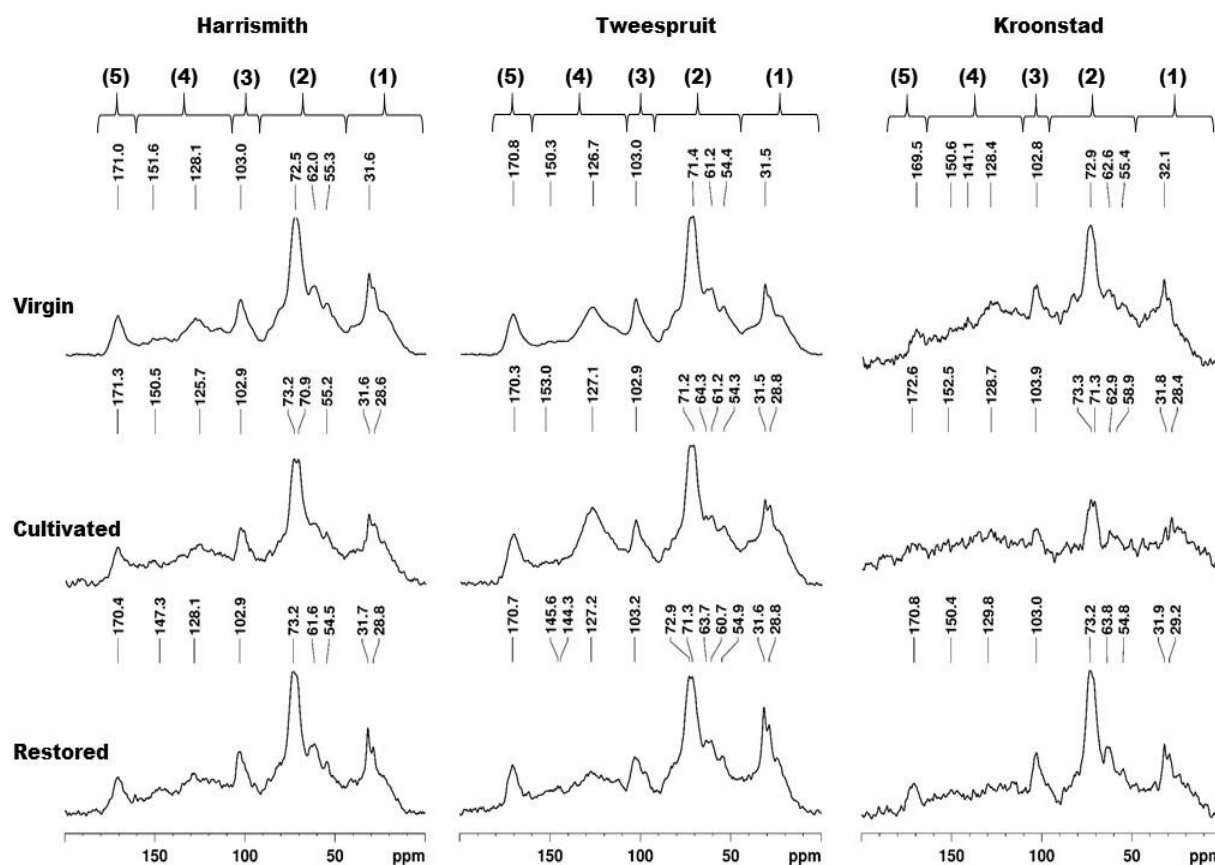


Figure 3.5 Carbon-13 nuclear magnetic resonance spectra of organic carbon (C) in the 0-200 mm soil layer of virgin, cultivated and restored soils in the Harrismith, Tweespruit and Kroonstad agro-ecosystems. (1), alkyl C; (2) O-alkyl C; (3), di O-alkyl C; (4), aromatic C; (5), carbonyl C.

Table 3.2 Content of organic carbon (C) functional groups (%) in the 0-200 mm layer of soils under native grasslands, cultivation and secondary pasture management in the three semi-arid agro-ecosystems

Functional groups	Harrismith			Tweespruit			Kroonstad		
	Virgin	Cultivated	Restored	Virgin	Cultivated	Restored	Virgin	Cultivated	Restored
Alkyl C	21	20	19	19	20	21	20	25	21
O-alkyl C	56	55	59	57	49	53	45	34	50
Aromatic C	17	21	18	19	26	19	28	30	23
Carbonyl C	6	5	4	6	6	6	7	11	6

Table 3.3 Effect of land use change on the extent of organic carbon (C) decomposition as indicated by humification (HI) and polymerization (PI) indices as well as alkyl C/O-alkyl C in the 0-200 mm layer of virgin, cultivated and restored soils across three semi-arid agro-ecosystems; Harrismith (HS), Tweespruit (TW) and Kroonstad (KR)

Land use	HI			PI			Alkyl C/O-alkyl C		
	HS	TW	KR	HS	TW	KR	HS	TW	KR
Virgin	0.23±0.01a	0.26±0.01a	0.21±0.10ab	0.37±0.01a	0.53±0.02a	0.40±0.21ab	0.38	0.33	0.44
Cultivated	0.18±0.02ab	0.15±0.03b	0.18±0.03ab	0.28±0.02ab	0.23±0.03b	0.28±0.04b	0.36	0.4	0.71
Restored	0.21±0.04a	0.25±0.02a	0.15±0.05b	0.29±0.04ab	0.52±0.09a	0.27±0.10b	0.32	0.42	0.41

Values presented are means ± standard deviation of three replicates. Means with different letters in a row or column indicate significant difference at $P < 0.05$ except for alkyl/O-alkyl C ratio, which was not subjected to any statistical analysis

3.3.5 Extent of decomposition

Changes in land use had variable effects on the extent of SOC decomposition as indicated by HI, PI and alkyl C/O-alkyl C ratios (Table 3.3). Significant ($P < 0.05$) changes in HI and PI values were recorded in Tweespruit, where cultivated soils (0.15 ± 0.03) had lower levels of these indices compared to virgin (0.26 ± 0.01) and restored (0.25 ± 0.02) soils. Comparisons between agro-ecosystems show that secondary pasture management resulted in a higher level of HI and PI in Harrismith and Tweespruit relative to Kroonstad. The alkyl C/O-alkyl C ratio was almost the same in Harrismith and Tweespruit regardless of land use, but higher in the cultivated soils compared to virgin or restored soils in Kroonstad. Similarly, cultivated soils of the Kroonstad agro-ecosystem had a higher alkyl C/O-alkyl C ratio compared to their counterparts in the Harrismith and Tweespruit regions.

3.4 Discussion

3.4.1 Soil organic and inorganic carbon

A decline in SOC as a result of cultivation of primary grasslands has been reported in many earlier studies, and losses in the magnitude of 47-59% are common in semi-arid regions (Lobe *et al.*, 2001; Seddaiu *et al.*, 2013; Kotzé *et al.*, 2016), but should not be generalized as the effects of tillage may be site specific. These results are consistent with those obtained in the temperate region of Northeast China (Sun *et al.*, 2012) and tropics to subtropics of Brazil (Dieckow *et al.*, 2009), respectively where 30 and 53% losses of SOC in the upper soil layers following conversion of primary grasslands into arable cropping were reported. These responses reaffirm that losses of SOC in the arable lands are mostly due to disruption of soil structure and breakdown of aggregates, which ultimately expose physically protected SOC to microbial attack (Von Lützow *et al.*, 2006; Lal *et al.*, 2015). In addition, the six-month fallow period included in a rotation cycle is another factor that probably led to lower SOC levels in the cultivated lands of the three agro-

ecosystems since plant residues were grazed by animals, returning less quantities to the soil (Lobe *et al.*, 2001; Janzen, 2006). Removal of crop residues by grazing animals left the cultivated soils bare and susceptible to erosion.

Signs of SOC losses by wind erosion were reported previously in the cultivated soils of the sampled agro-ecosystems (Lobe *et al.*, 2001; 2002), and beyond doubt SIC was also partly lost through the same mode. Usually a decrease in SOC is associated with an increase in SIC formation (Sanderman, 2012; Shi *et al.*, 2012), but was not the case in the present study. As such, it was the effect of soil mixing by tillage that contributed to SIC loss as it exposed the buried carbonate rich subsoil to the surface where it became prone to erosion (Wu *et al.*, 2009; Wang *et al.*, 2010). Rapid SOC decomposition in the cultivated soils followed by increased production of CO₂ dissolved carbonates (Wu *et al.*, 2009; Wang *et al.*, 2010), while the absence or lower concentrations of Ca²⁺ and/or Mg²⁺ restricted SIC re-precipitation and accrual (Bronick & Lal, 2005). Similar findings were reported by Shi *et al.* (2012), who indicated that higher temperatures in the Inner Mongolian grasslands stimulated production of soil CO₂, which subsequently constrained SIC formation and accrual compared to the lower temperatures in the Tibetan grasslands.

Contrary to the findings of this study, Mikhailova and Post (2006) indicated that applications of inorganic fertilizers and manure in a cultivated temperate Russian Chernozem increased levels of SIC compared to unfertilized adjacent grasslands. This depends on the quantities of Ca²⁺ and/or Mg²⁺ present in the inputs (inorganic fertilizers and manure) because even in our case inorganic fertilizers, including limestone ammonium nitrate, which is said to contain around 8% of divalent base cations (Loke *et al.*, 2014), were administered in the cultivated soils. Droppings of animals grazing crop residues during the fallow period were not adequate to elevate levels of Ca²⁺ and Mg²⁺ in the cultivated soils. Generally, base cations were lower considering slightly more

acidic soil conditions in the cultivated lands relative to adjacent primary grasslands (Table 3.1) and therefore adversely affected SIC formation and accumulation therein (Shi *et al.*, 2012).

Due to Harrismith and Tweespruit being in the cooler Highveld and Kroonstad in the warmer Highveld, expectations were that greater SOC and SIC losses would be in the cultivated Kroonstad soils. Contrary to the expectations, the higher SOC losses occurred in the cool-moist Harrismith (59%) and cool-dry Tweespruit (57%) with soils slightly higher in clay content, relative to warm-drier Kroonstad (47%). Although soil, climate and management conditions usually act together to determine the extent of SOC and SIC losses, these losses seemed to be more related to the initial SOC and SIC levels. The higher ($P < 0.05$) SOC concentrations in the virgin soils in Harrismith and Tweespruit relative to those in Kroonstad, explain greater SOC losses recorded in these two agro-ecosystems.

Chronosequence studies (Lobe *et al.*, 2001; Sun *et al.*, 2012) also support our presumption that SOC in the cultivated Harrismith soils (for 20 years) was still at the initial stage of decomposition where losses are mostly from the labile fraction. Sun *et al.* (2012) reported a decline in SOC by 22% from original values in the first 18 years of soil cultivation after which the rate decreased to a steady rate of 4% between 50 and 200 years. Lobe *et al.* (2001) added that beyond 17 years of cultivation, SOC losses derive from the stable fraction. This implies that SOC losses in Tweespruit soils that have been cultivated for 85 years were claimed from the humic fraction. The highest SIC losses (90%) in Tweespruit were due to prolonged cultivation (85 years) that stimulated dissolution of carbonates and destabilization of SIC re-precipitation processes (Shi *et al.*, 2012; Lal *et al.*, 2015). Slightly higher SIC in the virgin soils of Tweespruit relative to their counterparts in the Harrismith and Kroonstad agro-ecosystems partially contributed to such losses.

Many studies conducted under different climates and soil conditions (Bach *et al.*, 2010; Preger *et al.*, 2010; Lauer *et al.*, 2011; Kösters *et al.*, 2013; Baer *et al.*, 2015) conform with our results that revealed possible reversal of historic SOC and SIC, after croplands were reverted to secondary

perennial pastures (Figure 3.2). Secondary pasture management provided a permanent cover to control erosion-induced losses of SOC and SIC, which were suspected to have occurred in the sampled agro-ecosystems (Lobe *et al.*, 2001; 2002) and excluded intensive cultivation to improve soil structure and protect newly added SOC or formed SIC (Bronick & Lal, 2005; Von Lützow *et al.*, 2006). However, soil structure, recovery and stabilization of SOC and SIC are modulated by soil texture (Von Lützow *et al.*, 2006). Climatic conditions may also contribute in the recovery of SOC and SIC (Preger *et al.*, 2010).

According to Bach *et al.* (2010), reversal of the lost SOC components due to human intervention in the sand dominated soils of the Prairie ecosystems in Nebraska, USA seemed like an impossible process. On the contrary, our results indicated rapid SOC and SIC recovery in the sandy soils of the Kroonstad agro-ecosystem. In fact, the amount of reinstated SOC in Kroonstad was 21-24% higher than that restored in the slightly clayey soils of Harrismith and Tweespruit, which is quite surprising because Kroonstad has a warm-dry climate known to delay plant biomass turnover. Nevertheless, Baer *et al.* (2015), after comparing SOC recovery rates in the sandy soils of the South African Highveld and US Tallgrass Prairie, indicated higher recovery rates in the Highveld, which they attributed to lower initial C contents. The findings of Preger *et al.* (2010) also revealed rapid, but incomplete SOC recovery by sand-size fraction compared to silt- or clay-size fractions under secondary pasture management. However, they urged that SOC recovery in the sand-size fraction reflected more on the lability of the recovered SOC than sequestration, meaning the recovery was only temporary and therefore SOC could be lost at any given time when the soil is brought under cultivation. Higher evaporation in Kroonstad possibly withdrew base cations from deeper layers and deposited them in the surface soil for SIC formation. The warm-dry conditions constrained dissolution of SIC and rapid decomposition of SOC.

3.4.2 Labile carbon fractions

The labile pool, which is highest during the initial stage of decomposition, is characterized by a suite of readily decomposable organic materials with relatively rapid turnover rates (Gregorich *et al.*, 1994; Von Lützow *et al.*, 2006). They are, therefore, early indicators of SOC changes and can be relied on for monitoring short-term changes in soil quality as a result of land use, and be used to influence SOC management decisions (Gregorich *et al.*, 1994; Janzen, 2005). According to results of this study, POXC was the most sensitive fraction compared to CWEC and HWEC. The higher ($P < 0.05$) POXC concentrations in the virgin soils of the Harrismith, Tweespruit and Kroonstad agro-ecosystems suggest less stable and susceptible SOC to loss upon cultivation or introduction of any other form of soil disturbance (Brar *et al.*, 2015).

Regardless of the agro-ecosystem, POXC decreased significantly ($P < 0.05$) as grassland soils were put under intensive cultivation (Figure 3.3). The response of POXC to prolonged cultivation agree with literature (Ghani *et al.*, 2003; Culman *et al.*, 2012; Vázquez *et al.*, 2016) and the aggregate hierarchy theory that cultivation of virgin soils disintegrates macro-aggregates within which labile fractions (e.g. POXC) are held, leaving behind less than 0.5 mm aggregate size fractions (Janzen, 2005; Von Lützow *et al.*, 2006; Sun *et al.*, 2012). Exposed readily digestible POXC to the decomposer community declined and the remainder was either occluded within micro-aggregates rendering it inaccessible to microbial decomposers (Lobe *et al.*, 2001; Von Lützow *et al.*, 2006; Sun *et al.*, 2012), and/or immobilized and stored by soil microbes (Baldock *et al.*, 1992; Lobe *et al.*, 2002). This indicates that soils may not completely be deprived of labile C fractions, as such microbial immobilization of labile C fractions could be another mechanism for C sequestration.

Understandably, this fraction can be kept 'alive' through constant supply of fresh residues. Recycling of organic materials replenishes POXC and improve soil biological fertility. This suggests that the fallow period included in the rotation cycle should be replaced with cover crops as it offsets the build-up of POXC in the cultivated soils (Lobe *et al.*, 2001; Kösters *et al.*, 2013; Brar *et al.*, 2015). Microbial decomposers continue to mineralize labile C during the fallow period, while no or less inputs are returned. This disrupts the balance between inputs and outputs (Sun *et al.*, 2012), and thus terrestrial C cycling (Janzen, 2005). According to Brar *et al.* (2015), an eight-year fallow period resulted in a loss of 24% SOC in the cultivated soils, therefore we are of the opinion that six-month fallow periods are long enough to affect a sensitive POXC.

Losses of POXC followed a similar pattern exhibited by SOC, which basically indicated how the initial POXC levels affected the net POXC, after primary grasslands were converted into arable cropping. Highest POXC losses were detected in Harrismith (52%) and lowest in Kroonstad (27%), while being intermediate in Tweespruit (38%). Harrismith has conducive climatic (cool and moist) conditions that presumably elevated microbial activity and diversity, thus POXC decomposition as opposed to Tweespruit and Kroonstad (Vázquez *et al.*, 2016).

Alternatively, reversion of cultivated lands to secondary perennial pastures reflected the build-up of labile C fractions, especially in the Harrismith and Kroonstad agro-ecosystems (Figure 3.3). Reinstated POXC in Harrismith and CWEC in Kroonstad were stimulated by increased plant biomass production that provided cover to control erosion and drying-rewetting cycles, and thus losses of these C fractions (Lal *et al.*, 2015). Differences in plant residue quality between arable lands and secondary pastures could be another major contributory factor to the observed POXC and CWEC responses. Higher labile plant residues under secondary perennial pastures provide a substrate for a wide range of soil microbes, which can subsequently modulate sequestration of labile C fractions through immobilization (Baldock *et al.*, 1992; Lauer *et al.*, 2011). Higher levels

of CWEC were probably a carryover as the warm-dry conditions in Kroonstad constrained rapid losses in the cultivated soils before reverted into perennial pastures.

Significantly ($P < 0.05$) higher reinstated POXC concentrations in the Harrismith agro-ecosystem relative to the other two regions indicates that soil texture and climate may have contributed to the recovery of labile C fractions (Bach *et al.*, 2010; Baer *et al.*, 2015). Indeed, higher mean annual rainfall and clay content of the restored soils in Harrismith, respectively improved phytomass production needed to replenish the lost POXC by cultivation and provided protection against erosion and microbial degradation (Von Lützow *et al.*, 2006; Bach *et al.*, 2010; Preger *et al.*, 2010). On the other hand, restored soils of the Kroonstad agro-ecosystem were quicker to recover CWEC, exceeding that in the virgin soils. While this response could be attributed to vegetational differences, it also indicates that regardless of the soil or climatic conditions, reversion of cultivated soils to secondary pastures has a potential to restore previously lost labile fractions to levels close to those prevailing in the primary grasslands. In other words, exclusion of tillage in agricultural landscapes can maintain a balance between C inputs and outputs and therefore restore disrupted global C cycling (Janzen, 2005). This revolutionary act can minimize losses of labile C fractions, where their fragile nature to microbial attack has turned soils into a source rather than a sink for atmospheric CO₂.

3.4.3 Humic substances

Humic substances, which represent 40-60% of SOC, are recalcitrant in nature due to their structural complexity and multiple bonds that can be cleaved by a special group of soil microbes (Kögel-Knabner, 2002; Von Lützow *et al.*, 2006; Guimarães *et al.*, 2013). Owing to their selective preservation, humic substances can persist for centuries to millennia in the soil, and thereby sequester atmospheric C. However, the findings of this study proved otherwise as continuous soil cultivation resulted in a substantial ($P < 0.05$) loss of C_{EX}, C_{HA} and C_{FA} compared to the primary grasslands in all the chosen agro-ecosystems (Figure 3.4). In the same agro-ecosystems, Lobe

et al. (2005) also observed a rapid replacement of old SOC (from grasses) by crop-derived C (disregarding maize), 20 years after conversion of grasslands into arable cropping. Related to this, are the results of Lobe *et al.* (2001) and Sun *et al.* (2012) that revealed that after 20 years of arable cropping, losses of SOC predominantly arise from humic substances.

Around 37-76% losses of humic substances have also been reported elsewhere in the semi-arid to arid (Vázquez *et al.*, 2016) and tropical environments (Spaccini *et al.*, 2006) as a result of conversion of natural terrestrial ecosystems into arable lands. The loss of humic substances under arable cropping are often associated with dispersion of micro-aggregates and exposure of the stable C to microbial degradation (Von Lützow *et al.*, 2006). Thus, regular cultivation destabilizes processes leading to SOC stabilization by accelerating mineralization, while slowing down humification and polymerization processes (Sun *et al.*, 2012; Kotzé *et al.*, 2016). Vázquez *et al.* (2016) are of the impression that losses of humic substances signify intensive decomposition, which over time decreases as SOC approaches a steady state. Sun *et al.* (2012) shared similar sentiments after detecting non-significant changes in C_{HA} as a result of long-term cultivation. The C_{FA} fraction continued to decrease by 7% after 200 years of soil cultivation because it contains appreciable quantities of labile compounds compared to C_{HA} (Sun *et al.*, 2012; Guimarães *et al.*, 2013; Seddaiu *et al.*, 2013; Moussadek *et al.*, 2014; Kotzé *et al.*, 2016).

In the current study, C_{HA} was the most lost fraction, accounting for 68% in Harrismith, 76% in Tweespruit and 54% in Kroonstad. The lost C_{EX} and C_{FA} ranged from 37 to 61% and 31 to 58%, respectively, with the highest detected in Harrismith and lowest in Kroonstad. Increased C_{HA} losses in the Tweespruit region could be attributed to a number of factors including original levels of C_{HA} as well as duration of cultivation (Lobe *et al.*, 2001; Sun *et al.*, 2012), which is much longer than in both Harrismith and Kroonstad. While greater C_{EX} and C_{FA} losses were recorded in Harrismith, that did not come as a surprise because these humic fractions consist of substantial amounts of labile compounds, which under the cool-moist conditions can be broken down more

rapidly than in the warm-dry conditions in Kroonstad with sandier soils (Kotzé *et al.*, 2016). The drainage system of sandier soils combined with high evapotranspiration can deplete water in the surface soil and therefore inhibit diffusion of extracellular enzymes responsible for decomposition of humic substances (Davidson & Janssens, 2006).

In general, losses of these humic substances as a result of prolonged arable cropping suggest that recalcitrance of humic substances is relative and not absolute as was previously believed (Von Lützow *et al.*, 2006), and therefore explain why the concept of selective preservation has to be reviewed. Thus, stabilization and persistence of humic substances in the soil depend on land use, availability and activity of humus-decomposing microbes and associations with the mineral phase (Lobe *et al.*, 2002; Kögel-Knabner, 2002; Von Lützow *et al.*, 2006).

Regular additions of organic C inputs can delay decomposition and formation of humic substances, especially where tillage has been excluded. Although decomposition under secondary pasture management can be slower compared to cultivated soils, our results indicated that within 18-25 years of secondary pasture management, a significant ($P < 0.05$) increase in C_{EX} , C_{HA} and C_{FA} was evident throughout the sampled agro-ecosystems. Moussadek *et al.* (2014) also indicated that 5 years after the introduction of no-tillage in the cultivated lands, resulted in an accumulation of SOC containing higher levels of highly stable humic fractions (C_{HA} and humin) relative to conventional tillage. Besides processes that alleviate rapid decomposition e.g. chemical/physical protection, exclusion of tillage provides soil cover and encourages continuous additions of organic inputs to recharge the stable C fraction (Moussadek *et al.*, 2014; Kotzé *et al.*, 2016). Rapid gains of humic substances suggest that perennial grasses in the former cultivated soils were richer in the precursors (e.g. lignin) of humic substances compared to the rotated maize, wheat and sunflower (Von Lützow *et al.*, 2006; Abril *et al.*, 2013). This statement seems consistent with Helfrich *et al.* (2006) who found higher lignin contents and aromaticity in grasses relative to maize residues.

The quality and quantity of organic inputs could play a critical role to improve humic substances; however, the magnitude of increase was site specific (Spaccini *et al.*, 2006; Abril *et al.*, 2013). This study is a typical example, as secondary pasture management recovered 6% higher C_{EX} in Harrismith and Kroonstad than that reinstated in Tweespruit. In Kroonstad again, restored C_{FA} was 13% higher than that in Harrismith and 27% higher than that in Tweespruit. Higher recovery of C_{EX} in Harrismith relative to Tweespruit is probably due to higher rainfall that subsequently improved biomass production, while in Kroonstad the warm-dry conditions impeded rapid decomposition of labile residues that ended up refilling C_{EX} and C_{FA} fractions (Spaccini *et al.*, 2006; Sun *et al.*, 2012; Guimarães *et al.*, 2013; Seddaiu *et al.*, 2013; Moussadek *et al.*, 2014; Kotzé *et al.*, 2016). On the other hand, secondary pasture management reinstated 31% C_{HA} , which was 16% higher (on average) than in Harrismith or Kroonstad. This was probably due to a greater C_{HA} saturation deficit in the Tweespruit agro-ecosystem (Baer *et al.*, 2015). According to Janzen (2005) these gains do not reflect C sequestration, but a mere fill up of the empty spaces caused by intensive cultivation.

3.4.4 Structural composition of soil organic carbon

Changes in the molecular structure of SOC is often affected by the degree of decomposition and the quality of litter returned to the soil (Baldock *et al.*, 1992). Unfortunately sampling of plant litter was not done to determine molecular structural composition of the plant residues. This kind of data is crucial to understand the fate of plant residues following changes in land use. Again since the data on the intensities of these SOC functional groups were not subjected to statistical analysis, we may not confidently make strong conclusions, but a mere suggestion on how land use change affected SOC structure.

Regardless, results of this study revealed a decline in polysaccharides as designated by O-alkyl C when the primary grasslands were converted into arable cropping. Polysaccharides are easily digestible and form the most readily available energy source for a wide range of microbial

decomposers (Gergorich *et al.*, 1994; Kögel-Knabner, 2002; Von Lützow *et al.*, 2006), hence O-alkyl C has always been the first to lose intensity every time virgin soils are brought under cultivation (Mathers *et al.*, 2007). In fact, when cultivation intensifies the extent of SOC decay increases, resulting in a loss of O-alkyl C, which coincides with an increase in alkyl, aromatic and carbonyl C (Baldock *et al.*, 1992; Helfrich *et al.*, 2006; Carvalho *et al.*, 2009; Dieckow *et al.*, 2009).

However, O-alkyl C in the cultivated soils of the Harrismith agro-ecosystem remained virtually unaltered despite the cooler-moist climatic conditions that favour high microbial activity and diversity. Explanations to this phenomenon could be that: 1) microbial decomposers are not just important for SOC mineralization, but can immobilize labile C and incorporate it in their own tissues (Baldock *et al.*, 1992; Lauer *et al.*, 2011); and/or 2) slightly higher clay content of the cultivated Harrismith soils compared to their counterparts in the Tweespruit and Kroonstad preserved labile C and constrained its accessibility to the decomposer community (Von Lützow *et al.*, 2006; Preger *et al.*, 2010).

An increase in alkyl C as the extent of SOC decomposition increased was due to highly stable polymethylene structures of hydrophobic compounds such as lipids, waxes and cutins to mention but a few (Kögel-Knabner, 2002; Von Lützow *et al.*, 2006). It is the hydrophobic nature of these compounds and association with the soil mineral phase that prevent their accessibility to microbial decomposers (Baldock *et al.*, 1992; 1997; Kögel-Knabner, 1997; Von Lützow *et al.*, 2006). However, a higher increase in alkyl C was detected in Kroonstad with sandier soils. Probably SOC therein was richer in hydrolysable cutin and suberin, which could not be broken down because of the warm-dry soil conditions as opposed to Harrismith and Tweespruit (Mathers *et al.*, 2007).

Lignin was the major contributor to aromatic C, which increased with an increase in decomposition following cultivation of grasslands in all the three agro-ecosystems. However, noticeable increases were evident in Tweespruit (from 19 to 26%). Lignin could last for years in the soil and

have been indicated as the major occupant of humic acids (Kögel-Knabner, 2002; Carvalho *et al.*, 2009). Possibly lignin was not the major constituent of C_{HA}, which was the most lost humic fraction in the cultivated soils of Tweespruit compared to other regions (Dieckow *et al.*, 2009).

Similar to aromatic C, carbonyl C is not commonly used as an indicator of the extent of SOC decomposition due to its high variability in the soil (Baldock *et al.*, 1992; 1997; Skjemstad *et al.*, 2001; Mathers *et al.*, 2002; Mathers and Xu, 2003). Carbonyl C can decrease (Harrismith), increase (Kroonstad) or remain constant (Tweespruit) as decomposition processes proceed further, and this could be due to the lability or recalcitrance of organic compounds resonating within the carbonyl C chemical shift (Kögel-Knabner, 1997; 2002; Mathers *et al.*, 2007).

A constant supply of fresh organic materials under secondary pasture management reinstated carbohydrate C lost due to intensive soil cultivation. This has been extensively discussed under different environments though resemblance of primary grasslands specifically has not been reported in many studies (Bach *et al.*, 2010; Preger *et al.*, 2010; Baer *et al.*, 2015). In this study, secondary pasture management improved labile C as indicated by O-alkyl C, exceeding those recorded in the primary grasslands, particularly in Harrismith and Kroonstad. Even so, higher restored O-alkyl C was detected in Kroonstad (16%) compare to the other two regions (4%) because of the greater C saturation deficit (Baer *et al.*, 2015) and sandier soils which are characterized by rapid SOC turnover rates in the former (Preger *et al.*, 2010; Kotzé *et al.*, 2016). Alkyl, aromatic and carbonyl C intensities remained lower or unaltered compared to those in the cultivated and virgin soils, which indicates regular additions of labile residues and delayed humification (Vázquez *et al.*, 2016). This contradicts with responses of humic substances observed in the restored relative to cultivated soils. These inconsistencies are probably caused by efficiency differences between the chemical and spectroscopic characterization procedures (Baldock *et al.*, 1992; Kögel-Knabner, 1997; 2002).

3.4.3 Extent of decomposition

The HI, PI and alkyl C/O-alkyl C values are good indicators of the degree of lability or humification of SOC (Baldock *et al.*, 1992; Abril *et al.*, 2013). Usually the higher HI and PI values are said to signify aromaticity of C_{HA} and the extent of SOC humification with less proportion of labile C_{FA} (Sun *et al.*, 2012; Guimarães *et al.*, 2013; Vázquez *et al.*, 2016). However, in this study higher HI and PI values in the virgin and restored soils relative to the cultivated soils in Tweespruit indicated that the primary and secondary grasses contained little proportion of labile compounds compared to the rotated crops (maize, wheat and sunflower residues) in the cultivated lands (Helfrich *et al.*, 2006). In other words, an increase in HI and PI values were not due to increased C_{HA} as a result of decomposition, but because of the residue quality (Baldock *et al.*, 1992). On the other hand, greater HI values in the restored soils of Harrismith and Tweespruit compared to Kroonstad suggest higher C_{HA} in the latter (Figure 3.4), thus humified SOC therein. Since the secondary pastures were dominated by the same plant species e.g. *Eragrostis curvula* across the three agro-ecosystems, it is obvious that decomposition and humification processes occurred, but were lower in Kroonstad due to warm-dry soil conditions (Baldock *et al.*, 1992; Davidson & Janssens, 2006). The alkyl C/O-alkyl C ratio exhibited a different pattern compared to HI and PI values, and therefore indicated that SOC in the cultivated soils of Kroonstad were more humified than SOC in the virgin and restored soils or cultivated soils in Harrismith and Tweespruit. The differences are probably due to differences in the used analytical procedures (Baldock *et al.*, 1992; Kögel-Knabner, 1997; 2002). The contradictions between indicators of the extent of humification were evident, but because HI and PI values were subjected to statistical analysis, alkyl C/O-alkyl C ratio can be disregarded when drawing up a conclusion.

3.5 Conclusion

This study demonstrated the capability of secondary pasture management to restore substantial quantities of soil C fractions lost when grasslands were brought under intensive cultivation and

improve SOC quality, especially labile C (e.g. CWEC and O-alkyl C) to resemble primary grasslands. Restoration of these C fractions and SOC quality was modulated by climate and soil conditions in combination with the features of secondary pasture management (e.g. permanent cover, exclusion of cultivation and fallow periods and continuous additions of plant litter), and levels of the C fractions in the cultivated soils before reversion into perennial pastures. Higher O-alkyl C in the restored soils which also remained virtually unaltered in the cultivated soils of Harrismith indicate that soil microbes can store C through immobilization of labile C. This could contribute to intermediate- to long-term C sequestration. However, microbial immobilization of labile C fractions has not been explored adequately in soil science research, and therefore warrants further investigations. In general, these semi-arid agro-ecosystems have shown vulnerability to degradation, and therefore continuous cultivation beyond this point can result in irrevocable damage. Adoption of secondary pasture management or conservation agriculture are the only possible options to reverse soil degradation in the cultivated lands not only in central South Africa, but also in other areas susceptible to degradation as a result of regular cultivation.

Chapter 4

Changes in soil carbon fractions induced by grazing regimes in commercial grassland and savanna rangelands of South Africa

Abstract

Effects of grazing in commercial rangelands on soil properties have not been extensively studied under dry environments. We evaluated the impact of changes in rangeland conditions along a grazing gradient on soil carbon (C) fractions and soil organic carbon (SOC) structure in South African commercial grassland and savanna ecosystems. Three farms with two rangeland conditions were selected in both ecosystems. Soil samples were taken in the 0-50 mm layer from rangelands in good and poor conditions and analyzed for SOC, soil inorganic carbon (SIC), labile and humic C fractions. The SOC structure was determined with ^{13}C NMR spectroscopy. Indices of the extent of SOC decomposition were calculated. Rangeland conditions did not influence soil C fractions, probably due to rotational grazing practices. On average, good and poor rangeland conditions in the clayey and cool-moist grassland ecosystem resulted in 2-4 times higher C fractions compared to their counterparts in the sandy and hot-dry savanna ecosystem. In the grassland ecosystem, poor rangeland condition resulted in 7% lower O-alkyl C and 1-5% higher alkyl, aromatic and carbonyl C relative to the good rangeland condition, indicative that decomposition occurred in this ecosystem however its effects were masked by a higher MAR. In the savanna ecosystem, SOC quality was influenced more by litter quality than decomposition.

Key words: ^{13}C NMR spectroscopy, Carbon, Clay content, Climate, Grazing gradient, Vegetation

4.1 Introduction

Semi-arid to arid regions claim over 70% of South Africa's total land surface (Smet & Ward, 2006; Directorate Statistics and Economic Analysis, 2016). Due to low-erratic rainfall events and high

temperatures, at least 80% of these regions is used for livestock production under either communal or commercial farming systems (Directorate Statistics and Economic Analysis, 2016; Smet and Ward, 2006), with most of the livestock production coming from commercial farms (Directorate Statistics and Economic Analysis, 2016). However, besides the booming urban and industrial encroachment, commercial rangelands are continuously lost to degradation. In fact, there is a worldwide outcry that escalating demand for food and fibre as well as the harsh environmental conditions have imposed an immense pressure on rangelands, in the process threatening the sustainability of the ecosystems to deliver goods and services (Conant *et al.*, 2001; Conant, 2010).

Rotational grazing in closed paddocks remained the exclusive resource management strategy in the South African commercial farms since the 19th century when rangelands were surveyed and distributed among individuals (Kotzé *et al.*, 2013). Rotational grazing and controlled stocking rates prevented rangeland deterioration and improved ecosystem services, including forage production and recovery, basal cover and product quality for both local and international markets (Smet & Ward, 2006). However, recent studies have reported detrimental changes in the botanical composition accompanied by soil desertification in commercial rangelands not only in South Africa, but also in other parts of the world (Reeder & Schuman, 2002; Sousa *et al.*, 2012; Kotzé *et al.*, 2013; Sandhage-Hofmann *et al.*, 2015). Such changes may be induced among other factors by fluctuating stocking rates and differences in forage preferences by animals (Conant, 2010). Water scarcity in many parts of the world, which triggered erection of artificial water points for livestock, is another source of uneven grazing regimes in the commercial rangelands (Vetter, 2009).

Animal species and stocking rates, which vary from one farm to another, can modify the rangeland condition. However, declining rangeland condition in commercial livestock farms seems to be more pronounced around artificial water points, and as such, does not necessarily reflect animals'

forage preferences (Smet & Ward, 2006; Kotzé *et al.*, 2013; Sandhage-Hofmann *et al.*, 2015). High animal activity within the vicinity of watering points has been identified as the primary cause of vegetation degradation in commercial rangelands (Vetter, 2009). Thus, animals' regular visits to the water points result in intensive grazing and trampling within this vicinity and continue to destroy the existing seed bank (Kotzé *et al.*, 2013; Sandhage-Hofmann *et al.*, 2015). Reduced regenerative and reproductive potential of vegetation around the water points also renders the soil more vulnerable to erosion and desertification (Lu *et al.*, 2015).

Majority of studies carried out to examine the effect of grazing on rangeland degradation focused primarily on botanical composition and vegetation productivity (Smet & Ward, 2006; Lu *et al.*, 2015). Vegetation composition is influenced mostly by precipitation and grazing regimes, and could be used to assess short-term rangeland degradation (Vetter, 2009). Soil properties on the other hand, have been given little attention, yet they can be seen as reliable indicators of long-term rangeland condition (Smet & Ward, 2006; Kotzé *et al.*, 2013). Soil properties control the cycling and supply of nutrients and water necessary for the vegetation to flourish (Lu *et al.*, 2015). Soil carbon (C) for example, is one of the most widely used monitoring tools to assess changes in soil quality and land use effects not only in arable landscapes, but also in the natural terrestrial ecosystems due to its inherent relation with other chemical, physical and biological soil properties. As a key soil quality indicator and ecosystem regulator, soil C should be safeguarded at all costs.

Soil C can be divided into soil organic (SOC) and inorganic (SIC) carbon, with the former consisting of organic materials at different stages of decomposition e.g. labile, slow and stable (Von Lützwow *et al.*, 2006; Du Preez *et al.*, 2011b), while the latter represents total carbonates present in the soil either derived from parent material (lithogenic) or formed during soil reactions (pedogenic) (Wang *et al.*, 2010; Shi *et al.*, 2012; Jin *et al.*, 2014). Labile C represents a pool of readily digestible compounds, which are an important energy source for soil organisms, a nutrient reservoir and serves as early indicators for soil management changes, owing to their sensitive

nature and rapid turnover rates (Von Lützow *et al.*, 2006; Jianping & Wei, 2017;). Slow C consists of precursors to stable C, also referred to as humified C (humic substances) – an agent of soil structural formation and stability due to its high affinity for mineral particles, which also contribute to its recalcitrance to microbial degradation (Von Lützow *et al.*, 2006; Kotzé *et al.*, 2016). The selective preservation of humic substances, which is a function of their complex structure, is the main reason why stable C is seen as a potential sink for atmospheric carbon dioxide (CO₂) (Von Lützow *et al.*, 2006). Soil inorganic C is also important for soil aggregation and nutrient cycling in terrestrial ecosystems (Bronick & Lal, 2005). Dissolution or formation of SIC could be considered a C sequestration mechanism, especially when CO₂ and calcium (Ca²⁺) or magnesium (Mg²⁺) involved in these processes are derived from soil respiration and non-carbonate minerals, respectively (Sanderman, 2012; Shi *et al.*, 2012).

Several studies have been conducted in grassland and savanna ecosystems, and many have demonstrated that these ecosystems could be a prominent reservoir for atmospheric C, if managed properly due to their large geographic coverage e.g. one-third of the earth's total land area (Batjes, 1996; Conant *et al.*, 2001; Yong-Zhong *et al.*, 2005; Lal *et al.*, 2015). Minimum disturbance and continuous production of the plant biomass enhances the formation of soil aggregates and the buildup of C, thereby controlling erosion induced processes (Janzen, 2005; 2006; Conant, 2010). Kotzé *et al.* (2013) reported a decline in macro-aggregates accompanied by losses of SOC and total nitrogen (TN) around the water points due to animal trampling in the semi-arid grassland ecosystem of South Africa. Similar observations were recorded by Sandhage-Hofmann *et al.* (2015) in the arid savanna ecosystem. In both cases, SOC and TN deteriorations were less pronounced in commercial farms where overgrazing is kept minimal compared to communal rangelands. Nevertheless, SOC is relatively insensitive to management effects and its response to changes in rangeland conditions could take time to be detected

(Janzen, 2005; 2006; Sousa *et al.*, 2012). As such, quantitative evidence on different fractions is required for better decision making on soil C management.

Vázquez *et al.* (2016) evaluated SOC chemical and spectroscopic changes due to land use in the arid ecosystems of central Argentina and indicated that information regarding the combined use of qualitative and quantitative characterization of SOC is still scarce, particularly in the dryland ecosystems. In fact, to the best of our knowledge, no study has ever reported on chemical and solid state spectroscopic characteristics of SOC driven by grazing regimes in the grassland and savanna ecosystems of South Africa. Application of both chemical and spectroscopic SOC characterization techniques provide a more profound understanding of changes induced by decomposition-humification processes than when either of the methods is solely used (Vázquez *et al.*, 2016). Solid state cross polarization magic angle spinning (CPMAS) ^{13}C nuclear magnetic resonance (NMR) spectroscopy together with classical extraction methods can improve our knowledge of the type of C existing in the soil and in turn help to predict whether the accumulated C is a potential source or sink for atmospheric CO_2 (Janzen, 2005). Though results obtained with these two analytical tools can be complementary, it should be noted that either of these procedures may give lower or higher results due to their efficiency differences (Baldock *et al.*, 1992). Kögel-Knabner (1997) also urged that results obtained with wet chemical extraction methods are in most cases inconsistent with those given by ^{13}C NMR spectroscopy, presumably due to degradative effects of wet extraction methods or SOC enriched with humified C, which may be somewhat resistant to chemical degradation.

Unlike SOC and its fractions, SIC has received little attention yet it also plays a critical role in biogeochemical cycling of C, particularly in the arid to semi-arid environments where its concentrations may exceed those of SOC (Batjes, 1996; Wu *et al.*, 2009; Jin *et al.*, 2014). Carbon transfers between the atmosphere and rhizosphere involve both SIC and SOC, and therefore should all be considered in soil C studies (Wu *et al.*, 2009; Shi *et al.*, 2012), though some

researchers indicated negligible effects of SIC on atmospheric C sequestration (Jin *et al.*, 2014). Despite that, Bronick & Lal (2005) showed that SOC and SIC are both involved in soil structural formation, which implies that the presence of one could stimulate accumulation of the other. Some authors on the other hand, found that a decrease in SOC as a result of decomposition leads to precipitation and accrual of SIC whereby two moles of bicarbonate are consumed, while one mole of CO₂ is released (Wang *et al.*, 2010; Shi *et al.*, 2012; Jin *et al.*, 2014). However, Sanderman (2012) indicated that this reaction can only improve SIC accumulation provided there is a supply of Ca²⁺ or Mg²⁺. Concentrations of these base cations are often higher in arable landscapes where fertilization and liming are often part of soil management (Mikhailova & Post, 2006; Wu *et al.*, 2009), as such it is still not clear how altered rangeland conditions by grazing intensity affect SIC.

The objective of this study was to evaluate the effect of rangeland condition (poor and good) on different soil C fractions and SOC structural composition in the upper 0-50 mm of grassland and savanna ecosystems under commercial livestock farming. Selected grassland and savanna ecosystems are respectively located in Thaba Nchu in the Free State Province and Kuruman at the boarder of the Northern Cape and North West Provinces. These areas are characterized by low rainfall and high temperatures that have resulted in low phytomass production and turnover, rendering commercial rangelands vulnerable to degradation due to low soil resilience (Kotzé *et al.*, 2013; Sandhage-Hofmann *et al.*, 2015). However, these two ecosystems differ in terms of soil clay content, and therefore their degree of degradation may differ (Kotzé *et al.*, 2016). As a result, the two ecosystems were also compared to determine the influence of local conditions on rangeland degradation since the response of soil C fractions to livestock grazing can also reflect differences in site-specific conditions (Vázquez *et al.*, 2016).

We hypothesized that the measured C fractions and SOC quality will decrease with increasing grazing intensity along the grazing gradient, from the water points to further into the rangeland. It is also the hypothesis of this study that the clayey grassland soils will resist degradation compared

to the sandy savanna soils. To test these hypotheses, selected soil samples from good and poor rangeland conditions of grassland and savanna ecosystems were analyzed for soil C, SOC, SIC, labile C fractions, humic substances and SOC functional groups.

4.2 Material and Methods

4.2.1 Site description

Study sites are located in the grassland ecosystem, near Thaba Nchu in the Free State Province (latitude 28°-29°S, longitude 26°-27°E; with an altitude of 1400 to 1600 m above sea level) and in the savanna ecosystem, near Kuruman at the border of the Northern Cape and North-West Provinces, on the fringe of the Kalahari desert (latitude 27°-28°S, longitude 22°-24°E; with an altitude of 1047-1161 m above sea level). The locations of the Thaba Nchu and Kuruman ecosystems in South Africa are displayed in Figure 4.1.

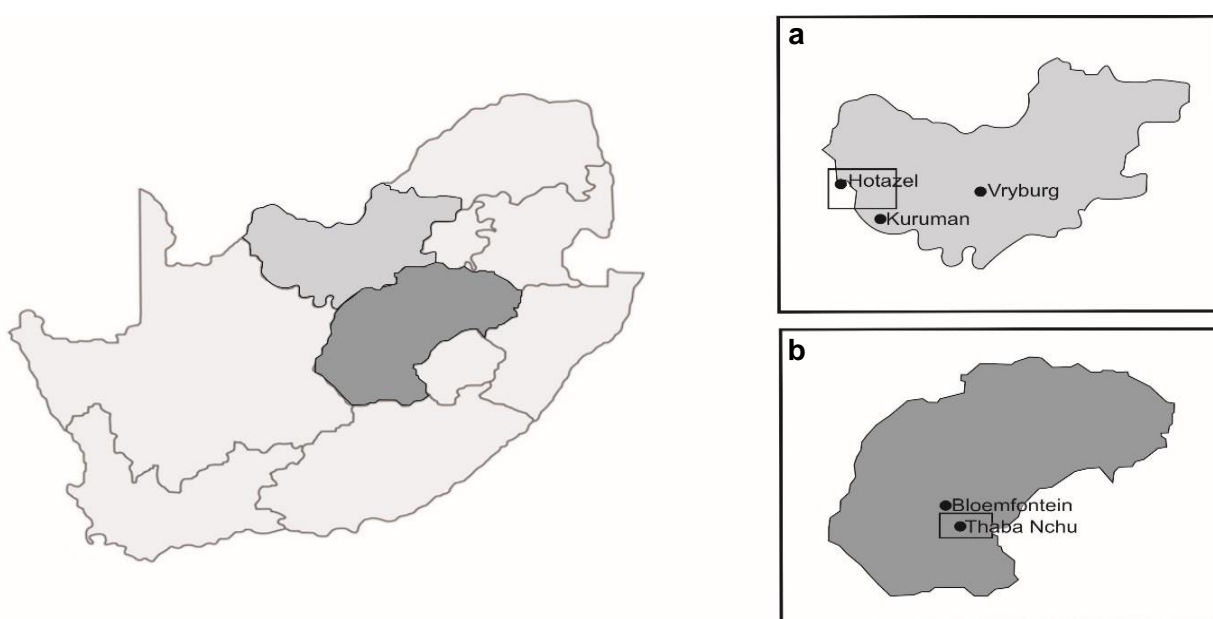


Figure 4.1 Location of study sites in the savanna ecosystem near Kuruman (a) and in the grassland ecosystem near Thaba Nchu (b), South Africa.

The vegetation at Thaba Nchu is classified as Moist Cool Highveld Grassland (Bredenkamp & Van Rooyen, 1996), with a mean annual temperature (MAT) of 14.8 °C, mean annual rainfall (MAR) of 553 mm and mean annual class A-pan evaporation (MAE) of 1832 mm. In this semi-arid climate 70% of rainfall occurs in summer between January and March. Soils in this grassland ecosystem were classified as Lixisols (IUSS Working Group WRB, 2014), with more than 15% clay in the A-horizons and more than 25% clay in the B-horizons and no obvious signs of wind or water erosion. The vegetation was dominated by a single layer of perennial C₄ bunchgrass (e.g. *Themeda triandra*, *Eragrostis superba* and *Cymbopogon plurinodis*) with sparsely distributed trees (e.g. *Acacia karoo* and *Rhus ciliata*) (Bredenkamp & Van Rooyen, 1996). The amount of grass cover depended on the degree of grazing, among others. Frost, fire and grazing maintained the grass dominance and prevented establishment of trees.

The vegetation at Kuruman belongs to the Kalahari thornveld and shrub busveld (Tainton, 1999). Kuruman has an arid climate with a MAT of 17.5 °C, MAR of 355 mm and MAE of 2050 mm. Most of rain falls in summer between October and March. Soils in this savanna ecosystem are deep Arenosols with aeolian origin underlain by calcrete (IUSS Working Group WRB, 2014), with less than 4% clay. The vegetation is characterized by a fairly well developed tree stratum with *Acacia erioloba*, *Acacia haematoxylon* (≤ 2 m height) and some *Boscia albitrunca* as the dominant trees (Van Rooyen & Bredenkamp, 1996). The shrub layer was dominated by individuals of *Grewia retinervis* and *Rhus tenuinervis* (Van Rooyen & Bredenkamp, 1996). The dominant grass species included *Eragrostis lehmanniana*, *Aristida meridionalis*, *Centropodia glauca* and *Stipagrostis amabilis* (Van Rooyen & Bredenkamp, 1996).

4.2.2 Rangeland management and sample selection

Commercial livestock farming is the dominant rangeland management system in South Africa. Farms used for this activity are well developed, market-oriented and typically managed using

rotational grazing systems at moderate stocking rates, with the sole purpose of producing high quality animals and their products, which can be marketed. Stocking rates ranged from 6.40 ha LSU⁻¹ in the grassland ecosystem to 14.20 ha LSU⁻¹ in the savanna ecosystem. Commercial livestock farming is under threat of rangeland degradation, as such it was found ideal to identify associated problems and strategies that can sustainably restore rangeland resources. Soil samples were selected from the collection made in March 2010 and 2011 by Kotzé (2015), and a brief description on how sampling was carried out follows.

In both ecosystems, three farms were sampled, treating each farm as an independent replicate. For each farm a representative grazing gradient was selected, starting nearby artificial water points to a little further in the rangeland. The length of the grazing gradients within farms differed depending on the size of the camp, but the gradient belonged to a single camp. The gradients included six single plots, each 10 x 10 m in size. These plots were defined exclusively through grass quality conditions, using a similar technique as Van Der Westhuizen *et al.* (2005), independent of bare patches or bush encroachment. Indicator grass species defined on-site by plant experts for the purpose of rangeland condition assessment were identified. Rangeland conditions were identified as poor, moderate and good.

In both ecosystems, the technique by Van Der Westhuizen *et al.* (2005) was used to identify indicator grass species for the purpose of rangeland condition assessment. In the grassland ecosystem, the undisturbed open grassland (good rangeland condition) was dominated by the climax grass *Themeda triandra* (Redgrass), and only a few other species occurred. With increasing grazing pressure, *Eragrostis spp.* (Weeping lovegrass) replaced *T. triandra* and became therefore dominant in the subclimax, moderate rangeland condition stage. In the sacrifice area, under poor rangeland conditions, pioneer annual grass species such as *Aristida congesta* (Tassel bristlegrass) and *Cynodon dactylon* (Couchgrass) increased in proportion to *Eragrostis spp.* Bare patches were common in poor rangeland conditions, although they also occurred under

moderate and good rangeland conditions. In the savanna ecosystem, the dominant indicator grass species is *Stipagrostis spp.* (Bushman grass) for good rangeland condition, *Eragrostis spp.* (Curley leave, Lehmann's love grass) for moderate rangeland condition and *Aristida spp.* (Tassel three-awn) and *Schmidtia kalahariensis* (Kalahari sour grass) for poor rangeland condition. *Acacia* species were also dominant in this ecosystem with the area being affected by bush encroachment.

Along a center line, soil samples were taken every 2 m using a 50 mm diameter auger and combined into a composite sample for three depth intervals, respectively (0-50, 50-100 and 100-200 mm). The composite samples were air-dried, sieved (< 2 mm) and prepared for further analyses. However, because the effects of rangeland conditions are normally more concentrated in the 0-50 mm soil layer, only samples from this layer in the poor and good rangeland conditions were selected for this study. As indicated earlier, the length of the grazing gradients differed with the size of camps, but distances between the six plots were kept constant within each sampling site. Moderate rangeland conditions were not necessarily present in all the grazing gradients, hence we selected samples only from poor and good rangeland conditions. Regardless of the ecosystem or rangeland condition, bulk densities of the sampled soils did not differ significantly (Table 4.1). As a result corrections of C concentration values with bulk density resulted in negligible changes in the actual contents (stocks), hence results of this study were presented in concentrations.

Table 4.1 Soil properties of the rangeland whose condition was classified as poor or good in the grassland and savanna ecosystems

Ecosystem	Rangeland condition	Bulk density (g cm ⁻³)	pH (H ₂ O)	Calcium (mg kg ⁻¹)	Magnesium (mg kg ⁻¹)	Clay (%)
¹ Grassland	Poor	1.36±0.05	6.6±0.5	2300±960	529±70	35
	Good	1.38±0.03	6.4±0.2	1800±250	519±18	35
² Savanna	Poor	1.54±0.03	6.9±0.5	331±45	53±13	2.6
	Good	1.53±0.08	6.2±0.4	299±108	50±25	3.9

¹Kotzé *et al.* (2013); ²Sandhage-Hofmann *et al.* (2015)

4.2.3 Laboratory analysis

Analyses were carried out to determine soil C, SOC, SIC, permanganate oxidizable C (POXC), cold (CWEC) and hot (HWEC) water extractable C, humic substances (extractable humic substances (EX), humic (HA) and fulvic (FA) acids) and organic C functional groups.

4.2.3.1 Soil carbon

Soil C was analyzed by dry combustion (Nelson & Sommers, 1982) with a TruSpec Leco CN analyzer. Approximately 0.43 g sieved air-dried encapsulated soil samples were placed in a loading head, and one by one dropped into a 950 °C hot furnace and flushed with oxygen for rapid and complete combustion. Combustion gases were then passed through a secondary furnace (850 °C) for further oxidation before collected in a collection vessel where oxygen was injected and mixed with combustion gases. These gases were purged through a CO₂ infrared detector, which measures C as CO₂.

4.2.3.2 Soil organic and inorganic carbon

Soil organic C was measured with a modified Mebius procedure (Nelson & Sommers, 1982). Briefly, 0.5 g of sieved air-dried soil was weighed in a 150 ml glass beaker and reacted with 10

ml of 0.5 N potassium dichromate ($K_2Cr_2O_7$) and 15 ml of concentrated sulphuric acid (H_2SO_4). Samples were placed on a preheated sand bath at a temperature of 130 °C for 10 minutes. Samples were then removed from the sand bath and 35 ml of deionized water was added to each. Excess $K_2Cr_2O_7$ was titrated with 0.2 N ferro-ammonium sulphate [$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$] until the end point was reached, which was detected by millivoltmeter with a platinum electrode. Soil inorganic C was calculated as the difference between soil C and SOC.

4.2.3.3 Permanganate oxidizable carbon

Permanganate oxidizable C was analyzed according to Culman *et al.* (2012). Briefly, 2.5 g of sieved air-dried soil was weighed into 50 ml falcon tubes, wherein 18 ml deionized water and 2 ml 0.02 M $KMnO_2$ solution were added. The tubes were shaken for 2 minutes at 240 oscillations per minute on an oscillating shaker and then centrifuged for 5 minutes at 906 x g for the soil to settle down. Immediately thereafter, 0.5 ml of the supernatant were transferred to new 50 ml falcon tubes and diluted with 49.5 ml deionized water before reading the sample absorbance on a spectrophotometer at 550 nm wavelength to obtain oxidizable C.

4.2.3.4 Cold and hot water extractable carbon

Cold and hot water extractable C were determined according to Ghani *et al.* (2003). In short, 3 g air-dried soil samples were transferred to 50 ml falcon tubes and extracted with 30 ml of deionized water. Samples were shaken for 30 minutes on an end-over-end shaker and centrifuged for 20 minutes at 1233 x g. The supernatants were filtered through a 0.45 μm cellulose membrane filter into separate vials for C analysis. A 30 ml aliquot of deionized water was again added to the sediments in the same tubes and shaken on a vortex shaker to suspend the sediments. The capped tubes were then left in a hot-water bath at 80 °C for 16 hours. Samples were again shaken on a vortex shaker and centrifuged for 20 minutes at 1233 x g. The supernatants were filtered through a 0.45 μm cellulose membrane filter. Carbon in both extracts was determined according

to the modified Mebius procedure, where 5 ml of the extracts was used instead of soil. Carbon obtained from the first extraction was referred to as C_{WEC}, while that from the second extraction was classified as H_{WEC}.

4.2.3.5 Humic substances

The sequential procedure of Schnitzer (1982) was slightly modified to extract and fractionate humic substances. Briefly, 5 g of sieved air-dried soil was weighed out in falcon tubes and reacted with 30 ml extraction solution [0.1 N sodium hydroxide (NaOH) and 0.1 M sodium pyrophosphate decahydrate (Na₄P₂O₇·10H₂O)]. The contents were shaken on an oscillating shaker for an hour and centrifuged at 906 x g for 15 minutes. Insoluble material contained in the supernatant was isolated from soluble alkaline material (extractable humic substances). Soluble alkaline material was then precipitated with 0.05 N H₂SO₄ acid to fractionate humic and fulvic acids. Carbon concentrations of extractable humic substances (C_{EX}) and humic acids (C_{HA}) were determined with the Mebius procedure (Nelson & Sommers, 1982). Carbon in fulvic acids (C_{FA}) was calculated as the difference between C_{EX} and C_{HA}. Humification index (HI = C_{HA}/SOC) and polymerization index (PI = C_{HA}/C_{FA}) were calculated according to Abril et al. (2013).

4.2.3.6 Nuclear magnetic resonance spectroscopy

Bulk soil samples were pre-treated with hydrofluoric acid (HF) to remove magnetic materials, concentrate organic C and increase the signal-to-noise ratio of the resultant NMR spectra as recommended by Skjemstad *et al.* (2001) and Mathers *et al.* (2002). Briefly, 5 g of ground sieved air-dried soil was weighed into 50 ml falcon tubes and 45 ml of 2% HF was added. The tubes were shaken on end-over-end shaker for 8 (4 x 1 hour, 3 x 16 hours and 1 x 64 hours) successive times. Samples were then centrifuged after every extraction at 671 x g for 20 minutes and the supernatant was filtered through a 5 mm Millipore Durapore membrane filter to recover the light fraction. After the final extraction, residues together with the light fraction trapped on the

membrane filter were washed 5 times with deionized water, oven dried at 75 °C and ground to powder using a mortar and pestle for NMR analysis.

Whole HF-treated soil samples were packed in cylindrical zirconia rotors before analysis. The NMR analysis was done on a 400 MHz Bruker AVANCE III spectrometer equipped with a 4 mm VTN multinuclear double resonance magic angle spinning probe, operating at room temperature. The ^{13}C NMR spectra were recorded at 100.6 MHz, using the CPMAS technique. A rotating speed of 14 000 Hz was used with a contact time of 1 minute, a recycle delay of 1 second and an acquisition time of 12.8 minutes. All the spectra were recorded with 86 016 scans and baseline corrected. The following ranges were integrated: 0–50 ppm (alkyl-C), 50–110 ppm (O-alkyl C), 110–160 ppm (aromatic C), and 160–180 ppm (carbonyl C).

4.2.4 Statistical analysis

Statistical analyses were performed with SPSS version 24 software package (SPSS Inc.). A two-way analysis of variance (ANOVA) was carried out to determine the influence of land use, agro-ecosystem and their interaction on soil C fractions. Means were compared with Tukey's honestly significant difference (HSD_T) post hoc tests, at 95% confidence level. All data were tested for normality and homogeneity using Shapiro-Wilk and Levene's test, respectively before carrying out ANOVA. For ^{13}C NMR spectra, three replicate soil samples per treatment were mixed thoroughly and subjected to NMR spectroscopy, as composite samples due to high costs and time needed to obtain spectra, as such no statistical analyses were performed.

4.3 Results

Although rangeland conditions varied along the grazing gradient from poor to good as indicated by plant species, the measured soil C fractions within the grassland or savanna ecosystem did not change significantly ($P < 0.05$) except for SIC, which was higher in the poor compared to the

good rangeland condition in the grassland ecosystem (Figure 4.2-4.4). Surprisingly concentrations of most of these C fractions in the poor rangeland condition were slightly higher than those recorded in the good rangeland condition irrespective of the ecosystem. However, the focus will be on comparing the rangeland conditions between the two ecosystems, which varied substantially in terms of site-specific conditions: MAR, MAT, clay content and vegetation. For SOC structural composition, results within and between ecosystems will be presented.

4.3.1 Soil organic and inorganic carbon

The SOC and SIC contents were significantly higher ($P < 0.05$) in the poor rangeland condition of the grassland ecosystem compared to the poor rangeland condition of the savanna ecosystem (Figure 4.2). Grassland soils under the poor rangeland condition contained 22.56 ± 5.44 g SOC kg⁻¹ and 9.71 ± 3.34 g SIC kg⁻¹, while soils under the poor rangeland condition of the savanna ecosystem had 5.10 ± 1.16 g SOC kg⁻¹ and 1.17 ± 0.24 g SIC kg⁻¹. In the good rangeland condition, SIC did not exhibit significant ($P < 0.05$) changes between the grassland and savanna ecosystems, while grassland soils accumulated 77% more SOC than the savanna soils.

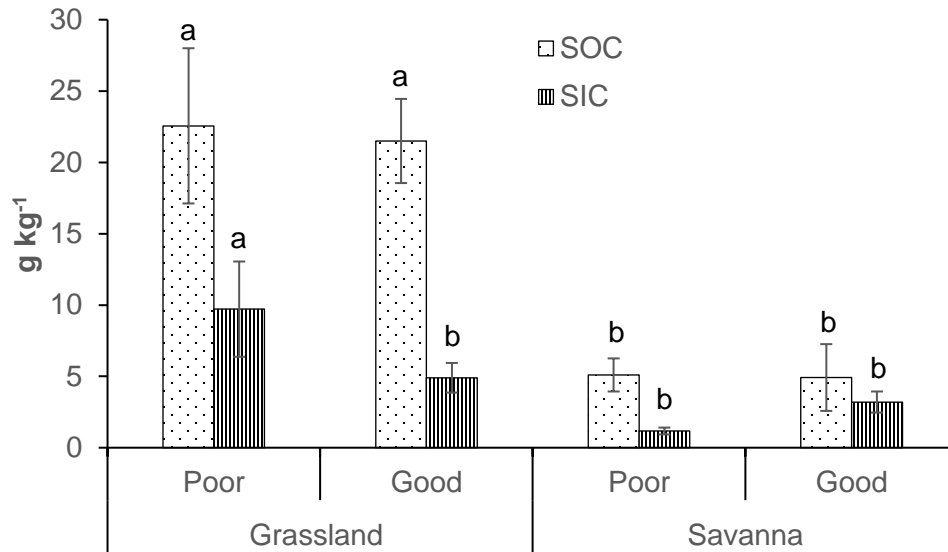


Figure 4.2 Response of soil organic (SOC) and inorganic (SIC) carbon to rangeland conditions in the grassland and savanna ecosystems. Significant differences ($P < 0.05$) are indicated by different letters for each fraction. Vertical bars with horizontal caps indicate standard deviation.

4.3.2 Labile carbon fractions

Labile C fractions were also affected by the ecosystems, exhibiting similar behaviour displayed by SOC (Figure 4.3). The CWEC, HWEC and POXC concentrations were larger ($P < 0.05$) in the grassland relative to the savanna soils regardless of the rangeland condition. In the poor rangeland condition, grassland soils had 2613 ± 481 g CWEC kg^{-1} , 2160 ± 702 g HWEC kg^{-1} and 730 ± 30 g POXC kg^{-1} , whereas savanna soils contained 1247 ± 109 , 890 ± 182 and 370 ± 61 g kg^{-1} of CWEC, HWEC and POXC, respectively. Under the good rangeland condition, CWEC, HWEC and POXC were 2660 ± 352 , 2123 ± 803 , 747 ± 78 g kg^{-1} , respectively in the grassland ecosystem and 997 ± 121 , 880 ± 151 and 303 g kg^{-1} , respectively in the savanna ecosystem.

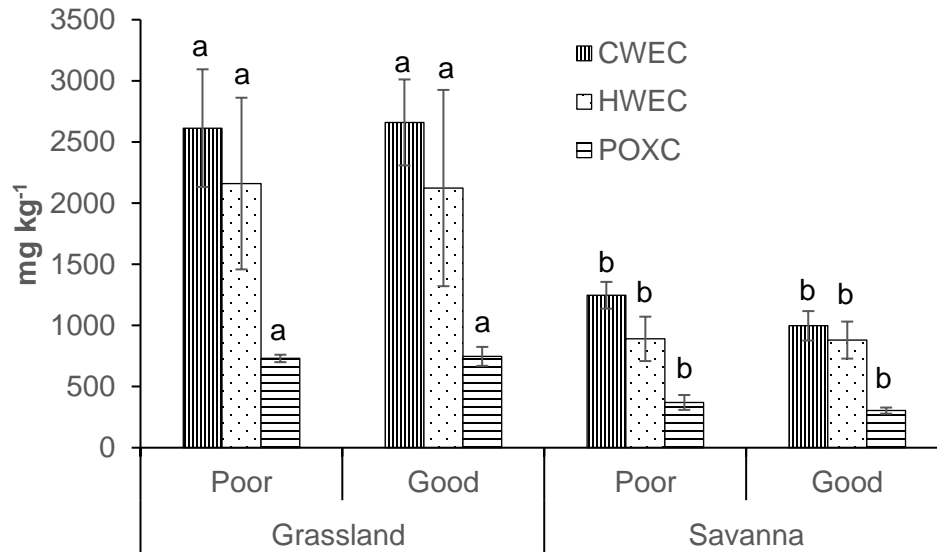


Figure 4.3 Changes in labile C fractions (CWEC, cold water extractable carbon; HWEC, hot water extractable carbon; POXC, permanganate oxidizable carbon) as influenced by rangeland conditions in the grassland and savanna ecosystems. Significant differences ($P < 0.05$) are indicated by different letters for each fraction. Vertical bars with horizontal caps indicate standard deviation.

4.3.3 Humic substances

Grassland and savanna ecosystems also had significant effects ($P < 0.05$) on humic substances (Figure 4.4). The poor rangeland condition resulted in a lower C_{EX} , C_{FA} and C_{HA} in the savanna relative to the grassland ecosystem. The C_{EX} , C_{FA} and C_{HA} varied from 3.67 ± 0.11 , 2.78 ± 0.55 and 0.88 ± 0.08 g kg⁻¹, respectively in the savanna ecosystem to 13.97 ± 3.68 , 9.57 ± 2.45 and 4.40 ± 1.30 g kg⁻¹, respectively in the grassland ecosystem. Similar patterns were observed in the good rangeland condition where grassland soils had 13.39 ± 1.00 , 8.96 ± 0.72 and 4.43 ± 0.30 g kg⁻¹ of C_{EX} , C_{FA} and C_{HA} , respectively, while savanna soils contained 3.08 ± 0.08 , 1.88 ± 0.63 and 1.20 ± 0.20 g kg⁻¹ of C_{EX} , C_{FA} and C_{HA} , respectively.

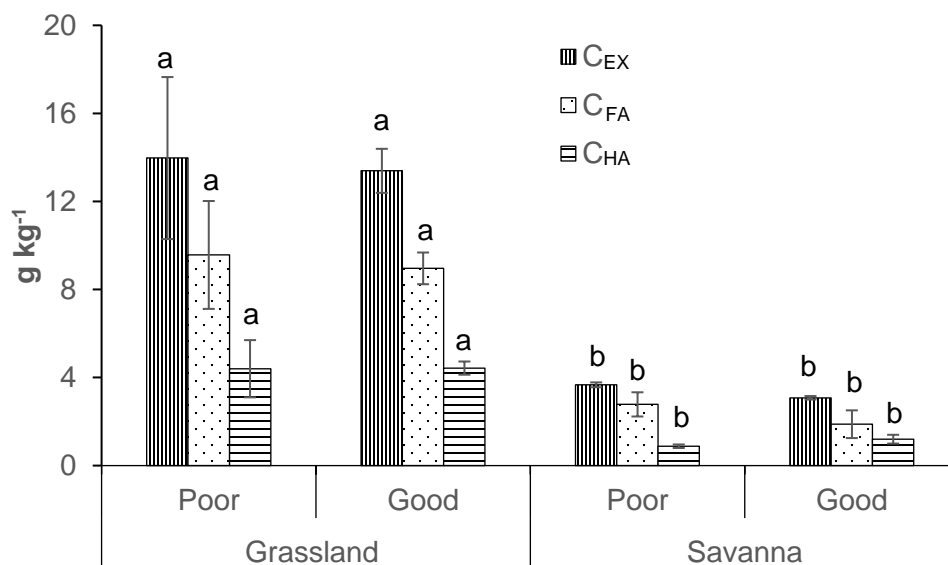


Figure 4.4 Effects of rangeland conditions on humic substances (C_{EX} , extractable humic substances; C_{FA} , fulvic acids; C_{HA} , humic acids) in the grassland and savanna ecosystems. Significant differences ($P < 0.05$) are indicated by different letters for each fraction. Vertical bars with horizontal caps indicate standard deviation.

4.3.4 Structural composition of soil organic carbon

Changes in SOC structural composition as indicated by the CPMAS ^{13}C NMR spectra are shown in Figure 4.5. According to these spectra, all the soils contain the major four organic C groups; alkyl C (0-50 ppm), O-alkyl C (50-110 ppm), aromatic C (110-160 ppm) and carbonyl C (160-180 ppm), irrespective of the rangeland condition or ecosystem and the interpretations were done according to Baldock *et al.* (1992; 1997) and Kögel-Knabner (1997, 2002). The 0-50 ppm region was dominated by aliphatic C in the long chain polymethylene structures arising at 28-32 ppm. Distinctive shoulders appearing towards 24 ppm of the savanna ecosystem (both good and poor rangeland condition) spectra represent aliphatic C in the short chain polymethylene structures. In the 50-110 ppm chemical shift region, dominant signals occurred within the ranges; 55-60, 60-74 and 100-104 ppm. Peaks at 55-60 ppm signify the presence of methoxyl C of lignin origin or alkyl

C bonded to nitrogen (N) in protein structures. Resonances at 60-74 ppm represent carbohydrate C of cellulose and hemicellulose structures, while peaks occurring in the vicinity of 100-105 ppm could be attributed to anomeric C1 of carbohydrate structures. Broad and poorly resolved resonances in the 122-134 ppm of the 110-160 ppm chemical shift region are typical of lignin-derived aryl C (C- or H (hydrogen)-substituted aromatics). In the 160-180 ppm region, peaks resonating around 171 ppm are indicative of carboxyl, amide and/or ester C.

These functional groups followed the order; O-alkyl C > alkyl C > aromatic C > carbonyl C, except in the poor rangeland condition of the savanna ecosystem where aromatic C was greater than alkyl C (Table 4.2). Good rangeland condition resulted in a higher O-alkyl C, but lower alkyl, aromatic and carbonyl C compared to the poor rangeland condition in the grassland ecosystem. The trend changed in the savanna ecosystem, with soils under the good rangeland condition having lower O-alkyl and carbonyl C, but higher alkyl and aromatic C relative to the poor rangeland condition.

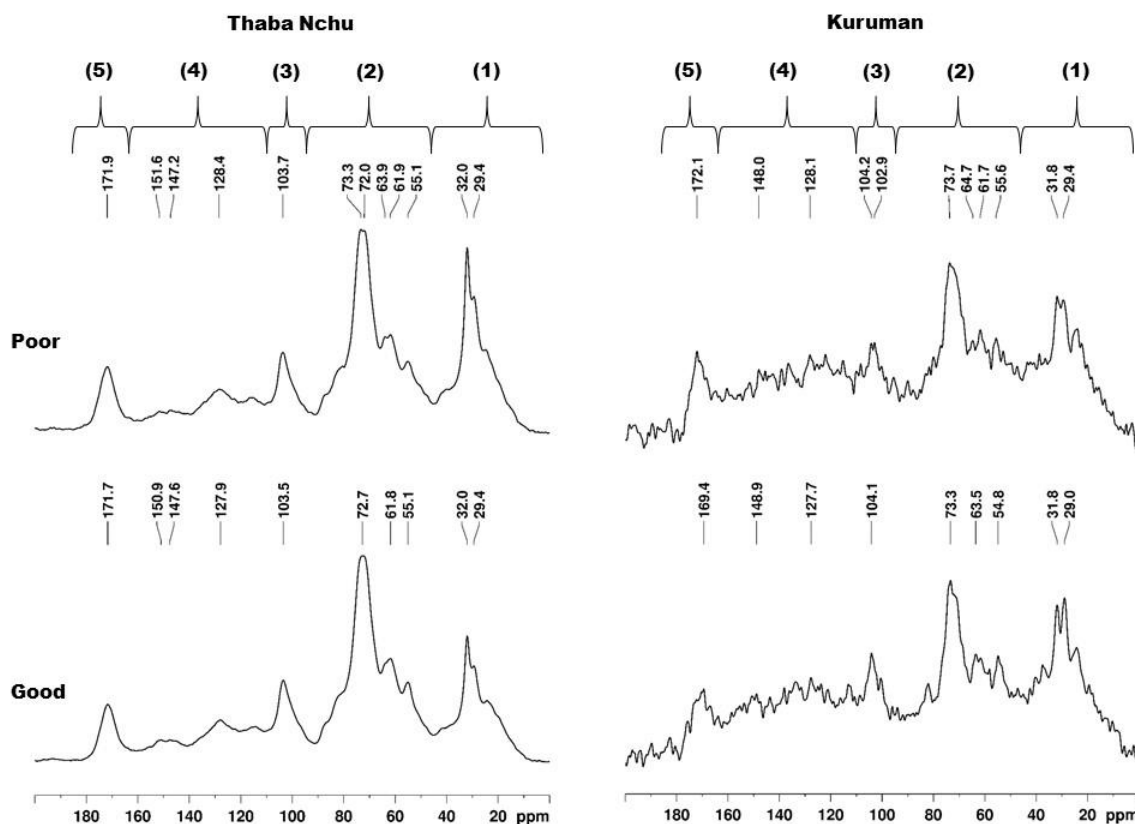


Figure 4.5 Cross polarization magic angle spinning (CPMAS) ¹³C nuclear magnetic resonance (NMR) spectra of soil organic carbon (SOC) in the 0-50 mm soil layer of poor and good rangeland conditions in the grassland (Thaba Nchu) and savanna (Kuruman) ecosystems. (1), alkyl C; (2) O-alkyl C; (3), di O-alkyl C; (4), aromatic C; (5), carbonyl C.

Although soils under both good and poor rangeland conditions in the two ecosystems contained all the major C types, their proportions varied with rangeland condition and ecosystem (Table 4.2). Inconsistent results were recorded when the grassland was compared with savanna ecosystem, mainly due to vegetational differences. Comparisons across the two ecosystems showed that grassland soils had 8-19% higher O-alkyl C and 10-11% lower aromatic C than savanna soils, regardless of the rangeland condition. Good rangeland condition in the grassland ecosystem resulted in lower alkyl and carbonyl C compared to the good rangeland condition in

the savanna ecosystem. On the other hand, alkyl and carbonyl C increased in the poor condition of the grassland ecosystem relative to the poor condition in the savanna ecosystem.

Table 4.2 Structural changes in soil organic carbon induced by rangeland condition of the grassland and savanna ecosystems

Functional groups (%)	Grassland		Savanna	
	Poor	Good	Poor	Good
Alkyl C	26	20	24	28
O-alkyl C	53	60	45	40
Aromatic C	15	14	25	26
Carbonyl C	7	6	6	6

4.3.5 Extent of decomposition

Neither the humification (HI) nor polymerization (PI) indices as markers of the degree of decomposition differed significantly within or between the grassland and savanna ecosystems, regardless of whether rangelands were in a poor or good condition (Table 4.3). The alkyl C/O-alkyl C ratio also revealed that the extent of decomposition was almost similar between the poor and good rangeland conditions in the grassland ecosystem, but slightly lower in the poor compared to the good rangeland conditions in the savanna ecosystem.

Table 4.3 Influence of rangeland condition on indices of the degree of soil organic matter decomposition in the grassland and savanna ecosystems

Ecosystem	Rangeland condition	HI	PI	Alkyl C/O-alkyl C
Grassland	Poor	0.20±0.04a	0.46±0.06a	0.48
	Good	0.21±0.03a	0.50±0.13a	0.34
Savanna	Poor	0.17±0.06a	0.30±0.12a	0.53
	Good	0.28±0.13a	0.68±0.22a	0.70

Except for Alkyl C/O-alkyl C ratio, values presented are means ± standard deviation of three replicates. Means with different letters in a column indicate significant difference at $P < 0.05$. HI, humification index; PI, polymerization index.

4.4 Discussion

Despite that there were no significant differences in the measured C fractions between rangeland conditions within the grassland or savanna ecosystems, interestingly most of the measured fractions were slightly higher in the poor compared to the good rangeland condition (Figure 4.2-4.4). Such responses could be attributed to higher deposition of animal excreta in the poor rangeland condition where animal activity was also higher as opposed to the good rangeland condition (Reeder & Schuman, 2002; Smet & Ward, 2006; Kotzé *et al.*, 2013; Sandhage-Hofmann *et al.*, 2015;). Higher animal traffic and trampling in the poor rangeland condition also seem to have incorporated most of the aboveground C into the soil. In other words, in the good rangeland condition where there was less animal trampling, a considerable proportion of the system C was still intact in plant stands, and thereby restricting aboveground C recycling into the soil (Reeder & Schuman, 2002; Yong-Zhong *et al.*, 2005; Lu *et al.*, 2015).

Animal trampling and treading on the other hand, destroys soil aggregates and limit soil C occlusion and stabilization (Kotzé *et al.*, 2013; Lal *et al.*, 2015; Sandhage-Hofmann *et al.*, 2015). Lower labile C fractions in the poor rangeland condition of the grassland ecosystem can confirm

that. This implies that a further decline in refractory C fractions (humic substances) in the poor rangeland condition could be expected over extended periods unless reseeding of perennial grass species around water points is practiced during rest periods (Vetter, 2009; Lal *et al.*, 2015). Therefore, it is accepted that rangeland conditions along the grazing gradients within the ecosystem can have profound additional influences on C fractions over time. Poor rangeland conditions usually have lower shoot/root ratio due to overgrazing, and that has a detrimental effect on the C flow through the atmosphere-plant-soil continuum (Conant *et al.*, 2001; Reeder & Schuman, 2002; Lu *et al.*, 2015).

Alternatively, results of this study revealed significant changes in soil C fractions only between grassland and savanna ecosystems. Comparisons between these two ecosystems showed that differences in grazing intensities had little impact on the measured C fractions. In fact, significant differences in the measured C fractions of the grassland and savanna ecosystems were mainly due to site-specific conditions: MAR, MAT, clay content and vegetation in particular.

4.4.1 Soil organic and inorganic carbon

A decline in SOC in the savanna ecosystem was accompanied by lower SIC, which occurs in the soil as carbonates derived from parent material (lithogenic carbonate) and/or synthesized during soil processes (pedogenic carbonate) (Sanderman, 2012; Shi *et al.*, 2012; Lal *et al.*, 2015). Lower levels of SOC fractions in the savanna ecosystem perhaps prompted SIC dissolution rather than precipitation (Bronick & Lal, 2005; Mikhailova & Post, 2006). Natural terrestrial ecosystems commonly have lower SIC compared to adjacent croplands where fertilization, liming and irrigation are sometimes part of the management (Mikhailova & Post, 2006; Jin *et al.*, 2014). However, deposition of animal dung and urine in the grassland and savanna ecosystems cannot be ignored, especially in commercial farms where supplementary feeds are administered (Table 4.1).

An increase in SIC in the grassland ecosystem could be stimulated by accrued SOC, which was also influenced by improved phytomass production, cooler-moist climate and higher clay percentage when compared to the savanna ecosystem (Bronick & Lal, 2005). Wang *et al.* (2010), who found that SOC increased, while SIC decreased with an increase in rainfall in the natural terrestrial ecosystems of Inner Mongolia indicated that higher rainfall improved plant biomass production, a primary source of SOC, but led to leaching losses of SIC. In the Loess Plateau of China, Jin *et al.* (2014) found that grassland ecosystems had higher SOC, but lower SIC compared to the forestlands; however, they indicated that grasslands generates more pedogenic carbonates, which is an important SIC fraction as far as C sequestration is concerned. Thus, increased SOC with an increase in precipitation gradient could also be held accountable for SIC responses to grassland and savanna ecosystems (Wu *et al.*, 2009; Shi *et al.*, 2012).

Normally when SOC increases, decomposition and the release of carbon dioxide (CO₂) and base cations (e.g. Ca²⁺ and Mg²⁺), which are also ingredients in the formation of SIC, are deterred (Janzen, 2005; Wu *et al.*, 2009; Sanderman, 2012; Shi *et al.*, 2012; Ahmad *et al.*, 2015). This could be another factor to explain results obtained by Wang *et al.* (2010) and Jin *et al.* (2014). For the sake of the current study on the other hand, it could be inferred that an increase in rainfall improved plant biomass and SOC in the grassland ecosystem, which subsequently stimulated SIC precipitation (Bronick & Lal, 2005; Wu *et al.*, 2009; Ahmad *et al.*, 2015). Thus, higher SOC and soil water content can influence weathering of primary carbonates, and the released CO₂ during microbial and root respiration can then react with Ca²⁺ or Mg²⁺ from animal feeds and/or exchange complex of the clay colloids, resulting in SIC formation (Bronick & Lal, 2005; Wu *et al.*, 2009; Shi *et al.*, 2012). Higher SIC and SOC fractions in the grassland ecosystem could further imply that SOC protected SIC or vice versa as both play a key role in soil structural formation and stability (Bronick & Lal, 2005; Von Lützow *et al.*, 2006; Lal *et al.*, 2015). In other words, soil aggregation, which is a function of both SIC and SOC (Bronick & Lal, 2005; Lal *et al.*, 2015) as

well as CO₂ transfers between SIC and SOC can act as a sink for atmospheric C (Ahmad *et al.*, 2015; Sanderman, 2012).

4.4.2 Labile carbon fractions

The CWEC, HWEC (Ghani *et al.*, 2003) and POXC (Culman *et al.*, 2012) as components of the labile C pool have been used as early indicators of soil quality changes due to their sensitivity to land use. These labile C fractions have been shown to have strong correlations with microbial biomass, which suggests their importance as substrate for soil microbes (Gregorich *et al.*, 1994; Ghani *et al.*, 2003; Culman *et al.*, 2012; Vázquez *et al.*, 2016). Concentrations of CWEC, HWEC and POXC distinguished effects of grassland and savanna ecosystems, and therefore reflected the quantity and quality of C inputs (Vázquez *et al.*, 2016). Therefore the higher levels of these labile C fractions in the grassland soils suggest that the cool-moist climate in the grassland ecosystem improved biomass production enriched with appreciable quantities of labile residues as opposed to a hot-dry climate and woody plant residues in the savanna ecosystem (Jianping & Wei, 2017). Woody plants apparently have longer life spans and their contribution to SOC is seasonal or decadal (e.g. deciduous species) compared to grasses (Scholes & Archer, 1997). In the dry forest of western Argentina, Abril *et al.* (2013) found that sites with woody plant material had significantly higher labile C relative to those with grass species, indicative that responses of labile C could be site-specific. Labile C increased with an increase in precipitation from 100 to 350 mm, but decreased in areas where MAR was 500 mm due to leaching and/or microbial decomposition.

In our study, an increase in MAR in the grassland stimulated vegetation growth and net biomass production, resulting in higher litter returns to the soil compared to the hot-dry savanna ecosystem. Vegetation coverage, which was also higher in the grassland (71±14% in the poor and 73±4% in the good rangeland condition) compared to the savanna (27±7% in the poor and

47±4% in the good rangeland condition) ecosystem (Kotzé *et al.*, 2017) not only contributed to SOC, but also protected SOC and its labile fractions against erosion induced processes (Sousa *et al.*, 2012; Kotzé *et al.*, 2013). Sandhage-Hofmann *et al.* (2015) reported a loss of fine silt particles in the savanna ecosystem due to lower vegetation cover. Sousa *et al.* (2012) also indicated the importance of vegetation cover in alleviating the trampling impact of grazing animals and improving the infiltrability of soils, thereby controlling erosion losses of soil and associated C. Yong-Zhong *et al.* (2005) reported soil coarsening induced by accelerated wind erosion in the degraded Inner Mongolian grasslands. Coarse-textured soils have lower spatial protection for labile C fractions compared to fine-textured soils (Yong-Zhong *et al.*, 2005; Von Lützwow *et al.*, 2006).

Thus, clayey soils of the grassland ecosystem preserved SOC and its labile C fractions (POXC, CWEC and HWEC), and regulated their accessibility to the decomposer community as opposed to sandy soils in the savanna ecosystem (Yuan *et al.*, 2012; Sandhage-Hofmann *et al.*, 2015). While the presence of *Acacia* vegetation in the savanna ecosystem probably provided enough N to accelerate decomposition rates that led to lower labile C fractions, especially during moist soil conditions in summer when most of rainfall occurs, slower turnover rates of organic inputs were certainly the major constraint to labile C accrual (Bach *et al.*, 2010; Du Preez *et al.*, 2011b). Several authors have acknowledged that organic C recovery in coarse textured soils is as rapid as C loss (Snyman & Du Preez, 2005; Vetter, 2009; Du Preez *et al.*, 2011b). Therefore, considering the arid climatic and sandy soil conditions prevailing in the savanna ecosystem, turnover rates of plant biomass and accumulation of labile C fractions were constrained compared to the grassland ecosystem with a cooler-moist climate and higher soil clay content (Snyman & Du Preez, 2005; Jianping & Wei, 2017; Kotzé *et al.*, 2017;).

4.4.3 Humic substances

Similarly to labile C, changes in humic substances in the terrestrial ecosystems are usually associated with the degree of decomposition and/or litter quality (Baldock *et al.*, 1992; Abril *et al.*, 2013). In other words, higher ($P < 0.05$) accumulations of C_{EX} , C_{FA} and C_{HA} in the grassland ecosystem suggest that humification processes occurred more rapidly leading to increased levels of C_{EX} , C_{FA} and C_{HA} compared to the savanna ecosystem. During humification labile C fractions decline as they become transformed into stable humic substances (Sousa *et al.*, 2012), but surprisingly the measured labile C fractions were also higher ($P < 0.05$) in the grassland ecosystem. It may be true that during humification labile C fractions were lost in the grasslands, but cool-moist climates stimulated rapid plant biomass turnover and therefore masked the effect of humification processes on labile C fractions (Vázquez *et al.*, 2016). On the other hand, labile C fractions could be microbially immobilized and re-synthesized, thus increasing their concentrations in the soil (Baldock *et al.*, 1992). These statements probably hold truth according to Kotzé *et al.* (2017), who in the same ecosystems found higher microbial enzyme activities in the grassland compared to savanna soils. This increases the possibility that an increase in humic substances in the grasslands was the result of microbial transformation of organic materials entering the soil and that labile C fractions were incorporated in microbial tissues, thus preventing their loss out of the soil system.

Regular additions of organic materials to the soil favour microbial activity and thus the formation of humic substances (Bach *et al.*, 2010; Vázquez *et al.*, 2016). Good vegetation cover as a result of higher MAR and clay content in the grassland ecosystem also preserved humic substances against losses through erosion and microbial degradation (Von Lützow *et al.*, 2006; Janzen, 2005; Lal *et al.*, 2015; Kotzé *et al.*, 2017). Bach *et al.* (2010) highlighted that soil texture was instrumental in the recovery and storage of SOC and its components during Prairie restoration in semi-arid Nebraska, USA. The Prairie ecosystems with sandier soils demonstrated that recovery and

stabilization of SOC was nearly impossible compared to silty-clayey soils regardless of vegetation type. In the semi-arid South African Highveld Grasslands, Kotzé *et al.* (2016) indicated that accumulation of humic substances in the native grasslands corresponded with MAR and clay gradient. Their results revealed that warm-dry sandier soils slowed down microbial activity and humification processes compared to cool-moist soils with higher clay content, and that was probably the case in this study.

Contrasting results were obtained by Abril *et al.* (2013) who indicated that an increase in humic substances did not proportionally relate with rainfall gradient, but the quality of plant residues. According to their results, higher contents of humic substances were recorded in areas with higher woody plant residues compared to areas covered with grasses. Obviously, woody plant species are richer in lignin, which is the major source of humic substances than grasses (Kögel-Knabner, 1997; 2002), but the higher proportion of sand particles and low vegetation cover in the savanna ecosystem subjected humic substances to losses by erosion (Sandhage-Hofmann *et al.*, 2015). Even so, the similarity between their (Abril *et al.*, 2013) results and ours is that higher humic substances were found in areas where labile C fractions were also higher, indicative that continuous additions of readily digestible C enhance microbial activity and accelerate the formation of humic substances. Nevertheless, Sousa *et al.* (2012) indicated otherwise as humic substances and labile C displayed opposite trends in response to changes in grazing intensity. The overgrazed sites had lower labile C fractions, but higher humic substances compared to sites where animals were excluded in an attempt to regenerate vegetation.

Humic substances are recalcitrant in nature and their association with the soil mineral phase or occlusion within micro-aggregates possibly increased their persistence in the soil (Von Lützow *et al.*, 2006). The loss of labile C fractions usually results in a decline in microbial activity and diversity, as a result humic substances, which are not a good energy source to a wide range of the decomposer communities, can then persist for longer periods in the soil (Kögel-Knabner,

2002; Von Lützow *et al.*, 2006; Kotzé *et al.*, 2016). Thus, the residence times of humic substances in the soil are not only determined by their selective preservation, but also by availability and activity of humus-decomposing organisms, soil texture and land use (Kotzé *et al.*, 2016; Vázquez *et al.*, 2016). In general, management systems that deplete labile C fractions without replenishment can also deprive soils of humic substances overtime. From a C sequestration point of view, soil management systems should improve organic inputs and preserve both labile and recalcitrant C fractions (Sousa *et al.*, 2012; Abril *et al.*, 2013).

4.4.4 Structural composition of soil organic carbon

Structural characterization of SOC with solid-state ^{13}C NMR spectroscopy showed the major four C types: O-alkyl, alkyl, aromatic and carbonyl C (Figure 4.5), which differed in terms of their contributions to SOC (Table 4.2). Proportions of these functional groups in the SOC depended on the chemical composition of the inputs and/or degree of decomposition (Baldock *et al.*, 1997). Results of ^{13}C NMR spectroscopy revealed that increased animal trampling around drinking points in the poor rangeland condition of the grassland ecosystem induced intimate interactions between mineral particles and C inputs, thereby stimulating SOC decomposition (Janzen, 2005). Carbohydrates, which are labile in nature, were the dominant compounds in the 50-110 ppm region. Carbohydrate C was affected first during the initial phase of decomposition as revealed by a decline in O-alkyl C in the poor as opposed to the good rangeland condition, thus resulting in the higher signal intensities of refractory alkyl, aromatic and carbonyl C. Accumulation of the latter three, but most importantly alkyl C, is often associated with their primary recalcitrance and *in situ* synthesis by the decomposer community (Baldock *et al.*, 1992).

Several studies (Conant *et al.*, 2001; Yong-Zhong *et al.*, 2005; Conant, 2010; Sousa *et al.*, 2012) including results obtained in the grassland ecosystem (Table 4.2) indicated that high animal activity and overgrazing in the poor rangeland condition around water points, enhanced SOC

decomposition as opposed to the good rangeland condition. In other words, decomposition and the chemical composition of the added inputs influenced the chemistry of SOC in the grassland ecosystem (Baldock *et al.*, 1992). However, trends evolving in the savanna ecosystem emphasized the probable contribution of heterogeneous (tree-shrub-grass) vegetation composition to SOC structural changes along the grazing gradient (Sandhage-Hofmann *et al.*, 2015).

Compared to the good rangeland condition, higher O-alkyl C as well as lower alkyl and aromatic C in the poor rangeland condition suggest that organic C inputs (animal excreta and vegetation) therein were enriched with labile C compounds such as polysaccharides than lignin and poly(methylene) derived compounds (Mathers & Xu, 2003; Helfrich *et al.*, 2006). Thus, due to lower woody cover and litter in the poor rangeland condition, it is possible that the organic inputs rich in easily decomposable organic materials were the major source of SOC (Reeder & Schuman, 2002; Helfrich *et al.*, 2006; Mathers *et al.*, 2007; Abril *et al.*, 2013). Again, lower vegetative cover in the poor rangeland condition (Conant, 2010; Sousa *et al.*, 2012; Sandhage-Hofmann *et al.*, 2015; Kotzé *et al.*, 2017) possibly subjected free woody fragments (from tree and shrub) in a sandy soil to wind erosion, detrimentally affecting recycling of plant-derived aromatics, waxes and lipids into the soil (Helfrich *et al.*, 2006).

Highly variable behavior of carbonyl C as the extent of litter decomposition increases has been reported in several studies (Baldock *et al.*, 1992; 1997; Kögel-Knabner, 1997; 2002; Mathers *et al.*, 2003; Mathers & Xu, 2003). Carbonyl C can increase, decrease or remain virtually unaltered as humification processes proceed further, and due to such inconsistencies has been excluded as an index of the degree of litter decomposition (Baldock *et al.*, 1992; 1997; Mathers *et al.*, 2003; Mathers & Xu, 2003). Baldock *et al.* (1992) attributed such unpredictable behavior to differences in the chemistry of organic inputs and availability and activity of organisms responsible for the breakdown of carbonyl C-containing material. Carbonyl C within the vicinity of 171 ppm derives

from aliphatic amide, carboxyl group, lignin and hemicellulose (Kögel-Knabner, 1997; 2002; Helfrich *et al.*, 2006). Differences in the amount and decomposability of these carbonyl C sources explain its variable nature in the soil (Mathers *et al.*, 2007). Though there were minor changes in carbonyl C between soils under the good and poor rangeland condition in the savanna ecosystem, it followed the same pattern as displayed by O-alkyl C, suggesting that carbonyl C was derived mainly from hemicellulose. In other words, higher carbonyl C concentrations in the poor rangeland condition of the savanna ecosystem further confirmed that indeed SOC structural composition was influenced by litter quality and not decomposition.

Results of the ^{13}C NMR spectroscopy to a certain extent conformed to those obtained with classical extraction methods, which is not common owing to efficiency differences between these analytical procedures (Baldock *et al.*, 1992; Kögel-Knabner, 1997). These similar trends were only evident within individual ecosystems, but not between ecosystems, presumably due to vegetational, soil textural and climatic differences. Thus, irrespective of the rangeland condition, higher O-alkyl C in the grassland compared to savanna ecosystem imply that besides polysaccharide enriched organic inputs in the grassland ecosystem (Helfrich *et al.*, 2006), higher clay content of the Lixisols trapped labile compounds within aggregates or through organo-mineral associations compared to Arenosols of the savanna ecosystem (Baldock *et al.*, 1997; Yong-Zhong *et al.*, 2005). It is also fair to speculate that a cooler-moist climate in the grassland relative to savanna ecosystem facilitated rapid O-alkyl C turnover rates through increased plant biomass and/or microbial re-synthesis (Baldock *et al.*, 1992).

Lower aromatic C in the grassland ecosystem could indicate that most of lignin was converted into humic substances as shown earlier in section 4.1 compared to the savanna ecosystem. In other words, an increase in aromatic C in the savanna compared to the grassland ecosystem, irrespective of the rangeland condition as well as variable results displayed by alkyl and carbonyl C when the two ecosystems were compared, reflected the influence of litter composition more

than that of MAR, MAT, clay content or litter decomposition (Helfrich *et al.*, 2006; Mathers *et al.*, 2007).

4.4.5 Extent of decomposition

The HI and PI obtained within and across the selected ecosystems indicated that the degree of humification was within the same ranges, regardless of the rangeland condition or ecosystem (Table 4.3). The alkyl C/O-alkyl C ratios recorded in the grassland ecosystem also confirmed that the degree of decomposition was similar under both poor and good rangeland conditions irrespective of the ecosystem. This is consistent with the non-significant changes in labile C fractions and humic substances between the rangeland conditions. However, a slight decline in the good compared to the poor rangeland condition in the grassland ecosystem suggest that SOC under the former was less humified (Baldock *et al.*, 1992). In other words, decomposition contributed to SOC structural changes in the grassland ecosystem (Baldock *et al.*, 1992; Mathers *et al.*, 2007). From the savanna ecosystem perspective where SOC derives from tree, shrub and grass components, alkyl C/O-alkyl C ratio as an index of humification is less useful (Mathers *et al.*, 2007). Thus, according to alkyl C/O-alkyl C ratios in the poor and good rangeland conditions, the structural composition of SOC was influenced by the quality of litter rather than decomposition (Baldock *et al.*, 1992).

5.1 Conclusion

Grassland and savanna ecosystems are the basic and cheapest source of animal feeds in most developing countries, including South Africa where most of animal production comes from commercial farms. Rotational grazing as the rangeland resource management system in commercial farms allows vegetation to regenerate during several months of animal exclusion, and this was reflected by non-significant changes in the measured C fractions along the grazing gradient. However, higher animal activity in the poor rangeland condition induced litter

decomposition, affecting SOC quality as indicated by a decline in labile O-alkyl C with concomitant increase in recalcitrant alkyl C compared to the good rangeland condition, particularly in the grassland ecosystem. In the savanna ecosystem, SOC structural composition was influenced by the heterogeneous vegetation rather than decomposition. Results of this study further indicated that responses of SOC, SIC, labile C fractions and humic substances proportionally corresponded with an increase in MAR and clay content, which were lower in the savanna and higher in the grassland ecosystem regardless of the rangeland condition. However, the SOC structural composition between the two ecosystems was affected more by vegetational than soil textural and climatic differences. In general, this study revealed that duration of grazing or withdrawal of animals from camps should not be fixed, instead be informed by availability of rangeland resources. Soil textural and climatic differences should also be used to determine animal withdrawals from camps and stocking rates as they play a critical role in vegetation and soil restoration. Thus understanding the interactions between rangeland management, soil and climate are essential.

Chapter 5

Synthesis and general conclusions

5.1 Summary

Soil degradation is particularly acute in the semi-arid to arid terrestrial environments due to high temperatures, low-erratic rainfall and high agricultural intensification. The interactions of these factors on soil C loss or restoration is still not well understood despite a myriad of soil C studies. In fact, there is a knowledge gap regarding the influence of land use systems on the relationship between the quantity and quality of soil C specifically in the context of South Africa. As thus, this study sought to determine the extent of soil degradation under different agricultural activities using different soil C fractions and SOC molecular composition as indicators and explore possible counter measures that could foster development of soil protection strategy and policy through which resilience of dryland ecosystems could be increased. Due to timeous availability of updated records on soil management for accurate and quality data, commercial farms were chosen for this study.

5.2 Synthesis and theoretical implications

Because of the diverse nature of this study, the key findings are chapter specific and have been synthesized and integrated within the respective empirical chapters:

- **Chapter 2:** Long-term effects of wheat production management practices on some carbon fractions of a semi-arid Plinthustalfs.
- **Chapter 3:** Dynamics of soil carbon concentrations and quality induced by agricultural land use in central South Africa.
- **Chapter 4:** Changes in soil carbon fractions induced by grazing regimes in commercial grassland and savanna rangelands of South Africa.

Chapter 2: Soil C loss is the most dominant cause of soil degradation in arable landscapes and the role of crop residues as the source of soil C is still not appropriately communicated with farmers in many countries, including South Africa. This communication breakdown as a result of limited research and practical results has manifested in bad residue management choices, as farmers continue to dispose of crop residues by burning to ease tillage operations and control pests and diseases. Chapter 2 evaluated effects of wheat production management practices on different C fractions in the 0-50 mm soil layer of a semi-arid Plinthustalf in a long-term trial established in the Eastern Free State near Bethlehem. Applied management systems in this trial included two methods of straw management (unburned and burned), three methods of tillage (no-tillage, mouldboard ploughing and stubble mulch) and two methods of weed control (chemical and mechanical weeding) in a factorial arrangement. However, some combination treatments that were not representative of conservation agriculture were excluded in this study.

Results showed that straw management and weed control methods in the ploughed plots did not induce significant changes to the measured soil C fractions, as such it is obvious that the degree of tillage was the major determining factor of the directional change (accumulation or loss) for the measured C fractions. It appears that incorporation of unburned or burned straw by mouldboard ploughing increased decomposition rates following residue-soil contact and exposure of physically protected C fractions to microbial decomposers compared to treatment combinations that included no-tillage. Decomposition over extended periods also altered SOC quality by depriving the ploughed soils of POXC, CWEC and O-alkyl C, and therefore demonstrated that these C types were the most sensitive and affected labile fractions as a result of soil management changes. Farmers can therefore rely on these labile C fractions to assess and monitor short-term changes in soil quality not only because of their sensitive nature, but also because they are a readily available source of energy for microbial communities and a reservoir for plant nutrients.

It is believed that an increase in C_{EX} and C_{FA} in no-tillage combinations implied that decomposition occurred but at a very slow rate, which could improve nutrient use efficiency. In other words, adoption of no-tillage could be a basis for improving soil fertility and crop production while reducing the production costs usually incurred during tillage operations and purchasing of inputs, especially in developing countries where there is low income and limited credit availability to most farmers. Lack of soil disturbance in no-tilled unburned plots obviously stabilized processes leading to formation of SIC and accrual of SOC, including labile and humic fractions. Positive correlations between SIC and most SOC fractions were also an indication that surface placement of wheat straw in no-tillage favoured accumulation and protection of SIC against erosion as opposed to unburned or burned straw in the ploughed plots.

Although prolonged cultivation seemed to have disrupted formation of SIC and humic substances, non-significant responses of C_{HA} , HI, PI and only a slight change in alkyl C or alkyl C/O-alkyl C ratio are indicative of similar degree of decomposition across the applied treatment combinations. Either decomposition was not the only mode of soil C loss or there were no losses at all in the ploughed treatments. If the latter is true, it means the application of no-tillage improved the quantity and quality of soil C and demonstrated the potential to sequester C in the 0-50 mm soil layer and improve soil resilience to the changing climate. But if the former holds true, then these results would be supporting the perception that some researchers (as reviewed by Janzen, 2005; 2006) have about C sequestration under no-tillage. They are of the impression that though no-tillage reduces fuel emitted C by excluding some of the tillage operations, it is only capable of filling C reserves emptied by intensive soil cultivation coupled with less returned crop residues. Unfortunately adjacent primary grasslands were not sampled, which could serve as reference and reveal whether adoption of no-tillage is a C sequestration mechanism or not.

These findings are consistent with some local (Wiltshire & Du Preez, 1993; Kotzé & Du Preez, 2007; Loke *et al.*, 2012) and international (Bakht *et al.*, 2009; Carvalho *et al.*, 2009; Chen *et al.*,

2009; Sequeira & Alley, 2011; Guimarães *et al.*, 2013) studies conducted under similar or different agro-ecological settings. However, the magnitude of increase for the investigated soil C fractions under no-tillage combination was lower presumably due to long-term wheat production under monoculture. Rotation of high biomass producing crops with legumes is perhaps needed to improve concentrations of accumulated C fractions. Climatic and soil variability are among other factors that influence C restoration, as such to realize the full potential of no-tillage as a C sink perhaps it should be tested under different climatic and soil conditions.

Chapter 3: Reversion of degraded arable lands to grasslands is one of the oldest management strategies used to regenerate soils' productive capacity. However, its popularity in South Africa escalated at least three decades ago when the government engaged farmers in five agro-ecosystems: Harrismith, Tweespruit, Kroonstad, Koppies and Vryburg to revert soils that have been cropped for over a century back to secondary perennial pastures. Several studies were conducted in these agro-ecosystems; however, information on the response of different soil C fractions and SOC composition to land use change is still scarce. Chapter 3 evaluated effects of land use change on different soil C fractions and SOC molecular composition across three semi-arid agro-ecosystems (Harrismith, Tweespruit and Kroonstad) in central South Africa. Selected soil samples were collected from primary grasslands, croplands and secondary pastures at the 0-200 mm depth in each agro-ecosystem.

Although all the three agro-ecosystems belong to the summer-rainfall region, they tend to differ slightly with climate and soil texture, which may affect net soil C loss or gain. Harrismith is classified as a Cool-Moist Highveld Grassland, Tweespruit as Cool-Dry Highveld Grassland and Kroonstad as Warm-Dry Highveld Grassland. Soils in Kroonstad are sandier compared to those in Harrismith. The clay percentage of Tweespruit soils was intermediate of that in Kroonstad and Harrismith soils. Despite the slight differences, these site-specific conditions had a profound influence on initial concentrations of C fractions as reflected in the virgin soils, with the highest

concentrations observed in Harrismith followed in a descending order by Tweespruit and Kroonstad.

Conversion of primary grasslands to arable cropping significantly depleted most of the measured soil C fractions (SOC, SIC, POXC, C_{EX} , C_{FA} and C_{HA}) and the magnitude of loss generally followed the order: Harrismith > Tweespruit > Kroonstad, indicative that losses of these fractions were not only modulated by soil mixing and breakdown of aggregates, but also by initial concentrations of the fractions in question. With increasing duration of cultivation of primary grasslands, molecular composition of SOC also changed, effecting a decline in O-alkyl C with concomitant increase in alkyl and aromatic C, especially in the cool-dry Tweespruit and warm-dry Kroonstad agro-ecosystems. Although the quantitative and qualitative loss of SOC may also be through other modes e.g. erosion and limited inputs during fallowing, these results revealed that decomposition was the major source of these losses. In the Harrismith agro-ecosystem, contradictions between POXC (which decreased significantly as a result of prolonged cultivation) and virtually unaltered O-alkyl C (which represents all labile C fractions including POXC) is normal considering efficiency differences between the chemical extraction methods and solid state CPMAS ^{13}C NMR spectroscopy. The response displayed by O-alkyl C could imply high microbial immobilization and preservation of labile compounds, which probably could not be extracted efficiently with KMn_2O_4 .

A decline in humic fractions, especially C_{HA} as a result of recurrent cultivation also decreased HI and PI, suggesting that cultivation destabilized humification and polymerization of SOC, particularly in Tweespruit. Contrarily, the alkyl C/O-alkyl C ratios revealed that the degree of SOC decomposition was more or less the same in Harrismith and Tweespruit, but increased in Kroonstad when virgin and cultivated soils were compared. This further attests that SOC fractions can increase, decrease or remain constant due to the interactive effects of land use, management and other site-specific conditions (Abril *et al.*, 2013; Vázquez *et al.*, 2016). As indicated earlier,

the combined use of different analytical procedures with different strengths can also give contradicting results (Kögel-Knabner, 1997; 2002).

Furthermore, the observed results underscored the ability of secondary pastures to restore historic soil C fractions and SOC quality, suggesting that soil degradation is a reversible process. However, the degree of recovery exhibited variable trends unlike the losses, probably due to saturation deficits, vegetation, duration of soils under secondary pasture management or the narrow differences in climatic and soil conditions. Regardless, restoration of SOC, SIC, CWEC and O-alkyl C was more rapid in the sandier Kroonstad soils. In fact, SIC, CWEC and O-alkyl C were high enough to represent primary grasslands. Restoration of POXC was higher in Harrismith; however, similarly to SIC, CWEC and O-alkyl C in Kroonstad the signal intensity of O-alkyl C indicated that secondary pasture management not only reinstated lost labile C fractions, but also added new ones. These in a way contradict with results of Preger *et al.* (2010), who indicated that SOC under secondary perennial pastures across these agro-ecosystems approached equilibrium state. Nonetheless, it would be reasonable to replicate samples particularly for solid state CPMAS ^{13}C NMR spectroscopic and statistical analyses in order to confidently assess whether or not the contradictions are due to differences in analytical procedures. Delayed accumulation of humic substances or alkyl and aromatic C could be a marker of SOC stabilization in the restored soils. However, long-term studies (> 30 years) on the dynamics of recalcitrant fractions under secondary pasture management can better our understanding on these contradictions.

Chapter 4: Rangelands constitute the basic and cheapest source of animal feeds in South Africa and probably in other parts of the world. Rangeland ecosystems are continuously degrading due to overgrazing, which lately seems to be gaining momentum in the commercial farms where rotational grazing in closed camps is the sole rangeland resource management system. Many studies conducted in the rangeland ecosystems focused their attention more on vegetation

composition than soil properties. Chapter 4 investigated effects of rangeland conditions (ranging from poor to good) along the grazing gradient, starting around the water points to a little further into the rangeland on different soil C fractions and SOC structural composition in the commercial semi-arid grassland and arid savanna ecosystems.

Changes in rangeland conditions from poor to good did not affect soil C fractions under investigation. However, higher animal activity and trampling in the poor rangeland conditions incorporated enough aboveground plant biomass and animal excreta to slightly increase most of the C fractions compared to the good rangeland conditions. Deposition of animal excreta in the poor rangeland condition overshadowed the deleterious effects of animal trampling on soil C fractions, which on the long-term can induce soil coarsening and therefore reduce the protection capacity of these soils. On the other hand, these results showed the impact soil texture, climate and vegetation had on the measured C fractions as changes became significant when the two ecosystems were compared, irrespective of the rangeland conditions. As revealed by soil C fractions, the cool-moist clayey grassland ecosystem seemed more resilient to degradation than the hot-dry sandy savanna ecosystem.

This study further showed that qualitative composition of SOC was affected, especially in the grassland ecosystem, by regular animal visits to the water points. A decline in O-alkyl C with an increase in recalcitrant alkyl C indicated that constant animal trampling induced SOC decomposition and loss of labile C fractions in the poor rangeland conditions. However, the loss of labile C fractions due to decomposition was not so severe as revealed by the similar level of alkyl C/O-alkyl C ratio, HI and PI. Molecular composition of SOC in the savanna ecosystem was depended more on litter quality than decomposition or environmental factors (climate and clay content), and this was evident even when the two ecosystems were compared. The heterogenous vegetation composition (grass-shrubs-trees) in the savanna ecosystem had variable effects on SOC quality.

Based on the overall results, loss of vegetation cover in the savanna ecosystem increased the risk of erosion losses of soil C (Sandhage-Hofmann *et al.*, 2015; Kotzé *et al.*, 2017). The savanna ecosystem also showed higher vulnerability to desertification than the grassland ecosystem. As such the use of obsolete general standards of stocking rates and animal withdrawal timeframes should be avoided and aligned with climatic, vegetation and soil conditions to ensure adequate vegetation recovery before subsequent grazing. In that way, vegetation cover and accumulation of soil C can be improved and soil degradation reversed even in the arid sandy savanna ecosystem.

Overall, this thesis underpins healthy biogeochemical C cycling in the native and arable managed ecosystems. However, irrespective of land use, results of this study demonstrated that the compositional variability of soil C influenced the fate or accrual of C fractions. Depending on site-specific conditions and land use, losses or accumulations of C fractions occurred at different rates. Excluding soil cultivation or reverting degraded arable lands to secondary pastures and controlling animal activity and grazing within the proximity of water points in rangelands improved labile C fractions, which subsequently were encapsulated by recalcitrant fractions and probably immobilized by soil organisms or stored in aggregates (Baer *et al.*, 2015).

The dilution effect on C fractions of the soils sampled at 0-200 mm layer (Chapter 3: Harrismith, Tweespruit and Kroonstad) did not seem to bring much impact as the concentrations of the investigated C fractions therein did not differ markedly with those obtained from soil samples collected at 0-50 mm layer (Chapter 2: Bethlehem and Chapter 4: Thaba Nchu and Kuruman) regardless of land use. Although this phenomenon has not been articulated comprehensively in soil science literature, it is possible that C of the top soils in the Harrismith, Tweespruit and Kroonstad agro-ecosystems reached saturation levels quicker than that in the other regions and therefore moved down the profile to fill the empty C spaces. Soil textural, climatic and vegetational differences could be another explanation to such unusual observations.

5.3 Limitations and recommendations

Inadequate funding support is one of the major challenges facing the research community in Africa and this study was not spared. However, the implications that inadequate research funding had on this study can be addressed in the future.

- For this study, soil samples were selected from previous collections by other researchers and some samples were too old that their analysis perhaps underestimated concentrations of the measured soil C fractions. Although there were no significant changes in SOC as a result of the increased duration of sample storage, it is believed that labile C fractions, which were however not tested against their counterparts due to lack of such data, could be affected.
- At the time of sampling, plant biomass (above- and below-ground), which is of utmost importance in soil C studies, was not considered. Knowledge of the amounts of returned biomass to the soil and determination of molecular composition would have strengthened the empirical results on SOC quantity and quality. Replication of soil samples for solid state CPMAS ^{13}C NMR spectroscopic analysis was also a necessity in order to make compelling arguments in this study.
- At this point it is not known whether SIC present in the studied sites is lithogenic, pedogenic or a combination, as such the use of C isotopes is recommended for future studies since these SIC fractions have different impacts on accumulating atmospheric CO_2 .
- Knowledge of the C location and understanding mechanisms of C stabilization in the soil are also pertinent under the current climatic conditions as they also affect C cycling in terrestrial ecosystems. Carbon cycling is a global issue that cannot be addressed by a single discipline of science. Inter- and intra-disciplinary approaches are also highly recommended because commercial farmers are mostly interested in making profit out of

their investment, but pay little attention on the effects of their interventions on the soil and environment. Collaborative efforts with other disciplines can convince them to adopt sustainable management strategies to make profit while preserving non-renewable resources like soil.

5.4 Conclusion

This study indicated that the complexity of soil degradation in the dryland ecosystems is aggravated by the interaction of natural (climatic, soil and vegetation conditions), land use and management factors. Understanding the interactive effects of these factors on soil C quantity and quality is imperative and a prerequisite to devise land use and management strategies that can improve net accumulation and storage of soil C and reverse degradation. As far as soil degradation and food production are concerned soil C quantity and quality are equally important, and as such this study provided guidelines for improved management of both these aspects under different agricultural activities in the face of a changing climate. This study further demonstrated that consequences of intensive soil cultivation and animal impact over extended periods can deprive soils of humic substances, which their restoration under semi-arid to arid environments could be far-fetched owing to slower turnover rates of plant biomass and recalcitrant biomolecules. Most importantly, the overall results revealed that replacement of cultivation with no-tillage, reversion of degraded arable soils to secondary perennial pastures and informed decisions on animal withdrawals from the paddocks as a rangeland resource management system can restore historic C, improve SOC quality and reverse soil degradation in these drought prone terrestrial ecosystems.

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