THE EFFECT OF LONG-TERM NO-TILL CROP ROTATION PRACTICES ON THE SOIL ORGANIC MATTER FUNCTIONAL POOLS

By

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DECLARATION

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ABSTRACT

Total soil organic matter (SOM) and its different functional pools (fractions) are important attributes of the physical, chemical and biological quality of the soil and are seen as key factors in the evaluation of the sustainability of management practices. Until now, limited information was available regarding soil C accumulation and stabilization under conservation tillage managed soils in the Western Cape grain production regions of South Africa. Long-term field experiments investigating different crop and crop/pasture rotation systems under no-tillage were initiated in 2002 at the Tygerhoek Research Farm of the Western Cape Department of Agriculture, near Riviersonderend, Overberg, Western Cape, South Africa. The study site enabled us to compare the following five dryland cropping systems; permanent Lucerne (100% pasture), Medic-Medic-Wheat (MM<u>W</u>) (67% pasture; 33% crop), Medic-Medic-Wheat-Wheat (MM<u>W</u>W) (50% pasture, 50% crop) and two 100% cropping systems (continuous cropping) in different phase [Wheat-Barley-Canola-Wheat-Barley-Lupin (WBC<u>W</u>BL4 & <u>W</u>BCWBL1)]. The numbers "1" and "4" in rotation code refers to the first and fourth crop planted in the cropping system, respectively. The underlined crop in rotation code represents the crop that was on the field at time of sampling. Natural vegetated soil (non-cultivated area) acted as a reference for this study. In 2012, soil samples were taken at four depth increments; 0-5, 5-10, 10-20, 20-30 cm.

The objectives of the study were to investigate the effect of long-term crop/pasture rotation systems on: i) the total soil organic carbon (SOC) storage under different cropping systems, ii) the SOC and N content in different functional pools (fractions); free particulate organic matter (fPOM) fraction (labile fraction), occluded particulate organic matter (oPOM) fraction (moderately stabile intra-aggregate C) and mineral-associated fraction (stabile fraction), (iii) the main C stabilizing mechanisms operative in these soils and (iv) the relationship between the extent of C sequestration and crop yields.

After 11 years, the medic-wheat rotations had the highest total SOC contents (15.2-18.6 g kg^{-1} in 0-30 cm depth, P ≤ 0.05), compared to the continuous cropping (13.3-14.1 g kg^{-1} in 0-30 cm depth), permanent lucerne pasture (15 g kg^{-1}) or natural vegetated soil (13.2 g kg^{-1}). Higher belowground C inputs through roots and the lower extent of disturbance in the 0-10 cm depth are the main reasons for higher total C content in the wheat-medic systems compared to the other systems.

The contribution of the fPOM fraction (labile C) to total C content in the cultivated treatments (6-9%) was lower than the natural vegetated soil (13%) in the 5-10 cm depth. The fPOM fraction is the most sensitive soil organic C and N pool to detect changes due to management practices, which include quantity and quality of OM inputs, extent of physical disturbance, and fertilization. The medic-wheat rotations had the highest C (1.37-1.74 g kg^{-1} in 5-10 cm depth) and N (0.107-0.110 g kg^{-1} in 5-10 cm depth) contents in the fPOM fraction of the cultivated treatments. Compared to the natural vegetated soil, the cultivated treatments had a lower C content in the oPOM fraction (moderately stabile fraction) and concomitantly a lower aggregate stability. On average, the oPOM fraction only contributed 0.4-2.4% to total C content at all sites. A significant positive correlation (R^2= 0.77) was found between C occluded in aggregates (oPOM fraction) and aggregate stability with the highest aggregate stability found in the medic-wheat rotations of the cultivated treatments. The major part (85-93%) of the SOC was associated with the mineral fraction (stabile fraction) in the natural vegetated and agricultural soils. The MMWW treatment contained the highest C content (18.7 g kg^{-1}, 5-10 cm depth) in the mineral-associated fraction and the two continuous cropping systems the lowest (14.2-14.7 g kg^{-1}, 5-10 cm depth) of the cultivated treatments. A significant positive correlation was found between mineral-associated SOC fraction and clay (R^2 = 0.74) and Fe-oxide (R^2 = 0.57) content. This helps explain the large mineral SOC fraction found in these soils and is the dominant SOM stabilization mechanism operative in these shale-derived soils. The mineral-associated organic matter is probably predominantly sorbed to the clay minerals (illite, kaolinite and sesquioxides) via ligand exchange resulting in very strong organo-mineral associations. Physical protection via occlusion in aggregates is not a dominant C stabilizing mechanism in these soils. The C:N ratios of the fractions decreased in the order fPOM > oPOM > mineral with a C:N ratio below 10 in the mineral fraction indicative of humified organic matter.

The MMW and MMWW treatments produced higher wheat yields in 2012 with a significant positive correlation found between total soil C and N, and yields obtained. In a higher quality soil, higher agronomic production is expected. Findings in this study enabled us to conclude that due to effect of cropping system and soil properties, the MMWW treatment had the highest total SOC content, which included highest labile C and N content and highest.

OPSOMMING

Totale grond organiese materiaal (GOM) en die verskillende poele (fraksies) is belangrike eienskappe van die fisiese, chemiese en biologiese kwaliteit van grond en word gesien as belangrike faktore in die evaluering van die volhoubaarheid van bestuurspraktyke. Tot nou was beperkte inligting egter beskikbaar rakende grond koolstof akkumulasie en stabilisering in gronde onderhewig aan bewaringslandbou in die graanproduserende streke van die Wes-Kaap. In 2002 is langtermyn plaasskaal wisselbou proewe op die Tygerhoek Navorsingsplaas van die Wes-Kaapse Departement van Landbou, naby Riviersonderend in die Overberg (Suid-Afrika) geloots. Die studiegebied het dit moontlik gemaak om die volgende vyf droëland gewasverbouing stelsels (behandelings) te vergelyk: permanente Lusern (100% weiding), Medic-Medic-Koring (MM<u>K</u>) (67% weiding, 33% gewas); Medic-Medic-Koring-Koring (MM<u>K</u>K) (50% weiding, 50% gewas) en Koring-Gars-Kanola-Koring-Gars-Lupien (KGK<u>K</u>GL4 & KGKKGL1) (100% gewas). Die nommers "1" en "4" in rotasiekode verwys na die eerste en vierde gewas geplant in die rotasie stelses, onderskeidelik. Die onderstreepte gewas in rotasiekode verteenwoordig die gewas in die veld toe monsterneming plaasgevind het. Grond onderhewig aan natuurlike plantegroei (onbewerkte gronde) het gedien as verwysing vir hierdie studie (sesde behandeling). In 2012 was grondmonsters geneem op vier verskillende dieptes; 0-5, 5-10, 10-20 en 20-30 cm.

Die doelwitte van die studie was om ondersoek in te stel oor die effek van langtermyn gewas/weiding wisselboustelsels op: i) die storing van totale grond organiese koolstof (GOK) inhoud onder verskillende verbouingstelsels, (ii) die GOK en stikstof inhoud in die verskillende funksionele poele (fraksies); vrye fraksie (VF), ingeslote (intra-aggregate) fraksie (IF) en mineraalgebonde fraksie (MF), (iii) die hoof koolstof stabiliserings meganismes in werking in hierdie gronde (iv) die verhouding tussen die omvang van koolstof sekwestrasie en opbrengste.

Na 11 jaar het die medic-koring rotasies die hoogste totale koolstof inhoud gehad (15.2-18.6 g kg^{-1} in 0-30 cm diepte, P ≤ 0.05), in vergelyking met volgehoue verbouing met kontantgewasse (13.3-14.1 g kg^{-1} in 0-30 cm diepte), permanente weiding (15 g kg^{-1}) en natuurlike plantegroei (13.2 g kg^{-1}). Hoër ondergrondse koolstof insette deur wortels en die mindere mate van versteuring in die 0-10 cm diepte is die vernaamste redes vir die hoër totale koolstof inhoud in die gewas weiding stelsels.

Die bydrae van die vry fraksie (labiele koolstof) tot totale koolstof inhoud in die bewerkte behandelings (6-9%) was laer as die van natuurlike plantegroei (13%) in die 5-10 cm diepte. Hierdie fraksie is die sensitiefste poel van organiese koolstof en stikstof om veranderinge weens effek van bestuurspraktyke, wat die kwantiteit en kwaliteit van OM insette, mate van versteuring en bemesting insluit, op te spoor. Die medic-koring rotasies het die hoogste koolstof (1.37-1.74 g kg^{-1} in die 5-10 cm diepte) en stikstof (0.107-0.110 g kg^{-1} in die 5-10 cm diepte) inhoud in die vrye fraksie gehad van die bewerkte behandelings. In vergelyking met die grond onder natuurlike plantegroei, het die bewerkte behandelings 'n laer koolstof inhoud in die ingeslote fraksie (gematigde stabiele fraksie) gehad weens 'n laer aggregaat stabiliteit. Die ingeslote fraksie het gemiddeld net 0.4-2.4% bygedra tot die totale koolstof inhoud in al die behandelings. 'n Beduidende positiewe korrelasie (R^2= 0.77) was gevind tussen intra-aggregate koolstof (ingeslote fraksie) en aggregaat stabiliteit met die hoogste aggregaat stabiliteit in die medic-koring rotasies van die bewerkte behandelings. Die grootste deel (85-93 %) van die totale GOK inhoud hou verband met die mineraal fraksie (stabiele fraksie) in beide die natuurlike plantegroei en landbougrond. Die MMKK behandeling (18.7 g kg^{-1}, 5-10 cm diep) het die hoogste koolstof inhoud in die minerale fraksie gehad met die twee 100 % gewas wisselboustelsels (14.2-14.7 g kg^{-1}, 5-10 cm diepte) die laagste van die bewerkte behandelings. 'n Beduidende korrelasie tussen minerale koolstof (mineraal fraksie) en klei (R^2 = 0.74) en Fe-oksied (R^2 = 0.57) inhoud is ook gevind wat die groot bydra van die mineraal fraksie tot totale koolstof inhoud help verduidelik. Dit is ook die dominante GOK stabiliserings meganisme in werking in hierdie skalie-afkomstige gronde. Dit blyk dat die mineraal geassosieerde OM oorheersend aan die klei minerale (kaoliniet, illiet and seskwioksiedes) adsorbeer d.m.v ligand-uitruiling wat baie sterk organiese-mineraal komplekse vorm. Fisiese beskerming d.m.v. insluiting binne aggregate is nie 'n dominante koolstof stabiliserings meganisme in hierdie gronde nie. Die C:N verhouding van die fraksies het afgeneem in die volgorde VF> IF> MF met 'n C:N verhouding onder 10 in die mineraal fraksie wat 'n aanduiding is van gehumufiseerde OM.

Die MMK en MMKK sisteme het hoër koring opbrengste in 2012 tot gevolg gehad en beduidende positiewe korrelasies was gevind tussen totale koolstof en stikstof en opbrengste. In 'n hoër kwaliteit grond word hoër opbrengste verwag. Bevindinge in hierdie studie het gelei tot die gevolgtrekking dat a.g.v. die rotasie sisteem en grond eienskappe, het die MMKK behandeling die hoogste totale koolstof inhoud gehad. Dit sluit die hoogste labiele koolstof en stikstof inhoud asook die hoogste stabiele koolstof inhoud in.

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LIST OF ABBREVIATIONS

ECEC	Effective cation exchange capacity
C	Carbon
fPOM	Free particulate organic matter
oPOM	Occluded particulate organic matter
POM	Particulate organic matter
OM	Organic matter
SOM	Soil organic matter
SOC	Soil organic carbon
MMW	Medic-Medic-Wheat
MMWW	Medic-Medic-Wheat-Wheat
N	Nitrogen
WBCWBL	Wheat-Barley-Canola-Wheat-Barley-Lupin

CHAPTER 1

GENERAL INTRODUCTION AND RESEARCH AIMS

Long-term field experiments investigating different crop/pasture rotation systems under conservation tillage were conducted by Elsenburg, Western Cape Department of Agriculture, at Tygerhoek Research Farm near Riviersonderend, situated in the Overberg region of the Western Cape, South Africa. In 2012 the trial was in its 11th year of a planned 20 years. Dryland crop choice and productivity in this region is limited as the rainfall is highly variable and unpredictable and can therefore lead to unreliable crop yields. Together with climate, a decline in soil organic carbon (SOC) as a result of agricultural practices, especially under dryland cultivation, can have severe negative impacts on the quality and productivity of soils. Several factors (e.g. climate, quantity and quality of biomass input, soil properties) affect the SOC content in arable soils but only management practices can be controlled. Conversion to conservation agricultural practices (e.g. no-tillage and crop rotation) that can enhance the SOC pool are now increasingly being adopted, as it can improve soil quality, increase agronomic productivity and thereby advance global food security (Lal, 2011).

The most common dryland crops produced in this region are barley, wheat and canola while lupin is commonly used as a legume in different rotation systems. Although the use of crop-pasture rotation systems in general have almost disappeared due to the specialization of grain crop production (Salvo et al., 2010), medics and lucerne, both legume pastures, is still extensively applied by local farmers in their crop rotation sequence. The different crop rotation sequences that local grain farmers are currently applying is thus continuous crop, crop-legume (cover crop) and crop-pasture systems. They are also increasingly switching over to conservation tillage (no-till) practices in attempt to restore SOC. However, little information is currently available regarding the most suitable crop sequence for dryland wheat production under no-tillage. The soils used for crop production in this region are typically shallow (typically less than 50 cm deep) and contain considerable amounts of coarse fragments, which also make them challenging to cultivate and to study.

Although several scientific studies on the effect of prolonged cropping on SOC content have been carried out in South Africa (du Toit et al., 1994; Dominy et al., 2002; Smit 2004; Lobe 2005), studies in the Overberg region are non-existent. To our knowledge, no work has been conducted on the combined effects of conservation tillage (no-tillage) and crop rotation practices on soil organic matter (SOM) functional pools in the agricultural soils of the

Overberg. The effect of soil management practices on C storage is climate, crop and soil specific; therefore, it is imperative to investigate these effects in specific geographic areas, as results from other climatic regions will most likely not be relevant. Furthermore, the majority of the previous studies only focused on the effect of management practices on total SOC content, which does not provide any indication on the proportion of the SOC that is actually active or stabilized by association with the mineral fraction. Decomposition of organic C can be slowed down by different stabilization processes. They are complex and entail an understanding of chemical, physical and biological interactions between organic components and the mineral matrix (Kögel-Knabner & Kleber, 2012). The interaction of SOM with minerals and also its chemical properties allows it to be divided into different functional pools, with each pool containing unique functional characteristics and turnover rate and contributing differently to total SOC (von Lützow et al., 2007). Density fractionation (Golchin et al., 1994a; Sohi et al., 2001; Cerli et al., 2012;) is a very common and effective technique used for quantifying the amount of C stored in different functional pools, ranging from active (labile) to passive (stable). Soil organic matter and its different pools play an important role in optimizing crop production, minimizing negative environmental impacts and improving soil quality and soil sustainability (Freixo et al., 2002).

The first objective of this study was to investigate the effect of long-term no-till crop rotation practices on total soil C sequestration. It involved understanding the underlying reason for differences in soil C sequestration by examining selected soil and plant properties. A second objective was to examine the relationship between the extent of SOM sequestration and crop yields. These first two objectives were addressed in Chapter 3. The main objective of this study was to investigate the effect of long-term no-till crop rotation practices on the C and N content and distribution in the SOM functional pools. This objective was addressed in Chapter 4. This involved the fractionation of total SOM into different functional pools (fractions) in order to investigate the role of each fraction in soil quality, as well as, the mechanisms by which C is stabilized. Elucidating the mechanisms responsible for SOC stabilization was carried out by examining the relationships of stable C (intra-aggregate and mineral-bound) with selected soil properties known to play a role such as aggregate stability, clay content and metal-oxide content.

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CHAPTER 2

LITERATURE REVIEW – SIGNIFICANCE AND FACTORS CONTROLLING SOIL ORGANIC MATTER STABILIZATION

2.1. Introduction

This literature study covers the latest scientific literature on soil organic carbon (SOC) stabilization. The current gaps in knowledge will also be highlighted in this review. Understanding the contribution of stable SOC to total SOC is helpful in approximating the long term effect of different land use types and climate on C cycling and SOC dynamics (Falloon & Smith, 2002).

The SOC pool is one of the largest on the global scale (Jobbagy & Jackson, 2000) and can be enhanced either through increased C inputs or decreased C losses (Figure 2.1). According to Fischlin et al. (2007) soils store almost three times more C in soil organic matter (SOM) than found in both the atmosphere or in living plants. However, due to the climate, low crop yield, removal of crop residues due to grazing, and fallowing to advance water storage and control weeds, most agricultural soils in semi-arid regions are known for its low soil C content (Rasmussen et al., 1998). Hence, it is important to identify and quantify the effect of different management systems (e.g. crop rotation, tillage) on soil C stabilization to help prevent C losses, and therefore degradation in soil quality (Rasmussen & Albrecht, 1997).

Conversion to more recommended management practices (e.g. conservation practices) can enhance the SOC pool, improve soil quality and productivity and thereby progress food security (Lal, 2011). Many researchers, including Lal (2011) agree that SOM, which is a complex mixture and affects various soil properties, is one of the primary indicators of agricultural sustainability and soil quality. Not only does it affects the soil quality but CO_2 (end product of SOM) is also one of the major greenhouse gases responsible for global warming. Soils however, have the potential to act as either a source or a sink for carbon dioxide (CO_2) depending on the land use and management as they have a direct influence on the rate of SOM mineralization (Lal, 2011).

Sequestering atmospheric CO_2 in agricultural land has received a lot of interest due to concerns about global warming. The soil C stabilizing mechanisms have therefore received much interest (Torn et al., 1997; von Lützow et al., 2006) as a good understanding of these

mechanisms is necessary to develop management practices that increase C sequestration in soils (Marschner et al., 2008).

The components of SOM range from undecomposed plant and animal tissues (living component) to partially degraded compounds to stable brown and black material, known as humus (non-living component). Dissolved organic matter forms also part of the non-living component. Humus is usually the largest proportion and contains no evidence of the anatomical structure of the material from which it was derived (Johnstone et al., 2009). Soil organic matter chemical properties and interactions with the mineral matrix allow them to be placed in different SOM functional pools with different turnover rates (Kögel-Knabner & Kleber, 2012). The three major SOM functional pools, each with their own chemical and physical properties that can be isolated are: (i) the free particulate organic matter (fPOM) fraction which resembles recent litter inputs and usually have younger C than other fractions (active pool); (ii) an occluded or intra-aggregate POM (oPOM) fraction, generally older than fPOM fraction released by disruption of soil aggregates (intermediate/passive), and (iii) a heavy or mineral-bound fraction (mineral), comprising of organic C tightly bounded or sorbed to minerals containing the oldest C (passive pool) (Golchin et al., 1994a; Marin-Spiotta et al., 2008; Cerli et al., 2012). Studies using isotope tracers have shown longer residence times for C associated with minerals (mineral and oPOM fractions) than the fPOM fraction (Marin-Spiotta et al., 2008). An effective seperation of SOM fractions of different stability is necessary to understand the SOC stabilization mechanisms operating under specific soil and climate conditions.

Soil water and temperature control turnover of C in soils, but other factors like size and physicochemical properties of C inputs through litter and roots, its distribution within soil matrix and its interaction with clay surfaces are all factors that can modify turnover rates (Oades, 1988). The total amount of organic matter (OM) in soil depends thus on soil properties, climate, C input and on the rate at which existing SOM decomposes (Johnstone et al., 2009). Outputs (oxidation and erosion) are increased by destabilization processes and decreased by stabilization processes (Figure 2.1) (Sollins et al., 1996). All these factors contribute to the dynamic equilibrium C value, specific to the soil type and farming system (Johnstone et al., 2009).

The effect of management practices on SOM functional pools and the possible stabilizing mechanisms involved, as well as the importance of C sequestration for sustainable agriculture was reviewed in this study in order to obtain a better understanding of the effect of

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management practices (e.g. crop rotation and no-tillage) on SOC stabilization to ensure food security via a sustainable soil.
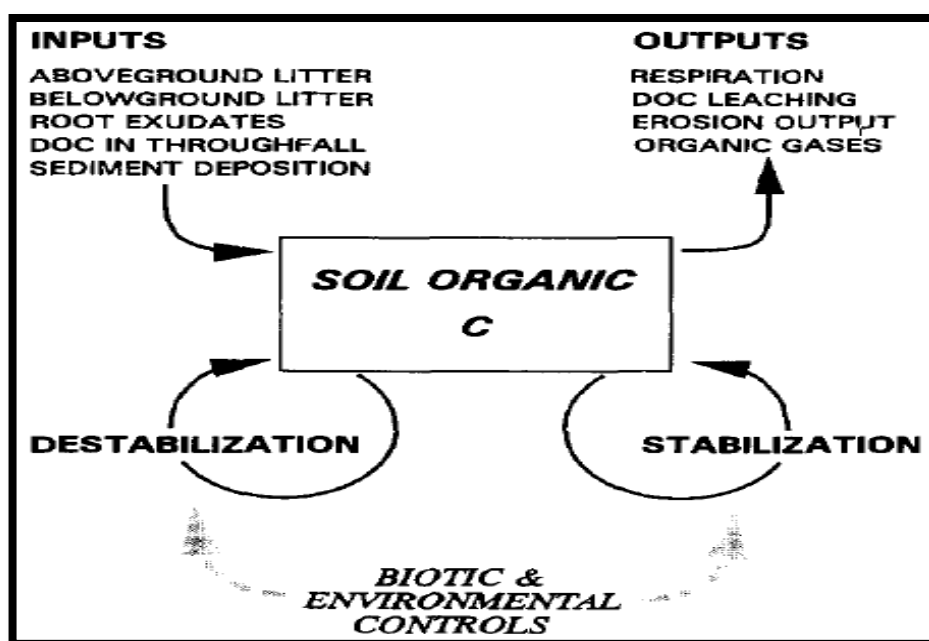
Figure 2.1 The dynamic equilibrium soil C value due to difference between long-term organic C additions and losses: From Sollins et al. (1996). Reprinted with permission from Elsevier.

2.2. The importance of C sequestration in soils

Carbon sequestration according to Paustian et al. (2000) is a reduction in CO_2 emissions in agricultural soils via an increase in soil C storage through different SOM pools. The rate of soil C sequestration in soils however, depends on soil morphology (Baldock & Skjemstad (2000), climate (White et al., 2009), farming system and soil management (Lal, 2004). There are concerns about the low levels of OM in many of the cropland soils (Johnstone et al., 2009). Consequences of severe depletion of SOC pool are low agronomic yield, soil structure degradation and low use efficiency of added input and therefore it is essential to increase the SOC pool in soils to improve the soil quality and to increase agronomic production (Lal, 2011). Soil quality is defined as "the capacity of the soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality and promote plant and animal health" (Doran & Parkin 1996 cited in Lal & Bruce 1999). Food production in developing countries is estimated at 1223 million Mg and it must increase by $2.5\% \, y^{-1}$ between 2000 and 2025 to fulfill the needs of an increased population and expected change in diet (Lal, 2006). There are several options to try and fulfill these needs but the one based on

increasing soil quality and thereby productivity by enhancing the SOC pool has many benefits (Lal, 2006).

Figure 2.2 displays the possible effect of depletion in SOM and therefore, this brutal cycle has to be broken by improving soil quality through C sequestration. According to Thomson et al. (2008) the Intergovernmental Panel on Climate Change (IPCC) has estimated that approximately 40 Gton of C could be sequestered in agriculture soils over 50-100 years by just improving agriculture practices.
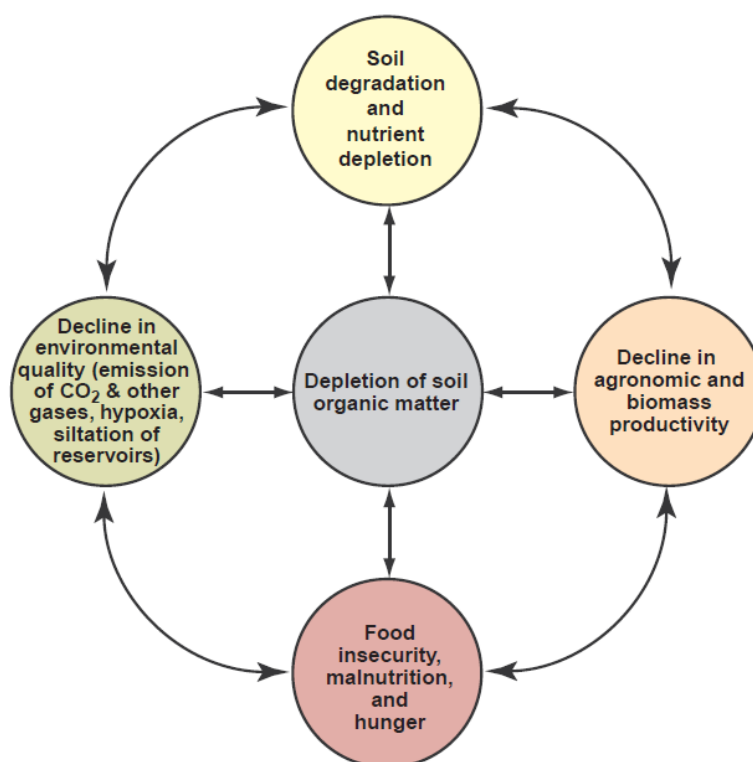
Figure 2.2 The brutal cycle of depletion in soil organic matter: From (Lal, 2004). Reprinted with permission from AAAS.

Soil C dynamics is thus important for soil fertility, sustainable agriculture systems, crop productivity and protecting the environment. The SOC pool contributes to soil fertility both directly and indirectly. Directly it releases important inorganic nutrients and trace elements while it decomposes and indirectly it increases the soil cation exchange capacity (CEC) and water holding capacity while it also improves the structure of the soil. High organic C levels are also important to ensure active microorganism populations which are necessary for sustainable crop production systems (Lal, 2011). Figure 2.3 is a good summary of how the quality and quantity of SOC pool can enhance the quality of the soil chemically, physically and biologically. It is thus obvious that agricultural practices must aim to enhance C

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sequestration as this is a strategy to achieve food security through improvement in soil quality as well as lowering CO_2 emissions from soil (Lal, 2006).
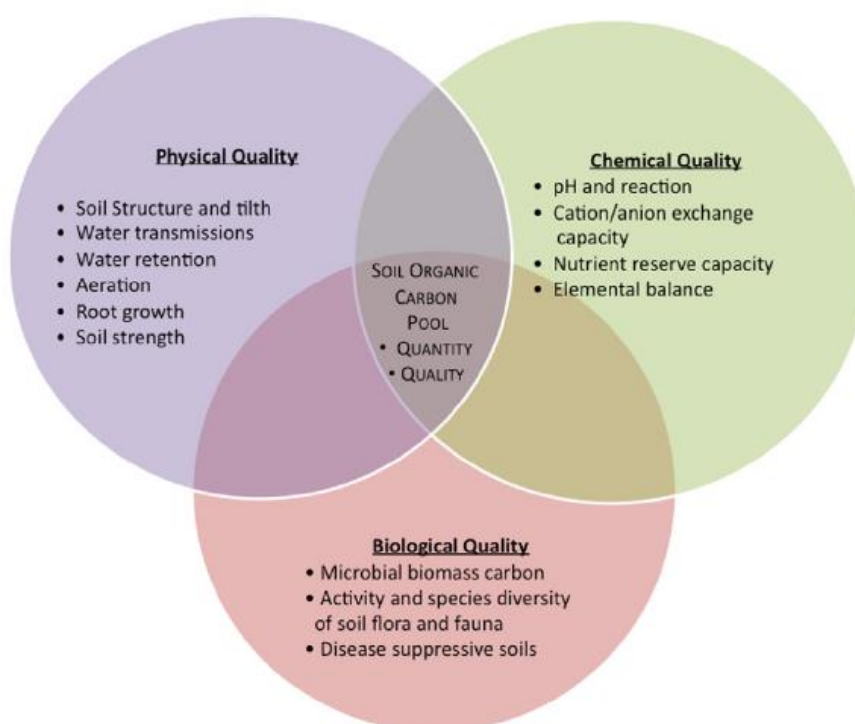
Figure 2.3 Soil quality enhancement by increase in soil organic carbon pool in agricultural soils: From Lal (2011). Reprinted with permission from Elsevier.

2.3. Carbon stabilization mechanisms in soils

When plant litter enters the soil mineral horizon it undergoes microbial decomposition and possible stabilization through interactions with soil mineral particles and aggregates, but the mechanisms of SOC stabilization and destabilization as well as the factors controlling it, is not fully understood (Wagai et al., 2009). The terms "labile" and "stable" are used to indicate important functional differences between turnover times of SOC pools. "Labile" represent the active pool and has a turnover time of a few years (easily mineralizable fraction) whereas "stable" represents the intermediate and passive pool with turnover times of decades and centuries, respectively (Krull et al., 2003; Marín-Spiotta et al., 2008).

The active pool is composed of fresh plant residues (roots and shoots), faeces, and faunal and microbial residues (von Lützow et al., 2006). According to Marschner et al. (2008) selective preservation of recalcitrant compounds doesn't explain longer term stabilization of SOC. Fast turnover rates of lipids and lignin (recalcitrant compounds) and slow turnover time rates for polysaccharides and proteins (labile organic compounds) indicate the importance of other

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protective mechanisms (Marschner et al., 2008). Mechanisms which can contribute to the passive pool are the protection of organic C within aggregates (Christensen, 1996) and the interaction with mineral surfaces (e.g. ligand exchange, cation bridging, weak interactions) (Torn et al., 1997). Organic C that is not physically protected is very susceptible to breakdown when land is disturbed and it turns over much more quickly than C bound to soil minerals. Marchner et al. (2008) found that SOC with turnover time's equivalent to the passive pool was only found in mineral associations.

Various authors have thus proposed and reviewed the different mechanisms for how organic C is stabilized in soil (Christensen, 1996; Sollins et al., 1996; Krull et al., 2003; von Lützow et al., 2006) and each of them had their own theories relating to the protection and stabilization of SOC against microbial decomposition and other losses. Decomposition of organic C can be slowed down by different stabilization processes, which are complex and entail an understanding of chemical, physical and biological interactions between organic components and the mineral matrix. These mechanisms can be broadly categorized into three groups; (i) chemical recalcitrance, i.e. selective preservation of OM due to its molecular composition, (ii) physical protection e.g. by occlusion in aggregates and (iii) interaction with soil minerals (von Lützow et al., 2007). Much effort has gone into elucidating the relative importance of these mechanisms for soil C content, or easier stated, how much C a given soil can protect against decomposition. Stabilization of SOC via these mechanisms is very important for C sequestration in soils (Krull et al., 2003). More than one of these mechanisms may operate together to various degrees in soil or even within an individual soil horizon (von Lützow et al., 2007).

2.3.1   Chemical recalcitrance

The chemical characteristics of the organic matter (OM) substrate, e.g. their elemental composition, presence of functional groups, and molecular conformation, can stabilize organic matter against microbial decomposition or degradation (Sollins et al., 1996). In later stages of decomposition, selective preservation is less important. This was shown by the longer turnover times for potentially labile organic compounds (polysaccharides and proteins) than for potentially recalcitrant compounds (lignin, lipids) (Marschner et al., 2008). The recalcitrance of plant litter and rhizodeposits is known as primary recalcitrance whereas the transformed humified nature of the organic matter as well as the recalcitrance of microbial and faunal products relates to secondary recalcitrance (von Lützow et al., 2006).

1.3.1.1. Primary recalcitrance

The non-humic constituents of SOM contributes to primary recalcitrance as it has identifiable physical and chemical properties, and consists mainly of known classes of biochemistry, such as carbohydrates, proteins, peptides, amino acids, fats, waxes and low molecular weight acids (Derenne & Largeau 2001; von Lützow et al., 2006).

There are four major groups of biomolecules in which organic matter can be allocated to e.g. polysaccharides (e.g. cellulose, hemicellulose, chitin), proteins, lipids (e.g. waxes, cutin, suberin), and lignin. Biomolecules, such as lipids and lignin are recalcitrant fractions as they contain alkyl structures and aromatic rings respectively, and are therefore less easily degraded in the early stages of decomposition than the other two groups of biomolecules (Derenne & Largeau, 2001; Krull et al., 2003). Lignin is considered as an important precursor of humic substances (Derenne & Largeau 2001). From this, one can assume that the litter quality and therefore vegetation (different proportions of biomolecules) can play a significant role in the rate of leaf litter decomposition and nature of substances and thus also the amount of CO_2 released to the atmosphere. However, studies with CPMAS ^{13}C NMR and pyrolysis techniques have verified that lignin is not so stable in the soil in the long term as soil microbial communities can and will degrade any type of organic residues entering the soil (Kiem & Kögel-Knabner, 2003).

1.3.1.2. Secondary recalcitrance

Secondary recalcitrance refers to selective degradation of microbial products, humic polymers and charred material (von Lützow et al., 2006). The humic components of SOM are regarded as the most resistant compounds and although accumulation of C is not indefinite, humic substances have been accumulating on the surface of the soil for a very long time. Stable organic C consists mainly of humic substances, which are complex high-molecular-weight organic molecules made up of phenolic polymers produced from the products of biological degradation of plant and animal residues (Baldock & Nelson, 2000). The polymers formed (process of humification) have a unique chemical structure compared to plant polymers and are therefore not easily degradable by microbes and their enzymes, making them recalcitrant which leads to a long residence time in the soil.

Black C (charred material) which originates from incomplete combustion of organic materials has a high stability in the environment compared to other types of organic C substances as it has an estimated mean residence time of about 10 000 years in soil (Swift

2001). Kuzyakov et al. (2009) reported that the mean residence time of black C produced from perennial rygrass is in the range of millennia, which supports the findings of Swift (2001). Due to this long residence time in soils, formation of black C is regarded as a stabilization process. However, according to Bird et al. (1999) black C has a mean residence time of only a few decades in a well-aerated tropical soil environment and these inconsistent findings related to the degradation of charred material leads to the conclusion that the stability of black C in soils depend on several factors (e.g. pyrolysis process, biomass residues, environment and soil conditions).

2.3.2. Physical protection

Physical protection plays a major role in C sequestration (Christensen, 1996). Spatial inaccessibility of C in soil micro-pores are one of the most important physical protection mechanisms in controlling the long-term stabilization of C (von Lützow et al., 2006). Physical protection is particularly effective in soil environments with high contents of clay and fine silt-sized particles (more physical protection) as C stabilization increases with decreasing aggregate size. Soil temperature and moisture also plays a role in the extent of physical protection (Krull et al., 2003). Organic C accessibility is reduced by the following processes: (i) occlusion of organic C by aggregation, (ii) intercalation within phylosilicates, (iii) hydrophobicity and (iv) encapsulation in organic macromolecules (von Lützow et al., 2006).

2.3.2.1. Occlusion of OM by aggregation

One of the most important processes in C sequestration is the formation of aggregates. This allows C to be included and thereby making it inaccessible to decomposing microorganisms (Christensen, 1996). Observations of increased SOM mineralization following the disruption of aggregates served as evidence for this statement (Six et al., 2000). Protection will thus be greatest where aggregate stability is high and aggregate turnover is low. Aggregation is the stabilizing mechanism that is potentially most vulnerable to disturbance. Organic matter spatially protected by occlusion within aggregates is shielded against decomposition and stabilized due to restricted accessibility for microorganisms and their enzymes, and restricted aerobic decomposition due to limited oxygen and extracellular enzymes (von Lützow et al. 2006). Accessibility is the stabilizing mechanism controlling the size of the slow or intermediate pool of C turnover models, but not the dominant control of the passive pool according to Baldock & Skjemstad (2000). There is an inverse relationship between

aggregate size and C turnover time, with the highest reported turnover times occurring in the smallest aggregates (<20 μm) (John et al., 2005).

2.3.2.2. Intercalation within phyllosilicates

This is a difficult concept to understand because it is sometimes unclear whether the phyllosilicates function as adsorbents for C or if they represent physical barriers between enzymes and SOM. This is illustrated by the fact that clay content often correlates with SOM content, but not with turnover time (Kleber et al., 2005). The chemical characterization and quantification is also very unreliable because no specific methods exist to determine the organic C intercalated in the interlayers of expandable phylosilicates (von Lützow et al., 2006). However, it is possible for organic ligands to intercalate into the interlayer spaces of expandable phyllosilicates at low pH where a small degree of dissociation is found (Violante & Gianfreda, 2000).

2.3.2.3. Hydrophobicity

Decomposition rates are very dependent on soil moisture as the living conditions of microorganisms is restricted by the absence of water (Goebel et al., 2005). A lack of surface wettability (hydrophobic) would therefore limit the accessibility and interaction of OM with microorganisms. In addition it enhances aggregate stability (Bachmann et al., 2008) and further contributes as stabilizing mechanism of C via occlusion within aggregates (Goebel et al., 2005). Goebel et al. (2005) also found evidence for great stability of hydrophobic OM itself.

2.3.2.4. Encapsulation in organic macromolecules

Encapsulation involves the protection of labile OM from degradation (von Lützow et al., 2006) as the labile OM is encapsulated in the network of recalcitrant polymers or humic pseudo-macromolecules (Zang et al., 2000). Humified organic C represents the most persistent pool of organic C in soil and therefore, any OM encapsulated in the hydrophobic interior domains of such molecules will be well protected and stabilized with mean residence time of several centuries (Piccolo, 1999).

2.3.3. Interaction with soil minerals and metal ions

In natural environments a large amount of the organic C is represented as mineral-associated organic matter. The protection of OM against decomposition due to sorption to minerals is assumed to be because of strong chemical bonds that limit desorption (Mikutta et al., 2007).

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Sorption of organic matter to minerals is among the most important mechanisms by which organic C is stabilized against decomposition (Kalbitz et al., 2005) as longer turnover times for OM associated with silt and clay than other soil fractions has been found (Eusterhues et al., 2003; Kalbitz et al., 2005). Kalbitz et al. (2005) revealed that mineralization of OM was reduced by 20% due to sorption of soluble OM to subsoil material. Degradation can also be slower due to the sorption of the enzyme on the clay mineral rather than the OM itself (Demaneche et al., 2001). Mikutta et al., (2007) came to the conclusion that the more mechanisms simultaneously involved, the more resistant the sorbed OM is to decomposition.

2.3.3.1. Ligand exchange

An important mechanism for the formation of strong organo-mineral associations in ligand exchange is the displacement of OH/water groups on mineral surfaces by organic functional groups (carboxyl and phenolic OH groups) of the OM (Gu et al., 1994; Mikutta et al., 2007). Carboxylic acids are most abundant in soil at a pH between 4.3 and 4.7 and therefore complexation of OM to mineral surfaces via ligand exchange is highest at low pH (Kögel-Knabner & Kleber 2012). In acid soils where hydroxyl groups of minerals is protonated, ligand exchange can take place between hydroxyl groups of Fe, Al, and Mn-oxides and edge sites of phyllosilicates and organic carboxyl and phenolic OH groups (Gu et al., 1994).

2.3.3.2. Polyvalent cation bridges

Binding of organic anions to negatively charge surfaces in soils can occur when polyvalent cations are present on the exchange complex. Polyvalent cations play thus a major role in the retention of OM on both organic (e.g. OM itself) and inorganic (e.g. clay minerals) colloids. Polyvalent cations can act as a bridge between these two charged sites by neutralizing the negatively charged mineral surface and acidic functional group of the OM (e.g. COO^-) (von Lützow et al., 2006). The most predominant polyvalent cations are Ca^{2+} and Mg^{2+} in neutral and alkaline soils and Fe^{3+} and Al^{3+} in acid soils (von Lützow et al., 2006). If an organic molecule has multiple functional groups, more than one point of attachment to the clay particle is possible. Cation bridges forms a weaker type of bond than ligand exchange. These cations also play an important role in the structure of both organic and inorganic colloids as the swelling of clays is restricted by these polyvalent cations (Oades, 1988).

In a study done by Mikutta et al. (2007) he proved through his sorption experiments, using $CaCl_2$ and NaCl as electrolyte solutions, that the presence of $CaCl_2$ enhances sorption of OM

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via Ca-bridging as his results displayed larger sorption in $CaCl_2$ than in NaCl. However, the effect of Ca^{2+} on OM sorption was more prominent in a neutral pH than in a low pH.

2.3.3.3. Weak interactions

Hydrophobic interactions occur via Van der Waals forces where hydrogen bonds are formed partially via interaction of hydrogen atoms with positive partial charge, with other partially negatively charged (O or N) atoms (von Lützow et al., 2006).

Non-expandable layer silicates (kaolinite) or quartz particles without layer charge have only weak-bonding affinities. Due to the presence of hydroxyl and polar groups in organic matter, a linkage between the molecule and minerals with very low layer charge can be formed via hydrogen bonding or van der Waals forces (Quiquampoix et al., 1995). At low pH, hydrophobic interaction is more favourable because then the hydroxyl and carboxyl groups of OM are protonated and the ionisation of carboxyl groups is inhibited (von Lützow et al. 2006).

2.3.3.4. Interaction with metal ions

Metal ions that can potentially stabilize OM are Ca^{2+}, $Al^{3+,}$ and Fe^{3+} and heavy metals (von Lützow et al., 2006). According to Oades (1988), the high OM content that is sometimes found in calcareous soils can be attributed to the effect of Ca^{2+} ions. In podzols, the interaction of OM with Fe and Al plays a major role in the stability of OM (Nierop et al., 2002).

The effect that metals have on the stabilization of organic matter is still unclear. It can be either attributed to changes in the quality of the substrate by forming a complex with metals, or direct toxic effects of metals on soil microorganisms or enzymes (von Lützow et al., 2006). Possible changes of soil OM caused by metal complexation will decrease their availability to soil enzymes (McKeague et al., 1986). Metals can also affect the stability of dissolved organic matter (DOM) by precipitation, making it more stable than the remaining DOM (Nierop et al., 2002).

2.4. Effect of management practices on SOM stabilization and distribution

The mineralization rate of SOM is mainly affected by (i) the chemical and physical environment of the soil, which includes soil climate and availability of nutrients, (ii) the molecular composition of the SOM and (iii) the physical accessibility of the organic matter to the microbes and enzymes (Paustian et al., 2000). Soil preparation, specific crop rotation

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system, SOM quality, soil texture and climate are thus some of the factors that contribute to SOC losses and gains (Salvo et al., 2010). The effect of most of these factors can however be controlled by the type of agricultural management practices applied e.g. no-tillage and rotation systems.

Conservation tillage (minimum-, no- and zero-tillage) can potentially contribute to reducing greenhouse gas emissions within the agricultural sector and various studies have made the conclusion that by reducing tillage, soil C increases (Arshad et al., 1990; Machado & Silva, 2001; Bhattacharyya et al., 2012). A Brazilian study by Sisti et al. (2004) suggested that OM accumulation also increased by using a legume in the rotation system. From these findings it is possible to conclude that different crop rotation and tillage practices can play a vital role in the stabilization of soil C and thereby increase soil quality and partially mitigating the current increase in atmospheric CO_2. However, the relative contribution of these two factors is dependent on both soil and climate conditions as Sombrero & Benito (2010) found that after 10 years of management in semi-arid region, the tillage system (conventional vs. no-till) had a greater effect on SOC than crop rotation.

According to Lal & Bruce (1999), the SOC content is increased by adopting crop rotation and no-till procedures that retain crop residues close to the surface of the soil and attributed it to increasing biomass production and crop residue retention. A review of different studies on the effect of crop rotation and tillage practices on SOC distribution and stabilization as well as the mechanisms involved is discussed in detail in the next few sections.

2.4.1. Tillage practices

2.4.1.1. General

Conservation tillage, which includes both minimum and no-tillage, has been found to increase soil C sequestration and soil productivity and thereby also contributing to the role of soil as a C sink (Sombrero & Benito, 2010). By minimizing soil disturbance and increasing aggregate stability, conservation tillage decreases the mineralization of OM which results in higher C stocks and C stabilization than with conventional tillage (Bhattacharyya et al., 2012). Conservation tillage can be defined according to the Conservation Information Center (CTIC, 2004) as any tillage and planting system that leaves 30% or more crop residues on the surface after planting and thereby reduces soil and/or water loss compared to conventional tillage.

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Fine intra-aggregate particulate organic matter from micro- and macroaggregates in the surface soil can act as a potential physical indicator of C-sequestration (Six et al., 2000). Terra et al. (2006) (cited by Salvo et al., 2010) found that SOC (0-15 cm depth) was 17% lower under continuous cropping under no-till than SOC in pasture rotations for the same period of time (8 years). This can be attributed to less disturbance of the soil with pasture in the rotation which can result in higher aggregate stability. No-till practices can also contribute to an increase in SOC by increasing C inputs (higher crop biomass) due to better soil water conservation (Cantero-Martínez et al., 2007). The higher input of crop residues plays an important role in aggregation as it acts as an energy source for microorganisms which are capable of producing polysaccharides which is very effective for soil aggregation.

Sombrero & de Benito (2010) found that the organic C in the 0-30 cm layer was only 7% higher in no-till than conventionally tilled soils after 6 years but at the end of a 10-year period it was 25% higher for no-till and this indicates that time is also an important factor that plays a role in the effect of conservation tillage on C sequestration and that the positive effect of conservation practices will only show after a number of years. From this study done in a semi-arid region in Spain, it is clear that conservation tillage raised the SOC content (Figure 2.4). Under dryland Mediterranean conditions, no-tillage increased SOC with a maximum annual SOC sequestration rate estimated to occur 5 years after adoption of no-till (Figure 2.5a). More than 75% of the total SOC sequestered however, was gained during the first 11 years after no-till adoption on a loamy texture soil (Figure 2.5b)(Álvaro-Fuentes et al., 2012). West & Post (2002) also estimated a maximum annual SOC sequestration after 7 years since adoption of no-tillage and pointed out that the duration of C sequestration depends on the climate, ecosystem, land-use history and management. The rate of increase in SOC content through adoption of conservation management practices follows a sigmoid curve as it achieves maximum, 5-20 years after adoption and continuous until SOC achieves another equilibrium (Lal, 2004). The reasons for this phenomenon is that crop residues are incorporated into the soil much slower through soil fauna under no-till systems compared to conventional tillage systems which may contribute to the lack of C sequestration over the first few years in a water-limited region (Six et al., 2004). Another possible reason according to Álvaro-Fuentes et al. (2012) is the decline in crop yields after the first few years of no-tillage which results in lower C inputs. Different SOC sequestration durations in Mediterranean conditions can be due to low C inputs, soil water- limiting conditions and elevated soil temperatures.
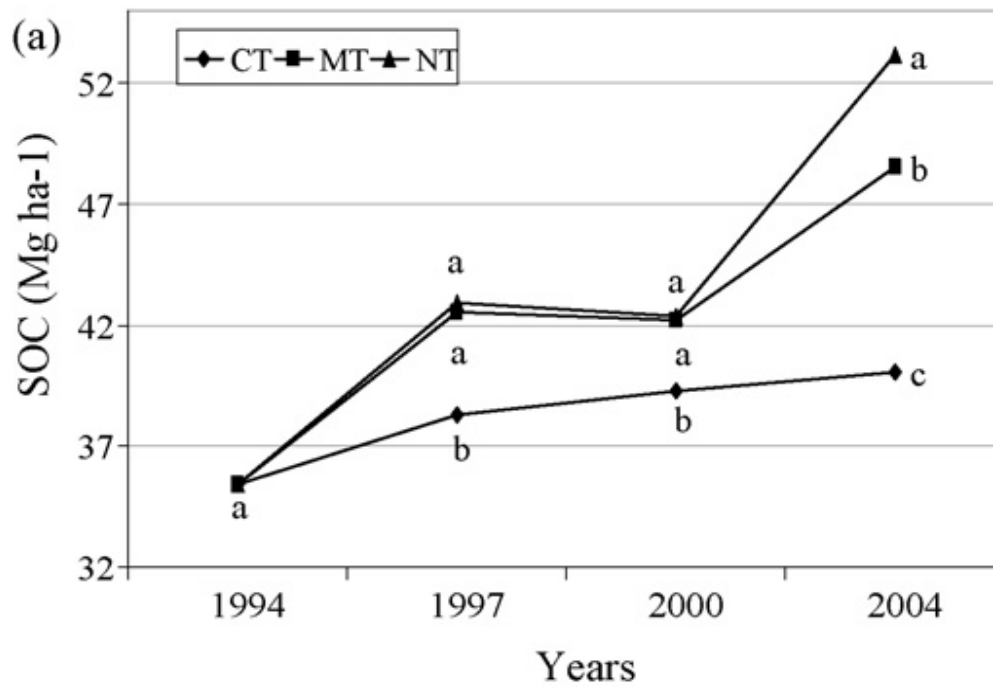
Figure 2.4 Soil organic C (SOC) levels in the 0-30 cm layer from 1994-2004 with CT, conventional tillage; MT, minimum tillage; NT, no tillage: From Sombrero & de Benito (2010). Reprinted with permission from Elsevier.
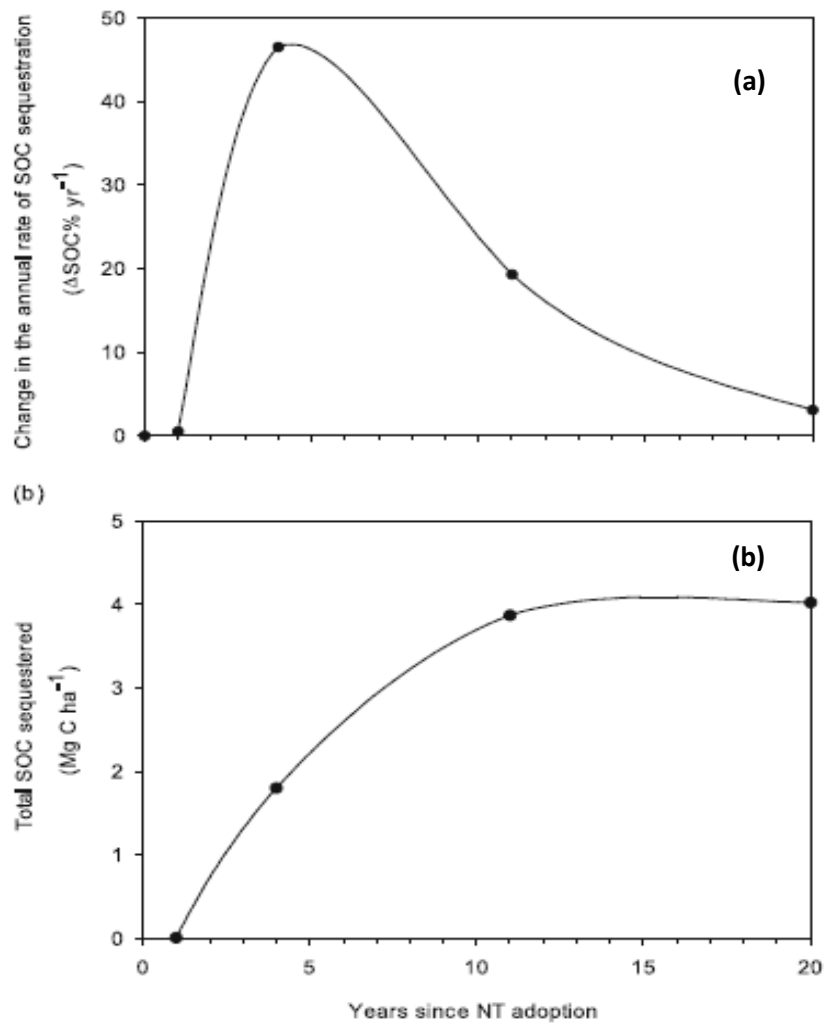
Figure 2.5 (a) Percentage change in the yearly rate of soil organic C (SOC) sequestration in the 0–30 cm soil layer over the 20-yr period after the adoption of no-tillage (NT); (b) Total SOC sequestered in the 0–30 cm soil layer after NT adoption: From Álvaro-Fuentes et al. (2012). Reprinted with permission from Springer.

2.4.1.2. Mechanisms

In order to understand the mechanisms involved in the responses of SOC to tillage practices, fractionation techniques have to be used to evaluate the effect of long-term no-tillage on soil aggregation and SOC fractions (Huang et al., 2010). The stabilization of SOM in soil aggregates is an important mechanism for long-term sequestration of C in SOM (Christensen, 1996; Verchot et al., 2011) and good correlation between aggregate stability and SOC dynamics have been found (Sohi et al., 2005). Results obtained by Sohi et al. (2005) also confirmed that SOM within aggregates contains more microbial products and more resistant C as compared with SOM in the light fraction (inter-aggregate).

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Usually by increasing the proportion of C-rich macroaggregates in soils, C sequestration can be enhanced, but long-term sequestration depends on stabilization of C in microaggregates (Six et al., 2000). The genesis and dynamics of these microaggregates however is still uncertain and different models have been proposed (Verchot et al., 2011). Several authors, including Christensen (1996) and Six et al. (2000) suggested that no-till practices that minimize macro-aggregate turnover enhances the formation of stable microaggregates within the macroaggregates, and therefore ensure long-term C sequestration via physical occlusion of the microaggregates protecting it from microbial breakdown. Microaggregates form within macroaggregates as the fine organic matter becomes encrusted with clay particles and microbial products. This model (1) (Figure 2.6) also suggests that the increase in macroaggregate turnover caused by tillage lead to the formation of less new free microaggregates, compared to no-tillage, when the binding agents in macroaggregates degrade and results in the loss of macroaggregate stability and the release of microaggregates. Macroaggregates are initially formed by the encapsulation of organic matter (Model 1) (Six et al., 2000).
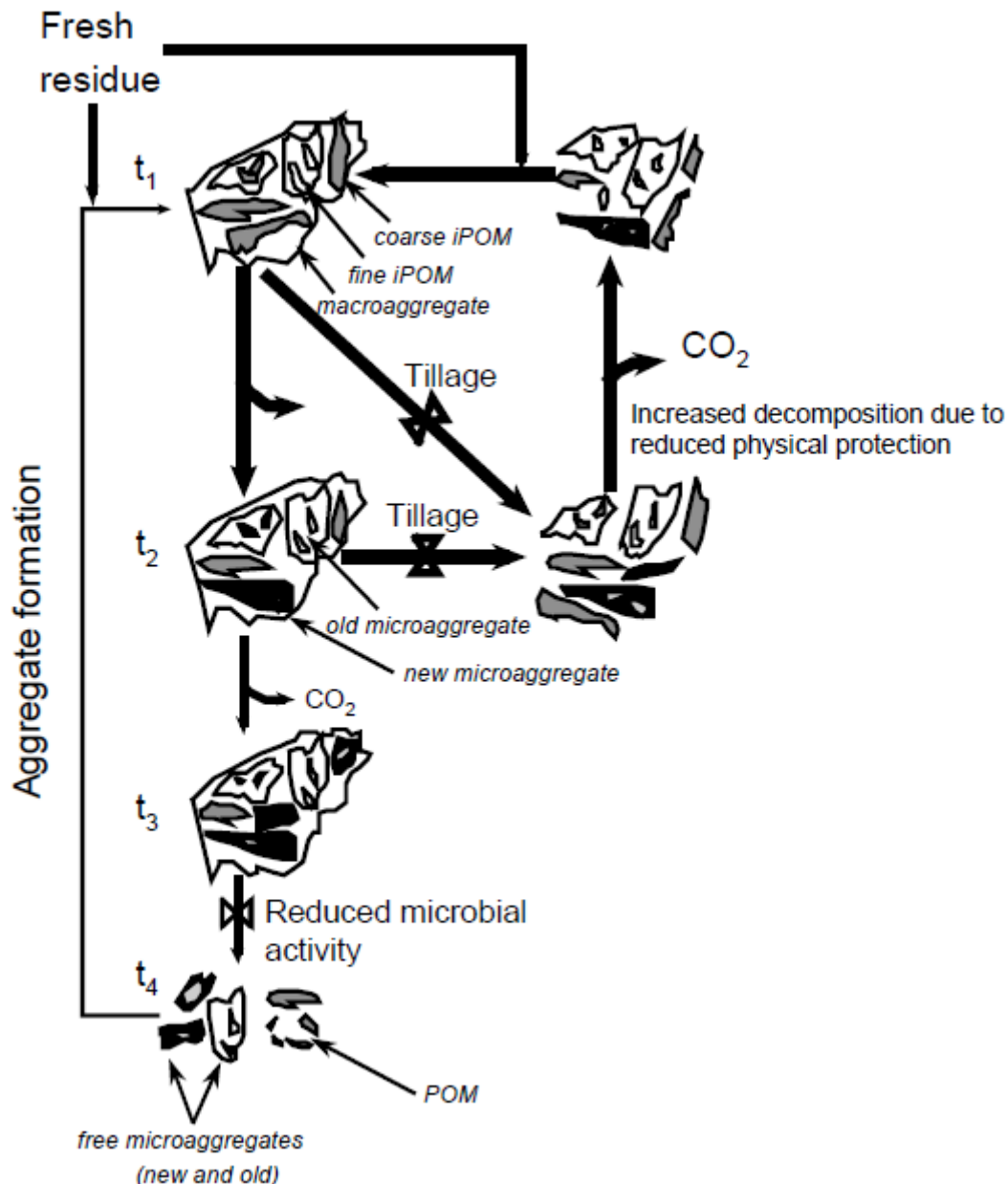
Figure 2.6 The model (1) that shows the "life cycle" of a macroaggregate and the formation of microaggregates: From Six et al. (2000). Reprinted with permission from Elsevier.

Another model for aggregate formation is a process where the microaggregates are formed through interaction between mineral surfaces and organic matter with no real protection in the early stages of microaggregate formation (Lehmann et al., 2007). The microaggregates are then later incorporated into macroaggregates as they form through the occlusion of plant derived organic matter (Model 2). Results obtained by Verchot et al. (2011) (Figure 2.7) and Huang et al. (2010) showed that the second model supports C stabilization and aggregate formation much better. Similar results were obtained by Mupambwa & Wakindiki (2012) which stated that microaggregates formed first followed by macroaggregates. The

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microaggregates showed equal or greater decrease in ^{13}C compared to the macroaggregates, suggesting that microaggregates are not protected by macroaggregates as suggested in the first model (Figure 2.6). Verchot et al. (2011) thus concluded that the stable microaggregates form through the interaction between mineral surfaces and organic ligands.
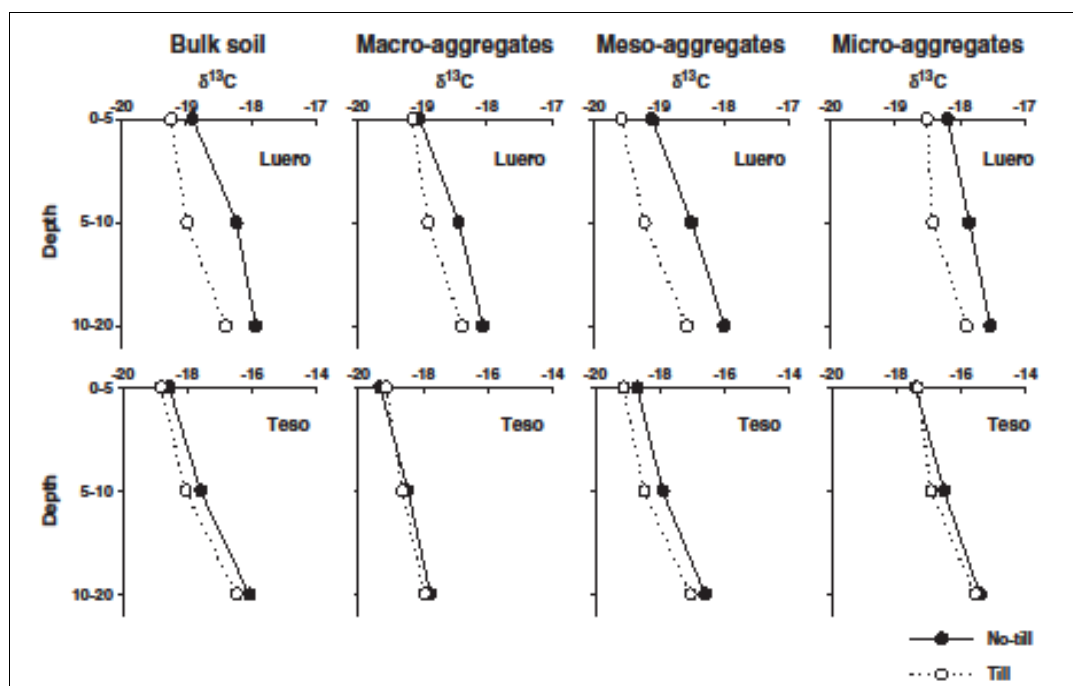
Figure 2.7 The effect of tillage on 13C values in the bulk soil and in each fraction by depth: From Verchot et al. (2011). Reprinted with permission from Elsevier.

2.4.1.3. No - Tillage vs. Conventional tillage

A lot of research has been conducted (Huang et al., 2010; Sombrero et al., 2010) to evaluate the effect of conservation tillage vs. conventional tillage on C sequestration and significant differences, favouring conservation tillage, have been found. Tillage frequency, depth and intensity all have an influence on how much the soil is disturbed.

According to West & Post (2002) no-tillage minimize soil disturbance which leads to a higher aggregate stability and thereby decreases the mineralization of organic matter. Retaining crop residues with no-till systems also contribute to aggregation. An increase in aggregate stability due to no-till practices encourages the formation of recalcitrant SOM fractions within micro- and macroaggregates with experiments showing a much slower turnover time of SOM in no-till vs. intensive tillage (Paustian et al., 2000).

However, results of a study (11 year experiment) done by Chen et al. (2009) showed that the particulate organic matter (POM) and other labile fractions of OM were much higher in the

no-till systems than in conventional tillage, only in the surface soil and not in the subsurface soil. The same trend was observed for the total SOC content, as the different tillage treatments only had an effect in the 0-15 cm layer, with SOC being much higher in this layer when no-till was applied. In the 15-30 cm layer no differences were observed. Àlvaro-Fuentes et al. (2008) also found that no-tillage increased the SOC content only at the soil surface (0-10 cm) as in deeper soil layers, more SOC accumulated under conventional till than under no-till. Sombrero & de Benito (2010) found a similar trend in the vertical distribution of the soil organic C content after 10 years (Figure 2.8). These trends can be attributed to the fact that crop residues penetrates to deeper depths with conventional ploughing while crop residues in no-tillage remains on the surface (Sombrero & Benito 2010).
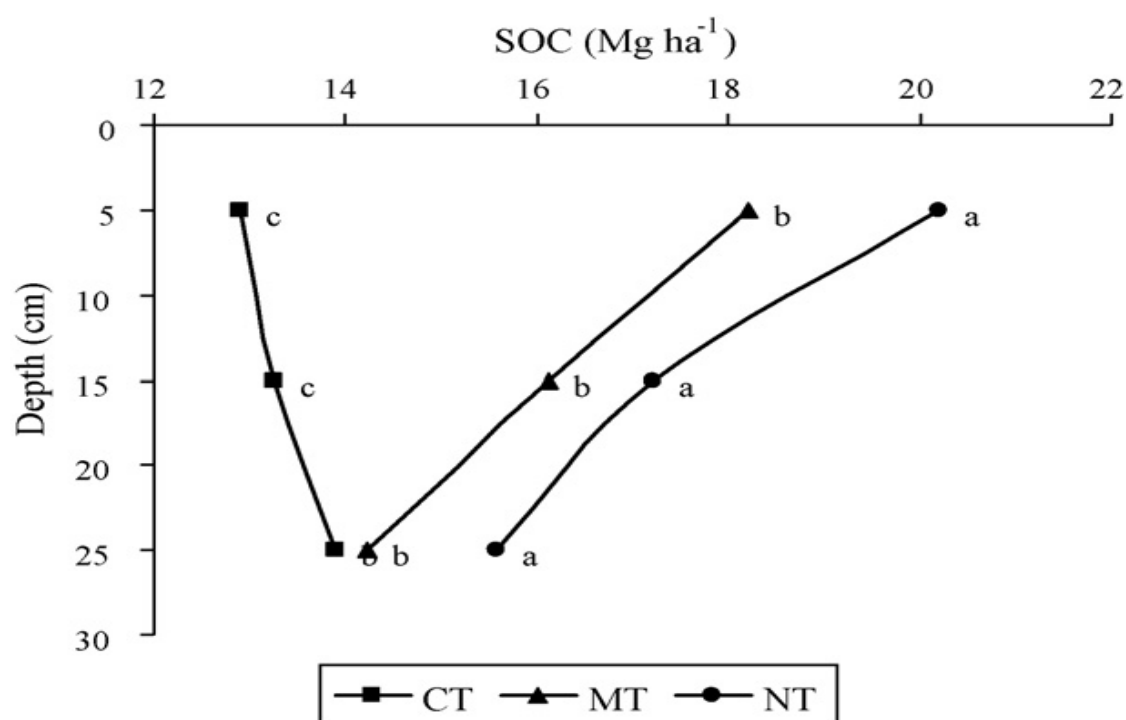
Figure 2.8 Vertical distribution of the soil organic C content after 10 years for each tillage system. CT, conventional tillage; MT, minimum tillage; NT, no tillage: From Sombrero & de Benito (2010). Reprinted with permission from Elsevier.

The overall differences obtained between the two tillage systems can be explained by the breakdown of aggregates under conventional tillage and thereby exposing the protected organic matter to microbes and their enzymes and increases the loss of labile C (Chen et al., 2009). Plant residues retained in the no-till system could also enter the labile pools and act as a substrate for microorganisms. Huang et al. (2010) found an 18.1% increase in the

concentration of total SOC compared with conventional tillage under a long-term maize monoculture with no-till causing larger C concentrations in macroaggregates (>2000 and 250-2000 μm) and microaggregates (53-250 μm) relative to conventional tillage. According to Arshad et al. (1990) no-till don't only increase the quantity of OM but also improves the quality of OM as OM under no-till contained more carbohydrates, amino acids, and amino sugars.

In general, no-till systems have higher SOC than tillage systems but a meta- analysis done by Luo et al. (2010) in different climates found that adopting no-till did not enhance total C stock down to 40 cm but only in the first 10 cm. The rates of accumulation are thus variable, since the amount of SOC stabilized is not only dependant on how the soil is managed, but also on its mineralogy, climatic conditions, quantity of residues, and N inputs (Salvo et al., 2010).

2.4.2. Crop rotation systems (C input)

2.4.2.1. General

According to Jordan et al. (1997) crop rotation is a key component in reduced and integrated systems of production. The type of crop, crop rotation, and the quality and quantity of crop residues play an important role in the soil C content (Wright & Hons, 2005) and different arable crop rotations can thus have different effects on SOM (Johnstone et al., 2009). Mechanisms that have been suggested resulting in the positive effect of crop rotation diversity on SOC content are reduced pest abundance, greater microbial biomass, increased diversity of water and nutrient demands, and increased diversity of rooting depths and residue decomposition rates (Grant et al., 2002).

The quality of organic matter is assessed on its C:N ratio where high-quality organic matter has a low C:N ratio and low-quality organic matter a high C:N ratio. High-quality organic matter will thus have a higher mineralization rate and essential nutrients (N, P, & K) will be more rapidly available and it will enhance biological activity. The C:N ratio of material added to soil determines whether N will be released or fixed in SOM as the material decomposes (Johnstone et al., 2009). The N contents of crop residues play thus an important role in C and N sequestration as crops with low-quality residues have a greater potential for C and N sequestration (Wright & Hons, 2005). However, according to Abberton (2010), legumes (lucerne, medics and lupines) have a potentially significant role to play in increasing soil C sequestration and for a number of years the potential importance of legumes in many

agro-ecosystems has been recognized. By enhancing C sequestration in the soil, soil fertility and biomass are increased and thereby increasing the soil C sink capacity which leads to a reduction in greenhouse gas emissions (GHG). The role of legumes in supplying N through fixation is increasingly being seen as important and beneficial in terms of overall GHG balance (Abberton, 2010).

Soil organic matter is not a homogeneous substance as it is composed of different biomolecules with different chemical composition and structure which will affect the recycling rates. Schmidt et al. (2011) proposed that C stability rather depends on its biotic and abiotic environment and that the molecular structure of plant inputs plays a secondary role in determining C residence times over decades to millennia. However, there is scientific evidence which indicates that planting alternative crops/pastures in a crop rotation system is potentially more economically and biologically sustainable than using a fallow system (McEwen et al. 1989).

Crops such as canola and lupines are ideally suited to rotation with wheat as it has the potential to improve soil structure and, in the case of lupines, provide N to the following wheat crop. Pastures can play a significant role in decreasing soil degradation processes which increases its productive capacity (Salvo et al., 2010) and are therefore linked with environmental sustainability and productivity. In a study done by Garcia Préchac et al. (2004) they found that on the Abruptic Argiudoll (silt loam), the SOC was reduced by 7.5 % after 5 years of continuous cropping (CC) with no-tillage (NT) while the NT-based crop-pasture rotation had 6 % greater SOC than the original content (Figure 2.9). The crops were directly grazed. Salvo et al. (2010) speculated that more SOC was humified in the systems including pastures in rotation due the contribution of N from legumes and supports their hypothesis that mixed crop-pasture systems has a beneficial effect on the stabilization of C in the soil. According to work done by Agenbag & Maree (1989) in the Swartland, South Africa the advantages due to higher SOC will be reached sooner if wheat is rotated with legumes than in all wheat years.
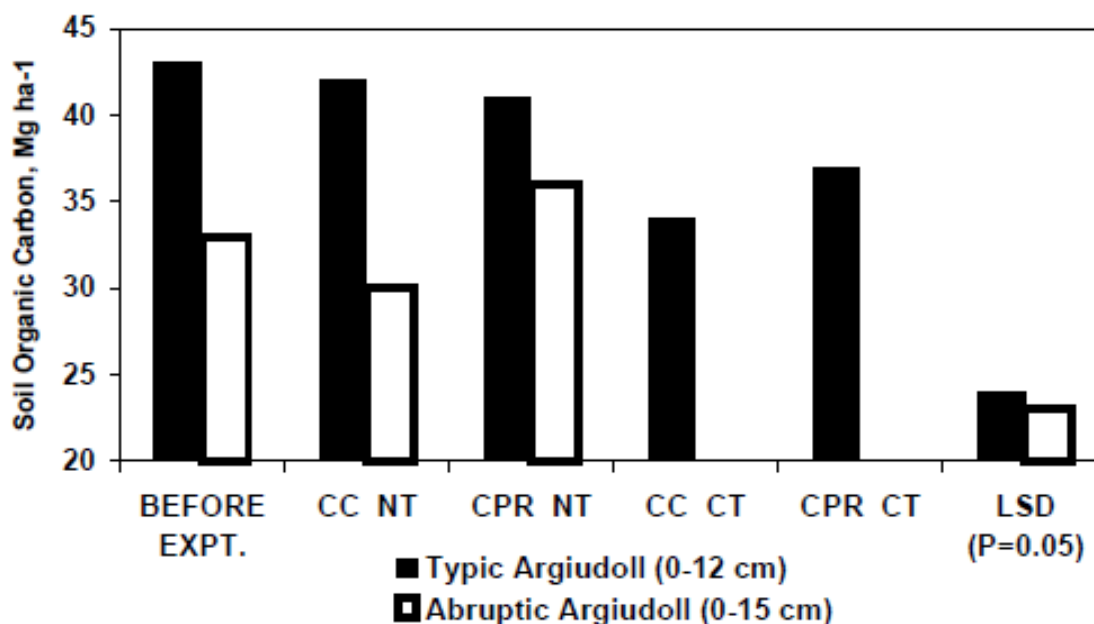
Figure 2.9 SOC content of two experiments after 6 years of crop–pasture rotation (CPR) and continuous cropping (CC) under no-till (NT) and conventional tillage (CT), as well as before experiments started: From Garcia Préchac et al. (2004). Reprinted with permission from Elsevier.

Salvo et al. (2010) also found that under conventional tillage, the continuous cropping had 17% less mineral-bound SOM (0-3 cm depth) than under crop-pasture rotation and related this finding to the fact that the soil was undisturbed during the pasture cycle which reduced oxidation. Under no-till, crop-pasture rotations had 12.5 % more mineral-bounded SOM (0-3 cm) than the continuous cropping systems but no differences in total SOC (0-3 cm) between the continuous cropping systems and the crop-pasture systems rotations were found. An experiment done in Typic Argiudoll (Colonia-Uruguay) for 26 years, crop-pasture rotations with conventional tillage retained soil productivity and produced between 18 and 26% higher yield than continuous cropping systems. The topsoil under continuous cropping had an average loss of SOC of 540 kg ha^{-1}year^{-1} and the SOC loss under crop-pasture rotation was 80 kg ha^{-1}year^{-1} (Figure 2.10) (Garcia Préchac et al., 2004).
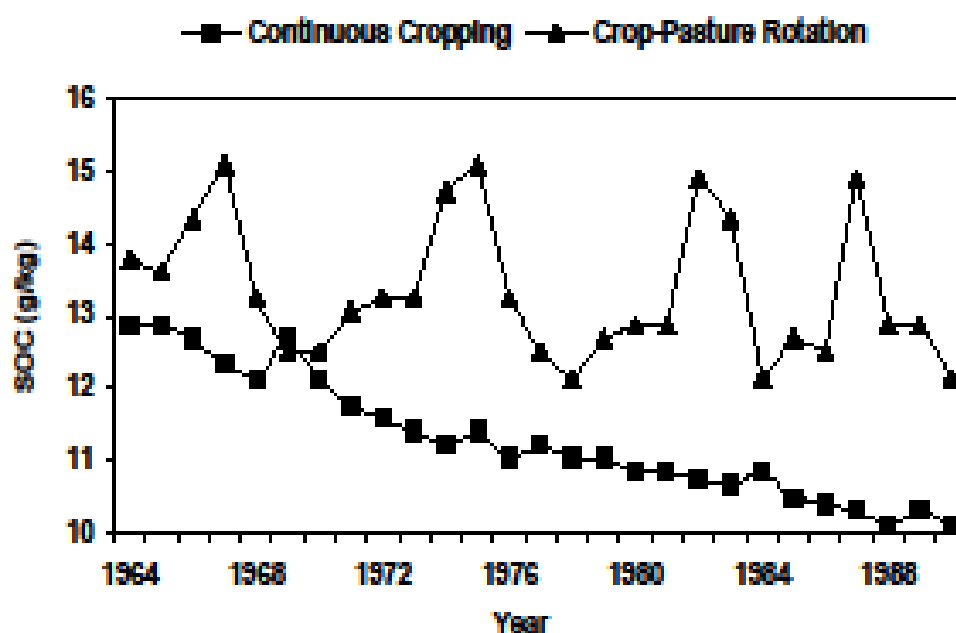
Figure 2.10 Change in SOC concentration (0–20 cm depth) from 1964 to 1990 in two different cropping systems with conventional tillage in a Typic Argiudoll (adapted from D´ıaz-Rosell´o (1992, 1994)): From Garcia Préchac et al. (2004). Reprinted with permission from Elsevier.

Litter dynamics (chemistry and quality) vary with crop type, and are likely to control the rates of litter decomposition and SOC accumulation (White et al. 2009; Salvo et al., 2010). In South Africa less than 10% of grain crops planted annually are legumes and this is despite the fact that Bloem et al. (2009) found that maize yields intercropped with legumes were comparable with those from crops that gained from additional fertilizer (54 kg ha^{-1} at planting and 54 kg ha^{-1} N as top dressing). The beneficial effects of legumes are reduced N inputs in the subsequent grain crop and possible increase in C stabilization. Soil organic carbon and its size fractions which act as C indicators suggest that crop-pasture rotations, especially in conventional tillage systems, would be preferred rather than continuous cropping systems (Salvo et al., 2010). The use of legumes as a pasture in a rotation system is thus worthy of consideration to enhance C sequestration and thereby soil quality and sustainability (Abberton, 2010).

2.4.2.2. Effect of roots

The contribution of roots to soil C pools has been mostly ignored, although it is a vital factor that plays a role in whether the plant-tissue C will be mineralized to CO_2 or accumulates as stabilized SOM. Plant roots represent a significant, but poorly understood source of C inputs (van Vleck & King, 2011). In the topsoil, OM is mainly derived from plant residues that are

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mixed into surface soils by tillage or by soil fungi whereas in the subsoils, plant roots and leached dissolved organic matter is the main sources of C input (von Lützow et al., 2006). The input of OM from roots is thus a very important factor and different root systems of different crops can have an effect on the stabilized C content, especially in the subsoil (Schmidt et al, 2011). It is specifically important in this study as different crop rotation systems and their effects on C stabilization will be compared. Most of the C found in soils is derived from belowground inputs (plant roots and rhizosphere) as it is retained in soils much more efficiently and is transformed into the substances found in the soil (Schmidt et al., 2011). In annual cropping systems, due to slower decomposition, root-derived C (detrital root biomass and more labile root exudates) contributes nearly twice as much C to stable SOC pools as does aboveground residue (Rasse et al., 2005). Root inputs have more opportunity for physicochemical interactions with soil particles and therefore greater chemical and physical protection than many aboveground inputs being mineralized in the litter layer. Fine root (less than 2 mm diameter) decomposition of legumes leads to enhanced root N release and net soil N mineralization. This can have a significant influence on N and C cycling (Fornara, Tilman & Hobbie, 2009). There are three main mechanisms that are involved in protecting root C in soils (Rasse et al., 2005).

2.4.2.2.1.   Mechanisms (Rasse et al., 2005)

Chemical recalcitrance (Figure 2.11)

The aromatic compound lignin found in plant litter is usually responsible for its selective preservation (Palm et al., 2001) with tannins, suberins and cutins all constituents included in lignin (Rasse et al., 2005). In certain species it was found that the lignin content of roots is on average more than double than that of shoots. Root and shoot lignin has the same molecular structure which indicates that the quantity of lignin is the main potential driver for different degradation between roots and shoots (Rasse et al., 2005). According to Waid (1974) tannins are very resistant to biodegradation and the high content tannin substances that are found in roots of cereals contribute to their slow rate of decomposition. The other two constituents of lignin, cutins and suberins are also very recalcitrant plant molecular structures in soils. According to Bernards (2002) cutins is only found in aboveground material and is therefore a shoot specific compound while suberin is a good tracer of root activities. Although quite different in chemical composition, these two molecules can thus be used as biomarkers in studies to determine how above- and belowground material contributes to soil organic matter.

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Analysis that was done on this root and shoot biomarkers confirmed the dominance of root C in soil (Mendez-Millan et al., 2010).

Physical protection through the interaction with soil minerals (Figure 2.11)

Oades (1995) proposed that the close interaction of root material with the soil minerals is the main mechanism through which root C is protected, but then, ploughing would increase the stability of aboveground C when it is incorporated into the soil. Several other studies shown (Salvo et al., 2010; Bhattacharyya et al., 2012) that ploughing, if anything, leads to a decrease in stabilized C. Roots enjoy thus certain activities that stimulate the interaction between root compounds and soil minerals. Due to the negative charge of the organic acids (usually labile compounds) produced by plant roots, cation bonding can play an important role be enhancing sorption to the mineral phase which inhibits degradation (Jones, 1998). In the subsoil the mineral surfaces are not yet saturated with organic matter and therefore root C sorption and stabilization is much more effective at deeper soil depths (Rasse et al., 2005).

Physical protection through occlusion within aggregates (Figure 2.11)

Soil aggregation can be improved by roots as they enhance microbial biomass which produces a type of glue that acts as a binding agent and the roots itself can also hold soil particles together (Jastrow et al., 1998). Organic C inside soil aggregates is limited for microbial decomposition. Organic matter input from root hairs, mycorrhiza, and fine roots contribute at a scale equivalent to that of C that is physically protected (Rasse et al., 2005). This scale is thus very related to physical protection of soil organic matter and according to Six et al. (2002a) the two main mechanisms are (i) the inaccessibility of fine pores to microbial decomposers and, (ii) the anoxic conditions found in these fine pores.
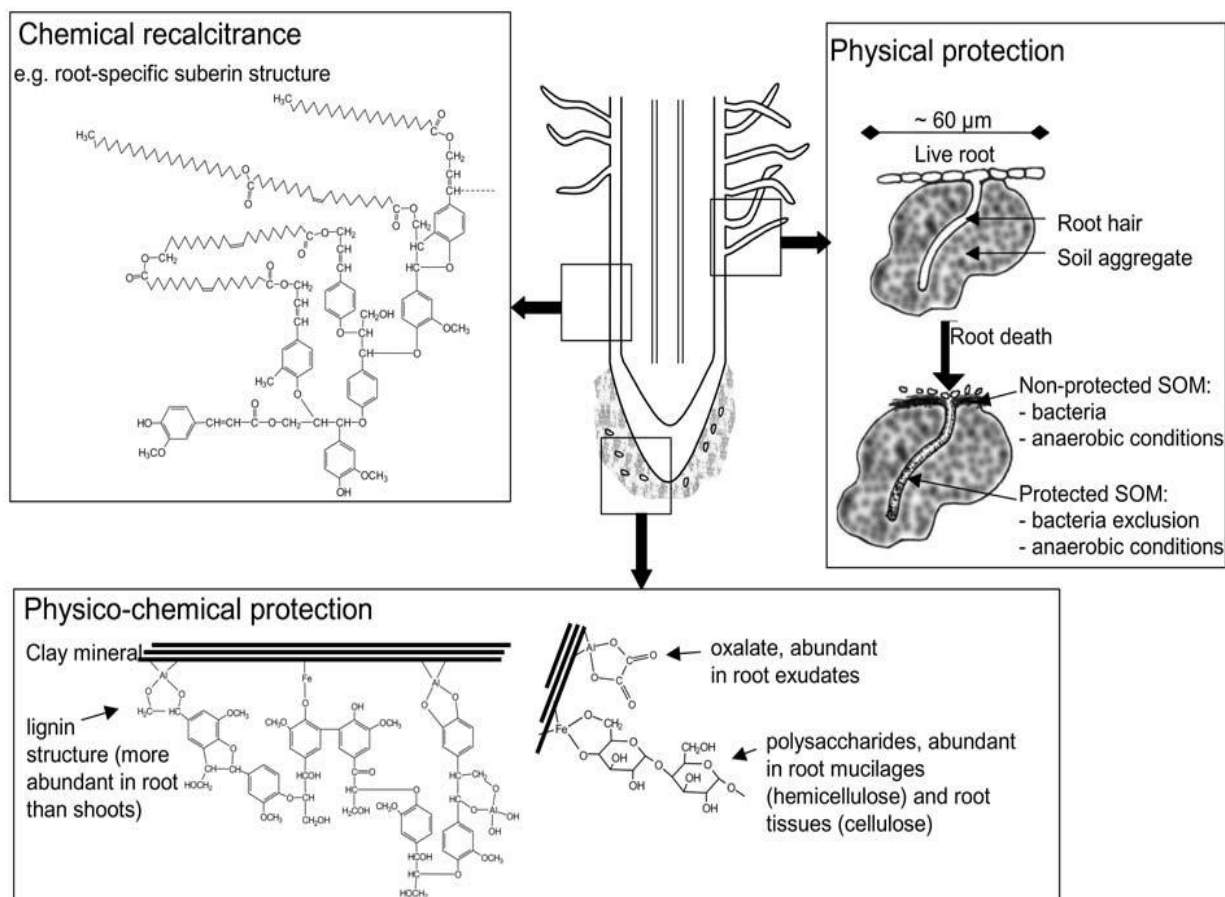
Figure 2.11 Illustration of the main mechanisms resulting in the protection of root C in soils: From Rasse et al. (2005). Reprinted with permission from Springer.

2.5. Effect of Mineralogy and Texture on soil C dynamics

According to Baldock & Skjemstad (2000), the extent of protection of potentially labile SOM is mostly influenced by soil texture and mineralogy. However, the effect of soil texture and mineralogy on SOM is still not completely understood. Multiple studies suggested that the amount of C in the soil is not only dependant on soil silt and clay content but also on surface area and reactivity of mineral soil particles (Kiem & Kögel-Knabner, 2002; Six et al., 2002a). Soil clay minerals and pedogenic oxides have been recognized as important parameters influencing the storage of SOC by mineral associations as mineral associated SOM is characterized by slower turnover rates in soil C models compared to the free particulate organic matter fraction (Kleber 2005; von Lützow et al., 2006).

Soil texture (clay content) can affect the storage of C through direct and indirect mechanisms as studies have shown that increased clay contents is associated with increased aggregate stability. By increasing the aggregation, clay content affects soil C stabilization indirectly by occlusion within aggregates and thereby making them inaccessible to microbial degradation.

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Kölbl & Kögel-Knabner (2004) found that more C was occluded within aggregates with increasing clay content as they found a 72% correlation between occluded particulate organic matter (POM) and soil clay content. The effect of clay content on occluded POM is most prominent between 5 and 30% and declines with increasing clay content. The relationship between clay concentration and total SOC content ($R^2 = 0.78$) has also been found sufficiently strong by Nciizah &Wakindiki (2012) which was attributed to the formation of strong chemical bonds between the clay and organic particles (chemical protection). Clay affects thus chemical protection of soil C stocks directly and physical protection (occluded within aggregates) of soil C stocks indirectly, whereas unprotected C and biochemically protected C are independent of soil texture. Schjonning et al. (1999) suggested that the effect of texture on soil water-holding capacity and soil structure should be considered when interpreting results of differently textured soils.

Although there is evidence that clay concentration may explain the amount of SOC stabilized (Kölbl & Kögel-Knabner, 2004; Nciizah & Wakindiki, 2012), sometimes SOC is better related to factors such as extractable aluminium, allophane content, or specific surface area (Krull et al., 2003). Mineralogy plays an important role in the accumulation of SOC as it defines the capacity of soil minerals to adsorb and protect organic C. Clay-sized particles, like layer silicates, sesquioxides, short-range ordered Fe-oxides and amorphous Al-oxides provide the largest surface onto which OM can adsorb (Mikutta et al., 2007). Quartz which is known for having a very low specific surface area was found to have a negative relationship ($R^2 = -0.74$) with SOM while hematite, known for a high specific surface area had a strong positive relationship ($R^2 = 0.83$) with SOM (Nciizah & Wakindiki, 2012). According to Torn et al. (1997) mineral activity, rather than mineral texture, is a better predictor of the residence time and turnover time of stable SOC. The effect of minerals on C stabilization also depends on the pedogenic environment as this will result in variations in pH, wetness, OM chemistry, cation availability and many other environmental controls.

Inconsistencies still exist about the effect of mineralogy and texture on SOM but soil texture, which is closely linked to soil mineralogy, is according to Baldock & Skjemstad (2000) one of the most important factors influencing SOM concentrations in soils.

2.6. Effect of climate on SOC content

Climate is generally considered the most important soil-forming factor governing the SOM content as it controls the type of plants and productivity which in turn affect the quantity and quality of inputs to the SOC pool as well as its susceptibility to microbial decomposition. The SOC pool to a 1 m depth ranges from 30 tons ha^{-1} in arid climates to 800 tons ha^{-1} in organic soils in cold regions and this acts as proof that climate plays an important role in the amount of C stored in soils (Lal, 2004). Semi-arid regions are known to have high evaporation and low annual precipitation and these soils therefore usually have a low C content (White et al., 2009). Soil organic C stocks generally increase as the temperature decrease with variation in temperature and precipitation being the most important controlling factors for SOC cycling over regional to global scale (Post et al., 1982). White et al. (2009) proposed the timing and frequency of precipitation to be the dominant factors of climatic control for SOC cycling in arid and semi-arid ecosystems.

2.7. Soil Respiration

Soil respiration is the production of CO_2 and includes respiration of plant roots, the rhizosphere, soil microbes and fauna. It is the primary way that C moves from the soil back to the atmosphere as CO_2 flux from soils to atmosphere is one of the largest fluxes in the global C cycle (Schimel, 1995). Soil respiration makes up more than 50% of total ecosystem respiration with soil respiration varying with latitude, from 80 g C m^{-2} y^{-1} in deserts to 800-2000 g C m^{-2}y^{-1} in tropical forests (Raich & Potter, 1995).This indicates that total ecosystem respiration roughly equals gross primary productivity. Management practices, especially tillage, also play an important role in regulating the functional capacity of soil as a net sink or source of CO_2 (West & Post, 2002) as soil CO_2 efflux is not only sensitive to temperature and moisture but also to disturbance (Keith & Wong, 2006). It was found that soil and crop type also play an important role in respiration rates in agricultural fields (Lohila et al., 2002).

One hypothesis, often use to explain reductions in decomposition and heterotrophic respiration (decomposition of SOM to CO_2 by microbes), is abiotic stabilization of SOM as soil respiration is inhibited in a soil that has a high clay content (Telles et al., 2003). In a study where the mineralization of ^{14}C-labelled was monitored for 8 years by Ladd et al. (1985) on soils having similar mineralogies but different clay contents, the soil organic C remaining at the end of the study were proportional to soil clay content as clay minerals had a stabilizing effect on SOM. Similar results were found by Lohila et al. (2002) as soil

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respiration in a sandy soil was slightly higher than that in the clay soil confirming what was found by Ladd et al. (1985). Lohila et al. (2002) also found differences in respiration rates between soil types with peat soils producing 2-3 times more CO_2 than mineral soils attributing this finding to the organic C content of the soil which was 4 times higher in the peat soils. Crop type also plays an important role in CO_2 efflux as Lohila et al. (2002) found respiration was higher in grass than in the potato plots. A higher root biomass and annual C input together with more microorganisms utilizing the root exudates was a possible reason for perennial grasslands producing more CO_2 than potatoes although not studied by Lohila et al. (2002). Tufekcioglu et al. (2001) also observed higher soil respiration rates in grasslands than crop fields and attributed it to a higher SOC content, greater fine root biomass and higher soil moisture content. The effect of different crop rotation practices on soil respiration rates have been studied by Omonode et al. (2007) as they found a shift from continuous corn to a corn-soybean rotation resulted in lower soil respiration rates. However, the relative importance of more diverse rotation shifts on soil respiration in a semi-arid region is still unknown and needs attention.

A good correlation between the SOC stored in the labile fraction and CO_2 efflux was found by Janzen et al. (1992) as C in the light fraction (fPOM) has a turnover time of less than 10 years. However, there are some limitations for soil respiration being an indicator for SOM decomposition: (i) difficult to divide soil respiration into its two sources, heterotrophic respiration (decomposition of SOM to CO_2 by microbes) and autotrophic respiration (respiration from live plant roots), and (ii) mostly only a small fraction of total SOM contributes to respiration due to microbial decomposition (Kuzyakov, 2006). Not all of the CO_2 coming from the soil is thus soil derived and as a result of these limitations, an increase in soil respiration do not necessarily indicate an increase in SOM decomposition, but also an increase in root respiration and therefore, respiration measurements do not always provide information about the large, stable C pools. Studies have shown that autotrophic respiration is more dependent on plant biomass and gross primary productivity, whereas heterotrophic respiration is more dependent on the quantity and quality (C:N ratio) of soil organic matter (Bond-Lamberty et al., 2004). Heterotrophic respiration is thus very dependent on soil management practices applied (tillage and crop rotation) as this can affect the amount of C stabilized in the soil as well as the quality of the organic matter.

2.8. Research Methodologies for studying C stabilization mechanisms in soils

Soil organic matter consists of various functional pools, defined by their characteristic turnover times and pool sizes (von Lützow et al. 2007; Sequeira & Alley, 2011). It is important to be able to predict C turnover as a function of management and environmental changes and therefore knowledge of the size and fluxes of these pools are necessary.

The different SOM functional pools, each with their specific chemical and physical properties are stabilized by different mechanisms which results in different stability and turnover rates (von Lützow et al., 2006). Since the persistence of specific compounds may also be due to other stabilization mechanisms, such as physical protection or chemical interactions with mineral surfaces, recalcitrance is difficult to assess methodologically (Marchner et al., 2008). Turnover time can be defined as the time interval between entering and leaving a reservoir and in the case of C chemistry it is the time between assimilation of C-atom and the release of the same C-atom as respired CO_2 (Kögel-Knabner & Kleber, 2012).

2.8.1. Isolation of specific SOM fractions

There are several fractionation methods that can be used to isolate the different SOM functional pools. To understand the SOC stabilization mechanisms it is necessary to attain an effective separation of SOC fractions of different stability as factors like climate, soil type and properties, mineralogy, land use and management practices play a major role in the dominance of one mechanism over the other (Basile-Doelsch et al., 2009).

There are three major SOM functional pools that are of great importance in soil C stabilization studies and in particular this study; (i) the free particulate organic matter (fPOM) fraction which resembles recent litter inputs and usually have younger C than other fractions (active pool); (ii) an occluded or intra-aggregate POM fraction (oPOM), which is generally older than fPOM fraction released by disruption of soil aggregates (intermediate/passive), and (iii) a heavy or mineral-bound fraction (mineral), comprising organic matter tightly bounded or sorbed to minerals containing the oldest OM (passive pool) (Marin-Spiotta et al. 2008). The oPOM and mineral fraction consist largely of microbial processed materials as well as partially degraded plant detritus (less recognizable structure) and therefore varies widely in C:N ratio among soils (Wagai et al., 2009). Significant changes in the amounts of fPOM caused by cultivation and climate may imply that this fraction is largely composed of labile C and nutrients (John et al., 2005). Isolation of these three pools from whole soils would

facilitate their relative contribution to the total organic C and quantification of organic C contained in these pools. Therefore, separating fractions of OM with different biochemical properties and functional significance has received a lot of interest in the last 20 years (Cerli et al., 2012). To elucidate the possible chemical and physical mechanisms responsible for the stabilization of SOC it is necessary to examine the relationships of the stable mineral fraction and oPOM fraction with selected soil properties such as clay content, sesquioxides, exchangeable cations and aggregate stability.

A critical review of all the possible methods recommended by various authors e.g. Golchin et al. (1994a); Sohi et al. (2001); von Lutzow et al. (2007); Jagadamma & Lal (2010); Cerli et al. (2012) regarding isolation of different SOC fractional pools have been done. It must be able to identify SOC pools that behaves more or less the same regarding deterioration, formed by specific stabilization mechanisms (Bruun et al. 2004). The objective of fractionation according to von Lützow et al. (2007) is: "To reduce the chemical, physical, and C cycling time variation in the fractions compared to the bulk soil". Various combinations of fractionation methods can be used for this purpose. A common approach is physical fractionation in combination with ultrasonic dispersion (Sohi et al., 2001; Cerli et al., 2012).

2.8.1.1. Physical fractionation

Physical fractionation techniques have been used to isolate stabilized SOM functional pools from labile SOM functional pools (John et al., 2005) as these techniques helps detect the C fraction sensitive to soil management practices (Six et al., 2002a). Soil organic carbon is subdivided into different pools according to physical properties as this emphasizes the implication that spatial location is an important factor in determining OM turnover. Physical fractionation techniques involves a combination of either density fractionation or particle size fractionation and ultrasonic soil dispersion (Christensen, 1992; Golchin et al.,1994a; Sohi et al., 2001; Cerli et al., 2012). The effectiveness of soil dispersion procedures is crucial for both particle and density fractionation methods. It is unpractical to separate precisely all and only SOM of the same reactivity or specific age so Christensen (1996) suggested that it is rather more suitable to model the measurable than to measure the modelable pools.

2.8.1.1.1. Density fractionation

This method is used to isolate SOM that is not strongly associated with soil minerals from organo- mineral complexes as the aim of the method is to achieve active pools (SOM not associated with organo-mineral) and intermediate and passive pools (organo-mineral

complexes) (von Lützow et al., 2007). The light fraction (fPOM) isolated by this method contains mostly easily decomposable plant debris while the dense fraction represents the mineral-associated and thus protected parts of OM. Organic materials in the dense fraction (> 2 kg L^{-1}) consist mostly of carbohydrates and aliphatic structures and a narrow C:N ratio leading to the conclusion that minerals are able to protect OM rich in N, suggesting a microbial origin for this fraction while the light fraction predominantly contains easily decomposable plant debris (Golchin et al.,1994b).

When used as a pre-treatment, density fractionation is very useful in isolating different SOM functional pools (von Lützow et al., 2006). In combination with sonication it is a useful approach because it is an easy method to obtain important insights into the chemical characteristics of isolated fractions in a variety of soils (Wagai et al., 2009).

Several high density solutions have been proposed for this purpose, including sodium polytungstate ($Na_6(H_2W_{12}O_{40}).H_2O$) (von Lützow et al., 2006) and sodium iodide (NaI) (Sohi et al. 2001) solutions. The fPOM fraction is isolated by simply suspending a sample of soil in a high density solution and decanting the light (floating) material. Plant material that is relatively free of mineral particles (fPOM) can be isolated by flotation in a liquid adjusted to 1.6 - 1.8 g cm^{-3} (Wagai et al., 2009). The remaining soil residue is disaggregated by sonication to release the occluded OM and is called the occluded particulate organic matter fraction (oPOM) (Sohi et al., 2001). This fraction contains much finer organic particles of similar composition than the first fraction (Golchin et al., 1994b) and it is held within aggregates and therefore doesn't float in density solution unless the aggregates are disrupted (Cerli et al., 2012). The organic matter tightly bound or sorbed to minerals (heavy fraction) with a density above 2 g cm^{-3}, can be collected as the residual, dense soil pellet (Marin-Spiotta et al., 2008). Organo-mineral complexes have a lower density than those of pure minerals but higher than pure organic particles (Christensen, 1992). Separating soil samples into different density fractions has often been used as a technique to investigate the mechanisms responsible for OM stabilization.

2.8.1.1.2. Particle-size fractionation

According to Christensen (1992) particle size fractionation is based on SOM fractions that are associated with different particle sizes (sand, silt, clay) and therefore also different mineralogical which effects SOM turnover rates. Clay-sized particles provide a large surface area and reactive sites where SOM can be sorbed. Sorption is an important stabilizing

mechanism and the SOM associated with the clay and silt fraction will be assigned to the intermediate and passive pool whereas the SOM within the sand fraction is allocated to the active pool. The slower C turnover rates associated with the clay fraction compared to the sand fraction can be contributed to a chemical change in OM quality, an increase in spatial inaccessibility and the adsorption of OM on mineral surfaces (von Lützow et al., 2007). According to Sohi et al. (2001) it is necessary to do density isolation of the light (particulate) SOM fraction(s) prior to particle-size fractionation to avoid confusion of the particulate SOM with that associated with mineral particles.

2.8.1.1.3. Ultrasonic dispersion (sonification)

Organic matter substrate that is occluded in soil aggregates are inaccessible to microbial attack and provides thus a large amount of physical protection (von Lützow et al. 2006). Physical disruption of soil aggregates exposes OM that was formerly inaccessible to microbial attack. It is thus necessary for the purpose of liberating the occluded OM prior to isolation with heavy solutions and can be practically simulated by ultrasonic dispersion, sometimes referred to as sonication or ultrasonication. After fPOM fraction is separated by flotation in NaI, the oPOM fraction can be floated from the remaining dense material via sonication procedures. The energy applied is designed to break stable soil-aggregates releasing the oPOM (Sequeira & Alley, 2011). Optimal level of sonication energy is very important and the effect of sonication level on oPOM depend on the soil properties and density of liquid (Sohi et al., 2001).

2.8.1.2. Chemical fractionation

Stable organic matter can be defined as the material that survived destruction by chemical treatment. According to von Lutzow et al. (2007): "Chemical fractionation procedures are based on the extraction of SOM in aqueous solutions with and without electrolytes, in organic solvents, on the hydrolysability of SOM with water or acids, and the resistance of SOM to oxidation." Hydrogen peroxide (H_2O_2), sodium hypochlorite (NaOCl) and disodium peroxodisulfate ($Na_2S_2O_8$) are the most acknowledged chemical reagents for this purpose (Mikutta et al., 2005) and results obtained by Helfrich et al. (2007) indicated that treatments with H_2O_2 and $Na_2S_2O_8$ were the most effective ones in isolating stable SOM on different land uses. In contrast, treatments with $Na_2S_2O_8$ and NaOCl were considered more effective than with H_2O_2 in isolating SOC (Mikutta et al., 2005). Humic substances are very stable in

soils due to its very complex structures (von Lützow et al., 2007). Oxidation with hydrogen peroxide (H_2O_2) is an appropriate tool for isolating a functionally passive OM pool if POM or light fraction (LF) is separated prior to the treatment because fresh aliphatic plant material is also resistant to H_2O_2 oxidation. Correlations between total Fe oxides in subsoil horizons and SOC that is resistant to H_2O_2 oxidation, suggests that the old fraction is protected through interactions with soil minerals (von Lützow et al., 2007). According to Mikutta et al. (2005) oxidative treatments selectively remove a younger C fraction (less protected) and allow the isolation of an older, non-oxidisable, refractory C pool.

2.8.2. Characterization of SOM fractions

Solid-state CPMAS ^{13}C NMR spectroscopy techniques can be efficiently used to study and investigate the variation in chemical composition of the isolated fractions as well as the fresh litter input of the different crops used in the crop-rotation systems. ^{13}C NMR spectroscopy permits direct chemical characterization of organic materials in soil (Baldock et al., 1992). Application of NMR spectroscopy in this study will be useful in identifying ^{13}C atoms of the isolated SOM fractions from the density separation. To collect the ^{13}C NMR spectra for the mineral fraction may not be possible because of paramagnetic ions, such as iron that can be present in the soil which can interfere with spectral readings. There are several chemical shift regions that represent C-types: 0-45 (alkyl); 45-65 (methoxyl); 65-95 (O-alkyl); 95-110 (di-O-alkyl and some aromatic C); 110-145 (aromatic); 145-165 (phenolic) and 165-220 ppm (carboxylic and Cyl C) (Baldock et al., 2004).

2.8.3. Loss of C by soil respiration

The NFTSS (non-flow-through steady-state) chamber technique using chemical absorption of CO_2 by soda lime (Soda-Lime Method) is a practical method for field measurements as an 82 % correlation has been found between this method and the infrared gas analyzer (IRGA) (flow-through non-steady-state). With the soda-lime method it is possible to determine cumulative respiration over a long period of time across a large, heterogeneous site and it is relatively cheap and the equipment is easy to use (Keith & Wong, 2006). The application of this method doesn't yield exact measurements, but it allows one to compare the soil respiration rates among the different crop rotation systems (Keith & Wong, 2006).

2.9. Conclusion and Gaps in Knowledge

A lot of research has been done on the effect of tillage and crop rotation practices on SOC stabilization. However, there is still some uncertainty as various studies shows different results. In general, conservation practices (crop rotation and no-tillage) have been observed to contribute to the role of soil as a C sink rather than a source, especially in the first few centimetres of the soil.

Due to the fact that climate, soil conditions and C input a major role play in the C content of soils, there is a gap in knowledge on how these practices affect soil C stabilization and distribution in the grain production regions of the Overberg. Understanding the mechanisms involved in the stabilization of C in these soils is of utmost importance as it can help identify management practices that contribute to a higher quality soil. A lot of research has been done in tropical regions trying to figure out how C is stabilized in these soils but little research has been done in semi-arid regions across the world. As it is known, semi-arid soils differ significantly from tropical soils in mineralogical and chemical composition as well as climate dependent chemical processes such as weathering and biodegradation and therefore it is necessary to elucidate C stabilization in these soils as no research has been done in this region.

Through effective isolation of the different SOM functional pools, the vacant gaps in knowledge relating to different SOM pools and C stabilizing mechanisms operating in the soils of the Overberg can be addressed. We will know what the contribution is of stable C (mineral-bound C) to total C (stable & labile C fractions) which will allow us to estimate the effect of the different crop/pasture rotations under no-tillage on SOC dynamics. By isolating total C into its different fractions it will also allow us to elucidate the stabilizing mechanism operating in these soils. This will enable us to determine whether C sequestration in these soils is controlled by management practices (e.g. no-tillage, crop rotation) or whether soil properties (texture, mineralogy) and climate is the dominant factors controlling C stabilization.

Current knowledge suggests that if the right management practices are implemented, agricultural soils can reach higher SOC equilibrium within several decades. Soils are now in the 'front line' of global environmental change and we need to be able to predict how they will respond to agricultural systems (cropping practices and tillage) so that we can better understand their role in the earth system and ensure that they continue to provide for

humanity and the natural world (Schmidt et al., 2011). A soil parameter that can be used for prediction is SOC as it is directly related to the sustainability and quality of the soil.

CHAPTER 3

THE EFFECT OF CROP ROTATION AND SELECTED SOIL CHARACTERISTICS ON TOTAL SOIL CARBON CONTENT IN CONSERVATION-TILLAGE MANAGED SOILS

3.1. Introduction

With a yearly precipitation of 400-450 mm, limited moisture is the main constraint for dryland agriculture in the grain production area of the Overberg. Dryland agriculture in this region is mainly focused on cereal and canola production. With increasing production costs (e.g., fuel), and highly variable climatic conditions, it is of utmost importance to identify sustainable agricultural production systems to try and counter these negative impacts. Low rainfall can also cause a low soil organic matter (SOM) content due to low crop biomass production which limits residue input into the soil. Soil organic matter content is an important factor in dryland agriculture as it directly and indirectly affects the quality and productivity of soils through its influence on several physical (e.g. soil water holding capacity, aggregate stability), chemical (e.g. cation exchange capacity, nutrient supply) and biological (e.g. microbial activity and biomass) properties (Lal 2004; 2006; 2011). Severe depletion in SOM will lead to: (i) soil degradation and nutrient depletion, (ii) decline in agronomic and biomass productivity, (iii) food insecurity and, (iv) decline in environmental quality by the emission of CO_2 while a degraded soil and a low biomass input will lead to a low SOM content (vice versa effect) (Lal 2004). Application of management practices that can contribute to C sequestration in soils is thus necessary. According to Àlvaro-Fuentes et al. (2008) the SOM content in semi-arid climates can be enhanced by the application of recommended agronomic management practices (e.g. no-tillage). This can mainly be attributed to reducing SOM decomposition and/or increasing residue inputs (Paustian et al., 2000). However, no-tillage has to be combined with the establishment of diverse and high C input cropping systems. No-tillage as an isolated system may probably not produce the positive results as expected from the accumulation of soil C (Sisti et al., 2004; Conceicão et al., 2013). According to Lal & Bruce (1999) crop rotation and no-till management that retain crop residues near the surface of the soil, can increase the SOC by raising biomass production and crop residue retention. Manipulating the cropping systems can thus improve soil quality and sustainability through enhancement of SOM.

Conservation tillage can contribute to the soil functioning as a C sink (Sombrero & Benito 2010; West & Post 2002) by minimizing soil disturbance and thereby increasing the aggregate stability (decrease in decomposition of SOM) (West & Post, 2002). An increase in aggregate stability due to no-till practices also enhances SOM concentration within aggregates. This causes an increase in residence time of SOM and is therefore an effective agricultural practice to increase C sequestration in soils and thereby productivity (Paustian et al., 2000). The effect of conservation tillage on C sequestration however is time dependant and will only show after ± 10 years (West & Post, 2002; Sombrero & Benito, 2010; Álvaro-Fuentes et al., 2012). Possible reasons for this phenomenon are the slow incorporation of crop residues under no-till systems through soil fauna. This may contribute to the lack of C sequestration over the first few years in a water-limited region (Six et al., 2004). Another possible reason is a decline in crop yields (lower C inputs) after the first few years of no-tillage.

The effect of cropping systems on SOM can also be very slow, especially in semi-arid regions (Masri & Ryan 2006). Wright & Hons (2005) stated that the type of crop, the rotation sequence and the quality and quantity of crop residues are important for soil C content. Knowledge of the primary input of each crop (shoots and roots) in a rotation system, together with C:N ratio of each crop allows the estimation of the C inputs to the soil and therefore the amount of C that can potentially be sequestered. Plant roots represent a significant, but poorly understood source of C inputs (van Vleck & King, 2011). Most of the C found in soils is derived from belowground inputs (plant roots and rhizosphere) as it is retained in soils much more efficiently and is then transformed into the substances found in the soil (Schmidt et al., 2011). In annual cropping systems, due to slower rate of decomposition, root-derived C contributes twice as much C to stable soil organic C pools as does aboveground residue. This was attributed to root inputs that have more opportunity for physico-chemical interactions with soil particles (clay) than many aboveground inputs being mineralized in the litter layer (Rasse et al., 2005). Soil texture therefore also plays an important role in soil C stabilization as Nciizah & Wakindiki (2012) found a strong relationship between clay concentration and total soil organic C (SOC) content ($R^2 = 0.78$). This can be attributed to the formation of strong chemical bonds between the clay and organic particles (chemical protection).

The use of a fallow period in the grain production areas of the Overberg is not economical and water-conserving fallowing is thus replaced by continuous cropping and crop-pasture rotations. Legumes therefore have a potentially significant role to play in increasing soil C

sequestration in rotation systems as the potential importance of legumes has been recognized for a number of years (Sisti et al., 2004; Masri & Ryan, 2006; Abberton, 2010). According to Agenbag & Maree, (1989), the advantages due to higher SOC will be reached sooner if wheat is rotated with legumes than in all wheat years. Pastures also present a significant potential to decrease soil degradation processes and recovering of its productive capacity which is linked with environmental sustainability and productivity (Salvo et al., 2010). Garcia Préchac et al. (2004) found that SOC was reduced by 7.5% after 5 years of continuous cropping (CC) with no-tillage (NT) while the NT-based crop-pasture rotation had 6% greater SOC than the original content. It is therefore expected that the use of legume pastures in a rotation system can possibly contribute to a more sustainable system in dryland production.

When more C is adsorbed into the ground than is released as CO_2, C accumulates in the soil and this refers to the term soil C sequestration. The role of soil and plant properties in C sequestration in these soils remains to be elucidated as well as their relative importance. Therefore, a study was initiated in the dryland grain production area of the Overberg. Farmers are currently applying different crop rotation systems, ranging from continuous crop to crop/legume to crop/pasture and they are also increasingly switching over to conservation tillage practices in attempt to restore SOC, but the effect of these practices on the maintenance and improvement of the SOC content is still unknown. Detailed studies on the effect of common cultivated crops and pastures (e.g. canola, medics, lucerne, lupine, barley and wheat) under no-tillage on total SOC content are limited as no data is currently available. There is also a lack of knowledge on the biological and economic sustainability of crop and crop-pasture rotation systems in this region. Research on soil C sequestration for a specific soil/climate/cropping system is thus necessary.

The main objective of this component of the study was to investigate the effect of long-term (11 years) no-till crop rotation practices, ranging from 100% pasture to 100% crop, on total soil C sequestration. It involved understanding the underlying reason for differences in soil C sequestration by examining selected soil and plant properties. A further aim was to examine the relationship between the extent of C sequestration and crop yields. Long-term experiments provide valuable information regarding the effect of management practices on soil productivity.

3.2. Material and Methods

3.2.1. Study area

A long-term field experiment at the Tygerhoek Research farm, situated just outside Riviersonderend, Overberg, Western Cape, South Africa (34° 09' 32" S, 19° 54' 30" E) was used in this study. This region is a well-known dryland grain production area. The Köppen-Geiger climate classification for Riviersonderend is BSk (cool semi-arid climate). This type of climate tends to be located in temperate zones and at lower latitudes it tends to have precipitation patterns more similar to Mediterranean climates. Riviersonderend has a low annual rainfall of 450 mm rain per year with most of the rain falling during winter (60% of annual rainfall), but summer rainfall is not unusual (40% of annual rainfall). The long-term average annual temperature is 17.5°C (ARC-ISCW, 2013).

Table 3.1 Long-term monthly climate data for Tygerhoek Research Farm.

Parameter	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Maximum Temperature (°C)	29.1	29.6	28.7	24.8	21.6	18.6	17.9	18.4	21.4	24.4	26.4	27.9
Minimum Temperature (°C)	15.9	16.4	15.1	12.3	9.4	6.1	5.2	5.7	7.6	10.2	12.4	14.4
Rainfall (mm)	25.6	29	32.7	50.4	41.9	42.9	45.2	52.4	33.9	38.6	31.6	28.3

This long-term field experiment was already in its 11th year when soil samples and data were collected in 2012. Rolf Derpsch, a specialist in conservation agriculture stated at a recent conference held in Strand, South Africa, that the consolidation phase of no-tillage is 10-20 years after it has been initiated. It is in this phase where higher C content, cation exchange capacity and soil water can be expected due to application of conservation agriculture (no-till and crop rotation practices) (Derpsch R. 2013.Personal communication).

Most of the cereal crops are planted from May and harvested from mid-October to November as the climatic conditions are suitable for grain production in winter. The soils are very shallow, highly weathered shale-derived soils (30-40 cm depth) with a loamy texture and high content coarse fragments (> 2mm) with soil parent material in this region comprised of Bokkeveld shales (Ellis F. 2012.Personal communication). Due to these properties, the soils

were very challenging to work on. Sampling as well as the analysis of some of the soil properties were very demanding and needed extra attention. The experimental site of the crop rotation systems lies on a middle to lower foot slope with a slope of $\pm$ 5%. The natural vegetated soil, used as reference in this study, lies about 500 m further down the slope (Figure 3.1).
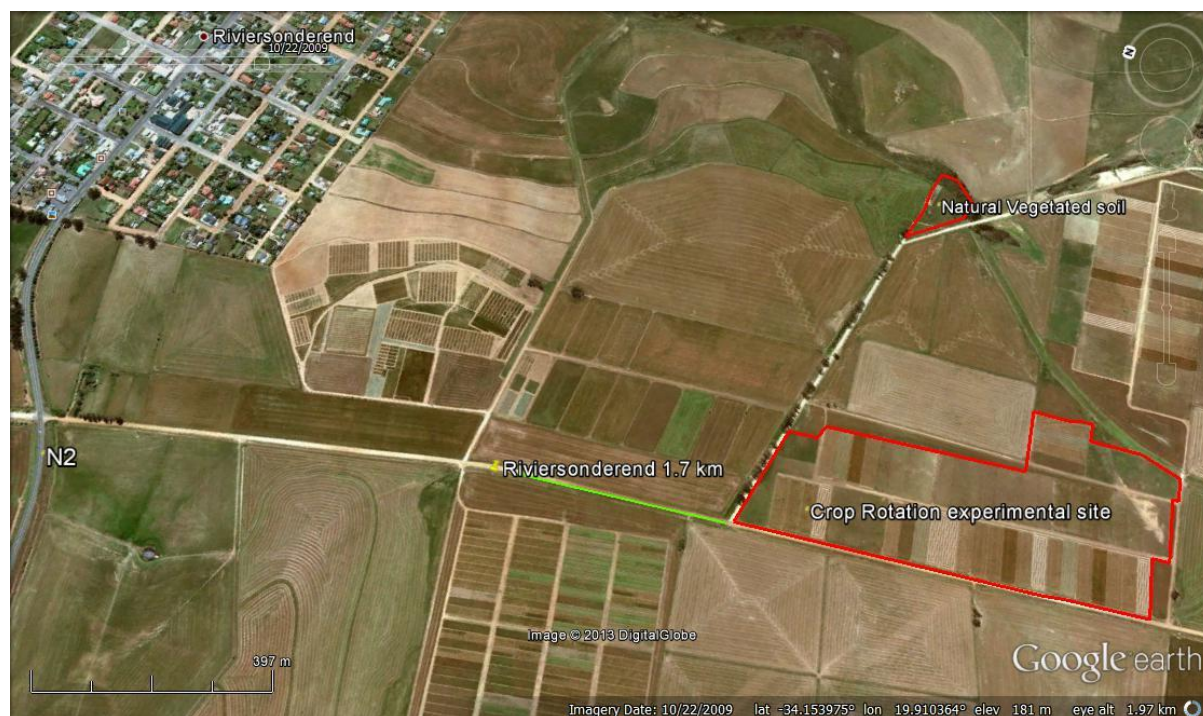
Figure 3.1 The location of the study site at the Tygerhoek Research Farm, Riviersonderend.

3.2.2. Experimental design

Five crop/pasture rotation systems ranging from 100% pasture to 100% crop (Figure 3.2) were included in this study. Each of these rotation systems acted as a treatment with three replicates (A, B & C) within each treatment. Natural vegetation acted as a reference (treatment 6) in this study to compare the effect of conservation agriculture managed soils with **natural vegetated** soils in terms of soil C sequestration (Table 3.2). The crop rotation treatments were all similarly managed with no-till applied to the management of all treatments. Crop residues were retained following harvesting. No-tillage means that the soil is left undisturbed until planting and then planted with a tine, no-till planter (a no-till Ausplow fitted with knife-openers and presswheels). Pastures were grazed by sheep throughout the year on a rotation basis while the stubble of cereal crops was grazed in the off season.

Treatment 1 consisted of a **lucerne** (*Medicago sativa*) pasture (100% pastures) that has been undisturbed since the start of the trial in 2002. The medic-medic-wheat (**MMW**) treatment

(2) is a 67% pasture, 33% crop rotation system and is disturbed once in every four years when wheat (*Triticum aestivum*) is planted. After medics (*Medicago spp*) have been established (planted) for the first time it is able to re-establish itself within a rotation system, if managed correctly. When medics are rotated with wheat, the medics are suppressed in the year when wheat is planted, and then in the following year, the medics spontaneously regrow again without any disturbance of the soil. The medic-medic-wheat-wheat (**MMWW**) treatment (3) is a 50% pasture, 50% crop rotation system and is disturbed twice every four years when wheat is planted. Two 100% cropping systems (continuous cropping) in different phase were also included in this study; a wheat-barley-canola-wheat-barley-lupin (**WBCWBL4**) (treatment 4) and a **WBCWBL1** system (treatment 5). The number "4" in the rotation code refers to the fourth crop planted in the cropping system, wheat planted after canola (*Brassica napus*) (non-legume) while the number "1" in the rotation code refers to the first crop planted in the cropping system, wheat planted after lupin (*Lupinus luteus*) (legume). The soil in both these treatments is disturbed annually during planting. The underlined crop (letter) in the rotation code represents the crop that was on the field at the time of sampling or data recording.

Table 3.2 The different treatments used in this study at the Tygerhoek Research Farm.

Treatment	Camp size (ha)	Rotation System (Treatment)		Crop planted in 2012
1	0.32	Lucerne	100% pasture	Lucerne
2	0.25	MMW	67% pasture; 33% crop	Wheat
3	0.25	MMWW	50% pasture; 50% crop	Wheat
4	0.25	WBCWBL4	100% crop	Wheat
5	0.25	WBCWBL1	100% crop	Wheat
6	± 0.12	Reference	Natural	Natural vegetation

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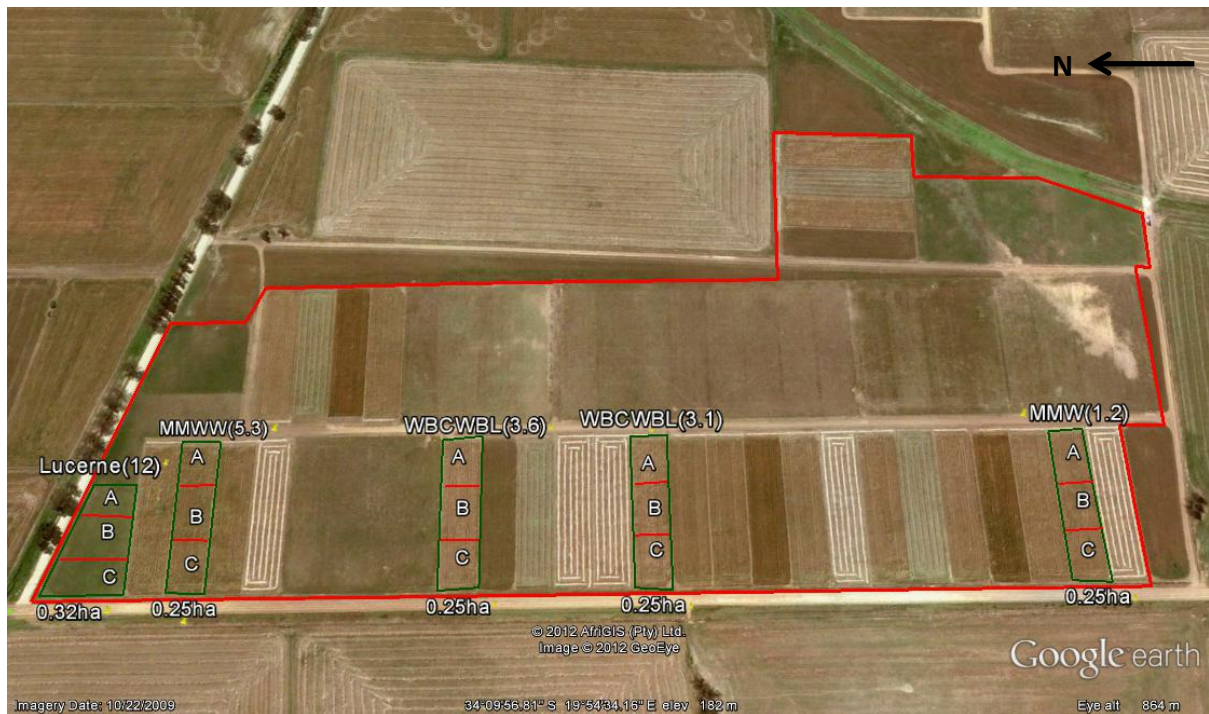
Figure 3.2 Experimental design specifying the different crop rotation systems included in the study.

(a) (b)

Figure 3.3 Digital images of the (a) WBCWBL4 rotation system and (b) Natural vegetated soil at the Tygehoek Research Farm.

3.2.3. Classification and morphological description of soil

A total of 18 soil profile pits were excavated in six different treatments and classified according to the Taxonomic system for South Africa (Soil Classification Working Group, 1991) with soil morphological characteristics recorded in the field.

Glenrosa soil form (Orthic A – Lithocutanic B) (Figure 3.4a) was the dominant soil form at the experimental site while an Oakleaf soil form (Orthic A – Neocutanic B – unconsolidated material without signs of wetness) (Figure 3.4b) was found in Treatment 2 and 6. The profiles were very shallow and were seldom deeper than 600 mm with the A-horizon depth of about 200 mm. The A-horizon is bleached with a clay content ranging between 15 and 25%. The B-horizon (Neocutanic and Lithocutanic) has a yellowish brown colour (10YR 5/6) in the dry state, and a clay content ranging between 20 and 30%. In summer these soils are very hard as the excavator struggled to dig deeper than 600 mm. In the appendix, a detailed description of each soil form is given. According to the FAO classification (IUSS Working group WRB, 2006), the glenrosa soil form is a Leptic Cambisol Skeletic while the oakleaf (natural treatment) is classified as a cutanic Leptic Luvisol and the oakleaf (MMW treatment) as cutanic Leptic Luvisol Skeletic.

Table 3.3 The soil form that dominated in the various treatments.

Treatment	Rotation System		Soil Form
1	Lucerne	100% pasture	Glenrosa
2	MMW	67% pasture; 33% crop	Oakleaf
3	MMWW	50% pasture; 50% crop	Glenrosa
4	WBCWBL4	100 % crop	Glenrosa
5	WBCWBL1	100 % crop	Glenrosa
6	Reference	Natural	Oakleaf

(a)

(b)

Figure 3.4 Digital images of the soil profiles that dominated the experimental site at Tygerhoek Research Farm (a) Glenrosa soil form and (b) Oakleaf soil form.

3.2.4. Soil sampling and preparation

Soil samples for this study were collected in June/July 2012 from three different replicate sites (A, B & C) located within each of the six treatment blocks (camps) at 0-30 cm depth. Samples were divided into four depth increments: 0-5, 5-10, 10-20, 20-30 cm. At each replicate site approximately 30 cores per depth were taken within a 20 m radius using a steel pipe (4 cm in diameter) and hammer. The pipe was marked at the particular depth increments. When the pipe had been inserted into the desired depth, the pipe was pulled out of the ground and the soil collected in a plastic bag. After being transferred to the laboratory, the samples were air dried and sieved through a 2 mm sieve. All soil cores were bulked (3.5 kg of soil), and thoroughly mixed with a soil splitter. Unsieved soil was also preserved for aggregate stability determination while the rest of the samples were stored in marked bags. All laboratory soil analysis was performed on air-dried, sieved soil unless otherwise specified.

To avoid determination of litter material and because the most significant differences between cropping systems are expected to appear in the top 10 cm of the soil, the 5-10 cm soil depth in each treatment were mainly used for carrying out different analysis. However, for the lucerne and WBCWBL4 treatments all four depths (0-5, 5-10, 10-20 & 20-30 cm) were analysed.

3.2.5. Quantification of coarse fragments

The quantification of the coarse fragments in these shallow, shale-derived soils was much more complicated to determine compared to soils in other regions. Normally, the amount of coarse fragments in an air-dried soil is quantified by separating the fine fraction (< 2 mm) from the coarse fraction (> 2 mm) using pre-crushing in a large mortar and pestle to break up large aggregates and then sieving the soil through a 2 mm mesh sieve. However, because these soils are shale-derived it is important not to crush the soil samples before sieving as the crushed shale fragments (soft) could have an influence on certain soil chemical and physical analyses. As the soil samples were not crushed prior to sieving, a substantial amount of fine soil (< 2 mm) was not separated as it had adhered to smaller shale fragments to form clods bigger than 2 mm during the drying process. The soil sampling process had to take place when the soil was relatively moist as it was too difficult to sample during the dry season which lead to the formation of clods during drying. This resulted in the coarse fragment percentage to be estimated as high as 80% using only sieving in some of the treatments, which is not realistic for these soils although a high coarse fragment percentage is present. To

correct for this and to make sure that the fine soil (< 2 mm) sample is representative, it was necessary to take a representative subsample of the course fragments and use an ultrasonic bath (UR 1, Retsch Gmbh & CO.KG.,Germany) for ± 3 min to loosen the soil from the coarse fragments in each sample. The soil was then wet sieved through a 2 mm mesh sieve and left to dry in a drying room (35°C). The soil that was recovered from the course fragments was weighed and the coarse fragment percentage was corrected for each soil sample.

3.2.6. General soil characterization

3.2.6.1 Mineralogical composition

The mineralogical composition of the soils was examined using clay separation and X-ray diffraction (XRD) analysis of Ca-saturated samples (Whittig and Allardice, 1986) at 45 kV and 30 mA, using the Cu Kα radiation. The x-ray diffraction (XRD) method is the most widely used technique in the identification of clays but this method is not applicable to analysis of amorphous or non-crystalline materials. Each mineral is characterized by a specific atomic arrangement, creating characteristic atomic planes that can diffract (reflect) x-rays. This diffraction pattern is used as a fingerprint in the identification of mineral species. The clay mineral composition was only determined in two different depth increments (5-10 cm and 20-30 cm) in the lucerne treatment as similar clay mineralogy is expected in the other treatments as the parent material is the same as well as the climatic conditions.

The total free Fe-oxide content of the soils was determined using the citrate-bicarbonate-dithionite (CBD) extraction method according to Jackson et al. (1986).

3.2.6.2. pH

Soil pH was measured in distilled water and in 1M KCl at a 1:2.5 soil to solution ratio (White 1997). The suspensions were shook on a horizontal shaker for 30 minutes and were allowed 30 minutes standing time before the reading was recorded. A calibrated *Metrohm, Swissmade,* 827 *pH lab* electronic pH meter was used.

3.2.6.3. Exchangeable cations

Exchangeable basic cations (Ca, Mg, Na, and K) were determined according to Thomas, (1982), using 100 ml 1M NH_4OAc and 10 g of soil (10:1 ratio). About 40 ml of NH_4OAc solution was added to 10 g of soil allowing standing for an hour after which it was transferred to a 5.5 cm Buchner funnel. The funnel was connected to a suction flask and the soil solution was passed through a Whatman 40 filter paper. Ten ml portions of NH_4OAc were added to

final volume of 90 ml to transfer remaining traces of soil. The extract was then made up to 100 ml by adding fresh NH_4OAc.

Exchangeable acidity was also determined according to Thomas (1982) using 10 grams of soil and 25 ml of 1M KCl. It was left for 30 minutes and then transferred through a funnel fitted with Whatmann 42 filter paper. The filtrate was collected in a 250 ml Erlenmeyer flask as an additional volume of 125 ml KCl followed in 25 ml increments. It was then titrated by 0.01M NaOH until pink endpoint was reached (phenolphthalein used as indicator). Exchangeable Al was estimated by recording the titer for NaOH, adding 10 ml of 1M KF to the filtrate, and titrated with 0.1 M HCl until pink colour disappeared.

3.2.6.4. Total C and N analysis

Dry combustion was used to determine total C & N using a Eurovector Elemental Analyzer (Eurovector Instruments & software, Italy). This method ensures the oxidation of all organic C. It is considered the most accurate method. The Eurovector instrument is capable of simultaneous determination of C, N, H and S in soils (Nelson and Sommers, 1996). Since all the samples tested free of carbonates, the measured total C content was equivalent to the organic C content.

3.2.6.5. Particle size distribution

Particle size distribution was performed on a 40 g soil sample (< 2 mm) from each of the sampled depths for the lucerne (100% pastures) and 100% crop rotation systems (WBCWBL4). For the other treatments, particle size distribution was only done in the 5-10 cm soil depth. The method that was used was adapted from Gee & Bauder (1986).

The samples were chemically pre-treated by removing the organic matter (OM) using 35% by volume H_2O_2 solution. The mass loss after OM was removed was recorded (base mass). The soils tested negatively for carbonates using 10% HCl. Secondly, the soil samples were dispersed by adding 10 ml Calgon solution and mechanically stirring the mixture for 5 minutes. The clay and silt fractions were then washed through a 0.053 mm mesh sieve into a 1 dm^3 sedimentation cylinder. The various fractions were weighed and reported as a percentage of the base soil mass. The silt and clay fractions were determined using the sedimentation technique and a Lowey pipette.

3.2.6.6. Aggregate stability

The wet sieve technique was used to examine the relative stability of soil aggregates of the six different treatments according to Kemper and Rosenau (1986). The stability of aggregates

were determined in each of the four depths for the lucerne and WBCWBL4 treatments while the aggregate stability was only determined in the 5-10 cm depth increment for the other treatments. The principle of this technique relies on the fact that unstable aggregates will break down more easily than stable aggregates when submerged in water. The analysis was carried out under laboratory conditions using the Eijkelkamp E-365-08.13 wet sieving apparatus (Eijkelkamp Agrisearch Equipment, Netherlands). Four grams of macroaggregates (2 mm < aggregates < 2.8 mm) of duplicated samples of each replicate in each treatment were hand-picked to avoid determination of small coarse fragments (Figure 2.5). The aggregates in the sieve were raised and lowered in distilled water for 3 min ± 5 s with these cans containing water unstable aggregates. The remaining aggregates were then raised and lowered in NaOH or $Na(PO_3)_6$, depending on pH of soil sample, for ± 10 min using a rubber until all aggregates disintegrated. These cans contain the water stable aggregates. Both the weighed cans (containing stable and unstable aggregates) were allowed to dry in oven until the water has evaporated. The weight of the material in each can was determined.
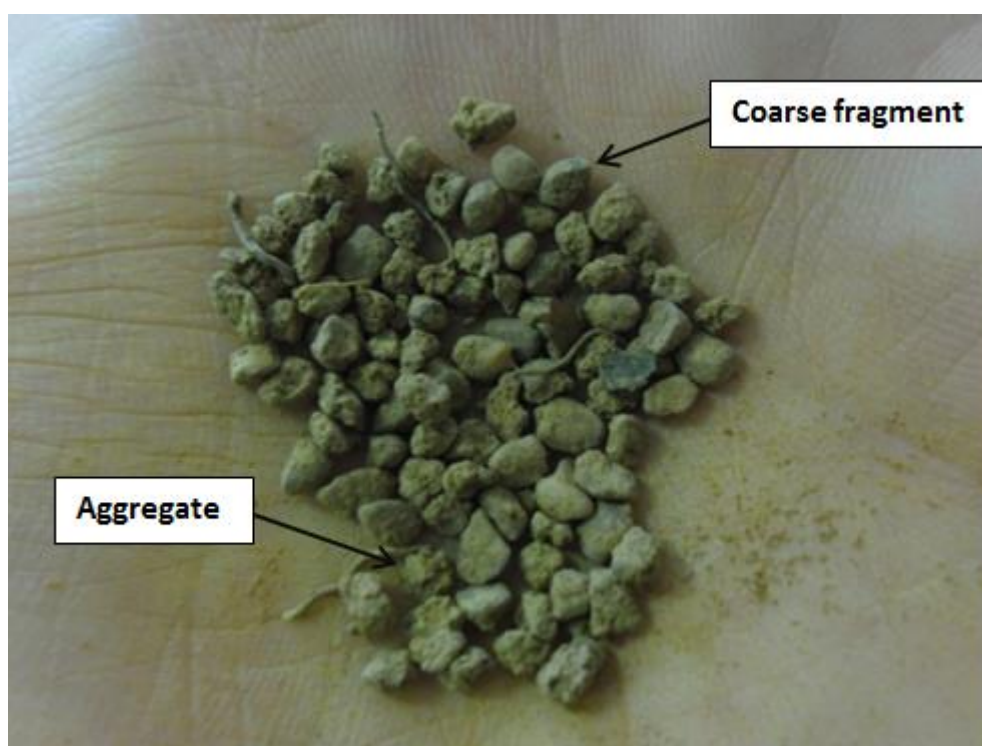
Figure 3.5 Sub-sample of soil (2-2.8 mm) showing visual difference between course fragments and actual soil aggregates. The soil aggregates were hand-picked and used for aggregate stability determination by wet-sieving.

3.2.6.7. Bulk density

Field bulk density was determined using a combination of the clod (Grossman & Reinsch 2002) and core method (Grossman & Reinsch 2002) depending on the suitability of the two methods in the specific treatments and depths. In these soils the clod method was the preferred method due to the extremely high amount of coarse fragments.

The bulk density of the 0-5, 5-10 and 10-20 cm layers in all treatments as well as the 20-30 cm layer of treatment 1 (MMW) and treatment 6 (Natural), both containing a Neocutanic B horizon, were determined using the clod method as it was possible to sample clods in these treatments and depths. Undisturbed clods were thus sampled in each of these depths in the different treatments. To make sure that the bulk density obtained via this method was representative of the whole area, a duplicate sample (clod) in each replicate (A, B & C) in each depth was sampled. The clods were oven dried for 24 h at 105°C and the weight was recorded. The clod was dipped in paraffin wax (70°C) for a few seconds after the clod has been secured with thread. The clod was then let dry before the weight of the clod + wax (air-dried) was recorded after which the sample was weighed again when it was completely suspended in water. The density of the water and paraffin wax was known and used in calculating the density of the clod.

The bulk density of the 20-30 cm layer of the lucerne, MMWW, WBCWBL4, WBCWBL1 treatments were all determined based on the core method describe by Grossman & Reinsch (2002) as it was impossible to sample clods in the Lithocutanic B horizon. The same steel pipe (4 cm diameter) that was used for soil sampling and root quantification was used for this method. The volume of the pipe as well as the dry mass of the soil in the pipe was recorded to determine the bulk density of the soil (g cm^{-3}). The sand cone method (Blake & Hartge, 1986) was initially used to try and determine the bulk density of the Lithocutanic B horizon but this method proved to be inaccurate, possibly due to the soil containing high amount of coarse fragments. It was not always possible to fill the hole perfectly with the sand due to the gaps caused by the coarse fragments (stones) and therefore an alternative method (core method) had to be used to determine the bulk density as accurately as possible.

3.2.7. Above- and belowground C inputs (quantity and quality)

3.2.7.1. Quantity

The root distribution and density (root biomass) in the four depth increments were determined for wheat, medics, barley, canola, lucerne and lupine using the sequential soil

core method (Samson & Sinclair, 1994). Samples were taken just after harvest in November 2012. For this study it was important to know the amount of organic matter restored after a season via the roots of each crop. As the rooting system of each crop differs, it can have different effects on the distribution and stabilization of soil C.

In each replicate (A, B & C) the mass of aboveground material (kg) per square meter of each of these crops was cut off at ground level after harvest and dried for 2 days at 60°C and weighed. Three soil cores were also extracted from each replicate (A, B & C) for each crop using a steel pipe (4 cm in diameter). One core was taken within a row while the other two were taken in-between the rows (Figure 3.6). The pipe was marked at the particular depth increments (0-5; 5-10; 10-20; 20-30 cm). When the chosen depth had been achieved, the pipe was pulled out of the ground and the soil collected in a bag. The volume and the mass of the soil in each core were thus known. The soil was allowed to air dry before weighed. Five representative subsamples (5 g) were extracted from the weighed sample and the roots and coarse sand particles were separated from the fine sand particles, clay and silt using a gentle water stream through a 0.250 mm mesh sieve. The coarse sand particles and roots remaining on the sieve were separated using density fractionation (roots floating in water). The method used was based on root sampling methods used by Crawford et al. (1997), Qin et al. (2004) and Samson & Sinclair (1994).

The average root density (kg m^{-3}) and distribution as well as above-ground inputs (kg ha^{-1}) of each crop in the different treatments were compared.

Figure 3.6 Sequential core method used to determine root density and distribution.

2.2.7.2. Quality

The quality (C and N) of each crop residue above- and belowground were determined with the dry combustion method using the Vector elemental analyser (Nelson & Sommers, 1996). After harvest, each crop was sampled (Figure 3.7). The aboveground (shoots) and belowground (roots) residue were separated and the C and N content of the shoots and roots of all the crops were determined.

Figure 3.7 A wheat sample that was used for carbon and nitrogen analysis.

3.2.8. Soil Respiration Rate (CO_2 efflux)

To characterize the loss of C from the different treatments, the reliable and cost-effective soda-lime method was used to quantify soil respiration (Keith & Wong, 2006). This method is an easy and practical method to use and also allows the determination of cumulative soil respiration over a long period of time. Soda lime granules consist of NaOH and $Ca(OH)_2$ and about 20 % absorbed water as water is necessary for chemical absorption of CO_2 to form Na_2CO_3 and $CaCO_3$ Keith & Wong, 2006). Carbonate formation is reflected in weight gain of granules.

$$2NaOH + CO_2 > Na_2CO_3 + H_20$$
$$Ca(OH)_2 + CO_2 > CaCO_3 + H_20$$
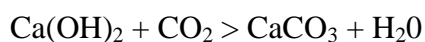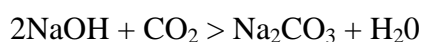
A correction factor (1.69) (Grogan, 1998) was also required to account for the water that is formed when soda-lime reacts with CO_2 and was used when estimating the CO_2 efflux.

Soil respiration was estimated using polyvinylchloride (PVC) chambers (drainage tubes) of approximately 12 cm in diameter. Three chambers (A, B & C) in each treatment were placed in the soil ($\pm$ 4 cm depth) for 3 to 4 weeks during two different climate periods allowing enough time to capture CO_2 loss from the soil (Figure 3.8). The first trial was carried out in

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September 2012 (Spring) while the soil was still very moist due to the winter rainfall period (May – August) and the second trial was carried out in March 2013 (Autumn) while the soil was very dry due to a dry, hot summer period (November-February). The soil and root derived respiration rates between different crop rotation systems and natural vegetated soil in the two different climate periods (spring and autumn) were determined and compared.

Briefly, ± 10 g of soda lime was weighed into a perforated tube (for a ground area within chamber of 0.00841 m^2), fixed with glass wool and then oven dried for 11 h at 100 °C. The dry weight of the soda and perforated tube was recorded and sealed within a zip plastic bag. An aeration tube (outer) filled with an unknown amount soda lime and fixed with glass wool was connected to the sealed lid of the chamber via an inlet tube (Figure 3.8a). This tube helps to prevent CO_2 from the atmosphere entering the chamber. At the field site the perforated tube (inner) (Figure 3.8b) was positioned (hooked) in the chamber for 3-4 weeks. After collection, the perforated tubes were again retained in sealed bags, transported back to the laboratory, oven dried and re-weighed. The difference in weight before and after the period in the field is the amount of CO_2 absorbed. Soil CO_2 efflux is measured in units of g C m^{-2} day^{-1}. During the sample preparation as well as chamber leakage, atmospheric CO_2 can also be absorbed by soda lime which is not part of the soil CO_2 efflux. To assess this, blank chambers with a sealed base, were treated in the same way as the sample chambers in the soil. The application of this method however, will not yield exact measurements, but it will allow one to compare the soil respiration rates among the different treatments (Keith & Wong, 2006).

(a)   (b)

Figure 3.8 (a) Soda lime trap used in the field with an outer to prevent CO_2 from the atmosphere entering the chamber; (b) Perforated tube (inner) hooked inside chamber containing ± 10 g soda lime.

3.2.9. Statistical analysis

Statistical analysis was done using SAS Interprize guide 5.1. Statistical differences between treatments were distinguished at the $P < 0.05$ level using Tukey's Studentized Range test. A one-way ANOVA was used for this completely randomized design.

3.3. Results and Discussion

3.3.1. Background soil information

The highest pH (KCl) values for the cropping systems were observed in the 0-5 cm layer with pH values ranging between 6.4 and 7 in contrary with the natural vegetated soil in the same depth which had a pH of less than 5.5. The pH, exchangeable cation and Fe-oxide values of all the treatments are shown in the appendix (Table A1 & A2). The higher pH in the 0-5 cm depth in the cropping systems is most likely due to lime addition and little soil disturbance. In the 5-10, 10-20 and 20-30 cm layers, the pH for both the cropping systems and natural vegetated soil ranged between 5.5 and 6.6. The soils tested free of carbonates and confirmed

the neutral pH values observed. Due to the neutral pH of these soils, the base saturation percentage ranged between 96 and 99%. It can therefore be concluded that very little Al and H ions occupied the exchange sites of the minerals and organic material. The effective cation exchange capacity (ECEC) ranged between 7 and 12 $cmol_ckg^{-1}$ soil. Taking the clay content into account ($cmol_ckg^{-1}$ clay) the ECEC ranged between 37 and 47. This points to the dominance of low activity minerals e.g., kaolinite (CEC 3-15 $cmol_ckg^{-1}$) or illite (CEC 10-40 $cmol_ckg^{-1}$) as it is difficult to determine the contribution of SOM to the ECEC of the soil. This was confirm by X-Ray diffraction (XRD) analysis as it showed that the clay mineral composition between two depth increments was largely similar comprising of a mixture of mainly kaolinite, illite/mica and quartz (Figure 3.9 & Table 3.4). The Fe-oxide content in the different treatments and depths ranged between 1.6 and 3.2% with the Fe-oxide content significantly related (P = 0.0010; R^2 = 0.50) to the clay content of the soil.
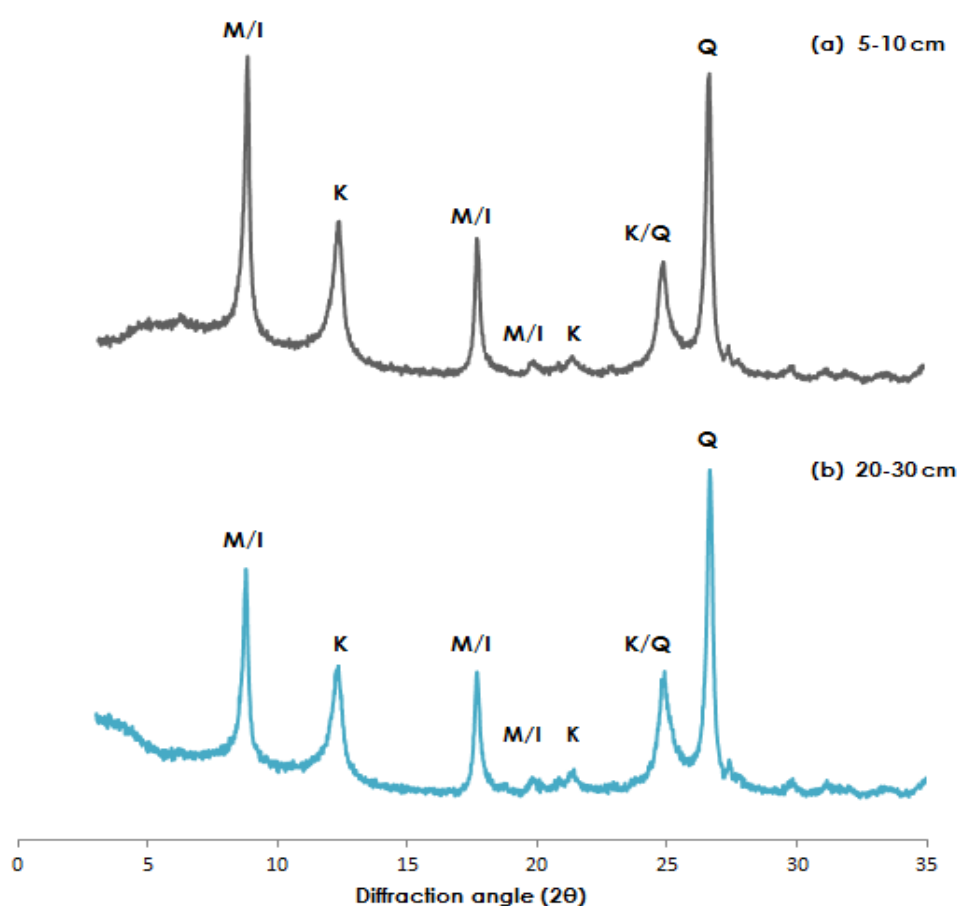
Figure 3.9 Glycol-solvated, Ca-saturated X-Ray diffractograms of selected depth increments, 5-10 and 20-30 cm. M/I = Mica or Illite, K = Kaolinite, Q = Quartz.

Table 3.4 Descriptive legend for the XRD-identified mineral peaks according to Tan (2011) and Whittig & Allardice (1986).

Diffraction angle (2θ)	d-spacing (Å)	Mineral
8.8	10.04	Mica and Illite
12.3	7.19	Kaolinite
17.6	5.01	Mica
19.7	4.5	Illite, Mica
21.1	4.21	Kaolinite/Quartz
24.8	3.59	Kaolinite
26.6	3.35	Quartz

3.3.2. Total (organic) Carbon

The total organic C contents of the bulk soil show typical values for agricultural soils with the lowest C content of 8.6 g C kg^{-1} found in the 20-30 cm depth of the WBCWBL4 cropping system and highest of 28.3 g C kg^{-1} in the 0-5 cm layer of the MMWW cropping system (Figure 3.10a). In a semi-arid climate, only small differences in SOC are expected between different rotation systems as annual C inputs are small and variable (Shrestha et al., 2013).
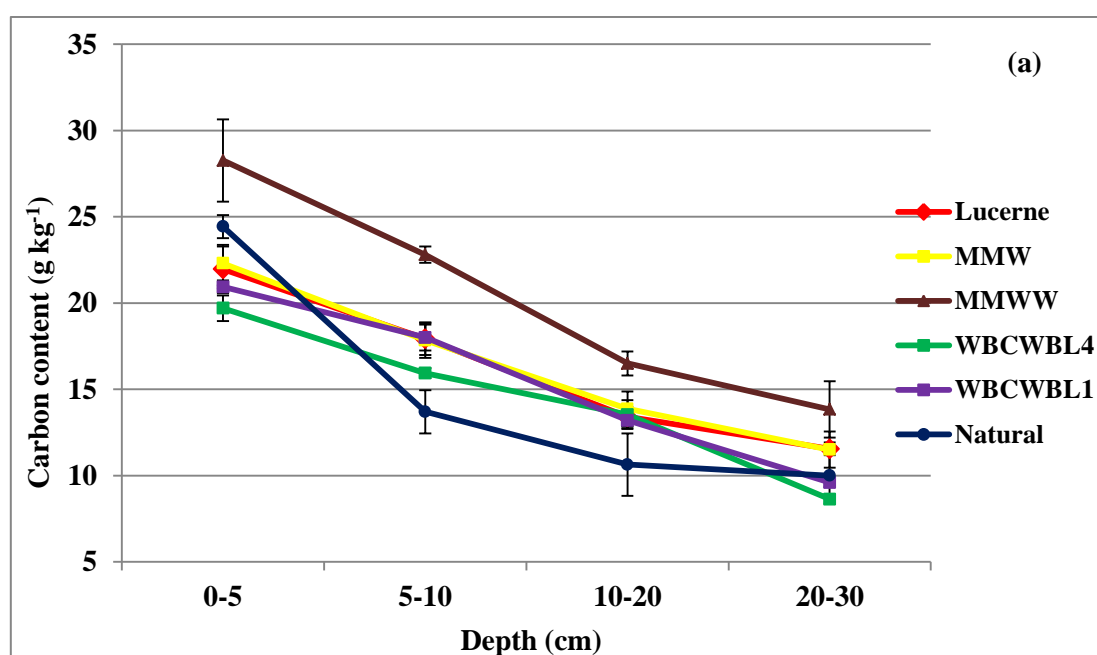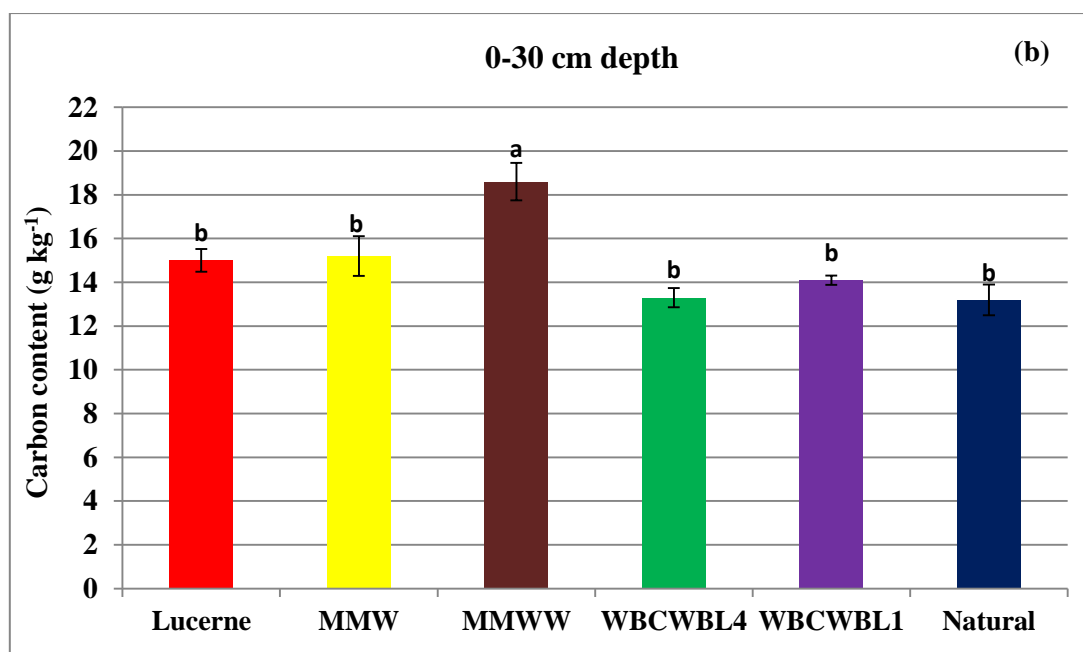
Figure 3.10 (a) Vertical distribution of total soil C between treatments; (b) average C content between treatments in the 0-30 cm depth.

Note: Error bars represent standard error, and alphabetic letters denote statistical differences between treatments according to Tukey's Studentized Range test at α = 0.05. Similar letters indicate lack of significant differences.

The pasture-crop and permanent pasture treatments resulted in higher soil C contents than the crop only or natural vegetated soils (Figure 3.10a & b). The soil C content of the MMWW treatment was found to be significantly higher (P = 0.0008) than all the other cultivated systems and the natural vegetated soil in the 0-30 cm depth, although not statistical different in each depth increment, but clear trends were observed (Figure 3.10a & b). The weighted average C content of the MMWW rotation system in the 0-30 cm depth was 18.6 g C kg^{-1} while the MMW rotation system and lucerne treatments contained an average C content of 15.2 g C kg^{-1} and 15 g C kg^{-1}, respectively, in the 0-30 cm depth. The two 100% crop rotation systems had the lowest C content of the cultivated soils with the WBCWBL1 and WBCWBL4 treatments containing an average of 14.1 g C kg^{-1} and 13.3 g C kg^{-1}, respectively, in the 0-30 cm depth. Compared to the natural vegetated soil (13.2 g C kg^{-1}), a higher C content was observed in all of the crop treatments.

In the 0-5 cm depth, the MMWW treatment had a significantly higher (P = 0.0061) C content than the lucerne, WBCWBL4 and WBCWBL1 treatments with values of 28.3 ± 2.4 vs. 22.0 ± 1.4, 19.7 ± 0.7 and 20.9 ± 0.1 g C kg^{-1}, respectively. The MMW treatment (22.3 ± 1 g C kg^{-1}) and natural vegetated soil (24.4 ± 0.7 g C kg^{-1}) did not differ significantly from the MMWW treatment. For the WBCWBL4 treatment, 30% less C was found than in the

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MMWW treatment in the 0-5 cm depth. The C content of the MMWW treatment (22.8 ± 0.8 g C kg^{-1}) in the 5-10 cm depth differed significantly (P = 0.0003) from all the other treatments in the same depth (Figure 3.10a). A significant decrease in C content for the natural vegetated soil was observed in this depth with 13.7 ± 1.3 vs. the 24.4 ± 0.7 g C kg^{-1} found in the 0-5 cm depth. The lucerne, MMW and WBCWBL1 treatments had similar C values in the 5-10 cm depth with 17.7 ± 1.1, 17.8 ± 1, 18 ± 0.8 g C kg^{-1} respectively. The WBCWBL4 treatment had the lowest C content of 15.9 ± 2.9 g C kg^{-1}. The 5-10 cm depth was the most important in terms of C accumulation in the soil, within the 0-30 cm depth evaluated in the study. In the 10-20 cm layers no significant differences were found between the treatments although the MMWW treatment still contained more C in this depth than the other treatments. The average C content between the treatments in the 10-20 cm depth was 13.5 g C kg^{-1}. The C content of the MMWW treatment (13.8 g C kg^{-1}) was significantly (P = 0.0368) higher than the WBCWBL4 treatment (8.6 g C kg^{-1}) and also higher than the other treatments in the 20-30 cm layer.

In all the treatments a general decline in total soil C with depth was observed with the most significant differences in C content between the cropping systems restricted to the 0-10 cm depth (Figure 3.10a). An average decline of 53% in C content in the different treatments was observed between depths 0-5 and 20-30 cm. The general decrease in SOC observed with depth can be explained by the fact that aboveground crop residues remain on the surface (0-10 cm) in no-till systems while the root systems of the different crops are also mostly concentrated at the 0-10 cm depth. In the 20-30 cm depth smaller differences in SOC between treatments were observed. The top 10 cm of the cultivated treatments is thus most greatly influenced by tillage, plant roots and aboveground crop residues. The severe decrease in SOC from the 0-5 to 5-10 cm depth in the natural vegetated soil can most likely be attributed to the shallow rooting system of the grasses that dominates the natural vegetated soil and also to the absence of cultivation that causes aboveground residues to concentrate in the litter layer (0-5 cm).

In light of other studies carried out in similar climate conditions, the C content in these soils is relatively high. In a wheat monoculture (no-tillage) Chen et al. (2009) found an average C content of 9.1 g C kg^{-1} after 11 years in the 0-30 cm depth; on the Canadian prairies in a wheat-oilseed-pulse (WOP) rotation system under no-tillage, Shrestha et al. (2013) found an average C content of 9.4 g C kg^{-1} (0-15 cm depth); Plaza-Bonilla et al. (2013) estimated an average C content of 14.7 kg C Mg^{-1} in the 0-30 cm depth after 11 years of no-till in a winter

cereal rotation and after 12 years Masri & Ryan (2006) also found that wheat/medic rotation increased soil organic matter in the 0-20 cm depth (13.8 g C kg^{-1} vs. 10.9-11 g C kg^{-1} for continuous wheat and wheat/fallow).

The total soil C values were also determined in 2003 for each of the treatments by the Western Cape Department of Agriculture which allowed us to determine the rate at which C increased (0-20 cm depth) in each rotation system (Figure 3.11). These are only approximate rates as the Walkley-Black method was used to determine C content in 2003 which probably underestimated the C value while the dry combustion method was used in 2012. These rates however will still provide a good indication of the effect of different rotation systems on C storage. After 8 years (2003-2011) the two crop-pasture rotation systems showed a much higher rate of soil C increase as the C content in the MMW rotation system increased by 0.79 g C kg^{-1}year^{-1} and the MMWW by 0.70 g C kg^{-1}year^{-1}. The C content in the WBCWBL4 and WBCWBL1 treatments only increased by 0.25 g C kg^{-1}year^{-1} and 0.4 g C kg^{-1}year^{-1} respectively while the C content in the lucerne treatment increased by 0.38 g C kg^{-1}year^{-1}. The rate at which C increases per year is important to consider when interpreting the long-term effect of different crop rotation systems. These data show the effect of crop type as well as the effect of disturbance on soil C content as all the treatments have been subjected to the same climate conditions and similar soil conditions, yet the C increase was different in the different treatments. To effectively increase the SOC content, the rate of input has to exceed the rate of loss from decomposition and leaching and in this study is best achieved by rotating crops with pasture. A dynamic equilibrium C value will however be achieved after a while, specific to the farming system, soil type and climate (Johnstone et al., 2009).
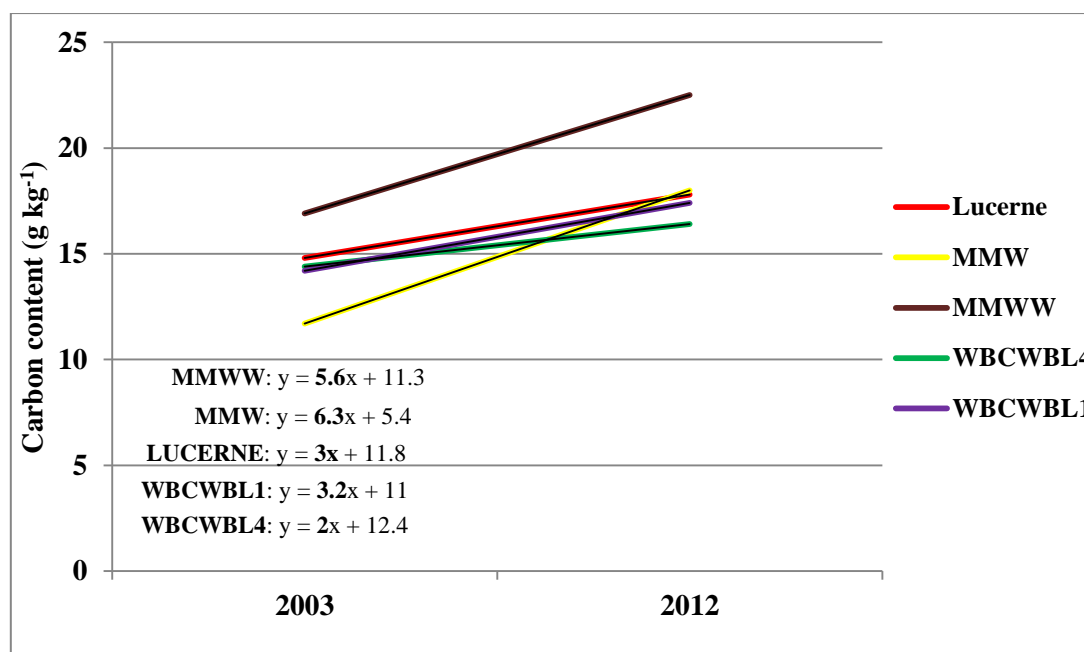
Figure 3.11 Change in C content observed in the different treatments from 2003-2012.

The differences in C content as well as the rate at which C increased per year in the different crop rotation systems, could be attributed to differences in C input (above- and belowground), the quality of C input (C:N ratio) as well as to differences in soil texture and aggregate stability, the latter influenced by soil disturbance of the no-till planter. At this stage however it was only speculation and it was therefore necessary to perform certain plant and soil analysis to try and define the differences obtained in soil C content between the different treatments.

3.3.3. Bulk density and carbon stocks

In order to be able to accurately measure soil C stocks, soil bulk density (g cm^{-3}) needed to be determined. Soil C stocks (Mg ha^{-1}) are a measure of the C content of soil mass per given area. The methods that were used to determine the soil bulk density included both the coarse (> 2mm) and the fine fraction (< 2mm). Due to the fact that C is mainly stored in the fine fraction, the density of the fine fraction had to be used for this calculation and therefore the density obtained for the bulk soil had to be corrected for the coarse fragment percentage (Table 3.5). Significant differences in C stock obtained using the density of the bulk soil and fine fraction is shown in Figure 3.13. The density of the fine fraction was much lower in each depth and treatment indicating the effect of coarse fragments (section 3.3.5.3) on soil density as coarse fragments consists of a different volume and mass than fine soil. The lucerne, MMW and natural vegetated treatments displayed a higher fine fraction density in the top 10

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cm, relative to the MMWW, WBCWBL4 and WBCWBL1 treatments. This could be attributed to less disturbances of the soil with no-till planter and also due to extensive grazing, leading to a higher density of the fine fraction through compaction. In the continuous cropping systems, the diverse rooting systems of the different crops can also cause lower soil compaction.

Table 3.5 The density of both the bulk soil and fine fraction (< 2mm) obtained for each depth in the different treatments.

Soil density (g cm^{-3})

Treatment	Depth (cm)							
	0-5		5-10		10-20		20-30	
	Bulk soil	Fine fraction	Bulk soil	Fine fraction	Bulk soil	Fine fraction	Bulk soil	Fine fraction
Lucerne	1.56 *bc*	**1.41** *a*	1.69 *a*	**1.47** *a*	1.65 *a*	**1.48** *ab*	1.70 a	**1.36** *a*
MMW	1.63 *ab*	**1.45** *ab*	1.70 *a*	**1.52** *a*	1.83 *a*	**1.62** *a*	1.80 a	**1.59** *a*
MMWW	1.63 *ab*	**1.33** *b*	1.64 *a*	**1.35** *a*	1.71 *a*	**1.39** *b*	1.70 a	**1.32** *a*
WBCWBL 4	1.60 *ab*	**1.30** *b*	1.63 *a*	**1.32** *a*	1.68 *a*	**1.46** *ab*	1.73 a	**1.35** *a*
WBCWBL 1	1.65 *a*	**1.38** *ab*	1.64 *a*	**1.38** *a*	1.67 *a*	**1.44** *ab*	1.73 a	**1.35** *a*
Natural	1.48 *c*	**1.39** *ab*	1.63 *a*	**1.52** *a*	1.71 *a*	**1.63** *a*	1.78 a	**1.64** *a*

Note1: The term **bulk soil** refers to the density obtained including both the fine fraction (< 2mm) and the coarse fraction (> 2mm) while the term **fine fraction** refers to the density obtained of only the fine fraction (< 2mm), therefore excluding coarse fragments (> 2mm).

Note2: The alphabetic letters denote statistical differences between treatments at specific depth according to Tukey's Studentized Range test at $\alpha = 0.05$. Similar letters indicate lack of significant differences.

The C stock (Mg ha^{-1}) values were obtained for the different treatments in each depth using the C content (g kg^{-1}) and both the bulk soil and fine fraction density (g cm^{-3}) for the calculation (Figure 3.12a & b). Although coarse fragments are part of the soil, they have a major effect on C stock values as the presence of coarse fragments dilutes the amount of C stored in a hectare of soil significantly (Figure 3.13). In each depth and treatment the C stocks were lower when density of bulk soil was used rather than density of fine fraction only; indicating the effect coarse fragments on soil C stocks. Therefore, to be able to accurately compare the effect of different rotation systems and natural vegetated soil on soil C sequestration (which is limited to the fine soil fraction), the effect of coarse fragment percentages in the different treatments had to be excluded (Figure 3.12b).

Figure 3.12b shows that the same trend that was observed for total C content (Figure 3.10a) was also observed for C stock in the different treatments. The most significant differences in C stock was found in the 0-5 and 5-10 cm depth increments between the MMWW crop-pasture system and the WBCWBL4 & WBCWBL1 cropping systems (P = 0.0074 and 0.0002 respectively). In the 0-5 cm depth the MMWW contained 18.7 ± 1.4 Mg C ha^{-1} vs. the 12.8 ± 0.5 and 14.5 ± 0.6 Mg C ha^{-1} of the WBCWBL4 and WBCWBL1 systems, respectively. In the 5-10 cm depth the MMWW contained 15.4 ± 0.4 Mg C ha^{-1} vs. the 10.5 ± 0.2 and 12.4 ± 0.3 Mg C ha^{-1} of the WBCWBL4 and WBCWBL1 systems, respectively. The C stock of the natural vegetated soil (16.8 ± 0.7 Mg C ha^{-1}) was similar than the MMWW system in the 0-5 cm layer but was significantly (P = 0.0002) lower (10.3 ± 0.8 Mg C ha^{-1}) in the 5-10 cm layer. The C stock values for the lucerne and MMW treatments was similar in the 0-5 (16.1 ± 0.7 vs. 15.4 ± 0.7 Mg C ha^{-1}) and 5-10 cm depth (13.6 ± 0.7 vs. 12.7 ± 0.4 Mg C ha^{-1}).
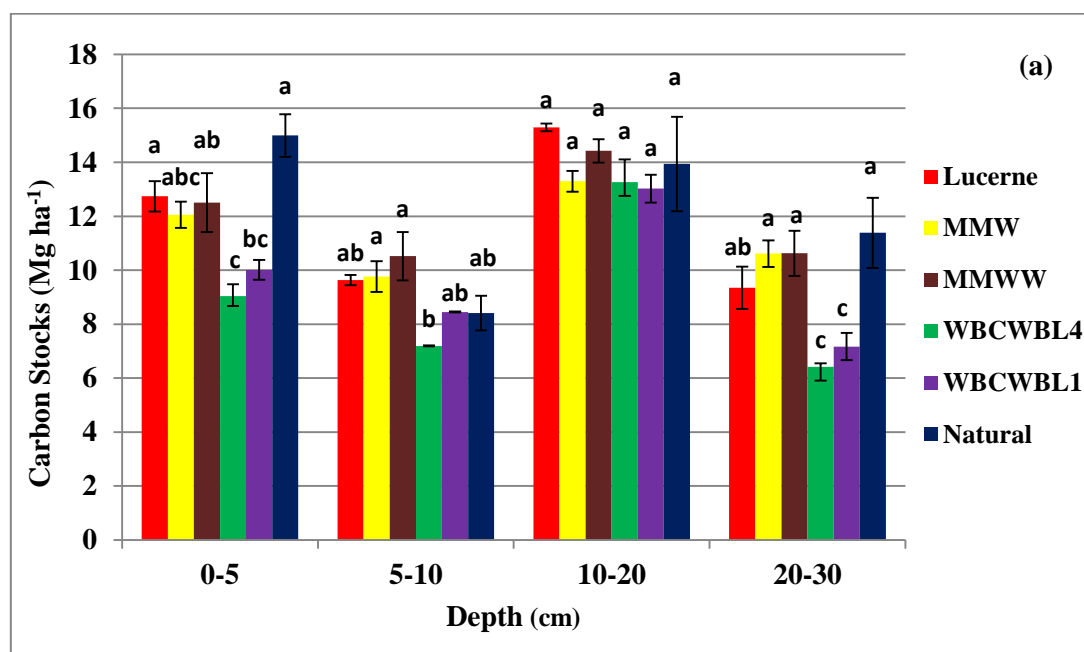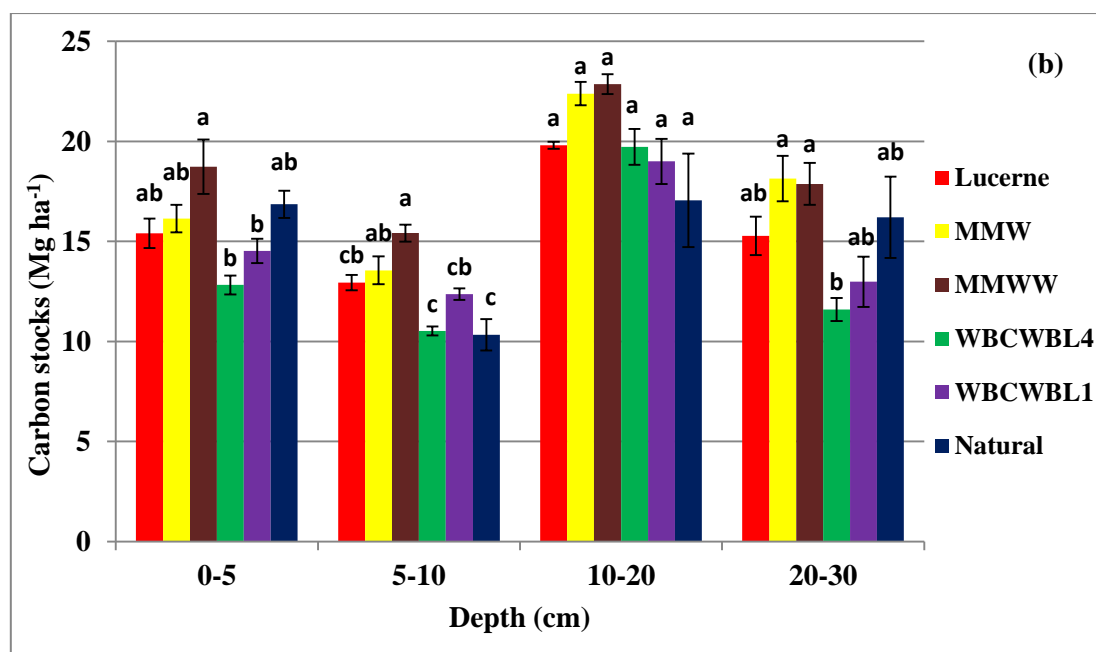
Figure 3.12 Distribution of C stocks obtained in the four depth increments (0-5, 5-10, 10-20, 20-30 cm) of each treatment for (a) bulk soil and (b) fine fraction (< 2mm).

Note1: Error bars represent standard error, and alphabetic letters denote statistical differences between treatments at specific depth according to Tukey's Studentized Range test at $\alpha = 0.05$. Similar letters indicate lack of significant differences.

Note2: The term **bulk soil** (coarse + fine fraction) refers to the carbon stocks obtained including the coarse fragments while the term **fine fraction** (< 2mm) refers to carbon stocks obtained excluding the effect of coarse fragments

The influence of cropping system was not restricted to a certain layer, but was extended in the 0-30 cm depth as is shown in Figure 3.13. This figure also shows the effect of coarse fragments on C stock values obtained between the different treatments in the 0-30 cm depth. The C stocks obtained for the bulk soil was significantly ($P \leq 0.0001$) lower than the C stocks obtained for the fine fraction in each treatment in the top 30 cm. To compare the effect of the crop/pasture treatments on soil C sequestration, the C stocks of fine fraction was used. The C stocks (0-30 cm depth) of the MMWW treatment (74.7 ± 2.5 Mg ha^{-1}) was significantly ($P = 0.0003$) higher than the WBCWBL4, WBCWBL1, lucerne and natural vegetated soil with C stock values of 54.7 ± 1.5, 58.9 ± 2.4, 63.4 ± 1.4 and 60.5 ± 2.4 Mg ha^{-1} respectively. The C stocks of the MMW treatment (70.2 ± 2.7 Mg ha^{-1}) was after the MMWW treatment the second highest of all the treatments. Contrasting the two extreme cropping systems under no-tillage, MMWW and WBCWBL4, the difference in C stock was 20 Mg ha$^{-1.}$ This is higher than some of the extreme differences found in several studies (Conceicão et al., 2013; Freixo et al., 2002) between conventional and no-tillage. This demonstrates the importance of

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cropping systems on C sequestration, especially in a semi-arid region. Both the crop-pasture systems contained more C than the natural vegetated soil, although only the MMWW treatment was significantly higher. These results suggests that the current crop-pasture rotation systems (MMWW and MMW) do enhance C sequestration compared to the natural vegetated soil while the 100% crop rotation systems compared well with the natural vegetated soil. The MMWW and MMW systems are thus more effective for C sequestration than the 100% crop rotation system and are therefore an important strategy to increase the quality and sustainability of the soil through a higher C content.
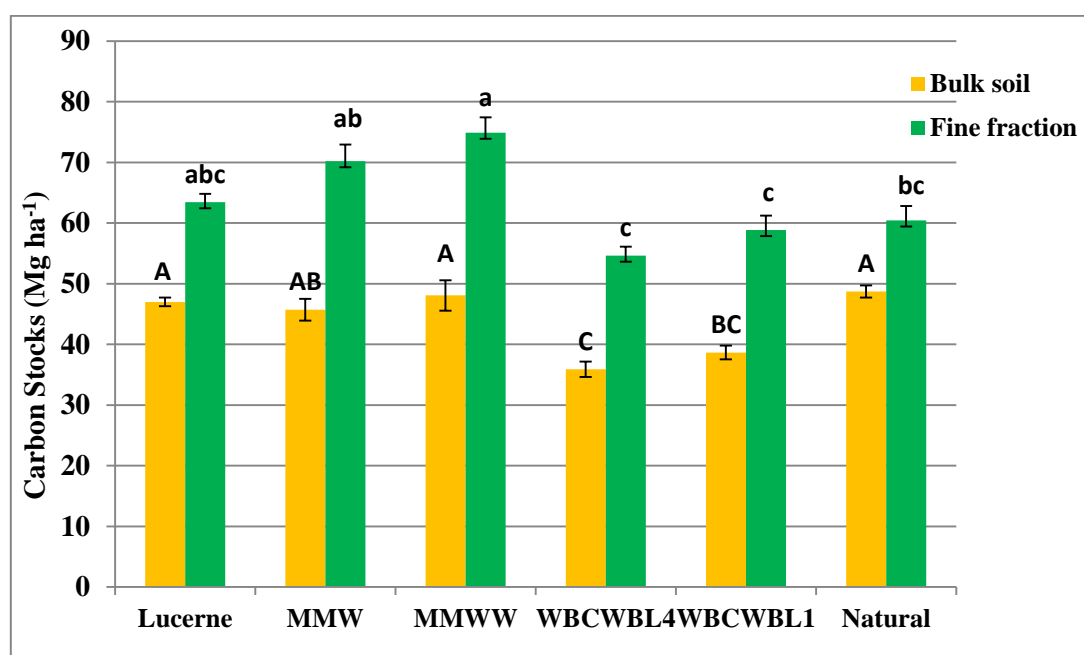
Figure 3.13 Total C stocks obtained for both the bulk soil and fine fraction of the different treatments in the 0-30 cm depth.

Note1: The **bulk soil** (coarse + fine fraction) refers to the C stocks obtained including the effect of coarse fragments while the **fine fraction** (< 2mm) refers to C stocks obtained excluding the effect of coarse fragments.

Note2: Error bars represent standard error, and alphabetic letters denote statistical differences between treatments in the bulk soil (Capital letters) and fine fraction (lower case letters) according to Tukey's Studentized Range test at $\alpha = 0.05$. Similar letters indicate lack of significant differences.

In light of other studies; Sombrero & Benito (2010) estimated 52.19 Mg ha^{-1} SOC for a cereal-legume rotation under no-tillage to a depth of 30 cm in a semi-arid area of Castile-Leon, Spain; also in semi-arid region (Mediterranean climate) Alvaro-Fuentos et al. (2012) estimated a C stock of 37.7 (0-30 cm depth) after 11 years of no-till application with barley and wheat in rotation; Jobbagy & Jackson (2000) estimated 62 Mg ha^{-1} SOC for a desert

ecosystem to a depth of 1 meter; Conceição et al. (2013) estimated 37.3 Mg ha^{-1} SOC in the 0-20 cm depth for legume based crop rotation system under no-tillage in the southern region of Brazil; Freixo et al. (2002) estimated 68.5 Mg ha^{-1} SOC in no-till wheat /soybean rotation in 0-30 cm depth vs. 76.2 Mg ha^{-1} SOC in forest, both situated in Brazil with a yearly precipitation of 1746 mm. The C stock values (bulk soil) obtained in this study compared well with other studies carried out in similar climate conditions.

3.3.4. Plant Properties

3.3.4.1. Quantity of Crop Residues

In agricultural soils, the SOM content is derived from C inputs in the form of crop residues on soil surface, root turnover during crop growth and from root material left in the soil after harvest (Wood et al., 1990; Haynes, 2005). A major input of C comes at the end of the growing season as the plants mature and therefore above- and belowground quantities of each crop was determined after harvest (Crawford et al., 1997). This will give an idea of the amount of C input of each crop and how each crop contributes in a specific cropping sequence. However, because the turnover (death and decomposition) of roots was not accounted for during the season in this study, the values obtained are only giving some indication of the belowground biomass of each crop.

Aboveground

No significant aboveground biomass differences were found between the different crops but clear trends were observed (Figure 3.14). Wheat added the most aboveground biomass to the soil (4400 kg ha^{-1}) with barley and lupin adding similar amounts ($\pm$ 4000 kg ha^{-1}) to the soil. The two pasture crops, medics and lucerne, added the least aboveground biomass ($\pm$ 2600 kg ha^{-1}). The low aboveground biomass input of these pastures is due to constant grazing during the year and also because the samples were taken mid-November (summer), which means it is after the growing season of medics as the medics are dying-off at this time of year. The reason for taking the samples at this late stage is because harvesting of the other crops only occurs in November and to make it possible to compare the different biomass inputs of the different crops, samples had to be taken at this time. The main interest was the amount of biomass input of each crop after harvest and the amount it adds to the soil before the next season which starts in May the following year. Sampling the medics earlier in the year (September) when these crops are growing vigorously and has the highest aboveground biomass, estimation of the total aboveground biomass will be incorrect. Medics are

constantly grazed thereafter and will affect the total biomass input to the soil. The aboveground biomass values (kg ha^{-1}) of canola, lupine, barley and wheat obtained after harvest in this study in 2012 correlates well with values obtained in the previous years (2002-2011) which was determined by Mr. Willie Langenhoven (Farm Technician of the Crop Rotation trials at Tygerhoek Research Farm). No correlation could be made between the pasture crops as lucerne biomass were not determined whereas medics biomass were determined but at a different time of the year. To compare aboveground biomass values to other studies is difficult as yields are dependent on both the climate and soil. In return it directly affects the amount of aboveground biomass added to the soil after harvest. The main focus was thus to compare the C input through aboveground residues between the different crops.
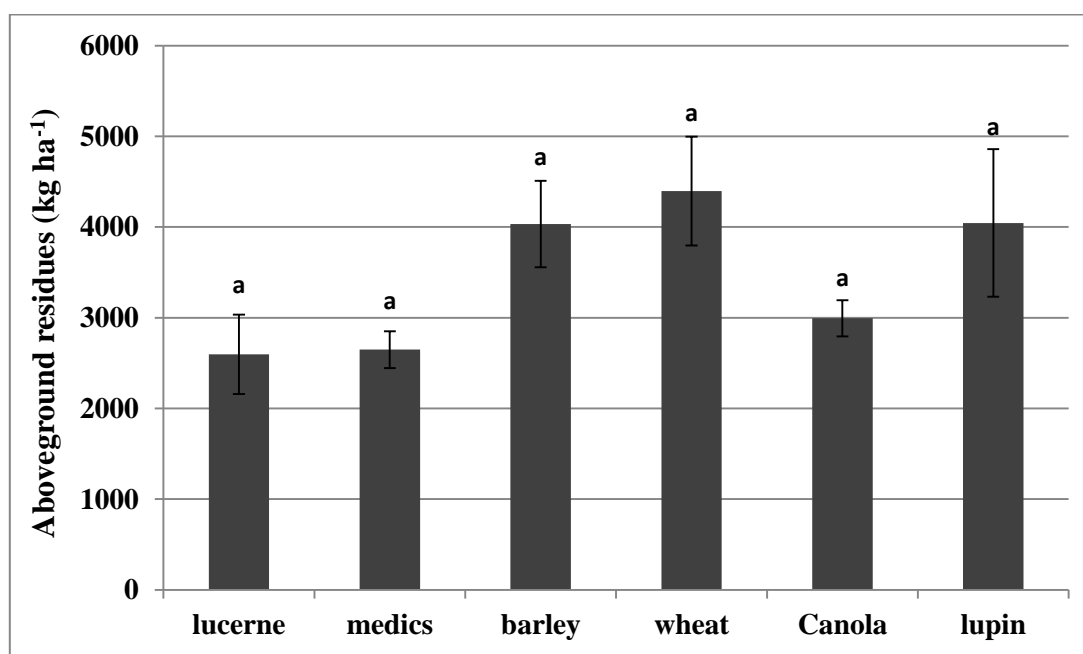
Figure 3.14 Aboveground biomass production for the different crops.

Note: Error bars represent standard error, and alphabetic letters denote statistical differences between crops according to Tukey's Studentized Range test at α = 0.05. Similar letters indicate lack of significant differences.

Both the MMWW and MMW treatments had higher soil C contents than the 100% crop rotation systems (WBCWBL1 & WBCWBL4) although the aboveground biomass of medics was very low compared to the crops in the 100% crop rotation system. This shows that the quantity of aboveground biomass may not be as important as the belowground input (Rasse et

al. (2005). However, the low aboveground biomass of lucerne corresponds well with the low rate of C increase per year for the lucerne treatment.

Belowground

The pattern of root development varied with soil depth and crop type (Figure 3.15 & 3.16). Lucerne had a significantly higher root biomass in each of the four sampling depths compared to the other crops, especially at 5-10 and 10-20 cm (Figure 3.15). The medic roots were concentrated in the 0-10 cm depth (65% of total medic root biomass). This corresponds well with Crawford et al. (1997) who also found in their study that 50-60% of the medic roots were concentrated in the top 10 cm. From these results it is concluded that medics consists of a shallow rooting system. In the 5-10 cm depth, except for lucerne, medics consisted of a much higher root density compared to the other crops which can therefore affect the SOM content of the MMWW and MMW treatments in this depth significantly. Rasmussen et al. (1989) also stated that medic has an extensive root system. It therefore contributes to soil organic matter in the root zone rather than through aboveground inputs.

The root development pattern for barley and wheat were similar in the 0-10 cm depth with lupine and canola having the same root distribution pattern in the 0-10 cm depth as both these crops consists of taproot systems (Figure 3.16). In the 10-30 cm depth, wheat had a higher root density than the barley with the root density of canola and lupin still very similar in this depth. On average, lucerne and medics had the highest root biomass density in the 0-30 cm depth. This means that these two crops added the most belowground biomass to the soil while barley added the least. Wheat, canola and lupin had a similar root biomass density in the 0-30 cm depth.
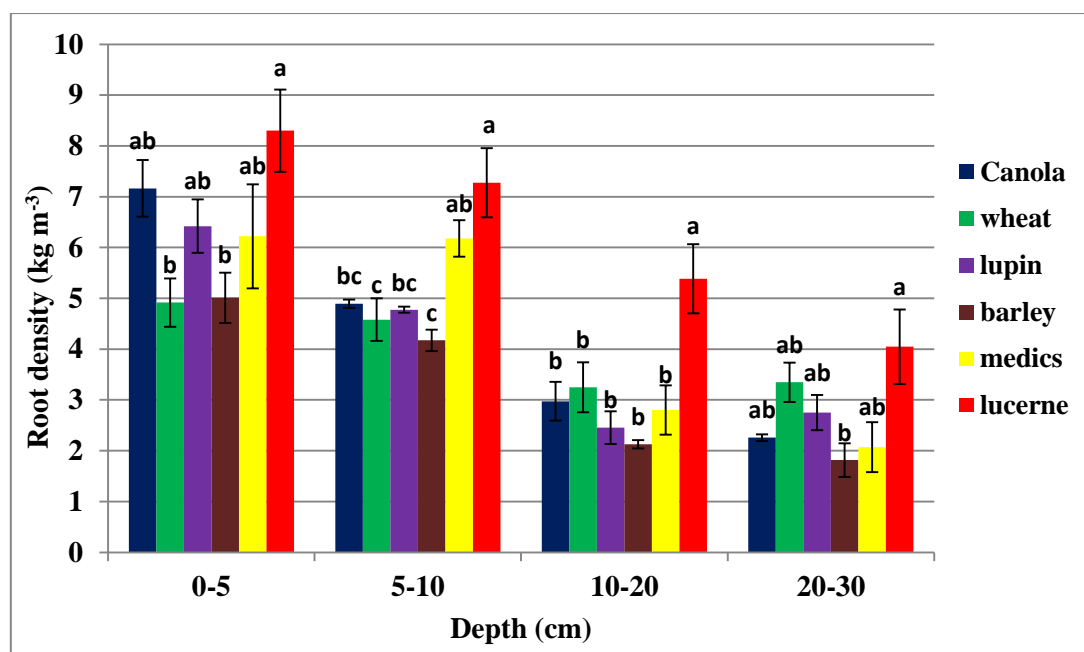
Figure 3.15 Root density and the pattern of root distribution within the four sampling depths of each crop.

Note: Error bars represent standard error, and alphabetic letters denote statistical differences between crops at specific depth according to Tukey's Studentized Range test at α = 0.05. Similar letters indicate lack of significant differences.

Crop roots are not only important for productivity by providing water and nutrients to the plant; it is also an important source of C input. However, to measure the actual crop root density is quite difficult, especially in these shallow soils with high coarse fragment percentage. Belowground inputs through roots represent a major input into the SOC pool. Rasse et al. (2005) stated that root-derived C contributes nearly twice as much C to stable SOM pools as aboveground residues. Despite the importance of belowground input of C through roots, it is quantitatively the least understood (Van Vleck & King, 2011). Because conservation of SOM is considered to be essential for sustainable agricultural activities, it was important to get a good idea of the amount of C input possible from each crop belowground (Crawford et al., 1997). This information can help farmers design appropriate crop rotation systems where crop species with different rooting systems can be arranged in the correct sequence (Gan et al., 2009). At the end of a growing season, roots are likely to represent the single largest pool of legume N for mineralization.

The greater belowground input of organic matter through fine roots can somewhat explain why the SOC content is higher in the crop-pasture rotations (MMW & MMWW) compared to continuous cropping (WBCWBL1 & WBCWBL4). Both the MMWW and MMW treatments

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showed the highest rate of C increase in 8 years which can partly be attributed to the higher fine root biomass of medics. Saying this, one would expect that the lucerne would have a much higher rate of C increase per year due to its high root biomass (higher than medics) but this is not the case as the C content of the lucerne only increased by 0.38 g C kg^{-1}year^{-1}. A possible reason for this is that medic (annual pasture) is able to re-establish itself (re-germinate) and therefore leads to the formation of new roots again (i.e. higher C inputs) in the following season. Lucerne on the other hand is perennial pasture specie which doesn't grow actively in winter and usually only becomes dormant. The roots therefore don't grow actively but only sustains the plant leading to lower belowground biomass input to the soil compared to the medics. There are also other factors playing a role in soil C dynamics e.g. rotation effects leading to different belowground inputs compared to continuous lucerne residue inputs. It has been stated by Wright & Hons (2005) that the amount of C stored in the soil doesn't only depend on the quantity of inputs but also on the quality (C:N ratio).

Figure 3.16 Digital images of the root systems of different crops; (a) canola; (b) lupin; (c) wheat; (d) barley.

3.3.4.2. Quality of Crop Residues

No significant differences in C content (39-44%) between the different crop residues both above- and belowground were found (Figure 3.17a). The lucerne and medics which are both legumes supply the most N through fixation as both of these crops had significantly higher (P ≤ 0.0001) N content compared to the other crops (Figure 3.17b). Lucerne shoots and roots had N contents of 3.96% and 2.08% respectively while the medic shoots and roots had a N content of 2.48% and 1.83% respectively. Lupin is also a legume but had a much lower N content both above- and belowground (0.61 & 1%). Canola and wheat (non-legumes) had the lowest N content both above- and belowground with values of 0.26 and 0.39% and 0.3 and 0.6% respectively. Interestingly, compared to wheat, barley had a high N content, especially aboveground with a N value of 0.57% vs. the 0.3% of wheat.

Due to the very high N content of the lucerne and medic aboveground residues, these crops had a significantly (P < 0.0001) lower C:N ratio (11 & 19 respectively) compared to the other crops. The C:N ratios of barley (73) and lupin aboveground residues were also significantly lower than wheat (165) and canola (162). Crawford et al. (1997) reported C:N ratio of 15-18 for medic roots which is similar to the C:N ratio of the medics obtained in this study. The C:N ratios of lucerne and medic roots (20 & 26 respectively) were significantly (P < 0.0001) lower than wheat (70) and canola (113) and much lower than barley (58) and lupin (Figure 3.17c). Lupin (also legume but intermediate N) shoots and roots had a C:N ratio of 71 and 49 respectively.
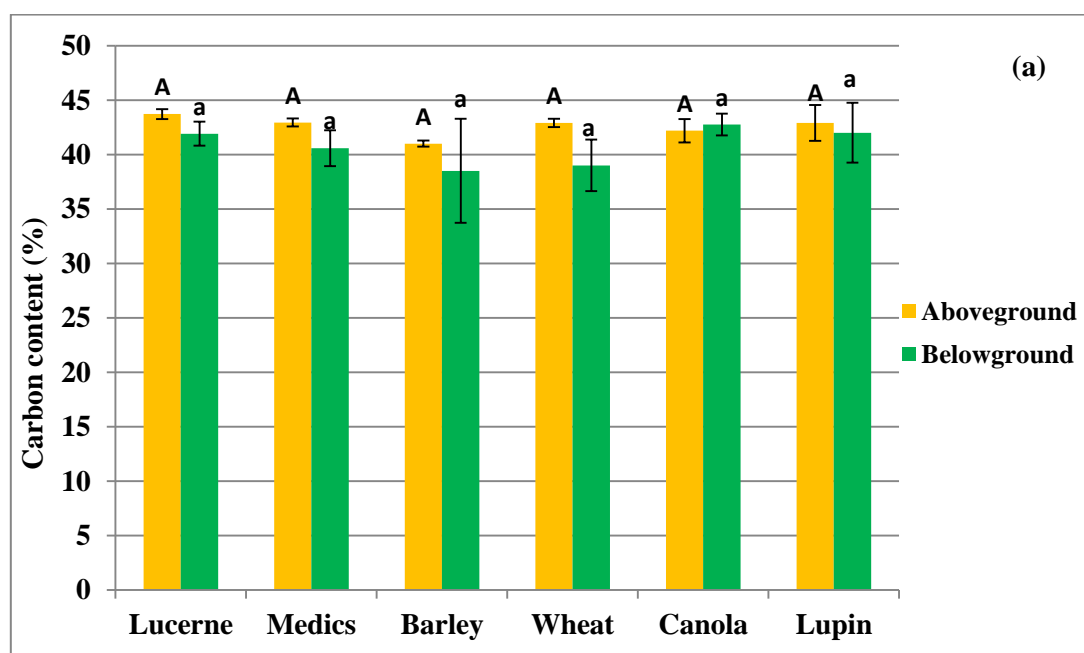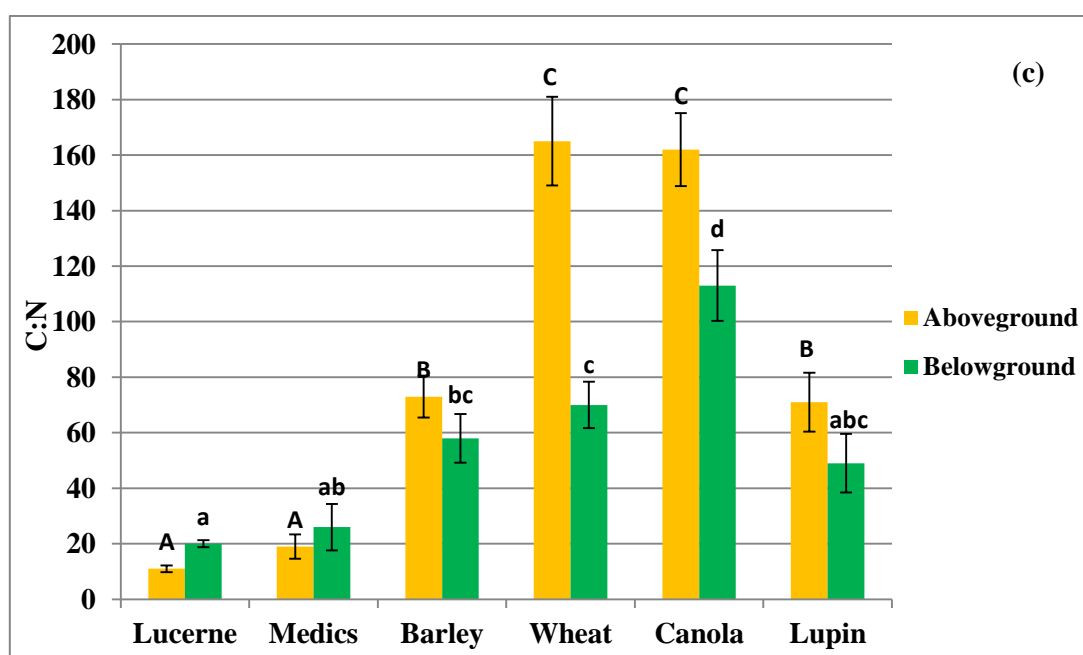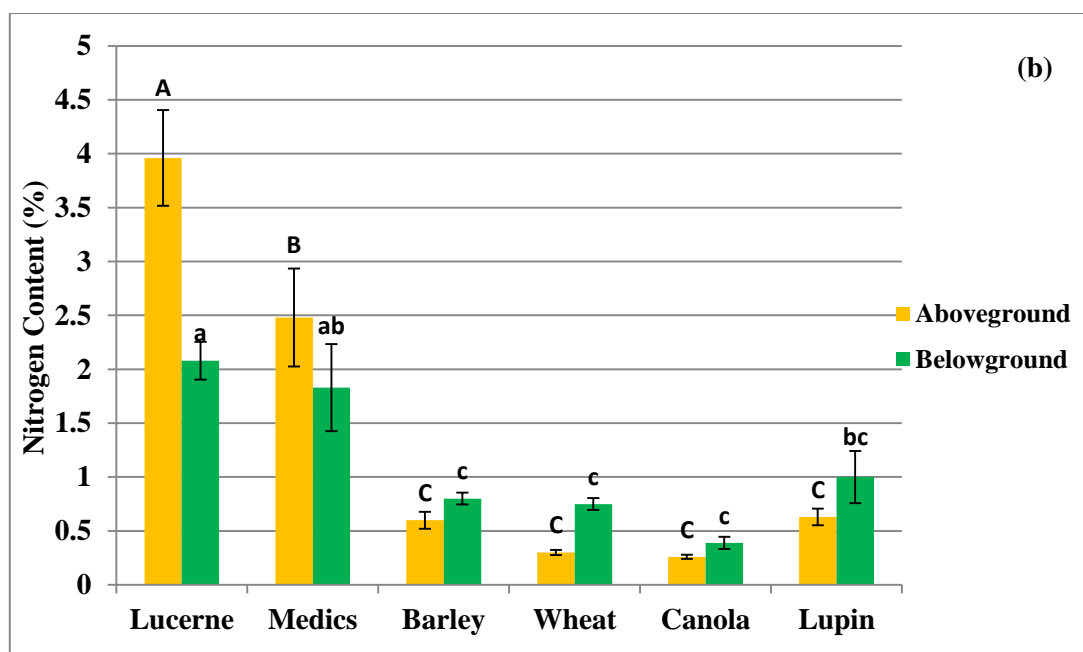
Figure 3.17 (a) The Carbon content of the different crop residues; (b) The Nitrogen content of the different crop residues; (c) The C:N ratio of the different crop residues.

Note: Error bars represent standard error, and alphabetic letters denote statistical differences between crops aboveground (Capital letters) and belowground (lower case letters) according to Tukey's Studentized Range test at α = 0.05. Similar letters indicate lack of significant differences.

The C:N ratios of the roots and shoots gave an indication of the quality of the input of organic material as it partly determines the rate of decomposition (Table 3.6) (Praveen-Kumar et al., 2003). Plants with high C:N ratio decomposes and release nutrients slowly whereas residues

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with low C:N ratio will decompose rapidly and thereby enhance microbial activity and the release of nutrients. It was therefore necessary to determine the C:N ratio of the different crops to be able to predict the rate at which it is expected to decompose. According to Praveen-Kumar et al. (2003), both lucerne and medic residues are moderately to highly decomposable while lupin is only slowly decomposable. Wheat and canola will have a very slow decomposition rate as it is the least decomposable of all the crops. Constant input of material with high C:N ratio can lead to N deficiencies in soil due to microbes competing with plants for N. Lucerne and medics adds a lot of N to the soil through fixation which is important for the subsequent crop (Burle et al., 1997). However, the low C content increase in the lucerne treatment since 2003, can partly be explained by the high susceptibility (very low C:N ratio) of the residues to be decomposed to CO_2.

Table 3.6 Residue quality based on C:N ratio (modified from Praveen-Kumar et al., 2003).

Residue quality	C/N
Highly decomposable	< 18
Moderately decomposable	18-27
Slowly decomposable	28-60
Least decomposable	>60

3.3.5. Soil Properties

3.3.5.1. Particle size distribution

Table 3.7 shows the particle size distribution obtained for the different treatments as well as the texture class at the specific sampling depths. Comparing the 5-10 cm soil depth, significant differences (P = 0.0014) in each of the size fractions were found between the natural vegetated treatment (14% clay) and the different cultivated treatments (21-26% clay). Within these cultivated treatments in the same depth the MMWW treatment contained highest clay content but no significant differences were found as the different size fractions were very similar. There were also no significant differences found between the lucerne and WBCWBL4 treatments in each of the size fractions at 0-5, 10-20 and 20-30 cm soil depths. General increases in clay content in the 20-30 cm soil depth (Lithocutanic B horizon) for both treatments were observed. The texture class in each soil depth of all the cultivated treatments was classified as loam except for the 20-30 cm soil depth in the lucerne and WBCWBL4 treatments which is clay loam while the natural vegetated soil in the 5-10 cm depth was classified as sandy loam. As expected, no significant differences in particle size distribution was found between the different crop rotation systems as the soil is very homogeneous

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(Glenrosa soil form) in this experimental area, except for the MMW treatment and natural vegetated soil where an Oakleaf soil form is dominant.

Table 3.7 Particle Size Distribution of the fine fraction (< 2 mm) for the different treatments.

Treatment	Depth	Clay (%)	Silt (%)	Sand (%)	Texture class
Lucerne	0-5	22	38	40	loam
	5-10	23	39	37	loam
	10-20	23	39	38	loam
	20-30	27	37	36	clay loam
MMW	5-10	21	32	46	loam
MMWW	5-10	26	41	33	loam
WBCWBL4	0-5	22	42	36	loam
	5-10	24	43	33	loam
	10-20	23	41	32	loam
	20-30	27	39	35	clay loam
WBCWBL1	5-10	22	43	35	loam
Natural	5-10	14	27	59	Sandy loam

Clay content can be a possible stabilizing mechanism as it can protect organic C against decomposition via strong chemical bonds (Kalbitz et al., 2005; Mikutta et al., 2007). This formed the reason why especially clay content was important to determine in this study as a significant relationship (P = 0.0002; R^2 = 0.60) was found between the clay content and total SOC in the 5-10 cm depth (Figure 3.18). An increase in total SOC was thus observed with an increase in clay content but a rather weak correlation (R^2= 0.39) was found between clay plus silt and SOC. This is in line with what Nciizah & Wakindiki (2012) found in their study with a 78% correlation between clay concentration and total SOM and a 38% correlation between clay plus silt and SOM. This was attributed to the formation of strong chemical bonds between clay types and organic particles (chemical protection) and not necessarily silt particles. The strong correlation between clay content and SOC suggests a possible reason for the high C content found in the MMWW treatment. Clay can also play an indirect role in soil C stabilization as clay content is usually associated with increased aggregate stability. An increase in aggregation due to higher clay content can lead to more C stabilized within aggregates as it is inaccessible to microbial degradation (Kölbl & Kögel-Knabner, 2004).
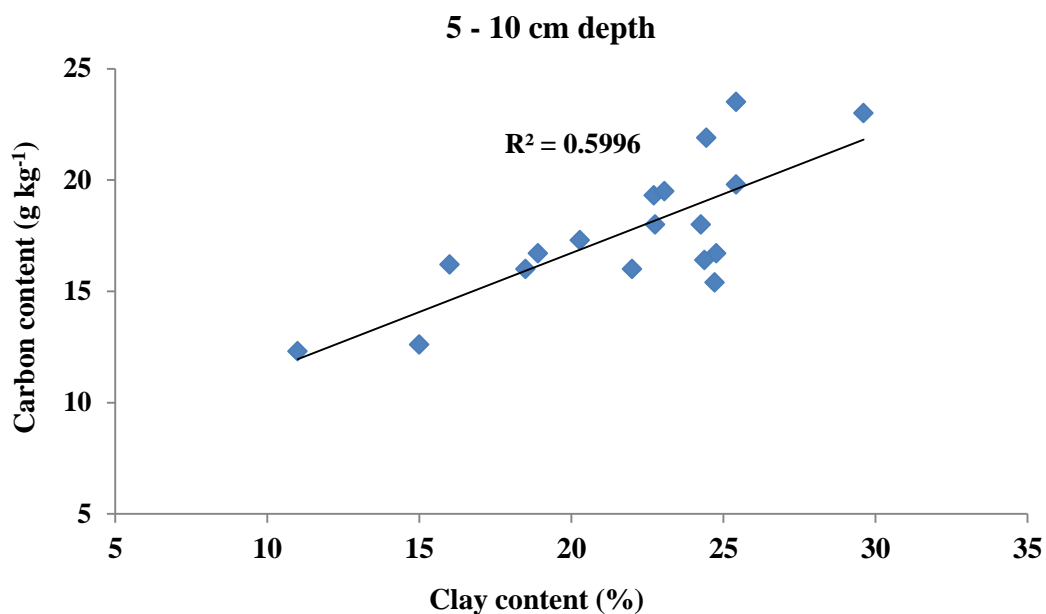
Figure 3.18 Relationship between clay content and total carbon content in the 5-10 cm depth increment.

3.3.5.2. Aggregate stability

A significant difference (P = 0.0036) in aggregate stability in the 5-10 cm depth was found between the natural vegetated soil and the different crop rotation treatments as the natural vegetated soil contained 65% water stable aggregates while the water stable aggregate percentage for the crop rotation treatments ranged between 39 and 50% (Figure 3.19a). Within the different crop rotation systems clear trends were observed but no significant differences were found. On average, the crop-pasture rotations (MMW & MMWW) had a higher aggregate stability than the two 100% crop rotations with the WBCWBL4 treatment containing lowest aggregate stability.

Figure 3.19b shows the water stable aggregates (%) obtained for the lucerne and WBCWBL4 treatments in all sampling depths. The aggregate stability in the 0-5 cm depth for the lucerne and WBCWBL4 treatments were significantly higher (P <0.0001) than the 5-10 cm depth but no significant differences were found between the two treatments in both the 0-5 and 5-10 cm depths. There were also no significant differences found between the two treatments in the 10-20 cm depth but in the 20-30 cm depth the lucerne treatment had a significant higher (P = 0.0278) aggregate stability than the WBCWBL4 treatment. A general decrease in aggregate stability was thus observed with depth for the WBCWBL4 and lucerne treatments up till 20

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cm whereas a severe increase in aggregate stability was found in the 20-30 cm depth increment for the lucerne treatment but not for the WBCWBL4 treatment.
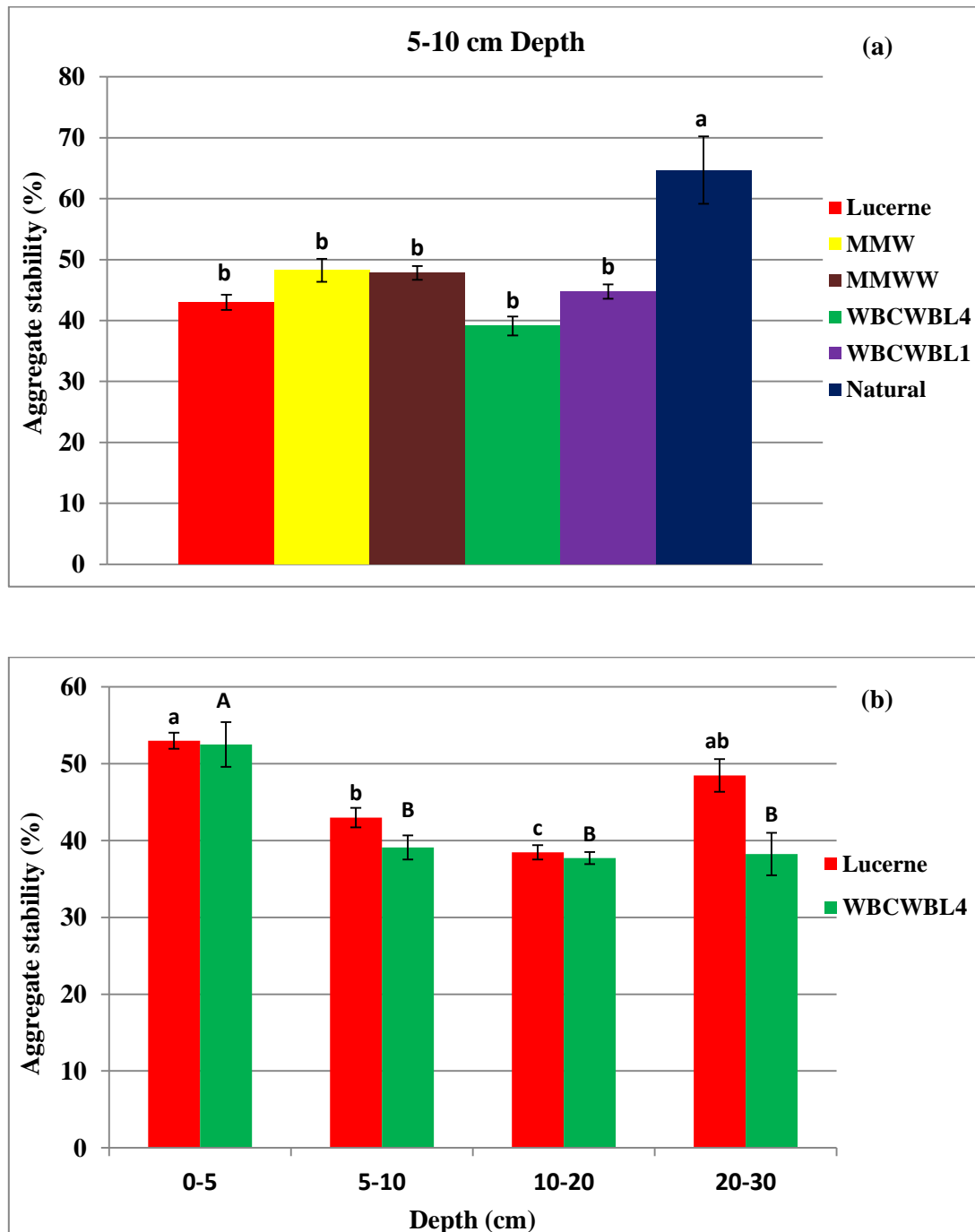
Figure 3.19 Water stable aggregate percentage for the (a) different treatments at the 5-10 cm depth and (b) lucerne and WBCWBL4 treatments in all four sampling depths.

Note1: In Figure 2.19a error bars represent standard error, and alphabetic letters denote statistical differences between treatments according to Tukey's Studentized Range test at $\alpha = 0.05$. Similar letters indicate lack of significant differences.

Note2: In Figure 2.19b Error bars represent standard error, and alphabetic letters denote statistical differences between depths in the WBCWBL4 treatment (Capital letters) and lucerne treatment (lower case letters) according to Tukey's Studentized Range test at $\alpha = 0.05$. Similar letters indicate lack of significant differences.

In a study done by Gale et al. (2000) they found that in no-till systems, root C was much more important in stabilizing small macroaggregates than C from aboveground residues. Aggregation is promoted by root growth by binding soil particles together and by stimulating soil microorganism's activity (Jastrow et al., 1998; Haynes & Beare, 1997; Six et al., 2002b). Root C input from root hairs and fine roots contributes at a scale equivalent to that of physically protected C (Rasse et al., 2005) and at this stage it is assumed that in the crop-pasture treatments more C is occluded and stabilized within the aggregates and thereby also promoting aggregate stability. These findings can thus help explain the differences in aggregate stability found between the different crop rotation systems under no-till in this study. A significantly higher root biomass obtained for medics and the fact that medics produce new roots in the following season can thus promote aggregate stability in the crop-pasture rotations compared to the 100% crop rotations. Although not determined, it is expected that the natural vegetated soil which is covered by grasses, has a high root biomass which contributes to the significant higher aggregate stability compared to the arable rotation systems. The significant higher aggregate stability in the 0-5 cm depth compared to the 5-10 cm depth can be partially attributed to higher C content (direct effect) as good correlation ($R^2 = 0.50$) was found between total C content and aggregate stability in these two depth increments for the lucerne and WBCWBL4 treatments. Higher C content can cause a higher soil microorganism's activity (Jastrow et al., 1998) resulting in the production of microbial bonding materials for aggregates (indirect effect). Legumes (lupine, medics and lucerne) have also been found by Haynes & Beare (1997) to enhance aggregate stability and contributed this to a different rhizosphere microbial population of legumes than that of non-legumes.

However, Golchin et al. (1995) found no correlation between total organic matter and aggregate stability but found a significant correlation ($R^2 = 0.86$) between organic matter occluded within aggregates and aggregate stability. No correlation was also found between aggregate stability and total soil C in the different treatments at the 5-10 cm layer indicating that only a small fraction of total soil C is possibly occluded in aggregates (Golchin et al., 1995). It is therefore important to fractionate total SOC into its different fractions to be able to predict how C influences aggregate stability directly and in return affects C stabilization

via spatial inaccessibility to microbial degradation (Christensen, 1996; von Lützow et al. 2006). The lack in correlation between total C and aggregate stability in the different treatments also shows that there has to be other factors that also play a role in aggregation. The natural vegetated soil had the lowest total C content in the 5-10 cm depth, yet the aggregate stability was highest in this treatment.

Aggregate stability is potentially very vulnerable to physical soil disturbance and this can also play a major role, even under no-tillage, as the pasture and crop-pasture rotations are disturbed to a much lesser extent than the 100% crop rotations which is disturbed every year when crops are planted. The natural vegetated soil has never been cultivated before which contributes to the high aggregate stability of these soils. However, because the lucerne treatment hasn't been disturbed the last 12 years one would expect this treatment to have the highest aggregate stability of all the cultivated treatments but this was not the case. The lucerne is frequently grazed (more often than any other treatment) during the year and has therefore low aboveground residue inputs. This together with the fact that lucerne doesn't re-germinate each year unlike medics, it produces lower C inputs through roots compared to the medics. The lower particulate organic matter input (easily accessible for microbes) in the lucerne treatment can cause lower microbial activity which leads to less production of microbial bonding materials for aggregates (Six et al., 2002b). According to García-Préchac et al. (2004) crop-pasture systems will promote aggregate stability due to no disturbance in pasture cycle and also due to the dense and fibrous grass root systems of pastures that include grass components.

As discussed in the previous section, higher clay content is usually associated with increased aggregate stability (Kölbl & Kögel-Knabner, 2004) and is therefore an important factor to consider when interpreting aggregate stability. Good correlation has been found between clay content and aggregate stability ($R^2 = 0.52$) in the 10-30 cm depth for the lucerne treatment and this helps explain the higher aggregate stability in the 20-30 cm depth than the 10-20 cm depth, although a decrease in C content with depth was observed. The clay content in the 20-30 cm depth (27%) is relative higher than the clay content in the 10-20 cm depth (23%) with the clay particles promoting aggregation. Although the clay contents in the lucerne and WBCWBL4 treatments in the 20-30 cm depth were similar, the lucerne treatment had a higher root biomass in this depth (section 3.3.4.1) compared to the WBCWBL4 treatment with the roots possibly promoting aggregation further in the lucerne treatment. For the 10-30

cm depth in the WBCWBL4 treatment no correlation was found between clay content and aggregate stability which excludes clay effect on aggregate stability in this treatment.

There are thus several factors that can affect aggregate stability in soils and it is important to consider all these contributing factors when interpreting differences in aggregate stability. The dominant effect on aggregate stability seems to be the extent of disturbance the treatments are subjected to as well as belowground inputs through roots. It is however, as observed in this study, not always possible to define differences in aggregate stability to a specific factor, but rather to a combination of various factors.

3.3.5.3. Coarse fragments

The coarse fragment percentages were obtained on a mass basis in the different treatments and four sampling depths (Figure 3.20). Because the soils are very shallow and shale derived a high coarse fragment percentage of mainly shale fragments was expected. This is a common feature for the soils in this region. The natural vegetated soil had a much lower coarse fragment percentage in each depth, compared to the arable soils, although not statistical different from all the arable treatments in all depths but clear trends were observed. Of the arable soils, the lucerne treatment had the lowest coarse fragment percentage in the 0-20 cm depth. In the 0-5 cm depth the natural vegetated soil (16%) differed significantly (P < 0.0001) from the MMWW, MMW, WBCWBL4 and WBCWBL1 treatments with coarse fragment percentages of 43, 32, 40 and 40% respectively while it didn't differ significantly from the lucerne treatment (24%). The same trends were observed in the 5-10 and 10-20 cm depth increments as in the 0-5 cm depth increment. In the 20-30 cm depth, the MMWW, lucerne, WBCWBL4 and WBCWBL1 treatments consist of a lithocutanic B horizon and therefore a general increase in coarse fragment percentage was found in this depth while for the MMW treatment which consists of a neocutanic B horizon no increase was observed. However, for the natural treatment which also consists of a neocutanic B horizon an increase in coarse fragments (34%) were found, although it was still significantly (P = 0.0037) lower than the MMWW, lucerne, WBCWBL4 and WBCWBL1 treatments with coarse fragment percentages of 52, 49, 55 and 54% respectively and much lower than the MMW treatment (47%). Overall, except for the MMW treatment, the coarse fragment percentage in the 0-20 cm depth for each treatment were more or less the same (Orthic A horizon) with a general increase observed in the 20-30 cm depth, especially in the treatments that consists of a lithocutanic B horizon.
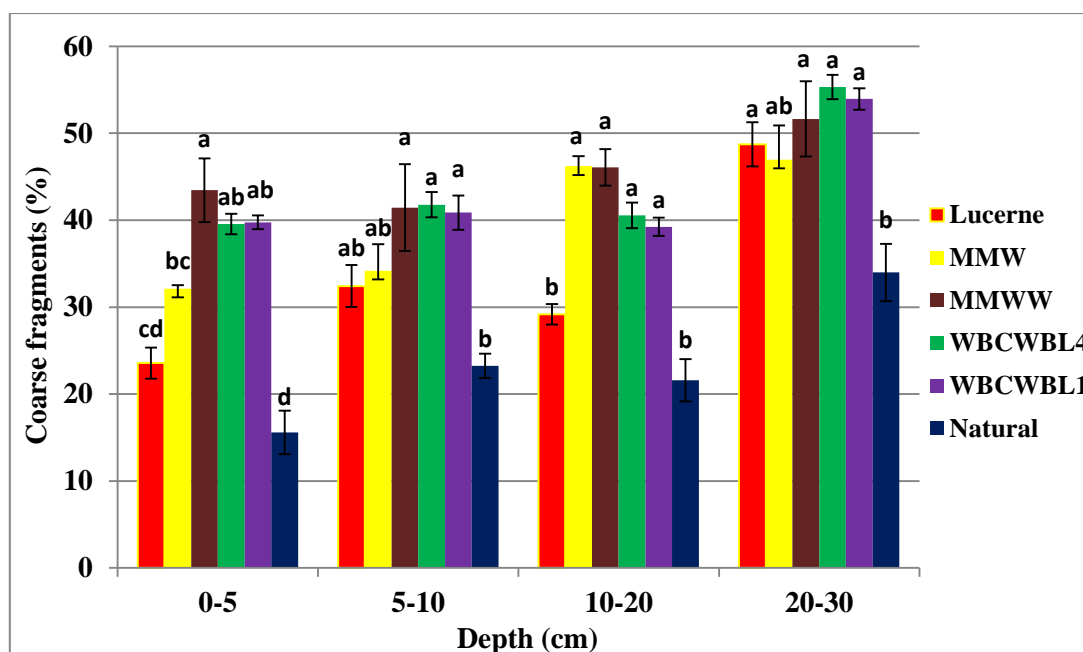
Figure 3.20 Coarse fragment percentage for the different treatments in the four sampling depths.

Note: Error bars represent standard error, and alphabetic letters denote statistical differences between treatments at specific depth according to Tukey's Studentized Range test at $\alpha = 0.05$. Similar letters indicate lack of significant differences.

It is clear that there are significant differences in the amount of coarse fragments found in some of the treatments. The natural treatment which is about 500 m further down the slope, do possess of more colluvial material than the cultivated treatments (field observation). Colluvial material consists dominantly of finer material (< 2 mm) and therefore the significant lower coarse fragment percentage found in this treatment, especially in the 0-10 cm depth. Another important contributing factor to the lower coarse fragment percentage is that the natural vegetated has never been cultivated before and therefore no coarse fragments have been brought up to the surface of the soil. In contrast, it is assumed that before 2002, deep tillage (conventional tillage) was applied in the agricultural soil which resulted in coarse fragments being brought up to soil surface from the Lithocutanic B horizon causing a higher coarse fragment percentage in the top 20 cm. The much lower coarse fragment percentage of the lucerne treatment (0-20 cm depth) compared to the other cultivated treatments can be due to the possibility of this land being under pasture before 2002 and therefore less disturbed. No-tillage which has been applied since 2002 would have no major effect on the distribution of coarse fragments with depth in the different treatments.

As most of the soil analysis is only performed on the fine fraction (< 2 mm) of the soil, variable coarse fragment percentages cannot be ignored as it can lead to misinterpretations

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and incorrect conclusions on especially soil C dynamics. Coarse fragments (> 2 mm) reduce the amount of fine fraction (< 2mm) per soil volume severely (Bornemann et al., 2011). Differences in the amount of coarse fragments in the different treatments affect therefore the volume of the fine fraction (< 2mm) of each treatment. A treatment with less coarse fragments will have more of the fine fraction per soil volume while a treatment with a high coarse fragment percentage will have a much lower fine fraction volume. The coarse fragments in this study therefore also had a direct influence on C stocks as C is mainly stored in the fine fraction of the soil (Figure 3.13). The higher the coarse fragment percentage, the more significant the effect of coarse fragments on C stocks was with the coarse fragments decreasing the amount of C stored. These results is in line with what Bornemann et al. (2011) observed in their study as they found a significant negative relationship (R^2 = 0.73) between SOC stocks and coarse fragments.

Another important reason for quantifying all the coarse fragments (> 2 mm) is to make sure that the fine fraction (< 2 mm) that is used for analysis is representative as more silt and clay particles tend to stick to the coarse fragments than the sand particles, influencing the particle size distribution of the soil. By sonication and wet sieving another 15-35% soil was regained which can have a major influence on results of several soil analysis.

3.3.6. Soil Respiration (CO_2 Efflux)

In the **September measuring period** the natural vegetated soil had a soil respiration rate of 1.68 g C m^{-2} day^{-1}, significantly higher (P = 0.0015) than the MMWW, WBCWBL4 and WBCWBL1 treatments with respiration rates of 1.25, 1.04, 0.96 g C m^{-2} day^{-1} respectively. The respiration rate of the MMW treatment was the highest of the crop rotation systems with a rate of 1.29 g C m^{-2} day^{-1} (Figure 3.21). The respiration traps were destroyed by grazing sheep in the lucerne treatment in September and therefore no data was collected. During the **March sampling period**, the exact same trends were found between the different treatments as was found in the September trial, except significantly (P = 0.0002) lower respiration rates were observed in each of the treatments. The natural vegetated soil still produced the most CO_2 during the trial with 1.13 g C m^{-2} day^{-1} leaving the soil. The lucerne treatment with a respiration rate of 1.09 g C m^{-2} day^{-1} produced significantly (P = 0.012) more CO_2 than the WBCWBL1 treatment with a respiration rate of 0.70 g C m^{-2} day^{-1}. The lucerne also produced more CO_2 than the other crop rotation systems with soil respiration rates of 0.78, 0.85 and 0.84 g C m^{-2} day^{-1} for the WBCWBL4, MMWW and MMW treatments, respectively.
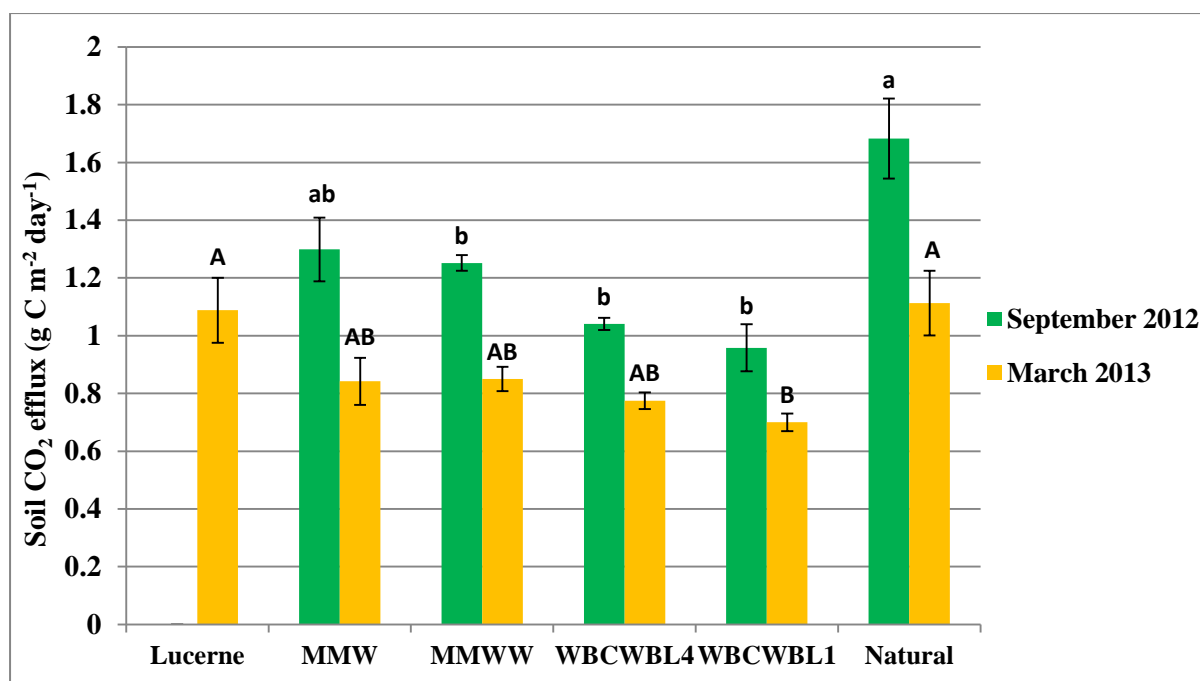
Figure 3.21 Respiratory CO_2 produced per day by the different treatments during two different climate periods.

Note: Error bars represent standard error, and alphabetic letters denote statistical differences between treatments in March 2013 (Capital letters) and September (lower case letters) according to Tukey's Studentized Range test at $\alpha = 0.05$. Similar letters indicate lack of significant differences.

The release of C as CO_2 by oxidation of soil organic matter has raised concerns about global warming and led to increasing interest in sequestering atmospheric carbon dioxide (CO_2) in agricultural ecosystems as soils have the potential to serve as either a source or a sink for CO_2, depending mainly on soil management practices applied (IPCC, 1996). However, the contribution of roots to soil respiration (autotrophic respiration) cannot be ignored (Kuzyakov, 2006) as Holt et al. (1990) found in a tropical semi-arid woodland that roots contributed up to 40% of the total soil respiration. There are thus several factors that affect soil respiration rates which include factors like soil moisture and temperature and also soil organic C quantity and quality (C:N), most of which can be influenced by soil management practices (Lohila et al., 2002).

Seasonal differences in soil respiration in arid regions are great where soil moisture may be a factor limiting microbial activity as Holt et al. (1990) found much lower respiration rates in the dry season compared to the wet season with soil moisture accounted for 82% of the difference and soil temperature only 7%. This finding compares well with what was found in this study as the respiration rate in the dry season (March) was significant lower than the respiration rate in September when the soils were still moist. Davidson et al. (1998) also

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found that soil respiration decreased from wet to dry seasons in different land uses. Soil temperature and water content are the factors that are most commonly related to temporal variation in CO_2 efflux from soils. Soil respiration is low in extreme (wet and dry) conditions while maximum respiration occurs at intermediate soil moisture conditions (Davidson et al., 1998). Absence of water restricts the living conditions of micro-organisms and would therefore lower decomposition rates which will lead to less CO_2 produced while high soil moisture content causes anaerobic conditions and also restricts microbial activity (Linn & Doran 1984). It is thus possible that the soil moisture content was closer to optimum in September as it is just after the rainy season.

Another possible reason for the difference in soil respiration rates between the different climate periods can be attributed to the contribution of roots (autotrophic respiration) as root activity in the March trial will be less prominent than in September as the roots have died off this time of year. In both these measuring periods the crop-pasture rotations (MMWW & MMW) had a higher CO_2 efflux than the 100% crop rotation systems (WBCWBL4 & WBCWBL1) which can be attributed to a combination of higher C content, higher belowground biomass input and lower C:N ratio (quality) of medics and lucerne roots. A greater amount of CO_2 is produced by the roots and the amount of microorganisms consuming the root exudates increases which enhance soil respiration activity (Lohila et al., 2002). Tufekcioglu et al. (2001) also attributed higher soil respiration rates to higher soil C content, higher soil moisture content and greater fine root biomass. A possible higher root biomass for the natural vegetated soil can also explain the high soil respiration rates observed in this treatment. Plant residues with a low C: N ratio are more easily mineralized (decomposed) than plants with a high C:N ratio (Crawford et al., 1997) and therefore produce more CO_2 as it is the end product of mineralization. Medics and lucerne roots have a C:N ratio of 26 and 20 respectively causing faster organic matter decay than the other crops with higher C:N ratios. The lucerne treatment produced the most CO_2 of the crop systems partly due to a high belowground biomass (autotrophic respiration) and an optimum C:N ratio for maximum decomposition (heterotrophic respiration). This is however only a hypothesis as no significant correlations have been found between soil respiration rates and root quantity and quality ($R^2 = 0.22$ & $R^2 = 0.21$). These weak correlations are mainly attributed to the combined effects of these two factors together with C content and soil climate conditions on soil respiration rates. A higher soil respiration rate found in the crop-pasture rotations

compared to the 100% crop rotation systems can also partly be attributed to higher microbial activity which means a possible enhancement of nutrient turnover and release.

In Chapter 4 a possible correlation between the labile C fraction (C not stabilized via occlusion in aggregates and by minerals) and soil respiration rate will be investigated as this will provide more information regarding the effect of current management practices applied on soil respiration. It will however, still be difficult to distinguish between autotrophic respiration and heterotrophic respiration and whether a higher respiration rate is due to a higher root biomass or due to more labile C that is more easily decomposed by microbes.

3.3.7. Relationship between SOM and wheat yield and quality

One of the objectives of this study was to see if there is any relationship between total soil C and winter wheat yields produced in 2012 in the grain production area of the Overberg as Lal (2006; 2011) and Àlvaro-Fuentes et al. (2008) and several other authors made the statement that soil quality and productivity can be improved by an increase in SOC content.

Figure 3.22a & b shows the significant relationship found between wheat yield and total soil C ($R^2 = 0.99$) and N ($R^2 = 0.94$) respectively in this study. The two crop-pasture rotations (MMWW & MMW), containing the highest soil C and N, produced the highest wheat yields in 2012 compared to the two 100% crop rotation systems (WBCWBL4 & WBCWBL1) which contained a lower C and N content. The MMWW rotation system had an average soil C and N content of 18.6 g C kg^{-1} and 2.1 g N kg^{-1} in the 0-30 cm depth and produced a wheat yield of 5900 kg ha^{-1} in 2012, the highest of all the rotation systems. The yield of the MMWW (50% pasture, 50% crop) rotation system was on average 37% higher than the yields obtained of 4300 and 4400 kg ha^{-1} in the WBCWBL4 and WBCWBL1 rotation systems, respectively. The MMW rotation produced the second highest yield of 4900 kg ha^{-1} with an average C and N values of 15.2 g C kg^{-1} and 1.7 g N kg^{-1} in the 0-30 cm depth. No differences were found in wheat yield produced after lupine (legume) and wheat yield produced after canola (non-legume) with soil C and N values of 14.1 g C kg^{-1} and 1.6 g N kg^{-1} and 13.3 g C kg^{-1} and 1.4 g N kg^{-1}, respectively. Table 3.8 is a summary of Figure 3.22a & b as it shows the average C and N measured in the three replications and four depths as well as the yield and quality of wheat in the different treatments. From this table it is clear that both total soil C and N had a positive effect on the quality (protein content) of the wheat. The wheat quality in the crop-pasture rotations was higher compared to the 100% crop rotation

systems and therefore, the wheat in rotation with pastures was graded higher than the wheat in rotation with only crops.
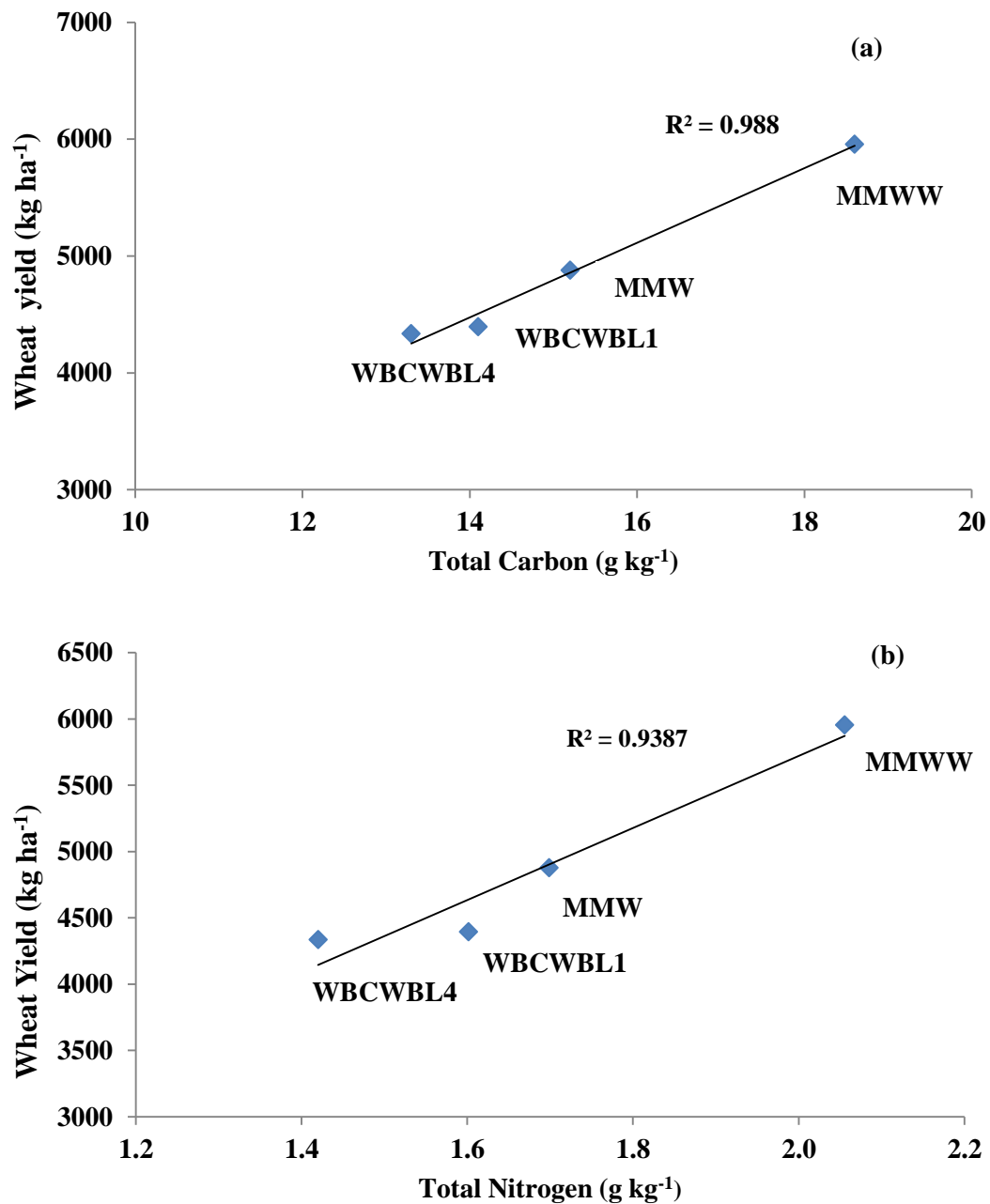
Figure 3.22 Relationship between 2012 wheat yields and (a) Total soil C in 0-30 cm depth; (b) Total soil N in 0-30 cm depth.

Table 3.8 Summary of the effect of soil carbon and nitrogen on wheat yield and quality in different rotation systems.

Treatment	Average C (g kg^{-1}) measured in 3 replicates and 4 depths	Average N (g kg^{-1}) measured in 3 replicates and 4 depths	Protein content of wheat	Wheat yield (kg ha^{-1})	Grade
MMW	15.2	1.7	13.2	4878	B1
MMWW	18.6	2.1	12.6	5955	B1
WBCWBL4	13.3	1.4	10.3	4335	B3
WBCWBL1	14.1	1.6	10.6	4394	B3

Medics (legume) supply N (N) through fixation (narrow C:N ratio) and are important for producing good wheat yields of high quality in the following year. Positive legume rotation effects on subsequent cereal yields have also been reported by several other scientists (Burle et al., 1997; Yusuf et al., 2009). Lupine, although a legume, has a rather high C:N ratio (low N content) compared to lucerne and medics (section 3.3.4.2) and this could explain the lack in difference in wheat yield and quality between the two 100 % crop rotation systems. The use of legume pastures (medics) in rotation with wheat possibly increased the quality of the soil directly (release of trace elements and N, P) and indirectly (increase cation exchange capacity, water holding capacity and microbial activity) by means of higher soil C content (Lal 2006; 2011; Àlvaro-Fuentes et al., 2008). According to García-Préchac et al. (2004), better soil quality is one of the main reasons for higher crop yields in crop-pasture rotations compared to 100% crop rotation systems. According to Lal (2011) an increase in agronomic production can be attributed to an increase in SOC content as it increases the use efficiency of energy-based inputs (e.g. fertilizers, pesticides) and the plant available water capacity. A higher water holding capacity is very important in a semi-arid region as water is the main limiting factor for dryland agriculture. Unfortunately, the plant available water was not determined in the different treatments as it was outside the scope of this study, but a higher aggregate stability found in the crop-pasture systems is expected to enhance the porosity of the soil and thereby its water holding capacity. Masri & Ryan (2006) also showed that legume-based rotations can improve the soil physical structure which affects soil water relations (hydraulic conductivity and infiltration). However, the effect of SOC on soil water retention tend to be greater in coarse textured compared to fine textured soils (Krull et al., 2004). Regression analysis showed a significant (P = 0.0005) linear relationship between total soil C and effective cation exchange capacity (ECEC) in the soil (R^2 = 0.72) in these

four treatments. This indicates that 72% of the variation in exchangeable cations retained in the soil is explained by variation in total soil C. Although the soils have high clay content (20-30 %), kaolinite seems like the dominant clay mineral and therefore provides very little binding sites for cations. The importance of SOC in increasing the ECEC of the soil is emphasized by the fact that the MMW treatment contained the lowest clay content of the cultivated treatments, yet it had higher ECEC than the lucerne and WBCWBL4 treatments. Organic matter plays thus an important role in retaining these cations in the soil and increases therefore the input efficiency of fertilizers. As discussed in the literature review, SOC affect several soil properties directly and indirectly but it is impossible to look at every property as it falls outside the scope of this study. A more comprehensive overview on the effect of SOC on soil quality and productivity can be reviewed in Lal (2004; 2006; 2011); Àlvaro-Fuentes et al. (2008).

3.4. Conclusion

After 11 years, significant differences in total SOC between cropping systems have been found, most notably in the 0-10 cm depth. The highest SOC content in the sampling depth (0-30 cm) was found in the MMWW treatment with a value of 18.6 g C kg^{-1}. This was significantly higher than all the other treatments. The WBCWBL4 and WBCWBL1 treatments had the lowest C content of the cultivated treatments in the 0-30 cm depth, 13.3 and 14.1 g C kg^{-1} respectively, and compared well with the natural vegetated soil (13.2 g C kg^{-1}). The C stock (Mg ha^{-1}) values of the bulk soil in the different treatments showed significant differences, but were less pronounced than the differences found in C stock values of fine fraction. This is indicative of the effect of coarse fragments diluting the C content per soil volume significantly and therefore, to exclude effect of coarse fragments, the effect of the treatments on soil C sequestration were determined using the C stocks obtained of the fine fraction. Medic in rotation with wheat (crop-pasture) was a better alternative to continuous cropping, even in no-till systems, since it stored more C (70.2-74.9 Mg ha^{-1} vs. 54.7-58.9 Mg ha^{-1} in 0-30 cm). The amount of SOC stored in the MMWW treatment was 20 Mg ha^{-1} higher than the amount of SOC stored in the WBCWBL4 treatment. Compared to the natural vegetated soil, the crop-pasture systems stored much more C in the soil whereas the C stored in the two 100 % cropping systems were similar than the natural vegetated soil. It was however important to consider C values obtained in 2003 (experimental trial started in 2002) to be able to directly determine the effect of disturbance and cropping systems on C content.

The MMW (0.78 g C kg^{-1} year^{-1}) and MMWW (0.70 g C kg^{-1} year^{-1}) treatments produced the highest rate at which C increased per year.

The high SOC content found in the present study, compared to other studies in agricultural soils of similar climate, especially in the MMWW treatment, shows the positive effect of current crops used in the specific crop sequences on C sequestration as the climate and soil aren't ideal for high soil organic C contents. The higher average C content found in the crop-pasture systems compared to the lucerne, 100% crop rotation systems and natural vegetated soil could be attributed to different soil characteristics, plant inputs and cultivation practices, which in turn is responsible for the accumulation of soil C in these conservation managed soils.

The soil in the MMWW treatment contained higher amounts of clay which offered protection to SOM against decomposition. A fairly strong linear relationship between clay content and total SOC was found ($R^2 = 0.60$) indicating that clay content could play an important role in SOC accumulation in these soils. However, to be able to determine the role of clay minerals in stabilizing C it is necessary to fractionate total soil C into a mineral-bound C fraction as free particulate organic C (carbon not associated with minerals) also contributes to total C content but is unaffected by the clay content (Kölbl & Kögel-Knabner, 2004).

Soil aggregate stability in the different land-uses was found to be decreasing in the following order: Natural vegetation > crop-pasture > pasture > continuous cropping. No correlation was found between aggregate stability and total SOC as several factors, other than total C, also influence the stability of aggregates, such as extent of soil disturbance. This statement was confirmed as the natural soil contained the lowest C in the 5-10 cm depth, yet it had the highest aggregate stability of all the treatments as well as the least amount of soil disturbance. The higher aggregate stability in the two crop-pasture systems could have enhanced the SOC content in these treatments but this statement is inconclusive at this stage as fractionation of C into different pools is necessary. From total SOC results obtained in this chapter, it seems that clay content is much more important for C accumulation than aggregate stability. This can probably be attributed to high clay content and low aggregate stability of these stony soils.

Plant properties also had a strong influence on the amount of SOC stored in each rotation system. This statement was confirmed as the MMW treatment showed the highest C increase since 2003, while its clay content was the lowest of all the cultivated treatments. This means

that although clay content seems to play an important role in C accumulation, the quantity and quality of C input is also important. The significant lower rate at which C increased since 2003 in the lucerne treatment compared to the crop-pasture systems, can partly be explained by the lower belowground inputs of C through roots. Although lucerne has a higher root density than medics, medics re-germinate from seed annually and leads to the formation of completely new roots (i.e. higher C inputs) each season. Lucerne becomes dormant in winter (roots are alive but not actively growing). Lucerne also has a very low C:N ratio and is therefore more easily decomposed by microbes leading to faster mineralization of organic C to CO_2. A higher soil respiration rate found for the lucerne, MMW and MMWW compared to the 100% crop rotation treatments confirmed this as medics also consists of a low C:N ratio. Medics in rotation with wheat (MMW & MMWW) produced the highest SOC content. This can be attributed to a combination of low C:N crops (medics) with high C:N ratio crops (wheat) with both crops consisting of well-developed rooting systems, especially medics in the 0-10 cm depth. The high aboveground input of high C:N residues from wheat would also contribute to the SOC content while the ability of medics to re-establish itself in the following season which leads to the formation of new roots (i.e. higher C inputs) is also an important contributing factor. Together with the effect that soil and plant properties have on SOC, the fact that the MMW and MMWW soils have been disturbed less than the WBCWBL4 and WBCWBL1 treatments is also a possible reason why the crop-pasture systems had more SOC. Although no-tillage is applied in all of the treatments, the tine planter still disturbs the soil in the 0-10 cm depth. Less disturbance of the soil could lead to more soil cover and this is known as a very important aspect of conservation agriculture.

SOM content is a primary indicator of soil quality due to its influence on chemical, physical and biological properties (Lal, 2011). In a higher quality soil, higher agronomic production is expected and this was the case in this study as the MMWW treatment, containing significant higher C also produced significantly higher wheat yields of higher quality than the other treatments. A 99 and 94% correlation was found respectively between soil C and soil N and wheat yields produced in 2012. The ability of medics to fix N seems to be a major reason for producing good yields in the following year. The higher aggregate stability in the crop-pasture systems could possibly enhanced the ability of the soil to absorb water (higher total porosity) which could lead to higher soil moisture content in the soils. A higher SOC content enhance the water holding capacity of the soil. This is very important, especially in the Overberg as the rainfall is low (± 450 mm) and the pattern very unreliable (summer rainfall

not unusual). The higher the SOC content the more soil water can be possibly stored during summer months. This together with the higher ECEC enhancing the efficiency of added fertilizers can also be a reason for the higher wheat yields obtained in these treatments. We conclude thus from these findings that crop-pasture rotations, in addition to being biologically and economically attractive (less fuel and less fertilizers), also enhance soil quality and promote soil sustainability in these stony soils. An increase in agronomic production is vital to enhance food security especially in developing countries like South Africa.

I think it is worthwhile for grain farmers in the Overberg to consider the use of legumes (medics) as a pasture in rotation systems. It can enhance C sequestration and thereby soil quality which can lead to higher crop yields. Pasture and crop-pasture rotation systems were more beneficial than 100% crop rotations due to; (i) a higher aggregate stability in topsoil due to less disturbance, (ii) larger quantity of belowground C input of pastures, (iii) the high % N of pasture residues both above- and belowground and therefore provides high quality organic matter (low C:N). A low C:N ratio enhances microbial activity and causes nutrients to become more rapidly available.

Investigation of the effect of the crop/pasture systems on SOM functional pools will form the basis of the study in the next chapter. This will enable elucidation of the extent and mechanisms of SOC stabilization in the different crop rotation systems. The relative contribution of the different SOM functional fractions to soil quality properties will also be investigated.

CHAPTER 4

THE EFFECT OF NO-TILL CROP ROTATION PRACTICES ON SOIL ORGANIC MATTER FUNCTIONAL POOLS AND STABILIZATION MECHANISMS

4.1. Introduction

Soil organic carbon (SOC) is sensitive to changes in climate and management, but how and on what timescale will it respond to such changes? Because the SOC pool is one of the largest on the global scale (Jobbagy & Jackson, 2000) it is important to understand C stabilization mechanisms as this information can be useful in attempt to minimize negative environmental impacts (e.g. CO_2 emission and soil erosion) and optimizing crop production. However, to be able to answer the question stated above, isolation of the total soil organic matter (SOM) pool into different fractions or pools, with each fraction (pool) containing distinct functional properties and turnover times, is necessary and has been a major research topic in the last few years.

The quantity and quality of C input and the rate of decomposition are the two vital factors controlling the amount of C stored in the soil (von Lützow et al., 2007). The quantity and quality of C input depends on the type of crop cultivated and/or crop rotation practices applied while the rate of SOM decomposition can be controlled by several stabilizing mechanisms operating in the soil. This can be directly affected by management practices (e.g. crop rotation & no-tillage). In general, no-tillage practices have been observed to contribute to the accumulation of C in soils by decreasing the mineralization rate of organic matter, especially in the surface soil. Several authors (West & Post 2002; Chen et al., 2009; Huang et al., 2010) contributed this to higher stabilization of organic matter within aggregates due to fewer disturbances. Retaining crop residues with no-till systems can also contribute to aggregation. According to Arshad et al. (1990) no-till doesn't only increase the quantity of organic matter (OM) but also improves the quality of OM, as OM under no-till contained more carbohydrates, amino acids, and amino sugars. No-tillage as an isolated system may however not always produce the positive results as expected in terms of accumulation of soil C as it has to be combined with the right crop rotation practices (Sisti et al., 2004; Conceicão et al., 2013). Therefore, the use of diversified and high input cropping systems (includes legumes) have to be considered as a management strategy together with no-tillage to enhance

C sequestration. Decomposition rates of SOM also depends on the mineralogy (Kalbitz et al., 2005; Mikutta et al., 2006; 2007) and texture (Kölbl & Kögel-Knabner 2004) of the soil as sorptive organic-mineral interactions are considered one of the most important mechanisms by which C is stabilized (Baldock & Skjemstad, 2000). A lot of studies have been carried out in temperate regions (von Lützow et al., 2006) with soils dominated by high activity clays while soils dominated by mainly kaolinite and illite still has to be investigated, especially those subjected to conservation management practices.

The chemical properties of SOM and its interactions with the abiotic mineral matrix allow them to be placed in different SOM functional pools with varying turnover rates (Kögel-Knabner & Kleber, 2012). The three major SOM functional pools that are of great importance in soil C stabilization studies (Golchin et al., 1994a; Sohi et al., 2001; Marin-Spiotta et al., 2008; Cerli et al., 2012) and in particular this study are; (i) the free particulate organic matter (fPOM) fraction which resembles recent undecomposed litter inputs and tends to have younger C than other fractions, (ii) an occluded particulate organic matter (oPOM) fraction which is generally older than fPOM fraction released by disruption of soil aggregates, and (iii) a heavy mineral-bound fraction (mineral), comprising organic matter tightly bounded or sorbed to minerals containing the oldest OM. The fPOM fraction that represents the labile C pool contains physically non-complexed OM and is seen as an early and sensitive indicator of SOC as this fraction usually responds the quickest to soil management changes (Haynes, 2005; Gregorich et al., 2006).

Decomposition of soil C can be slowed down by different stabilization processes. These processes are complex and entail an understanding of chemical, physical and biological interactions between organic components and the mineral matrix (Kögel-Knabner & Kleber, 2012). Two main possible soil C stabilization mechanisms that can contribute to SOM stabilization have been considered; (i) physical protection via occlusion in aggregates and (ii) chemical interaction with soil minerals (phyllosilicates and metal-oxides) (Sollins et al., 1996; von Lützow et al., 2006). More than one of these mechanisms may operate together to various degrees in soil (von Lützow et al., 2007). Both these C stabilizing mechanisms can contribute to the passive pool (oPOM & mineral fractions) through the protection of OM within aggregates (Christensen, 1996) and also its interaction with mineral surfaces (e.g. ligand exchange, cation bridging, weak interactions) (Torn et al., 1997; Mikutta et al., 2007).

In order to understand the SOC stabilization mechanisms operating under specific soil and climate conditions as well as management practices it is necessary to achieve an effective

separation of SOM functional pools of different stability and functional relevance. Density fractionation (Golchin et al., 1994a; Sohi et al., 2001; Cerli et al., 2012) is an effective technique in quantifying the amount of OM between (fPOM) and within soil aggregates (oPOM) as well as the amount of SOM associated with the minerals. This method is thus based on the extent and degree to which SOM is bound to minerals that regulates its dynamics. The fPOM fraction or labile fraction contains physically non-complexed OM which is not occluded within aggregates or bound to minerals. It can be separated by density using a certain liquid density as free OM is less dense than the mineral fractions (oPOM & mineral) (Gregorich et al., 2006). The fPOM fraction still contains much of the characteristics of the litter or root residues (wide C:N ratio) and it is in this fraction where chemical recalcitrance may have some stabilizing effect in the early stages of decomposition (von Lützow et al., 2006). This stabilization mechanism however was not investigated in this study. The C:N ratio of the mineral fraction is usually below 10 indicating an increasing degree of degradation as well as the ability of minerals to adsorbed and protect humified organic matter (Baisden et al., 2002). Separating soil samples into these three SOM functional pools allow investigation of the mechanisms responsible for C stabilization.

Soil organic matter (SOM) and its different pools play an important role in optimizing crop production, minimizing negative environmental impacts and improving soil quality and soil sustainability (Freixo et al., 2002). Therefore, total SOC needs to be fractionated into biologically meaningful C pools to account for the value of each pool to overall soil quality as total SOC content is only a coarse indicator of soil quality (Krull et al., 2004; Haynes, 2005). This formed the basis of the study as soil quality and sustainability are important to ensure food security. This can be achieved by applying management practices that enhance both the labile and stable C fractions. The stable C fractions contribute a major proportion to soil C sequestration while the labile C (fPOM), more easily decomposed, have an important role to play in enhancing nutrient availability and microbial activity. Several studies (Freixo et al., 2002; Chen et al., 2009; Salvo et al., 2010; Conceicão et al., 2013 ext.) have reported the effect of different management practices on SOM functional pools. However, there is still a gap in knowledge on how different crop rotation practises under no-tillage affect C stabilization and distribution in the grain production region of the Overberg.

Therefore, the main objectives of this component study were to evaluate the extent and mechanisms of soil C stabilization in the different crop/pasture rotation systems under no-tillage practices. This involved the fractionation of total SOM into different functional pools

(fractions) in order to investigate the role of each fraction in soil quality as well as the mechanisms by which C is stabilized. Elucidating the mechanisms responsible for SOC stabilization was carried out by examining the relationships of stable C (intra-aggregate and mineral-bound) with selected soil properties known to play role such as aggregate stability, clay content and metal-oxide content. Information on the binding mechanisms is mostly obtained from statistical correlations (Kögel-Knabner et al., 2008). The fPOM fraction (labile C) was also correlated with the soil CO_2 efflux in order to evaluate the contribution of heterotrophic respiration (decomposition of SOM to CO_2 by microbes) to total CO_2 efflux.

4.2. Material and Methods

Soils for this study were collected in June/July 2012 from three different replicates in each of the six treatments as described in detail on page 42-48. To avoid determination of the litter material (overestimation of fPOM pool size) and to ensure the carbon associated with minerals reflect the sorption capacities of minerals and metal oxides, subsoil samples were used for this study. The 5-10 cm soil depth in each treatment were thus used for the fractionation of total carbon in different pools and various other analysis (clay content, aggregate stability, metal oxides) in the same depth increment were carried out for correlation studies. However, for the lucerne and WBCWBL4 treatments all four depths (0-5, 5-10, 10-20 and 20-30 cm) were analysed for carbon fractions and relative soil properties in order to see how the different carbon pools varies between depth and treatment.

4.2.1. Density fractionation of SOM functional pools

The aim of the physical fractionation was to separate total SOM into three organic fractions using physical fractionation techniques with density separation and sonication, respectively. The SOM fractionation method used was modified from the procedure proposed by Golchin et al. (1994a); Sohi et al. (2001) and Cerli et al. (2012). The three fractions isolated were; (i) the free particulate organic matter (fPOM) (undecomposed material), (ii) the occluded particulate organic matter (oPOM) (intra-aggregate organic matter) and (iii) the heavy mineral-bound (mineral) (OM sorbed /bound to minerals) fraction.

The samples were fractionated using 5 g of soil and 25 ml Sodium Iodide (NaI) (1:5 soil to solution ratio) solution at a density of 1.6 g cm^{-3}. The rationale for using 1.6 g cm^{-3} is that OM density is generally less or equal to 1.5 g cm^{-3} (Golchin et al., 1994a). Cerli et al. (2012) also found the best results at this density for various types of soils. It was gently shaken by hand in a 50 ml centrifuge tube to ensure soil wetting and to avoid disruption of aggregates.

The suspension was then allowed to stand for 1 hour before centrifuging at 5600g for 20 min. The floating fPOM was transferred into a Millipore filtration funnel fitted with a 0.8 μm membrane and filtered under vacuum pressure. To ensure that all the fPOM has been quantitatively transferred, the centrifuging-filtering procedure was repeated three times for each sample. The retained fPOM fraction was then thoroughly rinsed with deionized water using a separate collector until the conductivity of the fresh water was less than 50 μS cm^{-1}. This fraction (fPOM) was allowed to air dry at below 40°C before weighed. The weighed samples were then ground using a mortar and pestle to ensure a homogeneous sample for analysis. The oPOM fraction within stable aggregates were released by resuspending soil residue with 25 ml of fresh NaI and the solution was then dispersed by ultrasound at an energy level of 200 J ml^{-1}. The suspensions were sonified with a sonicator (ultrasonic processor) (Qsonica Sonicators, Newton, USA) fitted with a probe tip (dimensions 13.8 cm x 1.3 cm) to a depth of 15 mm in suspension to disrupt the aggregates and attain the intra-aggregate organic matter occluded within.

The intra-aggregate fraction (oPOM) was recovered after centrifugation, using the same procedure described for the fPOM fraction. The sample residue (mineral-bound fraction) that remained after the fPOM and oPOM fractions were removed, was dialyzed in a Pierce (Perbio) SnakeSkin pleated dialysis tubing in a container of distilled water until the water tested free of salts with 0.1 M AgNO$_3^-$. It was then oven-dried at 40 °C for 72 hours.

A five decimal digital micro-scale was used to weigh the isolated pools and each pool was then analysed for C and N by dry combustion using the Euro Vector elemental analyser (Nelson & Sommers, 1996).

4.3. Results and Discussion

In Chapter 2 significant differences in total C accumulation between treatments were found. However, it was still unclear how much of the total C was labile or active and how much of the total C was actually stabilized within aggregates or associated with minerals. These fractions are seen as fine indicators of soil quality (Haynes, 2005). For example the fPOM pool releases nutrients and acts as a source of energy for the microorganisms but contribute little to soil CEC (Krull et al., 2004). To evaluate the soil quality and sustainability of a specific crop rotation system, it was therefore necessary to further divide total SOM into functional fractions. The mechanisms responsible for C stabilization in the different pools can also be investigated.

4.3.1. Distribution of Total Carbon in SOM functional pools

Despite working as carefully as possible during the fractionation process, the C recovered in the fractions ranged between 85 and 101% of total C of bulk soil. The average recovery of C was 93%. The residual C that was not accounted for in the particulate and mineral fractions can be considered as dissolved organic carbon (DOC) that was lost during the filtration process while collecting the particulate fractions. It can however also be attributed to materials stuck to filters as well as mistakes made when weighing these extreme small quantities of fraction and determining their C and N contents (inaccuracy of CN analyser). Due to uncertainty on how much of the lost C actually was DOC, the percentage of C in each fraction relative to total C was calculated using the total amount of C recovered and not the total amount of C in the bulk soil (Freixo et al., 2002; John et al., 2005). The percentage C recovered in each treatment and depth is shown in Table 4.1.

Table 4.1 Summary of SOC accumulation and percentage of this accumulation in the free particulate organic matter (fPOM), occluded particulate organic matter (oPOM) and mineral-bound organic matter (mineral) fractions, in the 0-5, 5-10, 10-20 and 20-30 cm layers in different no-till crop rotation practices.

Crop rotation	Carbon content (g kg^{-1})					% C Recovered	C in fraction, % of total C		
	fPOM	oPOM	mineral	Total C of fractions	Total C of bulk soil		fPOM	oPOM	mineral
0 - 5 cm									
Lucerne	1.7	0.24	19.3	21.2	22	96.5	8.0	1.1	90.9
WBCWBL4	2.3	0.17	17.5	20.0	19.7	101.4	11.5	0.9	87.6
5 - 10 cm									
Lucerne	1.2	0.1	16	17.3	17.9	96.6	6.9	0.6	92.5
MMW	1.4	0.16	14.1	15.7	17.8	88.0	8.9	1.0	90.0
MMWW	1.7	0.2	18.7	20.6	22.8	90.4	8.3	1.0	90.8
WBCWBL4	1	0.07	14.7	15.8	15.9	99.2	6.3	0.4	93.2
WBCWBL1	0.9	0.11	14.2	15.2	18.0	84.5	5.9	0.7	93.4
Natural	1.7	0.31	11	13.0	13.7	95.0	13.1	2.4	84.6
10 - 20 cm									
Lucerne	0.62	0.06	11	11.7	13.4	87.2	5.3	0.50	94.2
WBCWBL4	0.72	0.06	12	12.8	13.5	94.7	5.6	0.47	93.9
20 - 30 cm									
Lucerne	0.4	0.1	9.7	10.2	11.6	87.9	3.9	1.0	95.1
WBCWBL4	0.3	0.09	7.6	7.99	8.6	92.9	3.8	1.1	95.1

In all the treatments, the major part of the total SOC was associated with the mineral fraction at all depths. The organic C contained in the mineral fraction of the natural vegetated soil accounted for 85% of total organic C recovered at the 5-10 cm depth. The crop rotation treatments led to a higher percentage of organic C contained in the mineral fraction ranging from ca. 90- 93% in the 5-10 cm layer, ca. 88- 91% in the 0-5 cm layer, 94% in the 10-20 cm layer and 95% in the 20-30 cm layer of total recovered C (Figure 4.1 & 4.2). This indicates a general increase in the contribution of mineral-associated C to total C with depth. An increase in the proportion of the mineral fraction with depth is indicative of increased mineral association and protection with depth (Torn et al., 1997). The higher contribution of mineral C to total carbon in the cultivated treatments (90-93%) compared to the natural treatment (85%) can be mainly attributed to the higher clay content (Table 3.6). However, according to

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John et al. (2005), the lower input of organic matter and increased disruption of aggregates in agricultural soils leads to a loss of C in the fPOM and oPOM fraction and therefore, the proportion of C bound to minerals in forest or natural vegetated soil is lower due to higher free and occluded particulate organic matter. This corresponds well with what was found in this study.
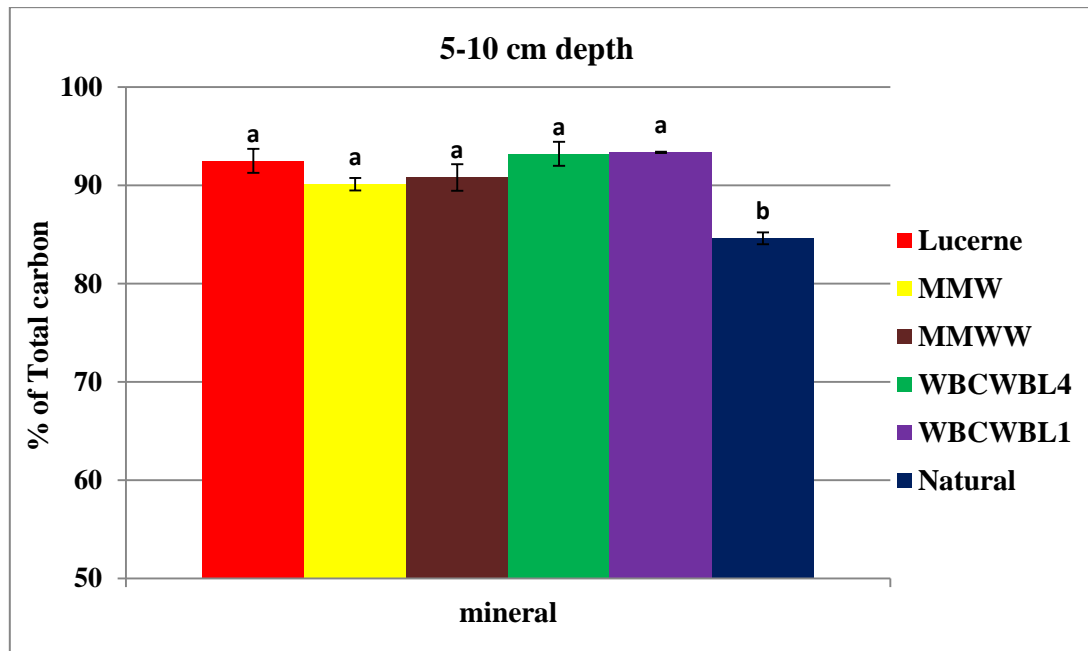
Figure 4.1 Relative contribution of organic carbon in the mineral fraction to total organic carbon of the different crop rotation treatments and natural vegetated soil at 5-10 cm.

Note: Error bars represent standard error, and alphabetic letters denote statistical differences between treatments according to Tukey's Studentized Range test at α = 0.05. Similar letters indicate lack of significant differences

Figure 4.2 Relative contribution of organic carbon in the mineral fractions to total organic carbon of the lucerne and WBCWBL4 treatments in the 0-5, 5-10, 10-20 and 20-30 cm layers.

Note: Error bars represent standard error.

The percentage of C contained in the fPOM fraction of the crop rotation treatments at the 5-10 cm layer accounted for ca. 5.9 - 8.9% of total C recovered while the fPOM fraction of the natural vegetated soil in the same depth accounted for 13% (Figure 4.4). The percentage of total C recovered contained in the fPOM fraction decreased with depth from 11.5% for the WBCWBL4 treatment in the 0-5 cm layer to 3.8% in the 20-30 cm layer with the lucerne treatment decreasing from 8% to 3.9% in the same depth increments (Figure 4.5a). A severe decrease was observe in the WBCWBL4 treatment from the 0-5 cm to 5-10 cm depth indicating the effect of aboveground material in the first 5 cm of the soil whereas the decrease in the same depth increments for the lucerne treatment was not as severe due to lower aboveground residues. Janzen et al. (1992) also reported that the light fraction of surface soil (0-7.5 cm) under wheat based rotations accounted for 2-17% of total SOC and stated that it depends largely on cropping systems (residue input and substrate decomposition). Typical photographs representing the free particulate organic matter (fPOM) in lucerne treatment and WBCWBL4 treatment appears in Figure 4.3. Clear visual differences (size) between the two treatments were observed.
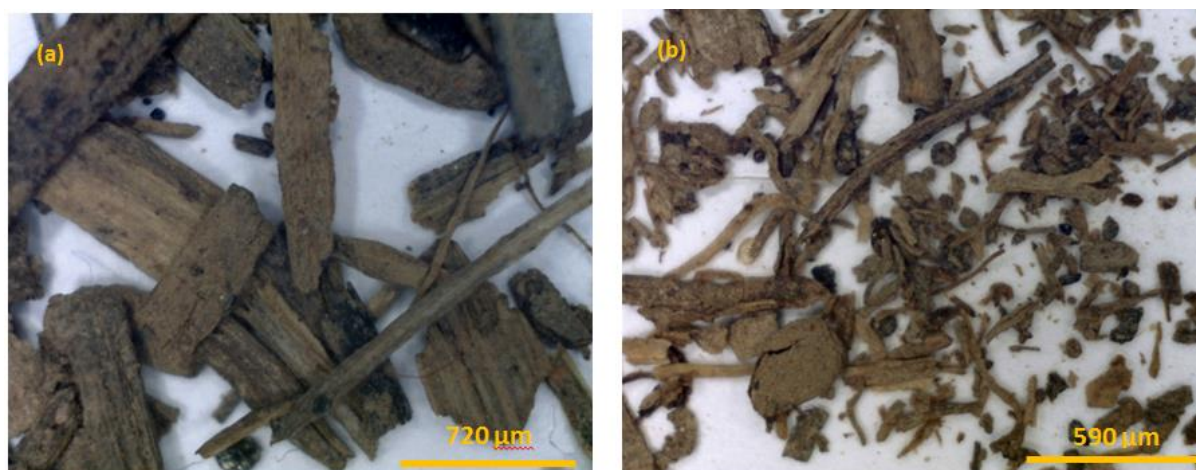
Figure 4.3 Digital images showing the difference in size of the free particulate organic matter (fPOM) in (a) WBCWBL4 treatment and (b) lucerne treatment.

The C stored in the occluded-POM fraction contributed the least to total C in the 5-10 cm depth and corresponds with the soil's weak aggregation (Figure 3.19). The oPOM fraction of the natural vegetated soil accounted for 2.4% of total C recovered which was the highest of all the treatments. The C that accumulated in the oPOM fraction of the crop rotation treatments accounted for ca. 0.4 - 1% of total C recovered (Figure 4.4). A general decrease in the contribution of C accumulated in the oPOM to total C with depth was observed up to the 20 cm layer whereas an increase was observed in the 20-30 cm layer for both treatments (Figure 4.5b). This trend is in agreement with the higher aggregate stability observed in the 20-30 cm depth. Due to the small contribution of the oPOM fraction to total C it is not considered as an important fraction in these soils.
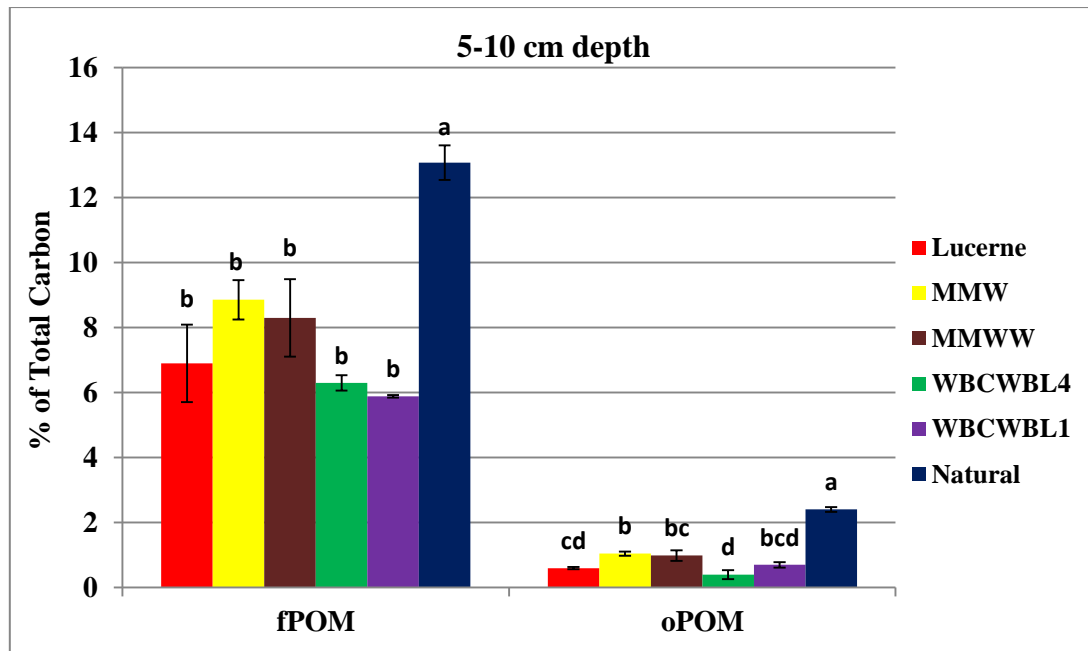
Figure 4.4 Relative contribution of organic carbon in the free-POM and occluded-POM fractions to total organic carbon of the different crop rotation treatments and natural vegetated soil at 5-10 cm.

Note: Error bars represent standard error, and alphabetic letters denote statistical differences between treatments according to Tukey's Studentized Range test at $\alpha = 0.05$. Similar letters indicate lack of significant differences.
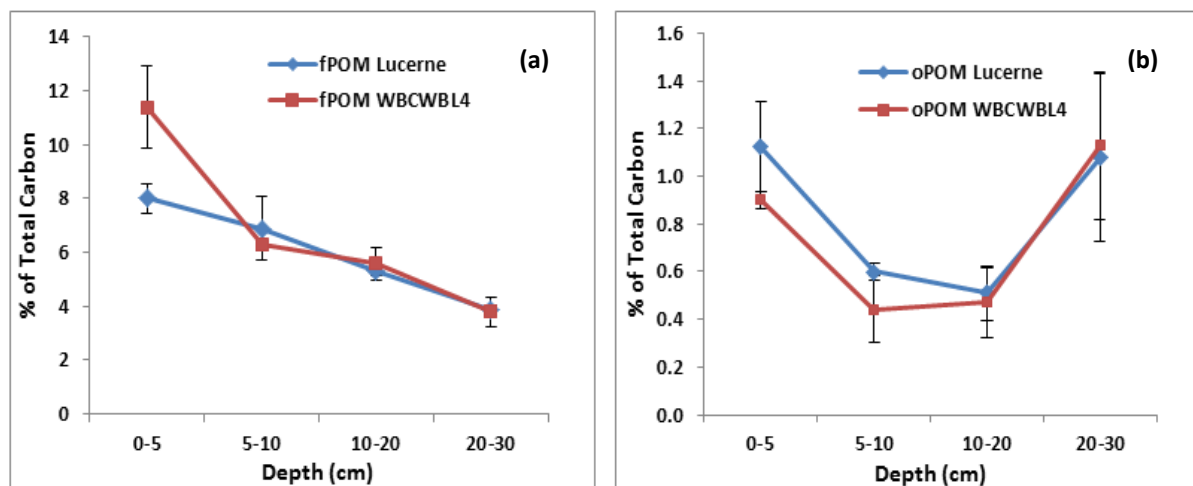
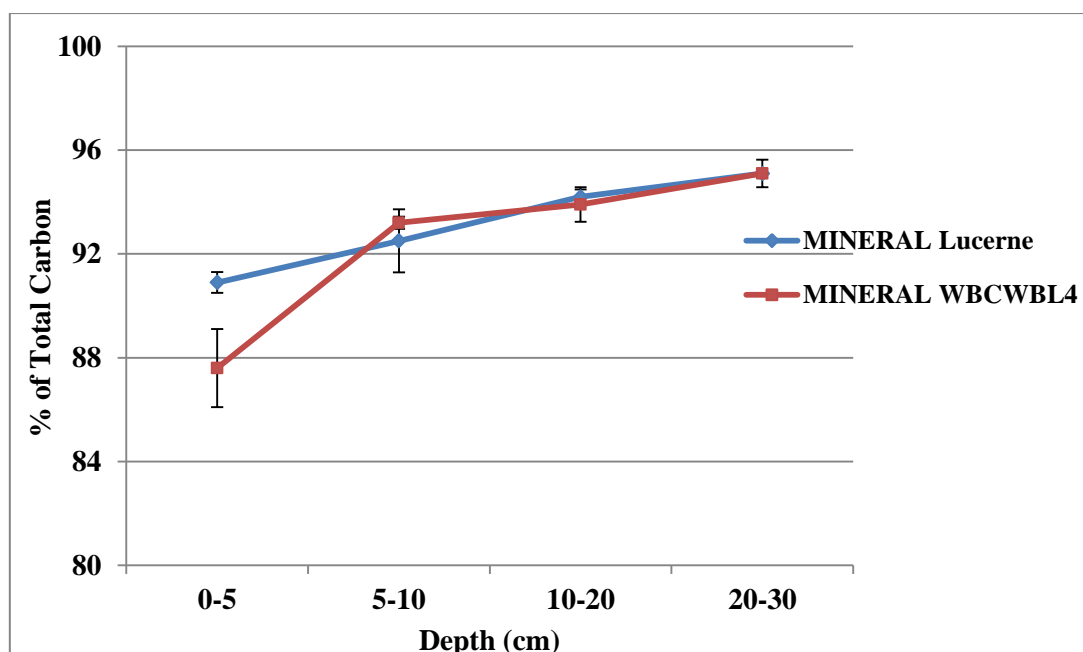**Figure 4.5** Relative contribution of organic carbon in the (a) free-POM and (b) occluded-POM to total organic carbon of the lucerne and WBCWBL4 treatments in the 0-5, 5-10, 10-20 and 20-30 cm layers.

Note: Error bars represent standard error.

Similar proportions were found by Freixo et al. (2002) in no-till crop rotation (wheat/soybean & wheat/soybean/vetch/maize) practices in Southern Brazil as the average mineral fraction accounted for 84 to 98% of total recovered organic C with the average fPOM accounted for 1.3 to 15% and the average oPOM for 0.3 to 1.4% of total recovered C in 0-5, 5-10 and 10-20 cm depths. The relative contribution of the fPOM and mineral fraction to total SOC found in this study was also in the same order of magnitude as was found by John et al. (2005) for continuous wheat and maize plots under conservation tillage. According to Gregorich & Janzen (1996), agricultural soils typically contain 20-30% C in the fPOM fraction and make up 2-18% of total C which correlated well with findings in this study.

4.3.2. Carbon and Nitrogen content of SOM functional pools

The fPOM and oPOM fractions composed of only ca. 0.13-0.75% and ca. 0.013-0.05% respectively of the soil mass in the different treatments and depths. The organic C content determined in each fraction was multiplied with the mass to obtain the C contents of the fractions on a whole soil basis (g C kg^{-1}). Significant treatment effects were observed on C contents in all three fractions while significant differences in N content were found in the fPOM fraction.

a) fPOM and oPOM fractions

The content of organic C in the fPOM fraction ranged between 0.9 and 1.7 g C kg^{-1} in the 5 - 10 cm layer for the arable treatments with the two crop pasture rotation systems (MMWW & MMW) containing the highest amount of C of the cultivated treatments. The MMWW and natural vegetated treatment (1.74 & 1.69 g C kg^{-1} respectively) contained significantly higher (P = 0.003) C contents than the WBCWBL4 and WBCWBL1 treatments (1 and 0.9 g C kg^{-1} respectively) with the MMW treatment containing 1.37 g C kg^{-1} in the fPOM fraction. The 100% pasture (lucerne) had a C content of 1.16 g C kg^{-1} in the fPOM fraction (Figure 4.6 & Table 4.1). As total and, especially, labile organic C content are climate and soil specific and also very sensitive to management, it is important that a comparison is made to a reference soil (natural vegetated soil) that is unaffected by agricultural management in order to determine the effect of management practices on C content. Compared to the natural vegetated soil, the amount of C in the fPOM fraction of the agricultural soil decreased on average by 27% with only the MMWW crop rotation system containing the same amount of C than the natural vegetated soil. The natural vegetated soil is covered mostly by grasses and

therefore it can be expected that the free particulate organic matter will be depleted when it is converted from "grassland" to arable rotation (Christensen, 1996).

The C content of the oPOM fraction ranged between 0.07 and 0.31 g C kg^{-1} for the different treatments in the 5-10 cm layer. The natural vegetated soil contained significantly (P < 0.0001) higher C than all the crop rotation treatments indicating the effect of cultivation on soil C occluded within aggregates. The MMWW treatment contained the highest C content (0.20 g C kg^{-1}) in the oPOM fraction of the arable treatments, significantly higher than the lucerne (0.07 g C kg^{-1}) and WBCWBL4 (0.065 g C kg^{-1}) treatments (Figure 4.6).
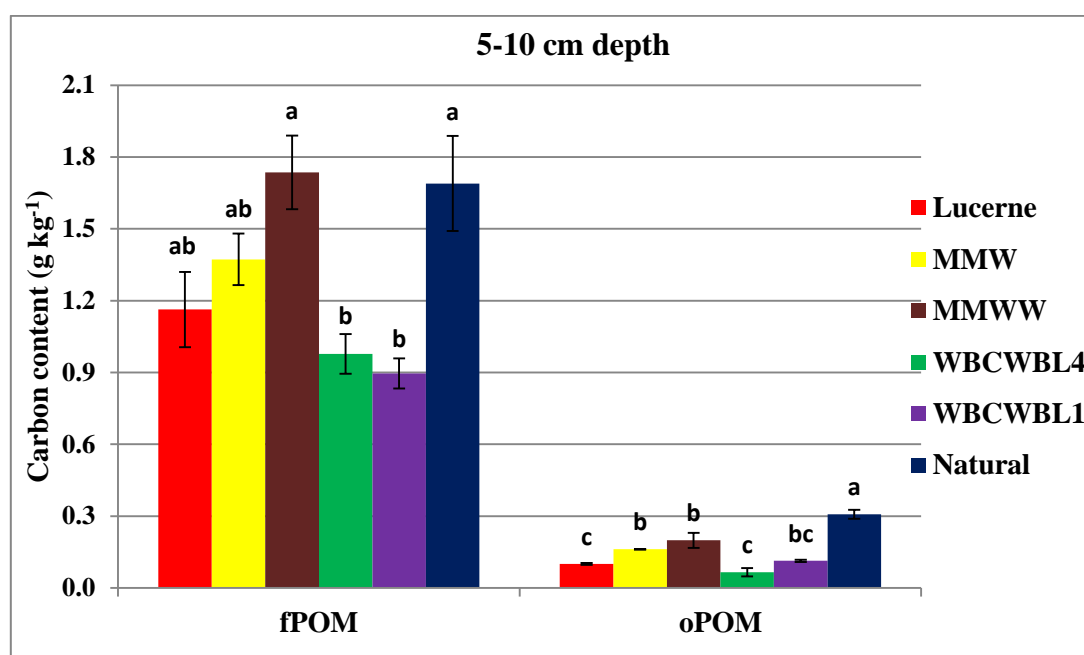
Figure 4.6 The carbon content recorded in the fPOM and oPOM fraction of the different treatments at 5 - 10 cm depth.

Note: Error bars represent standard error, and alphabetic letters denote statistical differences between treatments according to Tukey's Studentized Range test at α = 0.05. Similar letters indicate lack of significant differences.

On average across the WBCWBL4 and lucerne treatments the C content in the fPOM fraction decreased from 2.0 in the 0-5 cm to 0.67 g C kg^{-1} in the 20-30 cm depth. The C content of the oPOM fraction followed a different trend with depth than the other fractions as a general decrease in C was observed up till 20 cm (0.2-0.06 g C kg^{-1}) whereas an increase in C content was observed in the 20-30 cm depth (0.1 g C kg^{-1}). No significant differences in C content were found between the two treatments in the different fractions and depths but clear trends were observed (Figure 4.7).
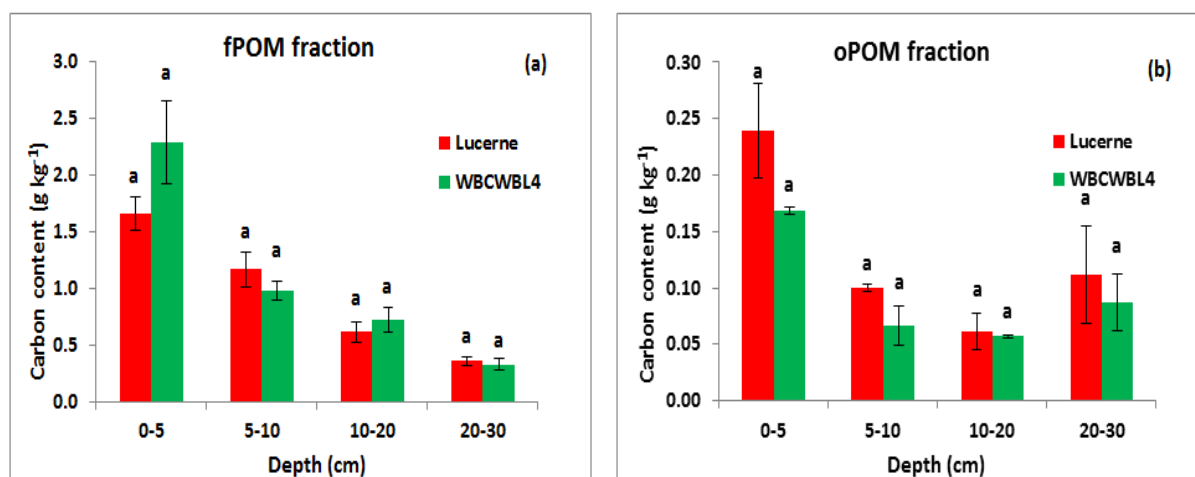
Figure 4.7 The carbon content obtained in the (a) fPOM fraction and (b) oPOM fraction of the lucerne and WBCWBL4 treatments at the four depth increments (0-5, 5-10, 10-20, 20-30 cm).

Note: Error bars represent standard error, and alphabetic letters denote statistical differences between treatments at specific depths according to Tukey's Studentized Range test at α = 0.05. Similar letters indicate lack of significant differences.

The N content in the fPOM fraction (5-10 cm depth) was found to be significantly higher (P <0.0001) in the lucerne, MMW and MMWW treatments compared to the 100% crop rotation systems and also higher, but not significantly, than the natural vegetated soil (Figure 4.8). The MMWW and MMW rotation systems contained N values of 0.111 g kg^{-1} and 0.107 g kg^{-1} respectively compared to the 0.045 and 0.042 g kg^{-1} in the WBCWBL4 and WBCWBL1 rotation systems respectively.

Figure 4.8 Nitrogen content in the fPOM fraction of the different treatments at 5-10 cm depth.

Note: Error bars represent standard error, and alphabetic letters denote statistical differences between treatments according to Tukey's Studentized Range test at $\alpha = 0.05$. Similar letters indicate lack of significant differences.

In the 0-5 cm depth, the 100% crop rotation system (WBCWBL4) contained more C than the 100% pasture (lucerne) in the fPOM fraction. This can be attributed to higher aboveground inputs of various crops versus the low aboveground inputs of lucerne contributing to lower particulate organic matter content, especially in the litter layer (0 - 5 cm). As was observed with the total C content, the C content in the fPOM fraction decreased with depth in both the WBCWBL4 and lucerne treatments, especially from the 0-5 to 5-10 cm layer as the 5-10 cm depth excludes the litter layer (Figure 4.7a). The amount of C occluded within aggregates (oPOM fraction) followed the same trend with depth as was observed with aggregate stability (Figure 4.7b). The higher the aggregate stability, the more carbon was occluded within the aggregates and/or vice versa as aggregate stability is largely affected by the amount of C in the soil. Carbon occluded within aggregates is physically protected against microbial degradation and this spatial inaccessibility is an important C stabilizing mechanism (section 4.3.5.1).

The fPOM fraction is a very sensitive indicator of the effects of cropping on SOM content and composition and reflects mainly short-term effects (Janzen et al., 1992; Freixo et al., 2002). The fPOM fraction represents an intermediate pool between undecomposed residues and humified SOM and consists mainly of fresh plant residues and therefore its amount

depend directly on the crop rotation adopted (quantity and quality of C input) (Gregorich et al., 2006). According to Haynes (2005) increases in particulate organic matter usually reflects greater above- and belowground inputs which is then expected to be translated into higher SOM contents in the longer term. However, according to Biederbeck et al. (1994) factors other than the amount of residues returned to the soil regulate the amount of POM. Factors, like temperature and moisture, especially in a relative dry region, can constrain decomposition of physically non-complexed OM. The extent of disturbance in the different treatments can also play an important role.

Bolinder et al. (1999) like Janzen et al. (1992) and Freixo et al. (2002) stated that both C and N content of the fPOM fraction is very sensitive indicators of the effect of agricultural management on SOM. This was confirmed in this study and is shown in Figure 4.9. Comparing total C and N content to the C and N content in the fPOM fraction in the 5-10 cm depth, greater differences were observed between cropping systems in the fPOM fraction. The two crop-pasture rotation systems contained significantly more C and N in the fPOM fraction than the two 100% crop rotation systems. The higher N content can be attributed to the quality of the lucerne and medic residues (high N) while the higher C content is attributed to higher belowground inputs of especially medic roots in the crop-pasture systems. The two crop-pasture systems were also disturbed to a much lesser extent since the start of this experiment 11 years ago compared to the 100% crop rotation systems. With the tine no-till planter that was used, the first 10 cm of the soil is still disturbed and therefore during planting time, it is well exposed (less soil cover) to oxidising conditions causing more C being mineralized to CO_2.

It is also evident (Figure 4.9) that the natural treatment contained the lowest total C content, but together with the MMWW treatment, contained the highest C content in the fPOM fraction. This is another indication that the fPOM fraction is more sensitive to management practices (tillage and crop rotations) than total C as the natural vegetated soil has never been subjected to any management. The lower input of OM in agricultural soils compared to the natural vegetated soil can also lead to a preferential loss of organic C from fPOM fraction. According to Neff et al. (2002) N additions to the soil (cultivated treatments) significantly increase decomposition rates of light soil C fractions (fPOM).

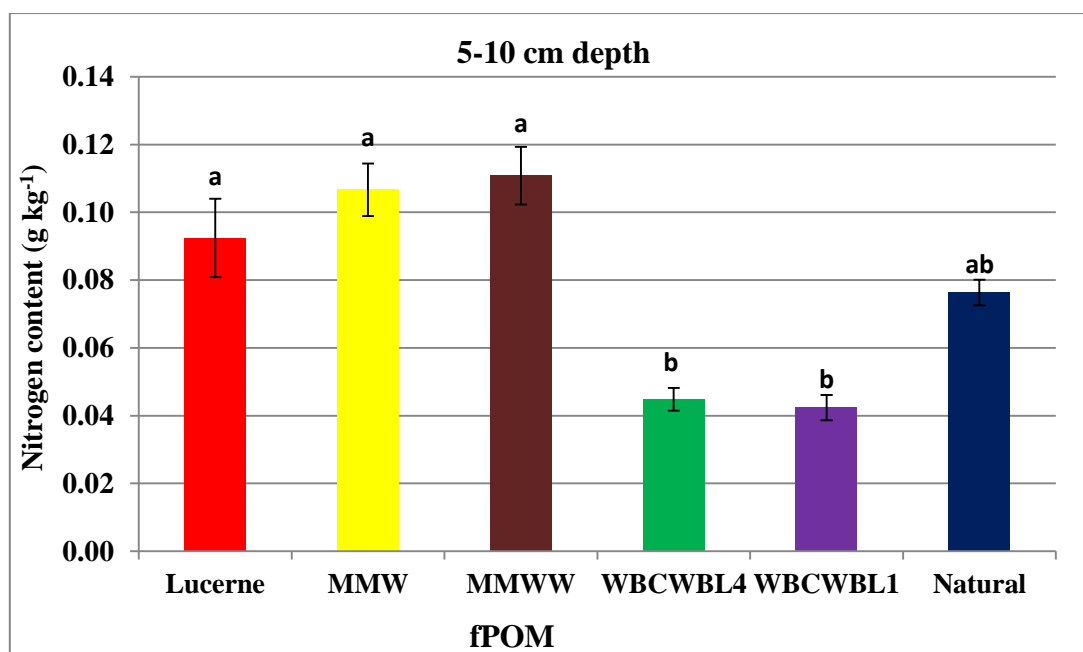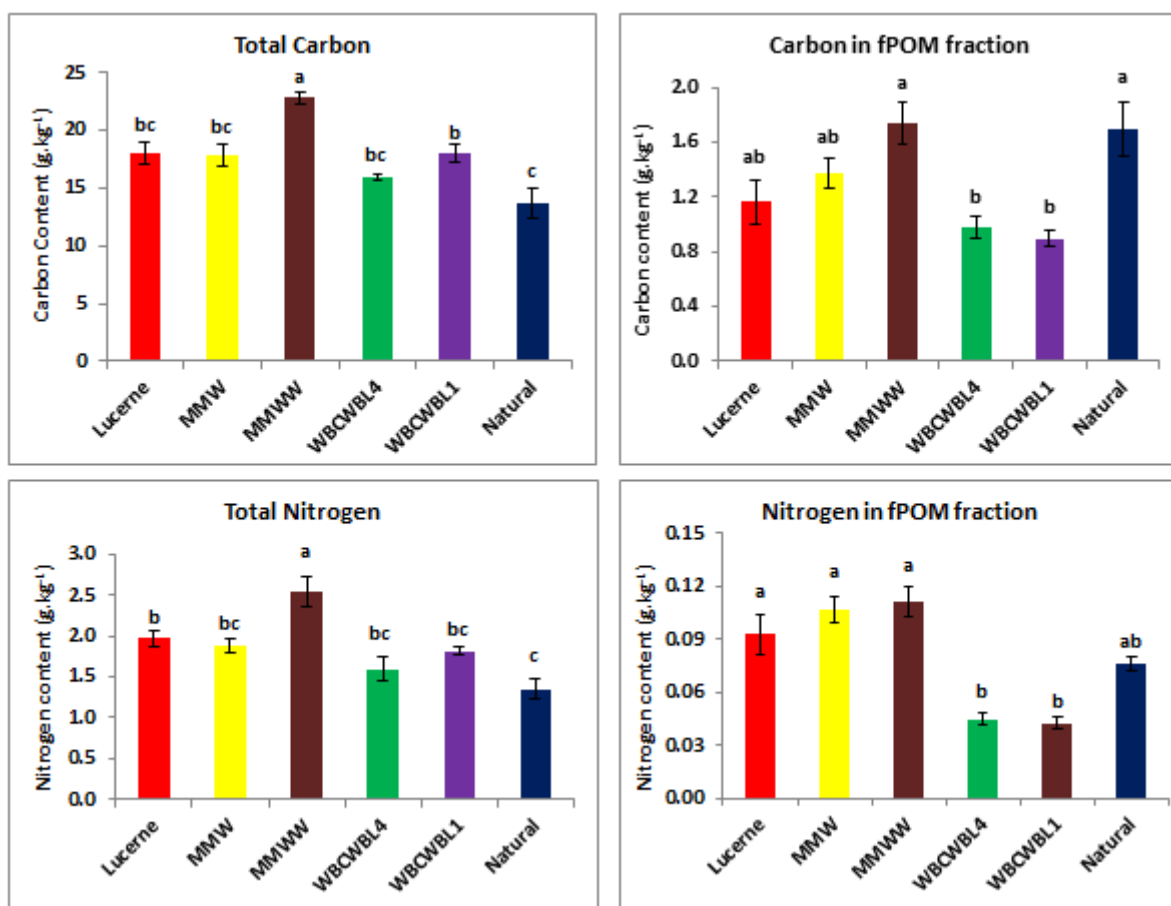**Figure 4.9** Comparison between total C and N contents and C and N content in the FPOM fraction in the different treatments at 5-10 cm depth.

Note: Error bars represent standard error, and alphabetic letters denote statistical differences between treatments according to Tukey's Studentized Range test at $\alpha = 0.05$. Similar letters indicate lack of significant differences.

This pool (fPOM fraction) is enrich with nutrients and C and is an important fraction that contribute to soil quality, although it represents only a small portion of the soil mass (Haynes, 2005). As discussed in Chapter 3, the yield and quality of the wheat was higher in the crop-pasture systems and were attributed to a higher total SOM content. However, according to Lal (2006), an increase in crop yields due to an increase in SOM pool is mainly related to an increase in the labile fraction (fPOM). The C in the labile or fPOM fraction serves as the primary energy source for heterotrophic organisms and therefore from a biological standpoint a very important pool (Gregorich et al., 2006). The active microbes also derive nutrients from the molecules in SOM and if the nutrients are not taken up by the microbes, they are available for plants, which means the higher the N content, as was found in the crop pasture and lucerne treatments, the more N will be available for the subsequent crop. The labile organic matter (fPOM) fraction can therefore be used as a monitor in changes in soil quality as it

reflect the diverse, but central, roles that organic matter have concerning soil properties and the ability of the soil to function (Haynes, 2005). According to Biederbeck et al. (1994), the C and N in the fPOM fraction may provide an early indication of future trends in OM response to cropping systems.

b) Mineral fraction

The content of organic C in the mineral fraction ranged between 11 and 18.7 g C kg^{-1} between the different treatments in the 5 - 10 cm layer. The MMWW treatment (18.7 g C kg^{-1}) contained significantly (P = 0.0022) higher C content than the MMW, WBCWBL1 and natural treatments with C values of 14.1, 14.2 and 11 g C kg^{-1} respectively. The lucerne and WBCWBL4 treatments contained values of 16 and 14.7 g C kg^{-1} (Figure 4.10 & Table 4.1). On average, across the WBCWBL4 and lucerne treatments the C content in the mineral fraction decreased from 18.4 in the 0-5 cm to 8.7 g C kg^{-1} in the 20-30 cm depth (Figure 4.11) following the same trend as total organic C (Figure 3.10a). No significant differences in C content between the lucerne and WBCWBL4 treatments were found except in the 20-30 cm depth where lucerne contained a significantly (P = 0.0072) higher C content.
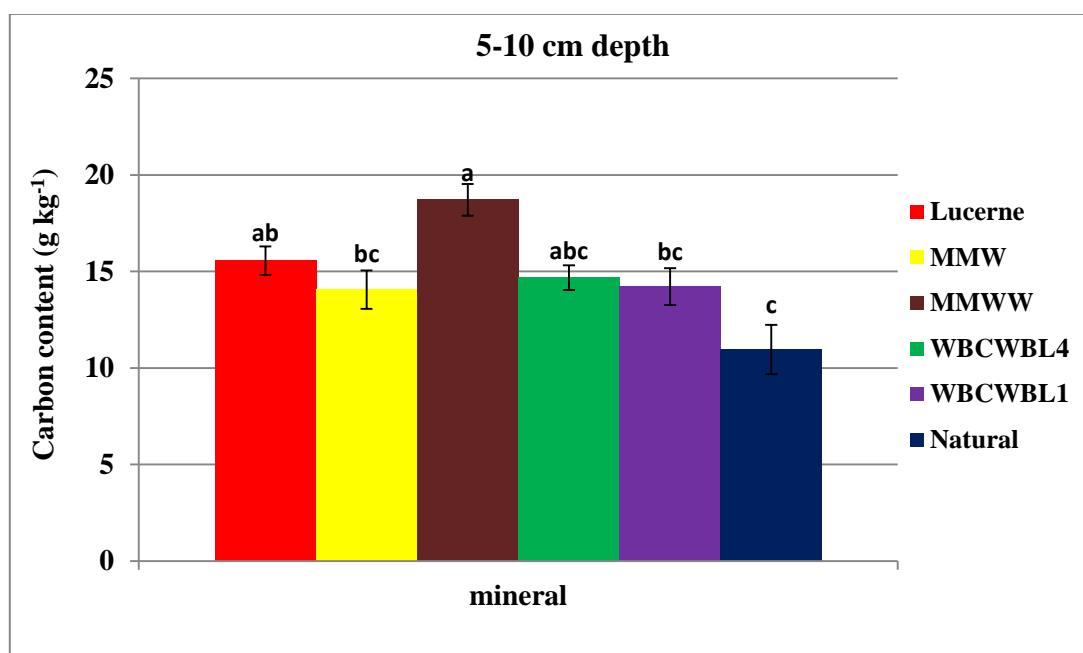
Figure 4.10 The carbon content obtained in the mineral fraction of the different treatments in the 5-10 cm depth.

Note: Error bars represent standard error, and alphabetic letters denote statistical differences between treatments according to Tukey's Studentized Range test at α = 0.05. Similar letters indicate lack of significant differences.

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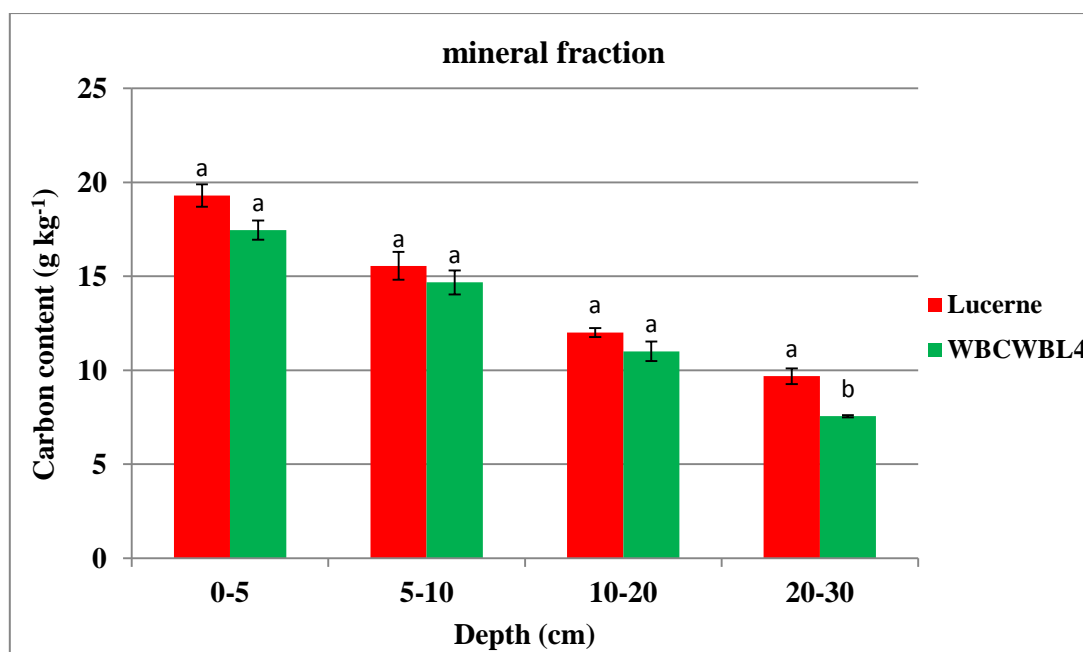
Figure 4.11 The carbon content obtained in the mineral fraction of the Lucerne and WBCWBL4 treatments at the four depth increments (0-5, 5-10, 10-20, 20-30 cm).

Note: Error bars represent standard error, and alphabetic letters denote statistical differences between treatments according to Tukey's Studentized Range test at α = 0.05. Similar letters indicate lack of significant differences.

These results obtained support the fact that the C in the mineral fraction determines the total C content of these soils. The significantly higher C content in the mineral fraction found for the MMWW treatment in the 5-10 cm depth can possibly be attributed to a higher clay content in this treatment since the same trend was observed as was found for the clay content in the different treatments (Table 3.6). Sequestered C is stored mainly in the mineral associated fraction and is therefore a very important long-term stabilized fraction for C sequestration. Kögel-Knabner (2008) also stated in her review that the interaction of organic C with mineral surfaces, especially with Fe-oxide surfaces, is the major control of long-term organic C preservation. The mineral fraction contains more humified SOM and can be a major sink for C storage in soil as the C stabilized via the minerals is not easily decomposed. This was partially verified by poor correlation ($R^2 = 0.04$) between mineral C content and soil respiration rate (CO_2 efflux) (Figure A5).

A significant (P = 0.0025; $R^2 = 0.55$) linear correlation was found between mineral C and effective cation exchange capacity (ECEC). This indicates that an increase in C in the mineral fraction enhances the ECEC of the soil (Figure 4.12). Weak correlations were found between ECEC and the C content of the fPOM ($R^2 = 0.22$) and oPOM ($R^2 = 0.22$) fraction (Figure A3

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& A4). This confirms Krull et al. (2004) statement that the fPOM fraction is important for certain soil functions but contributes little to the ECEC of the soil.
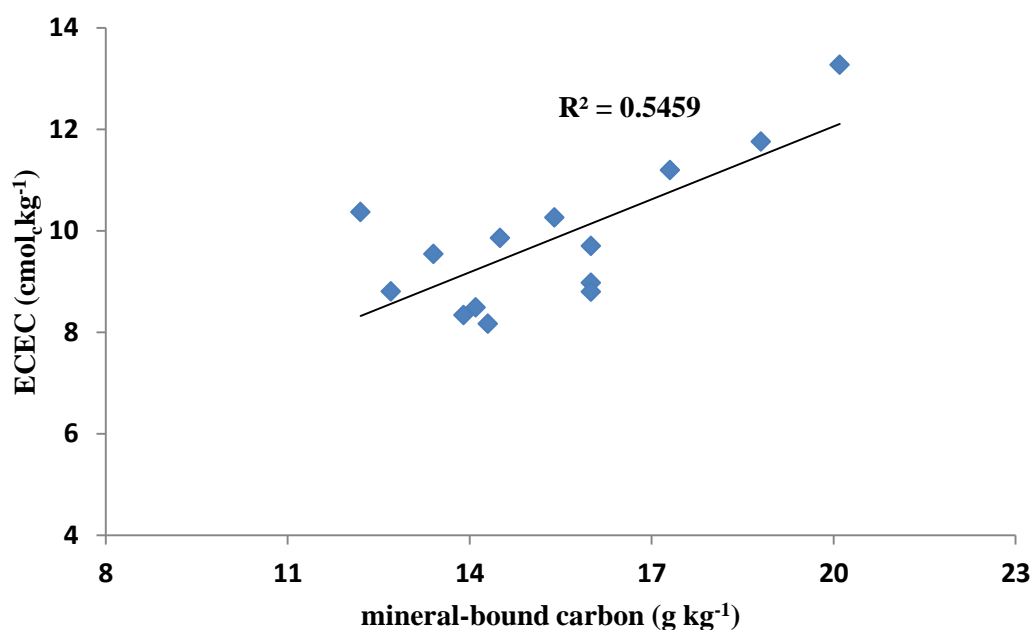
Figure 4.12 Relationship between mineral-bound carbon and ECEC.

4.3.3. C:N ratio of SOM functional pools

The C:N ratios of the isolated fractions in the different treatments were similar to what was reported by Tan et al. (2007), Gregorich et al. (2006) and Grünewald et al. (2006) in their studies, relating to organic matter characteristics and decomposition. The C:N ratio of the mineral fraction was consistently smaller than that of the POM fractions in all treatments and depths. The composition of the residues found in the fPOM fraction was characterized with a relative high C:N ratio (intermediate between bulk soil and plant tissue) ranging between 13 and 22 for the lucerne and WBCWBL4 treatments respectively in the 5-10 cm depth (Figure 4.13). This signifies the dominance of less decomposed plant components in this fraction (Gregorich et al., 2006) as it contains a mixture of the residues of plants, animals, and microorganisms at different stages of decomposition (Baisden et al., 2002). The fPOM fraction of lucerne and the two crop pasture treatments (MMW & MMWW) had a significant ($P < 0.0001$) lower C:N ratio compared to the 100% crop rotation systems (WBCWBL4 & WBCWBL1). This can mainly be attributed to the high N content of above- and belowground residues of lucerne and medics (both legumes). The lower C:N ratio observed in the fPOM fraction of the pasture and crop-pasture systems (legume pastures) compared to the 100% crop rotation systems will enhance the mineralization of organic N into inorganic N

(plant available N). This means that more N is available for the subsequent crop, in this case wheat, in the crop pasture systems. On average, the C:N ratio (18:1) of the fPOM fraction in the different treatments at a 5-10 cm soil depth was found to be much wider than the bulk (total) C:N ratio (10:1) in the same depth. The fPOM pool is seen as an intermediate pool between fresh plant residues and stabilized SOM (Gregorich et al., 2006).
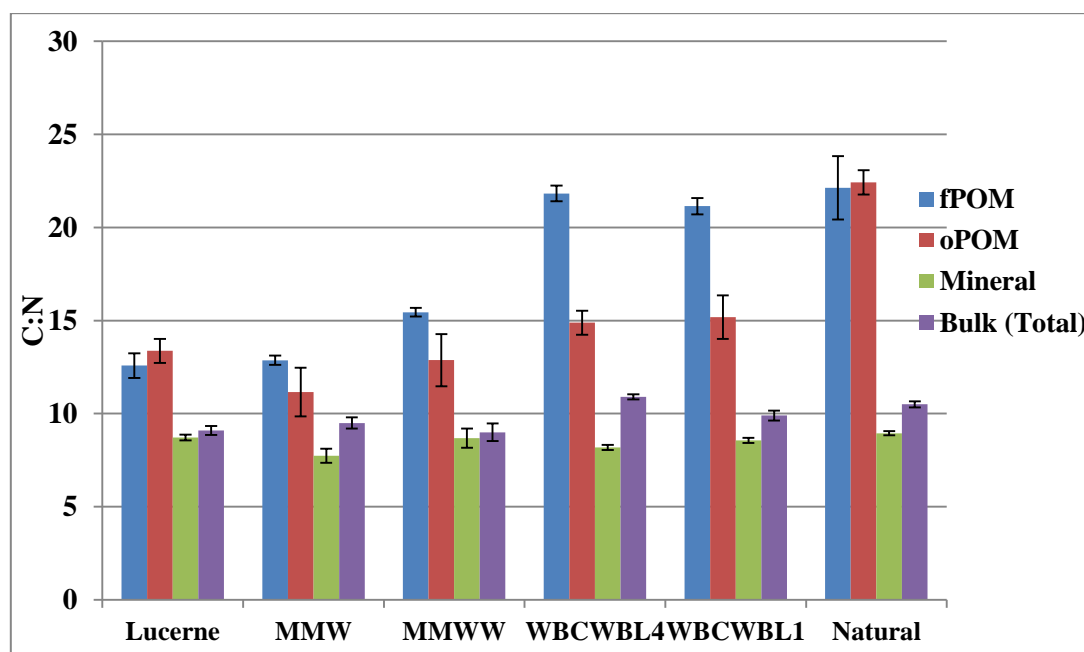
Figure 4.13 The C:N ratio of the three fractions; fPOM, oPOM, mineral and the bulk soil determined at the 5-10 cm layer of the different treatments.

Note: Error bars represent standard error.

A general decline in the C:N ratio from the fPOM fraction to the oPOM was detected except for the lucerne and natural vegetated soil where the C:N remained more or less the same than the fPOM fraction. A significant decline in the C:N ratio from the fPOM to oPOM was observed in the two 100% crop rotation systems. This general decline in C:N ratio from the fPOM to oPOM corresponds well with findings in other studies (Grunewald et al., 2006; Gregorich et al., 2006; Baisden et al., 2002) and suggest a stronger contribution of microbial biomass to the oPOM fraction as microbial products is rich in N. The decreasing C:N ratio in this order fPOM > oPOM > mineral also indicates an increasing degree of degradation and humification of the organic matter (Baisden et al., 2002; John et al., 2005). In all the treatments the C:N ratio of the mineral fraction was 9 and below indicating the ability of minerals to adsorb and protect well decomposed organic matter and microbial products

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(Golchin et al., 1994b). The low C:N ratio of the mineral fraction also indicates efficient isolation of the carbon fractions

The C:N ratio in the different depths of the lucerne and WBCWBL4 treatments is shown in Table 4.2. The C:N ratio varied between 11 and 25 for the fPOM and oPOM fractions while the ratio of the mineral fraction was generally smaller than the bulk soil in all depths. No real trend with depth was observed in all three fractions.

Table 4.2 C:N ratio of the three fractions; fPOM, oPOM and mineral and the bulk soil determined at the 0-5, 5-10, 10-20 and 20-30 cm layers of the lucerne and WBCWBL4 treatments.

Depth (cm)	fPOM		oPOM		Mineral		Bulk Soil	
	Lucerne	WBCWBL4	Lucerne	WBCWBL4	Lucerne	WBCWBL4	Lucerne	WBCWBL4
0-5	12.6	21.5	13.6	11.3	8.9	9	8.4	10.8
5-10	12.6	21.8	13.4	14.9	8.7	8.2	9.1	10.9
10-20	11.4	21.1	18.2	16.2	7.7	7.2	8.1	10.7
20-30	12.2	24.5	10.6	14.6	6.6	5.4	6.8	7.6

4.3.4. Relationship between characteristics of the fPOM fraction and total soil respiration (CO_2 efflux)

Soil organic matter in the fPOM fraction is physically non-complexed and is therefore easily accessible for microbes to decompose. A significant (P < 0.0001; R^2 = 0.74) linear relationship between the C content of fPOM fraction and total soil respiration was found (Figure 4.14). This is in line with Janzen et al. (1992) signifying that the fPOM fraction is a useful indicator of labile organic matter. A positive relationship was expected as the fPOM acts as substrates for mineralization of C and N (Haynes, 2005). Another important characteristic that contributes to the decomposability of organic material is the C:N ratio (Praveen-Kumar et al., 2003). A significant (P = 0.0011; R^2 = 0.67) linear relationship was also found between the C:N ratio of the crop rotation systems (MMWW, MMW, WBCWBL4 & WBCWBL1) and soil respiration rates obtained in September (Figure 4.15). The C:N ratio of the two crop-pasture systems was significantly lower than the continuous cropping systems and resulted in a higher CO_2 efflux. The lower the C:N ratio the more decomposable the material is (Praveen-Kumar et al., 2003) and this enhances microbial activity and the release of nutrients
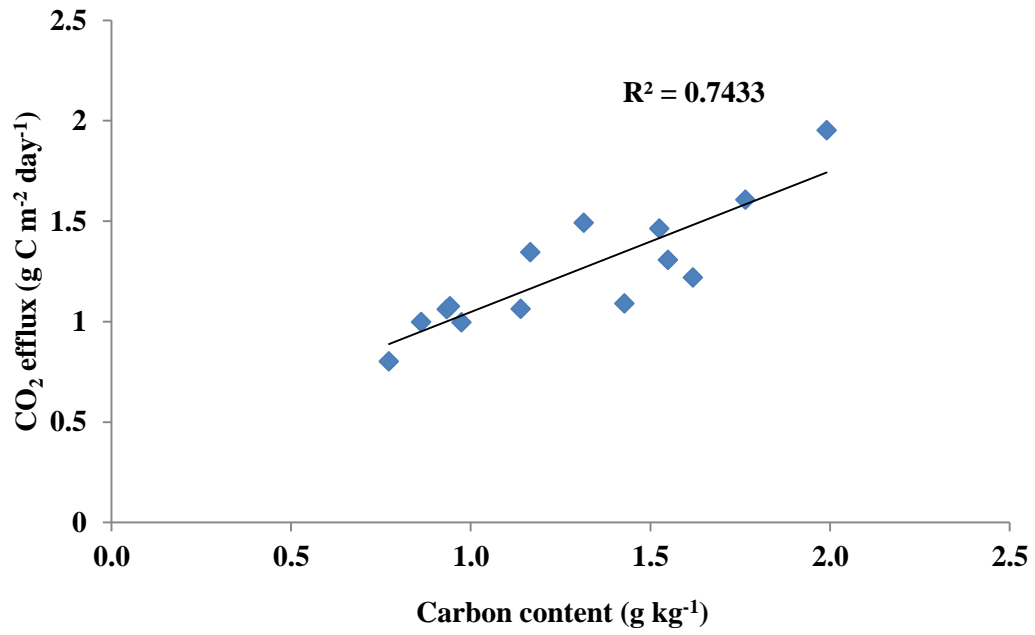
Figure 4.14 Relationship between carbon content (fPOM fraction) and CO_2 efflux (September) determined in the different treatments.
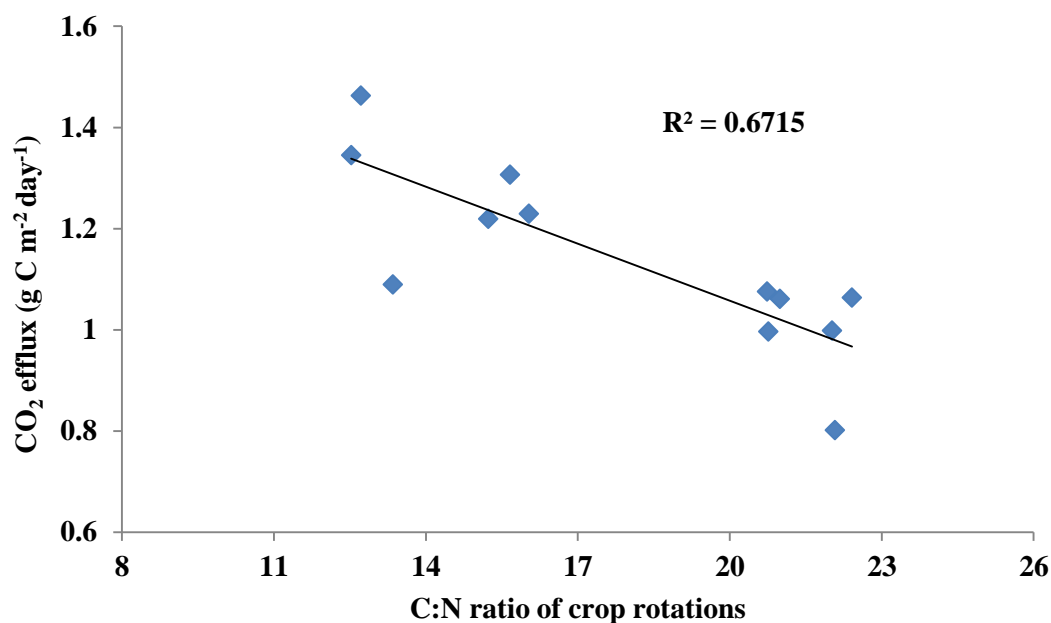
Figure 4.15 Relationship between C:N ratio of the different crop rotation systems and the CO_2 efflux (September).

However, contribution of autotrophic root respiration to total amount of CO_2 produced from soils cannot be ignored (Holt et al., 1990). In order to determine the rate of turnover of organic matter (heterotrophic respiration) the contribution of roots to total CO_2 release should actually be determined. This limits soil respiration rates being an indicator of SOM decomposition but Janzen et al. (1992) still found, like in this study, a correlation between the light fraction (labile carbon) and soil respiration rates. In four of the treatments (MM<u>W</u>W, MM<u>W</u>, WBC<u>W</u>BL4 & <u>W</u>BCWBL1) in this study, wheat was on the field at the time of sampling and therefore the effect of root respiration can be neglected.

4.3.5. Carbon stabilizing mechanisms

As already shown in this chapter, a large amount (± 90%) of the total SOM is stored in the stable mineral fraction due to stabilizing mechanisms operating in the soils. In order to elucidate the possible C stabilizing mechanisms responsible for SOC stability it was necessary to examine the relationship of the C content of the oPOM and mineral fractions with selected soil properties such as aggregate stability, clay content and metal-oxide content. Two main possible soil carbon stabilization mechanisms that can contribute to SOM stabilization have been considered; (i) physical protection via occlusion in aggregates and (ii) chemical interaction with soil minerals (phyllosilicates and metal-oxides).

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4.3.5.1. Physical protection via occlusion in aggregates

Spatial inaccessibility of OM in soil aggregates is an important mechanism in controlling long-term stabilization of C (von Lützow et al., 2006). Organic matter spatially protected by occlusion within aggregates is shielded against decomposition and stabilized due to restricted accessibility for microorganisms and their enzymes, and restricted aerobic decomposition due to limited oxygen and extracellular enzymes (von Lützow et al. 2006). Aggregation is the C stabilizing mechanism most vulnerable to disturbance.

No relationship was found between total C and aggregate stability in the different treatments at the 5-10 cm depth. This indicates that only part of the total soil C is involved in stabilizing aggregates. A significant ($P < 0.0001$; $R^2 = 0.77$) linear relationship was thus found between the amount of C occluded within aggregates (intra-aggregate carbon) and aggregate stability (Figure 4.16). Golchin et al. (1995) found similar trends in their study with a 86% correlation. This means that the higher aggregate stability found in the crop-pasture systems compared to the 100% crop rotation systems leads to more C being stabilized within soil aggregates. The natural vegetated soil had the highest C content in the oPOM fraction of all the treatments due to the significant higher aggregate stability compared to the agricultural soils. Due to the weak aggregation of these soils, only a small part of total C (0.4-2.4%) was occluded within aggregates and therefore only contributes a very small proportion to total C content. Physical protection via occlusion in aggregates is therefore not a dominant C stabilizing mechanism in these soils.
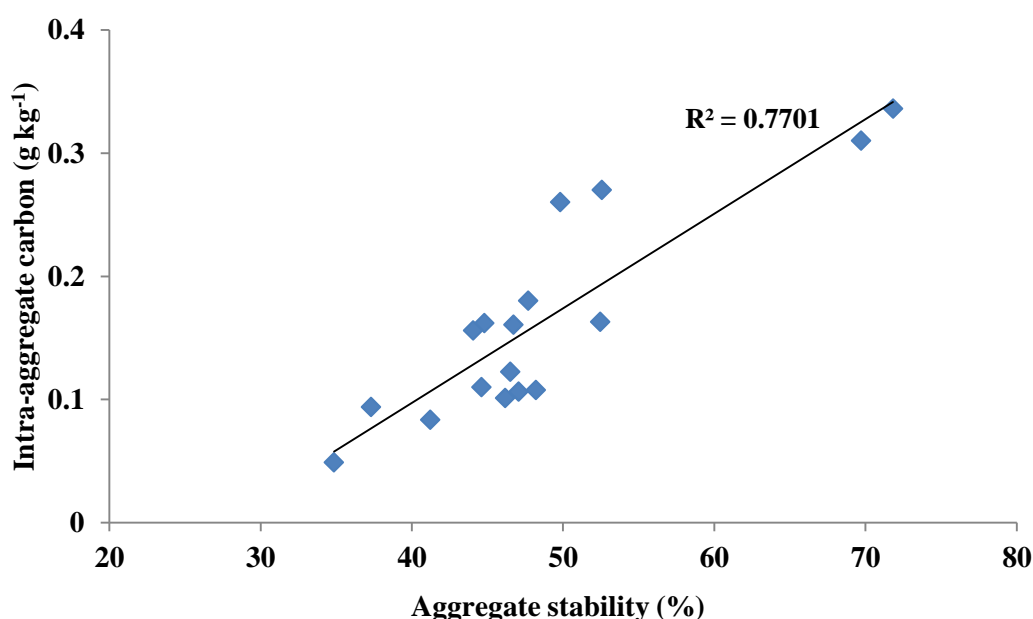
Figure 4.16 Relationship between carbon occluded within aggregates and aggregate stability (%).

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4.3.5.2. Chemical interaction with mineral particles

The clay mineral composition of the soil was dominated mainly by kaolinite, illite and quartz (Figure 3.9). Mineralogy plays an important role in the accumulation of SOC as it defines the capacity of soil minerals to adsorb and protect organic C through different stabilizing mechanisms (Krull et al., 2003; Kaiser & Guggenberger 2003; Mikutta et al., 2007). Each mineral matrix has a unique and limited capacity to stabilize organic matter (Baldock & Skjemstad, 2000) as it largely depends on the specific surface area (SSA) available for sorption (Kaiser & Guggenberger, 2003) as well as on the surface charge characteristics of the mineral.

Kaolinite is a 1:1 clay mineral with no permanent charge and a low specific surface area (SSA) (6-39 m^2g^{-1}) (Dixon 1977) and therefore this mineral has a low capacity to adsorb SOM. Quartz which is also known for having a very low SSA was reported by Nciizah & Wakindiki (2012) to have a strong negative relationship ($R^2 = 0.74$) with SOM while they found a significant relationship ($R^2 = 0.83$) between hematite and SOM. Hematite has a high SSA (45 to 110 $m^2 \ g^{-1}$) (Fontes & Weed 1996) that results in higher adsorption of SOM (Baldock & Skjemstad, 2000). According to Robert & Chenu (1992) the specific surface area of illite ranges between 50-100 m^2g^{-1}. Surface charge also varies among clay type. For kaolinite and sesquioxides the net surface charge is pH-dependent and becomes more negative with increasing pH while illite (2:1 clay mineral) has a permanent negative charge (isomorphic substitution) and is largely unaffected by pH. The cation exchange capacity of kaolinite range between 3-15 $cmol_ckg^{-1}$ while illite has a cation exchange capacity of 10-40 $cmol_ckg^{-1}$ at pH (H_2O) 7. Due to the low exchange capacity, sorption of SOM on kaolinite and illite will mainly take place at the broken edges of these minerals while Fe-oxides will also contribute considerably to the sorption of SOM in these soils.

The relative proportion of C associated with the mineral fraction increased with soil depth (section 3.3.1). Carbon sorbed to mineral surfaces (sesquioxides and clay minerals) is effectively stabilized (turnover times up to millennia) due to strong organo-mineral bonds. This reduces their susceptibility towards oxidative attack and also lowers their bioavailability (Kögel-Knabner et al., 2008). Long-term stabilization or preservation is thus determined by the interaction with mineral surfaces (Kögel - Knabner et al., 2008).

In this study a significant correlation has been found between mineral C and clay content (P < 0.0001; $R^2 = 0.74$) as well as mineral C and Fe-oxide content (P = 0.0005; $R^2 = 0.57$) in the 5-

10 cm depth (Figure 4.17 & 4.18 respectively). According to Eusterhues et al. (2003); Kalbitz et al. (2005); Mikutta et al. (2006) and Wagai & Mayer (2007) iron oxides play an important role in stabilizing SOM through sorption while illite and kaolinite, the two dominant clay minerals, also seems to contribute significantly to the stabilization of C in the soils investigated here. With increasing clay content, the amount of organic C associated with the mineral fraction increased whereas the amounts of C stored in the oPOM and fPOM fractions were not related to soil clay content. The mineral fraction represent a significant fraction of the total SOM, depending on the soil particle size distribution due to strong bonds formed with clay minerals while weak bonds are formed with sand particles (von Lützow et al., 2007).
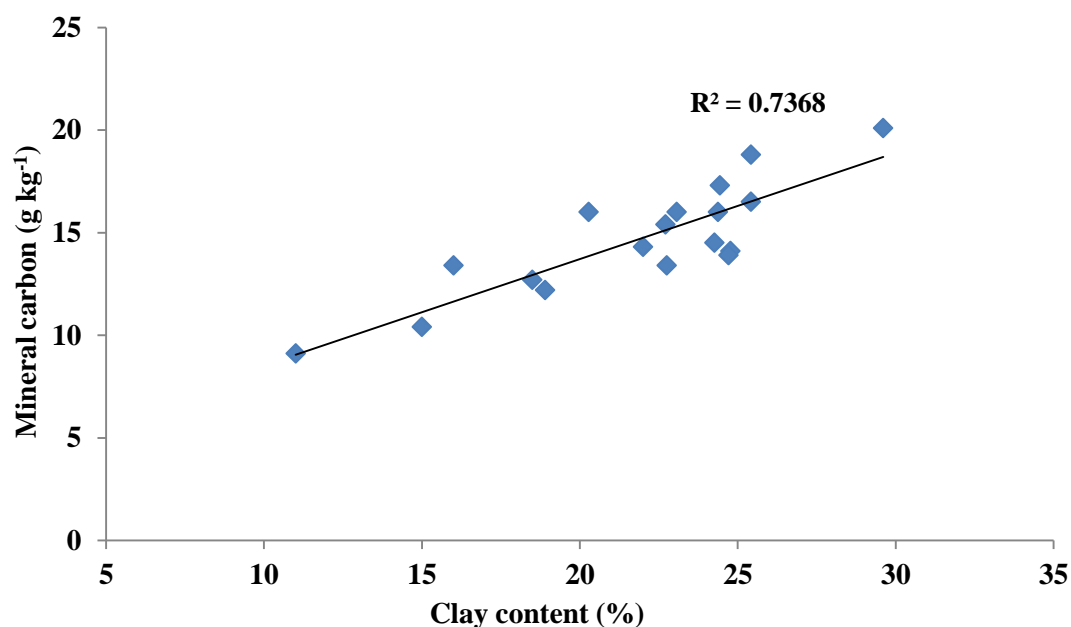
Figure 4.17 Relationship between mineral carbon and clay content (%).
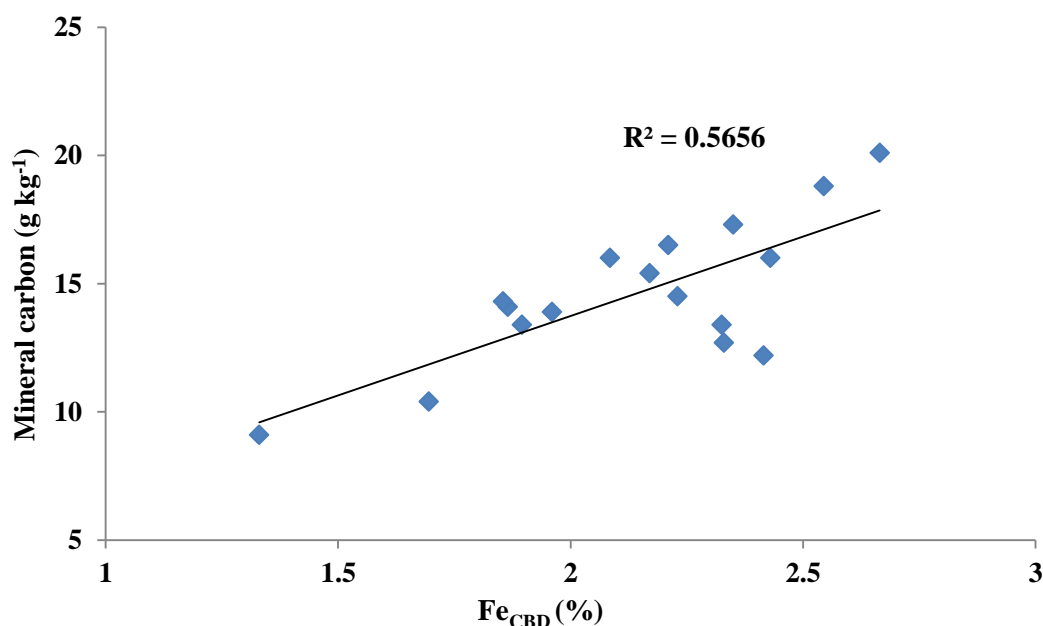
Figure 4.18 Relationship between mineral carbon and Fe-oxide content (%).

Organic matter can be bound to mineral surfaces through various mechanisms that can be considered for chemical interaction of OM with mineral particles (vön Lutzow et al., 2006). The major mechanisms by which organic matter adsorb onto minerals are: (i) displacement of surficial hydroxyl/water groups of minerals by organic functional groups (i.e., ligand exchange), (ii) cation-mediated bridging of OM to permanently negative-charged siloxane surfaces or to hydroxyls of phyllosilicates and oxides (cation bridging), and (iii) van der Waals interactions (vön Lutzow et al., 2006; Mikutta et al., 2007). According to Mikutta et al. (2007) organic matter is more resistant to mineralization if it is bound to minerals by ligand exchange compared to van der Waals forces and cation-bridges. In this study, the possible stabilizing mechanisms involved between minerals and organic matter will only be discussed built on theoretical assumptions which is based on the mineralogy of the soil and therefore the research of other authors.

Ligand exchange, Cation-bridging and Weak interactions

Anion chemisorption occurs on soil minerals that possess surface hydroxyl groups. Oxides and hydroxides of Fe, Mn and Al and edge sites of silicate clays are the most important minerals in this regard. Silicate clays such as kaolinite as well as oxides possess little or no permanent charge and for these minerals "edge" sites is very important (McBride, 1994). These soils are dominated by low activity clays (illite and kaolinite) and sesquioxides

resulting in a low cation exchange capacity. This causes ligand exchange being the dominant mechanism by which OM bind on the edges of kaolinite and illite and also on sesquioxides.

Chorover & Amistadi (2001) observed that high molecular weight aromatic constituents were preferentially sorbed to Fe-oxides (goethite) through ligand exchange. Gu et al. (1994) also found that organic matter was strongly sorbed to Fe-oxides via ligand exchange reactions and was the dominant interaction mechanism in acidic or slightly acidic conditions. Mikutta et al. (2007) found that 92% of forest floor organic matter interacted with goethite via ligand exchange at pH 4. Thus, ligand exchange occurs mostly in acid soils, rich in oxides according to several authors. However, the isoelectric points (i.e.p) for goethite is pH 8.4. This means that at the current soil pH ($\pm$ 7), goethite is still net positively charged (Chorover & Amistadi, 2001). Appel et al. (2003) also determined using three different techniques that the point of zero charge (p.z.c) for synthetic goethite ranged between 7.4 and 8.2. Anion sorption varies with pH, generally increasing with pH and reaching a maximum close to pKa for anions (Sparks, 2003). At pH of about 6-7, which is found in these soils, the carboxyl group will be deprotonated (COO^-). The dissociated functional group (carboxylate, $R-COO^-$) will thus be strongly attracted to the positive charged iron oxides (Chorover & Amistadi, 2001). Sorption of organic matter to Fe-oxides is very strong as only poor desorbability of OM bound to goethite was found, even under strong alkaline conditions (Kaiser & Guggenberger, 2007).

$$\equiv FeOH_2^+ + R\text{-}COO^- \leftrightarrow \equiv FeOOC\text{-}R + H_2O$$

$$\equiv FeOH^0 + R\text{-}COO^- \leftrightarrow \equiv FeOOC\text{-}R + OH^-$$
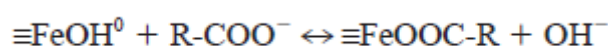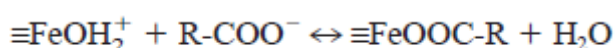
For kaolinite the point of zero charge (p.z.c.) was determined to average below pH 5 using different techniques (Appel et al., 2003) while Hussain et al. (1996) found that illite has p.z.c. at pH 2.5. According to Hussain et al. (1996) illite and kaolinite are negatively charged over the range from pH 2.5-11. This means that at the broken edges of the kaolinite and illite the functional groups such as O and OH will be deprotonated at soil pH causing the minerals to be negatively charged. However, maximum anion sorption still takes place close to the pKa values of the anions as was shown by Sparks (2003), irrespective of the negative charge on the broken edges of the minerals.

Cation bridging is the complexation of carboxyl groups of organic matter with surface bound polyvalent cations (Mikutta et al., 2007). Mikutta et al. (2007) found that sorption of OM on vermiculite (high cation exchange capacity) was mainly (78%) due to formation of Ca^{2+} bridges while OM on goethite (low cation exchange capacity), as mentioned previously, was

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mainly sorbed through ligand exchange. Due to the low exchange capacity of the dominant minerals in these soils, it is expected that cation bridging is not the prevailing mechanism operating in these soils. The clay minerals do not possess the capacity to adsorb large amounts of cations (Ca^{2+}, Mg^{2+}, Al^{3+}, Fe^{3+}) on their surfaces, and these cations can thus not sufficiently act as a bridge between the OM and clay minerals. Cation bridging will most likely play a minor role in the sorption of OM on minerals.

Van der Waals interactions of OM with mineral surfaces are more favourable at low pH when the functional groups of OM are protonated and the ionisation of carboxyl groups is inhibited (von Lützow et al., 2006). Organic matter bound via non-columbic interactions to minerals is easier to desorb and has a larger bioavailability. Kaolinite which is a non-expandable layer silicate doesn't have layer charge on its surface and therefore only weak-bonding affinities. A linkage between molecule and minerals with very low layer charge can be formed via hydrogen bonding (van der Waals forces) (Quiquampoix et al., 1995). Weak interactions can thus contribute to the sorption of OM on especially kaolinite surfaces but as mentioned, van der Waals interactions are more relevant in acidic and high-ionic strength soils.

Therefore, ligand exchange on the edge sites of kaolinite and goethite will most likely be the dominant carbon stabilizing mechanism operating in these soils. This corresponds with the significant correlation found between mineral carbon and the clay and Fe-oxide content.

4.4. Conclusion

Soil organic matter (SOM) and its different functional pools play an important role in contributing to a sustainable soil and optimizing crop production through an improvement in soil quality. The proportion of each pool to total SOM as well as the primary role that each pool plays in contributing to specific soil properties have been investigated in this chapter. The C stabilization mechanisms operating in these soils have also received a lot of attention in order to understand the relative importance of management practices and soil properties on soil C stabilization and therefore soil sustainability. A sustainable soil of high quality is vital to ensure food security due to an increasing population expected in the next few decades, especially in a developing country like South Africa.

After isolation of total SOM into its different pools it was found that the mineral fraction contributes on average ± 90% to total SOM in the different treatments. The content of organic C in the mineral fraction ranged between 11 and 18.7 g C kg^{-1} between the different treatments in the 5-10 cm layer with the MMWW treatment containing the highest C content

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in this fraction. The results obtained also support the fact that the C in the mineral fraction determines the total C content of these soils. The large contribution of mineral C to total C content in all of the cultivated treatments is promising as it shows that conservation agriculture is most likely contributing to a sustainable system. Carbon in this fraction is stabilized by clay minerals and this leads to long-term preservation in the soil. The cultivated treatments also compared well with the natural vegetated soil in terms of C accumulation in this fraction. However, lower input of OM in agricultural soils compared to the natural vegetated soil can also lead to a preferential loss of organic C from fPOM fraction. This increases the proportion of C bound to minerals in the cultivated treatments. The low C:N ratio (below 10) of the material in the mineral fraction in all of the treatments shows that it is dominated by humified organic matter. This is an indication of an active microbe population present in these soils as the microbes have the ability to break down the plant residues added to the soil into humus. The breaking down of plant material into humus leads to the release of important nutrients through mineralization. The humus contributes to soil quality by adding to the cation exchange capacity of the soil. Humus contains a lot of exchange sites and can therefore hold onto more nutrients. By enhancing the CEC, the efficiency of added fertilizers is increased.

The fPOM fraction contributed on average between 4-13% to total C content with a C:N ratio ranging between 13 and 22, an indication that this fraction is an intermediate pool between plant residues and humified organic matter. Although only a small fraction, it plays an important role in promoting certain soil functions. The C and N content in the fPOM fraction differed significantly between the different treatments. This fraction is known to be a sensitive indicator of total SOM that responds quickly to changes in management practices. The lucerne, MMW, MMWW and natural treatment contained higher C contents than the two 100% crop rotation systems (WBCWBL4 & WBCWBL1). This is mainly attributed to fewer disturbances in these treatments as well as to higher belowground biomass, especially of medic roots which was found to have a high fine root density in the 0-10 cm depth. Although no-tillage is applied, the first 10 cm of the soil is still disturbed and exposed to oxidative conditions during planting leading to higher mineralization rates in the two 100% crop rotation systems. The lucerne and two crop-pasture systems (MMW & MMWW) contained significantly higher N content in the fPOM fraction compared to the other treatments and shows the ability of legumes to fix N. The lucerne and medics residues are high in N and therefore add a lot of N to the soil. The fPOM fraction affects soil quality by enhancing

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microbial activity since the SOM in this fraction is easy accessible for microbes and the C an important source of energy. This was confirmed as a significant correlation (R^2 = 0.74) was found between C content and soil respiration rates as it is assumed that a higher soil respiration rate is partly due to a higher microbial activity (heterotrophic respiration). The lower C:N ratio in the lucerne, MMW and MMWW treatments also enhance microbial activity and nutrient availability. The fPOM fraction is enriched with nutrients (active source of N) and therefore contributes to the nutrient reserve capacity of the soil. The active microbes use the C for body maintenance and as energy to get hold of N for their own use and also to mineralize the extent N to plant available N (inorganic form). In the lucerne, MMW and MMWW treatments more N will be plant available (mineralized) due to higher N content and this can lead to better yields and yield quality. Higher yields observed in the MMW & MMWW treatments compared to the 100% crop rotation systems, with significant correlation found between yields and N content, confirmed this statement.

As reflected by the soil's weak extent of aggregation, the oPOM fraction only contributed between 0.4 and 2.4% of total C content. Due to no disturbance and grazing, the natural vegetated soil had the highest aggregate stability and also contained the highest proportion of C in the oPOM. A higher aggregate stability can lead to better structure, better soil porosity and therefore better soil water holding capacity. This is an important aspect in dryland production in semi-arid regions. Of all the cultivated treatments, the MMWW treatment contained the highest C in the oPOM fraction due to a higher aggregate stability.

The dominant C stabilization mechanisms operative in these soils was also established. The two main mechanisms that were investigated was physical protection via occlusion in aggregates and chemical interaction with minerals. A significant correlation was found between C occluded in aggregates (oPOM fraction) and aggregate stability with intra-aggregate C increasing with increasing aggregate stability. This confirms that more C is protected against decomposition in soils with higher aggregate stability and it also shows the effect of C on aggregation. A significant correlation between mineral C (mineral fraction) and clay and Fe-oxide content was also found in these soils which are partly responsible for the large contribution of the mineral-associated C fraction to total C. Thus the dominant stabilization mechanism operative in these soils is mineral stabilization. Due to presence of mainly low activity clays in these soils, it was concluded, that organic matter is dominantly sorbed to minerals via ligand exchange resulting in very strong organo-mineral associations.

From results obtained in this study, it can be concluded that amount of C stored in these soils is partly due to conservation agriculture practices applied and partly due the mineralogy and texture of the soil. Conservation agriculture causes favourable conditions for microbial activity which is responsible for the decomposition of plant residues into humus. The humus is then stabilized by the clay minerals (clay content and mineralogy) in the soil, properties that can't be affected by management practices. Therefore, both management practices and soil properties contribute to soil C sequestration, consequently soil sustainability. The MMWW treatment had the highest C and N content in the fPOM fraction, highest C content in the oPOM fraction and the highest C content in the mineral fraction of all the crop/pasture systems investigated in this study. This can be attributed to both the higher clay content in this treatment as well as the effect of the higher wide C:N above-ground inputs (wheat) and higher annual below ground inputs (wheat and medics). The lower frequency of physical disturbance due to planting in this cropping system also plays an important role (50 % medics, 50 % wheat).

I agree with García-Préchac et al. (2004) that better soil quality is the main reason for higher crop yields obtained in the crop-pasture systems compared to the 100% crop rotation systems. From results obtained in this study via fractionation of total SOM into different functional pools a better soil quality is mainly attributed to higher C and N contents in the fPOM fraction. This fraction plays an important role in specific soil functions. Due to the sensitivity of the fPOM fraction, valuable information was obtained regarding the effect of different crop rotation systems on SOM. It is clear that belowground inputs through roots (quantity and quality), the extent of disturbance of the soil and the constant use of legumes (medics or lucerne) in rotation systems are all vital factors controlling C and N content in the fPOM fraction. Biederbeck et al. (1994) stated that C and N content in the POM fraction may provide an early indication of future trends in OM response to cropping systems.

CHAPTER 5

GENERAL CONCLUSION AND FUTURE RESEARCH

Substantial increases for global food demand pose a huge challenge for producing enough food over the long-term. Application of conservation agriculture becomes vital as it has great potential to sequester C that can result in positive effects on soil quality, sustainability and productivity. To enhance soil quality and sustainability it is not only important that the soil contains enough total soil organic carbon (SOC), but the SOC content in the different functional pools (fractions) also plays an important role. Previous studies carried out in South Africa focused mainly on the effect of management practices on total SOC. It didn't provide any indication on the proportion of the SOC that is actually active or stabilized by association with the mineral fraction.

The first objective of this research project was to examine the long-term effect (11 years) of different crop/pasture rotations under no-tillage on total SOC storage. A further aim was to examine the relationship between the extent of SOM sequestration and crop yields. The main objective of this study was to investigate the effect of long-term no-till crop rotation practices on the C and N content and distribution in the SOM functional pools. This involved the fractionation of total SOM, using density fractionation, in order to investigate the role of each fraction in soil quality as well as the mechanisms by which C is stabilized.

The most significant effects of no-till crop/pasture systems on total SOM and its functional pools were found in the top 10 cm of the profile. After 11 years the inclusion of medic pastures in rotation with wheat (MMW and MMWW) had the highest total SOC content (15.2 - 18.6 g kg^{-1} in 0-30 cm depth, $P < 0.05$), compared to the continuous cropping (13.3 - 14.1 g kg^{-1} in 0-30 cm depth), permanent lucerne pasture (15 g kg^{-1}) or natural vegetated soil (13.2 g kg^{-1}). An average decrease of 52 % in total C content was observed from the 0 - 5 cm depth to the 20 - 30 cm depth emphasizing the importance of the first few centimetres in these soils for SOC sequestration. Coarse fragments were found to have a significant diluting effect on the amount of SOC stocks stored.

Higher belowground C inputs through roots (quantity and quality) and the lower extent of disturbance in the 0-10 cm depth are the main reasons for higher total C content in the crop-pasture systems compared to the other systems. The lower rate at which C increased in the lucerne treatment (0.38 g kg^{-1} year^{-1}) compared to the crop-pasture systems (0.7 - 0.78 g kg^{-1} year^{-1}) since 2003, is most likely due to lower belowground inputs of C through roots.

Although lucerne has a higher root density than medics, medics is able to re-establish from seed annually and leads to the formation of new roots again (i.e. higher C inputs). Lucerne on the other hand becomes dormant in winter and roots grow less actively. The continuous input of only lucerne residues with a low C:N ratio also means it is more easily decomposable, leading to a lower soil C content. A fairly strong linear relationship was also found between clay content and total SOC ($R^2 = 0.60$) indicating that the higher clay content in the MMWW treatment could play an important role in SOC accumulation in these soils. Aggregate stability had no significant effect on the total SOC content.

The contribution of the fPOM fraction to total C content in the cultivated treatments (6-9%) was lower than the natural vegetated soil (13%) in the 5-10 cm depth. Although only a relatively small fraction, it plays an important role in promoting certain soil functions (microbial activity and reserve nutrient capacity) and thereby contributing to soil quality. This fraction is the most sensitive pool of organic C and N to detect changes in total SOM due to effect of management practices (quantity and quality of OM inputs, extent of disturbance, fertilization). The medic-wheat rotations had the highest C (1.37 - 1.74 g kg^{-1} in 5-10 cm depth) and N (0.107 - 0.110 g kg^{-1} in 5-10 cm depth) contents in the fPOM fraction of the cultivated treatments. The C content in the fPOM fraction correlated positively with soil respiration rates ($R^2 = 0.74$). This C is easy accessible for microbes and therefore, together with high N content (low C:N ratio) enhance microbial activity. The fPOM fraction is also enriched with nutrients and contributes to the nutrient reserve capacity of the soil.

Compared to the natural vegetated soil, the cultivated treatments had a lower C content in the oPOM fraction due to a lower aggregate stability. On average, the oPOM fraction only contributed 0.4-2.4 % to total C content at all sites. A significant positive correlation ($R^2 = 0.77$) was found between C occluded in aggregates (oPOM fraction) and aggregate stability, with the highest aggregate stability found in the medic-wheat rotations of the cultivated treatments. Aggregate stability is an important soil physical property as it contributes to a higher soil water holding capacity.

The major part (85-93%) of the total SOC was associated with the mineral fraction (stable fraction) in the natural vegetated and agricultural soils. The total C content was thus mainly determined by the C content in the mineral fraction. The MMWW treatment contained the highest C content (18.7 g kg^{-1}, 5-10 cm depth) in the mineral fraction and the two continuous cropping systems the lowest (14.2-14.7 g kg^{-1}, 5-10 cm depth) of the cultivated treatments. A significant positive correlation was found between mineral-associated SOC fraction and clay

and Fe-oxide content which contributes to the large proportion of total SOC accumulated in the mineral fraction. The C:N ratios of the fractions decreased in the order fPOM > oPOM > mineral with a C:N ratio below 10 in the mineral fraction indicative of humified organic matter. The humus contributes to soil quality by adding to the cation exchange capacity of the soil as humus contains a lot of exchange sites that can hold onto nutrients and thereby increases the efficiency of added fertilizers.

The organic matter is most likely predominantly sorbed to the minerals through ligand exchange on the edge sites of kaolinite, illite and on iron oxides resulting in very strong organo-mineral associations. Long-term stabilization or preservation of C in these soils is dominantly determined by the interaction with mineral surfaces. Physical protection via occlusion in aggregates is not a dominant C stabilizing mechanism in these soils.

The MMW and MMWW treatments produced higher wheat yields in 2012 compared to the continuous cropping systems with significant correlations found between total C and N and yields obtained. However, according to Lal (2006), an increase in crop yields due to an increase in the SOM pool is mainly related to an increase in the labile fraction (fPOM). This is consistent with findings in this study. In a higher quality soil, higher agronomic production is expected.

Results of this study can thus now be used to predict how different dryland crop/pasture rotations under conservation tillage (no-tillage) affect the SOC content of the arable land in the grain production area of the Overberg. Through fractionation of total SOM into different functional pools, it is now known that the MMWW treatment contributed the most to soil quality. This can be attributed to both the cropping system (C input and extent of disturbance) as well as the soil properties. The MMWW treatment had the highest total SOC content, which included highest labile C and N content and highest stable C content.

Future Research

Research on the application of conservation agriculture (no-tillage and crop rotation) in South Africa is relatively new and therefore further research is necessary to build a strong scientific database. This would greatly contribute in facilitating the effect of different crop rotations under no-tillage on SOC in different climate and soil types. Research on the effect of conservation agriculture on C storage in the shallow shale derived soils of the Overberg has

now been widely covered in this study. However, similar studies is still needed in other dryland grain production areas of the Western Cape e.g. Swartland where farmers are also extensively switching over to conservation agriculture, but the effect of these practices on the maintenance and improvement of the soil organic C (SOC) content is still unknown. A lot of grain farmers in the Overberg are also extensively starting to apply a rotation system of 5/6 years lucerne followed by 5/6 years of different cash crops under no-tillage. There is thus still a gap in knowledge on how this rotation system would compare with MMW, MMWW and 100% continuous cropping systems in terms of SOC accumulation and stabilization. This will only be known when the different SOC functional pools are investigated.

Crop rotation and the integration of livestock production from pastures into crop rotation systems have been widely adopted by several grain farmers. This result in lower economic risk to grain farmers due to the unpredictable rainfall pattern that can lead to low crop yields. However, some concerns rose about the effect of livestock on the amount of C input of pasture residues and therefore the effect of grazing on the SOC content. In this study, the medic was subjected to grazing yet the MMWW and MMW crop-pasture systems still showed the highest total soil C content increase since the start of the experiment in 2002 and produced the highest wheat yields. It will be interesting to compare the effect of allowing and not allowing sheep to graze the pastures in crop-pasture rotation systems on SOC dynamics and yields. According to findings in this study, medics mainly contributed to soil C content through its roots.

Lastly, many grain farmers are still critical about the application of conservation practices, especially in the shallow, stony soils which is found in great parts of the Swartland and Overberg regions. It is therefore recommended that a study is carried out comparing the SOM functional pools under conventional and conservation practices which will provide valuable information regarding the effect of only management practices on SOM functional pools. This would exclude the effect of soil mineralogy and texture on the mineral fraction and a stronger conclusion about the effect of conservation practices on the mineral fraction can be drawn than was possible from this study.

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APPENDIX

Soil description, additional data and correlations

1. Soil descriptions

GLENROSA (Gs) SOIL FORM

Orthic A-horizon	**0**
	200 mm
Lithocutanic B-horizon	

Soil Family

2000 A-horizon bleached

  2100 B1-horizon not hard

    2110 No signs of wetness in B1

      2111 Non-calcareous B1

Parent material: shale   **Aspect**: East

Slope: 3 - 5 %   **Terrain unit**: middle to lower footslope

Slope form: convex   **Altitude**: 184 m

Soil form and family: **Gs 2111 (Overberg)**

Glenrosa soil form consists of an Orthic A horizon overlying a Lithocutanic B horizon. The depth of the A-horizon is ± 200 mm and is bleached as it meets the criteria of an E-horizon (Dry state: 10 YR 6/4). The A-horizon has a clay content of 20 - 25 % (loam). The lithocutanic B horizon consists of weathered rock (saprolite) but was not hard and had no signs of wetness (no pedogenic mottles) with a clay content of 25 - 30 % (loam/clay loam). The B-horizon was also non-calcareous with the colour of this horizon in the dry state; dry colour: 10 YR 5/6 (yellowish brown) and moist state; moist colour: 10 YR 4/6 (dark yellowish brown). A lithocutanic B horizon is recognized with respect to its structure, colour or consistence which still has distinct affinities with the parent rock. Furthermore, it grades into relatively unaffected and, eventually fresh rock, sometimes at very shallow depths. A

lithocutanic B horizon differs from the neocutanic B by containing saprolitic material (Soil Classification Working Group, 1991).

OAKLEAF (Oa) SOIL FORM

Orthic A	**0**
	200 mm
Neocutanic B	

Soil Family

2000 A-horizon bleached

  2100 Non-Red B-horizon

   2120 Luvic B1-horizon

Parent material: shale	Aspect: east
Slope: 5 - 7 %	Terrain unit: lower footslope
Slope form: convex	Altitude: 163

Soil form and family: **Oa 2120 (Patrysdal)**

Oakleaf soil form consists of an Orthic A horizon overlying a Neocutanic B horizon on unconsolidated material without signs of wetness. The depth of the A-horizon is ± 200 mm and is bleached as it meets the criteria of an E-horizon (Dry state: 10 YR 5/3) and has a clay content of 15 - 20 %. The Neocutanic B horizon is a luvic non-red B-horizon (clay content 20-25 %) and the material is subjected to hard setting. The colour of this horizon in the dry state; 10 YR 5/8 dry colour (yellowish brown): and moist state; moist colour: 10 YR 4/6 (dark yellowish brown). Materials in which Neocutanic B horizons are formed is usually of alluvial or colluvial origin and is found on certain landscape positions e.g. a footslope.
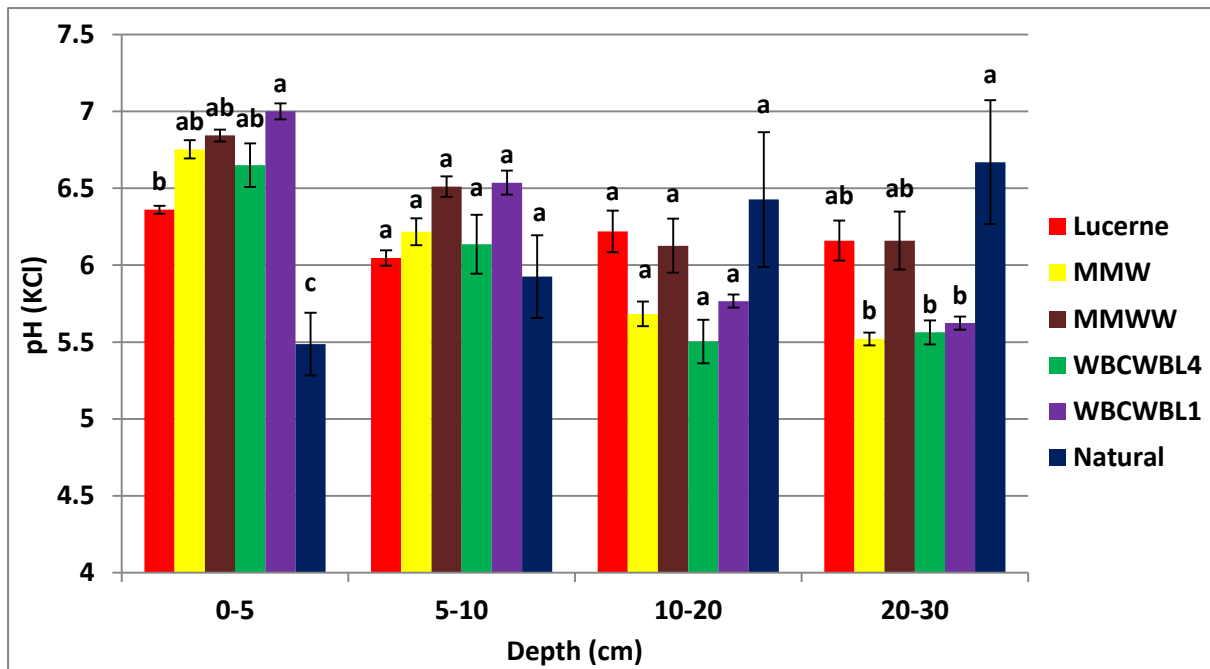
2. Additional data

Figure A1 The soil pH (KCl) for the different crop rotation systems and natural vegetated soil in the four sampling depths.

Table A1 General Soil Characteristics

Treatment	Depth	pH(H$_2$0)	pH(KCl)	Exchangeable Cations (cmol$_c$/kg)						ECEC value (cmol$_c$/kg)	BS (%)
				Ca	Mg	Na	K	H	Al		
Lucerne	0-5	7.17	6.36	8.151	1.380	0.177	1.657	0.077	0.167	11.61	97.9
	5-10	6.87	6.05	5.676	1.374	0.220	1.219	0.073	0.167	8.73	97.3
	10-20	7.14	6.22	5.839	2.145	0.303	0.817	0.062	0.17	9.33	97.6
	20-30	7.15	6.16	4.525	2.888	0.738	0.597	0.062	0.167	8.98	97.5
Natural	0-5	6.33	5.49	nd	nd	nd	nd	nd	nd	nd	nd
	5-10	6.83	5.93	2.645	2.910	1.021	0.567	0.098	0.2	7.44	96
	10-20	7.43	6.43	nd	nd	nd	nd	nd	nd	nd	nd
	20-30	7.71	6.67	nd	nd	nd	nd	nd	nd	nd	nd
MMW	0-5	7.39	6.75	nd	nd	nd	nd	nd	nd	nd	nd
	5-10	6.90	6.22	6.354	1.015	0.232	1.287	0.077	0.125	9.09	97.8
	10-20	6.44	5.68	nd	nd	nd	nd	nd	nd	nd	nd
	20-30	6.46	5.52	nd	nd	nd	nd	nd	nd	nd	nd
WBCWBL4	0-5	7.43	6.7	7.569	1.407	0.151	1.111	0.080	0.1	10.42	98.3
	5-10	6.94	6.14	6.238	1.234	0.188	0.946	0.060	0.067	8.77	98.2
	10-20	6.37	5.50	4.641	1.638	0.252	0.453	0.057	0.183	7.22	96.7
	20-30	6.79	5.56	4.608	1.692	0.271	0.460	0.063	0.133	7.23	97.3
WBCWBL1	0-5	7.66	7.0	nd	nd	nd	nd	nd	nd	nd	nd
	5-10	7.25	6.54	7.319	1.429	0.238	0.979	0.065	0.133	10.1	98.7
	10-20	6.57	5.77	nd	nd	nd	nd	nd	nd	nd	nd
	20-30	6.64	5.62	nd	nd	nd	nd	nd	nd	nd	nd
MMWW	0-5	7.35	6.84	nd	nd	nd	nd	nd	nd	nd	nd
	5-10	7.13	6.51	8.700	1.843	0.180	1.185	0.067	0.1	12.07	98.6
	10-20	6.86	6.13	nd	nd	nd	nd	nd	nd	nd	nd
	20-30	6.97	6.16	nd	nd	nd	nd	nd	nd	nd	nd

Table A2 Fe-oxide content (%) obtained per depth in the different treatments.

Treatment	Depth	Fe-oxide (%)
Lucerne	0-5	1.7
	5-10	2.0
	10-20	2.1
	20-30	2.5
MMW	5-10	2.2
MMWW	5-10	2.5
WBCWBL4	0-5	1.9
	5-10	2.1
	10-20	2.1
	20-30	3.2
WBCWBL1	5-10	2.3
Natural	5-10	1.6

Table A3 Average Total Soil C and N distribution and C:N ratio with depth between treatments.

| Treatment	Depth	Total C (%)	Total N (%)	C:N
Lucerne	0-5	**2.20**	**0.27**	**8.14**
	5-10	**1.77**	**0.20**	**9.00**
	10-20	**1.34**	**0.17**	**8.04**
	20-30	**1.16**	**0.14**	**8.45**
Natural	0-5	**2.44**	**0.21**	**11.82**
	5-10	**1.37**	**0.14**	**10.15**
	10-20	**1.06**	**0.11**	**9.97**
	20-30	**1.00**	**0.13**	**7.89**
MMW	0-5	**2.23**	**0.23**	**9.63**
	5-10	**1.78**	**0.19**	**9.47**
	10-20	**1.39**	**0.17**	**8.25**
	20-30	**1.15**	**0.13**	**8.73**
WBCWBL4	0-5	**1.97**	**0.18**	**10.69**
	5-10	**1.59**	**0.16**	**9.98**
	10-20	**1.35**	**0.13**	**10.33**
	20-30	**0.86**	**0.12**	**7.02**
WBCWBL1	0-5	**2.09**	**0.22**	**9.43**
	5-10	**1.80**	**0.18**	**9.91**
	10-20	**1.32**	**0.16**	**8.18**
	20-30	**0.96**	**0.12**	**8.18**
MMWW	0-5	**2.83**	**0.30**	**9.37**
	5-10	**2.28**	**0.25**	**8.96**
	10-20	**1.65**	**0.17**	**9.48**
	20-30	**1.38**	**0.16**	**8.40**
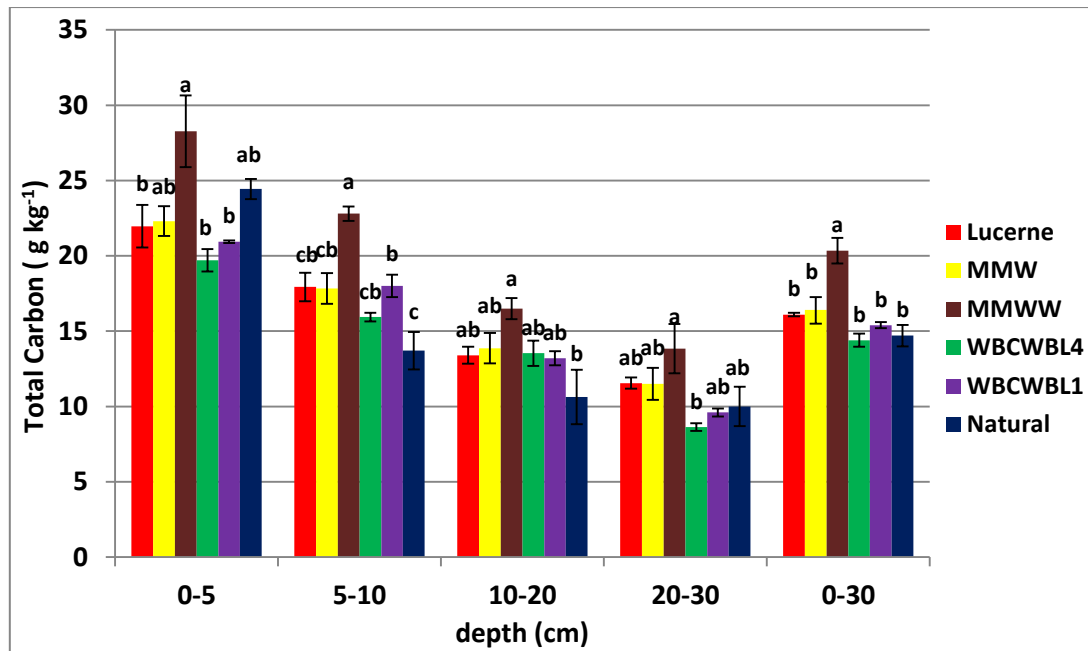
Figure A2 Significant differences (indicated by different alphabetic letters) found in C content between the different treatments

Table A4 C stocks obtained in both the bulk soil and fine fraction of the different treatments in each depth.

C stocks (Mg ha^{-1})

Treatment	Depth (cm)								Total (0-30)	
	0-5		5-10		10-20		20-30			
	Bulk soil	Fine fraction	Bulk soil	Fine fraction	Bulk soil	Fine fraction	Bulk soil	Fine fraction	Bulk soil	Fine fraction
Lucerne	13.52	16.56	9.49	12.70	15.53	20.20	9.69	15.28	**47.02**	**63.44**
MMW	12.06	16.14	9.76	13.55	13.36	22.42	10.57	18.14	**45.73**	**70.22**
MMWW	12.50	18.74	10.52	15.42	14.43	22.86	10.56	17.72	**48.08**	**74.89**
WBCWBL4	9.04	12.82	7.19	10.53	13.26	19.72	6.42	11.59	**35.92**	**54.66**
WBCWBL1	10.02	14.53	8.45	12.36	13.03	19.00	7.17	12.99	**38.66**	**58.87**
Natural	14.99	16.85	8.41	10.33	13.94	17.06	11.38	10.33	**48.72**	**60.46**

Note: The term **bulk soil** (coarse + fine fraction) refers to the C stocks obtained including the coarse fragments while the term **fine fraction** (< 2mm) refers to C stocks obtained excluding the effect of coarse fragments

Table A5 Root density (kg m^{-3}) of the different crops in the specific depths.

Treatment	Canola	wheat	lupin	barley	medics	lucerne

0-5	7.165	4.918	6.421	5.012	6.220	8.300
5-10	4.895	4.579	4.774	4.173	6.179	7.275
10-20	2.974	3.250	2.455	2.129	2.800	5.384
20-30	2.257	3.348	2.752	1.817	2.069	4.046

Table A6 Carbon and Nitrogen composition of roots and shoots of the different crops.

Crop	Aboveground (Shoots)			Belowground (Roots)			Combine (Roots + Shoots)
	C (%)	N (%)	C: N	C (%)	N (%)	C: N	C:N
Lucerne	43.72	3.96	11	41.91	2.08	20	16
Medics	42.94	2.48	19	40.58	1.83	26	23
Barley	40.98	0.6	73	38.5	0.80	58	66
Wheat	42.9	0.3	165	39	0.75	70	114
Canola	44.19	0.26	162	42.75	0.39	113	138
Lupin	42.9	0.63	71	42	1	49	60

Table A7 Soil respiration or CO_2 efflux determined in September 2012 and March 2013.

Treatment	September 2012 $g\ C\ m^{-2}\ day^{-1}$	March 2013 $g\ C\ m^{-2}\ day^{-1}$
A (MMW)	1.463	0.705
B (MMW)	1.089	0.986
C (MMW)	1.345	0.835
Ave	**1.299**	**0.842**
A (WBCWBL4)	1.061	0.749
B (WBCWBL4)	0.998	0.744
C (WBCWBL4)	1.063	0.833
Ave	**1.041**	**0.775**
A (WBCWBL1)	0.801	0.639
B (WBCWBL1)	0.996	0.736
C (WBCWBL1)	1.075	0.726
Ave	**0.958**	**0.700**
A (MMWW)	1.307	0.908
B (MMWW)	1.219	0.874
C (MMWW)	1.229	0.767
Ave	**1.252**	**0.850**
A (LUCERNE)	nd	1.313
B (LUCERNE)	nd	0.973
C (LUCERNE)	nd	0.979
Ave	**nd**	**1.088**
A (Natural)	1.491	1.177
B (Natural)	1.607	1.268
C (Natural)	1.952	0.895
Ave	**1.683**	**1.113**

Table A8 C: N ratio of the different pools (fractions) and bulk soil in the different treatments at 5-10 cm depth.

	Lucerne	MMW	MMWW	WBCWBL4	WBCWBL1	Natural
fPOM	12.6 **a**	12.9 **a**	15.4 **a**	21.8 **b**	21.1 **b**	22.1 **b**
oPOM	13.4 **a**	11.2 **a**	12.9 **a**	14.9 **a**	15.2 **a**	22.4 **b**
Mineral	8.7 **b**	7.7 **a**	8.7 **ab**	8.2 **ab**	8.6 **ab**	9.0 **b**
Bulk (Total)	9.1 **c**	9.5 **bc**	9 **c**	10.9 **a**	9.9 **abc**	10.5 **ab**

Note: Alphabetic letters denote statistical differences between treatments according to Tukey's Studentized Range test at $\alpha = 0.05$. Similar letters indicate lack of significant differences.
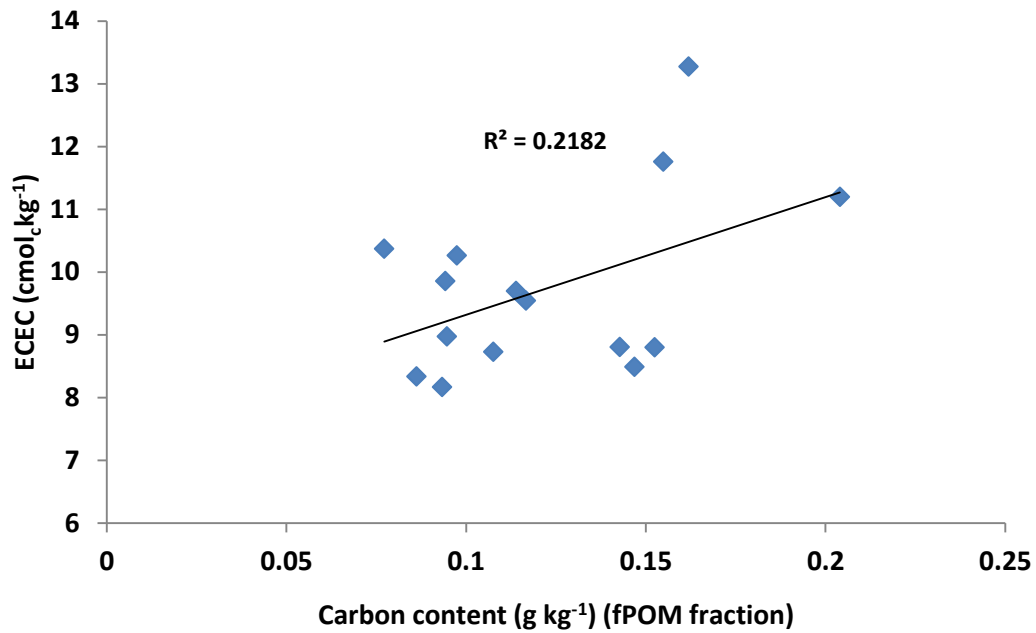
3. Additional correlations

Figure A3 Relationship effective cation exchange capacity and C content in the fPOM fraction in 5-10 cm depth.
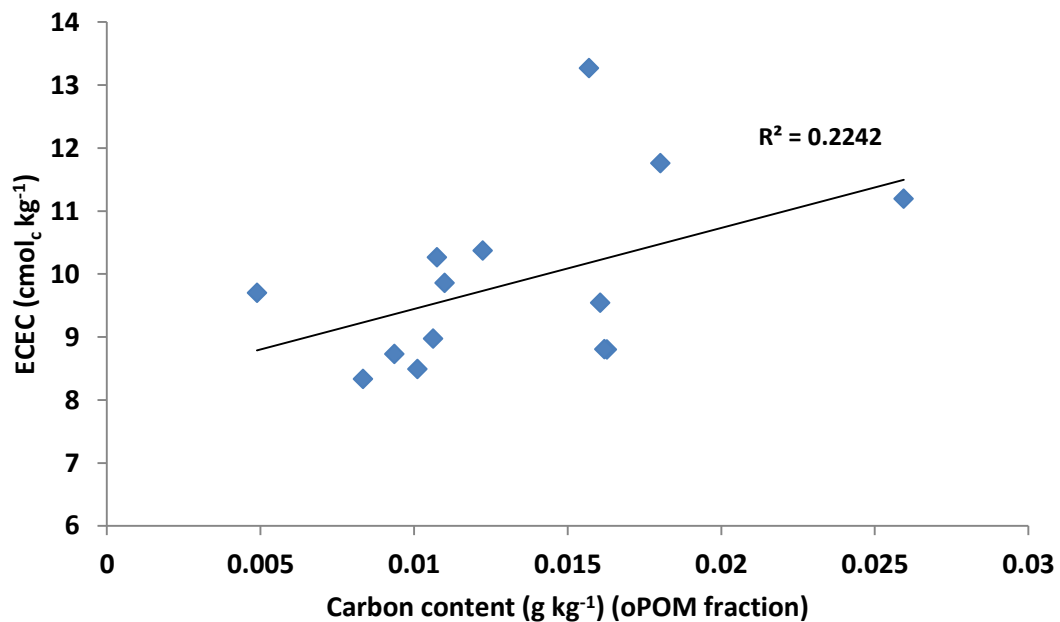

Figure A4 Relationship between effective cation exchange capacity and carbon content in the oPOM fraction in 5-10 cm depth.
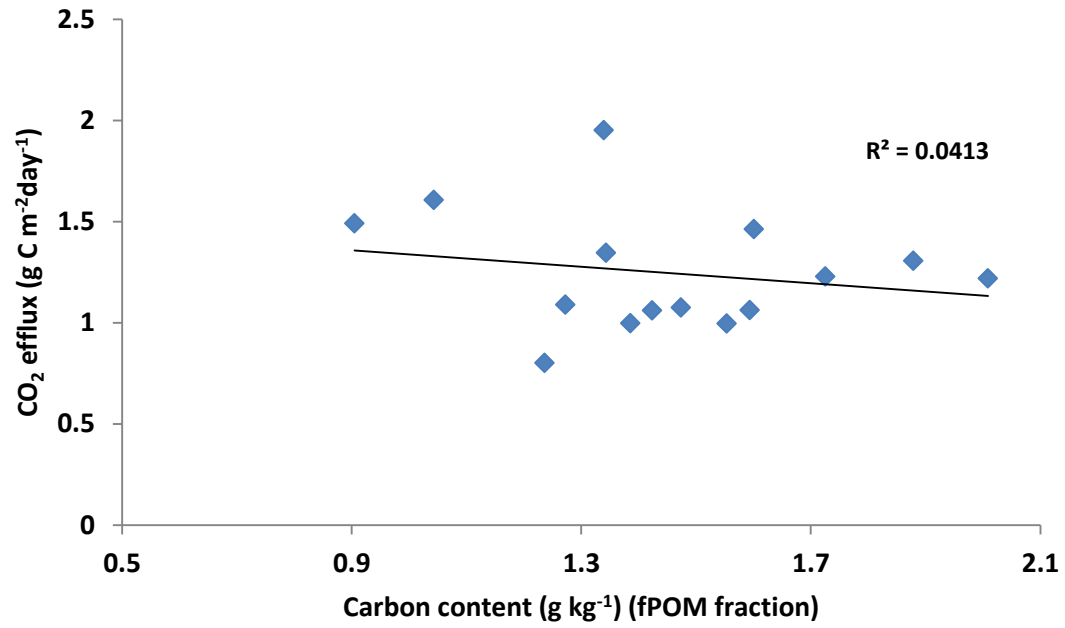
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Figure A5 Relationship between CO_2 efflux determined in September and carbon content in the mineral fraction in 5-10 cm depth.