SOIL ORGANIC CARBON, GLOMALIN RELATED SOIL PROTEIN AND RELATED PHYSICAL PROPERTIES AFTER 15 YEARS OF DIFFERENT MANAGEMENT PRACTICES IN A SUBTROPICAL REGION OF SOUTH AFRICA

by

CAROLINE MUBEKAPHI

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School of Agricultural, Earth and Environmental Sciences

College of Agriculture, Engineering and Science

University of KwaZulu-Natal

Pietermaritzburg

South Africa

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DECLARATION

I, Caroline Mubekaphi, declare that:

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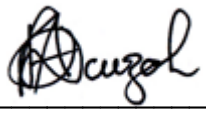

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ABSTRACT

Soil aggregation is an important mechanism, which plays a significant role in soil fertility as it decreases soil erosion and mediates air permeability, water infiltration, and nutrient cycling. Aggregation depends on a variety of aggregate binding agents, including carbon and its fractions, interrelating concurrently at different spatial scales. However, biologically active fractions of organic matter, such as microbial biomass carbon (MBC) and water-extractable organic carbon (WOC) could better reflect the changes in soil quality. Recent studies have highlighted the existence of a thermostable, water-insoluble soil glycoprotein operationally referred to glomalin-related soil protein (GRSP) that is crucial for preserving SOC. However, the relationship between SOM fractions and GRSP, and effects of different land uses on these parameters and relationships in humid environments are not clearly understood. The study sought to determine the relationships between soil organic carbon fractions, GRSP and aggregate stability under different management practices. The study was conducted on a farm located in the south-east of Howick, in the uMgungundlovu District Municipality, KwaZulu Natal province of South Africa. Soil samples were collected at 0-5, 5-10, 10-20 and 20-30 depths from three management practices i.e. long-term no till (NT), conventional tillage (CT), and native Forest (F). The native forest soils served as the control. Glomalin was assayed. The soils were further analysed for Ca, Mg, K, and Na, microbial biomass carbon (MBC), water soluble organic carbon (WSOC), soil bulk density (BD). Interaction between land-use and soil depth had significant effects on SOC content. There was general decrease in SOC as depth increased for all management practices except for no till, where no significant differences were observed in SOC across the four soil depth layers. The interaction between land-use and soil depth had significant effects ($p < 0.05$) on both easily

extractable (EE-GRSP) and total (T-GRSP) glomalin related soil proteins. The NT treatment had the lowest concentration of EE -GRSP than the other two treatments in the 0-5 cm depth whilst no differences among the management practices at deeper soil layers were observed. The concentration of T-GRSP for soils under forest and NT tended to decrease with depth, while in CT the 10-20 and 20-30 cm depths had higher concentrations than the 0-5 and 5-10 cm depths. Land use also had significant effects ($p < 0.05$) on soil aggregate stability. Soils under Forest were the most stable with an MWD of just over 3, whilst soils under Conventional Tillage had the lowest MWD value of 1.24. The observed aggregate stability was significantly influenced by GRSP as evidenced by a significant positive relationship between both EE-GRSP ($R^2 = 0.72$) and T-GRSP ($R^2 = 0.82$). Therefore, management practices that mimic natural forest favour the accumulation of SOC and T-GRSP and should be widely adopted.

Keywords: microbial biomass carbon, organic matter, water soluble carbon,

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LIST OF ABBREVIATIONS

AMF	Arbuscular mycorrhizal fungi
BRP	Bradford-reactive protein
BRSP	Bradford-reactive soil protein
BSA	Bovine serum albumin
EE-BRSP	Easily extractable Bradford-reactive soil protein
EE-GRSP	Easily extractable glomalin-related soil protein
EE-IRSP	Easily extractable immunoreactive soil protein
ELISA	Enzyme-linked immunosorbent assay
GRSP	Glomalin-related soil protein
Hsp	Heat shock protein
IRSP	Immunoreactive soil protein
PBS	Phosphate buffer saline
PSB	Phosphate solubilizing bacterium
SOC	Soil organic carbon
SOM	Soil organic matter
TG	Total Glomalin
T-GRSP	Total glomalin related soil protein

CHAPTER 1

INTRODUCTION

1.1 Background

Soil degradation is of major concern in most parts of Africa. Lal (1994) defined soil degradation as the loss of actual or potential productivity or utility of soil because of natural or anthropogenic factors. The most severe soil degradation, particularly erosion, occurs in communal croplands, grazing lands, and rural settlements of Africa (Laker, 2004). The ensuing loss of fertile top soil causes extreme degradation of soil quality (Lal, 2001) with negative effects on agricultural productivity (Scherr and Yadav 1996; Mukhebi et al., 2011; Blaikie, 2016). Le Bissonnais (2003) summarised the soil erosion process as a three-step event starting with aggregate disintegration followed by transportation and deposition by overland flow. Disintegration of a soil aggregate under the influence of raindrops is largely a function of aggregate stability (Le Bissonaiss, 1996; Amezketa, 1999). Soil aggregate stability thus gives a measure of the ability of aggregates to resist disintegration and maintain their integrity when exposed to breakdown forces (Le Bissonnais, 1996). Stable soil aggregates play a pivotal role in soil functions, such as, protection and stabilisation of organic matter, stabilisation of microbial community structure, control oxygen diffusion, regulate water flow (Six et al., 2000; Diaz-Zorita et al., 2002), determine nutrient adsorption and desorption (Linquist et al., 1997; Wang et al., 2001), and reduce run-off and erosion (Barthes and Roose, 2002).

Soil aggregation, the process by which aggregates of different sizes are joined and held together by different organic and inorganic materials and the stability thereof is a function of intricate interactions between biological, chemical and physical factors (Amezketa, 1999). This process is controlled by inherent soil properties such as texture, structure, SOM content, clay mineralogy, sesquioxides exchangeable cations, and water retention and transmission properties (Lal, 2001; Six et al., 2004). In addition, Norton et al. (2006) reported that aggregate stability also depends on $CaCO_3$, Fe and Al oxides. There is a plethora of literature widely highlighting the role of SOM and sesquioxides on soil aggregate stability (Barthès et al., 2008; Peng et al., 2015). However, there are contradictions in the role and interactions between the various aggregating agents in different soils especially soils varying in weathering levels (Bronick and Lal, 2005; Wei et al., 2016). It is generally agreed that SOM acts as a cementing agent, binding together primary particles in the aggregates, both physically and chemically, and hence increasing the stability of the aggregates and ultimately limiting their breakdown during wetting (Wuddivira and Camps-Roach, 2007). However, the role of SOM in stabilizing soil aggregates has been elaborated for young soils (Nciizah and Wakindiki, 2012). In contrast, its role in highly weathered soils is not clear (Denef et al., 2002), with most researchers suggesting that aggregation is less related to SOM in these soils (Six et al., 2000). Instead, it is generally hypothesised that in highly weathered soils iron oxides act as the cementing agents between the surfaces of clays and as charged discrete particles (Briedis et al., 2012). It is also often argued that where SOM is low, oxides of Fe and Al are the dominant factors responsible for soil aggregation (Barthes et al., 2015). The authors further reported that in tropical soils from sub-Saharan Africa and Brazil the Al containing sesquioxides

had a more important aggregating role than did SOM. Similarly, Duiker et al. (2003) highlighted that poorly crystalline iron oxides are more effective than free forms in stabilizing soil aggregates whilst Barthès et al. (2008) noted that Al-containing sesquioxides play a more significant role than SOM in the aggregation of tropical soils. According to Peng et al. (2015), Fe/Al oxides are the major agents in micro-aggregates while SOM plays a primary role in stabilizing the macro-aggregates in Ultisols. Moreover, where SOM is high it could interact with sesquioxides and the effects of such interactions on aggregate stability are not clearly understood (Peng et al., 2015).

It is generally agreed that consequences of unsustainable practices such as intensive long-term cultivation of highly weathered soils results in their degradation resulting in degradative processes such as soil acidification, SOM depletion and extreme erosion (Jien and Wang, 2013). However, highly weathered soils are known for their large content of 1:1 clay minerals and oxides, resulting in a variable (pH dependent) charge and both positive and negative surface charges at field pH (Oades and Waters, 1991; Six et al., 2002). Therefore, electrostatic interactions between oxides and 1:1 clay mineral can lead to aggregate formation through mineral–mineral bonding (Six et al., 2000; Denef et al., 2002) Hence the suggestion that aggregation is less related to SOM in highly weathered soils (Six et al., 2000 Duiker et al., 2003; Briedis et al., 2012).Instead, as earlier alluded to, the Al containing sesquioxides play a more important aggregating role than SOM (Barthes et al., 2015). However, there is also high possibility that the contribution of SOM in highly weathered soils could depend on the organic matter fractions including POM-C, water soluble C and the microbial fraction (Franzluebbers et al., 1995; Sainju et al. 2007). Therefore, measurement of total SOC alone does not adequately reflect soil quality and nutrient status

(Franzluebbers et al., 1995). Instead, measurements of biologically active fractions of organic matter, such as microbial biomass carbon (MBC) and water-extractable organic carbon (WOC) could better reflect the changes in soil quality and productivity that alter nutrient dynamics in these soils (Sainju et al., 2007). MBC is particularly important since it reflects a soil's ability to store and cycle nutrients and organic matter (Carter et al., 1999). Essentially, MBC is the pool of SOC that contributes cyclically to immobilization and release of minerals during formation and breakdown (Hassink, 1995). Moreover, MBC is related to biologically derived processes such as soil N mineralisation and soil aggregation. It is generally postulated that Microbial mucilages and polysaccharides produced by some groups of bacteria as well as many fungi can play an important role in the stabilization of aggregates (Gupta and Germida, 2015). Addition of crop residue rapidly stimulates microbial growth and activity which generate transient binding agents mostly polysaccharides in the first few weeks **(**Mupambwa and Wakindiki, 2012). These binding agents contribute to aggregate stabilisation.

In addition to the inherent soil aggregation agents discussed above alteration of soil conditions by tillage practices has complex effects on soil characteristics thereby affecting structural conditions (Borie et al., 2008), and number, diversity or activity of microorganisms. Decades of intensive agriculture have diminished SOM content, thereby reducing fertility and biodiversity of arable lands (Moore et al., 2004; Gardi et al., 2013). In contrast practices such as No-tillage (NT) which comprise land preparation with little or no soil surface disturbance the only disruption during planting (Bai et al., 2018) result in enhancements in soil quality in the upper soil layer by improving soil structure and enhancing soil biological activity, nutrient cycling and

reducing bulk density (Hamza and Anderson, 2005). This improves soil water holding capacity, water infiltration, water use efficiency (Islam and Weil, 2000; Pittelkow et al., 2015) and aggregate stability (Aziz et al., 2013). Accumulation of organic matter and nutrients near the surface under NT produces beneficial effects on soil physical, chemical and biological properties (Beare et al., 1997; Tebrugge and During, 1999), including enhanced rhizosphere biological activities (Kladivko, 2001). Fungal biomass is enhanced in the topsoil under NT (Frey et al., 1999), including arbuscular mycorrhizal fungi (AMF), which are important mediators of soil aggregation (Borie et al. 2008). Many reports have shown that AMF are able to counteract soil degradation by increasing the stability of soil aggregates (Bethlenfalvay et al., 1999; Miller and Jastrow, 2000) through the combined action of extraradical hyphae and their exudates and residues (Miller and Jastrow, 1992, 2005). The AMF are mutualistic symbionts living in association with roots of most terrestrial plants and they influence soil fertility and plant nutrition (Smith and Read, 2008).

Soil aggregation by AMF is through the combined action of extraradical hyphae exploring soil to form an aggregate network and an insoluble, hydrophobic, recalcitrant glycoprotein, called “glomalin” operationally known as glomalin related soil protein (GRSP) which has binding properties (Bedini et al., 2009). Haddad and Sakar (2003) reported that GRSP detaches from the hyphae, moves into the soil, and becomes a distinct component of the SOM. GRSP is an immunoreactive glycoprotein, which is produced by hyphae of AMF (Wright et al., 1996). Wright and Upadhyaya (1996) described GRSP using a monoclonal antibody Mab32B11 raised against crushed AMF. GRSP is an insoluble in water and resistant to heat degradation and hence very stable (Wright and Upadhyaya, 1996). Several studies have shown a significant

relationship between the amount of GRSP present in soil and aggregate stability (Wright and Upadhyaya, 1998; Wright et al., 2007). However, (Piotrowski et al. 2004) argues that the relationship is not that close. They stated that mechanisms underlying aggregation were not explained by measuring root biomass and total hyphal lengths alone, signifying that other physiological or architectural mechanisms may be responsible. Hence the need for more studies.

Soil disturbance leads to increased hydrolysis of the GRSP molecule and reduced production of GRSP due to disruption of the network of mycorrhizal hyphae (Wright et al., 2000). Significant differences in soil glomalin have been observed among different land use types and soil layers (Tang et al., 2009). GRSP exhibits vertical distribution pattern, which decreases with increasing soil depth. Despite evident importance of glomalin in maintaining soil aggregate stability, little remains known about soil profile distribution patterns and influencing factors of glomalin under different land use type. Generally, GRSP levels are affected by cropping systems and land management practices such as tillage despite its recalcitrance (Wright et al., 2007). For instance, in a study to determine glomalin content in aggregate size classes from three different farming systems, Wright et al. (2007) observed greater GRSP under no tillage than chisel tillage and intensive tillage for the whole soil. Moreover, larger proportions of GRSP were noted in macro-aggregates of no tillage than chisel tillage. In another study, Wright et al. (2009) observed substantial increases in GRSP concentration 3 years after converting from conventional to no-till. Therefore, practices such as No-till are likely to enhance the concentration of GRSP in cultivated soil, which will in turn improve soil structural properties particularly aggregate stability. It is particularly

important to determine how management practices are likely to influence GRSP concentrations.

The importance of GRSP on C-sequestration and aggregate stability and its huge potential as a biotechnology approach to control soil degradation particularly erosion is evident. However, relationships between GRSP and SOM fractions, and effects of land uses on these parameters, their relationships and influence on soil aggregation, are not clearly understood especially in highly weathered soils. Therefore, this study sought to determine the relationships between GRSP, SOC, WSOC, and MBC and aggregate stability under different land uses in highly weathered soils.

1.2 Objectives

The general objective of this study was to determine the changes in GRSP, SOC, WSOC, and MBC and aggregate stability under different soil management a subtropical region of South Africa after 15 years. The specific objectives were:

1. To determine the effects of soil management and depth on the soil organic carbon and glomalin related protein (GRSP) in a subtropical region of South Africa after 15 years.
2. To determine the effects of soil management and depth on aggregate stability of soil in a subtropical region of South Africa after 15 years.
3. To determine the relationships between GRSP, soil organic carbon fractions and aggregate stability under different management practices in a subtropical region of South Africa after 15 years.

1.3 Hypothesis

i. Soil management and depth, significantly affects soil organic carbon and GRSP in a subtropical region of South Africa.

ii. Soil management and depth significantly increase aggregate stability in a subtropical region of South Africa.

iii. GRSP and aggregate stability increase with an increase in SOC, MBC and WSOC depending on management practices in a subtropical region of south Africa.

CHAPTER 2

LITERATURE REVIEW

2.1 Soil degradation in South Africa

Land degradation is one of the most serious global environmental issues, connected to food security, poverty, urbanization, climate change, and biodiversity (Reynolds and Stafford Smith, 2002; Scholes and Biggs, 2004). Soil erosion is a chief soil degradation problem, challenging land and water resource management throughout South Africa (Rosenberg,2007). Eroded soil particles carry vital plant nutrients such as nitrogen, phosphorus, potassium and calcium (Meenar et al., 2017), resulting in nutrient depletion and decline in overall soil productivity (Pimentel et al., 2006; Cronk et al., 2012). Approximately 60% of the land in South Africa is currently degraded (UNEP, 1997), with severely degraded areas being closely associated with the distribution of communal rangelands, precisely in the steeply sloping environments in Limpopo, KwaZulu-Natal, and the Eastern Cape (Rosenberg, 2010). Several communal areas in the Limpopo, North West, Northern Cape, and Mpumalanga provinces are also severely degraded (Rosenberg, 2007). Commercial farming areas with the most severe degradation are located in the Western and Northern Cape Provinces (State of the Environment South Africa, 2008).

The vulnerability of soil to erosion, is related to soil aggregate stability, and aggregate breakdown leads to detachment of particles and small aggregates, resulting in crusting, then runoff and transport of the particles (Le Bissonnais, 1996: Torri et al 1998). Soil aggregates can be defined as groups of soil particles that bind to each other more strongly than to adjacent particles (Lal et al., 2004; Kibblewhite et al., 2008; Siddique et al., 2017). Soil aggregate stability is the consequence of complex interactions between biological, chemical and physical processes in the soil (Levy et al., 2003; Six et al., 2004; Regelink et al., 2015), as well as other environmental factors such as climate. A number of authors have documented the importance of soil aggregate stability in the ecosystem as it is strongly correlated to soil services such as carbon storage (Balabane and Plante 2004; John et al., 2005), organic matter stabilization (Six et al., 1998), water holding capacity (Shukla et al. 2003) and resistance to erosion (Barthes and Roose, 2002). Raindrop impact triggers soil erosion through breakdown of aggregates and transportation of the resulting micro-aggregates and soil particles by flowing water (Lal, 2001). Disintegration of soil aggregates under the influence of raindrop impact is largely a function of aggregate stability (Le Bissonaiss, 1996; Amezketa, 1999).

South Africa is subject to soil erosion, owing to poor farming practices, climate and topography, south African soils are easily eroded (Garland et al.,1999; Laker, 2004). Soil degradation is perceived as more of a problem in KwaZulu-Natal, Limpopo and the Eastern Cape (Hoffman et al., 1999; Hoffman and Todd, 2000) and less of a problem in the Free State, Western Cape and Northern Cape (Le Roux et al., 2007). South Africa have soil parent materials that yield in soils inherently susceptible to various forms of soil degradation, such as crusting, compaction, and water and wind

erosion. Soils derived from basic igneous rocks, especially dolerite, have higher stability against erosion as compared with the majority of other soils, mainly those from sedimentary rocks of the Beaufort and Ecca groups (D'Huvetter, 1985). The Beaufort (i.e. shales and mudstone) and Ecca groups are associated with substantial amounts of magnesium, sodium and the clay mineral illite and as a result they produce soils with silt percentages. Moreover, they produce unstable duplex soils that are erodable. It is well known that high Mg give rise to very poor structure resulting in very compact soils and high erodable soils. sodium is by far the most dispersive major cation in soils. Soils in the Eastern Cape Province are dominated by quartz minerals and are prone to crusting (Mandiringana et al., 2005; Nciizah and Wakindiki, 2012). The low specific surface area of quartz promotes rapid soil organic matter (SOM) mineralisation resulting in poor aggregate stability (Buhman et al., 2006)

A number of researchers reported that there is an interaction between aggregation and clay content and its mineralogy (Lado et al., 2004; Denef and Six, 2005; Norton et al. 2006). Calcium ions associated with clay generally promote aggregation, whereas sodium ions promote dispersion (Siddique et al., 2017). Soils with at least five percent iron oxides, expressed as elemental iron, tend to have greater aggregate stability. Soils that have a high content of organic matter have greater aggregate stability (USDA,1996).

2.2 Factors affecting aggregate stability

Soil aggregation and soil structure are important properties of natural and managed environments (Miller and Jastrow, 1992). A stable soil structure is vital not only for

increasing soil productivity and soil quality but also improving nutrient availability and water use efficiency (Byung et al., 2007). The processes of aggregation depend on a variety of aggregate binding agents interacting simultaneously at different spatial scales (Six et al., 2004; Bronick and Lal, 2005).

Factors affecting aggregate stability can be grouped as abiotic, biotic and environmental (Chen et al., 1998). The stability of aggregates is affected by soil texture, the predominant type of clay, extractable iron, and extractable cations. Soil organic matter and texture particularly clay content are the chief abiotic binding agents in the formation and stabilization of aggregates (Duchicela et al., 2012; Portella et al., 2012), whereas soil microbes (bacteria and fungi) and plant roots have been reported as key biotic aggregating agents (Chaudhary et al., 2009; Duchicela et al., 2013). Plants contribute to water-stable aggregates, with exudates from roots and soil microbes contribute to the formation of microaggregates, whereas fine roots and mycorrhizal hyphae contribute to the stabilization of macroaggregates (Amezketa, 1999; Six et al., 2004). A study by Amezketa (1999) found that microaggregates comprise mostly of associations of free primary particles bound together by persistent binding agents that comprise of metal oxy(hydr)oxides, polyvalent cations, Ca- and Mg- carbonates, $CaSO_4$ and organic molecules (Amezketa, 1999). Six et al. (2004) and Bronick and Lal (2005) also reported that SOC and microbial biomass acts as important binding agents for aggregation. However, some interaction between binding agents may negatively influence soil aggregation. For instance, Manyevere et al. (2016) indicated that in arid areas soils with high clay content and high Na, on long and steep slopes were vulnerable to erosion. They further noted that soils with high fine sand and very fine sand content are extremely vulnerable, especially where the

clay content also contains quartz, which reduces the aggregating potential of the clay. These effects are particularly worse for soils low in organic matter.

Aggregates that have high water resistance tend to have high organic carbon content within aggregates and directly influences soil structure and physical properties (Simansky and Bajcan, 2014). Simansky (2011) noted that microaggregates tend to be easily eroded and more influenced by soil management compared to macro-aggregates. Particles in small aggregates (<0.25 µm) are bound by older and more stable forms of OM (Oades and Waters,1991; Bossuyt et al., 2001; Six et al., 2004). Microbial decomposition of fresh organic matter release less stable products that bind small aggregates into large aggregates (>2-5 µm). When the proportion of large to small aggregates increases, soil quality increases. Manyevere et al. (2016) reported that the role of texture, cations and organic matter are less important in subhumid and humid areas where oxyhydroxides of Fe and Al dictate the aggregation of soils. While the effects of organic matter in highly weathered soils could be minimal, the interactions of organic matter with oxyhydroxides of Fe and Al is a well-known mechanism (Lutzou et al., 2006). The effects of such interactions on aggregation is not clearly understood. Studying changes in organic matter fractions and soil aggregate stability, as a result of tillage management can give an opportunity to understand the role of organic matter in aggregation in highly weathered soils.

2.3 Organic Matter and its labile fractions

A number of researchers (Yu et al., 2015; Peng et al., 2015; Somasundaram et al., 2016) suggests that SOM can improve the formation of soil aggregates and increase

the mechanical stability of aggregates by binding soil mineral particles. The influence of SOM on aggregate formation and stability is proven, although the existing fractions act differently in these processes (Baldock, 2002). These fractions may be arbitrarily established on the basis of location, composition or stability degree of the organic material (Baldock and Nelson, 2000). Costa et al. (2004) found differential contributions of total organic and particulate organic carbon (more labile) in the formation of soil aggregates.

Labile organic matter pools can be considered as fine indicators of soil quality that influence soil function in specific ways and that are much more sensitive to changes in soil management practice (Haynes, 2005). Particulate organic matter (POM) is composed of readily available material for decomposition by microbial attack, e.g., leaves, roots and animal remain (Amezketa, 1999; Six et al., 2004). This fraction responds more sensitively to soil management changes (Vieira et al., 2007; Campos et al., 2011) than to the total soil organic carbon (OC) (Figueiredo et al., 2013; Quanying et al., 2014.). This is especially true for occluded POM that may be lost from soil aggregates due to intense cultivation (Golchin et al., 1994). The particulate fraction acts as a cementing agent, to stabilize macroaggregates and as intra-aggregate protection of the proper organic matter (Six et al., 2002).

Water soluble organic carbon (WSOC) is the fraction of organic carbon that is soluble in water and is either sorbed on soil or sediment particles or dissolved in interstitial pore water (Tao and Lin, 2000). The WSOC accounts for a small portion of the total soil organic carbon content (Tao and Lin, 2000; Ohno et al., 2007; Barbara ad Fabrizio,

2009). Some researchers (Boyer and Groffman, 1996; Stevenson, 1994) consider it as the most vital labile and mobile form in soil organic matter pools since it is the main energy source for soil microorganisms (Schnabel et al., 2002; Marschner and Kalbitz, 2003), a primary source of mineralizable N, P, and S, and it influences the availability of metal ions in soils by forming soluble complexes. The contribution of WSOC may not easily be separable from the contribution of soil microbial biomass.

Soils with more labile C tend to have a higher microbial biomass. Microbial biomass comprises mostly of bacteria and fungi, which decompose crop residues and organic matter in soil and make up 1-5% total SOC (Haynes, 2005). Microbial biomass carbon is a measure of the carbon (C) contained within the living component of soil organic matter (i.e. bacteria and fungi). Unlike total organic C, microbial biomass C responds quickly to management changes (Vema et al., 2011). A change to more disruptive practices can quickly deplete soil carbon in the topsoil, particularly microbial biomass.

Arbuscular mycorrhizal fungi (AMF) are among the most widespread and important components of the soil microbiota in natural and agricultural systems (Finlay, 2008). Rillig (2004) noted that AMF directly contributes to soil aggregate stability through a physical effect of a network around soil particles, and indirectly by means of the hyphal exudation glomalin as an aggregate binding agent. AMF physically stabilize soil through the enmeshment of soil particles by means of hyphal networks and the production of glomalin operationally defined glomalin-related soil protein (GRSP) Miller and Jastrow 2000, Rillig 2004). Wright and Upadhyaya (1998) and Rillig (2004)

reported that GRSP confers stability to soil aggregates (Wright and Upadhyaya, 1998; Rillig, 2004).

While different organic matter fractions make an essential contribution on soil chemical, biological and some physical properties, the relationships with aggregate stability are not clearly understood. Although the different fractions of soil organic matter vary with soil management, management effects on GRSP are not clearly understood, especially in humid regions where nutrient cycles occur at high rates.

2.4 Glomalin related soil protein

Glomalin was discovered by Sara E. Wright in 1996 at the Agricultural Research Service, United States Department of Agriculture (USDA) and was firstly mistaken for an unidentifiable constituent of soil organic matter (Nichols, 2002; Nichols and Wright, 2004). It was identified during attempts to produce monoclonal antibodies reactive with AMF. It was recognized as a unique fungal glycoprotein secreted only by spores and extraradical mycelium of arbuscular mycorrhizal fungi in the taxon Glomales, including the genera *Acaulospora*, *Entrophospora*, *Gigaspora*, *Glomus*, and *Scutellospora* (Wright et al., 1996). The protein was named glomalin, because it is secreted by fungi belonging to Glomales. Glomalin either coats on the surface of extraradical mycelium or remains as a component of spore and hyphal walls (Wright and Upadhyaya, 1996), probably released into the soil by mycelium turnover (Driver et al., 2005), where it subsequently contributes to linking soil particles and stabilizing aggregates (Rillig and Mummey, 2006). Driver et al. (2005) reported that glomalin is only released into the soil environment during AMF hyphal turnover, after the death of the fungus.

Glomalin is a hyphal glycoprotein produced on the hyphae and spores of AMF (Wang et al., 2015) as well as non-mycorrhizal-related heat-stable proteins, lipids, and humic

materials (Jia et al.,2006) that has been found to make a significant contribution to SOM and to play a key role in the process of soil aggregation (Ariza, 2008). Immediately after degradation of the mycorrhizal hyphae, glomalin enters into the soils where it gets incorporated into the soil organic matter pool (Driver et al., 2005). It was named glomalin-related soil protein (GRSP), as per a grouping of proteins of AMF and non-AMF origin, together with soil-related heat-stable proteins (Rillig, 2004; Gillespie et al., 2011). However recent studies claim that GRSP is a mixture of many compounds and cannot be related to AMF (Wang et al., 2015). For instance, Snhindler et al. (2007) discovered that GRSP has variances in the aromatic carboxyl groups, which exhibited similar nuclear magnetic resonance spectra to humic acid. Similarly, Gillespie et al. (2011) detected that GRSP is a mixture of proteinaceous, humic, lipidic and inorganic substances and with a consortium of proteins and other impurities.

Glomalin related soil protein exists in large amounts in soils and is certainly a distinct component of soil organic matter. The GRSP is insoluble in water and resistant to heat degradation and hence very stable (Wright and Upadhyaya, 1996). GRSPs resistant to most chemical used in routine and characterization methods (Wright and Upadhyaya, 1996). Moreover, it has high cation exchange capacity and high affinity for polyvalent cations (Seguel et al., (2013) and has the potential to immobilize high amounts of metals (Gonzalez-Chávez et al., 2004; Vodnik et al., 2008; Cornejo et al., 2008). Because it is glue-like in nature and attaches to horticultural film and soil surfaces, glomalin is likely hydrophobic in its native state (Wright and Upadhyaya, 1998, 1999). However, GRSP has not yet been biochemically defined but it is a N lined glycoprotein which comprises of 5% N, 36 to 59% C, 4 to 6 % hydrogen, 33 to 49% oxygen (Lovelock et al. 2004; Schindler et al., 2007), and 0.03 to 0.1% P (Schindler et

al., 2007; Singh, 2011). Rillig et al. (2001) found that glomalin accounted for 4 to 5% of total C and N in Hawaiian soils. In another study in the tropical forest soils of Costa Rica and Hawaii, Lovelock et al. (2004) noted that 3.2% of total soil C and 5% of soil N was in glomalin (Fig 2.1). However, both C and N declined linearly with increasing total concentration of glomalin in the soil, whereas the opposite was true for protein and C: N. Glomalin is dark red-brown color and soil after extraction loses the brown color associated with organic matter. According to Wright and Upadhyaya, (1998) the reddish colour of glomalin extracts may be due to the presence of Fe (0.8 - 8.8 %). Glomalin accumulation in soils is assumed to result from the insolubility, hydrophobicity and high Fe content of the molecule.

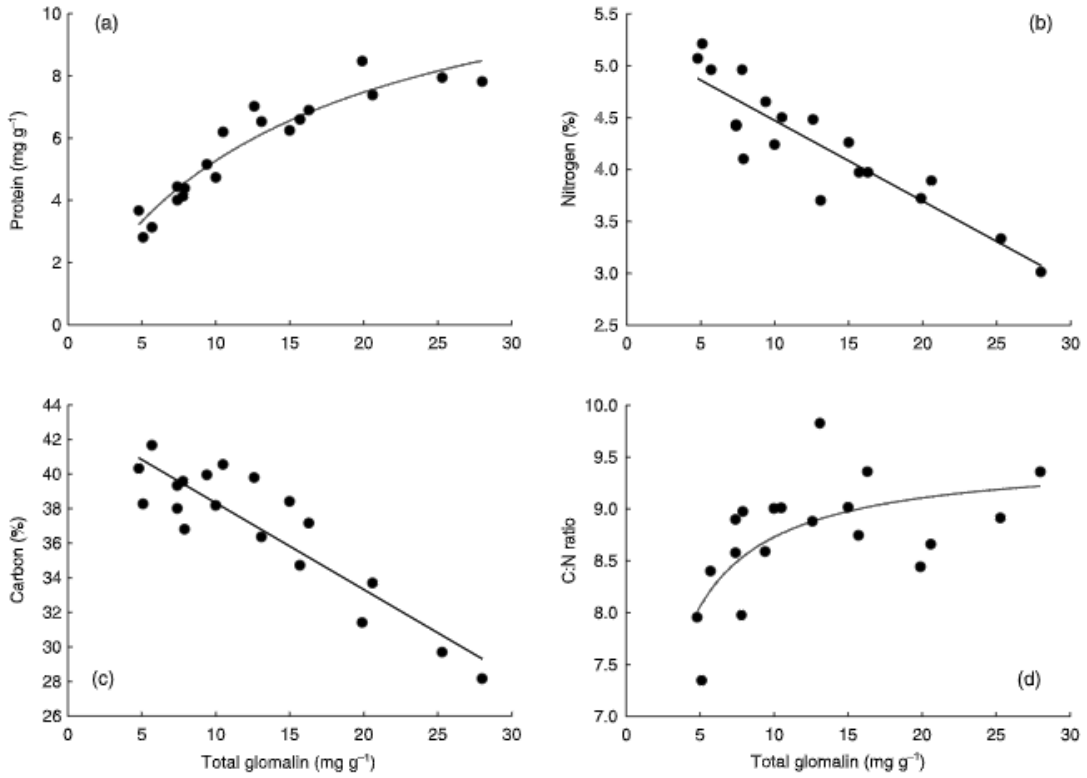

Figure 2.1: The protein (a), nitrogen (b)and carbon (c)concentration, and C : N ratio (d)of glomalin extracted from soils within old-growth tropical wet forest (Lovelock et al., 2004).

Hammer and Rillig (2011) characterized glomalin as a putative homolog of heat shock protein (hsp) 60. Wright and Upadhyaya (1998, 1999) and Nichols (2003) noted that GRSP is found abundantly (2 to 60 mg/ g) in a wide array of soils ranging from agricultural, grasslands, forest, desert and non-cultivated. The largest amount of GRSP has been reported in undisturbed forest soils, suggesting that tillage may affect the concentration of GRSP and associated aggregate stability. Rillig et al. (2001) reported concentrations of over 100 mg/g in Hawaiian tropical forests and up to 21 mg/g in woodland soils (Wright and Upadhyaya, 1996).

A number of authors have established the roles of glomalin in soil aggregate stabilization (Rillig et al., 2002; Wright et al., 2007). Glomalin contributes to the stabilization of aggregates by sloughing off hyphae onto the surrounding organic matter, binding to clays perhaps by means of cation bridging by iron, and providing a hydrophobic coating (Wright and Upadhyaya, 1999). The hydrophobic properties of glomalin contribute to aggregate stability by reducing the disruptive force of water movement within the pores of aggregates (Wright and Upadhyaya ,1998). Wright and Upadhyaya (1998) reported that glomalin content is highly correlated to soil aggregate stability and that the most stable aggregates had higher concentrations of extractable glomalin and immunoreactive extractible glomalin than less stable aggregates.

Nichols (2003) claims that the correlation between glomalin concentration and soil aggregation is possibly influenced by iron. Iron- and Al-(hydr) oxides are involved in aggregate formation by bridging organic matter with clay minerals hence contributing to the persistence of aggregates (Bird et al., 2002; Wright and Upadhyaya, 1998). This contribution could be of great importance particularly in high rainfall areas where soil Fe is high. Etcheverría (2009) showed that GRSP has the capacity to sequester substantial quantities of Al (4.2 to 7.5% by weight) in acidic soils of a temperate forest in southern Chile. Glomalin may have a crucial role in soil aeration and drainage, plant nutrient uptake, and productivity through its contribution in aggregation (Nichols and Wright, 2004; Rillig and Mummey, 2006). By virtue of its role in aggregate stability, glomalin enables soil C storage (Zhu and Miller, 2003, Rillig et al., 2004).

2.5 Relationship between glomalin and selected soil physicochemical properties

It is universally acknowledged that GRSP performs a vital role in making soil structure (Wright et al. 1996). The content of glomalin in soil particles is significantly correlated with aggregate stability (Driver et al. 2005, Koide and Peoples, 2013). GRSP have a role in both forming and stabilising microaggregates. Wright and Upadhaya (1998) assessed the association between the quantities of glomalin present in small aggregates (1-2 mm) with the water stability of those aggregates; results disclosed relatively strong part-whole correlations between the different fractions of glomalin. Correlation was relatively highly significant between aggregate stability and numerous fractions of glomalin, with the highest correlation coefficient found between

Immunoreactive easily extracted glomalin (IREEG) and aggregate stability. As in Wright and Upadhaya (1996) correlation between TG and soil carbon was very high ($r=0.82$, $p \leq 0.01$).

In a study on Bradford-reactive soil proteins and aggregate stability under abandoned versus tilled olive groves in a semi-arid calcisol, Hontoria et al. (2009) discovered that there was twice as much glomalin in stable than in unstable aggregates under different land management and abandonment. Emran et al. (2012) noted that after deposition of glomalin on soil particles soil micro- and macro-aggregates are progressively stabilized, so that larger contents of glomalin produce better structure. This in turn increases soil porosity, water storage and root development, and a more consistent resistance to surface crusting and sealing, and hence erosion (Wright and Anderson, 2000). Additionally, the ability of glomalin to maintain a stable structure can preserve organic compounds in soil microsites, which are able to protect organic carbon from mineralization processes, thereby favoring carbon sequestration against carbon dioxide production (Rovira and Vallejo, 2003).

Wang et al., (2015) noted that the relationship between GRSP composition and soil properties were not only in concentration but also its compositional characteristics. They state that SOC content ($R^2 = 0.89$) and N content ($R^2 = 0.62$) have great impact on both GRSP concentration and composition. Yang et al., (2017) noted that T-GRSP and EE-GRSP had significant positive relations ($P<0.01$) with SOC and TN. The highly significant correlation between SOM and GRSP confirmed that glomalin was a significant component of the soil organic matter, which was in agreement with the

findings of Rillig et al. (2003) who demonstrated that glomalin was a significant component of soil C and N and accounted for about 27% of SOM.

Neutral or slightly acidic soils are suitable for accumulation of GRSP due to it being suitable for plant roots and fungi. Soil pH directly affects effectiveness of AMF for improving plant viability and synthesis and secretion of GRSP from AMF (Wang et al., 2014). Soils low in pH have more TG due to increased organic activity (Bird et al., 2002). Fungi tend to prevail in more acidic soils. In higher pH soil environments, fungi still grow, nonetheless, they meet competition from bacteria and other organisms and as a result they may not be as active as they are in acid soils (Brady, 1990). Glomalin is produced by AMF, therefore, more protein is anticipated in the more acidic soils because of an increase in the activity of AMF and lower competition.

The C/N ratio is one of the indicators of soil aggregate stability (Bird et al., 2002). They established a strong, positive correlation between the C/N ratio and immunoreactive glomalin and noted that inorganic carbon demonstrated a significant positive correlation with the immunoreactive fractions of glomalin. Plants use inorganic carbon in the form of carbon dioxide from the atmosphere (Rillig et al., 1999). Plants in turn assign a great portion of their photosynthetically fixed carbon to root-infecting symbionts, such as mycorrhizal fungi (Rouhier and Read, 1998; Bonkowski, 2003). Because of this, there is improved nutrient uptake from the soil, causing the hyphae to become more active (Haddad and Sarkar, 2003) and therefore produce glomalin, which helps to improve soil structure, easing the passage of air and water and increasing resistance to erosion. This as well leads to an increased ability for the soil to hold on to valuable organic matter and soil carbon (Haddad and Sarkar, 2003). A

study by Fokom et al. (2013) in a humid forest zone south of Cameroon where he measured TG and C in soil from three-different land used systems (native forest, fallow and continuous growing fields systems), found a positive correlation between TG and C concentration in the three land use systems. This observation agrees with those from other studies showing strong correlation between TG and C pool in natural and cultivated soils (Rillig, 2003; Nichols and Wright, 2005). Similarly, in an evaluation of 12 acidic soils of tropical and temperate zone in America, TG was strongly correlated with soil C the essential atoms found in OM (R^2 = 0.84; Wright and Upadhyaya, 1996). Fokom et al. (2013) also noted a positive correlation between the EEGRP, carbon and nitrogen concentration (r.= 0.76, 0.55; P < 0.01) across the three systems. As a result, they concluded that the positive correlation might explain the insinuation of GRSP to the restoration of soil stock of C and N for the reason that glomalin molecules are made with a relatively high proportion of C and N atoms. The relationship between GRSP with soil C and N indicate that the factors that affect this soil component also affect SOC. However, the relationship between GRSP and different labile fractions of SOC is not clear.

2.6 Influence of soil management on aggregate stability

Soil aggregate stability is an important ecosystem property which deteriorates overtime due to agricultural practices. Soil aggregate stability is often deteriorated by agricultural practices through directly breaking the soil particles or indirectly by the disturbance of potential aggregate binding agents (Barto et al., 2010; Duchicela et al 2013). The main disruptors of aggregate stability are tillage (Six et al., 2002; Lal, 2013)., subsoil compaction due to equipment (Pulleman et al., 2003) and traffic from livestock (Oades, 1993). Tillage can disrupt soil aggregation in several ways: (i)

it brings subsoil to the surface, thereby exposing it to precipitation and freeze-thaw cycles, and (ii) it changes soil moisture, temperature, and oxygen level, thereby increasing decomposition and carbon loss (Six et al., 2000). An increasing number of studies have found that tillage results in degradation of aggregate stability (Wright et al.1999; Pikul et al., 2007). In addition, tillage alters soil microbial community dynamics (Jansa et al., 2002; Wang et al., 2010) and modifies AMF density and composition (Jansa et al., 2002; Yuan-Ying and Liang-Dong, 2007) which can disrupt soil aggregate stability.

In the view of Filho et al. (1998), NT with accumulation of plant organic residues on the surface improves aggregation by increasing organic carbon levels in the topsoil in so doing raising the percentage of aggregates > 2. mm. Reduced tillage or NT permits the possible recovery of soil aggregate binding agents such as soil micro-organisms and biochemical properties (Portella et al., 2012). In addition, cover cropping physically protect the soil form erosive forces such as water and wind while building up more SOM as they enhance biomass to the soil and also increase biological activity, thus improves soil aggregate stability (Liu et al. 2005; USDA, 2008).

2.7 Effects of management on soil organic matter and its fractions

Human activities also contribute to disruption of aggregate stability. When stable soils are subjected to continuous arable cultivation (CT) there is a deterioration in the physical properties which, has been shown to be a result of loss of organic matter and the stability of the aggregates (Six et al., 2002; Lal, 2013).In the view of, Borie et al. (2006) CT abrades the network of mycelium by mechanical breakdown of

macroaggregates, which reduces the content of SOM, microbial biomass and faunal activities (Sainju et al., 2009; Curaqueo et al., 2011). Farming systems that maximise organic matter return to soil and minimise soil disturbance tend to increase the microbial biomass. Minimising tillage increases microbial biomass by protecting soil aggregates formed by fungal networks pore spaces in the aggregates are an important habitat for the microbial biomass in soil.

The use of conservation agriculture (CA) has proved to be a viable alternative to improving soil quality. Conservation agriculture is achieved through (a) minimal soil movement, (b) permanent cover with crop residues or growing plants and (c) crop rotations (Thierfelder et al., 2009, Murungu et al., 2010). As a means of improving soil productivity and crop production, crop residues are left on the soil surface. However, when the gap between harvesting one crop and establishing the next is too long, cover cropping is recommended (Lal, 2015). Cover crops are important as they conserve N for grain crops; reduce soil erosion; and increase crop yields and reduce moisture stresses (Pretty 2008; Miller, 2017). Cover crops with deep roots can alleviate soil compaction in NT systems (Williams and Weil, 2004) and suppress weeds (Moyer et al., 2000; Triplett and Dick 2008; Lal, 2015). Moreover, cover crops impact soil chemical properties (Calegari and Alexander, 1998) and affect N mineralization and availability (Schomberg and Endale, 2004). Prevailing farming systems are not disturbed by introduction of winter cover crops. Cover crops such as canola (*Brassica napus*), hairy vetch (*Vicia villosa*), lupins (*Lupinus albus*), broad-beans (*Vicia faba*), Japanese raddish (*Raphanus sativus*) and black oats (*Avena strigosa*) are grown in the Eastern Cape (Allwood, 2006).The selection of the best cover crop species has been mainly based on their ability to produce high and persisting biomass and the

ability to meet part of the nutrient requirement of the subsequent crop. However, no single cover crop species is able to adequately achieve both objectives. Growing of a mixture of legume and non-legume cover crops (bicultures) has been identified as an ideal method of fulfilling both the objectives of cover cropping (Sainju et al., 2005; Odhiambo et al., 2001). In contrast some authors advocate for monocultures instead of bicultures (Haynes et al., 1997; Ramos et al., 2010). Organic matter (OM), one of the main products of cover crops, is the most widely acknowledged indicator of soil quality (Ramos et al., 2010). OM strongly influences soil structure stability and water retention (Holland, 2004). Decomposing plant material releases organic material that directly stabilizes soil particles. According to dos Reis Martins et al., (2009), easily hydrolysable polysaccharides, which originate from decomposing plant material, represent the most active binding agents in aggregation of soil particles.

Winter cover crops do not only protect the soil from direct raindrop impact, they also add to SOM. Land management systems like winter cover cropping that prevent soil disturbance, improve soil fertility, increase organic materials and decrease organic matter losses from the soil, significantly improve soil aggregation (Pagliai et al., 2004; Wei et al., 2006).

Improvement of SOM content of degraded agricultural soils could enhance physical and chemical properties, and biological activity (Salazar et al., 2011), and promote productivity of the soil. The SOM content influences, largely the activities of soil organisms, which in turn influence the SOC dynamics. Accumulation of organic matter and nutrients near the surface under no-tillage produces beneficial effects on soil physical, chemical and biological properties (Beare et al., 1997; Tebrugge and During,

1999), including enhanced rhizosphere biological activities (Kladivko, 2001). Fungal biomass including arbuscular mycorrhizal fungi (AMF), which are important mediators of soil aggregation (Borie et al., 2008). is enhanced in the topsoil under NT (Frey et al., 1999), Many reports have shown that AMF are able to counteract soil erosion by increasing the stability of soil aggregates (Bethlenfalvay et al., 1999; Miller and Jastrow, 2000) through the combined action of extraradical hyphae and their exudates and residues (Miller and Jastrow, 1992, 2005). The AMF are mutualistic symbionts living in association with the roots of most terrestrial plants and are vital for the soil–plant system as they influence soil fertility and plant nutrition (Smith and Read, 2008). However, there are limited studies on effects of CA on AMF and associated organic compounds in soil.

Soil aggregation by AMF is through the combined action of extraradical hyphae exploring soil to form an aggregate network and an insoluble, hydrophobic, recalcitrant glycoprotein, called “glomalin” which has binding properties (Bedini et al., 2009). Haddad and Sakar (2003) reported that the glomalin related soil protein (GRSP) detaches from the hyphae, moves into the soil, and becomes a distinct component of the SOM. By virtue of impacting soil aggregate stability, GRSP is a crucial component of soil that significantly affects its structure (Haddad and Sakar, 2003).

2.8 Effects of management on glomalin-related soil protein concentration

Glomalin has been found in agricultural, grassland, forest, desert, and non-cultivated soils (Nichols and Wright, 2004; Antibus et al., 2006; Bai et al., 2009). Pools of glomalin are responsive to ecosystem disturbances, these consist of factors of global change,

such as elevated atmospheric carbon dioxide, leading to increased GRSP concentrations (Rillig et al., 1999, 2000, 2001) or warming which was observed to lead to decreased levels of GRSP (Rillig et al., 2002) and land use change (Rillig et al., 2002), tillage practices (Wright et al., 1999), and crop rotation systems (Wright and Anderson, 2000). A study by Wright et al. (1999) on the conversion from plough till to no till in maize in relation to a control grassland on a single soil type, found that glomalin concentrations increased with transition to NT. They noted that the correlation amongst the two-varied depending on the sampling location but was generally high ($0.82 \leq r \leq 0.88$. $p < 0.001$). They discovered that the glomalin concentrations and aggregate stability were lower in all cases in the cornfield than in the surrounding grass. Similarly, Wright and Anderson (2000) found that aggregate stability correlated well with glomalin concentration under various crop rotations in the Great Plains. Whilst Wright et al. (1999) reported the highest correlation to be between T-GRSP and aggregate stability, Wright and Anderson (2000) observed the highest correlation between immunoreactive glomalin (IRTG) and aggregate stability. However, Franzluebbers et al. (1999) conducted a long-term study of conservation tillage and grazing on aggregate stability and soil glomalin concentration and found that these measures were weakly related.

Effects of CA, with no-till and residue retention relative to conventional tillage, on GRSP, are not clearly understood. These effects will have implications on aggregation of soils and overall soil quality. This is particularly essential in dryland CA systems in sub humid and humid areas, where the practice is more feasible due to high rainfall and the soils are also highly weathered, with high oxyhydroxides of Fe and Al. In South Africa, CA practices with no-till, residue retention and rotations, are practiced mainly

in high rainfall areas to support high biomass input. Aggregation in highly weathered soils is mainly due to the oxides of Fe and Al, and the effects of GRSP and other SOC fractions are not clearly understood under these conditions. The understanding of effects of management on GRSP and its relationship with aggregate stability and SOC fractions requires that the protein is extracted and analysed

Glomalin levels can also be affected by other management practices such as the application of fungicides and pesticides. According to Kabir (2005), implementation of these practices perhaps will recover soil physical properties at the macroscopic level, however this will in due course affect chemical and biological properties of soil at the microscopic level as well as AMF. A study by Rillig and Mummey (2006) in long-term grassland plots from which application of fungicide resulted in in the elimination of AMF, as a result GRSP concentrations were drastically decreased. In addition, Wilson et al. (2009) in their study that was done over a six years' period of suppressing mycorrhizal symbioses through fungicide application, discovered that easily extractable Bradford reactive soil protein (EE-BRSP) and Bradford reactive soil protein (BRSP) levels were reduced by 18% and EE-IRSP and immunoreactive soil protein (IRSP) reduced by 53 and 76%, respectively.

Most farmers are inclined to agricultural practices such as the application of and organic manures owing to the fact that they improve soil aggregation as well as other soil characteristics such as increases in porosity, infiltration capacity, hydraulic conductivity, and decreases in bulk density (Haynes and Naidu, 1998; Brar et al., 2015). Thus, there have been an increase in interests on how these practices affect glomalin. Long term application of manure and straw increased soil GRSP

accumulation in China (Dai et al., 2015). Their study concluded that long-term fertilisation significantly increased GRSP concentration. In contrast, a study by Lovelock et al. (2004) showed that soils in old growth forests of Costa Rica that were higher in residual fertility correlated with lower levels of T-GRSP and EE-GRSP. Thus, even though low nutrient status has been made known to improve AMF associations (Liu et al., 2000; Bohrer et al. 2001), high soil nutrient content can enhance glomalin production by increasing the fungal turnover (Lovelock et al., 2004a).

In addition to agricultural practices, abiotic and biological factors such as elevated CO_2 global warming, climate conditions, vegetation types, could affect GRSP concentration in soils. According to Rillig et al. (2002) elevated CO_2 can only indirectly affect AMF, since soil serves mostly as a buffer against changes in atmospheric gas composition. AMF are obligate symbionts and hence depend on their host plant for carbon and variations in the availability of carbon may affect the amount of glomalin that is produced (Hernandez 2001). A decrease in carbon below ground possibly will limit the carbon availability to AMF, which might result in lower rates of glomalin production (Rillig et al., 1999, 2000, 2001). Rillig et al. (2001) conducted a study on an irrigated sorghum field and found an increase in EE -GRSP and no change in T -GRSP in response to artificially elevated carbon dioxide, while Rillig et al. (2000) discovered an increase in EE -GRSP and T-GRSP along a naturally occurring carbon dioxide gradient near a carbon dioxide spring in New Zealand. Warming can directly affect AMF and the decomposition of their products (such as glomalin) but warming can also indirectly affect the fungi by altering carbon allocation from the host to the mycobiont (Rillig et al., 2002). They found that artificial warming in a grassland decreases glomalin concentration. Rillig et al., (2002) found that IRTG and IREEG decreased in

response to artificial climate warming, whereas T -GRSP and EE-GRSP did not change. Warming can increase decomposition of soil organic matter (Buol et al., 1990), consequently reducing glomalin pools in soil.

2.9 Extraction of GRSP.

Glomalin is usually fractionated into total glomalin related soil protein (T-GRSP), easily extractible glomalin related soil protein (EE -GRSP), Immunoreactive total glomalin (IRTG) and Immunoreactive easily extracted glomalin (IREEG).

Glomalin-related soil protein is extracted from field soil, roots, mesh strips or bags, or pot culture media (Wright and Jawson, 2001; Rillig, 2004; Wright et al., 2006). The extract solution is then used in further analyses such as the ELISA and Bradford total protein assay. Glomalin is extracted from hyphae and soil in sodium citrate solution by autoclaving for thirty to sixty minutes or more (Wright and Upadhyaya, 1996). A substitute to using an autoclave is to use a pressure cooker. This methodology was tested and verified to be possible by Wright and Jawson (2001). The procedure that is used in the extraction process varies depending on what fraction of glomalin is of interest; either easily extractable or total glomalin. Easily extractable glomalin related soil protein (EE -GRSP) is extracted with 20mM citrate, pH 7.0 at 121 °C for 30 minutes, whereas Total glomalin related soil protein (TG) is extracted with 50 mM citrate, pH 8.0 at 121 ◦C for an hour (Rillig, 2003) ,though, additional time may be required depending on the soil horizon (Wright and Upadhyaya, 1998).T-GRSP is extracted up until the supernatant is colourless or straw-coloured, which can be achieved after autoclaving for three to five cycles, though up to seven (Wright and

Upadhyaya, 1998) and nine (Rillig et al., 2003) extraction cycles have been reached. By means of a centrifuge, soil from which glomalin is extracted is pelleted soon after autoclaving to ensure the glomalin extract is free of soil particles when decanting the supernatant. For the reason that it is proteinous in nature, extracts are stored at 4˚C (Wright et al., 1996). They recommended that any analysis ought to be done within two to four weeks as glomalin does degrade.

Two detection methods are frequently used to quantify glomalin, that is the Bradford protein assay, yielding the EE-GRSP and the T-GRSP fractions, and an ELISA assay (Wright and Upadhyahya 1998), yielding the Immunoreactive easily extractable glomalin (IREEG) and Immunoreactive total glomalin (IRTG) fractions. (Rillig and Steinberg, 2002; Gadkar and Riling, 2006). Bradford assay, originally described by Dr.Marion Bradford in 1976, is one of the common methods to determine GRSP concentration. According to Wright et al. (1996) the Bradford essay depends on the formation of a complex between Coomassie brilliant blue G-250 dye and proteins in the acidic environment of the reagent, protein binds to the Coomassie dye. The colour changes are read by a spectrometer at a wavelength of 590nm (A590) as optical density can be related to protein concentration in GRSP extract using a standard of known concentration of protein. A number of studies (Jonathan and Javier, 2006; Schindler et al., 2007; Whiffen et al., 2007) have shown that polyphenolic compounds, such as soil tannins and humic acids, might be coextracted with glomalin and interfere with the Bradford quantification, indirect enzyme-linked immunosorbent assay (ELISA) is relatively specific (Wright et al., 1996).

Total glomalin quantified using ELISA is regarded as Immunoreactive soil protein (IRSP) and the easily extractable fraction is named easily extractable Immunoreactive soil protein (EE-IRSP) (Wright and Upadhyaya 1998; Rillig 2004b). The procedure involves using monoclonal antibody MAb32B11 developed against crushed spores of *G*. *intraradices* antibody as the primary antibody and biotinylatedanti-mouse IgM antibody as the secondary antibody (Wright et al., 1996). The procedure is done as explained by Adeleke (2010), MAb32B11 is added to the glomalin extract and subsequently binds to an antigenic site (i.e., a site in which antibodies are induced) of glomalin. A solution containing a protein (e.g., ExtAvidin) and an enzyme (e.g., peroxidase) is added, followed by the addition of fifty microliters of 2,2~-azino-bis -(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) colour developer. The protein molecules bind to the biotin in the anti-mouse IgM antibody, and the enzyme reacts with a substrate molecule in the colour developer to produce a blue-green colour. The degree of colour change is determined using a spectrophotometer at 405 or 410 nm and compared with a standard to calculate glomalin concentrations. The standard curve in a range of 0.005 and 0.04 µg is prepared using glomalin obtained from soil samples with 100% immunoreactivity (Wright et al., 1996; Nichols and Wright, 2004; Rillig, 2004).

Even though the Bradford assay is not specific for glomalin, positive and significant correlations are usually found between Bradford and ELISA values (Wright and Upadhyaya, 1996, 1998, 1999; Harner et al., 2004). Rosier et al., (2008) claims that the Bradford assay is more accurate than the ELISA technique for the reason that the Bradford assay entails less pipetting. Apart from this, the Bradford assay is economical and faster, and not as technical and laborious compared with the ELISA technique

(Adeleke 2010). Glomalin values attained from the ELISA technique are compared with the Bradford values to determine percentage of Immunoreactive protein in glomalin extract (Adeleke 2010). Immunoreactivity is calculated by dividing ELISA values by the Bradford values and multiplying by 100. A number of researchers (Wright et al., 1996; Nichols and Wright, 2004 Rillig, 2004) concluded that the higher the percentage, the more Immunoreactive the glomalin fraction.

CONCLUSION

Soil organic matter and glomalin, operationally referred to as GRSP, were shown to be important determinants of soil structural stability and hence and important indicators of soil health. It was also shown that just like SOM, GRSP quantities are negatively affected by such unsustainable practices like continuous conventional tillage. However, little has been reported on the relationship between SOM fractions particularly the labile fractions and GRSP, in humid regions where the interaction with sesquioxides and clays could result in stabilisation, is not clearly understood.

CHAPTER 3

MATERIALS AND METHODS

3.1.1 Site description

The study was conducted on a farm located in the south-east of Howick, in the uMgungundlovu District Municipality, KwaZulu-Natal (Fig 3.1)..The area lies at altitudes ranging 950 to 1540 m and receives average annual rainfall of up to about 1400 mm, with most rainfall occurring mainly during mid-summer. The topography is made up mostly from frequent occurrences of dolerite dykes that pierce Karoo system shale and often resulting in isolated hills within the general incline of the Drakensberg escarpment (Wiese et al. 2016). Most of the profiles that were identified were deep red apedal soils, with medium to high clay content. The soils are derived from Ecca shale and to a lesser extent dolerite. For the reason that the area of research is such a large and dissected area (1 Ha), a dominant soil form was not identified which could have been used as a reference for all the observations

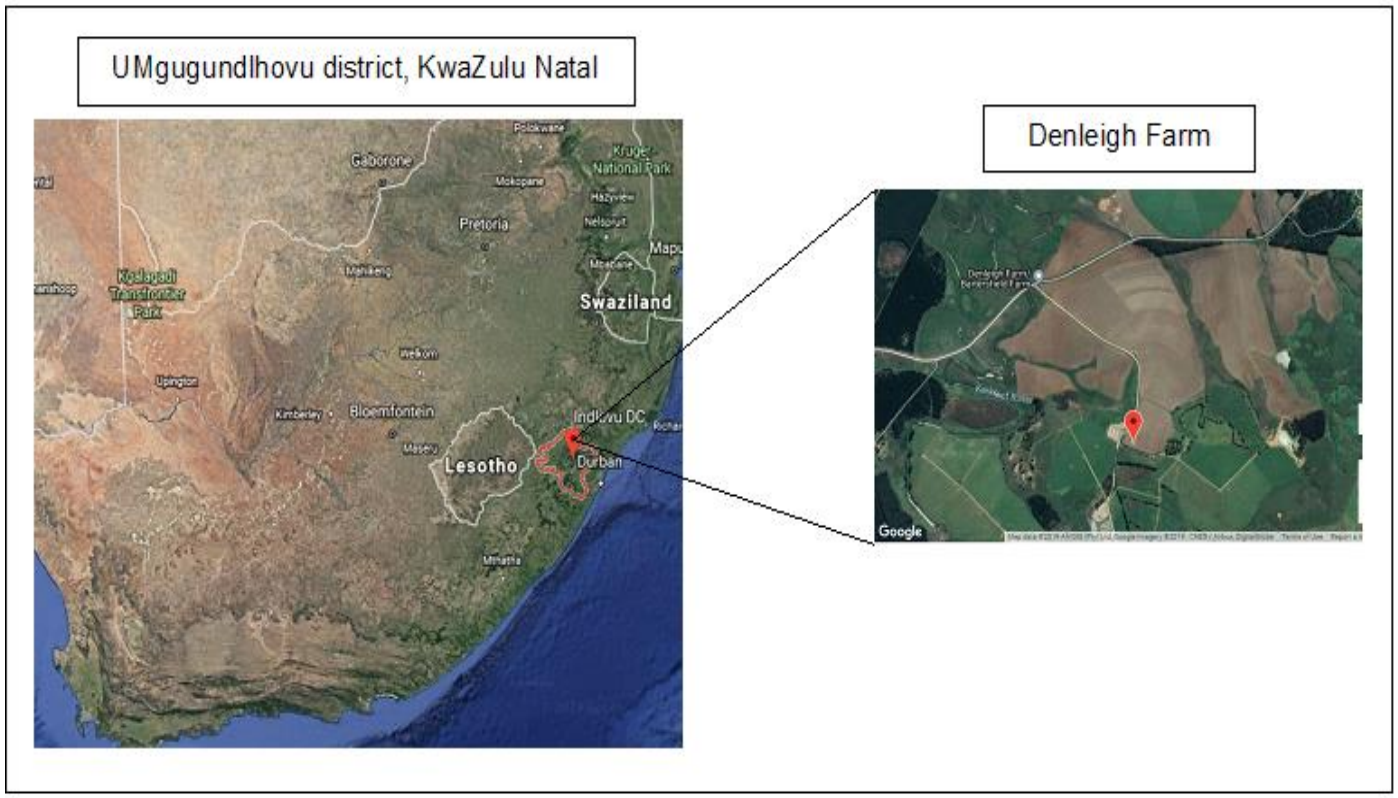

Figure 3.1: Map of study site

3.1.2 Soil management practices

The study site was situated in the Karkloof area, on a farm called Denleigh. No-till has been practiced on a portion of the farm for 15 years. These fields did not get ploughed at all, though the soil gets aerated with a special aerating implement to a 10 cm depth once in a decade. No weed control practices were practiced on the farm, neither chemical nor mechanical. After every three years Lime was applied with a spreader behind the tractor at a rate of between 2 – 3 tons per hectare. The lime was not get worked into the soil in any way after application. The soils were applied with nitrogen, first nitrogen application for the season was applied during the planting of maize *(Zea mays)* with the no-till planter and it varied between 40 – 50 kg/ha of nitrogen band placed with the seeds. After emergence of the maize plants the top dressing was split

into two applications of 60 – 70 kg of nitrogen per hectare each. The nitrogen was always applied in the form of urea with a 46% N. In total the farmer applied between 130 – 150 kg/ha of nitrogen each year during the maize growing season. Cover crops were planted within the first week of the maize harvest; these cover crops were either oats (*Avena sativa* or*x.Triticosecale* (a rye-wheat/triticale hybrid). Cattle were allowed to graze the cover crop from June to September each year and the following year's maize was planted in the mulch.

Conventional tillage has been practiced on a portion of the farm for over 15 years producing maize and soy beans *(Glycine max).* The plant residues were incorporated into the soil each year with the disc harrowing practices for maize and soy beans. After every harvest in May – June the fields got ripped up to 50 cm and disc harrowed to aerate the sub soil and break up all the clods on the soil surface. In total the conventional farmer aimed to apply 150 kg of nitrogen to the plants per growing season. With planting 20 – 30 kg of nitrogen was placed with the seeds in a mixture of urea and Monoammonium Phosphate. The planter was used to place the fertilizer in granular form in a band with the seeds during planting. Six weeks after planting when the plants have emerged successfully a top dressing was be applied. A tractor pulled a spreader and the spreader applied the fertilizer at a rate of between 100 – 120 kg/ha in a granular urea (46%) form. Lime was not applied often on this farm, in the last 10 years lime was only applied twice once in 2008 and once in 2014 only on selected fields as well. When lime was needed it was applied with a spreader behind the tractor at a rate of 2 tons per hectare and then worked into the soil with a disc harrowing practice. Where maize was planted for two seasons in a row soils were ripped once after harvest up to a depth of 50 cm. After the first rain late in winter or

early spring a mouldboard plough was used to plough the soils up to a depth of 20 cm. Depending on the soil moisture; the fields were disc harrowed up to a depth of 15 cm at least once to incorporate the stubble as well as prepare the seedbed. The native forest (F) lands are a home to the native trees and shrubs which are dense.

3.1.3 Soil sampling

Soil samples were collected at 0-5, 5-10, 10-20 and 20-30 depths from three management practices i.e. long-term no-till (NT), native forest (F), conventional tillage (CT) in August 2017. The native forest served as the control. Sampling was done following procedures described by Filho et al. (2016). Briefly, nine centering points were georeferenced in 100 m x 100 m grid sampling plot, at a distance of 30 m from each other and 20 m from the edge in each tillage system (Figure 3.2). Around the centering point, nine soil samples were collected and used for microbiological, physical, and chemical analyses of the soil. For samples intended for microbiological analysis, samples were collected from a depth of 10 cm, sieved using a <0.002 m sieve, and then kept refrigerated at 4 °C for analyses. For chemical analysis, samples were collected from a depth of 10 cm, air-dried, sieved (<0.004 m), and stored until analysis. For physical analysis, disturbed and undisturbed samples were collected (from a depth of 0.30m). Disturbed samples were air-dried, sieved (<0.004 m), and stored. Undisturbed samples were collected using a core sampler and kept intact and sealed to prevent loss of moisture and used to determine soil bulk density. Samples for aggregate stability determination were transported to the laboratory in rigid containers to avoid further disruptions of the aggregates (Le Bissonnais, 1996)

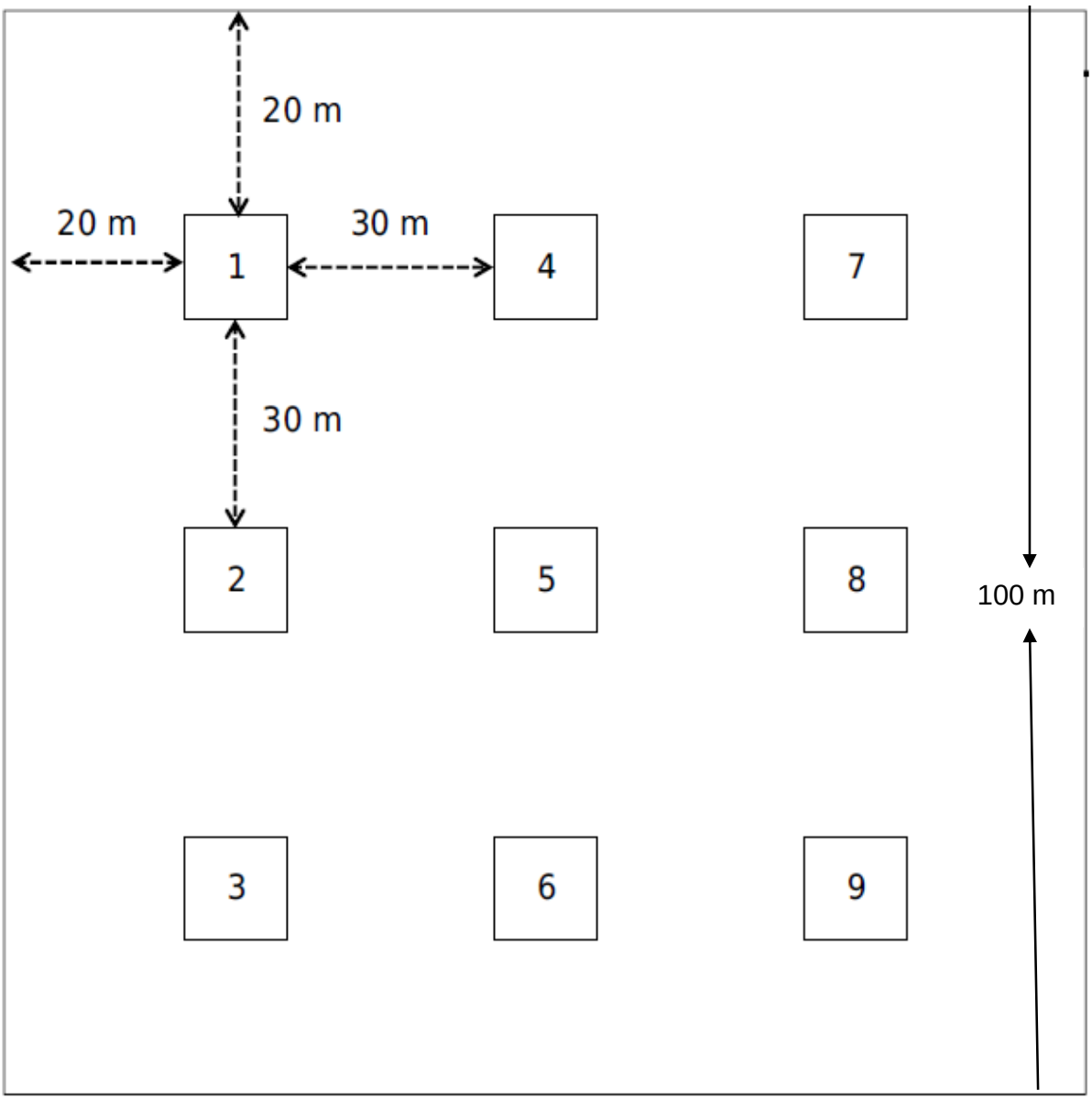

Figure 3.2: Soil sampling map

3.1.4 Soil characterisation

Soil pH was measured in water at soil-water ratio of 1:2.5, using a pH meter (model pH 25, Crison Instruments, South Africa) after shaking the suspensions for 30 minutes and equilibrating for 10 min (Okalebo et al., 2000). The dry combustion method was used to determine total C and N determined using a LECO® TruSpec C/N auto analyser (LECO Corporation, St Joseph, MI, USA). Available phosphorus was

determined using Bray-1 [0.03 M NH4F + 0.025 M HCl] at 1:10 soil/solution ratio (Okalebo et al., 2000). Exchangeable cations (Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}) were extracted with and ammonium acetate (pH 7). Briefly, a 5 g air dry soil sample was placed in a 100 cm^3 extraction bottle, 50 cm^3 of ammonium acetate cooled to 20 +/- 2 °C was added to the soil. The bottle was then shaken on a reciprocating shaker at 180 oscillations per minute for 30 minutes. The extract was then rapidly filtered through a buschner funnel with suction (Helmke and Sparks, 1996) and their concentrations determined using a Varian 700-ES Model inductively coupled plasma-optical emission spectrometer (ICP-OES, Varian, Inc., USA). Exchangeable acidity was determined using 1 mol dm^{-3} KCl on a volume basis. A 2.5 g soil sample was transferred into an extraction bottle into which 25 cm^3 of 1 mol dm^{-3} KCl was added. The mixture was stirred for 10 min at 400 rpm. The solution was filtered into a 150 cm^3 capacity Erlenmeyer flask. A 10 cm^3 aliquot was taken to which 10 cm3 deionised water was added. The solution was titrated with 0,01 mol dm^{-3} NaOH with phenolphthalein as indicator. A 10 cm^3 KCl blank was included. Extractable acidity was calculated using the formula below:

$$cmol\,(+)\,dm^{-3}\ soil = \frac{cm^3\ NaOH\ (sample) - cm^3\ NaOH\ (blank) \times\ f\ \times 100}{(cm^3)\ sample\ volume} \quad \text{[Equation 1]}$$

Where f = concentration of NaOH (mol dm^{-3})

Particle size distribution (7 classes) was determined after decomposition of organic matter with 30% hydrogen peroxide (H_2O_2), followed by dispersion with sodium hexametaphosphate. The sand fraction was separated by sieving and the coarse silt (0.05–0.02 mm effective diameter), fine silt (0.02–0.002 mm) and clay (<0.002 mm)

fractions were determined by the pipette method. The sand fractions were then separated by sieving, into coarse sand (2–0.5 mm), medium sand (0.5–0.25 mm), fine sand (0.25–0.1 mm), and very fine sand (0.1– 0.05 mm) (Soil Classification Working Group, 1991).

Soil bulk density was determined using the core method (Grossman and Reinsch, 2002). Briefly, soil samples were collected in triplicate at 0-5, 5-10, 10-20 and 20-30 depths in each of the three tillage treatments using a core sampler. The fresh weight of soil plus core was recorded. The cores along with soil sample were dried in an oven at 105°C for 24 hours. Dry bulk density was determined from the ratio of mass of dry soil per unit volume of soil cores using the following formula;

$$\rho_b = \frac{mass\ of\ oven\ dry\ soil\ (g)}{total\ volume\ of\ soil\ (cm^3)} \qquad \text{[Equation 2]}$$

3.1.5 Aggregate stability determination

The fast wetting method described by Le Bissonnais (1996) was used to determine the stability of aggregates between 3 and 5 mm diameters. The aggregates were oven dried at 40 °C for 24 h to ensure that all samples were at a constant matric potential. A 5 g sample of aggregates was gently immersed into a 250-ml beaker filled with 50 ml deionised water for 10 min. The water was then sucked off with a pipette to leave behind slaked aggregates. The slaked aggregates were then gently transferred onto a 0.053 mm sieve previously immersed in ethanol, to avoid re-aggregation during drying. The sieve was gently moved up and down in ethanol five times to separate the aggregates <0.053 mm from those >0.053 mm. The remaining <0.053 mm fraction

was oven dried at 40 °C for 24 h and its size distribution was measured manually. The aggregate fraction sizes were determined by sieving the soil in a net of sieves of 2, 1, 0.5, 0.25, 0.1- and 0.05-mm diameter. The soil in each sieve was then weighed and expressed as a percentage of the total weight of the soil. The aggregate stability was expressed by calculating the mean weight diameter (MWD) of the seven classes:

$$MWD = \sum_{i=1}^{7} \overline{x_i} w_i \qquad \text{[Equation 3]}$$

where w_i is the weight fraction of aggregates in the size class *i* with diameter $\bar{x}$. *xi* is mean diameter of each size fraction (mm) (Le Bissonnais, 1996).

3.1.6 Determination of water-soluble organic carbon

Water Soluble organic carbon (WSOC) was determined following the procedure described by McGill et al. (1986). The concentration of WSOC was measured from the top 5 cm only. Briefly, WSOC was extracted from field-moist samples within 24 h of sampling by shaking 10 g soil with 20 mL deionized water for 60 min, followed by centrifugation at 10 000 x g for 30 min. The supernatant was then filtered using 0.45 μm membrane filters. The filtrates were stored at - 10°C until they were analysed using the Walkley-Black Method (McGill et al., 1986)

3.1.7 Determination of soil microbial biomass and organic carbon.

The soil microbial biomass was determined using the chloroform fumigation – incubation technique outlined by Vance et al. (1987). Microbial biomass carbon was measured from the top 5 cm only. Two 10 g sieved (2 mm sieve) wet samples were weighed from each of the soil sample into small plastic containers and labelled (t_1) and (t_2). A third 10 g sample was weighed into a 125 ml watertight plastic bottle. Sample (t_1) was used for the determination of percent water content in order to express the results on a dry-weight basis. Sample (t_2) was fumigated with chloroform free alcohol in a desiccator prior to incubation, and subsequent extraction of dissolved organic carbon. Sample (t_3) was used for the extraction of dissolved organic carbon in unfumigated soil. Ten samples for fumigation were arranged simultaneously on a wire gauze in a vacuum desiccator containing 300 ml alcohol free chloroform (Hobbie, 1998) in a shallow dish beneath the gauze. The lid of the desiccator was closed and vacuum applied through a pressure pump until the chloroform evaporated. The tap on the desiccator was closed and placed in the dark for 5 days at room temperature. The desiccator was removed and each sample transferred into the respective 125 ml watertight extraction bottles. Fifty millimeters of potassium sulphate (0.5M) was added into each of the bottles, tightly stopped and shaken on a rotary shaker for 30 minutes. The samples were then filtered through a Whatman No.1 filter paper. Each of the extracts were analysed for dissolved organic carbon by titration following procedure described by Anderson and Ingram (1993).

The titration method was commenced with 30 minutes placement on a pre-heated block (150°C) of l litre glass beaker containing tap water and eleven test tubes; and

thereafter removed. Ten of the test tubes contained a mixture of 4.0 ml sample extraction, 1.0 ml of 0.0667 M $K_2Cr_2O_7$ and 5.0 ml conc. H_2SO_4; while the last (blank) tube contained similar mixture but without sample. The content of each heated tube was cooled together with one unheated blank tube and transferred into labeled 100 ml Erlen Meyer flasks and 4 drops of phenanthroline indicator added. Extracts were titrated with acidified Fe $(NH_4)_2SO_4$ solution through a green/violet color change to a red end point; ant the titre of each flask recorded. Estimation of extracted organic carbon was done according to the equation:

$$\%\ Organic C = \left[\left(A \times M \times 0.0037/g\right)\right] \times \left(\frac{E}{S}\right) \times 100 \qquad \text{[Equation 4]}$$

where

A = (mL Hb-sample) (mL Hb-Ml Hb/mL uHb + (Ml Hb-sample)

With Hb indicating heated blank and uHb indicated unheated blank.

M = Molarity of Fe $(NH_4)_2SO_4$ = 0.4/T

T = Standardised titre obtained daily by titrating 1.0 ml 0.0667M

$K_2Cr_2O_7$ in an Erlen Meyer flask with acidified Fe $(NH_4)_2SO_4$ for the purpose of establishing its molarity.

G = weight of dry soil

E = Extraction volume (50 ml K_2SO_4)

S = digest sample volume (4.0 ml)

Estimate of the microbial biomass C of each sample as described by Vance *et al*. (1987) using the equation:

Microbial biomass C = (Extracted C_{t2} – Extracted C $_{t3}$) x 2.64

3.1.8 Determination of GRSP Content

The glomalin-related soil protein (GRSP) was determined as easily extractable glomalin (EE -GRSP) and total glomalin (T-GRSP) according to the method of Wright and Upadhyaya (1998). Briefly, EE-GRSP was extracted from 1 g of air-dried and sieved soil (<2 mm) by suspending in 8 mL of 20 mM citrate buffer at pH 7.0 and autoclaved for 30 min at 121°C. Samples were centrifuged immediately at 5000 rpm for 15 mins. The protein in the supernatant were determined using a Bradford protein assay with bovine serum albumin as the standard (Wright and Upadhyaya, 1998). The EE-GRSP concentration in aggregates was calculated as:

$$EE-GRSP = \frac{(concentration\ of\ \ EE-GRSP)\times(volume\ of\ \ extract\ added)}{(initial\ mass\ of\ \ aggregates)} \quad \text{[Equation 5]}$$

The concentrations were expressed as mg g^{-1} dry soil aggregates.

The T-GRSP was obtained by repeated extraction from 1 g of air-dried soil with 8 ml of 50 mM citrate, pH 8.0 at 121^{o}C for 60 min. After each autoclaving cycle supernatant were removed by centrifugation at 5000 rpm for 20 min and stored. The extraction of T-GRSP was repeated until the glomalin content of supernatant was under the method detection limit (ca. 2 mg/ml). Extracts from each cycle were pooled, centrifuged at 10,000 rpm for 10 min to remove soil particles and then and protein in the supernatant were determined using a Bradford protein assay with bovine serum albumin as the

standard (Wright and Upadhyaya, 1998). The T-GRSP concentration of aggregates was likewise calculated as:

$$T-GRSP=\frac{\left(concentration\ of\ \ pooled\ T-GRSP\right)\times\left(total\ volume\ of\ \ T-GRSP\ extract\ added\right)}{\left(initial\ mass\ of\ \ soil\ aggregates\right)+EE-GRSP}$$

[equation 6]

The concentrations were expressed as mg g^{-1} dry soil aggregates.

3.1.9 Statistical Analysis

The data for WSOC and MBC were subjected to one-way analysis of variance (ANOVA), while a two-way ANOVA was used for organic C, glomalin related soil protein and aggregate stability, using JMP 12.1 (SAS Institute, 2016). Mean separations were done using Fisher's protected least significant differences (LSD) at $P < 0.05$. The coefficient of determination (R^2) was used to measure the strength of relationships between SOC and both EE-GRSP and T-GRSP, WSOC and both EE-GRSP and T-GRSP, MBC and both EE-GRSP and T-GRSP, aggregate stability and both EE-GRSP and T-GRSP as well as between aggregate stability and SOC, WSOC and MBC.

CHAPTER 4

RESULTS

4.1 Initial soil characterization

Some of the chemical and physical properties measured for the soils used in this study are shown in table 4.1. All management practices had acidic soils with pH values ranging between 4.9 and 5.5. The EC values varied from 3 to 5 mS/m and decreased in the order NT >CT> F. Soil P ranged between 1.73 and 39.36 mg/kg CT had medium P, NT hand low and F had the lowest P content. Forest soils had the highest OC content averaging 5.6% whilst soils under RT had the lowest with an average value of 2.04%. Clay content was highest in NT soil (23.1%) and lowest in soil under F (18.7%).

Table 4. 1 Selected physical and chemical soil properties of the three soil management practices after 15 years

Soil Property	Management practice		
	NT	CT	F
Exchangeable acidity (cmol /kg)	0.94 b	0.72 b	2.27 a
pH	5.5 a	5.4 a	4.9 b
EC (dS m^{-1})	5.0 a	4.43 a	3.33 a
CEC (cmol(+)/kg)	14.7 ab	9.9 b	17.4 a
P (mg/kg)	12.4 b	36.1 a	2.5 b
Clay (%)	23.1 a	21.0 a	18.7a
Coarse Silt (%)	18.8 a	6.60 c	11.8 b
Fine Silt (%)	31.05 a	19.6b	28.7a
Coarse sand (%)	2.42 b	6.83 a	7.70 a
Medium sand (%)	5.35 b	14.2 a	8.04 b
Fine sand (%)	10.7 b	19.3a	12.1b
Very fine sand (%)	9.61 a	7.9 b	9.57 f
OC (%)	3.8 b	2.9 c	5.6 a

Mean values and standard errors for the three land uses. Values in the same row with the same letter are not significantly different at $p < 0.05$. EC = Electrical conductivity, OC = Organic carbon, NT = Native forest, CT = Conventional tillage, F = Forest

4.2. Effects of soil management on soil aggregate stability

Land use had significant effects ($p < 0.05$) on soil aggregate stability measured as MWD. Soils under F were the most stable with an MWD of just over 3, whilst soils under CT had the lowest MWD value of 1.24 (Fig 4.1). The differences in MWD between NT and CT were statistically significant. MWD in F was more than twice that of CT (61%), whilst that of NT was 41 % less.No till was 34% greater than CT.

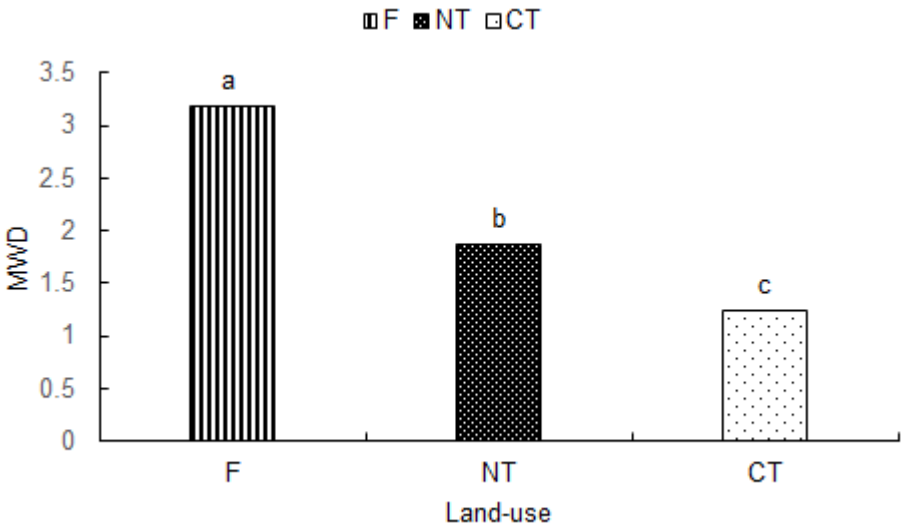

Figure 4. 1 Effect of soil management on aggregate stability in the top 0-5 cm depth. F = Native forest, NT = No till, CT = Conventional tillage.

4.3 Effect of land use and soil depth on soil organic carbon content

Both land-use and soil sampling depth had significant effects on SOC content ($p < 0.05$). However, the SOC content among the three land-uses varied with depth as indicated by statistically significant interaction between land-use and soil depth. There was a general decrease in SOC as depth increased for all land-uses except for NT,

where no significant differences were observed in SOC across the four soil depth layers (Fig 4.2). Within each soil depth, soils under F had the highest SOC content, whilst soil under CT had the lowest. The greatest percentage difference in the top 5cm was found between F and CT (45%) whilst F and NT had 35%.the same trend was observed for all depths, that is greater percentage difference was found between F and CT. The lowest percentage difference was observed in the top 10-20cm depth between NT and CT (16%). The greatest percentage difference was observed in the top 5cm between F and CT (51%).

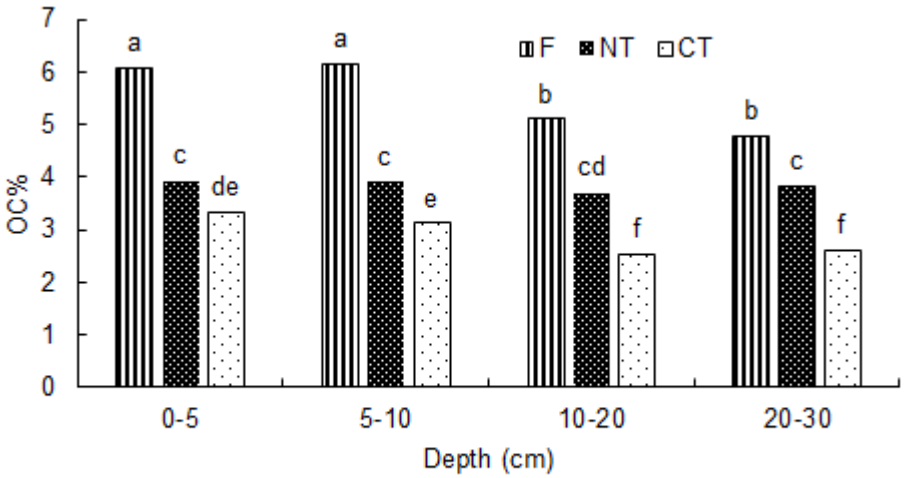

Figure 4. 2 Effects of soil management and depth on soil organic carbon. Treatment means with the same letter are not significantly different. F = Native forest, NT = No till, CT = Conventional tillage.

4.4 Effect of land use on microbial biomass carbon

Soil management had significant effects ($p < 0.05$) on MBC, with higher values under F than CT and NT, which had similar levels (Figure 4.3). forest had 34% more MBC

than CT which had 12% more MBC than NT. The greatest percentage difference was amongst F and NT (42%).

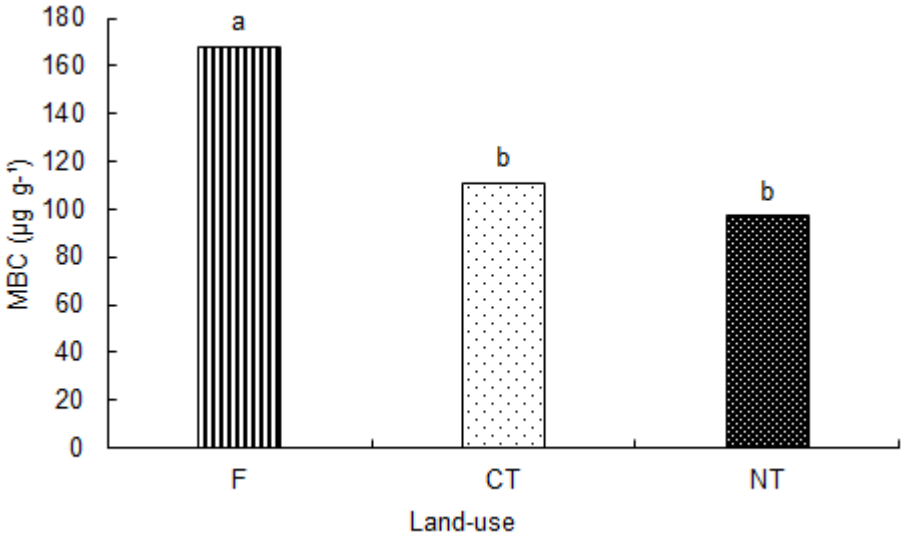

Figure 4. 3 Effects of soil management on microbial biomass in the 0-5 cm depth. Treatment means with the same letter are not significantly different. F = Native forest, NT = No till, CT = Conventional tillage.

4.5 Land use effects on water-soluble organic carbon

The WSOC significantly ($p < 0.05$) differed with land-use, with forest resulting in the highest concentrations of WSOC (200.63 µg g^{-1}), whilst NT had the lowest (92.52 µg g^{-1}) (Fig 4.4). However, there were no significant differences in WSOC concentration between NT and CT. Generally, concentrations of WSOC exhibited a similar response to that of MBC i.e. a gradual decrease in the following order F> CT >NT. The greatest percentage difference was obtained between F and NT (54%) and the least between CT and NT (24%). The difference between F and CT was 39%.

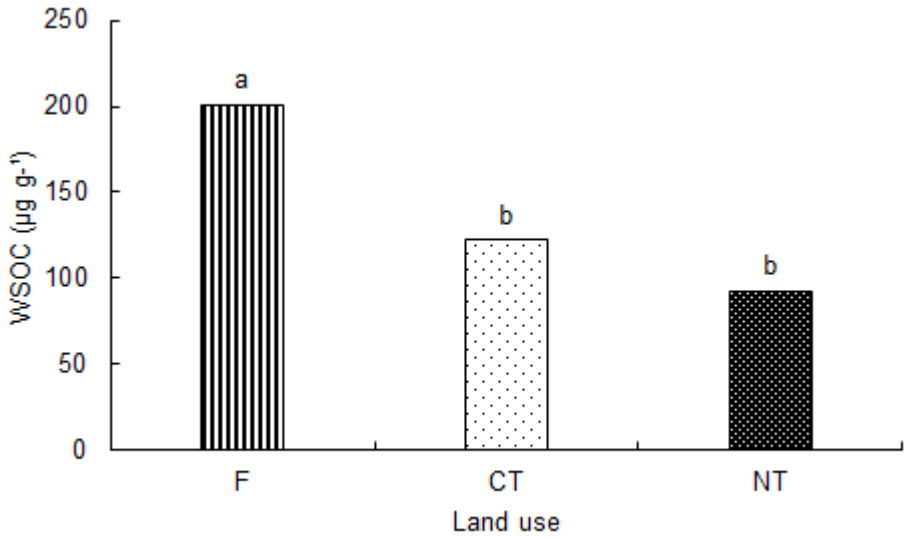

Figure 4. 4 Effect of soil management on water soluble organic carbon in the 0-5cm depth. Treatment means with the same letter are not significantly different. F = Native forest, NT = No till, CT = Conventional tillage.

4.6 Soil management effects on bulk density

Soil management and soil depth had significant interaction effects ($p < 0.05$) on soil bulk density. Soil bulk density was in the order CT = NT > F in the 0-5 and CT > NT > F in the 5-10 cm depths (Fig 4.5). Soil under CT had higher bulk density than both F and NT in both the 10-20 cm and 20-30 cm depths. There was no change in bulk density in the CT treatment with depth, while in the NT treatment the 0-5 cm depth had higher density than the other depths.

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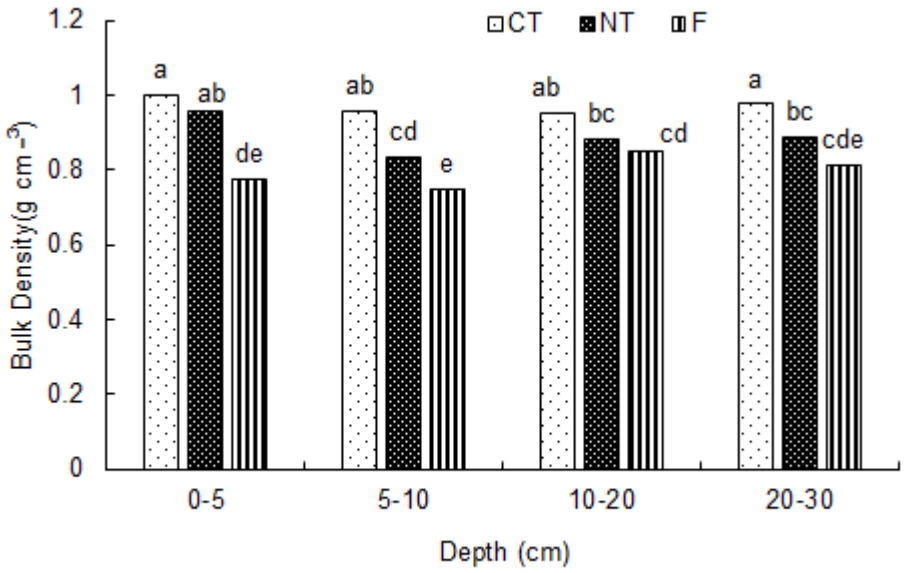


Figure 4. 5 Effect of soil management and depth on bulk density. Treatment means with the same letter are not significantly different. F = Native forest, NT = No till, CT = Conventional tillage.

4.7 Effects of soil management and depth on glomalin related soil proteins

The interaction between land-use and depth also had significant effects ($p<0.05$) on the concentration of easily extractable (EE-GRSP) and total (T-GRSP) glomalin related soil proteins (Figure 4.7). The NT treatment had the lowest concentration of EE -GRSP than the other two treatments in the 0-5 cm depth, and there were no differences among the management practices at deeper soil layers (beyond the 0-5 cm depth).

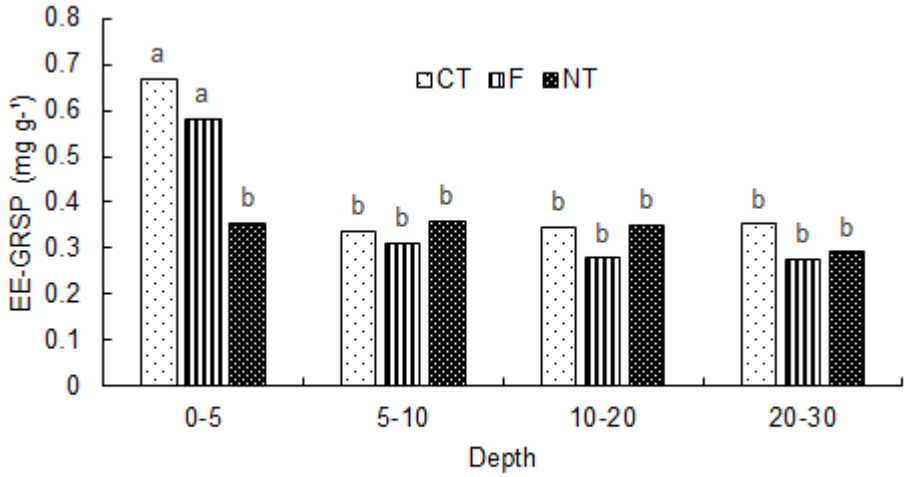


Figure 4. 6 Effect of soil management and depth on easily extractable glomalin related protein (EE-GRSP). Treatment means with the same letter are not significantly different. F = Native forest, NT = No till, CT = Conventional tillage.

Interaction between management practice and depth also had significant ($p < 0.05$) effects on T-GRSP concentration. For NT, the highest T-GRSP was observed in the 0-5 cm depth than other depths. No significant differences were observed on T-GRSP concentration across the remaining three soil sampling depths for NT. In contrast, under CT, the highest concentration of T-GRSP was observed in both the 10-20 cm and 20-30 cm depths than the 0-5 cm and 5-10 cm layers. The T-GRSP concentration was in the order F > NT > CT in the 0-5 and F>NT=CT in the 5-10 cm depth. The concentration of T-GRSP for soils under forest and NT tended to decrease with depth, while in CT the 10-20 and 20-30 cm depths had higher concentrations than the 0-5 and 5-10 cm depths. The concentrations were in the order CT=F>NT in the 10-20 cm depth and CT>F=NT in the 20-30 cm depth.

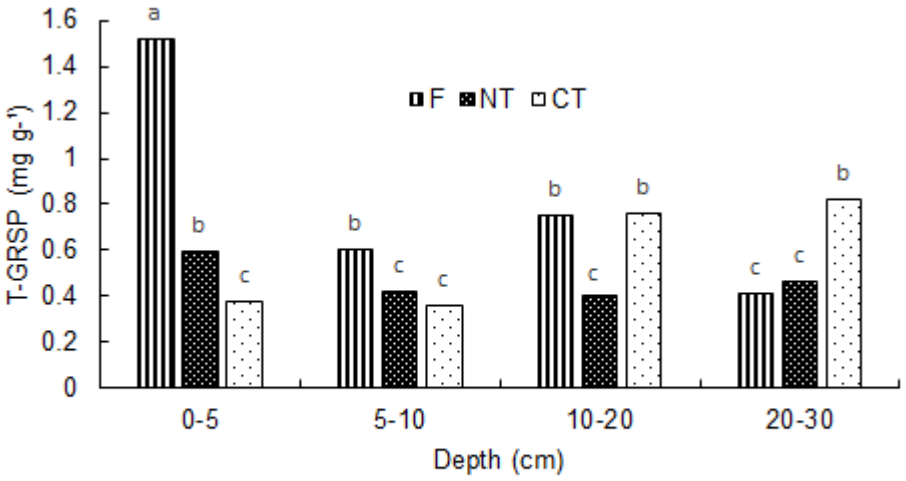


Figure 4. 7 Effect of soil management and depth on total glomalin related soil protein (T-GRSP). Treatment means with the same letter are not significantly different. F = Native forest, NT = No till, CT = Conventional tillage.

4.8 Relationships of glomalin related soil protein with organic C fractions and aggregate stability

There were strong positive linear relationships between SOC and both EE-GRSP (R^2 = 0.84) (Figure 4.8) and T-GRSP (R^2 = 0.83) (Figure 4.8). The linear relationships were moderate between WSOC and EE-GRSP (R^2 = 0.71) (Figure 4.10) and T-GRSP (R^2 = 0.6) (Fig 4.10). However, the relationships between MBC and EE-GRPSP (R^2 = 0.36) and T-GRSP (R^2 = 0.32) (Fig 4.9, were weak. There were strong positive linear relationships between aggregate stability and both EE -GRSP (R^2 = 0.72) and T-GRSP (R^2 = 0.82) (Figure 4.12). There were strong positive linear relationships between SOC and WSOC (R^2 = 0.71) (Figure 4.11).

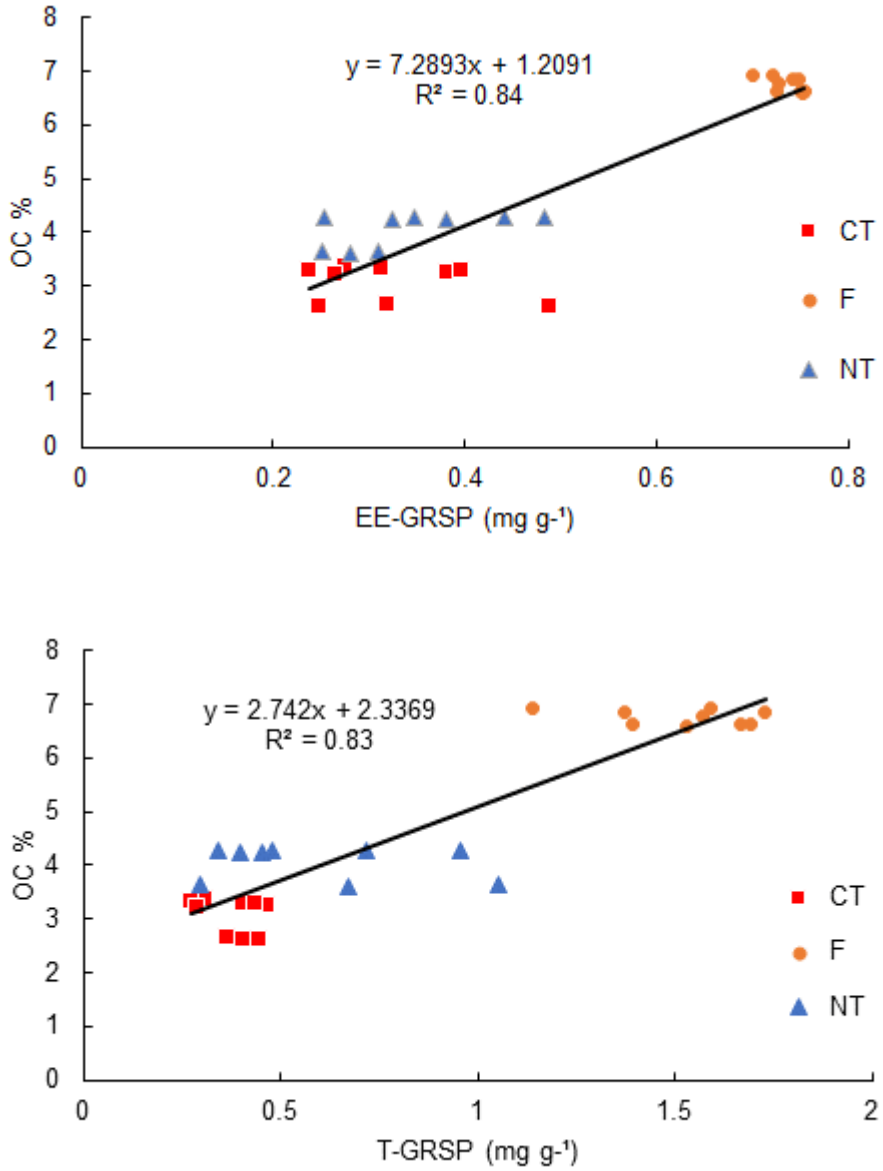
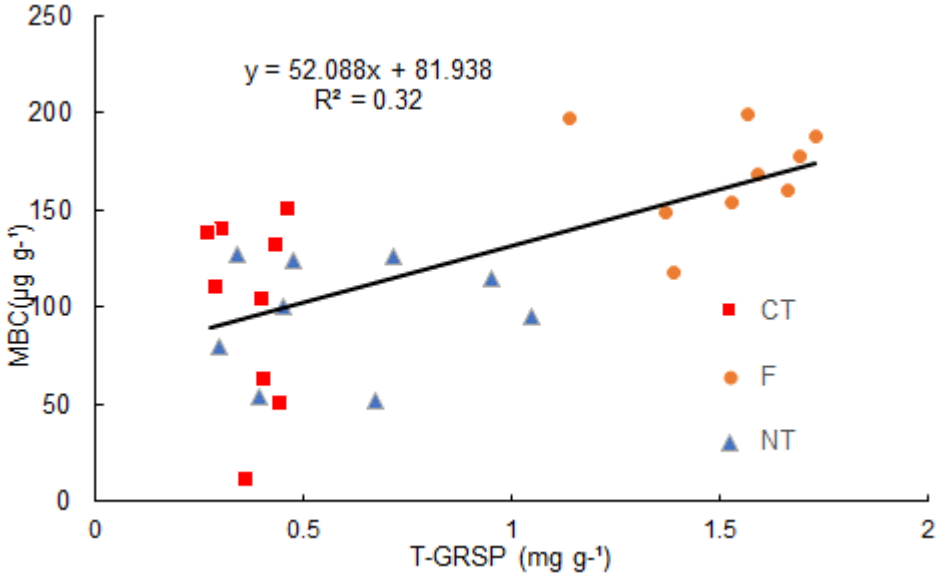


Figure 4. 8 Relationship between soil organic carbon with easily extractible (EE-GRSP) and total (T-GRSP) glomalin related soil protein. F = Native forest, NT = No till, CT = Conventional tillage.



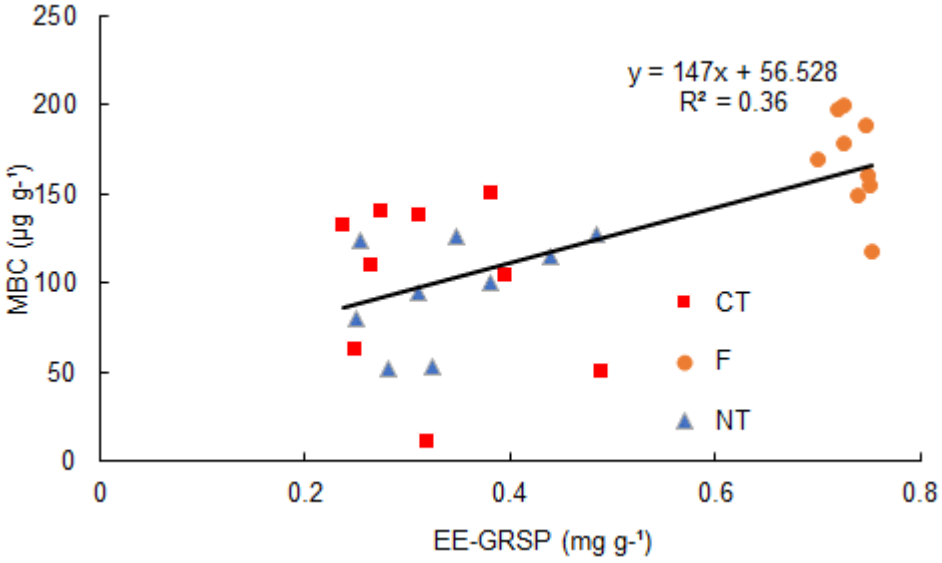


Figure 4. 9 Relationship between soil microbial biomass carbon with easily extractible (EE-GRSP) and total (T-GRSP) glomalin related soil protein. F = Native forest, NT = No till, CT = Conventional tillage.

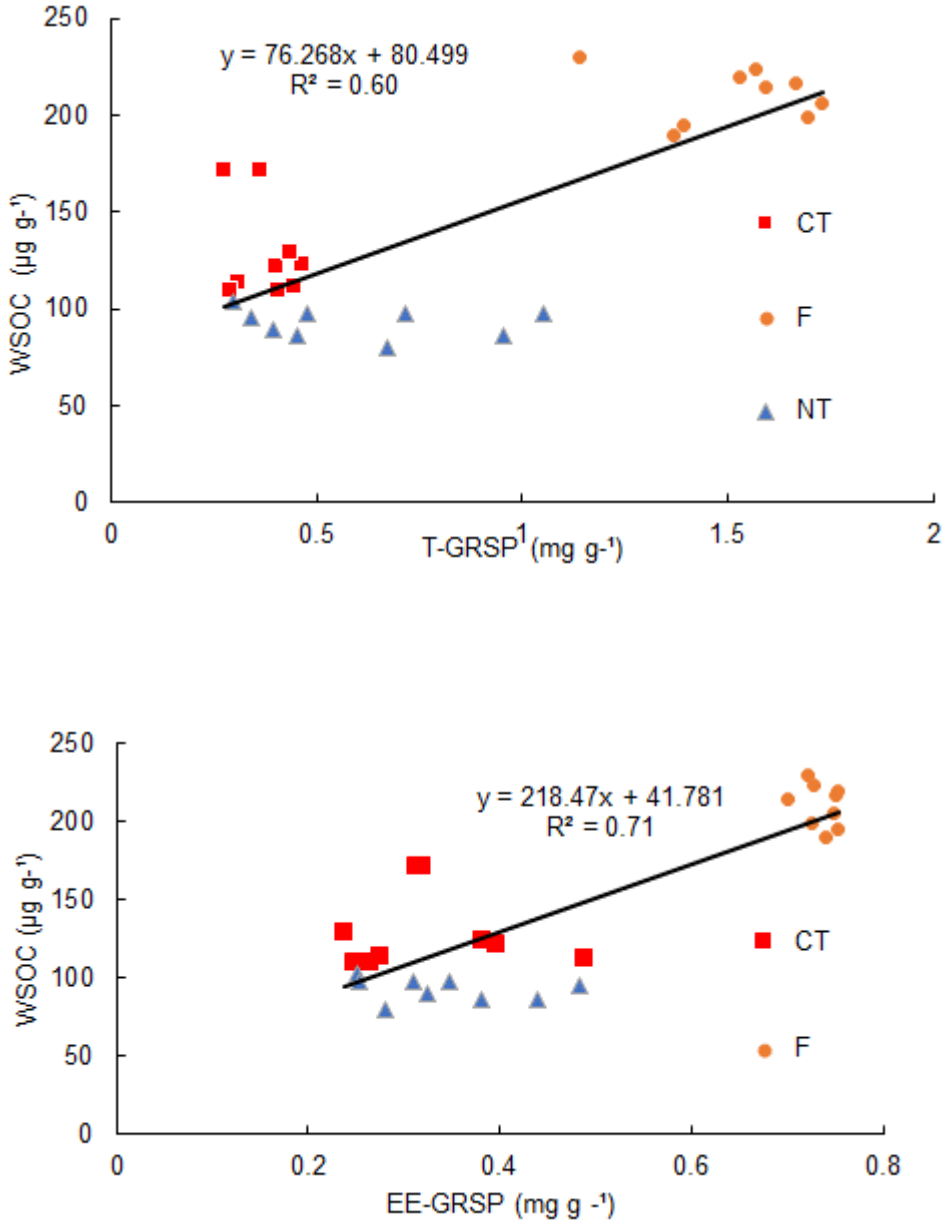


Figure 4. 10 Relationship between water soluble organic carbon (WSOC) and total (T-GRSP) and easily extractible (EE-GRSP) glomalin related soil protein in the top 0-5 cm depth. F = Native forest, NT = No till, CT = Conventional tillage.

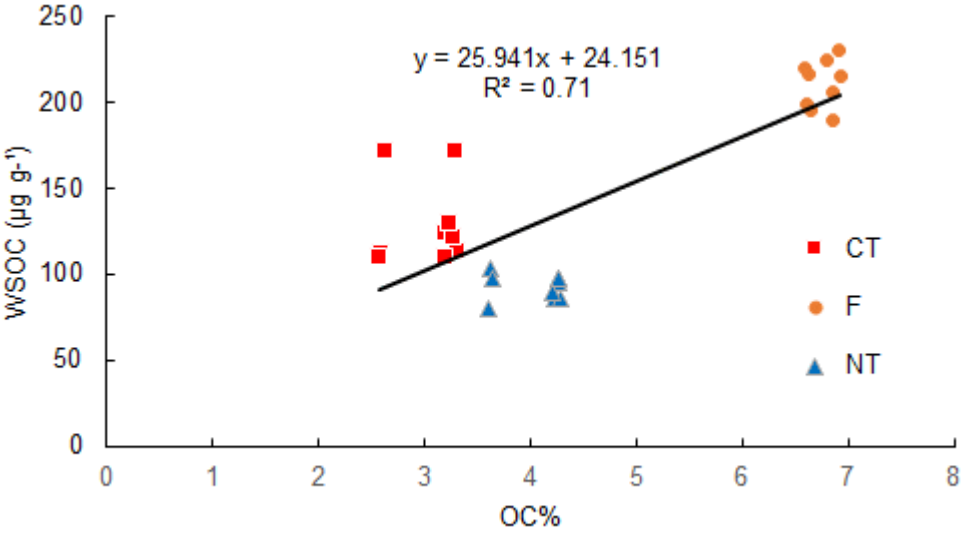
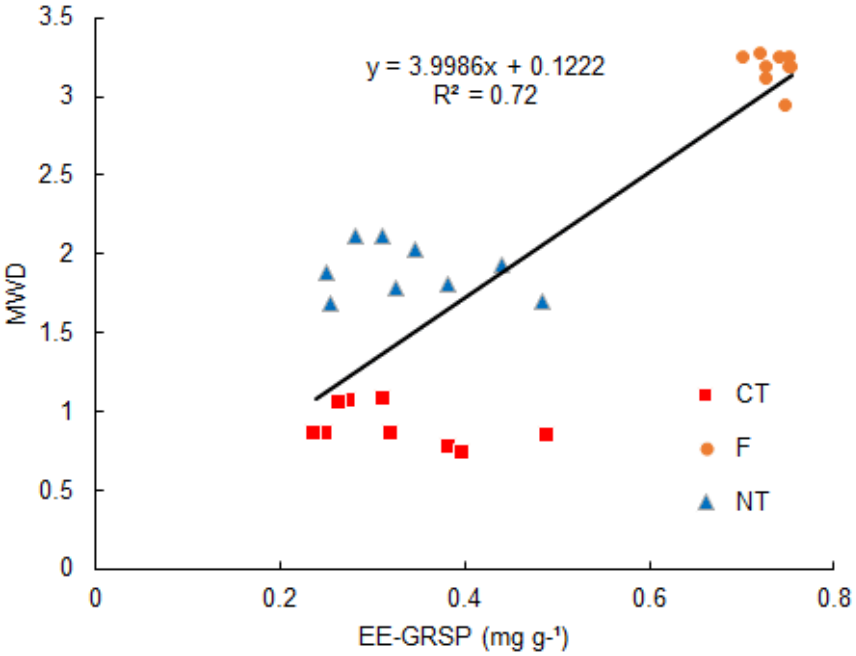


Figure 4. 11 Relationship between organic carbon content and WSOC in the top 0-5 cm depth. F = Native forest, NT = No till, CT = Conventional tillage.



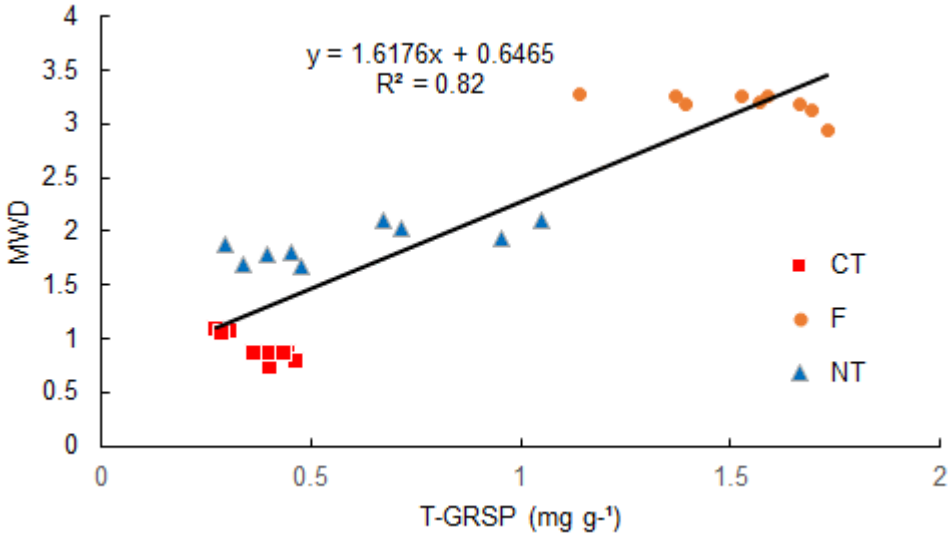
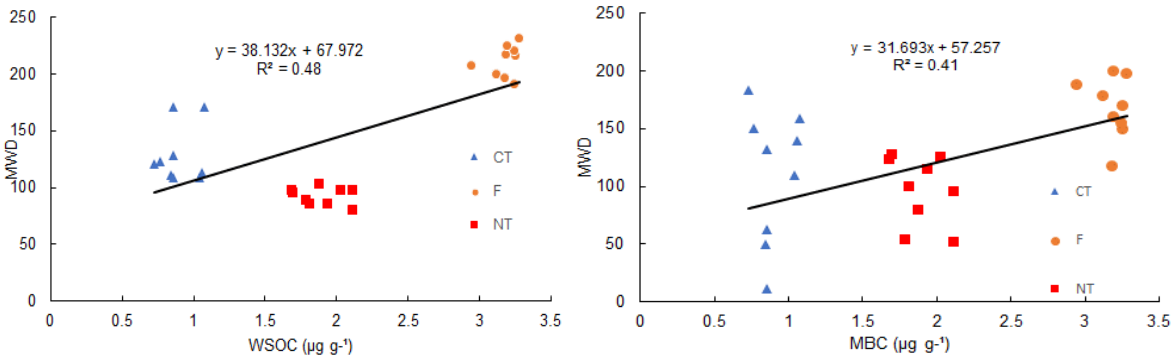


Figure 4. 12 Relationship between aggregate stability with EE-GRSP and T -GRSP in the 0-5 cm depth. F = Native forest, NT = No till, CT = Conventional tillage.

4.9 Relationships of aggregate stability with organic C fractions

There were strong positive linear relationships between aggregate stability and SOC (R^2 = 0.92). However, the relationships between aggregate stability and WSOC (R^2 = 0.48) and MBC (R^2 = 0.41) (Fig 4.13) were weak



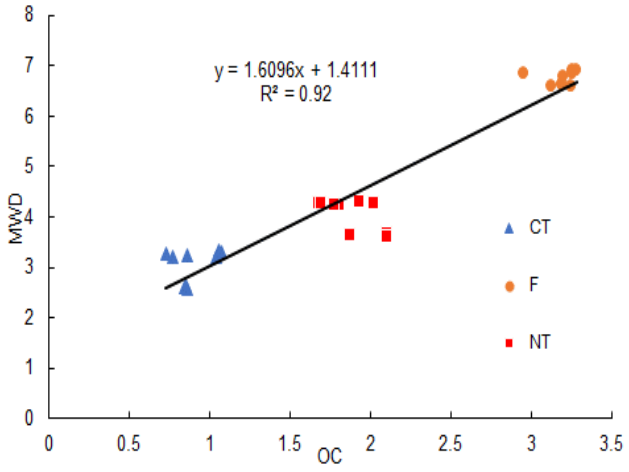


Figure 4. 13 Relationship between aggregate stability with organic C fractions in the top -cm depth. F = Native forest, NT = No till, CT = Conventional tillage.

CHAPTER 5

DISCUSSION

5.1 Soil organic carbon and glomalin related soil proteins effects on soil aggregate stability under different land-uses

Soil structure is an important indicator of soil quality due to its effects on water retention, infiltration capacity, porosity, and penetration resistance (Bronick and Lal, 2005), hence water availability to crops and susceptibility to erosion (Six et al 2000). Soil aggregate stability is a commonly used as an indicator of soil structure (Bronick, Lal, 2005; Six et al., 2000). Aggregate stability is related to MWD (Nimmo and Perkins, 2002), the higher the MWD the more stable the aggregates are (Le Bissonnais, 1996). Le Bissonnais (1996) classified aggregates into 5 groups i.e. when MWD is > 2.0 mm, very stable; 1.3-2.0 mm, stable; 0.8 to 1.3 mm, medium; 0.4-0.8 mm, unstable and < 0.4 mm, very unstable.

Land use and soil management have been shown to affect soil aggregate stability through their effects on soil aggregate binding agents and physical breakdown of macro-aggregates into micro aggregates (Six et al., 2002; Ashagrie et al., 2007; Emadodin et al., 2009). Increased soil disturbance through such practices as continual tillage has been shown to reduce aggregate stability through breakdown of macro aggregates into macro-aggregates (Ashagrie et al., 2007). In this study soils under F had the highest aggregate stability as shown by their higher MWD than the soils under other land uses with soil under Conventional Tillage having the lowest. Moreover, the

MWD values fell within the very stable to stable categories (Le Bissonnais, 1996). MWD for soils under F was more than two times higher than that for those under Conventional Tillage. Similar findings were reported by Emadi et al. (2008) and Saha et al. (2010). This was attributed to conventional tillage mechanically breaking down macro-aggregates into micro-aggregates (Six et al., 2000) and also disruption of binding agents such as roots, fungal hyphae, and by-products of microbial synthesis and decay (Kabir et al., 1997; Jastrow et al., 1998). On the other hand, reduced soil disturbance maintains the integrity of macro-aggregates (Jastrow et al., 1998) hence the higher MWD observed for soils under forest.

A study by Wright et al. (1999) examined the influence of no till and conventional tillage systems on aggregate stability and observed higher MWD under no till. This was attributed to a higher organic carbon content in soils under no till compared to those under conventional tillage. Similarly, in this study, higher SOC was observed in soils under native Forest and No Till than those under Conventional Tillage (Fig 4.3). This finding proposes that tillage can significantly decrease aggregate stability due to exposing SOM to decomposition. The resultant low SOM increases soil wettability resulting in slaking and rapid breakdown of soil aggregates upon wetting (Le Bissonnais, 1996). Under dry conditions, organo-mineral coatings may decrease the wettability of aggregate surfaces due to an increased hydrophobicity of the soil organic matter (Goebel et al., 2005; Vogelmann et al., 2013).

Soil organic carbon and microbial biomass all act as important binding agents for aggregation (Six et al., 2004; Bronick and Lal, 2005). Amongst these binding agents SOC is considered the most important agent for aggregate stability (Six et al., 2002). This is owed to processes such as direct binding of metals by CEC or SOM, or metal-cation bridges with SOC (Lal et al., 2007). Continuous tillage results in increased loss of organic matter by exposing it to decomposition by soil microbes and hence poor aggregation. In this study there were highly significant differences ($p < 0.0001$) in SOC among the different land uses as such propose that SOC was amongst the dominant mechanisms stabilizing soil aggregates. Soils under Forest had the highest SOC and MBC, which agreed with findings by Six et al. (2004) and Bronick and Lal. (2005) who showed that SOC and MBC act as binding agents for aggregates. Soil management practice had significant effects ($p < 0.05$) on both SOC and MBC. Conventional tillage had the lowest MWD because tillage interrupts the network of mycelium and mechanical breakdown of macro-aggregates (Borie et al., 2006), and reduces the content of soil organic matter, microbial biomass and faunal activities (Mikha and Rice, 2004; Sainju et al., 2009; Curaqueo et al., 2011).

The observed aggregate stability was also significantly influenced by GRSP as evidenced by a significant positive relationship between both EE-GRSP ($R^2 = 0.72$) and T-GRSP ($R^2 = 0.82$). This was in agreement with a number of studies that showed that GRSP significantly increases the stability of soil aggregates (Wright and Upadhyaya, 1998; Wu et al., 2015; Wright et al., 2007). Glomalin acts as glue, cementing aggregates together hence increasing stability. Moreover, glomalin is a hydrophobic glycoprotein which reduces wettability of aggregates hence increasing stability. Similarly, in this study, the highest MWD was observed in forest soil which

coincidentally had the highest EE-GRSP. Conversely, the lowest MWD was observed in conventionally cultivated soils, which had lower amounts of GRSP.

5.2 Effects of land use and depth on Soil Organic Carbon

Concentrations of soil organic carbon decreased significantly with depth under all land uses except for no till, which resulted in no significant OC changes with depth. This was in contrast to Zhao et al. (2015) who observed decreasing organic level with depth under No Till. This was attributed to stratification of OC due to non-perturbation. Similar results were obtained by Lopez-Fando and Pardo (2011) who noted that soil organic carbon in the 0 – 5 cm was 48 % and 60 % higher in No Till soils compared to conventional tillage soils after a period of 17 years. This was due to intensive use of mouldboard plough Likewise, Hernanz et al. (2009) observed 14% higher SOC in no-till soils than in conventional Tillage soils after a period of 20 years.

The top 5 cm depth had the highest OC with native Forest, no till (6.08%and 3.93% respectively) having higher concentrations than both CT (3.33%). Forest soils had approximately 35% more OC than no Till. In addition, F had the greatest OC for all depths studied. The results are similar to those found by Guggenberger and Zech (1999) who noted that soils under permanent vegetation with large returns of litter (forests and grassland) showed the greatest proportions of SOC.

SOC generally decreases with depth under no till because the non-disturbance of soil causes nutrients to accumulate in the top layers of the soil. Similar observations were

reported by Zhao et al. (2015) who reported a significant concentration of SOC in the surface-soil layers under no till due to high stratification ratios. High stratification ratios are key in soil carbon sequestration (Zhao et al., 2015). However, in this study, there were no differences in SOC among the four soil depths for soil under no till. SOC was however distributed relatively evenly through the soil profile under conventional tillage. This is probably due to the mixing effect of tillage operations which bury residues deep in the soil and expose any remaining residue to rapid decomposition.

5.3 Effects of land use on microbial biomass carbon

The microbial biomass of soil is recognised as a sensitive indicator of soil quality. It is highly sensitive to any changes in land-use due to alterations of natural soil characteristics brought about by tillage Kara and Bolat (2008). The decrease in tillage improves physical, chemical and trophic habitat characteristics of microorganisms, consequently enabling microbial growth and enhancing mycorrhizal hyphal density and effectiveness (Zhang et al., 2012). In a study on the effect of different land uses on soil MBC and nitrogen in Turkey, Kara and Bolat (2008) observed significantly higher MBC contents in forest than pasture and cultivated soils thus indicating differences in soil microbial activities. In this study, soils under forest had the highest MBC than the other two agricultural systems. Moreover, soil under forest had higher contents of SOC, which could have contributed to the high MBC content since SOC has significant influence on soil microbial activity (Wright et al., 2005). A significant positive correlation was observed between SOC and MBC in this study ($r^2 = 0.59$). Generally, soil under natural forests tend to have a higher accumulation of plant litter and fine roots which promotes growth of soil microbes. Furthermore, the higher MBC

under natural forest is possibly due to the significant influence non-disturbance has on the quality of soil root environment. It is generally agreed that the high contents of MBC generally indicate better soil quality (Xiangmin et al., 2014). Generally, active pools of SOM like microbial biomass, are important for soil metabolism, chiefly for the turnover of organic matter and the cycling of nutrients in soils. This fraction serves also as a short-term reservoir of plant nutrients (Fliebbach and Mader, 2000).

However, there were no differences in the MBC content of soils under no till and conventional tillage despite higher SOC in soils under NT than CT. This finding could be due to the formation of stable substances such glomalin, which are resistant to microbial degradation. In this study, a significant positive relationship was observed between SOC and both GRSP fractions. The similarity in MBC content between soils under no till and conventional tillage could also be due to the influence of such soil properties as soil pH, which was similar among these two land uses. Soil pH is known to correlate negatively with MBC, with maximum activity of microbial activity at about 6.5 (Acosta-Martinez and Tabatabatai, 2000). However, results in this study showed that the highest MBC was in forest soil which had the lowest soil pH. This indicates that other soil properties have more influence on MBC than soil pH.

5.4 Effects of land use on Water Soluble Organic Carbon

Water soluble organic carbon is one of the most important active fractions of SOC. It is the most labile and mobile form in soil organic matter pools and serves as a potential

nutrient source for soil microorganisms and plants (Gregorich et al., 2003). However, it is highly sensitive to any changes in land use. Buscot (2005) noted that WSOC in the top soil sustains microbial activity in the lower soil layers through its downward movement. Therefore, activities such as tillage that reduce soil organic matter inputs into the soil reduce the concentration of WSOC (Burton et al., 2007). Soon et al. 2007 reported higher WSOC under no till than Conventional Tillage. However, in this study, there were no differences in WSOC among Conventional Tillage and No Till although forest soils had much higher WSOC levels (Fig 4.4). The significantly higher levels of WSOC in forest soil were probably due to the high SOC levels observed in these soils (Fig 4.2). It has been widely reported that natural systems have less disturbance and high concentration of litter which accumulates at the surface thus high SOC levels (Novara et al., 2015). Lu et al. (2011) carried out a study aimed at analysing variations in WSOC under three types of alpine grasslands and study their relationships with environmental factors like moisture and temperature. They observed varying levels of WSOC within the same alpine area and concluded that environmental conditions like soil temperature and moisture are important factors influencing soil WSOC content. Some studies revealed that high soil temperature results in the utilisation of WSOC as a microbial substrate since the soluble fraction of organic carbon is the main energy substrate for soil microbes (Marschner and Bredow, 2002). The similarity in WSOC between soils under no till and conventional tillage contradicts most findings, which have shown greater accumulation of WSOC under no till. The lack of differences in content of WSOC between the two management systems could be due accumulation of fresh residues and less decomposition.

5.5 Effects of land use and soil depth on bulk density

The lower values of bulk density near the surface were likely associated with inputs of organic matter from vegetation and were particularly evident for forest soils. Low bulk densities were found under no till than Conventional Tillage at all soil depths apart from the top 0-5 cm depth. The generally lower bulk density under no till than Conventional Tillage at is consistent with findings from several other studies (Osunbitan et al.,2005). No tillage reduces soil disturbance as much as possible, which in turn maintains and improves soil structure. This is evidenced by the high aggregate stability observed under forest and no till than conventional tillage (Fig 4.1). The higher OC (Fig 4.2) MBC (Fig 4.3) and WSOC (Fig 4.4) under NT than Conventional Tillage also contributed to improved soil structure and hence lower BD values

Several authors noted that there was greater BD in cultivated soils than those under forests (Galantini. and Rosell 1997; Batjes and Dijkshoorn 1999). In all land-uses, bulk density except conventional tillage significantly varied with depth. Higher bulk density can be attributed to compaction and degradation of soil structure (Igwe, 2001).

5.6 The effect land use and soil depth on glomalin related soil proteins

Glomalin related soil protein, a component of soil organic matter, has received much attention since the first description by Wright and Upadhyaya (1996). This thermo-stable protein is reported to play significant roles in the stabilization of soil aggregates due to its recalcitrance nature (Treseder and Turner 2007). In this study, the EE-GRSP, was highest in the 0-5 cm depth in soils obtained from conventionally tilled and forest soils than no till. This contradicted some findings, which showed EE-GRSP is affected by tillage (Tang et al., 2009; Rillig et al., 2003). In contrast, T-GRSP was highest in soils under forest in both the 0-5 cm and 5-10 cm depths. This finding was

in agreement with most findings reported in literature. For instance, Rillig (2003) reported lower T-GRSP concentrations in agricultural soils relative to native forest and afforested soils. Mechanical disturbances by tillage decrease vegetation and AMF abundance, which in turn decreases glomalin production (Treseder and Turner 2007). Therefore, cropping systems and land management practices affect T-GRSP levels (Wright et al., 2007). In another study Wright et al. (1999) detected substantial increases in GRSP concentrations, after a 3-year period of converting from ploughed tillage to no-till. In addition, results on the conversion from conventional tillage to no till, showed that T-GRSP levels in the studied soil were lower than levels in undisturbed grassland. This was also attributed to tillage decreasing glomalin production and enhancing its decomposition by decreasing plant litter and AMF abundance (Treseder and Turner, 2007). T-GRSP was higher in the lower depth for conventional tillage due to indirect effects via soil physiochemical properties and soil nutrients (Wang et al 2017). In their study they concluded that in deeper soils the higher soil bulk density and lower soil water might have contributed to lower EE-GRSP but higher T-GRSP. Also, the deeper soils could directly result in a lower SOC, and this nutrient shortage in the deep soils in turn resulted in the higher T-GRSP.

Both EE-GRSP and T-GRSP were strongly and positively correlated with SOC at each depth (Fig 4.6and Fig 4.7), but negatively correlated with soil bulk density. The two glomalin fractions were also positively albeit weakly correlated to all SOC fractions. This positive relationship between both GRSP fractions and all measured SOC fractions showed that GRSP significantly contributes to SOC sequestration. However,

the poorer relationship between labile SOC fractions than total indicates that glomalin is more recalcitrant than these fractions. Zhang et al. (2017) observed higher recalcitrance index of GRSP than that of SOC, thus indicating that GRSP is vital for SOC sequestration. These results are similar to the findings of Rillig et al. (2003), Emran et al. (2012), Gispert et al. (2013) and; Vasconcellos et al. (2013), who noted glomalin to be positively correlated with soil C and N but negatively correlated with soil bulk density. Soil C highly correlated with glomalin across all soils and within each land-use type, indicating that glomalin may be under similar controls as soil C. The higher concentration of T-GRSP under forest, and no till observed in this study is consistent with numerous studies (Wright and Anderson 2000; Rillig et al. 2001; Harner et al. 2004; Staddon 2005). Glomalin production is usually greater under environments that favour AMF activity (Ryan and Graham 2002) or diversity (Helgason et al. 1998), such as minimal physical disruption of the soil (Wright et al. 1999; Borie et al. 2000; Wright and Anderson 2000), higher levels of plant diversity, or low to moderate soil fertility (Treseder and Allen 2002; Lovelock et al. 2004a; Treseder 2004). Minimal or no soil disturbance improves soil structure thus promoting actively growing mycelia, which freely penetrate the soil and produce higher amounts of total glomalin. These differences may be accredited to no soil disturbance in no till system, improving the amount and the activity of AMF hyphae in relation to conventional tillage (Cornejo et al., 2009; Kabir *et al*., 1997), and, subsequently, the levels of glomalin (Kabir, 2005). The pattern is similar to that observed by other authors. Similarly, Tang et al. (2009) found that GRSP decreased with increasing soil depth in farmland (1.60–2.94 mg/g), artificial grassland (1.82–3.18 mg/g), and orchard (1.41–1.91 mg/g). This was attributed to soil available phosphorus. In their study glomalin was significantly directly related with soil available phosphorus and protease (p<0.01). Hence, they concluded

that soil available phosphorus, to a large degree, determined the content and distribution of soil glomalin.

The highest amount of T-GRSP was found at the top layer (0-5 cm) in Forest (Fig 4.7), which had the lowest pH (4.9). Soils with lower pH had significantly higher concentrations of GRSP as a consequence of increased organic activity at lower pH values. Fungi tend to thrive better in acidic soils due to less competition from bacteria and other organisms. For the reason that glomalin is produced by AMF more GRSP is expected in the more acidic soil due to increase activity of AMF (Sarkar, 2003).

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

1. This study demonstrated that greater accumulation of GRSP could cause the accumulation of SOC, particularly under native forest. Therefore, land use practices that mimic natural forest favour the accumulation of SOC and T-GRSP and should be widely adopted.

2. This study also demonstrated that soil disturbance reduced SOC as evidenced by higher SOC in soils under forest and no till than those under conventional tillage. This observation was consistent at all the sampling depth used in this study. In addition, SOC decreased with depth for forest and conventional tillage whilst no differences were observed for No Till.

3. The study also showed that GRSP played a greater role in soil aggregate stability than labile fractions of SOC. This is especially true for soils under forest had the highest MWD values whilst soils under conventional tillage were the least stable. Therefore, minimizing disturbance in these soils is likely to increase their stability

4. GRSP concentrations in the sampled soils changed with changes in land use and soil depth. There washigher concentration of T-GRSP under forest and no till in soil under conventional tillage. It is therefore ideal to mimic natural systems in order to increase the concentration of this important natural glue.

So, agronomic practices like zero or minimum tillage may improve T-GRSP thus ultimately increasing soil structure

6.2 Recommendations

Clear understanding of the mechanism by which GRSP might influence soil physical characteristics is still lacking. It is necessary to determine the relationships between GRSP and such important soil properties as clay content across different soil types. It is also generally agreed that, mineralogy of the clay fraction has significant influence on soil behavior and interacts with other soil properties. It is therefore prudent that future studies focus on the influence of clay mineralogy on the concentration of GRSP

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