LONG-TERM FERTILIZER AND SUGARCANE RESIDUE MANAGEMENT EFFECTS ON STRUCTURAL STABILITY OF TWO SOIL TYPES IN SOUTH AFRICA

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DECLARATION

I, Sandile Siphesihle Mthimkhulu, certify that:

1. The research reported in this thesis is the result of my own investigation, except where otherwise indicated.
2. This thesis has not been submitted for any degree at any other university or institution.

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ABSTRACT

Under sugarcane production, soil aggregate stability (AS) is affected by the harvesting method i.e., burning, mulching and fertilizer application. This study combined mineralogical, biological, chemical and physical approaches to investigate the effect of these management techniques on a range of soil properties that may influence soil aggregation. The study site was located at the South African Sugarcane Research Institute (SASRI) at Mount Edgecombe near Durban, KwaZulu-Natal, South Africa. It is the oldest long-term, continuously monitored sugarcane production and soil management trial in the world, having been established in 1939. The area is characterized by summer (October to March) rainfall. Within the study site itself the dominant parent material was dolerite, with colluvial material in the south-western part of the lower slope. Due to the variation in topography, two soil types were identified. On the upper slope, the soil was classified as a Mollic Cambisol, locally known as Mayo form (Glenecho family). On the lower slope, the soil was a Mollic Nitisol, locally known as Bonheim form (Rockvale family). The trial is a split-plot factorial design arranged in a randomised complete block with four replicates for plots burned at harvest and eight replicates for all unburned plots. The main plot treatments are a) green sugarcane harvesting with all residues retained and spread evenly over the plot area (M), b) sugarcane burned prior to harvest (no foliage residue) with sugarcane-tops left scattered evenly over the plot area (BS) and c) sugarcane burned prior to harvest with all residue (sugarcane-tops) removed from the plots (BR). Split-plot treatments consisted of unfertilized (F0) and fertilized plots (F) receiving an annual application of 140 kg N, 28 kg P and 140 kg K ha^{-1} as 5:1:5 (46). From the 32 plots, 24 were selected including four replicates of each of the treatments.

Three replicate soil samples were collected with a spade at two soil depths (0-10 and 10-20 cm) from mini-pits in each of the 24 chosen plots. For soil AS determinations, samples were air-dried and sieved to collect soil aggregates between 2.8 and 5 mm and the mean weight diameter (MWD) determined. Some of the air-dried bulk sample was analysed for total carbon (Ct) and nitrogen (N), organic carbon (OC), pH, exchangeable calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K), aluminium (Al), soil texture (clay, silt and sand content), clay mineralogy, soil microbiological properties, phosphorus (P), zinc (Zn), copper (Cu), and manganese (Mn). Soil microbiological properties (the abundance and communities of bacteria and fungi) were measured on the 0-10 cm depth samples only. The saturated hydraulic conductivity (Ks), bulk density (ρ_b), water retention and available water capacity (AWC) were determined on undisturbed soil cores also collected from 0-10 and 10-20 cm soil depths.

Penetrometer resistance (PR) and apparent electrical resistance were measured in-field across the whole trial while the gravimetric soil moisture was measured in the laboratory and then mapped for the whole trial.

Mulching and burning as well as fertilizer application showed no clear relationship with the clay mineralogy of the investigated soils. The main clay minerals in both soils were high defect kaolinite, vermiculite and lepidocrocite. The main difference in mineralogy found was that the upper slope soil also contained talc, illite and interstratified vermiculite-smectite which were not present in the lower slope soil. However, differences in clay mineralogy between the two slope positions had no influence on the other measured soil properties. The OC and Ct increased non-significantly ($p > 0.05$) in M and BS compared to BR in both fertilized and unfertilized treatments suggesting that the soils might have reached their equilibrium in terms of carbon. A significant increase ($p < 0.05$) caused by M treatment was, however, observed in N. The Ct and N were generally significantly higher ($p < 0.05$) in the macroaggregates compared to the microaggregates (0.1 - 0.05 mm) in most treatments, showing the direct contribution of soil organic matter (SOM) to the stability of larger aggregates.

The Ca, Mg, pH and effective cation exchange capacity (ECEC) of the soils were similar between burned (BR and BS) and mulched (M) treatments but they decreased significantly ($p < 0.05$) in the fertilized treatments. Sodium concentrations were higher in the BRF0 and BSF0 treatments compared to the rest of the treatments. Potassium was significantly higher ($p < 0.05$) in MF0, and MF treatments compared to BSF0 and BRF0 treatments. The decrease in soil pH was mirrored by an increase in Al concentration and acid saturation in the fertilized treatments. These results could be due to the combined effects of basic cation mining by sugarcane plants, leaching of basic cations and their replacement by Al, mineralization of mulch leading to soil acidification, and oxidization of ammonium to nitrate. The higher concentration of P in the M treatments suggested that P resulted from both the fertilizer application and mineralization of SOM. High K accumulation came from the annual NPK fertilizer application.

The dsDNA significantly increased ($p < 0.05$) in M compared to BR in the F0 treatments and remained similar between M, BS and BR treatments in the F treatments. It decreased significantly ($p < 0.05$) in the F compared to the F0 treatments. Although fertilizer application had no effect, M treatment significantly ($p < 0.05$) increased the abundance of bacteria and decreased the abundance of fungi 16S rDNA copy numbers. Bacterial richness significantly (p

< 0.01) increased and decreased under mulching and fertilization, respectively, while the evenness decreased significantly ($p < 0.01$) in M and fertilized plots. Fungal richness significantly ($p < 0.01$) increased under M treatment in F0 treatments but showed no clear trend in the F treatments. Fertilizer application significantly ($p < 0.01$) reduced fungal richness. Burning and mulching showed no significant ($p > 0.05$) effect on fungal evenness though it was significantly ($p < 0.01$) decreased by fertilizer application.

The MWD increased slightly in the following order: BR < BS < M under F treatments at the 0-10 cm depth, but the differences were not significant ($p > 0.05$). These results were associated with the lack of differences or consistent increase in soil aggregating agents observed between M and burned (BR and BS) treatments. The MWD was significantly ($p < 0.05$) reduced by fertilizer application possibly due to the decrease in divalent exchangeable bases (Ca and Mg) and fungal richness observed in this treatment compared to the F0 treatments. In the absence of a correlation between OC and MWD, the multivariate analyses showed that fungi were the main factor influencing AS though some significant effects of exchangeable bases were also found. The changes in MWD possibly induced by fertilizer application showed no effect on PR and the decrease in PR observed in the M treatments was attributed to an increase in moisture (due to higher SOM) compared to the burned treatments. Similarly to PR, bulk density, water retention and AWC showed no clear relationship with MWD. Therefore, the higher water retention found in BS and M treatments was attributed to the direct effect of SOM. The saturated hydraulic conductivity (Ks) decreased significantly ($p < 0.05$) in the fertilized treatments following the decrease in MWD.

In conclusion, the long-term effect of mulching and burning on soil properties can be influenced by other external factors. In this study, the annual application of NPK fertilizer counteracted the impact of burning and mulching on AS and associated properties. Some of the properties were mostly influenced by soil type rather than sugarcane management practices. The annual application of NPK fertilizer also appeared to have led to increased acidification and soil structural deterioration (lower AS) under long-term sugarcane production regardless of the harvesting method practiced. Increasing additions of sugarcane residues are thus not necessarily sufficient to lead to improved soil structural stability and related soil properties.

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CHAPTER 1: INTRODUCTION

Soil aggregate stability (AS) is an indicator of the ability of soil aggregates to withstand the destructive action of wetting, raindrop impact and cultivation (Haynes, 1997). The AS generally serves as an indicator of soil structure (Haynes, 1997). Soil aggregation is normally mediated by soil organic carbon (OC), biota, clay content and type and exchangeable bases (especially Ca, Mg and Al) (Bronick and Lal, 2005). However, in soils with low carbon and exchangeable cations, the oxides of iron and aluminium and clay content and type play a dominant role in determining AS. High temperature and rainfall accelerates the leaching of exchangeable bases and breakdown of organic matter leading to reduced residence time for OC in the topsoil and lower soil aggregation (Bronick and Lal, 2005; Demarchi *et al*., 2011). Aggregate stability is a very important factor in the functioning of soil as it contributes to its ability to support and sustain the life of plants and animals (Bronick and Lal, 2005). High AS is important for improving porosity and decreasing erodibility and thereby contributing to improved soil fertility and agronomic productivity. Weakly aggregated soils usually have higher bulk density which leads to low infiltration rate and hydraulic conductivity and high risk of runoff and soil erosion. Stable aggregates promote balanced porosity against various stresses such as the impacts of raindrops, erosive forces and contraction and swelling caused by drying and rewetting. The mechanical breakdown by raindrop impact plays a major role under wet conditions as water weakens the soil aggregates (Le Bissonnais, 1996). The breakdown of aggregates may lead to a decreased infiltration rate to as little as 1 mm h^{-1}, which is often accompanied by surface sealing (Le Bissonnais, 1996).

The AS is also influenced by land management practices. For instance, under sugarcane, AS is affected by harvesting methods such as burning and mulching (green sugarcane). Traditionally, sugarcane is burned prior to harvesting to remove leafy, non-sucrose containing biomass. However, this can be detrimental to soil AS and nutrient availability due to the loss of soil organic matter (SOM) and nutrients through oxidation, particulate dispersal or volatilisation (Blair, 2000; Wiedenfeld, 2009). The destruction of SOM and reduced microbial activity under sugarcane subjected to pre-harvest burning is a major factor contributing to soil aggregate destabilization in the South African sugar industry (Graham and Haynes, 2006). An alternative to pre-harvest burning is green sugarcane harvesting where the leafy biomass is retained on the soil surface as a mulch, potentially increasing SOM and nutrient content (Robertson and Thorburn, 2003). This harvesting method is common in Australia, Brazil and parts of the West Indies (Graham *et al*., 2002). According to Wiedenfeld (2009), retention of sugarcane residues (mulch) may increase AS, nutrient and moisture conservation and reduce weed growth. Although mulch generally improves the soil properties related to AS, it can also interfere with fertilizer and herbicide application and lead to immobilization of nitrogen and phosphorus (Wiedenfeld, 2009). The mulch added to the soil during green sugarcane harvesting increases SOM that eventually mineralises into carbon, microbial material and relatively stable humus components (Verma *et al*., 2010). The effectiveness of SOM in improving AS can be influenced by management practices such as fertilizer application (Bronick and Lal, 2005). The application of fertilizers normally improves soil fertility and increases plant productivity, SOM and biological activity, which, in turn, improve AS (Haynes and Naidu, 1998). In contrast, Graham *et al*. (2002) reported that fertilization can alter the soil chemical properties in a manner that results in soil structure deterioration. Jung *et al*. (2011) also reported a decrease in plant biomass and AS with fertilizer application on Fragic Luvisols under switchgrass in Italy. Generally, the change in SOM affects the soil AS, which, in turn, influences soil strength, bulk density, water retention and saturated hydraulic conductivity. Thus, AS is an indirect measure of soil structure which indicates the health of the soil.

Although there has been extensive research on some of the soil characteristics and their effects on sugarcane yields, the interactive effect of various management practices and soil properties on AS as well as the main factor or factors controlling the AS under long-term continuous sugarcane cultivation are not yet fully understood. Thus this study was aimed at investigating the changes in various soil properties and their interactive effect on AS induced by 72 years of residue burning or mulching, with and without fertilizer application on a sugarcane trial. This study was conducted on a long-term (72 years-old at the time of sampling) sugarcane trial established by the South African Sugarcane Research Institute (SASRI) in 1939 at their research facility at Mount Edgecombe, KwaZulu-Natal. This trial offers the opportunity to investigate the long-term impacts of growing sugarcane and the associated trash management and continuous fertilizer use on soil properties. The present study involved the measurement of AS and the physical, chemical, mineralogical and microbiological properties associated with the structure modification induced by the long-term application of nitrogenous fertilizer and two sugarcane harvest residue management practices i.e. mulching and burning. The study further investigated how and to what extent, mulching and burning modifies soil aggregate composition and stability, and their influence on the soil water retention, in a dryland sugarcane production scenario. The results of the study will contribute to the understanding of soil and water conservation of much wider areas and will aid in the prevention of soil structure breakdown and erosion by indicating best management practices for sugarcane residues that maintain or improve AS.

The main hypotheses being evaluated are that:

1) Long-term sugarcane residue retention increases soil carbon and cations thereby improving soil properties such as AS and microbiology.
2) Long-term fertilizer application increases sugarcane biomass production thereby improving soil properties such as AS and microbiology.
3) Long-term fertilizer application increases soil acidification thereby affecting soil properties such as AS and microbiology.
4) Long-term sugarcane residue retention and fertilizer application increases soil carbon, cations and biomass production thereby increasing soil permeability and water retention and decreasing bulk density and soil strength.

The key objectives were thus to:

1) Compare the impacts of sugarcane burning at harvest against green sugarcane harvesting with residue retention (mulching), with and without fertilizer, on (a) AS of different granulometric fractions and (b) soil mineralogical and microbiological properties, and cation exchange reactions.

2) Determine the relationship between AS and the physicochemical and biological properties that may drive aggregate formation and stability.

The document is structured as follows:

Chapter 2: Presents a literature review on the different factors that affect soil AS, with emphasis on the effects of different levels of sugarcane residues and fertilization.

Chapter 3: Describes the study site and the field and laboratory methods used.

Chapter 4: Reports and discusses the impact of sugarcane crop residues and fertilization on clay mineralogy and selected soil physicochemical properties.

Chapter 5: Reports and discusses the response of soil microbial communities to sugarcane crop residues and fertilizer applications.

Chapter 6: Reports and discusses how the soil physical properties are influenced by sugarcane crop residues and fertilization.

Chapter 7: Presents a general discussion, and gives conclusions and recommendations.

Note: Some of the results in Chapters 4 and 6 have been published in an article entitled "The effect of 72 years of sugarcane residues and fertilizer management on soil physico-chemical properties" in Agriculture, Ecosystems and Environment (Mthimkhulu *et al*., 2016; Appendix A).

CHAPTER 2: THE EFFECT OF SOME SOIL PROPERTIES AND EXTERNAL FACTORS ON SOIL AGGREGATE STABILITY

2.1 Introduction

Soil aggregates are known as the secondary soil particles that are formed from the combination of mineral particles with organic and inorganic substances (Bronick and Lal, 2005). Tisdall and Oades (1982) stated that aggregates are divided into macroaggregates (> 250 micrometres) and microaggregates (< 250 micrometres) and their ability to resist when subjected to both internal and external stresses causing their disintegration is called aggregate stability (AS). The most common methods of measuring soil AS in agriculture are wet and dry sieving, while shear and axial compression are also used in other fields such as civil and environmental engineering.

On-going interactive effects of soil and external factors strongly influence AS. Numerous researchers have found clay content and type, soil organic matter (SOM), biota, cations and oxides to be the most important mediators of AS (e.g. Le Bissonnais, 1996; Wakindiki and Ben-Hur, 2002; Bronick and Lal, 2005; Mohanty *et al*., 2012). Demarchi *et al*. (2011) also affirmed that these are the main soil properties that contribute to AS and that microorganisms produce exudates that act as stabilizing agents. The effect of these soil factors under different crops is mostly influenced by external factors such as climate, topography, and management (Bronick and Lal, 2005; Mataix-Solera *et al*., 2011). Bronick and Lal (2005) stated that a decline in AS has been considered as a form of soil degradation which is often associated with land use and soil or crop management factors. Thus, interest in assessing AS under perennial crops such as sugarcane has increased.

Generally, perennial crops improve soil aggregation whereas annual row cropping often leads to soil structural degradation, mainly due to loss of ground cover and organic matter due to soil disturbance (Mohanty *et al*., 2012). Hartemink (1998) compared Fluvisols and Vertisols from Ramu valley in the Madang Province of Papua New Guinea and observed substantial deterioration in soil aggregate-related chemical and physical properties resulting from continuous sugarcane production. Souza *et al*. (2012) also reported a decrease and an increase in soil AS with burning and mulching, respectively, of sugarcane residues on an Oxisol at Paraguaçu Paulista, State of São Paulo, Brazil.

Other sugarcane management factors including land preparation, planting, liming and fertilization have been described as the key role players in the development and stabilization of soil aggregates (Graham *et al*., 2002). This review explores the impact of soil and external factors on AS and its critical role in other factors such as water holding capacity, hydraulic conductivity and resistance to erosion in the soil environment, under different cropping systems, with an emphasis on sugarcane.

2.2 Soil factors

2.2.1 Texture and clay mineralogy

Sand and silt are not as effective as clay in the formation of aggregates due to lower specific surface area and lower charge density compared to clay. The high specific surface area and surface charge of clay particles enable them to flocculate and bind with sand and silt to form stable aggregates (Williams, 1971; Bronick and Lal, 2005). Clay acts as an aggregating agent, binding particles together and so influences soil organic carbon (OC) decomposition (Bronick and Lal, 2005). This relationship can be associated with chemical stabilization of organic carbon (OC) through physical and chemical adsorption of OC onto clay particles (Miles *et al*., 2008; Razafimbelo *et al*., 2013). The adsorption of OC to clay particles reduces microbial decomposition of OC and, in turn, increases soil AS. Aggregate stability has generally been found to increase with increasing clay content (Wakindiki and Ben-Hur, 2002) but this does not necessarily indicate that coarse-textured soils lack aggregation. It was reported that any amount of clay present in a sandy soil may be drawn into interstices between larger particles by water menisci as the soil dries, which leads to the aggregation of clay particles at the micron scale (Jindaluang *et al*., 2013). Under fast wetting, high clay content in the soil aggregates may also increase the degree of differential swelling and the volume of entrapped air which, in turn, increases aggregate slaking (Wuddivira and Camps-Roach, 2007).

In other soils, an increase in clay content does not always indicate an increase in AS as the clay type is also very important in soil aggregation. Soil mineralogy has a significant impact on AS and dispersion, with its influence determined by the structure or morphology of each mineral (Green *et al*., 2002). A study by Lado and Ben-Hur (2004) reported lower mean weight diameter (MWD) in montmorillonitic soils even though they had higher clay content compared to soils that were dominated by non-phyllosilicate clays (Table 2.1).

Table 2.1: The relationship between the mean weight diameter (MWD), clay mineralogy and clay content in soils from different locations in Israel and Kenya (Lado and Ben-Hur, 2004).

Soil location	Country	Clay mineralogy	MWD (mm)	Clay (%)
Tunyai	Kenya	Kaolinite	2.80	64.0
NeveYa'ar	Israel	Montmorillonite	0.25	63.0
Netanya	Israel	Montmorillonite	0.31	10.0
Molo	Kenya	Non-phyllosilicates	0.84	30.4
Njoro	Kenya	Non-phyllosilicates	0.80	34.0

According to Wuddivira and Camps-Roach (2007), the increase in clay content plays a major role in increasing the AS in soils dominated by non-expanding, crystalline clays, such as kaolinite, that are less dispersive. However, the AS of soil consisting of high clay content dominated by swelling minerals, might be as small as or lower than that of a soil with low clay of the kaolinite type (Wuddivira and Camps-Roach, 2007). Aggregate stability is influenced mainly by polyvalent metal-organic matter complexes that form bridges between the negatively charged clay platelets in soils that are dominated by 2:1 clays (Six *et al*., 2000). However, AS is controlled by the minerals themselves rather than clay content in 1:1 clay dominated soils. Kay (1998) stated that in soils with coarse texture, the OC has a greater impact on AS, while with increasing clay content the clay type is more important than the amount in determining AS. However, it is still not clear if these findings are applicable in every soil regardless of management and climatic factors. The study that was conducted by Mohanty *et al*. (2012) showed poor soil aggregation in Vertisols in central India. These results suggested that a higher soil cation exchange capacity (CEC) may decrease AS due to an increased amount of hydrated cations and the degree of swelling in Vertisols.

The role of clay mineralogy on soil AS can be influenced by management practices such as fertilizer application (Tye *et al*., 2009). The study that was conducted by Velde and Peck (2002) at the University of Illinois under continuous corn cropping, and without fertilization, revealed that the extraction of potassium (K) for plant nutrition after 30 years led to an increase in the smectite content of interstratified minerals. Pernes-Debuyser *et al*. (2003) also reported that the addition of potassium fertilizer onto soils where plant growth is absent may result in an increase in the illitic component of the interstratified clays, potentially affecting soil aggregation behaviour. Though changes to clay minerals and their effect on soil AS have been reported for some crops, no reports have been found related to continuous sugarcane cropping.

2.2.2 Sesquioxides

Crystalline and nanocrystalline metal oxides and hydroxides are important aggregating agents in soils. The metal ions form bridges between mineral and organo-mineral particles (Briedis *et al*., 2012). Iron oxides act as a cementing agent between the surfaces of clays and as charged discrete particles in the case of many highly weathered, acid soils (Duiker *et al*., 2003; Briedis *et al*., 2012). The trivalent Al and Fe cations increase AS through cationic binding and formation of organo-metallic compounds and gels (Briedis *et al*., 2012). Interaction of Al and Fe with kaolinite can synergistically encourage aggregation with limited impact on OC, while oxides and hydroxides of Al interact synergistically with OC and dispersible clay to improve AS (Six *et al*., 2000; Molina *et al*., 2001; Duiker *et al*., 2003; Bronick and Lal, 2005; Ayaz *et al*., 2015). The role of sesquioxides on soil aggregation becomes very important in highly weathered soils that have low organic matter content. Igwe *et al*. (2013) reported that the effect of OC as an aggregating agent is dominated by Fe and Al in soils with low organic matter content.

2.2.3 Organic matter and carbon

Soil quality of agricultural land has been typically equated with SOM or its associated derivative, OC (Haynes, 1997). The SOM components are involved at various levels in the soil aggregate stabilization hierarchy, from the initial formation of basic organo-mineral complexes up to the stabilization of macroaggregates (Jenkinson and Rayner, 1970; Haynes, 1997). This complexation encourages soil physical aggregation processes and overall soil stability (Miles *et al*., 2008; Spaccini and Piccolo, 2013; Torres *et al*., 2013) and, in turn, the stable aggregates protect the SOM. The impact of SOM on the soil aggregation process is influenced by other factors such as the type and quality of the SOM, as well as the clay content and clay type. Piccolo and Mbagwu (1999) found that the addition of bio-labile organic material to soil has a short term (< 10 years) effect on soil aggregation processes while humified SOM improved soil aggregation over a long period (> 10 years). The below-ground SOM (roots) also plays a key role in soil aggregate stabilization. Souza *et al*. (2012) observed that the fasciculated root system of sugarcane produces an intense rhizosphere effect, and when the roots decompose, they release exchangeable cations and increase SOM which strongly promote soil aggregation. Roots enmesh and rearrange particles and release organic compounds that serve as glue to keep particles together. The microaggregates are formed from SOM attached to clay particles and polyvalent cations to form compound particles which are then combined with other microaggregate particles to form macroaggregates (Bronick and Lal, 2005). Such aggregation can be enhanced by earthworm activity although Haynes *et al*. (2003) reported that the impact of earthworm activity on soil aggregation is very low in the soils of the South African sugar industry compared to other soil aggregating processes. The SOM also influences the soil structure indirectly by increasing OC. The dynamics of AS development seem to be closely related with SOM storage in soils (Dominy *et al*., 2001). It has been frequently observed that undisturbed soils usually have higher OC, AS and saturated hydraulic conductivity when compared with their cultivated counterparts (Bronick and Lal, 2005). Land use and management are vital factors affecting OC accumulation and storage, as they control the magnitude of OC stocks and greatly influence the composition and quality of SOM (Ayoubi *et al*., 2012). Land use and management not only affect the total amount of SOM, but also influence the OC distribution within the various particle size fractions and the processes that influence its protection (Ayoubi *et al*., 2012).

Soil OC and AS mutually affect each other since OC is physically protected by its association with soil primary particles in aggregates; at the same time AS is enhanced by this association (Silva *et al*., 2007). This relationship is the reason for the frequently reported positive correlation between MWD and OC (Figure 2.1) (Chenu *et al.,* 2000). Silva *et al*. (2007) stated that poor soil AS observed under sugarcane cultivation could be associated with a reduction in the more labile fraction of the OC. The AS of soils that are dominated by free, light, particulate organic carbon (fPOC) is very low as this carbon fraction is highly susceptible to changes caused by soil management (Jindaluang *et al*., 2013). The heavy fraction of carbon including organo-mineral complexes consists of stable forms of OC with slower turnover rates compared to fPOC and therefore promotes higher AS. According to Bronick and Lal (2005) aggregation and OC concentration represent integrative effects of soil type, environment, plant species and land management practices. However, the mechanisms of the interaction between soil aggregation and OC are not clearly understood. As a result, there are still many conflicting views about this relationship in the literature.

The effect of OC on AS may also depend on the amount of OC present in the soil and the type of soil in question. The study conducted by Smith *et al*. (2015) showed that the AS of Vertisols increased with increase in carbon when the total organic carbon exceeded 2% in the soil. These results suggested that any increase or decrease in carbon below 2% total organic carbon does not affect the AS of a Vertisol. The OC is known to be less effective in controlling AS in Vertisols than other soil physical and chemical properties since slaking of aggregates upon rapid wetting of dry soils has been generally accepted as an inherent characteristic (Smith *et al*., 2015). While positive and significant relationships between OC and MWD are reported (e.g. Figure 2.1), the generally poor fit (R^2) of the regression line suggests that there are other factors (as previously discussed) that play a role in the stabilization of soil aggregates.

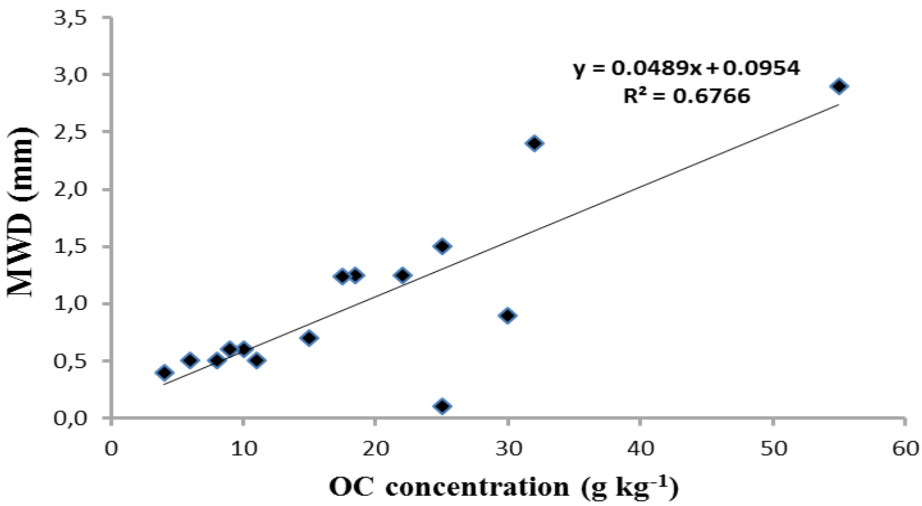


Figure 2.1: The relationship between soil organic carbon (OC) and mean weight diameter (MWD) in thick, humic, loamy soils (Vermic Haplumbrepts) in southwestern France (redrawn from Chenu *et al.,* 2000).

2.2.4 Exchangeable bases

Generally, AS increases with an increase in polyvalent exchangeable cations, especially magnesium (Mg) and calcium (Ca). Divalent cations, along with clay, will stimulate the precipitation of substances that act as binding agents for primary soil colloids and form bridges between SOM and clay forming stable microaggregates (Bronick and Lal, 2005). According to Virto *et al*. (2011), in an Aridisol, Ca originating from carbonate dissociation accelerated inter-molecular interactions between OC and soil colloids because of a cationic bridging effect. The formation of Ca bridges between organic and inorganic soil particles is the dominant factor in the long-term effect of the addition of Ca on the AS. Calcium ions inhibit clay dispersion and encourage aggregation of soil clay particles by replacing primarily monovalent sodium (Na) and K, and sometimes Mg ions on the exchange sites of clay particles (Wuddivira and Camps-Roach, 2007). The Ca can also be added to the soil through fertilizer application (Noble and

Hurney, 2000). However, a significant decrease in aggregate size distribution with fertilizer applications was observed under sugarcane at Mount Edgecombe, South Africa (Graham *et al.*, 2002). There was an increase in soil pH and aggregation in samples of the mulched and fertilized (R_3F_1) soil amended with $Ca(OH)_2$ and a decrease in aggregation with the use of KOH and K_2SO_4 (Figure 2.2). This decrease in aggregation was attributed to decreases in Ca and soil pH with an annual application of fertilizer (140 kg N ha^{-1}, 28 kg P ha^{-1} and 140 kg K ha^{-1}). The results obtained by van Antwerpen and Meyer (1998) also showed that loss of Ca and Mg and increase in K from the topsoil resulting from annual application of NPK fertilizer would encourage dispersion and a decrease in AS. The effect of change of soil pH on exchangeable cations is detailed in Section 2.2.5. It is worth noting that the cationic bridges involving Ca as the main bond-forming cation are generally common in the soils of temperate climatic areas where the natural occurrence of the cation is relatively high. The soils of tropical and subtropical areas are dominated by hydrogen (H) and Al cations under natural soil conditions, and are the main cations playing a major role in the bonding of colloids.

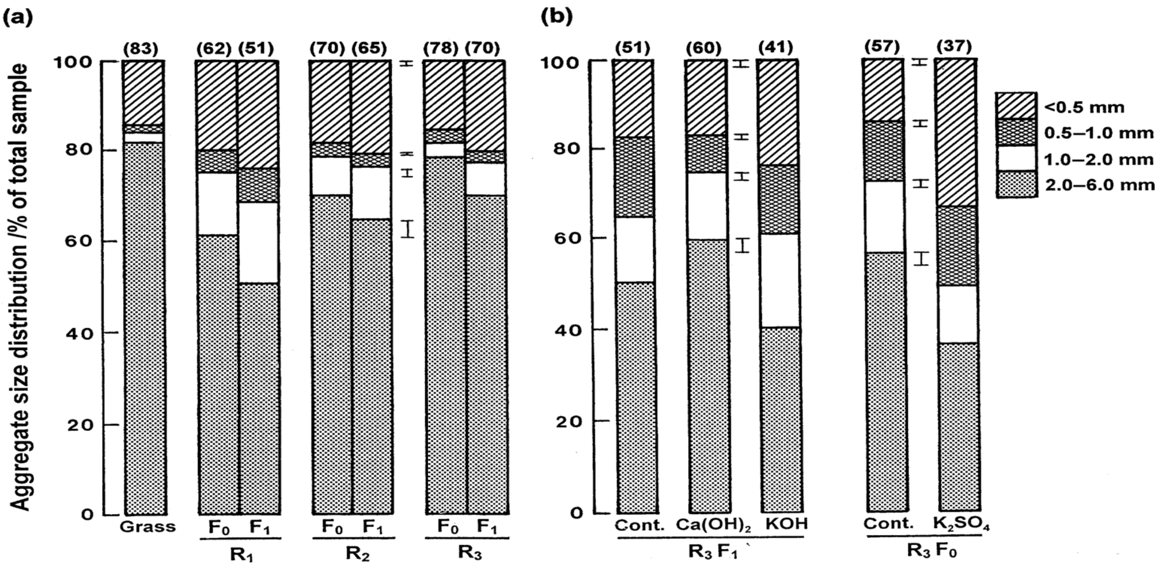


Figure 2.2: Long-term effects of calcium (Ca) and potassium (K) on the size of aggregates following wet-sieving of (a) the surface 2.5 cm of the different treatments and (b) the surface of the R3 treatment after 6 weeks of incubation with unamended control (Cont.), $Ca(OH)_2$, KOH or K_2SO_4. Grass: unfertilized grass; R1: burned with harvest residues removed; R2: burned with residues left on the soil surface; R3: green sugarcane harvested with mulch retention; F0: no fertilizer applied; F1: fertilized with N, P and K annually. Bars indicate standard errors of means for comparison between treatments. Values in parenthesis are percentages of aggregates remaining in the 2-6 mm category (Graham *et al.*, 2002).

2.2.5 pH

The soil pH is indirectly involved in the formation and stabilization of soil aggregates through its influence on various soil chemical and biological properties which normally play a key role in the soil aggregation process. Generally, a decrease in soil pH increases the solubility of Al in the soil solution, which then displaces Ca and Mg from the exchange sites of clay particles and thus decreases Ca and Mg concentrations in the soil. However, low pH soils are usually flocculated due to a high concentration of Al and high hydrogen ion activity in soil solution that promotes compression of the double layer and flocculation of clay particles (Haynes and Naidu, 1998). The attraction between Al oxides and negatively charged clay exchange sites, and bridging between SOM and clay surfaces may also promote flocculation. If the pH of a soil that was previously flocculated by Al is raised, the Al precipitates as hydroxyl-Al polymers, and as a result the repulsive forces between particles dominate and clay dispersion occurs (Haynes and Naidu, 1998). According to Six *et al*. (2004), an increase in pH of a variable charge soil leads to an increase in negative surface charges, leading to a dominance of repulsive forces between the clay particles, resulting in dispersion. A decrease in soil AS and OC, and an increase in microbial biomass C content have also been measured following the application of lime (Chan and Heenan, 1996). Chan and Heenan (1996) noted an increase in AS with increase in pH after only 1.5 years of lime applications.

2.2.6 Earthworms and termites

Earthworms exert direct and indirect effects on soil structure formation and stabilization. Due to their feeding activity they breakdown and redistribute the SOM vertically in the soil profile, change the size and activity of microbes in the soil, and thus strongly modify the soil AS (Ernst *et al*., 2009). All the SOM ingested by earthworms is mixed with inorganic material that passes through the gut and is excreted as casts (Six *et al*., 2004). Earthworm casts have been found to be more stable than the surrounding soil aggregates, especially when they have dried or aged (Six *et al*., 2004). The research by Jouquet *et al*. (2009) showed greater stability of the biogenic aggregates in comparison with physiogenic aggregates, especially in the larger aggregates (≥ 2 mm) which were associated with higher SOM content measured in the former. The stability of casts also develops from the microorganisms that proliferate in ingested materials in the gut and cast. These microorganisms deposit polysaccharides within the casts that form a gel-like substance which acts as a glue to bind particles into aggregates (Six *et al*., 2004; Ernst *et al*., 2009). When earthworms breakdown SOM, the OC released also contributes to the binding of soil colloids and stabilization of their casts. Earthworm casts are predominantly composed of clay particles reflecting their feeding preference for the finer material in a soil. According to Jouquet *et al*. (2004), the presence of a large amount of clay in the earthworm casts could also play a role in increasing the stability of the casts as explained in Section 2.2.1. However, the importance of earthworms on soil aggregation varies with species of earthworm and quality of organic material present in the soil (Bronick and Lal, 2005). A study conducted on a sandy loam Fluvisol under different crops showed that earthworms prefer feeding on SOM with lower C:N ratio (Ernst *et al*., 2009). Most earthworm species feed on lighter and more soluble organic compounds due to their poor digestive systems and thus it was found that the mean litter loss from maize residues was higher compared to oats (Ernst *et al*., 2009). Dlamini *et al*. (2001) studied the earthworms species found under sugarcane cultivation at Eshowe, KwaZulu-Natal and reported that *Pontoscolex corethrurus,* a widely distributed exotic species (endogeic group), made up about 70% of the earthworm community. This species was also found to be dominant in the sugarcane soils of northern Queensland (Spain *et al*., 1990). Endogeic earthworms play a major role in soil AS, compared to anecic earthworms, as they burrow and ingest soil (with preference for material high in organic matter) and then deposit their casts below-ground in burrows and other soil pores, thus promoting aggregation (Spain *et al*., 1990).

Termites generally modify their surrounding environment by increasing clay content and decreasing organic matter content and total porosity and thereby affect the AS (Cadet *et al*., 2004). The faecal pellets of termites are used together with salivary secretions to cement soil particles during the construction of the walls for their mound. It was reported that in the termite mound soil, 67% of clay aggregates consist of particles greater than 2 µm compared to 48% in the undisturbed soil (Orhue *et al*., 2007). The use of higher amounts of clay during the construction of the termite mound may increase the AS compared to the surrounding soils. Frageria and Baligar (2005) showed that termite activity increased exchangeable cations and pH of the mound soil and decreased Al in an Oxisol of the Cerrado region in Brazil. Increasing the cations may induce cationic bridging of soil colloids which improves AS. Increasing pH in the termite mound causes the dissolved carbonates and CO_2 to react with the cations present to form secondary carbonate coatings on primary soil particles. The effect of carbonates depends on the OC content and particle size distribution of the soil. According to Bronick and Lal (2005), carbonate coatings enhance the stability of macroaggregates by binding the soil colloids together where there is a low concentration of OC. Where carbonate concentrations are high in a soil they improve the protection of OC which leads to an increased AS. The decrease in aggregation that was observed in the silty soil with high carbonate content suggested that particle size distribution influences the role of carbonates in aggregation. The influence of termites in the distribution of particles can affect soil water-holding capacity and bulk density. The soils from termite mounds showed five times greater water-holding capacity compared to adjacent soils in India (Cadet *et al*., 2004; Orhue *et al*., 2007).

2.2.7 Fungi and bacteria

Among soil organisms, fungi have been found to be very important in the formation and stabilization of soil aggregates via both direct and indirect contributions. The direct effect is through the hyphae network that binds the soil particles and forces them together or aligns soil particles along the expanding hyphae (Siddiky *et al*., 2012; Tisdall *et al*., 2012). Indirectly, the arbuscular mycorrhizal fungi (AMF) secrete glomalin-related soil protein (GRSP) or polysaccharides that may glue and bind soil particles together (Rillig *et al*., 2005; Kohler *et al*., 2010; Siddiky *et al*., 2012). The improvement of soil aggregation also provides a conducive and protected environment for soil microorganisms and facilitates root oxygenation (Denef *et al*., 2001). Arbuscular mycorrhizal fungi also alter the community structure of microorganisms, both in their own surroundings and in the host plant rhizosphere (Rillig *et al*., 2005; Siddiky *et al*., 2012). An increase in AMF in the soil may increase the population of other soils organisms that feed on them. A combination of AMF and Collembola positively increased the proportion of water stable aggregates in an Albic Luvisol collected from the experimental farm of the Freie Universität Berlin (Figure 2.3) (Siddiky *et al*., 2012).

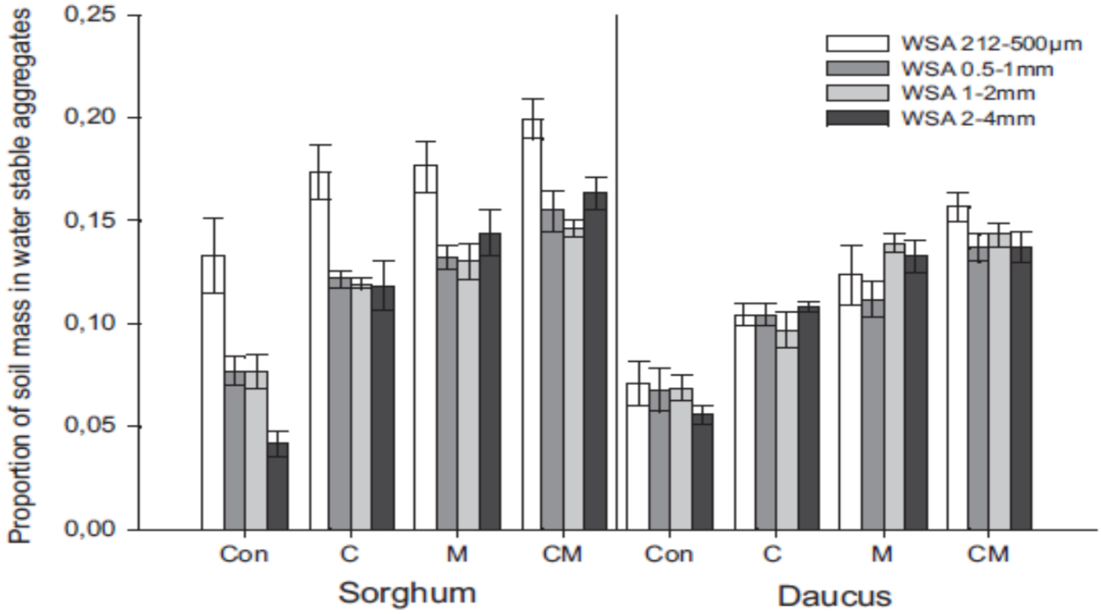


Figure 2.3: The effects of Collembola (C), arbuscular mycorrhizal fungi (M) and their interaction (CM) on the proportion of water stable aggregates (WSA) in four aggregate fractions of an Albic Luvisol under sorghum and Daucus (wild carrot) compared to the control treatment (Con) at the experimental farm of the Freie Universität Berlin (Siddiky *et al*., 2012).

Collembola are one of the most abundant groups of soil arthropods that feed on AMF. Collembola also improve the soil structure through their feeding behavior as they incorporate considerable amounts of SOM into faecal pellets, which increase the soil surface area and accessibility for bacterial and fungal utilization and thus increase decomposition (Rillig *et al*., 2005; Siddiky *et al*., 2012). A positive and significant relationship between soil microorganisms (total bacteria, anaerobes and fungi) and soil AS was reported (Andrade *et al*., 1998; Figure 2.4), although fungi showed a stronger effect (i.e. higher correlation coefficient) on AS than bacteria or anaerobes.

Fungi are known to be more effective soil aggregating and stabilizing microorganisms than other soil microflora according to Beare *et al*. (1997). Rillig *et al*. (2005) reported that fungi can increase microbial communities in their surroundings that are possibly involved in soil aggregation processes by exuding photosynthesis-derived carbon into the mycorrhizosphere which serves as food for them. The effect of total bacteria and anaerobes on AS accounted for approximately 40% and 43.5% only, respectively, though it was significant according to Andrade *et al*. (1998) (Figure 2.4). Since the correlation between AS and fungi was about 70%, it could be deduced that the contribution of the microorganisms to AS ranged from 40 to 70% and further soil aggregation was possibly due to other factors such as clay type and content, OC and other microorganisms that were not measured in the study. A negative correlation between fungi and AS at the beginning and the positive correlation observed at the later stage of the research conducted by Kihara *et al*. (2012) indicated that fungi only play a significant role in soil aggregation beyond a certain threshold (0.7 Simpson's index in their study). This relationship suggested that fungus species that are effective in the formation of macroaggregates thrive in low density and can be replaced by less effective fungus species as the diversity increases.

Kohler *et al*. (2010) conducted research in a saline soil and found no relationship between hyphae and AS and a negative relationship between GRSP and AS which was associated with the increase in Na concentration in the soil. González-Chávez *et al*. (2004) stated that the GRSP produced by fungi is very efficient in sequestering different toxic elements including Na that have negative effects on AS. Another research study by Caesar-TonThat (2002) reported that polysaccharides and GRSP rich soil treated with sodium tetraborate showed that Na can destroy long-chain polysaccharides and so disrupt soil aggregates.

The role of microorganisms on AS is also influenced by the environment and the amount of food available where they are found. Graham and Haynes (2006) measured AS and microbial biomass populations from the inter-rows and intra-rows of sugarcane and found higher AS and microbial biomass in the intra-rows. The intra-rows are usually moister and have more organic matter than the inter-rows making the environment more favourable for microorganisms and production of mucilage that cements the soil particles and microaggregates to increase AS. Microorganisms might have been adversely affected by environmental stress such as a sparsity of labile carbon (food for microorganisms) or water stress in the inter-rows in comparison with the intra-rows.

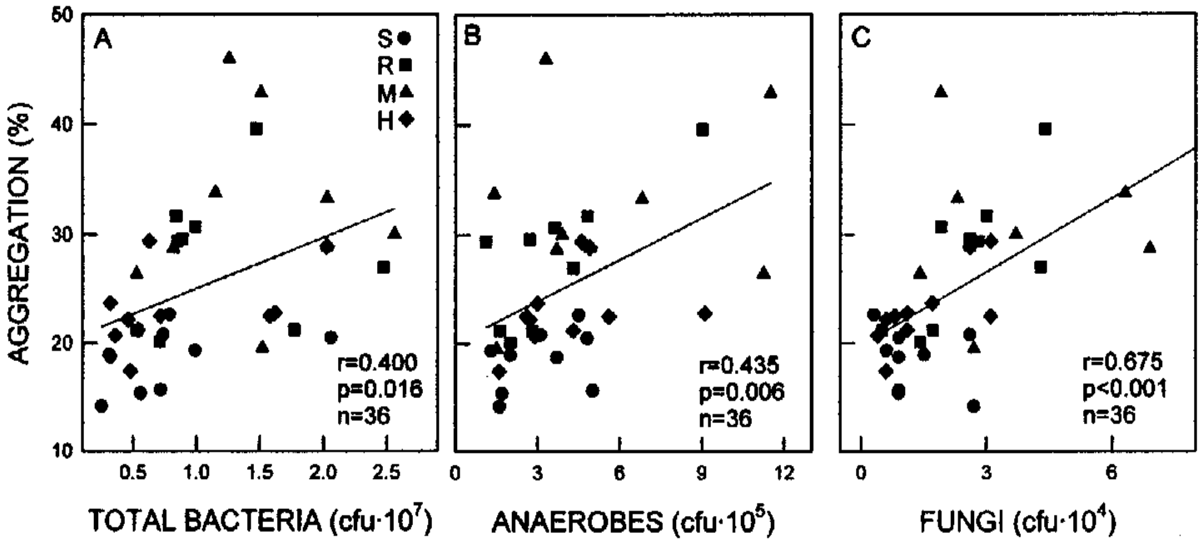


Figure 2.4: The relationship between soil microorganisms and soil aggregation measured under split-root sorghum plants grown in multi-compartment containers. The soils in the individual compartments were permeated by (M) arbuscular mycorrhizal roots and arbuscular mycorrhizal hyphae; (H) arbuscular mycorrhizal hyphae only; (R) non-arbuscular mycorrhizal roots; or (S) free of roots and arbuscular mycorrhizal hyphae as bulk soil. Data points indicate the number of colony-forming units of the groups of organisms assayed. The organisms found in the different compartments are represented by different symbols (Andrade *et al*., 1998).

2.3 External factors

2.3.1 Rainfall

Rainfall intensity and antecedent soil water play a key role in determining the effect of rainfall on AS. High intensity rainfall tends to break soil aggregates at the soil surface to form a dense impervious crust which is vulnerable to erosion (Jingi *et al*., 2011; Nciizah and Wakindiki, 2014). It is the kinetic energy applied in the form of raindrops that accounts for the greater part of the dispersion of soil particles and aggregate destabilization (Jingi *et al*., 2011). For example, a rainfall intensity of 60 mm h^{-1} significantly increased the destabilization of soil aggregates of a sandy clay loam soil compared to 45 and 30 mm h^{-1} intensities in soils of the Eastern Cape Province, South Africa (Nciizah and Wakindiki, 2014). Emerson (1967) reported that the vulnerability of soil aggregates to slaking may also be influenced by antecedent moisture after observing that fast wetting of a dry soil caused more slaking compared with fast wetting of a moist soil. Rapid immersion of aggregates in water causes the entrapped air pressure inside the pores of the aggregates to increase, resulting in explosive pressure release from within the aggregate, thus leading to disaggregation and dispersion (Emerson, 1967). The AS in an air-dry state is generally lower and much more variable compared to wetter soils due to antecedent water (Martinez-Mena *et al*., 1998). Knowing the role of antecedent moisture on AS has a significant implication for understanding the erosional response of soil, particularly in semi-arid areas. A study conducted on a Xeric Torriorthent in southeastern Spain recorded the frequency distribution of soil water content over a period of three years and showed that for about 60% of the time, soil water did not exceed 0.15 g g^{-1} (Martinez-Mena *et al*., 1998). Therefore, the lower AS of air-dried soils may suggest that erosional losses are likely to be greater in semi-arid regions than humid regions, where soil water content can remain high for most of the year (Martinez-Mena *et al*., 1998). The degree of soil aggregate disruption caused by the raindrops can be combated by the presence of mulch on the soil surface. In sugarcane production, spreading the sugarcane tops on the soil surface after harvesting or practicing green sugarcane harvesting increases the mulch and improves the protection of soil aggregates by intercepting the raindrops (Graham *et al*., 2002). Generally, mulch also increases organic matter and OC content which facilitate soil aggregation processes that enables the soil aggregates to withstand the disrupting force of the raindrops (Galdos *et al*., 2009).

2.3.2 Natural wetting and drying cycles

Exposing soil to cyclic wet-dry conditions induced by seasonal climatic changes is one of the most common natural processes resulting in reduced AS (Bronick and Lal, 2005). The wet-dry conditions lead to the breakdown of macroaggregates to microaggregates due to fractures produced during the shrinking and swelling process and the broken-down aggregates become more susceptible to dispersion and erosion (Singer *et al*., 1992; Imbufe *et al*., 2005). The reduced AS leads to slaking, low water infiltration rate and high run-off and reduced crop productivity (Imbufe *et al*., 2005). In contrast, other authors have reported a positive impact of wet-dry cycles on the AS of Vertisols at Linares, northeastern Mexico (Bravo-Garza *et al*., 2009). The application of wet-dry events improved the formation of larger water stable aggregates (>2 mm) in comparison with the constantly wet soil (Bravo-Garza *et al*., 2009). This positive impact of drying on AS was associated with additional intermolecular association between organic compounds and mineral surfaces and an increase in solid phase cohesion upon drying (Bravo-Garza *et al*., 2009). Improvement in AS with drying might have also been due to an increase in the number of contacts between particles as water menisci retract, and precipitation of organic and inorganic cementing agents after repeated drying events (Bravo-Garza *et al*., 2009). A study by Sarmah *et al*. (1996) suggested that the repetition of wet-dry cycles is the major process in regeneration of degraded structure in Vertisols, but detailed information on the number of cycles required is unknown.

2.3.3 Topography

The relationship between soil AS and topography is still not clearly understood although topography seems to have an indirect effect on the AS. Generally, south-facing slopes have higher AS compared to north-facing slopes in the southern hemisphere due to the differences in microclimates between these two positions (Bronick and Lal, 2005). Some studies have shown an increase in the rate of infiltration with increasing gradient (Janeau *et al*., 2003). These results have been attributed to weaker soil crusting on steeper slopes as raindrops strike the soil at a more acute angle, and thus with less kinetic energy per unit area of surface (Janeau *et al*., 2003) which may be an indication of better soil structure in that topographic position. In contrast, it has been generalized that sloping areas are more prone to erosion, especially in regions of intense, intermittent rainfall, and as a result, clay and OC are removed. However, rainfall erodes the OC from upslope and re-deposits it in lower-lying areas where it can be protected physically against decomposition via aggregation (Tang *et al*., 2010). Thus, more stable aggregates were observed in soils on the toeslope than on the shoulder slope (Tang *et al*., 2010; Table 2.2). High amounts of exchangeable cations were observed in footslope soils of southern Taiwan (Chun-Chih *et al*., 2004). In the same study, the Na concentration was inversely related topographically to the concentrations of exchangeable Ca and Mg. The accumulation of exchangeable divalent cations was also associated with a higher pH observed in the footslope soils. The reason for the relatively higher Na concentration in the summit soils could be that the vegetation may intercept airborne Na which is then transported into the soil via throughfall and stemflow (Chun-Chih *et al*., 2004). The high AS in the footslope soils is solely influenced by colluvial material (OC and exchangeable cations), but it is not clear what the relationship would be between AS and topography if OC and exchangeable cations were constant across the landscape.

Table 2.2: The mean weight diameter (MWD) of macroaggregates measured by dry-sieving from a Latosol developed from granite in Guangdong Province, China (Tang *et al*., 2010).

Soil depth (cm)	Slope position	MWD (mm)
0-20	Shoulder slope	3.63 (0.26)a*
	Toeslope	5.13 (0.31)b
20-40	Shoulder slope	3.29 (0.35)a
	Toeslope	4.07 (0.15)a

* Number in parentheses is the standard error. Different small letters in a column within the same depth (0-20, 20-40 cm) indicate significantly different values ($p < 0.05$).

2.3.4 Management impacts

2.3.4.1 In-field traffic

In-field traffic causes soil compaction which also contributes to degradation of the soil structure in agricultural fields. Research has indicated that compaction increases soil bulk density and mechanical strength, and decreases soil porosity, especially the proportion of macropores (Souza *et al*., 2014). During soil compaction, both static stresses and dynamic forces contribute, the latter caused by vibration of the engine and the attached implements and by wheelslip. In the case of sugarcane, new methods of planting and harvesting involve extensive use of machinery which, in turn, increases soil compaction and soil aggregate destabilization. The sugarcane harvesters exert high pressures on soils that are commonly moist with subsequent soil compression and shattering of aggregates. According to Bell *et al*. (2007), the reduction in soil AS under sugarcane is mostly due to the compacting effects of loaded machinery wheels on soil that is too wet, in addition to repeated shearing from tine or discs during tillage. The frequency of agricultural equipment traffic in sugarcane cultivation due to the application of fertilizers and herbicides, and during harvesting cause soil compaction, affecting both root growth and nutrient uptake (Perez *et al*., 2010; Homma *et al*., 2012).

2.3.4.2 Burning and mulching of residues

The burning of sugarcane before harvesting in South Africa is the main reason for loss of SOM that may lead to soil structure degradation (Graham and Haynes, 2005). The aim of burning is to facilitate the harvesting process. However, it is a practice that negatively affects soil AS as it reduces OC supply and exposes the soil to external factors (Hartemink, 1998; Torres *et al*., 2013). Due to problems that arise from burning of sugarcane during harvesting, mulching (green sugarcane harvesting) has been suggested by Wiedenfeld (2009) as a more sustainable alternative. Silva *et al*. (2007) and Souza *et al*. (2012) also observed higher values of SOM, OC, MWD and AS under the green sugarcane harvesting method compared to burning. Blair (2000) reported a significant reduction in soil AS under burned sugarcane compared to the undisturbed reference soil (under grassland) and an increase in AS in mulched compared to burned treatments (Table 2.3) on a Chromic Luvisol in Australia. The differences in MWD between the burned, mulched and reference plots were associated with low SOM in the burned compared to mulched and undisturbed soil. Burning may increase nutrients (including Ca) concentration in the soil leading to an increase in pH. However, this increase in soil fertility is a short-term benefit as the nutrients can be easily lost from burned soil through volatilization, leaching and erosion (Ball-Coelho *et al*., 1993).

Table 2.3: The mean weight diameter (MWD, mm) measured with immersion, tension wetting and dry sieving for the residue management and reference soils from Mackay, Queensland (Blair, 2000).

Method	Mulched sugarcane	Burnt sugarcane	Reference
Immersion wetting	0.479a*	0.368b	1.682
Tension wetting	0.801a	0.571b	2.578
Dry sieving	1.620a	1.480b	1.776

* Values in the same row within the same method of MWD followed by the same letter are not significantly different according to Duncan Multiple Range Test at ($p < 5\%$).

The mulch left on the soil surface after green sugarcane harvesting can affect the soil microclimate by modifying the soil thermal conductivities and reflection coefficients, which consequently influence air temperatures near the soil surface (Sandhu *et al*., 2013). Mulching has been found to have a negative effect on sugarcane due to frost damage compared to burning as the residence time of frost on the soil is longer under mulching (Sandhu *et al*., 2013). The direct effects of burning and green sugarcane harvesting on soil AS have not been reported, instead all researchers have reported on the effect of these methods on SOM, which is generally assumed to be related to all the other soil properties, including aggregation. A study that was done on forest soils in New South Wales, Central Mexico and Andalusia showed that fire can have a direct effect on soil AS (Zavala *et al*., 2010). Forest fires can increase AS through increase in water repellency which results in reduced slaking. Generally, slaking is caused by increased pore pressure in aggregates when water enters the aggregate by matric suction. The development of water repellent coatings reduces the attractive force between water and soil aggregates or particles, and increases the AS (Zavala *et al*., 2010). It was reported that burning of sugarcane before harvesting makes the topsoil hydrophobic and that this reduced soil hydraulic conductivity and increased the potential for runoff (Hartemink, 2008). Kornecki and Fouss (2011) also stated that the heat generated during the burning of sugarcane, when in contact with the soil, tends to encourage the formation of organic coatings on the soil particles and thus increases water repellency.

2.3.4.3 Fertilization

Generally, fertilization improves soil aggregation, but sometimes the effect is variable. Inorganic fertilizers may influence soil structural properties through changes in root development and soil chemical processes (Jung *et al*., 2011). Haynes and Naidu (1998) stated that the primary effect of fertilization is on increased plant productivity, OC and biological activity that, in turn, increase soil aggregation (Johnston, 1986; Bronick and Lal, 2005). Neff *et al*. (2002) also reported that an increase in plant residues and below-ground plant growth increase carbon and microbial activity which, in turn, improve AS. Stable aggregates usually have more nutrients and organic matter compared to less stable aggregates. Macroaggregates generally have more SOM and higher nutrient contents than microaggregates, are less vulnerable to erosion, and increase soil porosity. In some cases, fertilizers may decrease OC concentration, microbial communities and reduce AS. Abiven *et al.* (2007) stated that if nitrogen fertilizer application decreases the production of roots and fungal hyphae which are temporary binding agents of aggregates, then AS could be negatively affected.

A high amount of nitrogen can lead to the deterioration of soil structure (Table 2.4) through lowering root biomass and length. When ammonium-based nitrogen fertilizers are applied to the soil, the ammonium undergoes oxidation and releases H which increases the acidity of the soil. The acidifying effect of these nitrogen fertilizers could lead to a decrease in Ca and Mg concentrations (Section 2.2.5), microbial biomass and enzyme activities (Liu *et al*., 2011). A significant decline in microbial biomass content and enzyme activities over 31 years was observed in plots that were fertilized with nitrogenous fertilizers (Liu *et al*., 2011). According to Paradelo *et al.* (2013) a large accumulation of ammonium ions in soils as exchangeable cations can favour dispersion of soil colloids. When applied at high rates and under soil conditions unfavourable for nitrification, ammonium fertilizers enhance soil dispersion and surface crusting.

Table 2.4: The effect of fertilizer nitrogen (N) on mean weight diameter (MWD, mm) measured at three sampling depths under switchgrass in Fragic Luvisols at Milan, Italy (Jung *et al.*, 2011).

N rate (kg N ha^{-1})	0-5 (cm)	5-10 (cm)	10-15 (cm)
0	2.39 (0.21)	2.24 (0.17)a	0.81 (0.09)ab
67	2.64 (0.29)	2.03 (0.13)a	0.90 (0.06)a
202	2.29 (0.20)	1.48 (0.25)b	0.70 (0.10)b

Different small letters in a column indicate significantly different values ($p < 0.05$). Columns within the same depth with no letters indicate the lack of significant differences between the means.

2.3.4.4 Liming

The main purpose of applying lime is the remediation of acidity so that plants will not suffer from Al toxicity. Lime rates are commonly based on the amount needed to neutralize the exchangeable Al (Haynes and Naidu, 1998). When lime dissolves in the soil, the exchangeable divalent cations, Ca and Mg, adsorb onto the clay particle exchange sites, reduce the thickness of the diffuse double layer, and thus the repulsive forces acting between clay particles, and cause strong flocculation of clays and increased resistance to dispersion (Lehrsch *et al.*, 1993). However, lime incorporation can lead to the mineralization of the previously protected SOM through the disruption of aggregates which decreases water infiltration and increases soil erosion (Briedis *et al.*, 2012). These contrasting findings can be explained principally in terms of (1) the short-term effects of liming on dispersion of soil colloids, (2) the flocculating action of $CaCO_3$ and (3) the longer-term effects of liming on carbon returns to the soil (Haynes and Naidu, 1998). Lime may also improve soil structural properties indirectly by making the soil conditions more favourable for soil organisms. Grieve *et al.* (2005) found a greater abundance of larger enchytraeid genera following liming, with an increase in the mean number of individuals of *Eridericia* spp. from 2 377 m^{-2} in control plots to 13 839 m^{-2} in limed plots. These organisms contribute to water-stable aggregation through their casting activities.

2.3.4.5 Irrigation

Scarcity of water in many countries has forced farmers to use poor quality water to increase crop production. Udayasoorian *et al.* (2009) reported that the pressure to produce more food has meant that saline and alkaline waters are being increasingly diverted onto agricultural lands. Salinization is a common problem in arid and semi-arid regions where total water availability is limited and good quality water is required for high value uses, and thus poor quality water is often used for irrigation (Cucci *et al*., 2013). This water has a negative effect on both soil properties and plant production. Salinity and/or sodicity are common challenges in irrigated soils especially in areas of low mean annual rainfall and high evaporative demand (Rietz and Haynes, 2003; Ezlit *et al*., 2010). Poor irrigation water and drainage management are common causes of salinization and, as the water table rises, salts dissolved in the groundwater reach and accumulate at the soil surface through capillary movement (Rietz and Haynes, 2003). The exchangeable sodium percentage becomes detrimental to AS on Vertisols when greater than 15% (Ahmad and Mermut, 1996; Rietz and Haynes, 2003; Cucci *et al.,* 2013). Two contrasting soils (loamy sand and clay) that were investigated in Australia showed that high salt concentration decreased the AS (or MWD) regardless of the soil type (Ghadiri *et al*., 2004).

2.4 Conclusions

Aggregate stability changes with change in the soil environment, and different management practices affect aggregation and stabilization processes to different degrees. The AS is not a result of a single factor, but of the interactive effects of soil factors and external factors, including management. External factors, such as burning or mulching of sugarcane residues, may cause a change in SOM inputs and influence microclimate and thus microbial activity and AS. Burning of sugarcane residues reduces the SOM content and exposes the soil to wet-dry cycles which may increase or decrease the AS depending on the number of cycles. A decline in SOM makes conditions unfavourable for soil organisms to survive and multiply and this reduces the release of mucigels and other substances that promote the aggregation of soil particles. The extent to which SOM improves AS depends on inherent soil characteristics such as clay type and content that have the potential to limit the effect of OC. In comparison with OC, clay type plays a major role in AS in soils with high clay content and *vice versa* in sandier soils. In soils that have been fertilized for a long period of time, fertilization affects the soil pH, and salt and cation concentrations. Removal of organic matter by burning exposes the soil surface to raindrops that strike and breakdown the soil aggregates decreasing their stability and encouraging the formation of a soil crust that reduces water infiltration, and increases runoff and soil erosion. Although burning, mulching and fertilization seem to be the main external factors influencing soil AS, it has been observed that there are many other factors, both internal and external, that play a role. These factors include soil pH, exchangeable basic cations, oxides of Fe and Al, clay type, soil texture and wet-dry cycles.

Despite the extensive research that has been done to understand the relationship between sugarcane management practices and soil AS, there are many questions that are still not yet answered. There are much data available on the effects of mulching *vs* burning and their consequences for soil fertility and soil nutrient status but no study has yet combined the biological, mineralogical, chemical and physical approaches. The current literature does not report how much of the increase in water availability under mulched sugarcane fields is due to the improvement of soil structure. Although the impact of sugarcane residues management and fertilizer application on AS has been measured, there is a lack of information on how their effect on AS affect bulk density, water reserves and clay mineralogical properties under long-term continuous sugarcane cultivation. Numerous studies have shown that generally, higher concentrations of carbon and nitrogen are stored in macroaggregates compared to microaggregates but they have not indicated how burning and mulching and fertilization of continuous sugarcane cultivation affect the storage of carbon and nitrogen in the soil (i.e. in the microaggregates or macroaggregates). The influence of exchangeable bases (Ca and Mg) on soil AS has mostly been reported on alkaline and limed soils and it is not clear what their relationship might be in unlimed soil with low pH under long-term sugarcane production. Lastly, the main factor(s) influencing soil AS in a long-term continuously monitored trial subjected to continuous green sugarcane harvesting, burning and fertilizer application is still not known.

CHAPTER 3: MATERIALS AND METHODS

3.1 Site description and sampling

The study was conducted on the long-term, rainfed, sugarcane trial (known as BT1) established on the 25th October 1939 at the South African Sugarcane Research Institute (SASRI), Mount Edgecombe near Durban (31°02′41.0″ E, 29°42′10.7″ S). This trial is believed to be the world's longest running soil management field experiment under sugarcane (Graham *et al*., 2002). Sugarcane has been grown in BT1 (the plots used for this study) for an average of 8 years before replanting (i.e. seven ratoon crops after the initial planting). Graham *et al*. (2002) reported that for the first 30 years the land was tilled conventionally at re-planting, but thereafter a minimum tillage system was adopted in which old ratoons are ripped from the rows and the sugarcane re-planted within the rows. The mean annual rainfall recorded between 2005 and 2014 was approximately 950 mm although the annual rainfall has varied widely between 600 and 1300 mm (Figure 3.1a). The average minimum and maximum temperatures were 16.2 and 25.5°C, respectively (Figure 3.1b).

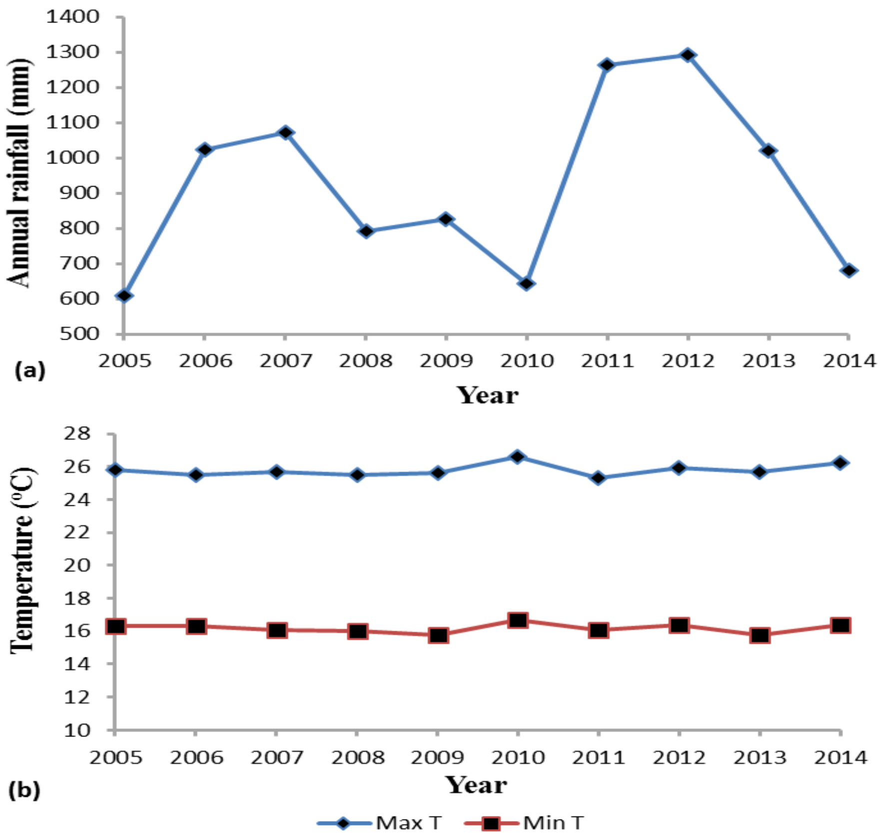


Figure 3.1: (a) The average annual rainfall and (b) the average minimum (Min T) and maximum (Max T) temperatures recorded between 2005 and 2014 at the BT1 sugarcane trial situated at SASRI, Mount Edgecombe.

The site is located on a south-west facing slope (13.5% and 18.5% at upper slope and lower slope, respectively). Exploratory soil pits were dug in the east side of the trial. On the upper slope, the soil was classified as a Mollic Cambisol (IUSS Working Group WRB, 2014), locally known as Mayo form (Glenecho family) (Soil Classification Working Group, 1991), with a dark (2.5YR 3/1 to 3/2) 50 cm thick A horizon extending to a dark reddish brown (2.5YR 3/3 to 3/4) AC transitional horizon overlying weathered dolerite. The profile contained a 5 to 10 cm thick stoneline at about 40 cm depth. On the lower slope, the soil was a Mollic Nitisol (deeper than the Mollic Cambisol found on the upper slope) (IUSS Working Group WRB, 2014), locally known as Bonheim form (Rockvale family) (Soil Classification Working Group, 1991), with the same A horizon as on the upper slope overlying a dark reddish brown to red (2.5YR 3/4 to 10R 3/6) B horizon (Figure 3.2; Appendix 3.1). The topsoil clay content was approximately 45% in both soil types.

Apparent electrical resistivity (AER) measurements (obtained using a RM15 Resistance Meter combined with an MPX-15 Multiplexer module), were taken at a total of 24 780 points at depths of 0.5 and 1.0 m across the entire BT1 experimental site (Figure 3.2a) and used to create maps using SURFER Golden software for each of the two depths (Figure 3.2b and c). The upper slope of the trial had higher AER than the lower slope and this was more distinct at 1 m depth (Figure 3.2b), with this approximately matching the change in soil classification down the slope.

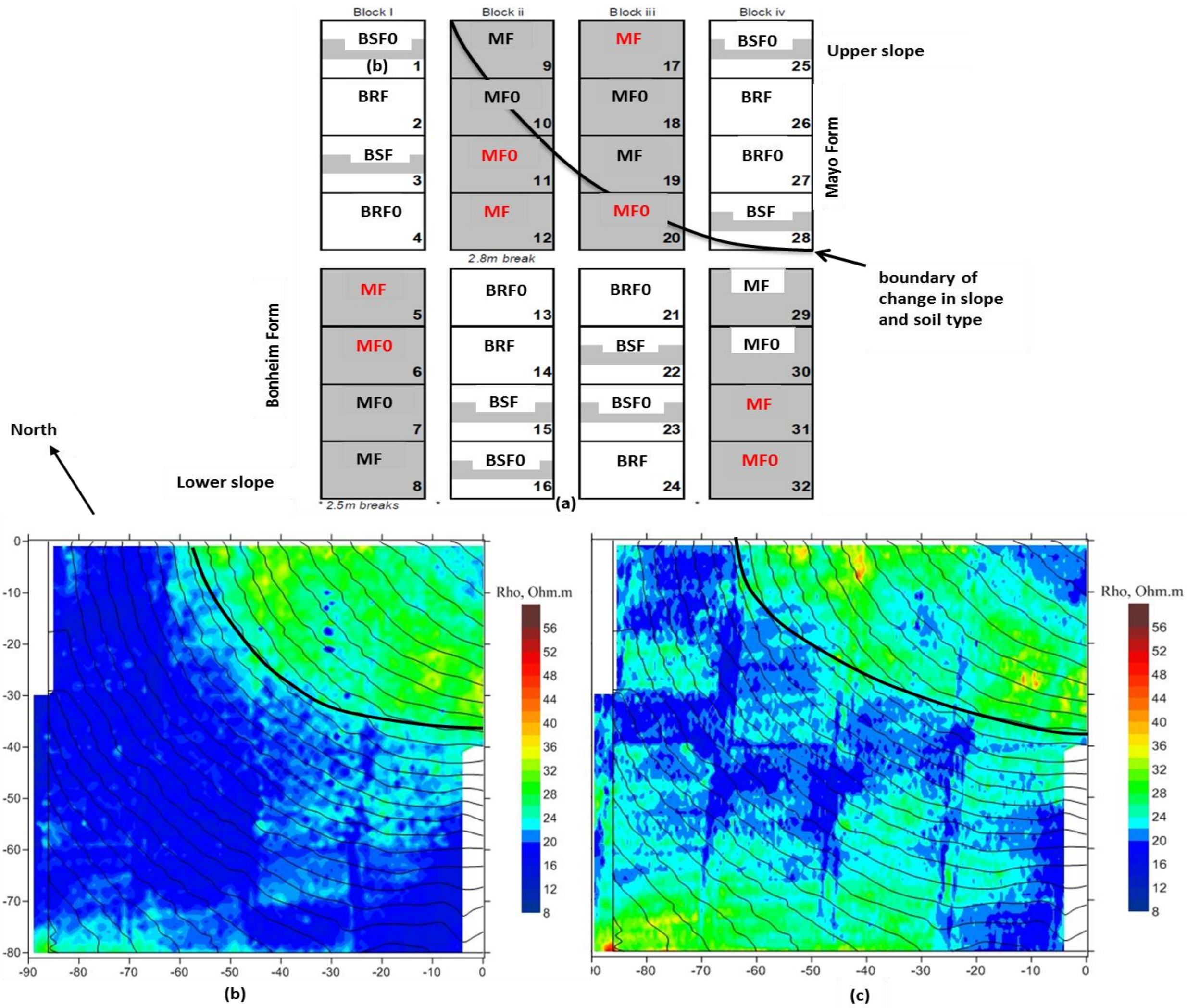


Figure 3.2: The (a) layout (treatments in red were not sampled) and (b) and (c) apparent electrical resistivity measured at 1 m and 0.5 m, respectively, of the BT1 sugarcane trial situated at SASRI, Mount Edgecombe. BRF0: burned with residues removed and not fertilized; BRF: burned with residues removed and fertilized; BSF0: burned with residues scattered and not fertilized; BSF: burned with residues scattered and fertilized; MF0: mulched and not fertilized; MF: mulched and fertilized.

The trial consists of 32 plots that are each 18 m long by 8.4 m wide and the sugarcane rows are 1.4 m apart. Each plot has seven rows lengthwise along the plot. The trial is a split-plot factorial design arranged in a randomized complete block with four replicates for plots burned at harvest and eight replicates for all unburned plots (Figure 3.2a).

The main plot treatments are:

a) green sugarcane harvesting with all residues retained and spread evenly over the plot area (M);

b) sugarcane burned prior to harvest (no foliage residue) with sugarcane-tops left scattered evenly over the plot area (BS); and

c) sugarcane burned prior to harvest with all residues (sugarcane-tops) removed from the plots (BR).

Sub-treatments consist of:

a) fertilized (F) and
b) unfertilized plots (F0).

On average, the amount of residues retained are 20 (MF) and 15 tons ha^{-1} (MF0) on the mulched treatments, and 3.2 (BSF) and 2.3 tons ha^{-1} (BSF0) on the burned treatments (van Antwerpen *et al*., 2001). There are no residues in the BRF and BRF0 treatments since the ash that remains after burning is usually blown away by the wind within days of harvest.

For this study all burned plots (representing two burned and two fertilizer management treatments, each replicated four times, giving a total of 16 plots) and half of the 16 unburned plots (four MF and four MF0 plots) were selected to obtain an equal number (4) of replicates per treatment. A total of 24 plots was therefore used for this study (Figure 3.2a). The fertilized treatments consist of 140 kg N ha^{-1}, 28 kg P ha^{-1} and 140 kg K ha^{-1} as 5:1:5 (46) at 670 kg ha^{-1} applied annually approximately 40 days after harvesting (van Antwerpen *et al*., 2001). The sugarcane was harvested every 24 months from the beginning of the experiment until 1966, then every 15 months between 1966 and 1987 and every 12 months since 1987 (van Antwerpen *et al*., 2001). The sugarcane variety that was planted at the trial site during the sampling time (February 2012) was N27.

Three replicate soil samples were collected at two depths (0-10 and 10-20 cm) from mini-pits, to avoid major disturbance in the trial, in each of the 24 chosen plots in February 2012. The 144 soil samples were carefully collected with a spade at each depth to avoid the shearing effects of an auger and all samples were carefully wrapped and transported to the University of KwaZulu-Natal's Pietermaritzburg Campus for analysis. The bulk samples were air-dried and about one third was used for aggregate stability (AS) measurements, while the rest was ground with a pestle and mortar and passed through a 2 mm sieve. Undisturbed soil cores (144) were collected by inserting a stainless-steel core ring (50 mm height and 75 mm diameter) into the soil using the core sleeve guide and hammer to insert the core ring to the correct depth. The 0-10 cm core samples were collected after removing the loose soil material from the soil surface. The excess soil protruding at the ends of each core after removal from the soil was removed in-field.

3.2 Analysis

3.2.1 Particle size distribution and clay mineralogy

The particle size distribution was determined by the pipette method (Gee and Bauder, 1986) on 48 samples with 4 replicates selected to be representative of the 24 plots investigated. For clay mineralogy (< 2 µm), the clay fractions were separated from 24 samples sampled at 0-10 cm through sedimentation after removal of organic matter by hydrogen peroxide followed by dispersion of the soil using sodium hexametaphosphate + sodium bicarbonate and ultrasound treatment (Brindley and Brown, 1980).

Clay mineralogy was determined by X-ray diffraction (XRD) carried out on oriented samples (saturated with $CaCl_2$) using a Panalytical X'Pert Powder diffractometer with Ni-filtered Cu-Kα radiation at 40 kV and 40 mA. The air-dried, glycerolated and heated (500°C for 3 hours) clay samples were scanned from 2° to 15° 2θ with a scanning step size of 0.01313° for 0.779 s per step (Klute, 1965).

3.2.2 Soil chemical properties

These were analyzed on all 144 bulk soil samples (with 4 replicates) collected that were air-dried and crushed to pass either a 2 mm mesh (pH, exchangeable bases, exchangeable acidity and extractable aluminium (Al), zinc (Zn), copper (Cu), manganese (Mn) and phosphorus (P)) or a 0.5 mm mesh (total carbon (Ct), total nitrogen (N) and organic carbon (OC)). Soil pH was measured in 1M KCl at 1:2.5 soil:solution ratio (Yeomans and Bremner, 1988). Exchangeable potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na) were extracted with 0.1M $SrCl_2$ (Hughes and Girdlestone, 1994) and measured by atomic absorption spectrophotometry (AAS, Varian SpectraAA-200). Aluminium and exchangeable acidity were extracted with 1M KCl (Hunter, 1974). The exchangeable acidity was measured by titration with sodium hydroxide and the extracted aluminium was measured with inductively coupled plasma emission spectrometry (ICP, Varian 720-ES). The effective cation exchange capacity (ECEC) was calculated as the sum of exchangeable base cations and acidity and the acid saturation (AS) was calculated as the ratio of acidity to the ECEC. The Ct and N were determined on both bulk samples and soil aggregate fractions of different sizes (obtained from the water stability test described in Section 3.2.4) using the automated Dumas dry combustion method on a LECO CNS 2000 analyzer (Matejovic, 1996) and the C:N ratio calculated. The readily oxidizable OC was determined by the acid dichromate wet oxidation procedure (Walkley, 1947). Extractable Zn, Mn, Cu and P were extracted with Ambic-2 solution (0.25 *M* NH_4CO_3 + 0.01 *M* Na_2EDTA + 0.01 *M* NH_4F + 0.05 g L^{-1} Superfloc (N100), adjusted to pH 8) (Manson and Roberts, 2000). Phosphorus was measured using the molybdenum blue method of Murphy and Riley (1962) and Zn, Cu and Mn were measured by AAS.

3.2.3 Soil microbiological properties

The soil microbial properties were only measured on bulk samples collected from the 0-10 cm depth from the 24 selected plots, with four replications. These analyses were carried out at the University of Paris-Est, Créteil.

3.2.3.1 DNA

The DNA extraction was carried out on a 25 g soil sample using the PowerSoil® DNA isolation kit (Mo-Bio laboratories, Carlsbad, CA, USA) according to the manufacture's instruction but omitting the last step that involves the elution of DNA with 100 µL of nuclease-free water (Ambion, Warrington, UK). The quality of DNA extraction was examined by electrophoresis on 1% agarose gels stained with GelRed (Molecular Probes, USA) using Gel Doc image analyzer (BioRad, USA) (White *et al*., 1990; Lerch *et al*., 2013).

3.2.3.2 Microbial abundance

The soil microbial communities were quantified with qPCR amplification targeting 16S rRNA gene for bacteria and the internal transcribed spacer (ITS) gene for fungi on a StepOneTM Real-Time PCR (Applied BioSystem, USA) as follows: each reaction was carried out using the pre-described primers 314F 5' 341F 5'-CCTACGGGAGGCAGCAG-3' and 534R 5'-ATTACCGCGGCTGCTGGCA-3' for 16S rRNA and ITS3 5'-GCATCGATGAAGAACGCAGC-3' and ITS4 5'-TCCTCCGCTTATTGATATGC-3' for ITS (White *et al*., 1990; Gardes and Bruns, 1993; Marchesi *et al*., 1998). In each reaction there was 1 ng of DNA template, 7.5 µL of Power SYBR® Green PCR Master Mix (Applied Biosystem, USA) and 0.1µM of each primer in a total reaction volume of 20 µL.

3.2.3.3 Microbial catabolic profiles

The soil moisture content was pre-adjusted to 40% water holding capacity to ensure that after the substrate was added, the moisture content was 60% of the soil's water holding capacity and then pre-incubated for two weeks. The community-level physiological profiles (CLPP) of the soil were determined by multiple substrate-induced respirations using the MicroResp™ method (Campbell *et al.*, 2003). The carbon substrate to be added was calculated to add a relative quantity of 10% of the SOC (Lerch *et al.*, 2013). The total substrate mineralization was estimated as the sum of carbon dioxide evolved for each substrate and catabolic evenness (E) was estimated using the Simpson-Yule index (Equation 3.1) (Magurran, 1988).

$$\mathbf{E = 1/\sum pi^2} \quad \text{(Equation 3.1)}$$

Where **pi** is the respiration response to the substrate **i** as a proportion of total substrate activity.

3.2.3.4 Microbial genetic structures

The amplification of 16S rRNA gene was carried out using the following primers: 63F (5'-CAGGCCTAACACATGCAAGTC-3') and 1389R (5'-ACGGGCGGTGTGTACAAG-3') (Marchesi *et al.*, 1998; Osborn *et al.*, 2000) for bacteria. Fungal internal transcribed spacers (ITS) were amplified using the primers; ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') (Gardes and Bruns, 1993) and ITS4 (5'-TCCTCCGCTTATTGATATGC) (White *et al.*, 1990). Bacterial purified PCR products (10 µL) were digested with 10 U of the restriction enzyme Alul and 1x restriction enzyme buffer (Roche, Hertfordshire, UK) in a total volume of 15 µL at 37ºC for 3 h. The fungal purified PCR products (10 µL) were desalted in the same way as the bacterial PCR products (Smith *et al.*, 2005).

3.2.4 Soil aggregate stability

Oven-dried (40ºC for 48 hours) soil aggregate fractions between 5 and 2.8 mm were used to measure the soil AS according to the AFNOR norm NF31-315 (AFNOR, 2005) (Appendix 3.2). This method constitutes three treatments i.e. water treatment (Wt), ethanol treatment (Et) and slow capillary wetting ethanol treatment (SCWEt) which represent a range of soil wetting conditions that can affect soil aggregate stability. The Wt imitates rainfall with >50 mm h^{-1} intensity on dry soil (to represent the effect of rapid water slaking); Et imitates rainfall with about 10 mm h^{-1} intensity on dry soil (to mimic slow (less aggressive) wetting of soil); and SCWEt represents aggregate stability when rainfall is deposited on aggregates that are already saturated with water (the effect of antecedent moisture) and is the least aggressive wetting method (AFNOR, 2005). The aggregates remaining on the sieve after dispersion were collected and dried at 40°C, and then gently sieved using a nest of six sieves: 2.00, 1.00, 0.50, 0.20, 0.10, and 0.05 mm. The AS is expressed as the mean weight diameter (MWD; Equation 3.2) calculated for each treatment (Wt, Et and SCWEt).

$$\mathbf{MWD\ (mm) = \sum [\mathit{d} \times \mathit{m}] / 100} \quad \text{(Equation 3.2)}$$

Where ***d*** is the mean diameter between the two sieves (mm), and ***m*** the weight fraction of aggregates remaining on the sieve (%).

3.2.5 Soil penetrometer resistance

Penetrometer resistance (PR) was measured using a Geotron PEN93 penetrometer (Geotron Systems, Potchefstroom) that recorded the resistance at 5 mm intervals to a depth of 60 cm (Plate 3.1). The penetrometer penetrated the soil at a rate of 1 000 mm per minute using a cone with a diameter of 20.27 mm and surface area of 320 mm^2. For this study, five points were measured in each plot in the inter-rows. All the readings were taken on 12 December 2013 to minimise differences in soil moisture contents that are due to variation in short-term weather.

Plate 3.1: The Geotron PEN93 penetrometer (Geotron Systems, Potchefstroom).

3.2.6 Water retention, saturated hydraulic conductivity and bulk density

The soil core samples were prepared and analysed for water retention, saturated hydraulic conductivity (Ks) and bulk density (ρ_b) according to Klute (1965). Briefly, a pre-weighed piece of nylon cloth and elastic band were placed onto the lower end of the soil core that had been trimmed level with the upper and lower surface of the core ring. All the samples were placed in a vacuum desiccator and slowly saturated with water through capillary wetting. When samples were completely saturated, the cores were weighed (0 kPa). After that, the soil cores were placed in various pressure pots and pressures of 2, 4, 6, 8, 10, 33, 100 and 1500 kPa were applied consecutively to mimic equivalent soil matric potentials from saturation to wilting point. All the samples were equilibrated for 48 hours and weighed at each respective pressure. For the determination of moisture content (θ_m) and ρ_b the soil samples were oven-dried at 105°C for 48 hours. Gravimetric moisture content was calculated using Equation 3.3 and mapped using SURFER Golden software for 0-10 cm soil depth while ρ_b and available water capacity (AWC) were calculated using Equations 3.4 and 3.5, respectively, for both 0-10 and 10-20 cm depth.

θ_m **(g g^{-1}) = [mass of water / mass of soil] * 100**……..…………..………..…….. (Equation 3.3)

ρ_b (g cm^{-3}) = [mass of soil / volume of soil]…………..……..…………..…..……..(Equation 3.4)

AWC (mm m^{-1}) = {[θ_m (%) (10 kPa) - θ_m (%) (1500 kPa)]/100}*ρ_b.................(Equation 3.5)

The Ks was determined directly after the measurements of water retention. It was measured using a brass permeameter (Plate 3.2) with the undisturbed soil core sample that was supported vertically on the outflow funnel and then water was admitted into the top of the permeameter (US Salinity Laboratory Staff, 1954). A fixed head of water was maintained (30 mm) in the top of the permeameter using a Marriott bottle system. The time water was first admitted and time taken to percolate through the base was recorded. At regular intervals (± 2 or 3 times a day) the amount of water percolating per unit time was measured. This was continued until the volume percolating in a fixed time remained constant. The Ks was calculated using Equation 3.6.

Ks (cm hr^{-1}) = [(V/ (A*t)) * (L / ΔH)]…………..………………………..…...….(Equation 3.6)

where V is the volume of water (mm) collected for time period of t (minutes), A is the cross sectional area of the core (mm^2), L is the length of the soil core (mm) and ΔH is the hydraulic head (mm).

Plate 3.2: A brass permeameter used to measure saturated soil hydraulic conductivity.

3.3 Statistical analysis

The overall differences between the treatment means were assessed using the general analysis of variance (ANOVA) for each depth separately. This statistical analysis approach was selected in preference to the split-plot design analysis (as per the original design of the trial) due to a) not all treatments were used in the present study, b) the use of general treatment structure increases the degrees of freedom for the treatments, which provides slightly more confidence, c) the original design does not adequately address trial site gradient found in the blocking, and d) the individual main effects were of limited interest, with the causal relationship between measured parameters being of primary interest in this study. The ANOVA was undertaken for Ct, OC, N, C:N, ECEC, acid saturation, exchangeable bases (Ca, Mg, Na and K), exchangeable acidity, pH, extractable Al, Zn, Cu, Mn and P, sand, silt and clay, MWD-Wt, MWD-Et, MWD-SCWEt, Ks, AWC, ρ_b, water retained at different matric potentials and PR (GENSTAT, 14th edition). The ANOVA was also performed to compare treatment means for the Ct and N distribution in the different sized aggregate fractions of soil using the statistical software package GENSTAT, 14th edition. Where significant ($p < 0.05$) overall differences (F-probability) between the treatment means were found, these were compared using least significant difference (LSD) comparisons at the 5% level of significance using Duncan's multiple range test (GENSTAT, 14th edition). To investigate the relationships between soil structure-related physical parameters, simple Pearson's correlations between MWD, Ks, PR, ρ_b and AWC were also done (GENSTAT, 14th edition). The results of the sub-samples (pseudo-replicates; n = 3) were averaged to provide a single variable estimate for each plot. The results of the true replicates (n = 4) across treatment plots were averaged and correlations were carried out between these.

For soil microbiological properties, the differences between the treatments in the ds DNA amount, fungal and bacterial abundance, richness and evenness as well as ratios were tested by ANOVA followed by Tukey's honest significant difference (HSD) test for 0-10 cm depth (R version 2.12.0; R Development Core Team, 2008). Redundancy analysis (R version 3.0.0; R Development Core Team, 2011) was used to establish the multi-variate relationship between variables (Na, K, Ca, Mg, Ct, C:N, pH, sand, silt, clay, fungi, bacteria and MWD) and also to determine the main factor influencing MWD for the 0-10 cm depth. The Monte-Carlo permutation test was performed to test if fertilizer had more effect on measured soil properties (excluding microorganisms) than mulching or burning of sugarcane at harvesting (R version 2.12.0; R Development Core Team, 2008) in both 0-10 and 10-20 cm, separately. For all the soil properties that were measured in both 0-10 and 10-20 cm, the statistical analyses were done separately between the two depths.

CHAPTER 4: EFFECT OF SUGARCANE RESIDUE AND FERTILIZER APPLICATION ON CLAY MINERALOGY AND SOME SOIL PHYSICOCHEMICAL PROPERTIES

Note: Some of the results of this Chapter have been published in an article entitled "The effect of 72 years of sugarcane residues and fertilizer management on soil physico-chemical properties" in Agriculture, Ecosystems and Environment (Mthimkhulu et al., 2016; Appendix A).

4.1 Introduction

Knowledge of clay mineralogy is very important for understanding soil aggregate stability (AS) (Wakindiki and Ben-Hur, 2002) and soil response to continuous additions or removals of residues and fertilizer application under long-term sugarcane cultivation. Although clay mineralogy is largely determined by the soil parent material and the degree of weathering, they can also be influenced by land management practices such as fertilizer applications (Pernes-Debuyser *et al*., 2003; Bronick and Lal, 2005; Khormali *et al*., 2015). A change in clay mineralogy affects other soil properties such as effective cation exchange capacity (ECEC), charge density, shrink-swell properties and dispersivity, and these, in turn, affect the carbon residence time in the soil (Bronick and Lal, 2005). The clay type and amount also influence the soil organic carbon (OC), exchangeable cations and soil pH. Understanding the relationship between clays and soil chemical properties depends on an ability to define the degree and nature of many soil solids and other surfaces encountered in soil microhabitats (Marshall, 1975). Little is known about the consequences of fertilizer application and sugarcane pre-harvesting practices and associated changes in soil organic matter (SOM) on clay mineralogical properties and soil chemical properties under continuous sugarcane cultivation in South Africa. This chapter investigates the differences in some soil properties that affect AS and soil structure as a response to three levels of sugarcane residues and fertilizer application in a long-term field experiment.

4.2. Materials and methods

The materials and methods used were given in Chapter 3.

4.3 Results

4.3.1 Particle size distribution and clay mineralogy

The average clay, silt and sand contents were 43.4, 33.5 and 23.2%, respectively, across all treatments and soil depths. There was no significant difference ($p > 0.05$; Appendix 4.1 and 4.2) between the treatments at both depths in terms of clay content (Table 4.1).

Table 4.1: The mean ($n = 4 \pm$ standard error) particle size distribution determined at 0-10 and 10-20 cm soil depth under different management treatments: BRF0: burned with residues removed and not fertilized; BRF: burned with residues removed and fertilized; BSF0: burned with residues scattered and not fertilized; BSF: burned with residues scattered and fertilized; MF0: mulched and not fertilized; MF: mulched and fertilized.

Treatment	Depth (cm)	Particle size distribution (%)		
		Clay (< 0.002 mm)	Silt (0.002-0.05 mm)	Sand (0.05-2 mm)
BRF0	0-10	43.6±1.0	30.9±2.0	25.3±1.7
	10-20	45.6±0.6	32.3±2.3	22.1±2.6
BRF	0-10	44.4±0.9	32.6±2.6	23.1±1.8
	10-20	41.4±1.5	34.9±0.6	23.7±1.6
BSF0	0-10	40.4±1.1	35.3±0.9	24.2±1.3
	10-20	41.3±1.5	36.4±0.9	22.3±1.2
BSF	0-10	43.4±2.8	35.1±1.6	21.5±1.9
	10-20	45.1±2.6	30.5±2.3	24.4±2.3
MF0	0-10	40.4±2.3	34.7±1.3	24.9±2.1
	10-20	43.5±2.6	34.1±1.7	22.4±1.8
MF	0-10	44.7±3.8	33.4±1.5	21.9±2.4
	10-20	46.6±3.5	31.4±1.8	22.1±1.9

Generally, both fertilized and unfertilized treatments as well as mulching and burning treatments showed no effect on the mineralogy of the investigated soil samples (Figures 4.1, 4.2, 4.3, 4.4 and 4.5). The clay minerals differed slightly between the upper and lower slopes, (Figure 4.5). Clay minerals that were present in the upper slope soil but absent in the lower slope included interstratified vermiculite-smectite, talc and illite, while those that were present in both slope positons were high defect kaolin, vermiculite and lepidocrocite (Figure 4.5). High defect kaolin was more pronounced in the lower slope. A small amount of illite-vermiculite was also measured in one MF treatment plot on the lower slope. The soil on the upper slope was shallow (± 50 cm deep) compared to the lower slope (± 70 cm deep).

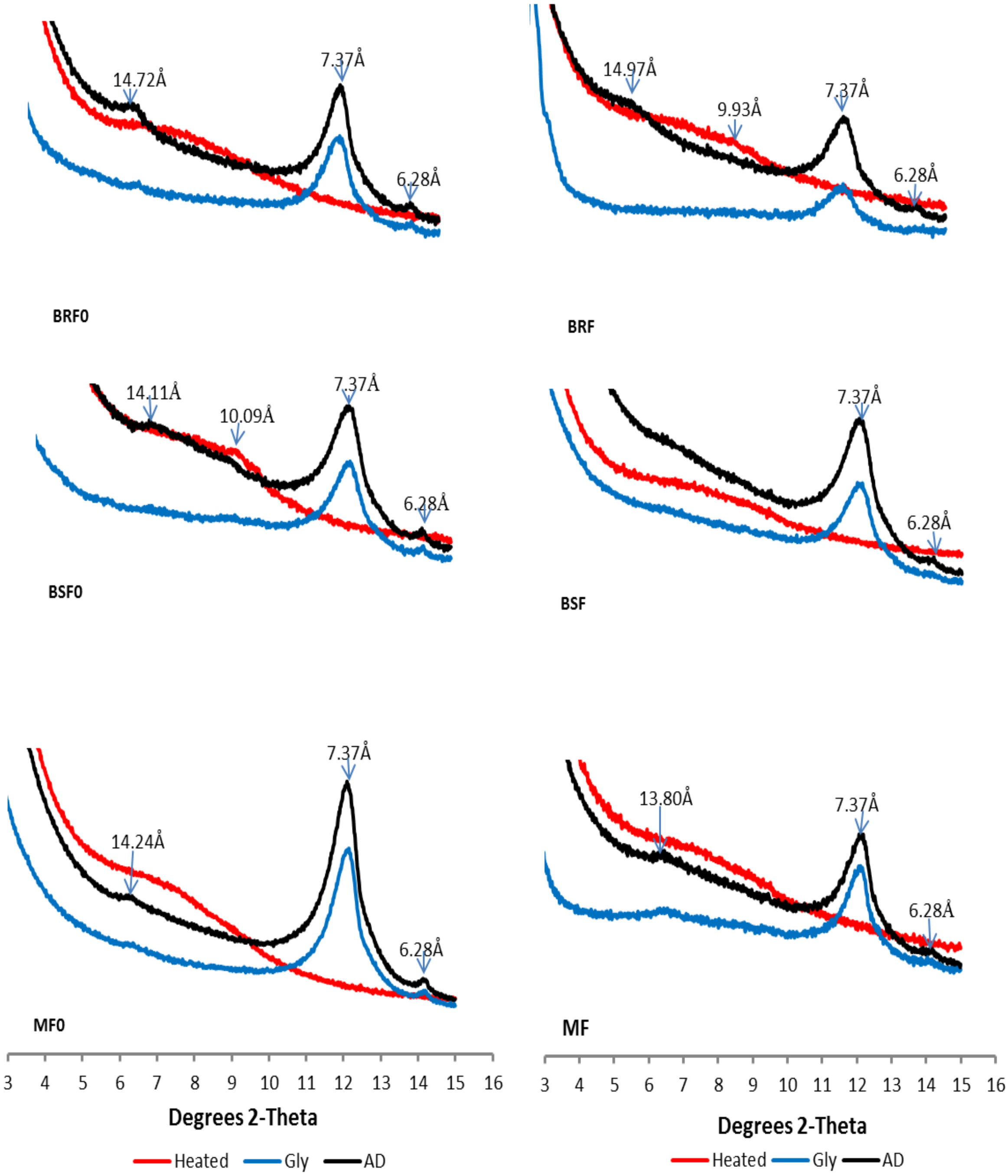


Figure 4.1: The X-ray diffraction traces of clay from the 0-10 cm soil depth of the different management treatments in Block I of the BT1 trial: BRF0: burned with residues removed and not fertilized; BRF: burned with residues removed and fertilized; BSF0: burned with residues scattered and not fertilized; BSF: burned with residues scattered and fertilized; MF0: mulched and not fertilized; MF: mulched and fertilized. Heated: heated at 550°C; Gly: glycerol; AD: air-dried. Clay minerals are as follows: 6.28Å: Lepidocrocite; 7.37Å: high defect kaolin; 14.72, 14.97, 14.24 and 14.11Å: Al-interlayered vermiculite; 9.93Å and 10.09Å: Illite; 13.80Å: interstratified illite-vermiculite.

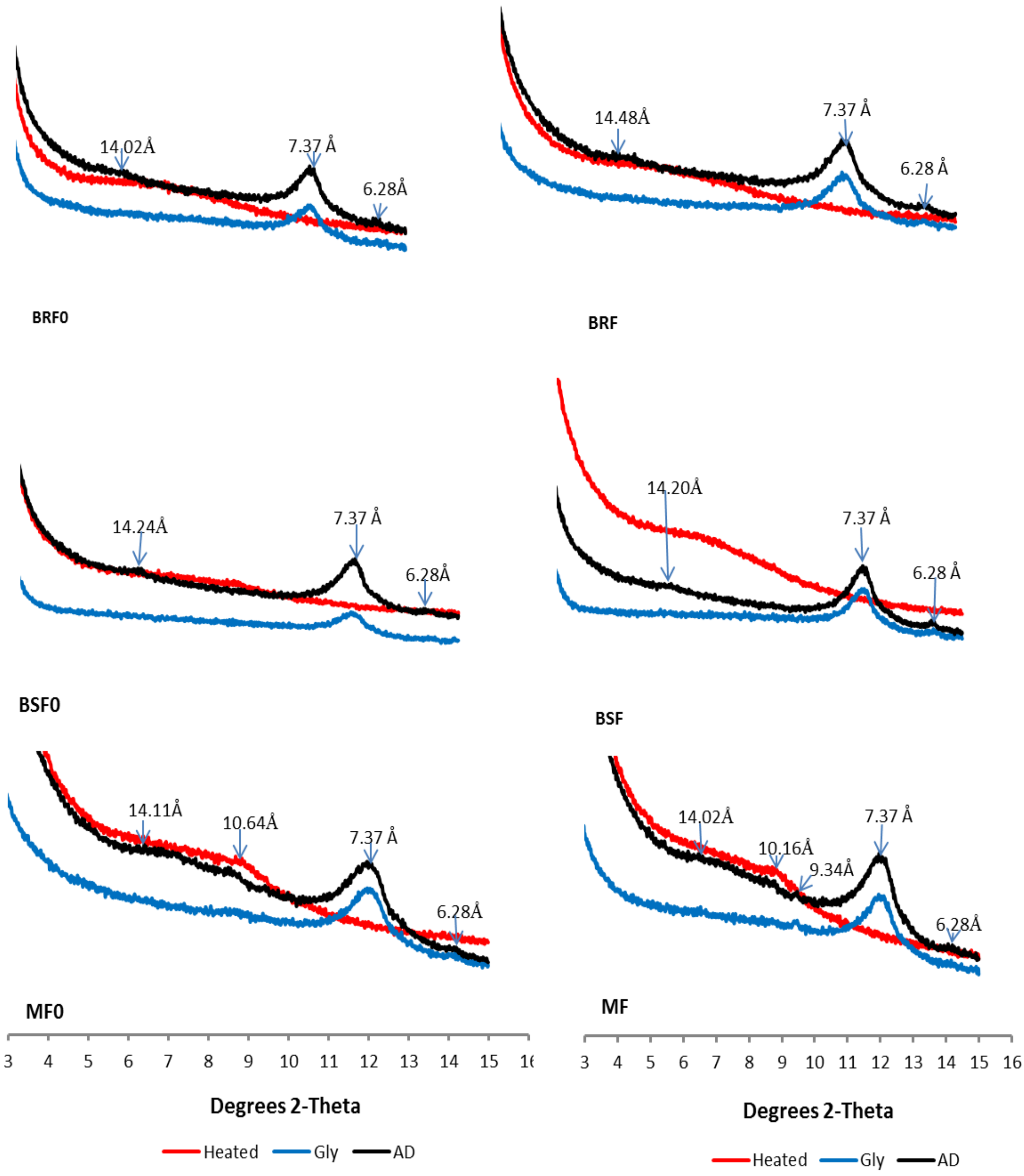


Figure 4.2: The X-ray diffraction traces of clay from the 0-10 cm soil depth of the different management treatments in Block II of the BT1 trial: BRF0: burned with residues removed and not fertilized; BRF: burned with residues removed and fertilized; BSF0: burned with residues scattered and not fertilized; BSF: burned with residues scattered and fertilized; MF0: mulched and not fertilized; MF: mulched and fertilized. Heated: heated at 550ºC; Gly: glycerol; AD: air-dried. Clay minerals are as follows: 6.28Å: Lepidocrocite; 7.37Å: high defect kaolin; 10.16: Illite; 14.02, 14.11, 14.20, 14.24, 14.48Å: Vermiculite; 9.34Å: Talc.

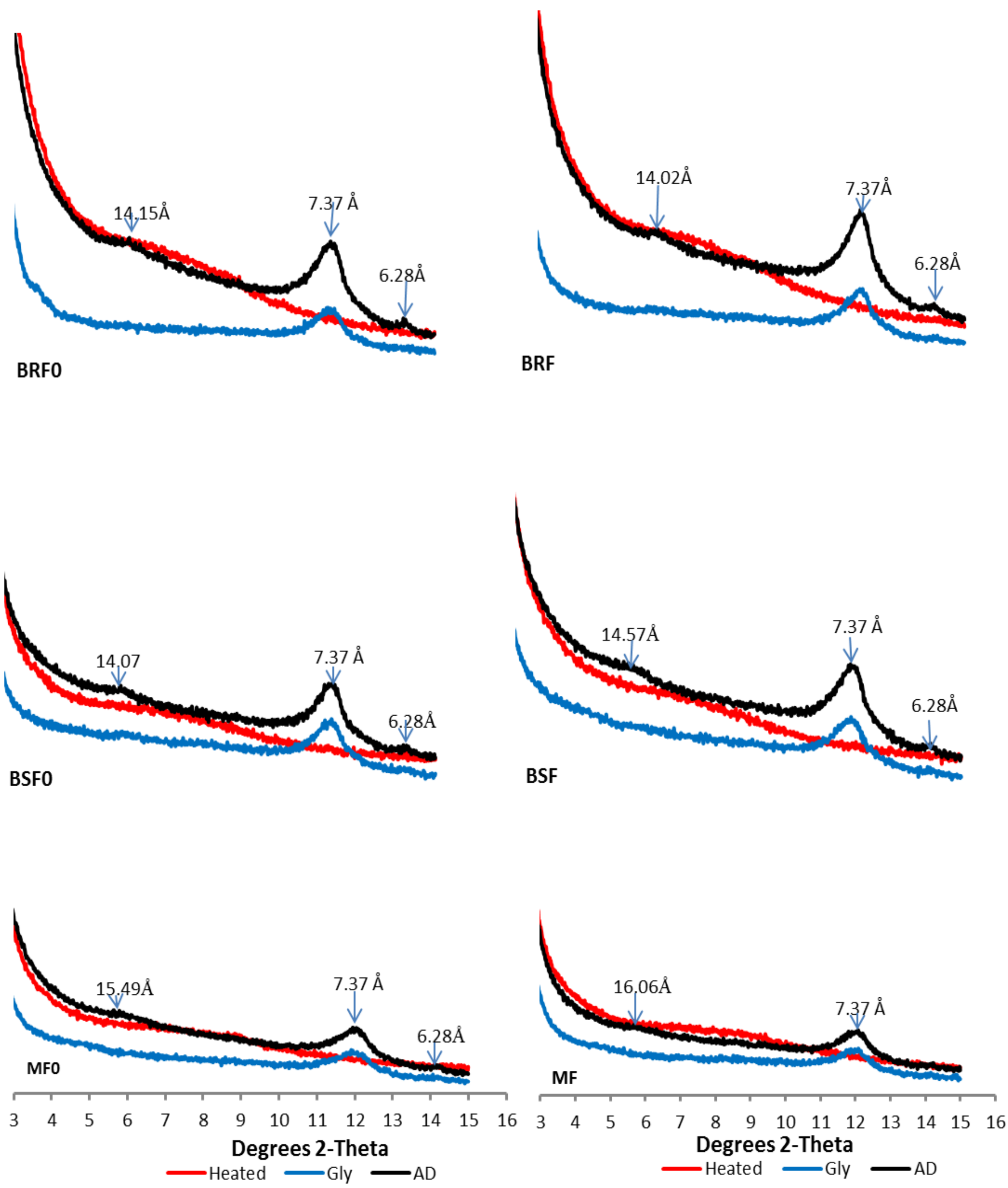


Figure 4.3: The X-ray diffraction traces of clay from the 0-10 cm soil depth of the different management treatments in Block III of the BT1 trial: BRF0: burned with residues removed and not fertilized; BRF: burned with residues removed and fertilized; BSF0: burned with residues scattered and not fertilized; BSF: burned with residues scattered and fertilized; MF0: mulched and not fertilized; MF: mulched and fertilized. Heated: heated at 550ºC; Gly: glycerol; AD: air-dried. Clay minerals are as follows: 6.28Å: Lepidocrocite; 7.37Å: high defect kaolin; 14.02, 14.07, 14.15, 14.57Å: Vermiculite; 15.49, 16.06Å: interstratified vermiculite-smectite.

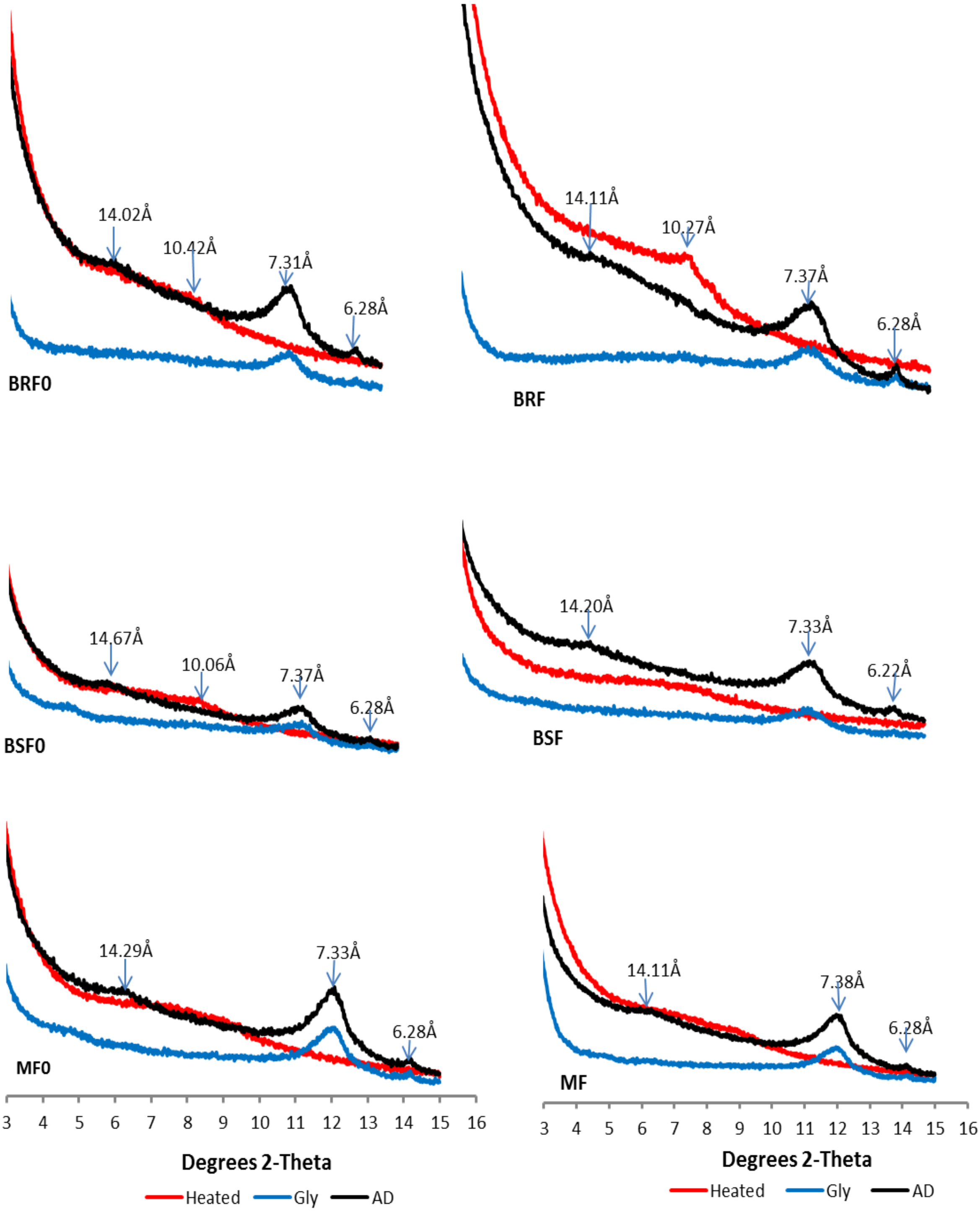


Figure 4.4: The X-ray diffraction traces of clay from the 0-10 cm soil depth of the different management treatments in Block IV of the BT1 trial: BRF0: burned with residues removed and not fertilized; BRF: burned with residues removed and fertilized; BSF0: burned with residues scattered and not fertilized; BSF: burned with residues scattered and fertilized; MF0: mulched and not fertilized; MF: mulched and fertilized. Heated: heated at 550°C; Gly: glycerol; AD: air dried. Clay minerals are as follows: 6.28, 6.22Å: Lepidocrocite; 7.31, 7.33, 7.37Å: high defect kaolin; 14.02, 14.11, 14.20, 14.67, 14.29Å: Vermiculite; 10.06, 10.27, 10.42Å: Illite.

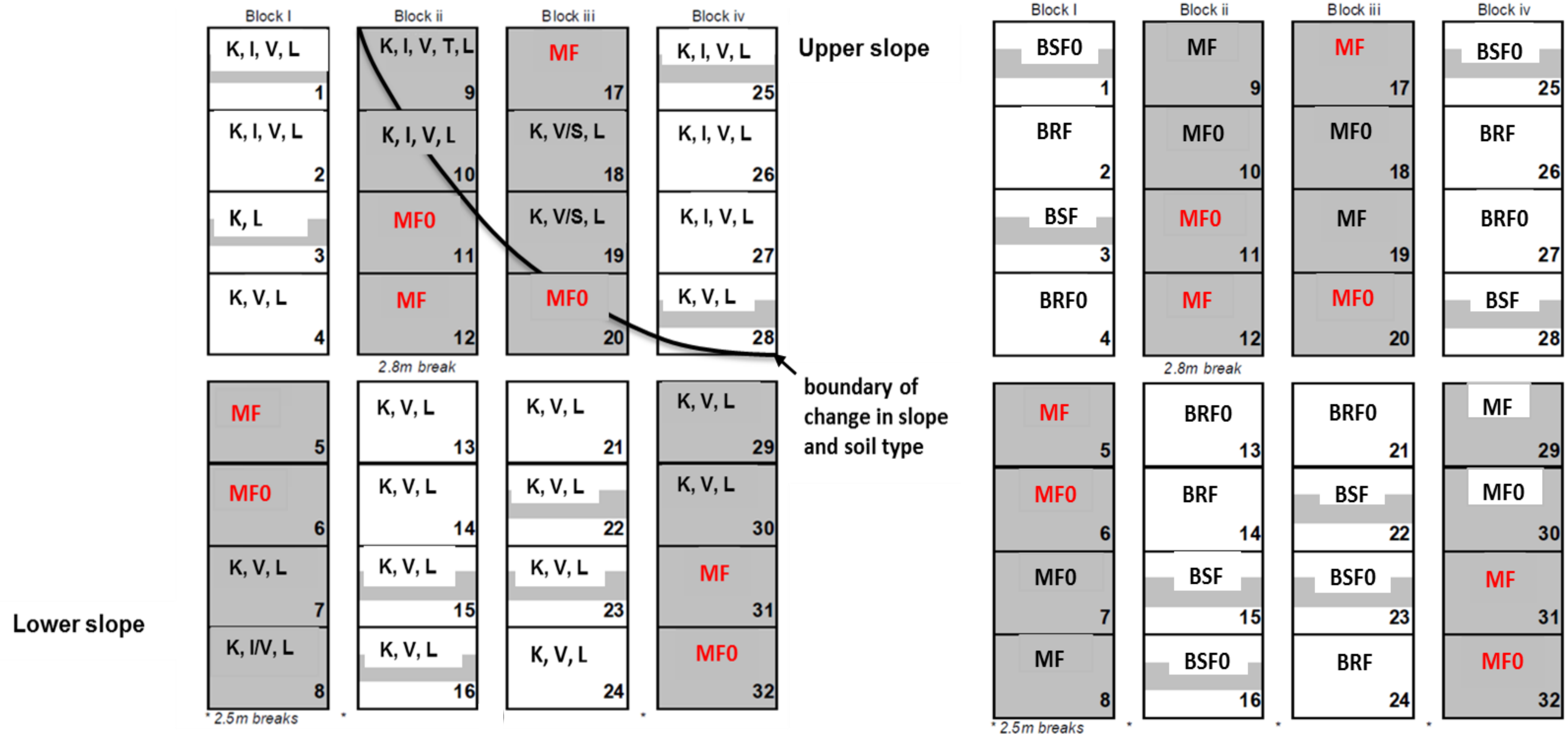


Figure 4.5: The clay minerals found in the 0-10 cm soil depth samples on the different management treatments (shown on the right of the diagram) at the BT1 sugarcane trial situated at SASRI, Mount Edgecombe. BRF0: burned with residues removed and not fertilized; BRF: burned with residues removed and fertilized; BSF0: burned with residues scattered and not fertilized; BSF: burned with residues scattered and fertilized; MF0: mulched and not fertilized; MF: mulched and fertilized. K: high defect kaolin; I: Illite; V: vermiculite; I/V: interstratified illite-vermiculite; V/S: interstratified vermiculite-smectite; T: Talc; L: lepidocrocite. Treatments in red were not sampled.

4.3.2. Carbon and nitrogen

The concentration of Ct was slightly higher under BSF0 (42 g kg $^{-1}$) compared to MF0 (41 g kg^{-1}) and BRF0 (36 g kg^{-1}) at 0-10 cm (Figure 4.6a). Although a slight effect of treatment on Ct was observed at 0-10 cm, no significant difference ($p > 0.05$; Appendix 4.3) was found between the treatments. No clear trends or significant differences ($p > 0.05$; Appendix 4.4) were observed at 10-20 cm (Figure 4.6b). There was a general increase in OC under M treatments at both depths (0-10 and 10-20 cm) (Figure 4.6c and d) though no significant differences ($p > 0.05$ at both 0-10 and 10-20 cm depth; Appendix 4.5 and 4.6) between the treatments were found. There was very little difference between Ct and OC although Ct tended to be slightly higher in both depths (Figure 4.6a to d). Nitrogen content increased significantly ($p = 0.01$; Appendix 4.7) in M compared to BR treatments in both fertilized and unfertilized treatments at 0-10 cm. At 10-20 cm, N was higher in the M treatments but not significantly ($p > 0.05$; Appendix 4.8) different from BS and BR treatments (Figure 4.6e and f). This higher N measured in the MF treatments compared to other treatments significantly decreased (0-10 cm ($p = 0.04$; Appendix 4.9) and 10-20 cm ($p = 0.02$; Appendix 4.10)) the C:N ratio at both depths ((Figure 4.6g and h)).

In the different aggregate fractions, the 0.1-0.05 mm aggregates had the lowest Ct and significant differences (Appendix 4.11) were observed in BRF0 ($p < 0.01$) and MF ($p = 0.01$) at 0-10 cm and MF ($p = 0.04$) at 10-20 cm only (Figures 4.7 and 4.8). Similarly to Ct, the lowest concentrations of N were also measured in the smallest aggregates from both unfertilized and fertilized treatments (Figures 4.9 and 4.10). Generally, there was a significantly lower N in the smallest aggregates compared to the largest aggregate fractions as non-significant differences ($p > 0.05$; Appendix 4.11) were only observed in BSF0 and BSF at 0-10 and MF0 and MF at 10-20 cm.

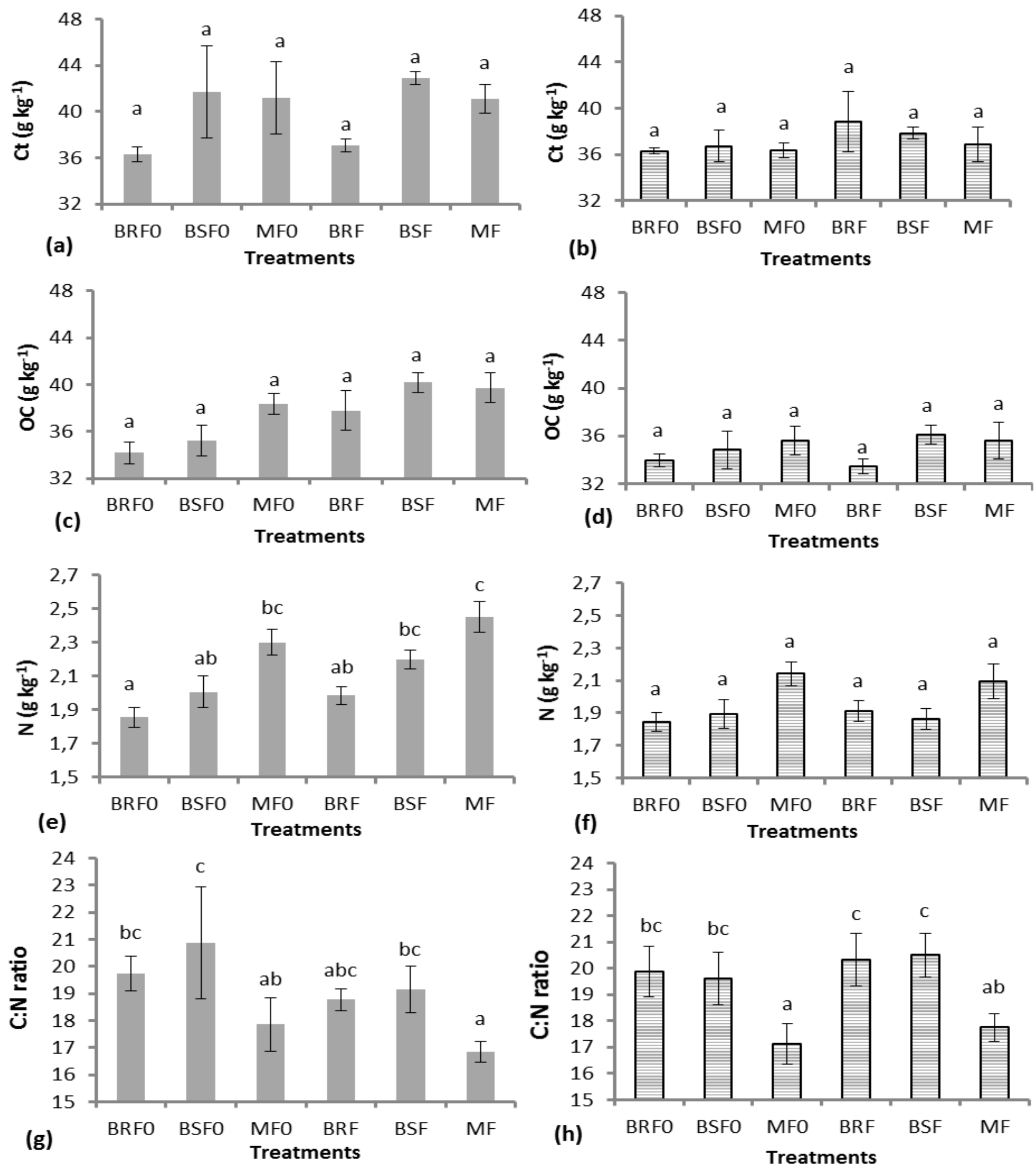


Figure 4.6: The mean (n = 4±standard error) Ct: total carbon, OC: organic carbon, N: total nitrogen and C:N ratio: carbon to nitrogen ratio at 0-10 cm (a, c, e and g) and 10-20 cm (b, d, f and h) soil depth under different management treatments: BRF0: burned with residues removed and not fertilized; BRF: burned with residues removed and fertilized; BSF0: burned with residues scattered and not fertilized; BSF: burned with residues scattered and fertilized; MF0: mulched and not fertilized; MF: mulched and fertilized. Means associated with the same letter are not significantly different at a given depth ($LSD_{5\%}$ (Ct: 0-10 cm = 7.50, 10-20 cm = 6.09, OC: 0-10 cm = 5.65, 10-20 cm = 5.93, N: 0-10 cm = 0.32, 10-20 cm = 0.38., C:N ratio: 0-10 cm = 2.38, 10-20 cm= 2.11)).

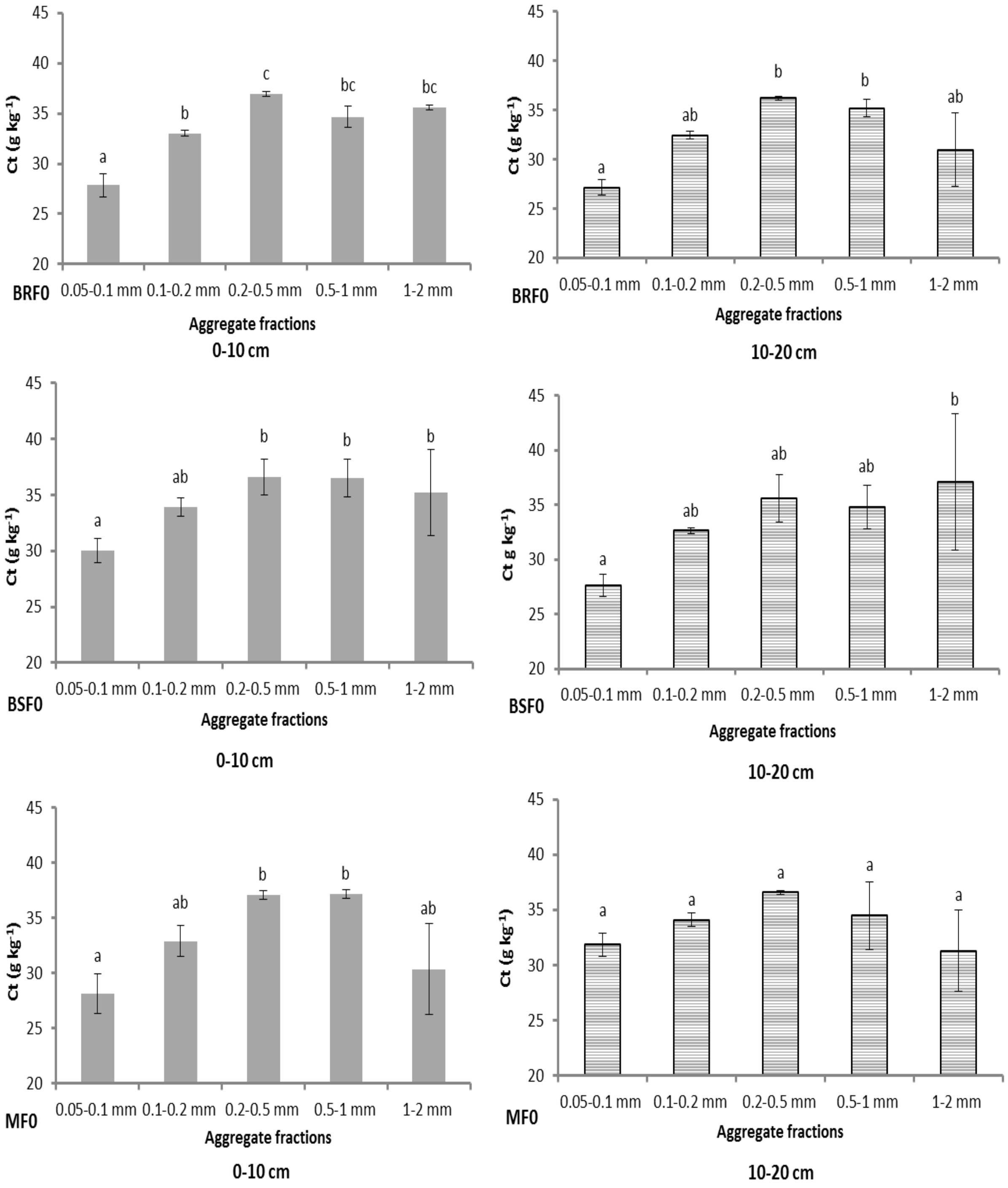


Figure 4.7: The mean (n = 3±standard error) Ct: total carbon in soil aggregate fractions of different sizes at 0-10 cm and 10-20 cm soil depth under different management treatments: BRF0: burned with residues removed and not fertilized; BSF0: burned with residues scattered and not fertilized; MF0: mulched and not fertilized. Means associated with the same letter are not significantly different ($LSD_{5\%}$ (BRF0: 0-10 cm = 2.96, 10-20 cm = 6.48, BSF0: 0-10 cm = 4.76, 10-20 cm = 8.75., MF0: 0-10 cm = 7.78, 10-20 cm = 7.61)).

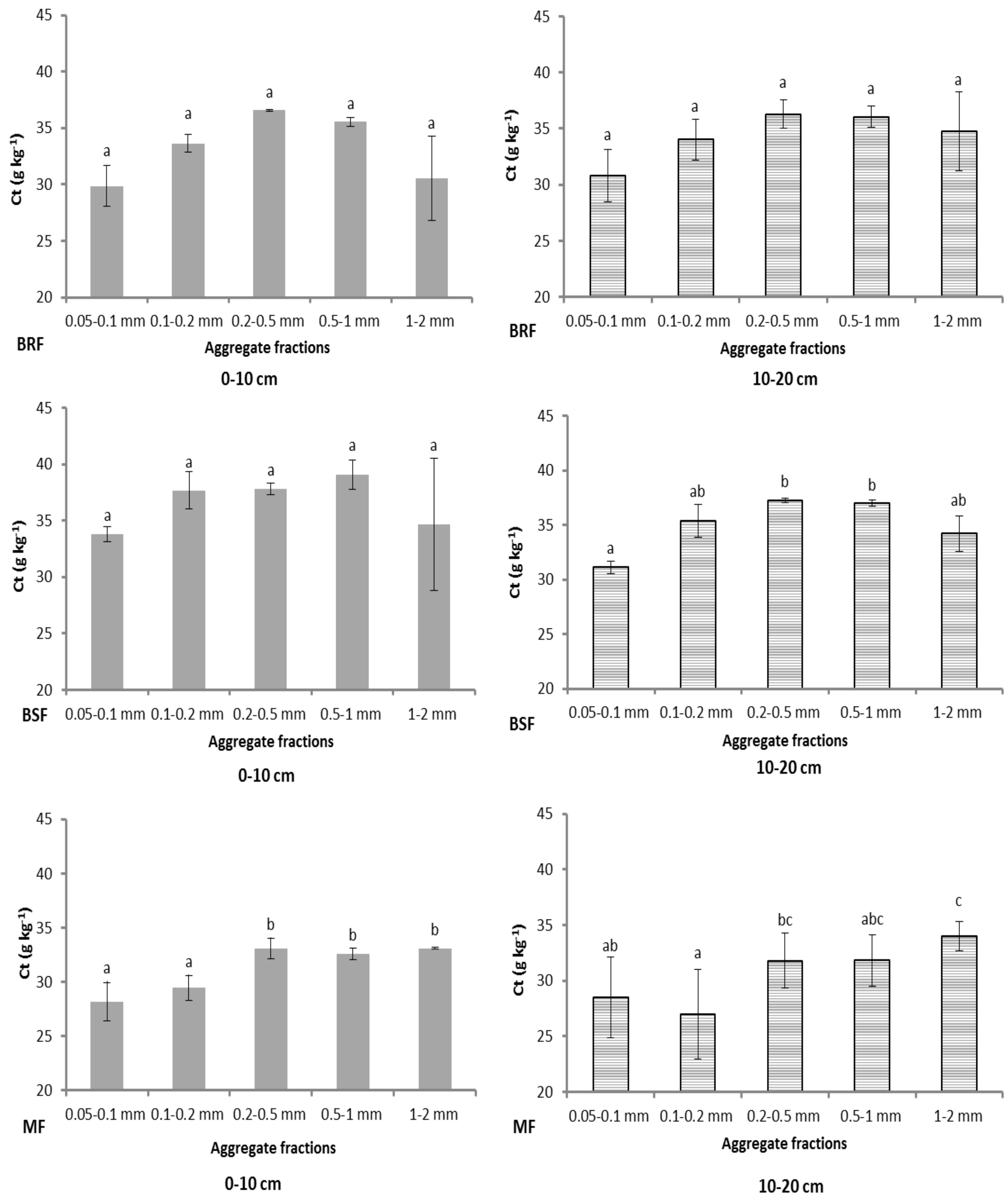


Figure 4.8: The mean (n = 3±standard error) Ct: total carbon in soil aggregate fractions of different sizes at 0-10 cm and 10-20 cm soil depth under different management treatments: BRF: burned with residues removed and fertilized; BSF: burned with residues scattered and fertilized; MF: mulched and fertilized. Means associated with the same letter are not significantly different ($LSD_{5\%}$ (BRF: 0-10 cm =7.63, 10-20 cm = 5.55, BSF: 0-10 cm = 10.93, 10-20 cm = 4.19, MF: 0-10 cm = 2.53, 10-20 cm = 4.57)).

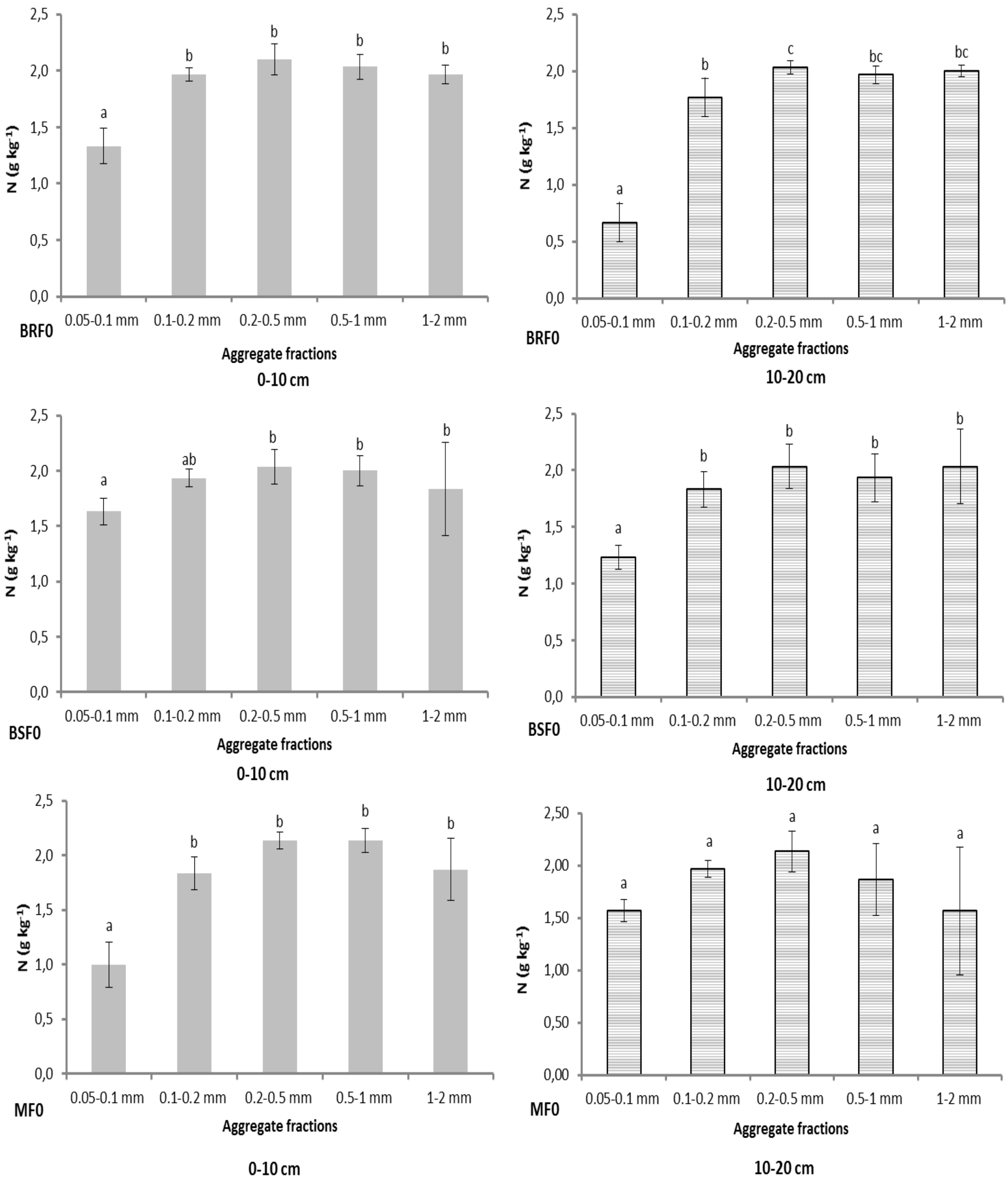


Figure 4.9: The mean (*n* = 3±standard error) N: total nitrogen in soil aggregate fractions of different sizes sampled at 0-10 cm and 10-20 cm soil depth under different management treatments: BRF0: burned with residues removed and not fertilized; BSF0: burned with residues scattered and not fertilized; MF0: mulched and not fertilized. Means associated with the same letter are not significantly different ($LSD_{5\%}$ (BRF0: 0-10 cm = 0.39, 10-20 cm = 0.22, BSF0: 0-10 cm = 4.76, 10-20 cm = 0.32, MF0: 0-10 cm = 0.71, 10-20 cm = 1.00)).

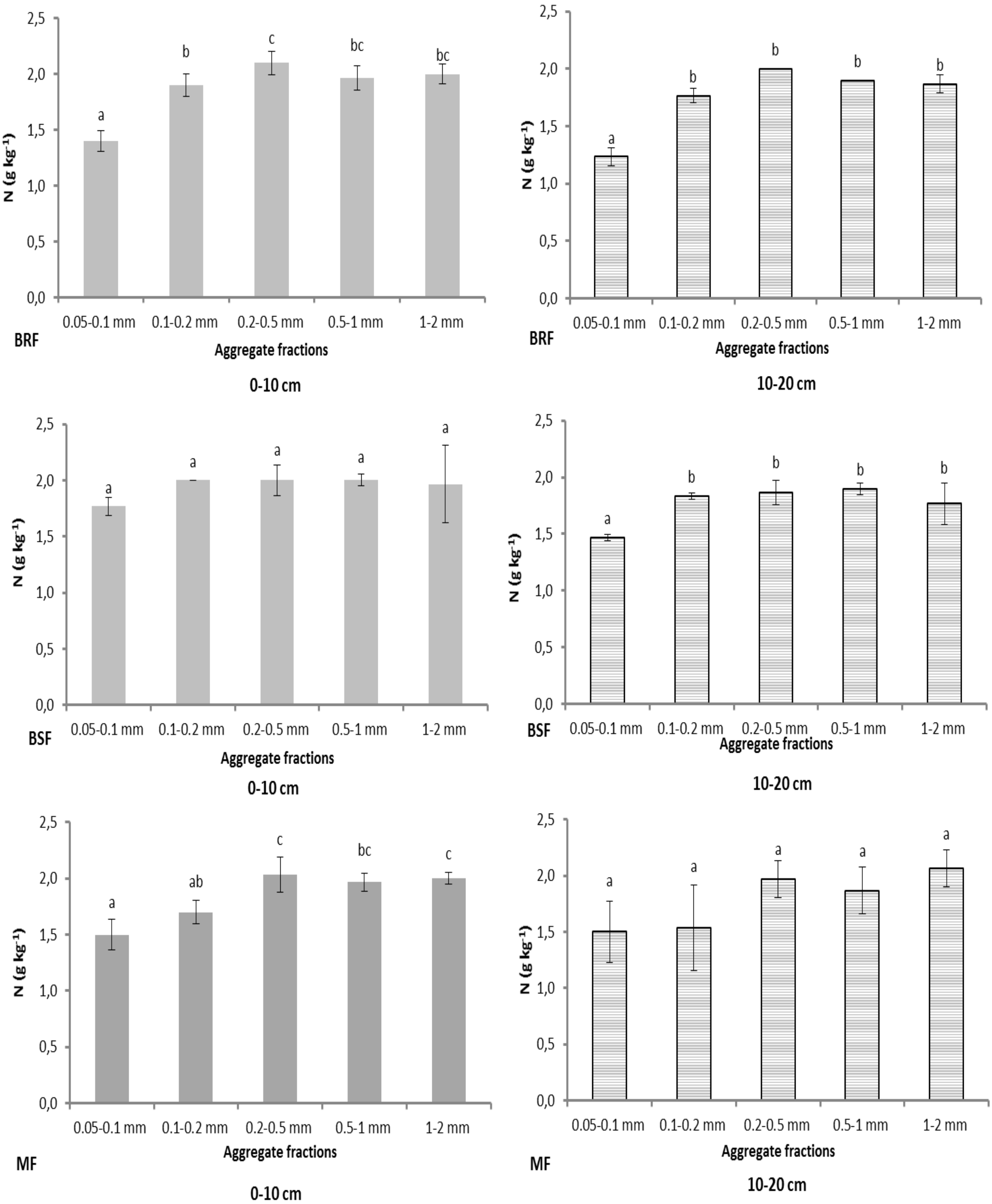


Figure 4.10: The mean (n = 3±standard error) N: total nitrogen in soil aggregate fractions of different sizes sampled at 0-10 cm and 10-20 cm soil depth under different management treatments: BRF: burned with residues removed and fertilized; BSF: burned with residues scattered and fertilized; MF: mulched and fertilized. Means associated with the same letter are not significantly different ($LSD_{5\%}$ (BRF: 0-10 cm = 0.13, 10-20 cm = 0.23, BSF: 0-10 cm = 0.50, 10-20 cm = 0.25, MF: 0-10 cm = 0.28, 10-20 cm = 0.58)).

4.3.3. Exchangeable cations, effective cation exchange capacity and pH

At 0-10 cm, the lowest and highest concentrations of Ca from the fertilized plots were measured in the MF (3.27 $cmol_c\ kg^{-1}$) and BRF treatments (4.32 $cmol_c\ kg^{-1}$), respectively (Fig 4.11a). At 10-20 cm depth, the highest Ca concentration was 6.37 $cmol_c\ kg^{-1}$ in the BSF treatment and the lowest was 4.83 $cmol_c\ kg^{-1}$ in the MF treatment. The concentration of Ca was similar between BR, BS and M treatments within each sampling depth in both fertilized and unfertilized treatments. There was generally a significantly ($p < 0.01$; Appendix 4.12) lower Ca content in the fertilized treatments compared to the unfertilized treatments (Figure 4.11a) in the 0-10 cm soil depth. This trend was, however, not reflected in the 10-20 cm depth across all the treatments and no significant differences ($p > 0.05$; Appendix 4.13) were found. Magnesium was also significantly ($p < 0.01$; Appendix 4.14 and 4.15) higher in unfertilized (3.61 $cmol_c\ kg^{-1}$) treatments compared to the fertilized plots (1.69 $cmol_c\ kg^{-1}$) at both depths and in all treatments (Figure 4.11c and d). Potassium was significantly higher ($p < 0.01$; Appendix 4.16) in fertilized plots (0.77 $cmol_c\ kg^{-1}$) as compared to unfertilized (0.47 $cmol_c\ kg^{-1}$) only under burned treatments at 0-10 cm depth (Figure 4.11e). There was no clear trend between the treatments in terms of K at 10-20 cm and the slight differences measured were not significant ($p > 0.05$; Appendix 4.17) (Figure 4.11f). Sodium was significantly ($p < 0.01$ at 0-10 and 10-20 cm; Appendix 4.18 and 4.19, respectively) higher in BRF0 and BSF0 compared to the rest of the treatments at both depths (Figure 4.11g and h).

The exchangeable acidity was significantly ($p < 0.01$; Appendix 4.20) higher in MF compared to BRF and BSF and also in fertilized compared to the unfertilized treatments at 0-10 cm (Figure 4.12a). At 10-20 cm, the exchangeable acidity was also higher in MF plots in comparison with BRF and BSF though the significant ($p < 0.01$; Appendix 4.21) differences were between fertilized and unfertilized treatments (Figure 4.12b). Similarly to exchangeable acidity, a significantly higher ($p < 0.01$; Appendix 4.22 and 4.24) Al concentration and acid saturation in MF was observed at 0-10 cm (Figure 4.12c and e). In the unfertilized treatments, both soil depths showed no effect of mulching on Al and acid saturation (Figure 4.12d and f; Appendix 4.23 and 4.25).

The pH was about 4.5 in unfertilized plots across BR, BS and M (Figure 4.12g and h). In fertilized plots the soil pH was about 3.5 at 0-10 cm depth and about 4.0 at 10-20 cm depth (Appendix 4.26 and 4.27). The average ECEC was 8.90 $cmol_c\ kg^{-1}$ in the fertilized plots and 11.49 $cmol_c\ kg^{-1}$ in the unfertilized plots, across all BR, BS and M plots at both depths. No significant differences were found between BR, BS and M in either fertilized or unfertilized plots (Figure 4.13a and b; Appendix 4.28 and 4.29).

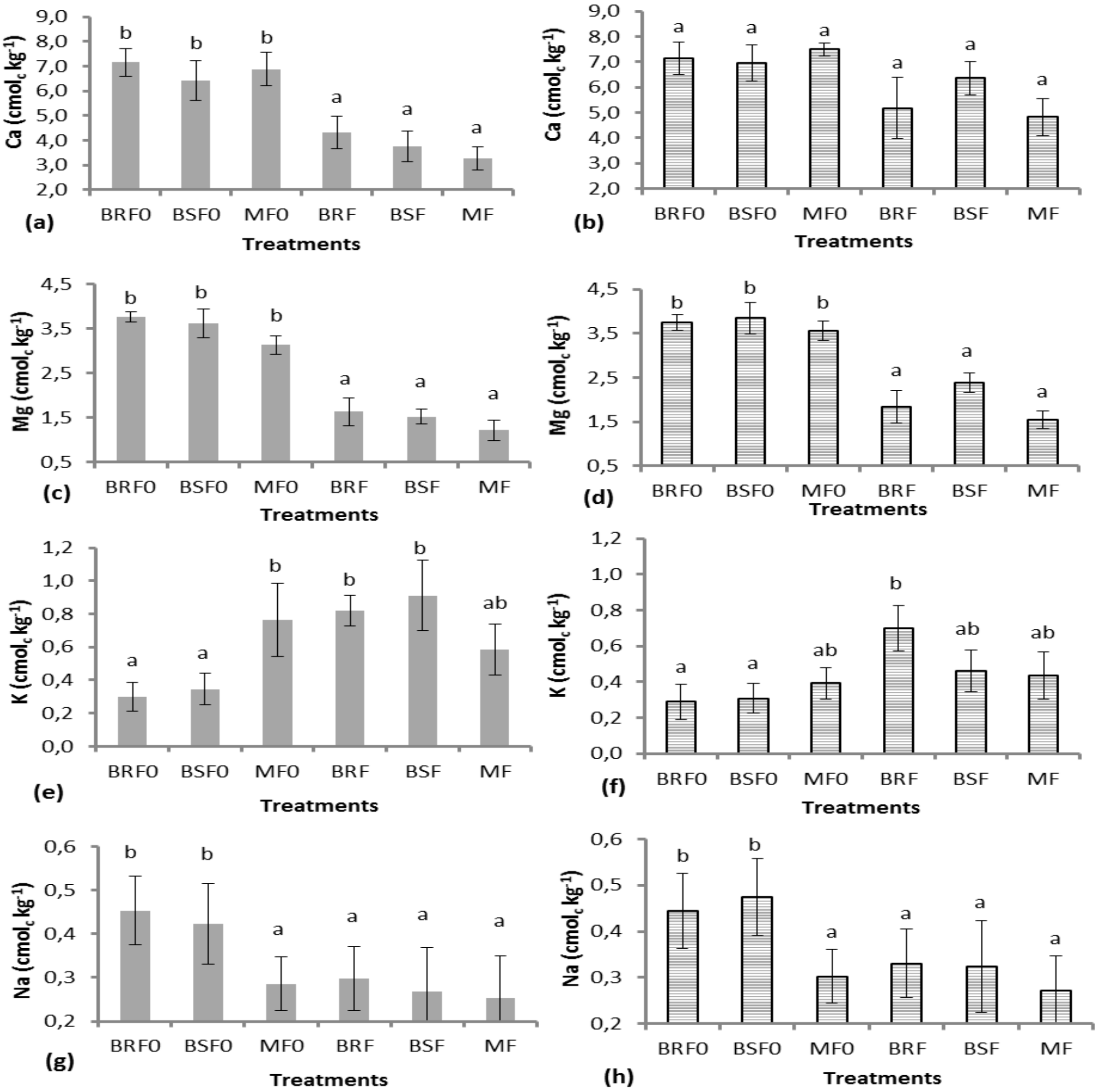


Figure 4.11: The mean (n = 4±standard error) exchangeable bases (Ca: calcium, Mg: magnesium, K: potassium, Na: sodium) measured at 0-10 cm (a, c, e and g) and 10-20 cm (b, d, f and h) soil depth under different management treatments: BRF0: burned with residues removed and not fertilized; BRF: burned with residues removed and fertilized; BSF0: burned with residues scattered and not fertilized; BSF: burned with residues scattered and fertilized; MF0: mulched and not fertilized; MF: mulched and fertilized. Means associated with the same letter are not significantly different ($LSD_{5\%}$ (Ca: 0-10 cm = 1.82, 10-20 cm = 2.55, Mg: 0-10 cm = 0.75, 10-20 cm = 0.90., K: 0-10 cm = 0.35, 10-20 cm = 0.29, Na: 0-10 cm = 0.11, 10-20 cm = 0.11)).

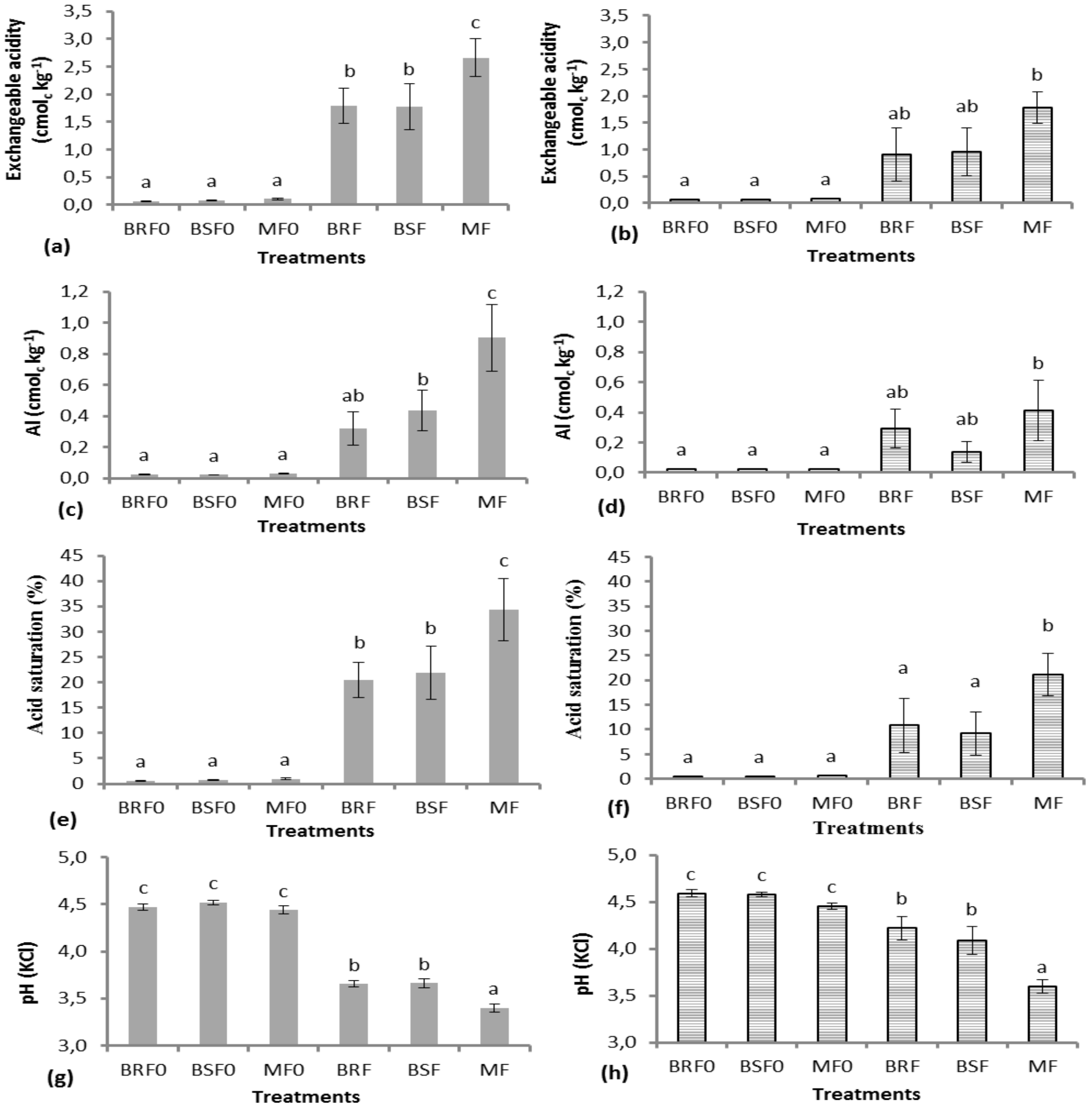


Figure 4.12: The mean (n = 4±standard error) exchangeable acidity, aluminum (Al), acid saturation and pH in potassium chloride (pH(KCl)) measured at 0-10 cm (a, c, e and g) and 10-20 cm (b, d, f and h) soil depth under different management treatments: BRF0: burned with residues removed and not fertilized; BRF: burned with residues removed and fertilized; BSF0: burned with residues scattered and not fertilized; BSF: burned with residues scattered and fertilized; MF0: mulched and not fertilized; MF: mulched and fertilized. Means associated with the same letter are not significantly different (LSD$_{5\%}$ (Exchangeable acidity: 0-10 cm = 0.76, 10-20 cm = 0.87, Aluminum: 0-10 cm = 0.36, 10-20 cm = 0.33, Acid saturation: 0-10 cm = 110.29, 10-20 cm = 10.11, pH(KCl): 0-10 cm = 0.13, 10-20 cm = 0.34)).

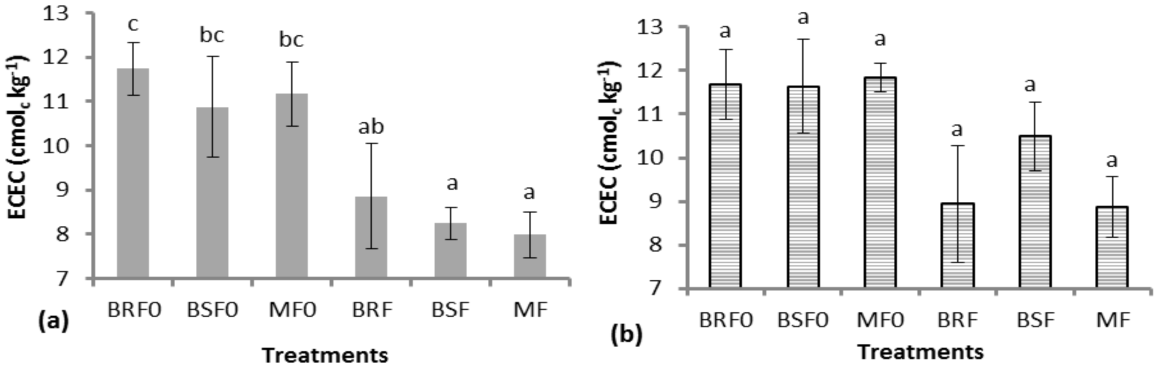


Figure 4.13: The mean (n = 4±standard error) effective cation exchange capacity (ECEC) measured at (a) 0-10 cm and (b) 10-20 cm soil depth under different management treatments: BRF0: burned with residues removed and not fertilized; BRF: burned with residues removed and fertilized; BSF0: burned with residues scattered and not fertilized; BSF: burned with residues scattered and fertilized; MF0: mulched and not fertilized; MF: mulched and fertilized. Means associated with the same letter are not significantly different ($LSD_{5\%}$ (ECEC: 0-10 cm = 2.43, 10-20 cm = 3.05)).

4.3.4 Micronutrients and phosphorus

The average concentration of Zn was significantly higher ($p < 0.01$) under BS and M treatments compared to the BR treatment on both fertilized and unfertilized plots at 0-10 cm (Appendix 4.30 and 4.31). The highest Zn concentration was 3.91 mg kg^{-1} (BSF), followed by 3.50 mg kg^{-1} (MF) and then 2.03 mg kg^{-1} (BRF) at 0-10 cm (Figure 4.14a). At 10-20 cm depth, significantly higher ($p = 0.047$) Zn was observed in the MF treatment (2.89 mg kg^{-1}) compared to all other treatment combinations (Figure 14b). Similarly to Zn, Mn also increased in the mulched (M > BS > BR) and fertilized plots (Figure 4.14c and d). There were generally no significant differences in Mn observed either between fertilized and unfertilized treatments or between burned and mulched treatments within fertilized and fertilized plots at both depths (Figure 4.14c and d; Appendix 4.32 and 4.33). Copper and P also were also higher in the mulched treatments (M > BS > BR). At 0-10 cm depth, Cu was significantly higher ($p < 0.01$) in MF (32.67 mg kg^{-1}) compared to BRF (12.71 mg kg^{-1}) (Appendix 4.34). The concentrations of Cu and P in the unfertilized plots were not significantly different between the burned and mulched plots (Figure 4.12f and h; Appendix 4.35 and 4.37). No significant differences were observed in the unfertilized treatments at both depths (Figure 4.14e and f). In the fertilized treatments, P was significantly higher ($p < 0.01$) under MF (14.85 mg kg^{-1}) compared to BSF (12.81 mg kg^{-1}) and BRF (10.93 mg kg^{-1}) at 0-10 cm (Appendix 4.36).

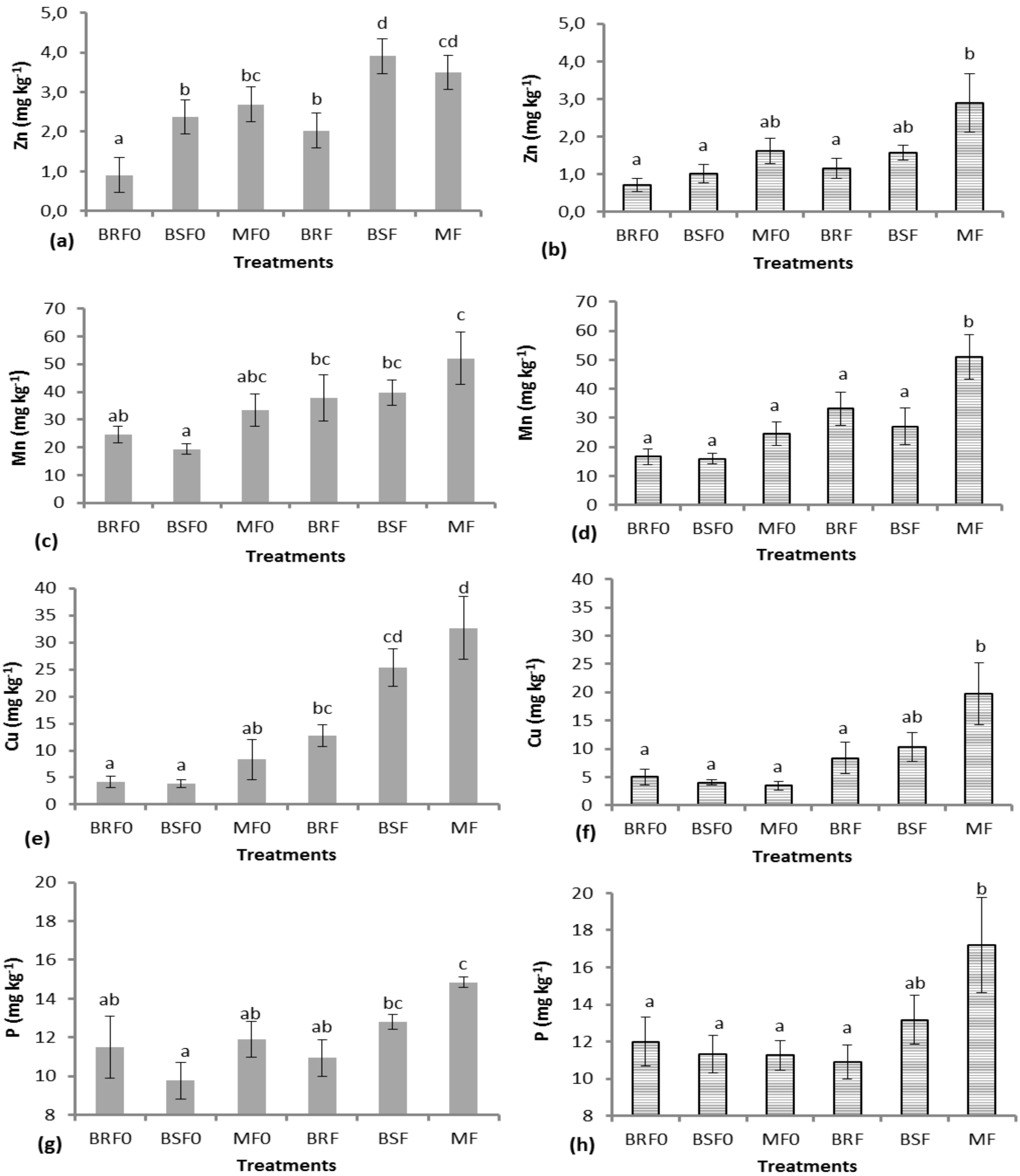


Figure 4.14: The mean (n = 4±standard error) micronutrients (Zn: zinc, Mn: manganese, Cu: copper) and P: phosphorus measured at 0-10 cm (a, c, e and g) and 10-20 cm (b, d, f and h) soil depth under different management treatments: BRF0: burned with residues removed and not fertilized; BRF: burned with residues removed and fertilized; BSF0: burned with residues scattered and not fertilized; BSF: burned with residues scattered and fertilized; MF0: mulched and not fertilized; MF: mulched and fertilized. Means associated with the same letter are not significantly different ($LSD_{5\%}$ (Zn: 0-10 cm = 0.98, 10-20 cm = 1.34, Mn: 0-10 cm = 18.43, 10-20 cm = 19.19, Cu: 0-10 cm = 10.25, 10-20 = 9.40, P: 0-10 cm = 3.42, 10-20 cm = 4.56)).

4.4 Discussion

4.4.1 Particle size distribution and clay mineralogy

The similarity in particle size distribution observed across the area reflects the dominant parent material (dolerite) at the study site on all the plots. According to Bronick and Lal (2005), soil texture is an inherent soil factor and is therefore mainly influenced by parent material rather than land use and management practices. Although the study conducted by Velde and Peck (2002) on the Morrow Experimental plots showed a significant change in clay mineralogy following 30 years of continuous corn production without fertilizer application due to high K extraction, the clay mineralogy at the study site (BT1) remained similar between the treatments despite 72 years of continuous sugarcane production. These contradictory findings could be associated with the soil textures. The BT1 is situated on high clay soils while the site used by Velde and Peck (2002) was on a silt loam soil which is generally less resistant to change compared to a high clay soil. According to van Antwerpen *et al*. (2001) the soils of BT1 showed no response to the applied treatments in the first 18 years of the trial establishment in terms of soil chemical properties and sugarcane yields suggesting that these soils have a high capacity to resist change. In the present study, the differences in clay mineralogy that were found between lower and upper slope could be associated with the influence of topography. Wilson *et al*. (2004) suggest that topographic position influences the allocation of water, translocation of materials and subsequently transformation of minerals. Generally, steeper slopes are dominated by shallow and immature soils due to erosive removals. The studies of Bühmann and Bühmann (1990) and Manassero *et al*. (2008) indicated that immature soils are dominated by clay minerals that have undergone little chemical weathering such as illite and random mixed-layered clays.

Lepidocrocite is generally associated with reducing conditions in the soil, often as a result of waterlogging. During the examination of soil pits, a 5 to 10 cm thick stoneline and signs of wetness were observed at approximately 40 cm depth and an increase in clay content was measured in the subsoil (±65%) compared to the topsoil (±45%) and it is possible that these factors might have caused waterlogging in the investigated soils since they have the potential to impede water movement in the soil. According to Fitzpatrick *et al*. (1985) and Tolpeshta and Sokolova (2013), waterlogging reduces Fe (III) to Fe (II) which is then rapidly oxidized on contact with air leading to the simultaneous precipitation of lepidocrocite. It has been reported that areas associated with lepidocrocite formation are characterized by a relatively high mean annual precipitation of 800-1200 mm (Fitzpatrick *et al*., 1985). The mean rainfall received at the BT1 trial is 950 mm (Section 3.1) which may encourage the formation of lepidocrocite.

4.4.2 Carbon and nitrogen

The lack of significant differences in Ct between the M, BS and BR treatments could be due to the presence of recalcitrant black carbon (Cb) in the BR and BS treatments. The presence of Cb might have also contributed to the generally higher Ct compared to OC observed across all the burnt treatments. The Cb increases with continuous burning of crop residues in agricultural systems (Rumpel, 2008). Generally, Cb can be easily removed by water erosion although it may remain in the soil for long periods during dry (low or no rainfall) seasons. According to Rumpel (2008), under less intense rainfall the Cb may be incorporated into the mineral soil, leading to long-term sequestration of carbon. In the study of Stewart *et al*. (2009), the absence of response in Ct to varying amounts of carbon input, over many years, was associated with carbon saturation.

After comparing the soil carbon status at five sugarcane study sites (Abergowrie (loam), Woodford Island (silty clay), Ayr (sandy loam), Mackay (loam) and Tully (silty clay) in the Australian sugarcane growing regions, Thorburn *et al*. (2012) concluded that changes in carbon in response to sugarcane residues are site specific. Following the observation of a lack of soil response to mulching treatments, Thorburn *et al.* (2012) stated that the decomposition rate of sugarcane residues is relatively slow and as a result their effects only become apparent in the long-term. However, the decomposition rate of sugarcane residues could not be used to explain the lack of differences between the treatments in the present study since the BT1 trial was established more than seven decades ago. Stewart *et al*. (2007) stated that smaller increases in Ct content with increased carbon input could be due to the decreased capacity of a high carbon soil to store further added carbon. In the present study, carbon decreased continuously from the inception of the experiment and reached a quasi-stable equilibrium at about 40 to 50 g C kg^{-1} (Appendix 4.38). The pattern of differences in OC were not clear between the treatments despite seven decades of mulching and/or higher yield resulting from continuous fertilizer application. The slight decrease in OC observed in the late eighties and nineties (Appendix 4.38) confirmed the findings of Stewart *et al*. (2007) who reported that the decrease in OC storage efficiency following a decline in SOM stability could possibly be due to the changes in the types, strengths, and turnover times of organo-mineral interactions with increasing carbon inputs.

Fontaine *et al*. (2004) demonstrated that the supply of fresh carbon may accelerate the decomposition of soil carbon and induce a negative carbon balance. Soil aggregates normally protect the soil carbon and thus limit the increase in soil carbon with increase in carbon inputs (Six *et al*., 2002; Chung *et al*., 2008; Kimetu *et al*., 2009; Stewart *et al*., 2009). The similarities in OC could again be due to the fact that the investigated soil has reached a carbon equilibrium. The dominance of kaolinite and illite in the clay fraction of the soils at the study site might have contributed to the reduction of the soil capacity to store further added carbon, causing the soil to reach a carbon equilibrium. According to Chan (2001), the low surface area of kaolinite and illite limits the ability of the soil to retain carbon. It thus appears that the long-term capacity of soil to store carbon is controlled by inherent soil properties, although management practices (such as residue mulching or burning) may result in a shift in the carbon inputs (Kool *et al*., 2007). Six *et al*. (2002) reported that climate can speed up the reduction of soil capacity to store carbon by accelerating weathering leading to an increased amount of 1:1 clays and Fe oxides. The presence of lepidocrocite (formed from Fe oxides) at the study site confirmed the presence of Fe oxides that are known to be strong flocculants (Duiker *et al*., 2003). Due to their strong flocculating characteristic, Fe oxides can further decrease the available surface for adsorption of OC and thus encourage the equilibration of carbon. In this study, continuous mulching has not resulted in correspondingly higher amounts of carbon storage, supporting this notion. The higher N in the mulched treatments could be a result of the decomposition of SOM and mineralization of organically bound N at 0-10 cm soil depth (Figure 4.6e and f) (Hartemink, 1998; Basanta *et al*., 2003). The low C:N ratio in M treatments (Figure 4.6g and h) reflects a high degree of C mineralization.

When comparing the Ct and N contents in the soil aggregate fractions, the highest Ct and N concentrations were found in the macroaggregate fractions (> 0.2 mm). Sodhi *et al*. (2009) found the greatest Ct concentration in the 2-1 mm aggregate size fractions which then decreased as the aggregates became smaller on a sandy loam soil (Typic Ustipsamment) after the application of compost at Ludhiana, Punjab, India. These findings could be due to the less decomposable SOM associated with macroaggregates and also the direct contribution of SOM to the stability of larger aggregates that result in only carbon and N-rich macroaggregates being able to withstand slaking (Sodhi *et al*., 2009). Similar results were obtained by Bongiovanni and Lobartini (2006) who measured 32.9 g kg^{-1} of Ct in macroaggregates and 23.5 g kg^{-1} in microaggregates from an uncultivated loamy Typic Haplustoll in the central Córdoba in Argentina. Nweke and Nnabude (2014) also observed the lowest Ct and OC in microaggregates from four different soil types from locations in the Nsukka area of south eastern Nigeria. Microaggregates generally have a larger specific surface area, therefore they expose more carbon to mineralization in comparison with macroaggregates.

4.4.3 Exchangeable cations, effective cation exchange capacity and pH

An increase in K and Al and decrease in Ca, Mg, Na, ECEC and pH on the fertilized plots can be associated with nitrogenous fertilizer application and higher organic matter. The reduction in pH could be a result of the combined effect of base cation mining by sugarcane plants (van Antwerpen and Meyer, 2002), leaching and being replaced by Al, increased mineralization of mulch which leads to soil acidification, and oxidization of ammonium to nitrate (Qongqo and van Antwerpen, 2000). A highly significant decrease in pH has been noticed in the experimental plots at Versailles after 80 years of application of different types of nitrogen fertilizers (Paradelo *et al*., 2013). According to Ng Cheong *et al*. (2009), the fertilizer-induced soil acidification process is associated with the release of two hydrogen ions per unit ammonium through the nitrification process. Hartemink (1998) also reported a substantial decrease in exchangeable cations on Vertisols under sugarcane production in Australia. A significantly high accumulation of K in the fertilized plots (BRF and BSF) and MF0 compared to BRF0 and BSF0 reflects the large amounts of K being added as fertilizer and some possibly recycled annually from ash (Graham *et al*., 2002). A long-term study at Rothamsted Research, UK demonstrated that up to 85% of P and 40% of K added as inorganic fertilizer over a period of 100 years had been retained in the soil (Johnston and Poulton, 1992). The organic matter (mulch) mineralization deposits hydrogen ions in the soil which also further increase the soil acidification. The decomposition SOM and release of N might have also contributed to the pH decrease under mulched treatments by adding organic acids (Williams, 1980).

4.4.4 Micronutrients and phosphorus

The increase in soil acidity caused the concentration of micronutrients such Zn, Mn and Cu to increase. Rutkowska *et al*. (2014) reported that the continuous application of nitrogenous fertilizers and high input of organic matter contribute to the reduction of soil pH leading to an enhanced mobility of Cu, Fe, Mn and Zn. These results are in agreement with the findings of Kumar and Balel (2011) who stated that organic matter reduces the precipitation of micronutrients into insoluble forms by supplying the chelating substances that increase their concentration in the soil. Sidhu and Sharma (2010) and Rutkowska *et al*. (2014) also reported that the available micronutrients increased with increases in organic matter. In contrast, Singh *et al*. (2010) reported an increase in Zn and a decrease in Cu and Mn with increasing organic matter additions through the application of farmyard manure. Micronutrients generally show high affinity to organic matter and therefore form stable bonds. The concentration of Zn in the soil increases under the influence of organic matter as it forms labile organic mineral complexes (Rutkowska *et al*., 2014). The higher concentration of P in the M treatments might suggest that P mostly came from the mineralization of organic matter (Sidhu and Sharma, 2010). The organic acids that are released during organic matter decomposition have the potential to compete with phosphate for adsorption to the soil particles, thereby decreasing adsorption sites for P (Li *et al*., 2008; Sidhu and Sharma, 2010; Kumar and Balel, 2011). The dissolved organic matter tends to cause clay particles to repel phosphorus leading to a higher concentration of soluble P in the soil solution (Li *et al*., 2008).

4.5 Conclusions

None of the treatments showed any consistent effect on the clay mineralogy in the present study and long-term fertilizer application has not resulted in a detectable change in the clay mineralogy. Clay mineralogy was mainly influenced by the topography that seemed to have strongly influenced soil type and the depth to the parent material. Although some studies have shown that mulching of sugarcane residues improves the soil chemical properties when compared with burning, this study has found that the impact of mulching may be counteracted by the nitrogenous fertilizer applied, especially at 0-10 cm depth. Carbon (both Ct and OC) was similar across all the treatments while Ca, Mg, pH and MWD were similar between BR, BS and M treatments, but significantly different between fertilized and unfertilized treatments. It was only total N, K, exchangeable acidity, Al, micronutrients (Zn, Cu and Mn) and P that were clearly increased mainly in the MF treatments but this could be due to the fertilizer applied. The data here suggest that the site (both upper and lower slope) has reached its carbon equilibrium for the given climate, site properties and biomass inputs. The concentration of C and N is higher in the macroaggregates than microaggregates suggesting that soil organic matter in the macroaggregates is less decomposable, and as such it contributes to the stability of larger aggregates resulting in only carbon and N-rich macroaggregates being able to withstand slaking. The lower C and N in the microaggregates compared to the macroaggregates is probably also related to the higher surface area of the microaggregates which exposes the carbon to a greater likelihood of decomposition. The annual application of NPK fertilizer also appears to have led to Mg, Ca and ECEC decreasing under long-term sugarcane production regardless of the harvesting method practiced. Increasing additions of organic matter thus do not always correspond to an increase in soil organic carbon and related soil chemical properties. The importance of these properties to soil structure is discussed in Chapter 6.

CHAPTER 5: EFFECT OF SUGARCANE RESIDUE AND FERTILIZER APPLICATION ON SOIL MICROBIAL ABUNDANCE AND COMMUNITY STRUCTURE

5.1 Introduction

In addition to the properties described in Chapter 4, soil microbiology plays a vital role in soil aggregation since soil microbial properties are known to be sensitive indicators of the soil organic matter (SOM) dynamics as they change relatively rapidly with a change in carbon supply (Graham and Haynes, 2005). Generally, soil microbial biomass and activity increase with an increase in SOM. Previous studies have shown that sugarcane crop retention or burning prior to harvesting play a major role in the soil organic carbon (OC) dynamics and the life of microorganisms in the soil (Bronick and Lal, 2005). Graham and Haynes (2006) reported a pronounced soil organic matter loss and a decrease in the size, activity and catabolic diversity of the soil microorganisms resulting from the pre-harvest burning of sugarcane. Most soil microbiological studies conducted under sugarcane in South Africa and other African countries have focused on measurements by non-molecular techniques such as basal respiration, fluorescein diacetate hydrolysis rate, arginine ammonification and phospholipid fatty acids analysis (Graham *et al*., 2002; Haynes and Graham, 2004; Graham and Haynes, 2005; Wallis *et al*., 2010).

Studies using molecular techniques to understand the behaviour of microorganisms under sugarcane are very scarce and Wallis *et al*. (2010) were the first researchers to use these techniques on the BT1 trial. In their study they used polymerase chain reaction-denaturing gradient gel electrophoresis of the 16S rDNA gene to measure the effect of sugarcane management practices on soil microbial community structure. However, their study only included the BRF0, BRF, MF0 and MF treatments causing difficulties in understanding the interactive effect of the different levels of mulching and continuous fertilizer application on soil microbial properties (Wallis *et al*., 2010). This chapter reports the effects of burning or mulching of sugarcane crop residues and fertilizer application on soil bacterial and fungal abundance and community structure with a view to gaining further understanding of aggregate stability in the soils of the study site.

5.2 Materials and methods

The samples used were those collected from the BRF0, BRF, BSF0, BSF, MF0 and MF treatments at 0-10 cm depth. The methods used were described in Chapter 3.

5.3 Results

5.3.1 Bacterial and fungal abundance and ratio and dsDNA amount

The dsDNA amount in the unfertilized treatments increased in the following order: BR (10.4 μg g^{-1}) < BS (12.2 μg g^{-1}) < M (14.4 μg g^{-1}) (Figure 5.1a). However, significant differences ($p < 0.01$) were only obtained between BR and M treatments. In the fertilized plots, the dsDNA amount was similar across all the treatments (BR, BS and M) but significantly ($p < 0.01$) lower compared to the unfertilized treatments (Figure 5.1a). Fertilizer application reduced the dsDNA amount by approximately 65% in comparison with unfertilized treatments. The abundance of bacterial 16S rDNA copy numbers was significantly ($p < 0.01$) increased with M treatment and decreased with BR treatment (Figure 5.1b). However, there were no significant differences ($p > 0.05$) between fertilized and unfertilized treatments. In comparison to BR and BS, the M treatment decreased the abundance of fungi 16S rDNA copy numbers significantly ($p < 0.01$) while fertilizer application resulted in no effect in both burned (BR and BS) and M treatments (Figure 5.1c). Although there was no significant ($p > 0.05$) difference, fertilized plots showed a slight decrease in the fungi to bacteria ratio compared to unfertilized treatments (Figure 5.1d). Moreover, the fungi to bacteria ratio significantly ($p < 0.01$) decreased in M plots compared to BR from 0.014 to 0.002 under F0 treatments and from 0.013 to 0.002 in the F treatments (Figure 5.1d).

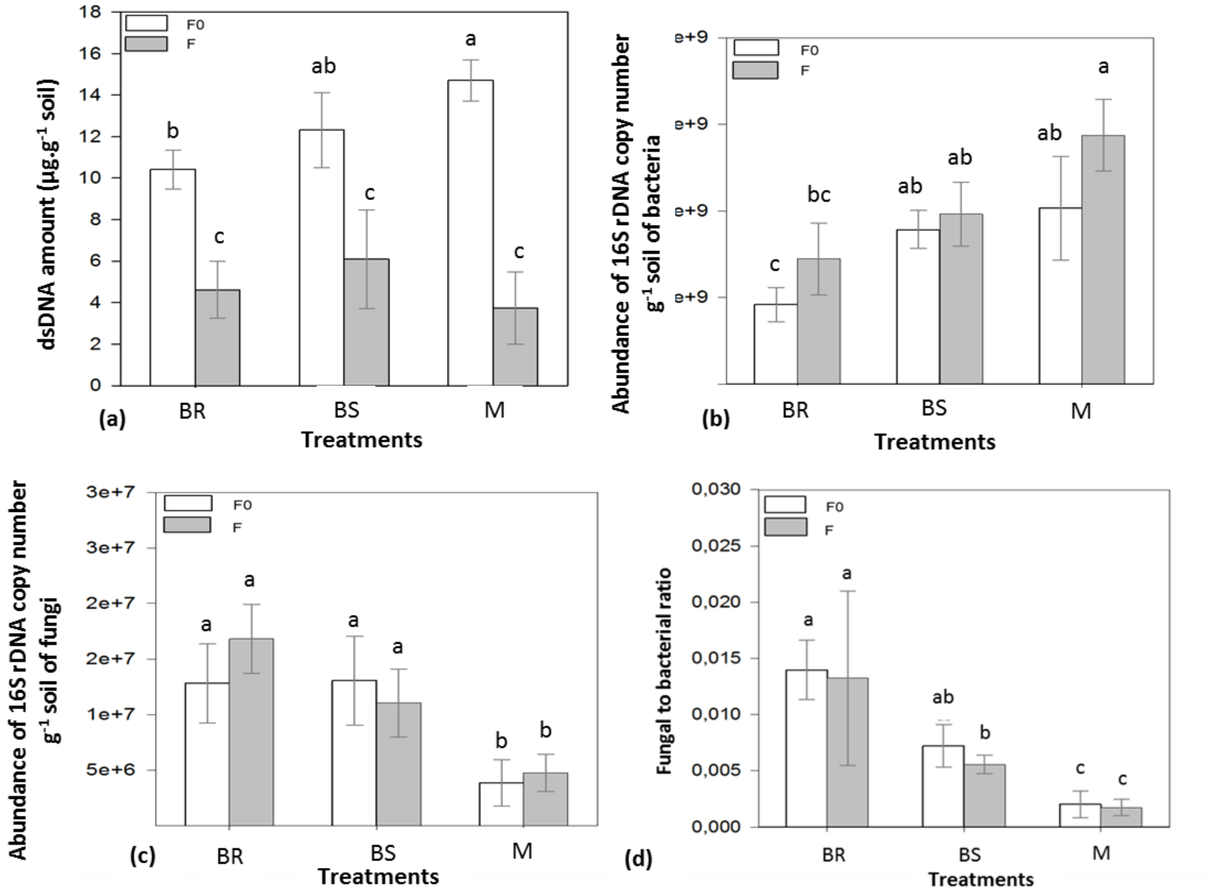


Figure 5.1: The mean (n = 4±standard error) of (a) dsDNA amount, and abundance of 16S rDNA copy numbers of b) bacteria, c) fungi and d) fungal to bacterial ratio at 0-10 cm soil depth under different management treatments: BR: burned with residues removed, BS: burned with residues scattered and M: mulched in both F0: not fertilized and F: fertilized treatments. Means associated with the same letter are not significantly different at p = 0.05.

5.3.2 Bacterial and fungal richness and evenness

Bacterial richness was similar between M and BS treatments in the unfertilized plots but significantly ($p < 0.01$; Appendix 5.1) higher compared to the BR treatment (Figure 5.2a). In the fertilized plots, treatment M was similar to BS and BR such that the significant differences ($p < 0.01$) were only observed between BS and BR in terms of bacterial richness. Although bacterial richness was similar between BS and M, it was slightly lower in the M treatments (Figure 5.2a). The bacterial evenness was similar in BS and M but significantly ($p < 0.01$; Appendix 5.2) lower compared to the BR treatment in both fertilized and unfertilized treatments (Figure 5.2b). Fungal richness significantly ($p = 0.01$) increased in the M compared to BR treatment under unfertilized treatments and showed no clear trend in the fertilized treatments (Figure 5.2c). Fertilizer application significantly ($p < 0.01$; Appendix 5.3) reduced fungal richness in BS and M treatments (Figure 5.2c). Burning and mulching showed no significant ($p = 0.64$ and $p = 0.19$, respectively; Appendix 5.4) effect on fungal evenness though it was generally significantly ($p < 0.01$; Appendix 5.4) decreased by fertilizer application (Figure 5.2d).

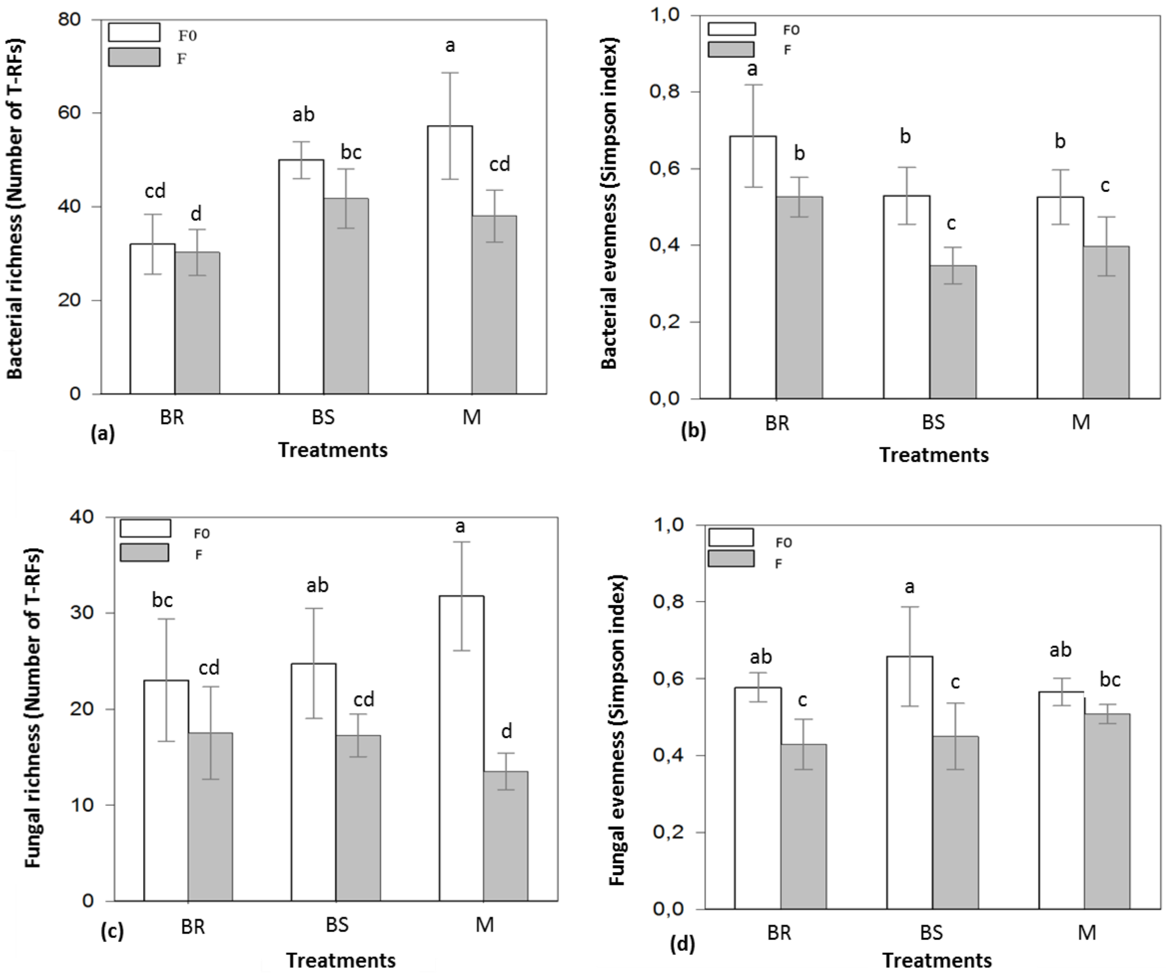


Figure 5.2: The mean ($n = 4$±standard error) of richness and evenness of bacteria and fungi at 0-10 cm soil depth under different management treatments: BR: burned with residues removed, BS: burned with residues scattered and M: mulched in both F0: not fertilized and F: fertilized treatments. Means associated with the same letter are not significantly different at $p = 0.05$.

5.4 Discussion

The increase in dsDNA recorded in the M in comparison with BS and BR treatments in the unfertilized treatments could indicate that the M treatment increased the amount of SOM which serves as the major energy and carbon source for microorganisms (Neumann *et al.*, 2013). The average dsDNA measured in the unfertilized (12.32 µg g^{-1}) plots was substantially higher than that measured by Franciolia *et al*. (2014) in a 30 year old pasture (7.2 μg g^{-1}). According to Wallis *et al*. (2010), the main factor limiting microorganisms under burned sugarcane is probably the shortage of available carbon leading to the growth of communities able to use recalcitrant humic substances. Graham and Haynes (2005) reported that an increase in the size and catabolic diversity of the soil microbial community in mulched plots could be due to higher SOM. The absence of this trend in the fertilized plots suggests that the number of organisms present in the soil was largely influenced by fertilizer application rather than the OC, as all the treatments were similar in terms of OC. These findings could also suggest that mulching during harvesting may be effective in increasing the total number of soil microorganisms under natural conditions (in the absence of inorganic fertilizer application). Fertilizer application reduced the dsDNA by approximately 65% showing that the majority of the microorganisms present in the soil were negatively affected by the increase in acidity that probably resulted from nitrogenous fertilizer application (Jiang *et al*., 2014).

The increase in the abundance of bacteria and decrease in fungal abundance in the M treatment could be attributed to the increase in food source (SOM from mulch) and soil moisture, and the nitrogen content that was significantly higher in the M compared to the BR treatment due to fertilizer application and SOM decomposition. According to Ramirez *et al*. (2012) the majority of bacteria have fast growth rates and rely on more labile carbon sources making them likely to increase in abundance with nutrient additions, while fungi that normally thrive under low nutrient conditions and grow more slowly, would decline. Blankinship *et al*. (2016) stated that fungi prefer drier conditions, therefore the increase in moisture observed in M treatments (Appendix 5.5) might have had an adverse effect on their growth. In contrast, another study that was conducted by Jiang *et al*. (2014) reported a decline in the abundance of bacteria in comparison with fungi following the addition of nitrogen. The lack of a significant effect of fertilizer application on fungal abundance could suggest that the majority of fungi are less sensitive to the change in soil pH compared to bacteria. These results are similar to those obtained by Jiang *et al*. (2014). Graham *et al*. (2001) and van der Wal *et al*. (2013) also reported that fungi are more tolerant to soil acidity compared to bacteria and low soil pH normally favours fungi over bacteria. The M treatment encouraged the dominance of bacteria over fungi in both fertilized and unfertilized treatments (Figure 5.1d). Tardy *et al*. (2015) reported that bacteria generally dominate the initial stages of decomposition while fungi dominate the later stages and this may be the cause of the increase and decrease in bacteria and fungi, respectively, with increase in SOM (M treatment) as shown in Figure 5.1b and c. Fungi are known to be more efficient than bacteria in decomposing recalcitrant OC compounds and the decomposability of the SOM added to the soil is likely to be a vital determinant of the sequential stimulation of bacteria and fungi in the early stages of decomposition (Tardy *et al.*, 2013; van der Wal *et al.*, 2013).

The generally negative relationship between bacterial richness and evenness measured in this study could indicate that where there is a small number of bacterial species present, they accumulate in relatively similar amounts, while where there is a relatively large amount of bacteria, a small number of species dominate (Haynes and Graham, 2004). This could suggest that the lack of microbial diversity results in a small community having a relatively small number of resilient groups and populations (Haynes and Graham, 2004). The richness and evenness of bacteria and fungi were both decreased by the fertilizer application suggesting that the number and diversity of microorganisms are negatively influenced by fertilization. It has been reported that a decline in microbial richness with fertilizer application indicates that soil fertilization results in a less diverse but more specialized soil microbial community due to increased acidity (Tiquia *et al.*, 2002; Wallis *et al.*, 2010).

5.5 Conclusions

All the treatments (BR, BS, M and F) showed a strong influence on the amount and diversity of microorganisms. An increase in total microorganisms (dsDNA) in M treatments showed that all soil microorganisms are associated with higher OC, likely due to the need for OC as a source of energy in order for them to grow. However, the positive effect of mulching may be counteracted by other land management practices such as the application of inorganic fertilizers in the long-term. The soil microbial community is strongly influenced by the decomposition stages of the SOM present. Bacterial abundance and richness was found to be significantly higher in the M plots compared to the BR treatments, whereas the opposite was observed in fungal abundance and richness. These results were attributed to the lower capacity of bacteria to decompose recalcitrant compounds compared to fungi. Although fertilizer application showed a negative effect on total microorganisms, it resulted in an increase in bacterial abundance and had no effect on fungi. The cause of these results is thought to be the increased nitrogen that is needed by bacteria to grow and the low sensitivity of fungi to the change in soil pH caused by the application of inorganic fertilizers. The relationship between these organisms and soil structural stability are investigated in Chapter 6.

CHAPTER 6: EFFECT OF SUGARCANE RESIDUE AND FERTILIZER APPLICATION ON SOIL PHYSICAL PROPERTIES

Note: Some of the results in this Chapter have been published in an article entitled "The effect of 72 years of sugarcane residues and fertilizer management on soil physico-chemical properties" in Agriculture, Ecosystems and Environment (Appendix A).

6.1 Introduction

Soil physical properties such as aggregate stability (AS) are strongly influenced by the chemical and microbiological properties of soil. According to Bronick and Lal (2005), soil AS is mediated by soil organic carbon (OC), exchangeable cations, amount and type of clay and the soil microbial biomass and activity present in the soil. Generally, increasing OC improves the AS and the capacity of aggregates to store carbon and nutrients (Jingi *et al*., 2011). Microorganisms and their products contribute to the formation of stable soil aggregates, which in turn control OC dynamics. High AS is important in decreasing bulk density (ρ_b) and enhancing porosity which result in higher aeration and an improved water regime in the soil. The porosity of the soil can be related to pore connectivity and then to dynamic properties such as saturated hydraulic conductivity (Ks). The presence of unstable soil structural units promotes the dispersion of soil particles and development of soil crusts and surface sealing that hinder water infiltration and decrease Ks (Le Bissonnais, 1996; Kimetu *et al*., 2009). The physical weakening of the soil surface aggregates may be the result of rainfall impact. However, the impact of rainfall on AS depends on the intensity and amount of rainfall. The intensity of rainfall affects the rate and degree of aggregate slaking, and degree of slaking decreases as the initial moisture content increases until saturation is achieved (Diego *et al*., 2006). Rapid wetting of soil by rainfall breaks down the macroaggregates first, since they are less stable than microaggregates, and exposes the protected carbon, facilitating rapid oxidation and attack by microorganisms of these binding agents (Jouquet *et al*., 2004).

The breakdown of soil aggregates may influence penetrometer resistance (PR). The PR and soil water content are interrelated, and both are influenced by the soil texture, AS and development and bulk density (ρ_b) (Otto *et al*., 2011). The main factors affecting PR are texture and soil water content (Rajaram and Erbach, 1999). Research conducted by Rajaram and Erbach (1999) showed that soil moisture content measured at the same time as PR and OC were significantly higher in mulched sugarcane plots compared to burnt plots in at least the upper 20 cm depth. This chapter reports the changes in some AS-related soil physical properties in response to sugarcane residue retention, burning, and also fertilizer application and their relationship with AS in a long-tern field experiment.

6.2 Materials and methods

The samples analyzed and the methods used were given in Chapter 3.

6.3 Results

6.3.1 Soil aggregate stability

Generally, continuous fertilizer application significantly ($p < 0.01$; Appendix 6.1 and Appendix 6.2) decreased the MWD-Wt in comparison with unfertilized treatments (Figure 6.1a and b). The MWD-Wt was significantly lower ($p < 0.01$; Appendix 6.1) in the BSF0 treatment (1.78 mm) compared to BRF0 (2.25 mm) and MF0 (2.52 mm) at the 0-10 cm depth (Figure 6.1a). Although the differences were not significant, the fertilized treatment showed an increase in MWD-Wt in the M treatment i.e., BRF (1.13 mm) < BSF (1.25 mm) < MF (1.54 mm) at 0-10 cm (Figure 6.1a). No significant differences in MWD-Wt at 10-20 cm depth were found between M and burned treatments on either fertilized or unfertilized treatments after the Wt (Figure 6.1b).

At 0-10 cm, the overall pattern of MWD-Et was the same as for MWD-Wt although the magnitude of the MWD was larger, probably reflecting the less aggressive nature of the ethanol treatment (Figure 6.1c). In the unfertilized treatments, the MWD-Et was similar between burned and mulched treatments at both depths but generally significantly (0-10 cm ($p < 0.01$; Appendix 6.3) and ($p < 0.01$; Appendix 6.4)) different between fertilized and unfertilized plots (Figure 6.1c and d). There were no significant ($p > 0.05$; Appendix 6.5 and 6.6) differences at both sampling depths in MWD-SCWEt treatment between mulched and burned treatments and also between fertilized and unfertilized treatments (Figure 6.1e and f). Generally, there was a significantly higher ($p < 0.01$) MWD in the unfertilized compared to the fertilized treatments (Figure 6.1) except for the MWD-SCWEt. The MWD-SCWEt was marginally higher compared to MWD-Wt and MWD-Et in both burned and mulched treatments and also in fertilized and unfertilized treatments (Figure 6.1).

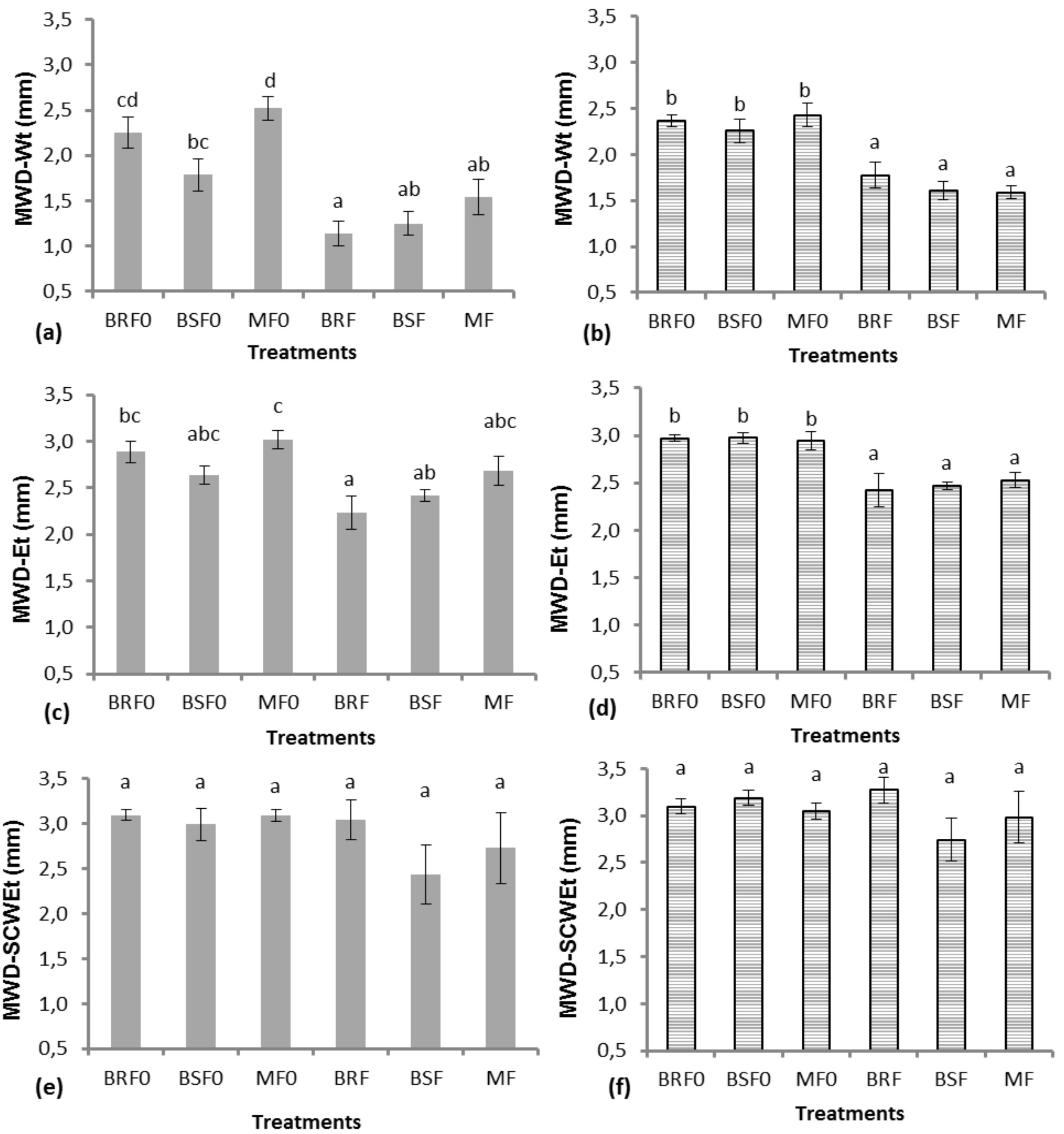


Figure 6.1: The mean (*n* = 4±standard error) for mean weight diameter measured with a) and b) water treatment (MWD-Wt), c) and d) ethanol treatment (MWD-Et) and e) and f) slow capillary wetting ethanol treatment (MWD-SCWEt) in samples from 0-10 (a, c and e) and 10-20 cm (b, d and f) soil depth under different management treatments: BRF0: burned with residues removed and not fertilized; BRF: burned with residues removed and fertilized; BSF0: burned with residues scattered and not fertilized; BSF: burned with residues scattered and fertilized; MF0: mulched and not fertilized; MF: mulched and fertilized. Error bars indicate the standard error, Means associated with the same letter are not significantly different ($LSD_{5\%}$ (MWD-Wt: 0-10 cm = 0.52, 10-20 cm = 0.36, MWD-Et: 0-10 cm = 0.44, 10-20 cm = 0.31, MWD-SCWEt: 0-10 cm = 0.69, 10-20 cm = 0.52)).

6.3.2 Bulk density, saturated hydraulic conductivity and soil water content

The ρ_b was similar across all the treatments ranging from 1.06 to 1.34 g cm^{-3} at both depths as no significant differences ($p > 0.05$; Appendix 6.7 and 6.8) were found, although there was a 0.15 to 0.2 g cm^{-3} decrease in fertilized treatments of BSF and MF treatments relative to their unfertilized equivalents (Figure 6.2a and b). The average Ks was significantly ($p = 0.01$; Appendix 6.9) higher (1.54 cm hr^{-1}) on the unfertilized treatments compared to the fertilized (0.56 cm hr^{-1}) (Figure 6.2c). The Ks trend at 10-20 cm was similar to 0-10 cm with no significant differences ($p > 0.05$; Appendix 6.10) measured (Figure 6.2d).

Generally, available water capacity (AWC) was similar ($p > 0.05$; Appendix 6.11 and 6.12) in all the treatments at both depths (Figure 6.2e and f). The water content at saturation point (0 kPa) was similar in all the treatments (Figure 6.3a). The BS plots showed higher water content at both F0 and F treatments in comparison with other treatments (Figure 6.3a). However, the significant increase in water content recorded in BS and M treatments was only observed in fertilized plots at matric potentials of 8, 10, 100 and 1500 kPa where BSF and MF were similar and significantly higher than BRF (Figure 6.3a; Appendix 6.13). At 10-20 cm depth, BS had higher water content compared to BR and M in both F and F0 treatments. In the unfertilized treatments, BSF0 was similar to BRF0 and significantly different from MF0 (Figure 6.3b; Appendix 6.13).

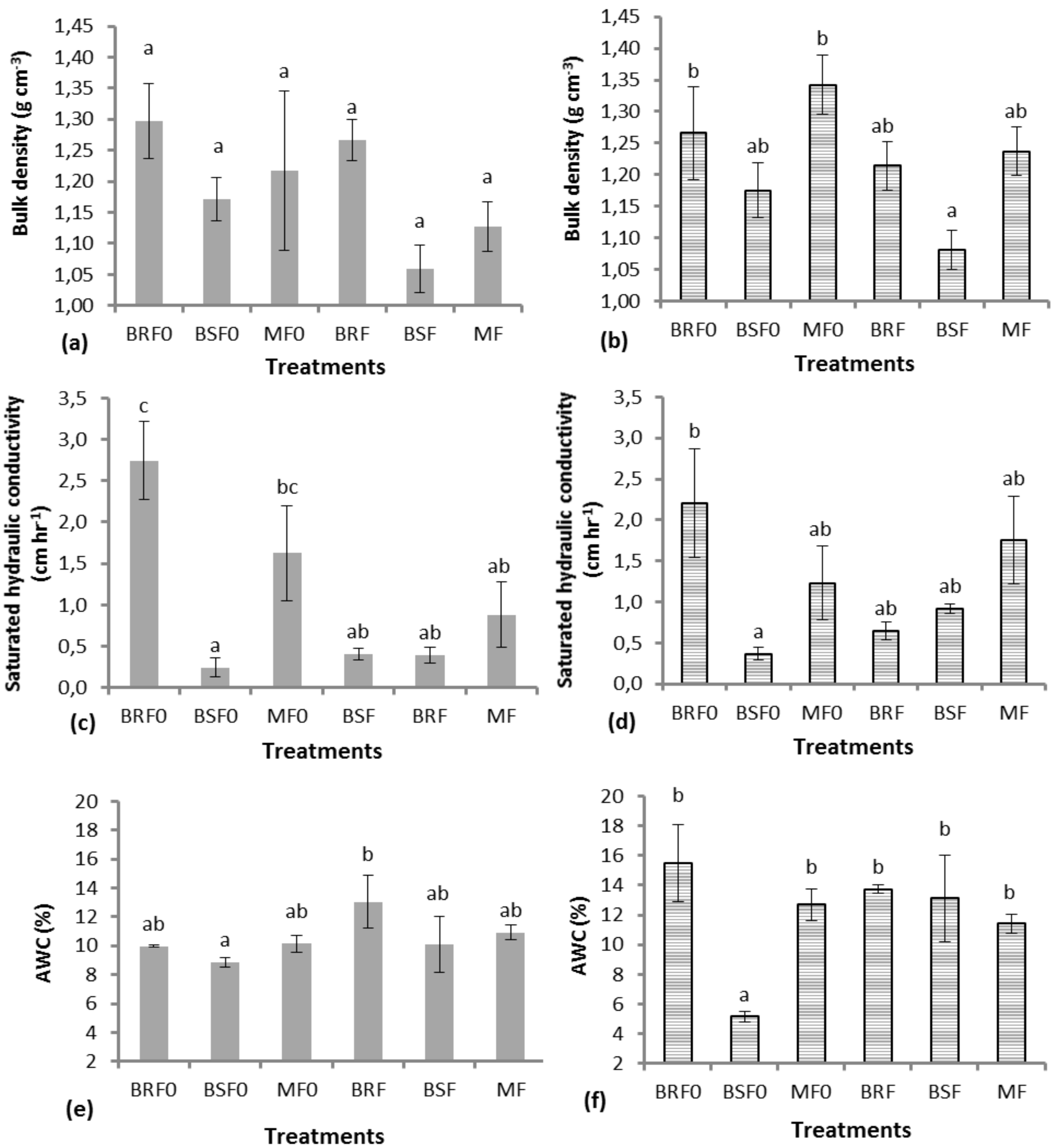


Figure 6.2: The mean (n = 4±standard error) for bulk density (ρ_b), saturated hydraulic conductively (Ks), available water capacity (AWC) measured in samples from 0-10 (a, c and e) and 10-20 cm (b, d and f) soil depth under different management treatments: BRF0: burned with residues removed and not fertilized; BRF: burned with residues removed and fertilized; BSF0: burned with residues scattered and not fertilized; BSF: burned with residues scattered and fertilized; MF0: mulched and not fertilized; MF: mulched and fertilized. Error bars indicate the standard error. Means associated with the same letter are not significantly different ($LSD_{5\%}$ (ρ_b: 0-10 cm = 0.24, 10-20 cm = 0.16, Ks: 0-10 cm = 1.27, 10-20 cm = 1.47, AWC: 0-10 cm = 3.80, 10-20 cm = 6.20)).

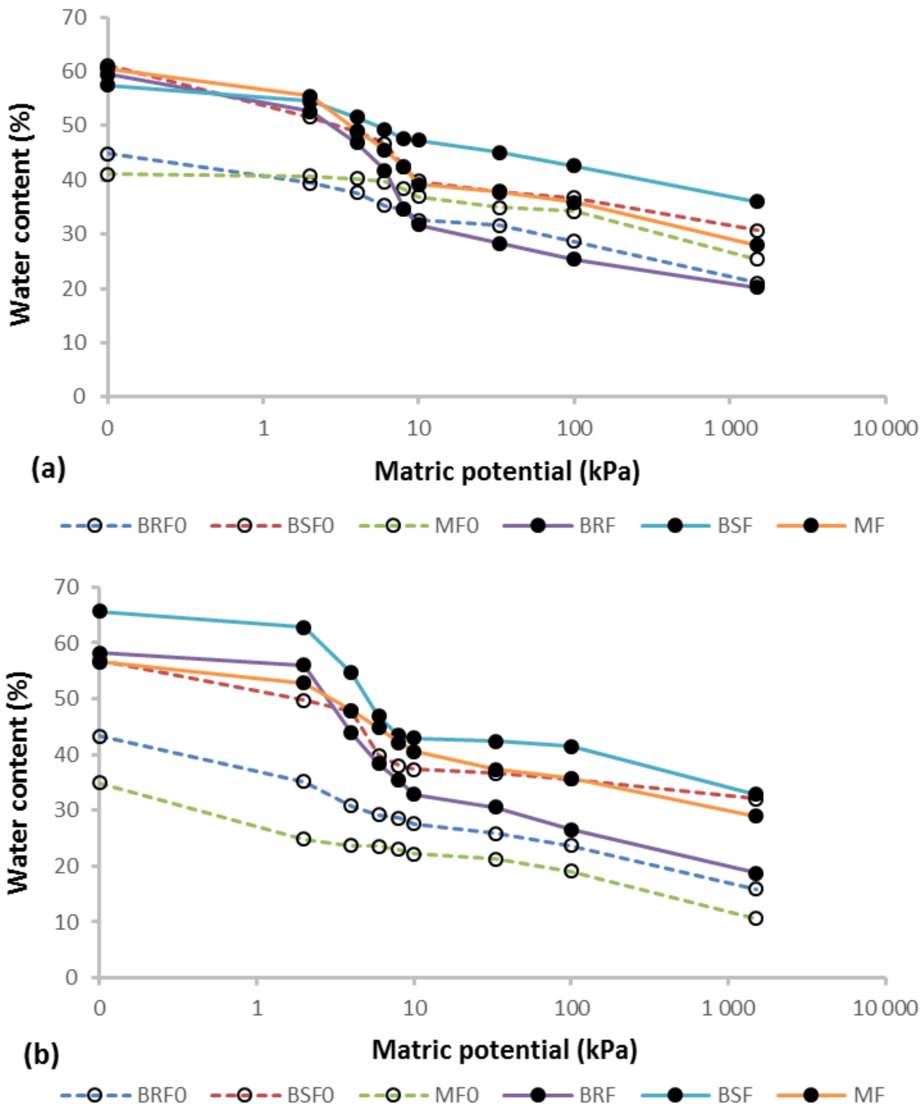


Figure 6.3: The mean ($n = 3$) volumetric water content measured at different matric potentials at (a) 0-10 and (b) 10-20 cm soil depth under different management treatments: BRF0: burned with residues removed and not fertilized; BRF: burned with residues removed and fertilized; BSF0: burned with residues scattered and not fertilized; BSF: burned with residues scattered and fertilized; MF0: mulched and not fertilized; MF: mulched and fertilized.

6.3.3 Penetrometer resistance

Mean penetrometer resistance decreased significantly ($p < 0.01$; Appendix 6.14) from 1 041 kPa (BRF0) to 782 kPa (MF0) in unfertilized treatments and from 955 kPa (BRF) to 770 kPa (MF) in the fertilized treatments at 0-10 cm depth (Figure 6.4a). There was generally no significant difference ($p > 0.05$; Appendix 6.15) between the treatments at 10-20 cm although the MF0 showed a significantly lower PR compared to BRF0 (Figure 6.4b). However, it is also worth noting that PR substantially decreased in the M treatments at 10-20 cm (Figure 6.4b).

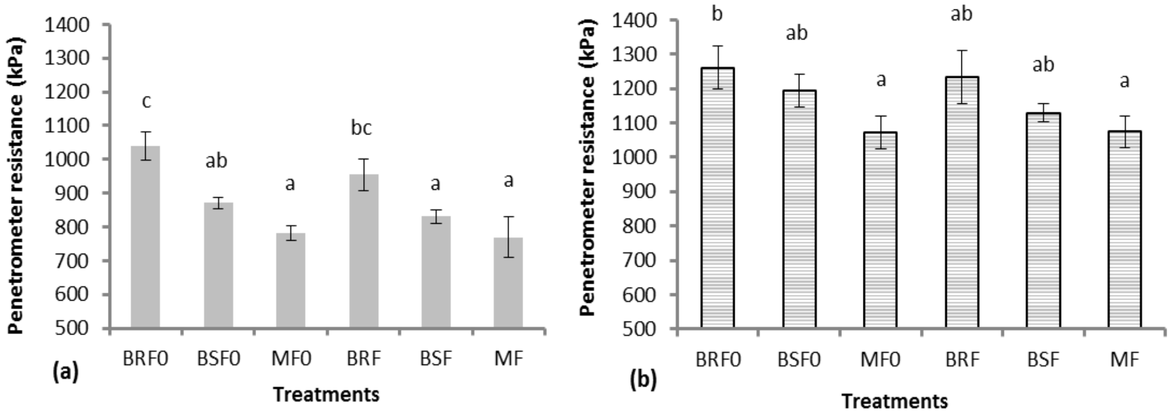
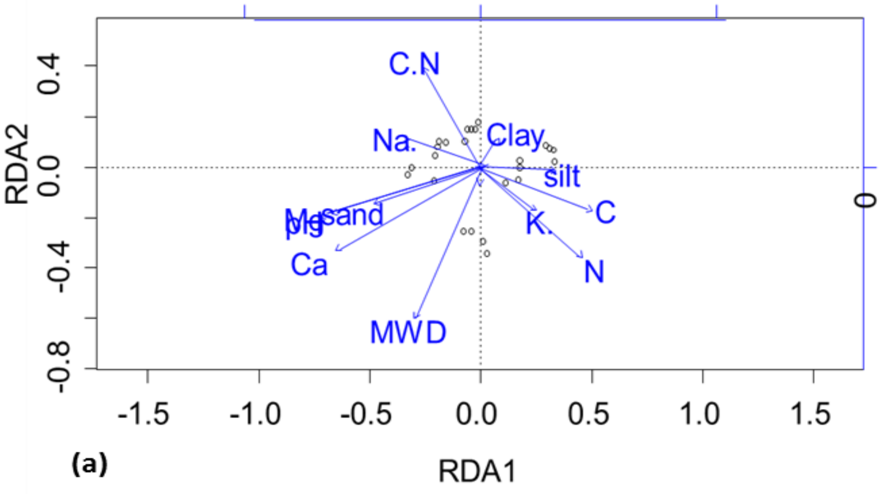


Figure 6.4: The mean (*n* = 4±standard error) penetrometer resistance at (a) 0-10 and (b) 10-20 cm soil depth under different management treatments: BRF0: burned with residues removed and not fertilized; BRF: burned with residues removed and fertilized; BSF0: burned with residues scattered and not fertilized; BSF: burned with residues scattered and fertilized; MF0: mulched and not fertilized; MF: mulched and fertilized. Error bars indicate the standard error, Means associated with the same letter are not significantly different ($LSD_{5\%}$ (0-10 cm = 113.64, 10-20 cm = 167.20)).

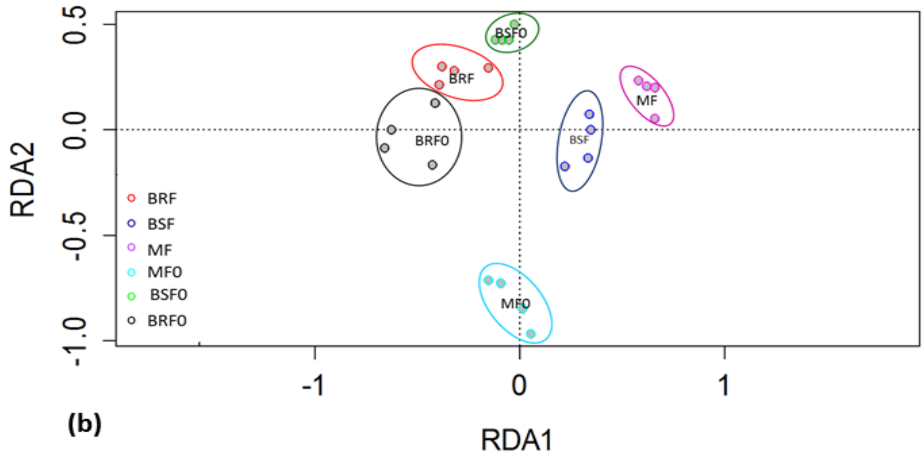
6.4 Discussion

6.4.1 Soil aggregate stability

The MWD-Wt recorded across all the treatments ranged from 1.3 to 2.5 mm suggesting that aggregates of the BT1 soils are stable according to the findings of Le Bissonnais (1996). Generally, there were no significant differences ($p > 0.05$) in MWD between burned and M treatments at both depths. These results are in agreement with those obtained by Torres *et al.* (2013) who also found no significant differences in MWD between burned and mulched sugarcane treatments to 10 cm depth in an Oxisol in Minas Gerais State, Brazil. All the MWD-related soil properties measured in the current study, except for fungal richness, such as clay mineralogy, OC, Ct, particle size distribution and exchangeable bases showed no significant differences between burned and M treatments at both depths and that could account for the similarities in MWD between the treatments. The slight increase in MWD-Wt and MWD-Et in BS and M treatments compared to BR that was observed could be associated with fungi that had a stronger correlation with MWD in comparison with bacteria (Figures 6.5 and 6.6). Increasing additions of crop residues as either BS or M treatment in comparison with BR might have provided a more favourable environment for faster multiplications of different species of fungi leading to an increase in MWD (Figure 6.7a). According to Bronick and Lal (2005), fungi grow as hyphae which increase MWD through the reorientation of clay particles and bridging of soil particles and microaggregates with extracellular polysaccharides. Therefore, factors that affect the amount of fungi such as pH (Figure 6.7b) tend to have an effect on the soil AS (or MWD).



(a)



(b)

Figure 6.5: The redundancy analysis (RDA) showing the effect fungi on mean weight diameter (MWD) in relation to (a) other soil properties and (b) different management treatments: BRF0: burned with residues removed and not fertilized; BRF: burned with residues removed and fertilized; BSF0: burned with residues scattered and not fertilized; BSF: burned with residues scattered and fertilized; MF0: mulched and not fertilized; MF: mulched and fertilized. C.N: carbon to nitrogen ratio, Na: sodium, Ca: calcium, Mg: magnesium, N: nitrogen, C: carbon, K: potassium measured at 0-10 cm soil depth.

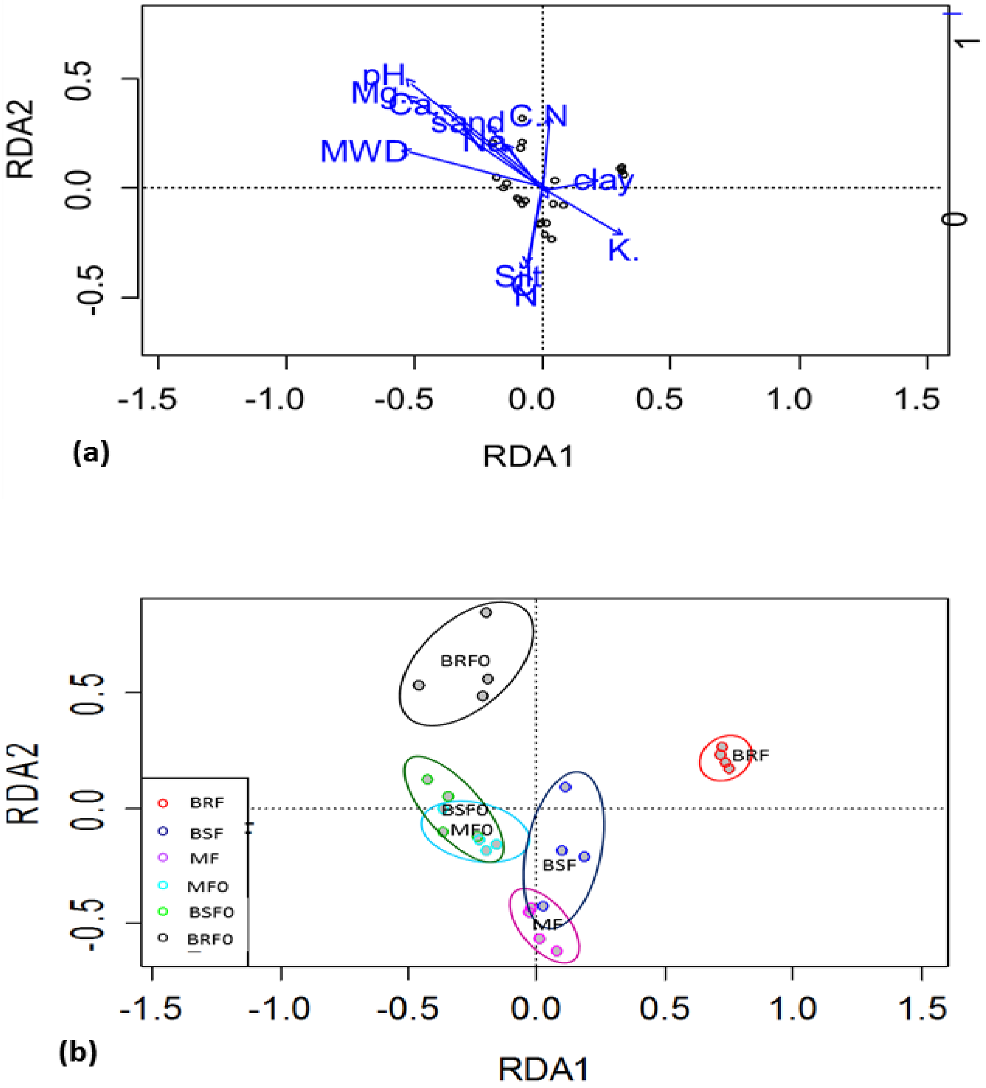


Figure 6.6: The redundancy analysis (RDA) showing the effect bacteria on mean weight diameter (MWD) in relation to (a) other soil properties and (b) different management treatments: BRF0: burned with residues removed and not fertilized; BRF: burned with residues removed and fertilized; BSF0: burned with residues scattered and not fertilized; BSF: burned with residues scattered and fertilized; MF0: mulched and not fertilized; MF: mulched and fertilized. C.N: carbon to nitrogen ratio, Na: sodium, Ca: calcium, Mg: magnesium, N: nitrogen, C: carbon, K: potassium measured at 0-10 cm soil depth.

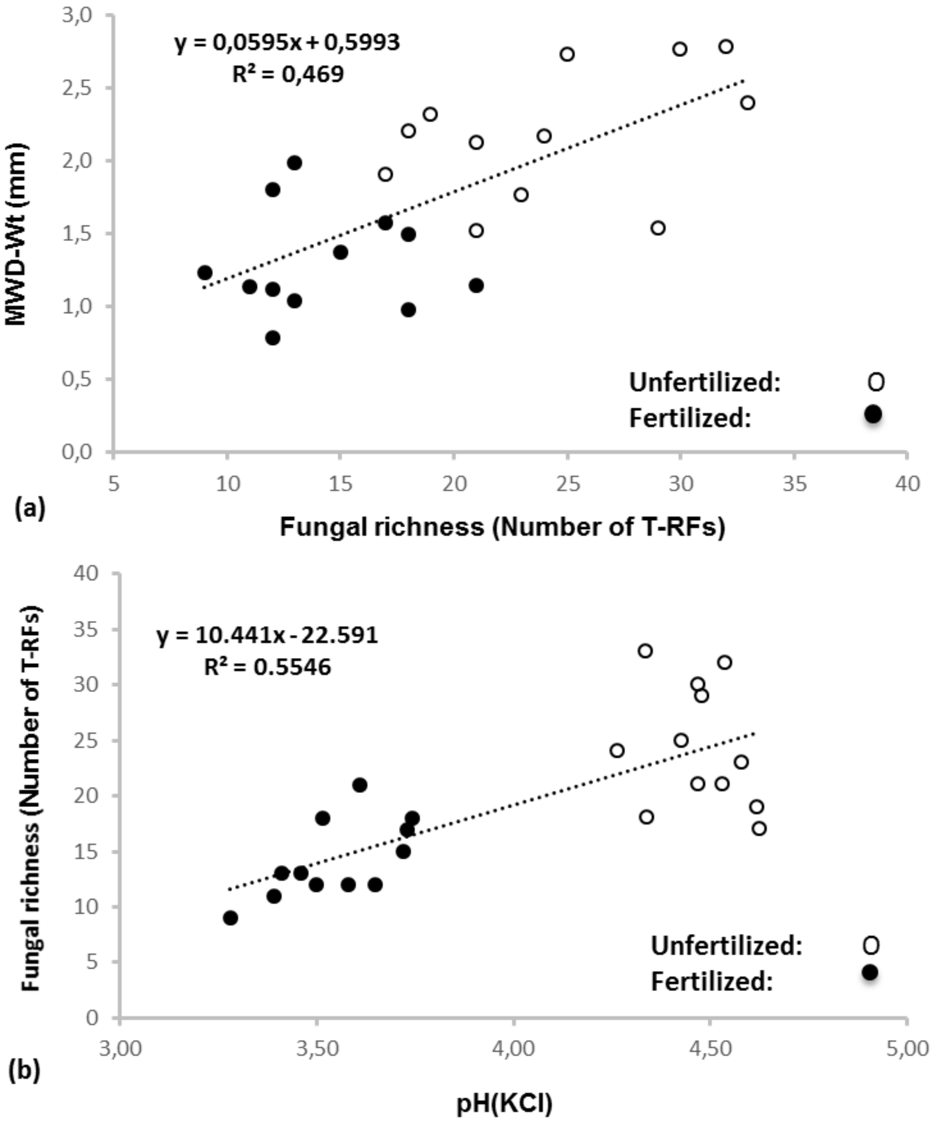


Figure 6.7: The relationship between fungal richness and (a) mean weight diameter measured after the water treatment (MWD-Wt) and (b) pH (KCl) at 0-10 cm soil depth.

The significant and positive relationship ($p < 0.05$, $r^2 = 0.60$) found between fungal richness and pH might confirm that increase of the latter caused an increase in the different species of fungi which possibly resulted in higher MWD (Figure 6.7a and b). The significantly higher MWD measured in unfertilized plots compared to fertilized plots could also be due to the effect of the Ca and Mg concentrations. Positive and significant relationships were found between MWD and exchangeable bases (Ca ($p < 0.01$, $r^2 = 0.70$) and Mg ($p < 0.01$, $r^2 = 0.70$)) and ECEC ($p < 0.01$, $r^2 = 0.60$) across all 24 sampled plots when analyzed as single-variate. The reduction in exchangeable bases was accompanied by a significant increase ($p < 0.01$) in exchangeable acidity and decrease in fungal richness that potentially decreased the MWD especially at 0-10 cm depth (Figures 6.6a and 6.7a). The Monte-Carlo test performed clearly indicated the significant differences ($p < 0.01$) between fertilized and unfertilized treatments in terms of MWD and related soil properties at both 0-10 and 10-20 cm depths (Figure 6.8). According to this test, about 34.1 and 24.5% of the observed variability was controlled by fertilizer application at 0-10 and 10-20 cm depths, respectively (Figure 6.8).

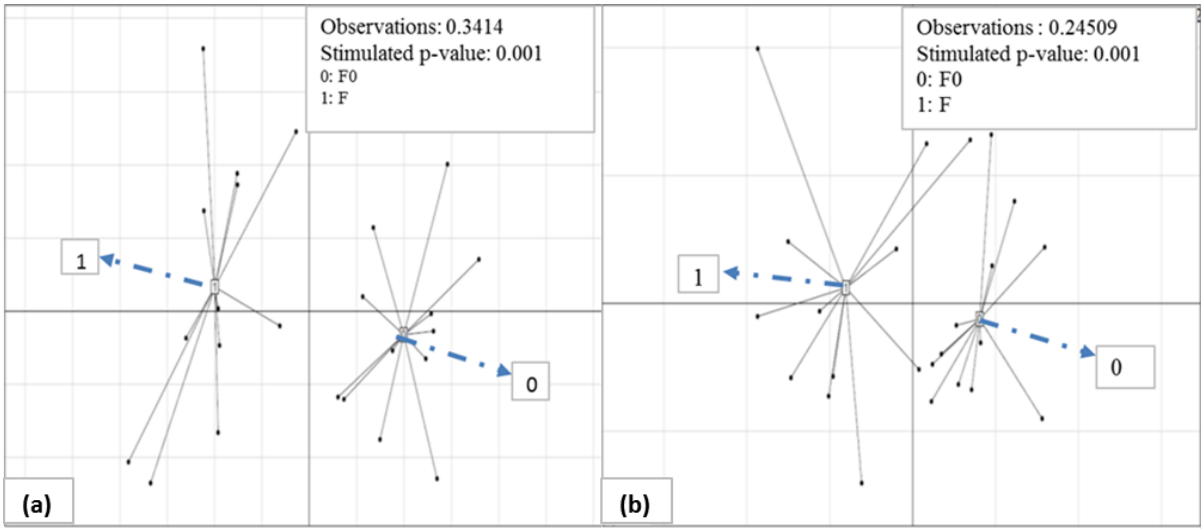


Figure 6.8: The Monte-Carlo test performed to show the distinction between fertilized (F) and unfertilized (F0) treatments at (a) 0-10 cm and (b) 10-20 cm soil depth. 34.1 and 24.5% of the observed variability for 0-10 and 10-20 cm depth, respectively, was controlled by fertilizer application.

The generally strong and weak correlations shown by fertilizer application and mulching, respectively, with the measured soil properties, suggest that the negative effects of fertilizer application were dominant over that of carbon additions (i.e. mulching) when these occurred in combination. The annual application of fertilizer might have encouraged Ca and Mg removal by the crop from 0-10 cm and leaching from 0-10 cm to 10-20 cm by reducing soil pH and increasing exchangeable acidity and K levels which potentially led to less stable aggregates at 0-10 cm (Graham *et al*., 2002). Paradelo *et al*. (2013) found a significant increase in clay dispersivity after 80 years of K fertilizer application in the experimental plots at Versailles. Haynes and Naidu (1998) reported that a high concentration of monovalent ions such as K may favour dispersion of soil colloids leading to deterioration of soil structure. Numerous researchers have found exchangeable cations (Ca and Mg) to be important soil binding agents through cationic bridging with clay particles that improves AS (Cook *et al*., 1992; Bronick and Lal, 2005; Graham and Haynes, 2005; Paradelo e*t al*., 2013). Some of the Ca and Mg that was removed by the crop from the upper layer (0-10 cm) might have possibly been exported to the mill with the sugarcane stalks and not returned to the soil.

The present study also confirmed the work of Le Bissonnais (1996) which reported that the stability of soil aggregates is also determined by the initial soil moisture content when external forces are exerted. The marginally higher MWD of aggregates from SCWEt indicate that pre-wetted soil aggregates are less susceptible to degradation compared to the air-dried soil aggregates regardless of the management treatment. Similar results were obtained by Liu *et al*. (2011) who stated that the slaking of soil aggregates is influenced by wetting rate i.e., the faster the wetting, the stronger the slaking forces and the larger the proportion of aggregates that undergo slaking. The slaking of air dry aggregates is also increased by the mechanical action of water moving within the aggregates (Liu *et al*., 2011). Le Bissonnais (1996) also stated that the slaking of soil aggregates decreases as the initial moisture content increases until saturation due to reduction in the volume of air that is entrapped during wetting and also to the reduction of matric potential gradients.

6.4.2 Bulk density, saturated hydraulic conductivity and soil water content

The higher Ks on the unfertilized compared to the fertilized treatments could be associated with the higher MWD-Wt and MWD-Et that showed a similar trend in these treatments. Continuous fertilizer application increased K and reduced the pH and exchangeable cations (especially Ca and Mg) resulting in the dispersion of soil particles and breakdown of aggregates which in turn decreased Ks. Ma *et al*. (1991) showed that ammonium fertilizer has the potential to cause soil particles to disperse and result in eventual crusting. According to Le Bissonnais (1996), an increase in soil aggregation (or MWD) might provide a better balance between macropores (between aggregates) and micropores (within aggregates) that influence the soil permeability and thus improve the Ks (Table 6.1). In the fertilized treatments, the Ks increased with mulch addition (BRF < BSF < MF) and aggregation suggesting that the rearrangement of soil particles improved water movement in the soil (Ekwe and Stone, 1995; Wuddivira and Camps-Roach, 2007). Generally, the improvement of Ks increases the AWC but there was a poor relationship between these two soil properties in the present study at both depths ($r = 0.13$ at 0-10 cm and $r = 0.44$ at 10-20 cm). Celik *et al*. (2010) reported that soil aggregation generally reduces ρ_b which, in turn, improves water retention and AWC of the soil. However, the absence of a consistent trend in the present study indicated that there was no significant effect of mulch addition under green sugarcane harvesting on ρ_b and AWC in both fertilized and unfertilized treatments. The large variability and unexpected significantly lower values of Ks in BSF0 which followed the MWD-Wt (0-10 cm) trend compared to other treatments (BRF0 and MF0) and the poor correlation between Ks and AWC may be attributed to the presence of colluvial material and cracks (visual observation) which normally lead to preferential flow of water. A higher apparent electrical resistivity was observed in some of the lower slope plots such as 7 (MF0), 8 (MF), 15 (BSF), 16 (BSF0), 23 (BSF0) and 24 (BRF), perhaps due to colluvial material, as shown in Figure 3.2. Yao and Hendrickx (1996) reported that preferential flow occurs predominantly in clayey soil with pronounced structure, such as the Mayo and Bonheim soil forms investigated in the present study.

Table 6.1: The relationship (r) between mean weight diameter (MWD) and available water capacity (AWC), bulk density (BD), saturated hydraulic conductivity (Ks), and penetrometer resistance (PR) measured at (a) 0-10 cm and (b) 10-20 cm soil depth.

(a)	**AWC**	1				
	ρ_b	0.1109	1			
	Ks	-0.1342	0.2700	1		
	MWD	-0.2489	0.0882	0.5024*	1	
	PR	0.0954	-0.1201	0.1619	0.4296	1
		AWC	ρ_b	***Ks***	***MWD***	***PR***
(b)	**AWC**	1				
	ρ_b	0.3050	1			
	Ks	0.4354	0.1507	1		
	MWD	0.0558	0.4056	0.2024	1	
	PR	0.3427	-0.2607	0.3090	0.2036	1

* = significant at $p < 0.05$.

In both fertilized and unfertilized treatments, water retention was generally similar between BS and M but higher compared to BR treatments suggesting that mulching of crop residues has a positive impact on soil water conservation, though the differences were not significant. With the poor correlations between water retention and MWD, it could be speculated that water retention by the mulch itself is a probable cause of the effect of organic matter on water retention, although the organic matter is known to modify the availability of adsorption sites of clay minerals to water (Rawls *et al*., 2003). These results are in agreement with those of Ball-Coelho *et al*. (1993) who reported higher soil water content in the mulch than in postharvest burned treatment. In their study, the increased water retained in mulched treatments was associated with reduced evaporation as well as greater root and fungal activity in the mulch than the burned treatments (Ball-Coelho *et al*., 1993). The absence and presence of significant differences in water retention between low (0 to 6 kPa) and high matric potential (8, 10, 100 and 1500 kPa), respectively, may indicate that though mulching plays a major role in improving soil water retained in the soil, the benefit of having mulch in terms of water retention may not be appreciated in cases where soils are saturated with water, especially in high clay soils. The mulch becomes more important in terms of conservation of water when the soil water decreases (i.e., under drought conditions) as the water decreases faster in the unmulched compared to the mulched treatments as a result of high evaporation (Mendoza *et al*., 2001). The general lack of significant differences between the treatments at both depths and the slight decrease in water retention in M compared with BS in both fertilized and unfertilized plots could be due to the texture (> 40% clay) of the investigated soils (Appendix 6.13). Rawls *et al*. (2003) also found similar results where water retention decreased with increase in organic matter in fine-textured soils with high clay content. The water retention of sandier soils is much more sensitive to changes in organic matter in comparison with clayey soils (Rawls *et al*., 2003). The initial organic carbon percentage of the present study site was about 5.5%, therefore slight changes in water retention even after 72 years of mulching supports the finding of Rawls *et al*. (2003) who found that sensitivity of water retention to changes in organic matter is low in soils with high initial organic carbon. The work of Rawls *et al*. (2003) revealed that additions of organic matter to high clay soils such as Vertisols tend to decrease bulk density and volumetric water content, though gravimetric water content may actually increase.

6.4.3 Penetrometer resistance

The significantly lower PR in M compared to BS and BR treatments in both fertilized and unfertilized treatments could be explained by the effect of organic matter. Generally, the effect of organic matter on PR is through the improvement of soil structure which leads to a decrease in PR (van Antwerpen and Meyer, 1997; van Antwerpen and Meyer, 1998). However, there was a poor relationship between PR and MWD in the present study. A strong correlation was observed between PR and gravimetric water content suggesting that the organic matter maintained higher moisture content which might have resulted in lower PR (Figure 6.9). The study of Ekwe and Stone (1995) found that PR decreases in soils with high organic matter. The presence of mulch resulted in higher soil water storage possibly by lowering losses through evaporation (Filho *et al*., 2014).

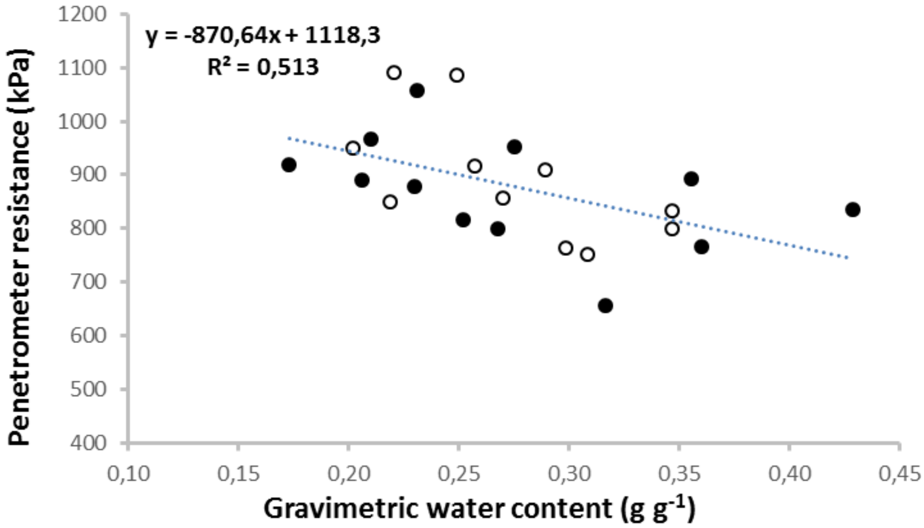


Figure 6.9: The relationship between penetrometer resistance and gravimetric water content measured at 0-10 cm soil depth.

Generally, PR and soil bulk density have a positive and very strong correlation that was not observed in the current study (Table 6.1). The PR showed some significant differences between the treatments, although bulk density was similar across all the treatments, possibly due to an increase in contact points between the clay particles. Similar results were reported by Filho *et al*. (2014) who stated that soil particles are more closely connected in highly cohesive soils and that reduces the chances that the penetrometer rod would find pores that permit a less restricted passage, as its entry into the soil is facilitated when a pore or root channel reduces the friction forces between rod and soil. The burning of crop residues might have continuously exposed the soil to more frequent wetting and drying cycles compared to mulching. More frequent wetting and drying cycles considerably increased the penetration resistance in the study that was conducted by Rajaram and Erbach (1999). The measured PR is considered low (lower than the sugarcane threshold), as overall for the trial at both depths it ranged between 875 and 1 161 kPa (Swinford and Boevey, 1984). Swinford and Boevey (1984) measured a significant decrease in sugarcane root density in their duplex soils with a penetrometer resistance of 2 800 to 3 200 kPa suggesting that this PR limits the growth of sugarcane roots.

6.5 Conclusions

There was a slight improvement in soil AS with increased additions of SOM in the fertilized treatments which was attributed to the positive effect of mostly fungal richness rather than bacteria on the soil structure, though the differences were not significant. The lack of significant effects of increasing SOM on most of the soil factors that generally drive MWD could be the cause of the non-significant difference seen in MWD between the mulched and burned treatments. However, fertilized treatments showed a significantly lower AS compared to the unfertilized treatments that corresponded with a decrease in Ca and Mg, and richness and evenness of both bacteria and fungi. Since the main significant differences in terms of AS i.e., basic cations (Ca and Mg) and soil microbial communities were only observed between fertilized and unfertilized treatments and not between burned and mulched treatments, it is suggested that the beneficial effect of mulching of sugarcane crop residues during harvesting on soil health can be counteracted by long-term fertilizer application. Both Ks and MWD slightly increased with increase in the amount of SOM in the fertilized treatments suggesting that mulch improved these soil properties, though its effect was not significant. The adverse effect of fertilizer application on soil structural stability was evident in the decrease in Ks following the trend of MWD in this treatment compared to the unfertilized treatment. However, MWD showed no correlation with ρ_b, AWC and water retention. The lack of the expected strong relationship between SOM and AS was probably due to the negative effect of fertilizer application. Moreover, AS was also not correlated to PR. Therefore, the decrease in PR under the mulched treatments compared to the burnt treatments was attributed to the increased moisture content which is probably caused by the increase in SOM. It was therefore concluded that (a) long-term use of fertilizer under sugarcane production has a detrimental effect on soil structure and that this has occurred regardless of the harvesting management method practiced, (b) increasing additions of sugarcane residues are not sufficient to lead to improved AS and related soil properties, (c) the AS-driven soil physical properties such as PR and water retention are not always related to soil structure as they may be directly influenced by SOM and (d) changes in water retention with additions of organic matter are influenced by soil texture and initial OC.

CHAPTER 7: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 Introduction

The long-term experimental site (BT1) at which the present study was conducted is a unique source of data providing information on the long-term sustainability of agricultural systems under continuous sugarcane production. This trial has a well-documented history, and as a result it is very important to sugar researchers for (a) the evaluation of new techniques and methods, (b) studying sugarcane mono-cropping and its effect on the surrounding environment and (c) measuring the effects of sugarcane management practices on sugarcane yields and possible dynamic changes in soil properties due to management of mono-culture sugarcane, some of which will only become detectable over extended periods of time. The long-term changes and impacts found in this study do not reflect much of the "text-book" theory about the role of organic carbon (OC) on aggregate stability (AS) and other soil physical properties such as saturated hydraulic conductivity (Ks), penetrometer resistance (PR), available water capacity (AWC) and bulk density (ρ_b). This has implications for management where it is usually argued that long-term mulching will increase OC continuously. Numerous researchers have generalized that continuous additions of organic matter result in a significant increase in OC which significantly improves the soil structure and related soil chemical, physical and microbiological properties (Le Bissonnais, 1996; Graham *et al*., 2002; Bronick and Lal, 2005).

In this investigation, it was hypothesized that:

1) Long-term sugarcane residue retention increases soil carbon and cations thereby improving soil properties such as AS and microbiology.
2) Long-term fertilizer application increases sugarcane biomass production thereby improving soil properties such as AS and microbiology.
3) Long-term fertilizer application increases soil acidification thereby affecting soil properties such as AS and microbiology.
4) Long-term sugarcane residue retention and fertilizer application increases soil carbon, cations and biomass production thereby increasing soil permeability and water retention and decreasing bulk density and soil strength.

The key objectives were thus to:

Compare the impacts of sugarcane burning at harvest against green sugarcane harvesting with residue retention (mulching), with and without fertilizer, on:

1) aggregate stability of different granulometric fractions, and

2) soil mineralogical, and soil microbiological properties, and cation exchange reactions; and to

3) determine the relationship between AS and the physicochemical and biological properties that may drive aggregate formation and stability.

7.2 The relationship between soil aggregate stability and selected inherent soil properties

There were no correlations between AS, soil texture and clay mineralogy found in this study. Soil texture and clay type were similar across all the treatments suggesting that burning and mulching, and fertilization had no effect on the inherent soil properties. According to Kay (1998), soils with high clay content are more resistant to change in mineralogy than those with low clay content and that could be the cause of the only small differences seen between fertilized and unfertilized treatments in the present study. These soil properties (texture and clay type) were mainly influenced by the single parent material (dolerite) that was found at the site, as well as the topography and soil depth. Though all the soils at the trial were dominated by high defect kaolin with lesser amount of vermiculite and trace amounts of lepidocrocite in the clay fraction, the upper slope soils had other clay minerals such as illite, talc and interstratified vermiculite-smectite that were closely associated with the depth to dolerite. The presence of lepidocrocite indicates waterlogging that was suspected due to the observed presence of signs of wetness just above the stoneline at about 40 cm (upper slope) and 50 cm depth (lower slope). Despite its effect on clay mineralogy and apparent electrical resistivity, the change in slope showed little influence on other soil properties measured in this investigation. The soil properties that showed a clearer relationship with AS were the chemical and microbiological properties.

7.3 The effect of soil chemical and microbiological properties on soil aggregate stability

In the absence of a clear relationship between OC, clay type and content and AS as determined by the mean weight diameter (MWD), the latter was, however, strongly related to exchangeable Ca and Mg, pH and fungal richness. The lack of correlation between MWD and carbon, both total (Ct) and OC and the small increase in carbon with relatively large increase in carbon input which suggested that the soil has reached carbon equilibrium. Other studies referred to the decreased capacity of soil to store further added carbon as carbon saturation (Chan, 2001; Six *et al*., 2002; Chung *et al*., 2008). The historical data recorded from BT1 between 1945 and 2012 shows that carbon has reached some quasi-stable equilibrium between 40 and 50 g C kg^{-1} (Appendix 4.38). Thorburn *et al*. (2012) compared the effects of retaining cane residues and burning on Ct at five (Abergowrie, Ayr, Tully, Mackay and Woodford Islands) different (in terms of soil texture and climate) sites and concluded that the response of soil carbon and related properties is site-specific. In their study, the increase in carbon following continuous addition of sugarcane residues at the oldest site, Abergowrie (17 years), was less than that which occurred at Ayr (9 years), Tully (6 years) or Mackay (5 years) (Thorburn *et al*., 2012). However, all these sites investigated by Thorburn *et al*. (2012) were on lower clay content soils in comparison with the soils of BT1 trial. Kay (1998) reported that in coarse-textured soils, carbon has a greater impact on AS, while with increasing clay content the clay type is more important than the amount in determining AS.

The MWD decreased with decline in pH, Ca, Mg and microorganisms (bacteria and fungi). Low pH renders Ca and Mg vulnerable to leaching as they are easily replaced by acid causing cations (H^+ and Al^{3+}) thereby decreasing the AS. Cations such as Ca and Mg form bridges between the clay and soil organic matter (SOM) particles encouraging the coming together of particles; thus loss of cations decreases soil aggregation. Another possible cause of the decrease in MWD at low pH was the decrease in the richness and evenness of bacteria and, especially, fungi. The redundancy analysis conducted showed that the main factor influencing MWD at the investigated site was fungi though some positive relationships between MWD and other properties (Ca, Mg, pH and bacteria) were observed (Figures 6.5 and 6.6). These findings support the notion that fungi play a very important role in soil aggregation through the production of sticky metabolites which contribute to the binding of soil particles and microaggregates to form stable aggregates (Bronick and Lal, 2005). The increase in AS increased the capacity of the soil to store Ct and N. This was evident in the generally higher amount of Ct and N found in the macroaggregates compared to microaggregates indicating that soil aggregation causes the SOM to be less accessible for decomposition and so provides a protective function to withstand slaking.

7.4 The effect of soil aggregate stability on soil physical properties

The MWD showed no clear relationship with ρ_b, AWC, water retention and PR at both soil depths (0-10 and 10-20 cm). The PR and water retention seemed to be influenced by the different amounts of SOM rather than the changes in MWD. The presence of SOM increases water retention and the gravimetric water content stored in the soil by protecting the soil from evaporation (Ball-Coelho *et al*., 1993) and thereby reduces PR. A significant increase in water retention in M and BS treatments that showed no clear relationship with MWD, leads to the conclusion that the water retention of mulch itself is a probable cause of the direct effect of mulch on water retention rather than the modification of MWD which normally improves the amount of water retained in the soil (Rawls *et al*., 2003). Despite the lack of relationship between AWC, water retention, ρ_b, PR and MWD, the decrease in the latter significantly decreased ($p < 0.05$; r = 0.50) the Ks showing that decrease in soil aggregation provided a poor balance between macropores and micropores that influence permeability and therefore reduce hydraulic conductivity. Generally, better balance between macropores and micropores increases AWC and decreases ρ_b and PR. However, these relationships were not observed in the present study.

7.5 Conclusions

The absence of a significant effect of mulch on some of the factors that are considered to have an impact on MWD such ECEC, Ca, Mg, Na, K, Al and C suggested that though retention of plant residues generally improves soil properties, their effect is influenced by other factors. The current study associated the lack of carbon response to the applied treatments with carbon equilibrium. The significant effects of residue retention were only observed on microorganisms especially in the unfertilized treatments. The significant differences between burned and mulched treatments that were observed in terms of microorganisms were between M and BR treatments, BS was generally similar to the M treatment. The overall results showed that mulch has little effect on the physicochemical properties of a Mayo and Bonheim soil form though they had been subjected to these treatments for more than seven decades. The main significant effects measured were between unfertilized and fertilized treatments where fertilizer application significantly decreased the MWD, Ca, Na, Mg, pH and richness and evenness of fungi and bacteria and increased Al, exchangeable acidity, K, N and micronutrients. Therefore, it was concluded that the effect of mulching was counteracted by the nitrogenous fertilizer applied especially at 0-10 cm depth.

This study has thus shown that (a) long-term use of fertilizers under sugarcane production has detrimental effects on soil structure (reduces AS), (b) this occurred regardless of the harvesting management method practiced, and (c) increasing additions of sugarcane residues are not sufficient to lead to improved soil structural stability and related soil properties. However, despite the lack of a general positive relationship between SOM and MWD, the presence of SOM increased the gravimetric water content and water retention and decreased PR. In addition, these results should be balanced against the effect of added fertilizer on production and yield of the crop and protection of the topsoil from erosion by mulching which were not part of the current study. At this site mulching has no clear benefit over burning as the soils have apparently reached equilibrium in terms of OC and, as the site does not undergo either extended periods of fallow or disruptive site preparation, soil degradation is less than might be expected under other forms of intensive agriculture. According to multivariate analysis, the main soil factor that influenced the MWD was fungi (Figures 6.5 and 6.6).

7.6 Recommendations

The lack of response in AS and carbon (Ct and OC) to varying amounts of carbon input, over many years, suggested that the investigated soils have reached equilibrium level. However, the mechanisms responsible for the development of carbon equilibrium levels in the soils of BT1 are still not clear. Therefore, it is recommended that further experiments are conducted to gain a better understanding of the mechanisms responsible for this phenomenon. The long-term carbon data (Appendix 4.38) presented in this study showed that carbon is gradually decreasing with time regardless of the management practices as the soil has reached carbon equilibrium level. Similar carbon (40-50 g kg^{-1}) content measured from different treatments correspond to either recalcitrant or strongly protected carbon in the soil, however, further studies should examine the abundance or pyrogenic carbon of nano to microaggregates occluded organic matter.

The unexpected higher carbon observed at the burnt treatments might have been caused by the hydrophobicity of soil aggregates. Therefore, examining soil aggregates for hydrophobicity may bring some insight about carbon dynamics of the soil investigated in the present study. The somewhat puzzling results found in the present study showing a lack of carbon response to 72 years of continuous addition of sugarcane residue raises the possibility of the further examination of the contribution of the aboveground and belowground biomass which will provide data to use in the calculation of cumulated carbon inputs from the inception of the experiment. Continuous measurements of the different types of carbon may provide an understanding of carbon dynamics that might explain the lack of significant response of AS and physicochemical properties to continuous carbon addition observed in the present study. It would also be useful to measure carbon concentration and stocks as well as AS, water retention, ρ_b, number of roots, Ks and microbiological properties at 10, 20, 30, 40 and 50 cm since deep roots might have an influence on some of these soil properties (Virto *et al*., 2012).

The significant decrease in AS in the fertilized treatments was associated with leaching from 10 to 20 cm and extraction of exchangeable cations (Ca and Mg) by the sugarcane. In the future, measuring the concentration of these cations from both soil and the whole fully-grown sugarcane stalk and leaves as well as the determination of nutrient mass balance might provide a better understanding of the removals and additions of cations in the soil. In addition, the concentration of cations could be measured at more depth intervals between 0 and 50 cm (i.e., 10, 20, 30, 40 and 50 cm) to investigate leaching and their role in aggregate stability. Although this study found that fungi were the main soil factor influencing the AS of BT1 soils, measuring the different types of fungi present in the different treatments and their relationship with other soil attributes will provide a better understanding of the AS dynamics of Bonheim and Mayo soils under continuous sugarcane production.

The present study concluded that mulching has no clear positive effect on AS and related soil properties in comparison with burning since the soil has reached equilibrium in terms of OC. It is recommended that future studies include an examination of production and yields of sugarcane to evaluate a) nitrogen uptake by the crop from decomposing mulch, b) potential reduction in nitrogen fertilizer application in mulched treatments, and c) protection of the topsoil from erosion by mulching to balance the findings of this study.

The soil assessment in the present study revealed that the experimental site is divided into two distinct sections, the upper slope (which takes up the northeast section) and the lower slope (which takes up the rest of the area), with some differences in terms of soil type, clay mineralogy and apparent electrical conductivity. It is therefore recommended that the differences between the treatments are assessed for each soil type separately and that different statistical methods are explored to find one that best suits this trial arrangement in future studies.

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APPENDICES:

Appendix A: Some of the results of Chapters 4 and 6 of this thesis have been published in an article entitled "The effect of 72 years of sugarcane residues and fertilizer management on soil physico-chemical properties" in Agriculture, Ecosystems and Environment 225, 54-61.

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The effect of 72 years of sugarcane residues and fertilizer management on soil physico-chemical properties

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ABSTRACT

This study, carried-out in KwaZulu-Natal, South Africa, investigated changes in selected soil properties and their effect on aggregation induced by 72 years of residue burning or mulching, with and without fertilizer application on a sugarcane trial arranged in a split-plot design with four replications. The main plot treatments were a) green cane harvesting with all residues mulched, b) cane burnt prior to harvest with cane-tops left scattered evenly over the plots and c) cane burnt prior to harvest with all the residues removed from the plots. Split-plot treatments consisted of fertilized and unfertilized plots. Soil samples for physico-chemical and aggregate stability analysis were collected at depths of 0–10 and 10–20 cm from 24 plots. In comparison with burning, significant effects of mulching were only observed on total nitrogen, exchangeable potassium, sodium and acidity and extractable aluminium, mainly at 0–10 cm. Aggregate stability estimated by mean weight diameter (MWD), exchangeable cations (especially calcium and magnesium), aluminium, exchangeable acidity and pH were significantly affected by fertilizer application. An increase in acidity and a decrease in MWD and exchangeable calcium and magnesium on fertilized plots were attributed to mining of nutrients by sugarcane, nitrification and subsequent base cation leaching. The significant positive correlation between calcium and magnesium and MWD, and the lack of correlation between organic carbon (OC) and MWD, indicated that bases contributed more to soil aggregation than OC. Total carbon and OC showed no differences across all treatments. It was concluded that (i) annual fertilizer applications may lead to soil structure deterioration under sugarcane regardless of the harvesting method practiced and (ii) increasing additions of organic matter (through mulching) do not always correspond to an improvement of soil aggregate stability and related soil properties.

1. Introduction

Sugarcane (*Saccharum officinarum* L.) is grown commercially in over 90 countries and nearly 20 million ha are harvested annually, with Brazil, India and China the main producers (Galdos et al., 2009). Sugarcane production is of major agricultural importance in South Africa, where much of the cane land has been converted from indigenous forest and grassland. Traditionally, sugarcane is burned prior to harvest in order to remove leafy non-sucrose containing biomass, but this can be detrimental to soil aggregate stability and nutrient availability due to the loss of organic matter and nutrients through particulate dispersal or volatilisation (Blair, 2000; Cerri et al., 2011; Wiedenfeld, 2009). In the South African sugar industry, the loss of soil organic matter (SOM) and the resulting reduced microbial activity under sugarcane subjected to pre-harvest burning are considered to be major factors contributing to soil aggregate destabilization (Graham and Haynes, 2005). Concerns have been raised regarding the loss of SOM occurring under continuous mono-culture sugarcane production (Dominy et al., 2001; Graham and Haynes, 2005; Kimetu et al., 2009; Paradelo et al., 2013; van Antwerpen and Meyer, 1996). For

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instance, van Antwerpen and Meyer (1996) reported a decrease in SOM, from 2.40% to 1.88%, and aggregate stability in the 0–150 mm layer in irrigated land under sugarcane compared to nearby non-irrigated, virgin grassland in northern KwaZulu-Natal, South Africa. Similarly, reductions of SOM induced by the change in land use from grassland to sugarcane were observed in Queensland, Australia (Bell et al., 2007; Blair, 2000; Wood, 1985). Numerous studies have associated the reduction in SOM with a decrease in soil aggregate stability (Barthes and Roose, 2002; Boix-Fayos et al., 2001; Le Bissonnais, 1996). Silva et al. (2007) reported such a relationship under long-term sugarcane production.

An alternative to pre-harvest burning is green cane harvesting, whereby leafy and non-sucrose containing biomass is retained on the soil surface as a mulch, potentially increasing SOM and nutrient content (Graham and Haynes, 2005). Numerous researchers have found that green cane (unburnt) harvesting and the retention of crop residues as a mulch can improve SOM content when compared to traditional burnt cane harvesting practices (Ball-Coelho et al., 1993; Blair, 2000; Graham et al., 2002; Torres et al., 2013; Vallis et al., 1996). According to Thorburn et al. (2012), mature sugarcane crops consist of a large amount of residues (13–20 t DM ha^{-1}) at harvest that contain about 42% carbon that can potentially be returned back to the soil under green cane harvesting.

However, little is known about the long-term consequences of fertilizer application and green cane harvesting (mulching), and associated changes in SOM on soil aggregation and structural stability under continuous sugarcane cultivation. A sugarcane management trial that was established in 1939 on the eastern seaboard of South Africa offered an ideal and rare opportunity to study the comparative long-term effect of conservation practices (no tillage and mulch residues) against continuous residue burning. Furthermore the long-term effects of mineral fertilizer inputs against no fertilizer application on soil physico-chemical properties were also assessed.

2. Materials and methods

2.1. Experimental site

The experimental site (BT1) is situated at the South African Sugarcane Research Institute (SASRI) at Mount Edgecombe near Durban, KwaZulu-Natal, South Africa (31°04′20″E, 29°04′20″S). It was established on the 25th October 1939 and is the oldest long-term, continuously monitored sugarcane production and soil management trial in the world (Graham et al., 2002). The climate of the region is humid subtropical and is characterized by summer (October to March) rainfall. The average annual precipitation is 950 mm, and the average annual temperature is 20.4 °C (Graham et al., 2002). The site is located on a south-west facing slope (13.5% and 18.5% at upper slope and lower slope, respectively). Control soil pits were dug in the east side of the trial. On the upper slope, the soil was classified as a Mollic Cambisol (IUSS Working Group WRB, 2014), locally known as Mayo form (Glenecho family) (Soil Classification Working Group, 1991), with a dark (2.5 YR 3/1 to 3/2) 50 cm thick A horizon extending to a dark reddish brown (2.5 YR 3/3 to 3/4) AC transitional horizon overlying weathered dolerite. The profile contained a 5–10 cm thick stoneline at about 50 cm depth. On the lower slope, the soil was a Mollic Nitisol (deeper than the Mollic Cambisol found on the upper slope) (IUSS Working Group WRB, 2014), locally known as Bonheim form (Rockvale family) (Soil Classification Working Group, 1991), with the same A horizon as on the upper slope overlying a red (2.5 YR 3/4 to 10R 3/6) B horizon. The $<2\,\mu m$ clay fraction of both soil types consists of mostly kaolinite, with a lesser amount of vermiculite and small amounts of lepidocrocite, and interstratified vermiculite-smectite and illite-vermiculite.

2.2. Experimental design

The complete trial is about 7200 m^2 (90 m × 80 m) composed of 32 plots (Fig. 1). Each plot of 175 m^2 is 18 m long and sugarcane is

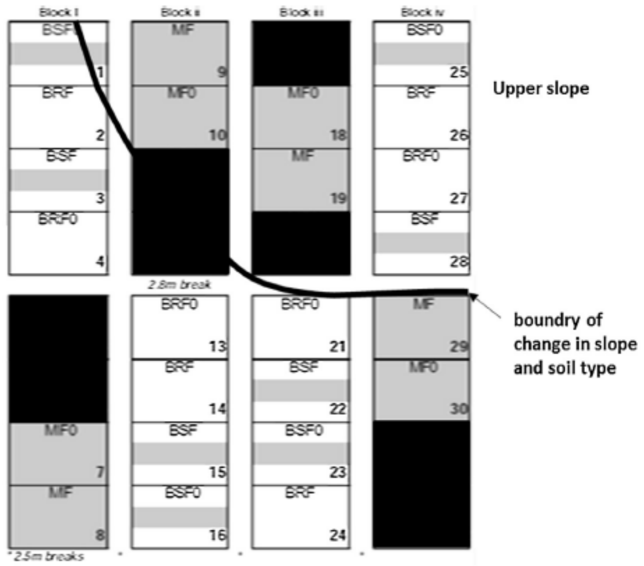

Fig. 1. The layout of the BT1 sugarcane trial situated at SASRI, Mount Edgecombe. BRF0: burnt with residues removed and not fertilized; BRF: burnt with residues removed and fertilized; BSF0: burnt with residues scattered and not fertilized; BSF: burnt with residues scattered and fertilized; MF0: mulched and not fertilized; MF: mulched and fertilized. Plots that are shaded black were not sampled for the present study.

planted in seven rows with a 1.4 m spacing. The trial is a split-plot factorial design arranged in a randomised complete block with four replicates. The main plot treatments are a) green cane harvesting with all residues mulched over the plot area (M), b) cane burnt prior to harvest with cane-tops left scattered evenly over the plot covering two thirds of the surface area (BS) and c) cane burnt prior to harvest with all residues removed from the plots (BR). Split-plot treatments consisted of fertilized (F) and unfertilized plots (F0) (Fig. 1). From the 32 plots, 24 were selected including four replicates of each of the treatments. From the 16 mulched plots, eight (four MF and four MF0) were selected in order to have the same number of treatments as the burnt plots.

The fertilized treatments consist of 140 kg N ha^{-1}, 28 kg P ha^{-1} and 140 kg K ha^{-1} as 5:1:5 (46) at 670 kg ha^{-1} applied annually approximately 40 days after harvesting (van Antwerpen et al., 2001). The annual mulch of cane crop residues on the green cane (unburnt) harvested plots is approximately 16 Mg ha^{-1} and the residues have a C:N ratio of about 120. The cane was harvested every 24 months from the beginning of the experiment until 1966, then every 15 months between 1966 and 1987 and every 12 months since 1987 (van Antwerpen et al., 2001). Three replicate soil samples were collected at two depths (0–10 and 10–20 cm) from mini-pits, to avoid major disturbance in the trial, in each of the 24 chosen plots in February 2012. The 144 soil samples were carefully collected with a spade at each depth to avoid the shearing effect of an auger.

2.3. Chemical analyses

These were performed on bulk soil samples that were air-dried and crushed to pass either a 2 mm mesh (pH, exchangeable bases, exchangeable acidity and extractable aluminium (Al)) or a 0.5 mm mesh (total carbon (Ct), total nitrogen (N) and organic carbon (OC)). Soil pH was measured in 1 M potassium chloride at 1:2.5 soil:solution ratio. Exchangeable potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na) were extracted with 0.1 M strontium chloride (Hughes and Girdlestone, 1994) and measured by atomic absorption spectrophotometry (AAS, Varian spectraAA-200). Aluminum and exchangeable acidity were extracted with 1 M KCl (Hunter, 1974). The exchangeable acidity was measured by titrating KCl with sodium hydroxide while the extracted aluminium was measured with inductively coupled plasma emission spectrophotometry (ICP, Varian 720-ES). The effective cation exchange capacity (ECEC) was calculated as the sum of exchangeable base cations and acidity. The Ct and N were determined in bulk samples and different aggregate fractions using the automated Dumas dry combustion method on a LECO CNS 2000 analyzer (Matejovic, 1996) and the C:N ratio calculated. The readily oxidizable OC was determined by the acid dichromate wet oxidation procedure (Walkley, 1947).

2.4. Physical analyses

Particle size distribution was determined by the pipette method (Gee and Bauder, 1986) on 48 samples selected to be representative of the 24 plots investigated. Soil aggregate stability was measured on all samples using the most aggressive water treatment method according to the French norm NF X 31-315 (AFNOR, 2005; Le Bissonnais, 1996). In brief, the samples were air-dried and sieved to collect about 45 g of aggregates between 2.8 and 5 mm in size. The collected aggregates were then oven-dried at 40 °C for 24 h to ensure all free water evaporated. About 5–10 g of aggregates was rapidly immersed in 50 mL of distilled water and allowed to stand for 10 min, after which the water was removed by pipetting. The aggregates were then transferred using an ethanol-filled wash bottle onto a 50 µm sieve immersed in a bucket of ethanol. The sieve was gently shaken side-to-side by hand 10 times. All the aggregates remaining on the sieve were collected, oven-dried at 40 °C for 48 h and then separated through a nest of six sieves (2, 1, 0.5, 0.2, 0.1 and 0.053 mm). The mass proportion of the size fraction of stable aggregates was calculated and results were expressed as the mean weight diameter (MWD) corresponding to the sum of the mass fraction remaining on each sieve multiplied by the inter-sieve size (Kemper and Rosenau, 1986) (Eq. (1)).

$$\mathrm{MWD(mm)} = \frac{\sum[d \times m]}{100} \quad (1)$$

where d is the mean diameter between the two sieves (mm); and m the weight fraction of aggregates remaining on the sieve (%).

2.5. Statistical analysis

The overall differences between the treatment means were assessed using analysis of variance (ANOVA) for a split-plot experimental design. This was done for Ct, OC, N, exchangeable bases (Ca, Mg, Na and K), pH and MWD. To investigate specific treatments differences between treatment means, post-hoc comparative tests were used at 5% level of significance (GENSTAT, 14th edition). The results of the sub-samples (pseudo-replicates; n = 3) were averaged to provide a single variable estimate for the plot. The results of the true replicates (n = 4) across treatment plots were averaged and correlations were carried out between these.

Principal component analysis (R version 3.0.0, R Development Core Team, 2011) was used to establish the multi-variate relationship between the measured variables Al, Na, K, Ca, Mg, Ct, C:N, pH, sand, silt, clay and MWD for both 0–10 and 10–20 cm soil depths. The variability between fertilized and unfertilized treatments and also between mulched and burnt plots was determined using the principal component analysis (R version 3.0.0, R Development Core Team, 2011).

3. Results

3.1. Carbon and nitrogen

The concentration of Ct was slightly higher under BSF0 (42 g kg^{-1}) and MF0 (41 g kg^{-1}) compared to BRF0 (36 g kg^{-1}) at 0–10 cm (Fig. 2a). However, no clear trend was observed at 10–20 cm (Fig. 2b). Although a marked effect of treatment on Ct was observed at 0–10 cm, no significant difference (p = 0.330) was found between the treatments. There was a general increase in OC under mulched treatments at both depths (0–10 and 10–20 cm) (Fig. 2c and d) though no significant differences (p = 0.184) between the treatments were found. Nitrogen content increased significantly (p = 0.006) in mulch plots in both fertilized and unfertilized treatments at 0–10 cm (Fig. 2e). At 10–20 cm, N was higher in the mulched treatments but not significantly different from the burnt treatments (Fig. 2f).

3.2. Exchangeable bases and pH

At 0–10 cm, the lowest and highest concentrations of Ca from the fertilized plots were measured in the MF and BRF treatments (3.27 $cmol_c\ kg^{-1}$ and 4.32 $cmol_c\ kg^{-1}$; respectively) (Fig. 3a). At 10–20 cm depth, the highest Ca concentration was 6.37 $cmol_c\ kg^{-1}$ in the BSF treatment and the lowest was 4.83 $cmol_c\ kg^{-1}$ in the MF treatment (Fig. 3b). The concentration of Ca was similar between BR, BS and M treatments within each sampling depth in both fertilized and unfertilized treatments. There was generally a significantly ($p < 0.001$) lower Ca content in the fertilized treatments compared to the unfertilized treatments (Fig. 3a) in the 0–10 cm soil depth. This trend was, however, not reflected in the

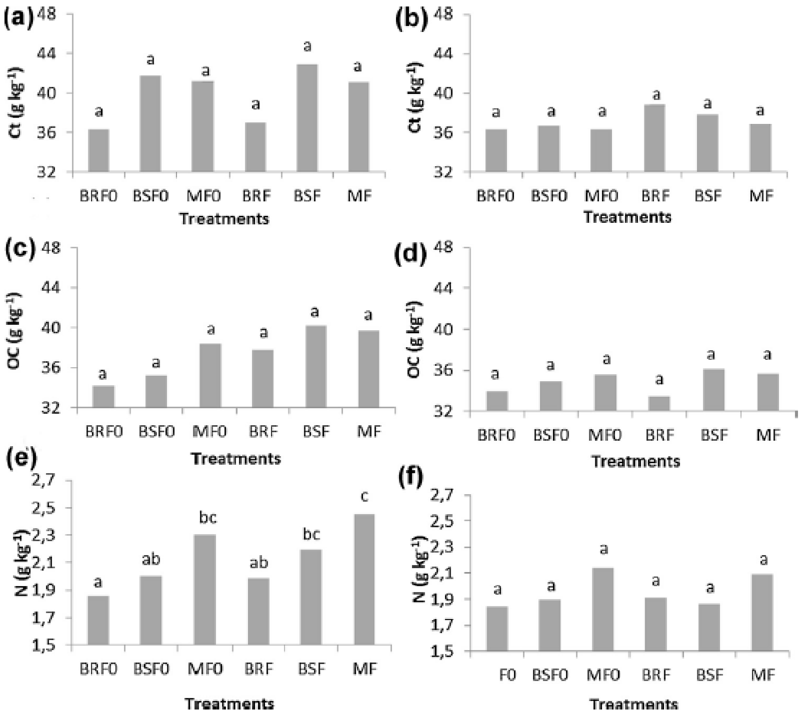

Fig. 2. The mean ($n = 4 \pm$ standard error) total carbon (Ct), organic carbon (OC) and total nitrogen (N) at 0–10 (a, c and e) cm and 10–20 cm (b, d and f) under different treatments: BRF0: burnt with residues removed and not fertilized; BRF: burnt with residues removed and fertilized; BSF0: burnt with residues scattered and not fertilized; BSF: burnt with residues scattered and fertilized; MF0: mulched and not fertilized; MF: mulched and fertilized. Means associated with the same letter are not significantly different ($LSD_{5\%}$ (Ct: 0–10 cm = 7.50, 10–20 cm = 6.09, OC: 0–10 cm = 5.65, 10–20 cm = 5.93, N: 0–10 cm = 0.32, 10–20 cm = 0.38)).

10–20 cm depth across all the treatments (Fig. 3b). Magnesium was also significantly ($p < 0.001$) higher in unfertilized (3.61 $cmol_c$ kg^{-1}) treatments compared to the fertilized plots (1.69 $cmol_c$ kg^{-1}) at both depths and in all treatments (Fig. 3c and d). Potassium was significantly higher ($p < 0.001$) in fertilized plots as compared to unfertilized only under burnt treatments at 0–10 cm depth and similar in the rest of the treatments (Fig. 3e and f). Sodium was significantly ($p < 0.001$) higher in unfertilized plots under burnt treatments at both depths (Fig. 3g and h).

The pH was about 4.5 in unfertilized plots regardless of treatment and depth (Fig. 4a and b). In fertilized plots the soil pH was about 3.5 at 0–10 cm depth and about 4.0 at 10–20 cm depth (Fig. 4a and b). At the 10–20 cm depth, MF treatments showed a significantly lower pH compared to burnt plots. The significant differences in the 0–10 cm depth were found only between fertilized and unfertilized treatments.

3.3. Particle size and aggregate stability

The average clay, silt and sand contents were 43.4, 33.5 and 23.2%, respectively, across all treatments and soil depths. There was no significant difference ($p = 0.691$) between the treatments and depths in terms of clay content.

The MWD was highly significant ($p < 0.001$) higher in the unfertilized treatments (average = 2.27 mm) than in the fertilized treatments (average = 1.48 mm) (Fig. 5a and b). Between the mulched and burnt treatments, the average MWD slightly increased with increase in OC at 0–10 cm in the fertilized treatment (BRF < BSF < MF) and either decreased or remained similar with fertilizer applications in all plots regardless of the treatments and depth. However, no significance differences were found between burnt and mulched treatments except between BRF0, BSF0 and MF0 at the 0–10 cm depth (Fig. 5a and b).

4. Discussion

4.1. Effects of mulching and burning

The lack of significant differences in Ct between the mulched and burnt treatments could be due to the presence of recalcitrant black carbon (Cb) in the burnt treatments. The Cb increases with continuous burning of crop residues in agricultural systems (Rumpel, 2008). Generally, Cb can be easily removed by water erosion although it may remain in the soil for long periods during dry (low or no rainfall) seasons. According to Rumpel (2008), under less intense rainfall the Cb may be incorporated into the mineral soil, leading to long-term sequestration of carbon. Another possibility for the lack of response in Ct concentrations to varying amounts of carbon input, over many years, may be that the soil has reached its saturation level for soil carbon. After comparing the soil carbon status at many sugarcane study sites, Thorburn et al. (2012) concluded that changes in carbon in response to sugarcane residues are site specific. Stewart et al. (2007) stated that smaller increases in Ct content with increased carbon input could be due to the decreased capacity of a high carbon soil to store further added carbon ('carbon saturation'). Fontaine et al. (2004) demonstrated that the supply of fresh C may accelerate the decomposition of soil C and induce a negative C balance. Soil aggregates normally protect the soil carbon and thus limit the increase in soil carbon with increase in carbon inputs (Chung et al., 2008; Kimetu et al., 2009; Six et al., 2002). The similarities in OC could again be due to carbon saturation of the soil. The dominance of kaolinite in the clay fraction of the soils at the study site might have contributed to the reduction of the soil capacity to store further added carbon, leading

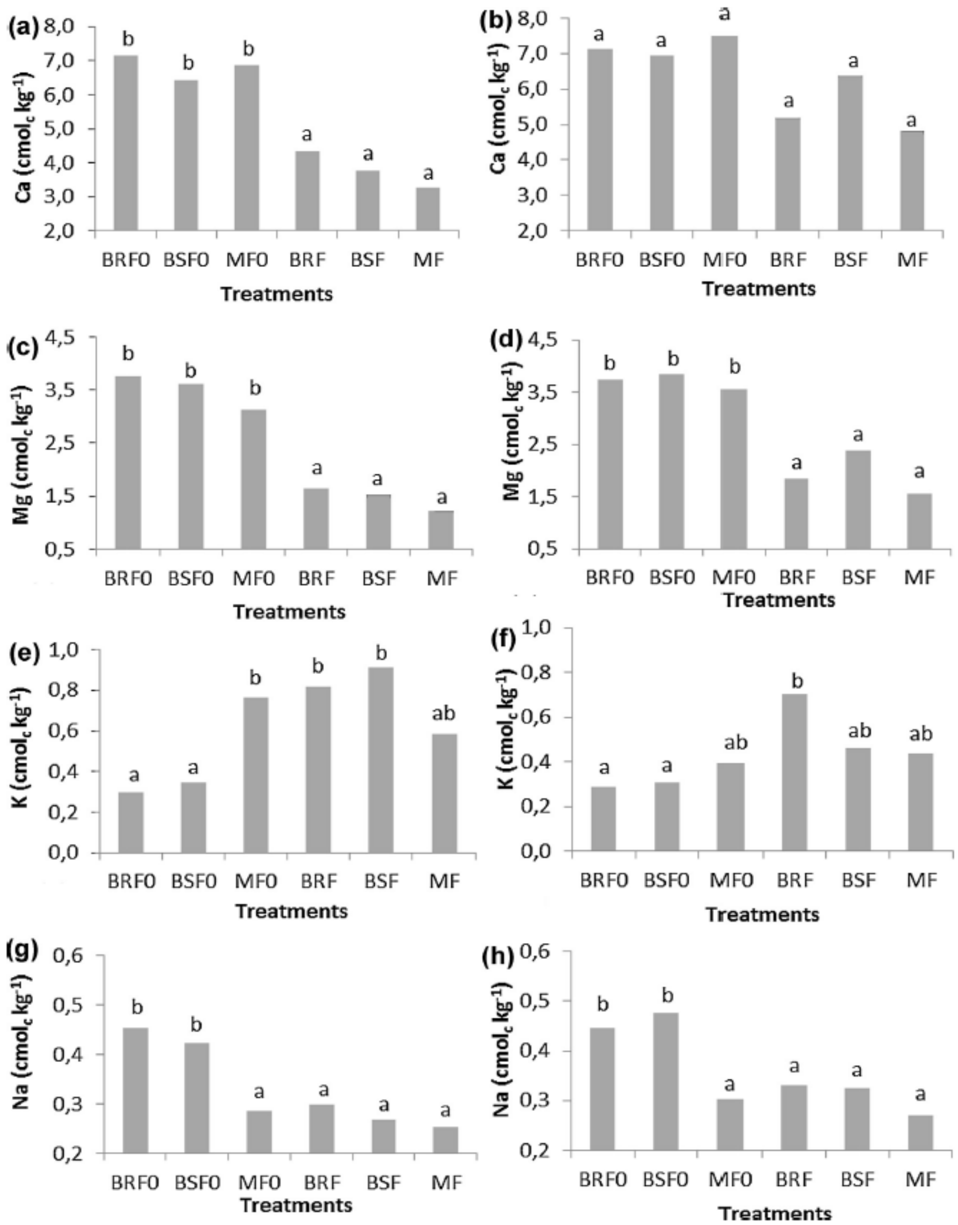

Fig. 3. The mean (n = 4 ± standard error) exchangeable bases (Ca: calcium, Mg: magnesium, K: potassium, Na: sodium) measured at 0–10 cm (a, c, e and g) and 10–20 cm (b, d, f and h) under different treatments: BRF0: burnt with residues removed and not fertilized; BRF: burnt with residues removed and fertilized; BSF0: burnt with residues scattered and not fertilized; BSF: burnt with residues scattered and fertilized; MF0: mulched and not fertilized; MF: mulched and fertilized. Means associated with the same letter are not significantly different ($LSD_{5\%}$ (Ca: 0–10 cm = 1.82, 10–20 cm = 2.55., Mg: 0–10 cm = 0.75, 10–20 cm = 0.90., K: 0–10 cm = 0.35, 10–20 cm = 0.29., Na: 0–10 cm = 0.11, 10–20 cm = 0.11)).

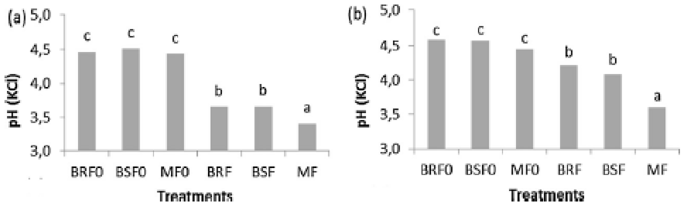

Fig. 4. The mean (n = 4 ± standard error) pH (KCl) at (a) 0–10 and (b) 10–20 cm under different treatments: BRF0: burnt with residues removed and not fertilized; BRF: burnt with residues removed and fertilized; BSF0: burnt with residues scattered and not fertilized; BSF: burnt with residues scattered and fertilized; MF0: mulched and not fertilized; MF: mulched and fertilized. Means associated with the same letter are not significantly different ($LSD_{5\%}$ (0–10 cm = 0.13, 10–20 cm = 0.34)).

to carbon saturation. According to Chan (2001), the low surface area of kaolinite limits the ability of the soil to retain carbon. It thus appears that long-term capacity of soil to store carbon is controlled by inherent soil properties while management practices (such as residue mulching or burning) may result in a shift in the carbon inputs, the absolute carbon storage threshold will reach an upper saturation limit for the given environmental conditions ("quasi-stable equilibrium"). In this study, continuous mulching has not resulted in correspondingly higher amounts of carbon storage, supporting this notion. The higher N in the mulched treatments is likely to be the result of the decomposition of SOM and mineralization of organically bound N at 0–10 cm depth (Fig. 2c) (Basanta et al., 2003; Hartemink, 1998). The larger amount of N and carbon released from SOM decomposition is stored and protected in the stable macroaggregates (Figs. S1–S4). The decomposition SOM and release of N might have also contributed in pH decrease under mulched treatments by adding organic acids (Williams, 1980).

Generally, there were no significance differences in MWD between burnt and mulched treatments (Fig. 5). These results are in agreement with those obtained by Torres et al. (2013) who also found no significant differences in MWD between burnt and mulched sugarcane treatments to 0–10 cm depth in Minas Gerais State, Brazil. Mulched and burnt treatments had no significant relationship with the measured soil properties at both depths (0–10 cm (p = 0.235), 10–20 cm (p = 0.827)) although mulching was hypothesized to improve soil carbon and root density, and thereby increase MWD and associated soil properties (Table S5). All the MWD related soil properties measured in this study such as OC, Ct, particle size and exchangeable cations showed only small differences or no significant differences between burnt and mulched treatments, and this could be the cause of their similar MWD. The similarity in particle size distribution observed across the area is associated with the dominant parent material (dolerite) at the study site on all the plots. According to Bronick and Lal (2005), soil texture is an inherent soil factor and is therefore mainly influenced by parent material rather than land use and management practices.

4.2. Effects of fertilization

There were no significant effects of fertilizer application on nitrogen and carbon (Ct and OC) possibly due to the nature of the investigated soil and carbon saturation as explained in Section 4.1. However, an increase in K and decrease in Ca, Mg, Na and pH on the fertilized plots can be associated with nitrogenous fertilizer application. The reduction in pH could be a result of the combined effect of base cation mining by sugarcane plants (van Antwerpen and Meyer, 2002), leaching and being replaced by Al and oxidization of ammonium to nitrate (Qongqo and van Antwerpen, 2000). A highly significant decrease of pH has been noticed in the experimental plots at Versailles after years of application of different types of nitrogen fertilizers (Paradelo et al., 2013). According to Ng Cheong et al. (2009), the fertilizer-induced soil acidification process is associated with the release of two hydrogen ions per unit ammonium through the nitrification process. Hartemink (1998) also reported a substantial decrease in exchangeable cations on Vertisols under sugarcane production in Australia. A significantly high accumulation of K in the burnt and fertilized plots reflects the large amounts of K being added as fertilizer and some possibly recycled annually from ash (Graham et al., 2002).

The significantly higher MWD measured in unfertilized plots compared to fertilized plots could be due to the effect of the Ca and Mg concentrations. Positive and significant relationships were found between MWD and exchangeable bases (Ca (p < 0.001, r^2 = 0.7) and Mg (p < 0.001, r^2 = 0.7)) and ECEC (p = 0.0016, r^2 = 0.6) across all 24 sampled plots. The reduction in exchangeable bases was accompanied by a significant increase (p < 0.001) in exchangeable acidity that potentially decreased the MWD especially at 0–10 cm depth. The principal component analysis (PCA) showed significant effects of fertilizer application on measured soil

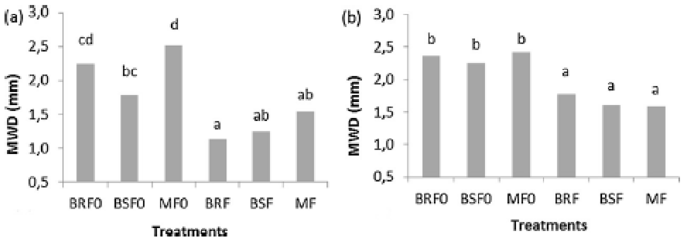

Fig. 5. The mean (n = 4 ± standard error) mean weight diameter (MWD) at (a) 0–10 and (b) 10–20 cm under different treatments: BRF0: burnt with residues removed and not fertilized; BRF: burnt with residues removed and fertilized; BSF0: burnt with residues scattered and not fertilized; BSF: burnt with residues scattered and fertilized; MF0: mulched and not fertilized; MF: mulched and fertilized. Means associated with the same letter are not significantly different ($LSD_{5\%}$ (0–10 cm = 0.52, 10–20 cm = 0.36)).

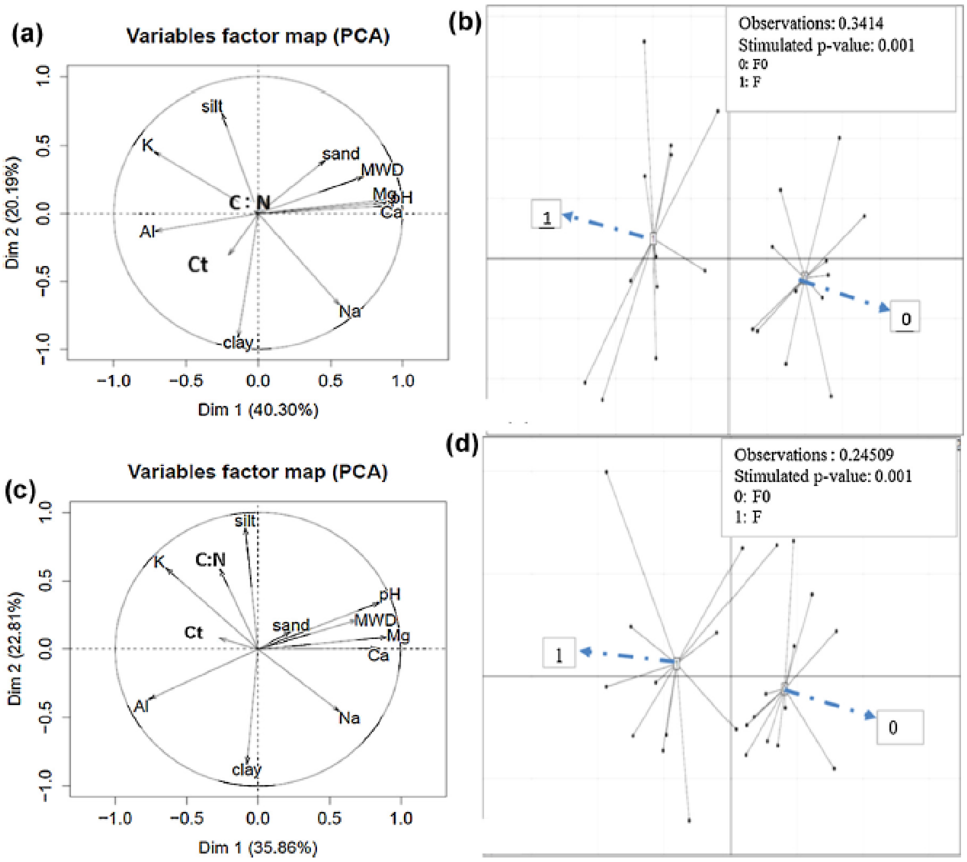

Fig. 6. Principal component analysis (PCA) for (a) and (c) the different soil properties influencing mean weight diameter, and (b) and (d) averages of all fertilized (F) and unfertilized treatments (F0) for 0–10 and 10–20cm depths, respectively.

properties, at both 0–10 (p=0.001) and 10–20 (p=0.001) cm depths (Fig. 6b and d) (Table S5). The PCA showed that about 34.1 and 24.5% of the observed variability was controlled by fertilizer application at both 0–10 and 10–20 cm depths, respectively. The generally strong and weak correlations shown by fertilizer application and mulching, respectively, with the measured soil properties, suggests that the negative effects of fertilizer application were dominant over that of carbon additions (i.e. mulching) when these occurred in combination. The annual application of fertilizer might have encouraged Ca and Mg removal by the crop from 0 to 10 cm and leaching from 0 to 10 cm to 10–20 cm by reducing soil pH and increasing exchangeable acidity which potentially led to less stable aggregates at 0–10 cm (Graham et al., 2002). Paradelo et al. (2013) found a significant increase in clay dispersivity after 80 years of K fertilizer application in the experimental plots at Versailles. Numerous researchers have found exchangeable cations (Ca and Mg) to be important soil binding agents through cationic bridging with clay particles that improves soil aggregate stability (Bronick and Lal, 2005; Cook et al., 1992; Graham and Haynes, 2005; Paradelo et al., 2013). Some of the Ca and Mg that was removed by the crop from the upper layer (0–10 cm) might have been exported to the mill with the sugarcane stalks leading to higher extractable Al at this depth (van Antwerpen and Meyer, 2002). This could be the reason for the lower base status and higher acidity at this depth.

5. Conclusions

This study has found that the impact of mulching may be counteracted by the nitrogenous fertilizer applied especially at 0–10 cm depth. Carbon (both Ct and OC) was similar across all the treatments while Ca, Mg, pH and MWD were similar between burnt and mulched treatments, but significantly different between fertilized and unfertilized treatments. It was only total N, K, exchangeable acidity and Al that were clearly increased in the mulched treatments but this could be as a result of the fertilizer applied. The data here suggest that the site (both upper and lower slope) has reached its maximum capacity ("saturation level") to sequester and store carbon and as a result the MWD is controlled by exchangeable bases (Ca and Mg) rather than carbon in both burnt and mulched treatments. This study has thus shown that (a) long-term use of fertilizers under sugarcane production has detrimental effects on soil structure (reduces aggregate stability), (b) this has occurred regardless of the harvesting management method practiced, and (c) increasing additions of organic matter do not always correspond to improvement of soil structural stability and related soil properties. However, these findings should be balanced against the effect of (a) added fertilizer on production and yield of the crop and (b) mulching on other soil properties such as water retention and protection of the topsoil from erosion. At this site mulching has no clear benefit over burning as the soils have apparently reached an equilibrium in terms of OC and, as the site does not undergo either extended periods of fallow or disruptive site preparation, soil degradation is less than might be expected under other forms of intensive agriculture.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.agee.2016.04.002.

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Appendix 3.1: Profile descriptions of the soils found in the (a) upper and (b) lower slope positions.

The soil of BT1 was previously classified as a Vertisol, locally known as Arcadia form, Lonehill family, in research studies conducted at this site. However, during the soil sampling for the current study, it was found that this study site consists of two different soil types neither of which conforms to the characteristics of a Vertisol.

Melanic A horizon: An A horizon of 50 cm depth with an average clay content of 43.4% in the upper 20 cm. It had a plasticity index of 25, an organic carbon content of 3.63% and angular blocky structure. The soil collected from this horizon was sticky. This horizon was dark (2.5 YR 3/1 to 3/2) with a progressive transition to a dark reddish brown (2.5 YR 3/3 to 3/4) AC horizon, with a very clear polyhedral angular structure overlying weathered dolerite. The profile has a 5 to 10 cm thick stoneline at about 50 cm depth between the A and the A/C horizon.

Lithocutanic B horizon: This horizon underlies the melanic topsoil via a stoneline at 50 cm. There was a mixture of brownish yellow (10YR 6/8) and dark (2.5 YR 3/1 to 3/2) colours that are possibly the result of illuviation causing the localization of clay and organic matter in the saprolite. The brownish yellow colour could also be the result of in *situ* weathering of dolerite. It showed no signs of wetness (hydromorphic features).

Appendix 3.1 continued.

(b)

The topsoil shared the same characteristics with the topsoil found on the upper slope.

Pedocutanic B horizon: This horizon (50-7-cm deep with ±50% clay) underlies the melanic A horizon via a stoneline (5-10 cm thick). It consisted of a medium to coarse angular blocky and non-calcareous B horizon. The boundary between this horizon and the melanic A horizon was abrupt with respect to structure, however, this was apparent mostly in the mulched plots. It was sticky and having a dark reddish brown to dark red (2.5 YR 3/4 to 10 R 3/6) in colour.

Appendix 3.2: Detailed method for soil aggregate stability (AFNOR, 2005).

Soil aggregate stability was determined according to the French norm NF X 31-515. The samples collected were air dried and sieved to collect about 45 g of aggregates between 2.8 and 5 mm in size. Prior to the analysis, collected aggregates were oven dried at 40ºC for 24 hours to remove all free water. This method combined three tests: water treatment (MWD-Wt), ethanol treatment (MWD-Et) and slow capillary wetting ethanol treatment (MWD-SCWEt). For the **MWD-Wt**, about 5-10 g of the collected aggregates was rapidly immersed in 50 mL of distilled water for 10 minutes. After that, the water was pipetted out. For the **MWD-Et**, a similar amount of aggregates was rapidly immersed in 50 mL of ethanol for 30 minutes. The ethanol was then extracted by siphoning and the aggregates transferred (using an ethanol wash bottle) into an Erlenmeyer flask containing 50 mL of distilled water. The flask was made up to 200 mL with distilled water, stoppered and gently shaken by hand end-over-end 10 times to slake all the unstable aggregates and then allowed to stand for 2-3 hours to allow the material to settle. The supernatant solution was then removed by pipette. For the **MWD-SCWEt**, a similar quantity of aggregates was capillary wetted with water using wet filter papers for 60 minutes.

After each test the aggregates were transferred using an ethanol-filled wash bottle onto a 0.053 mm sieve immersed in a bucket of ethanol. The sieve was gently shaken side-to-side by hand 10 times in the bucket. All the aggregates remaining on the sieve were collected, and dried at 40ºC for 48 hours. The aggregates were then poured into a nest of sieves stacked in the following sequence: 2.000, 1.000, 0.500, 0.200, 0.106 and 0.053 mm. The mass remaining on each sieve was weighed and the mass proportion of each size fraction of stable aggregates calculated. The results were expressed as a mean weight diameter (MWD) for each treatment corresponding to the sum of the mass fraction remaining on each sieve multiplied by the mean intersieve size:

$$MWD = \frac{\{(3.5*Pa)+(1.5*Pa)+(0.75*Pa)+(0.35*Pa)+(0.15*Pa)+(0.08*Pa)+(0.027*Pa)\}}{100}$$

Where Pa = mass of aggregates as a percentage of aggregates per sieve (using the mean intersieve size (mm)).

Appendix 4.1: The analysis of variance for clay content in the different management treatments: BRF0: burned with residues removed and not fertilized; BRF: burned with residues removed and fertilized; BSF0: burned with residues scattered and not fertilized; BSF: burned with residues scattered and fertilized; MF0: mulched and not fertilized; MF: mulched and fertilized at 0-10 cm soil depth.

Variate: Clay					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	89.62	29.87	1.35	
Treatments	5	75.29	15.06	0.68	**0.644**
Residual	15	331.24	22.08		
Total	23	496.14			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.2: The analysis of variance for clay content in the different management treatments **(**as shown in Appendix 4.1) at 10-20 cm soil depth.

Variate: Clay					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	29.42	9.81	0.37	
Treatments	5	98.03	19.61	0.73	**0.609**
Residual	15	400.64	26.71		
Total	23	528.09			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.3: The analysis of variance for total carbon (Ct) in the different management treatments (as shown in Appendix 4.1) at 0-10 cm soil depth.

Variate: Ct					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Rep stratum	3	184.11	61.37	2.48	
Treatments	5	143.00	28.60	1.15	**0.376**
Residual	15	371.87	24.79		
Total	23	698.98			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.4: The analysis of variance for total carbon (Ct) in the different management treatments (as shown in Appendix 4.1) at 10-20 cm soil depth.

Variate: Ct					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	37.44	12.48	0.76	
Treatments	5	19.88	3.98	0.24	**0.937**
Residual	15	245.19	16.35		
Total	23	302.52			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.5: The analysis of variance for organic carbon (OC) in the different management treatments (as shown in Appendix 4.1) at 0-10 cm soil depth.

Variate: OC

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	81.95	27.32	1.94	
Treatments	5	117.39	23.48	1.67	**0.202**
Residual	15	210.78	14.05		
Total	23	410.11			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.6: The analysis of variance for organic carbon (OC) in the different management treatments (as shown in Appendix 4.1) at 10-20 cm soil depth.

Variate: OC

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	68.89	22.96	1.49	
Treatments	5	22.08	4.42	0.29	**0.914**
Residual	15	231.93	15.46		
Total	23	322.89			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.7: The analysis of variance for total nitrogen (N) in the different management management treatments **(**as shown in Appendix 4.1) at 0-10 cm soil depth.

Variate: N

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	0.45472	0.15157	3.43	
Treatments	5	0.99721	0.19944	4.51	**0.010**
Residual	15	0.66303	0.04420		
Total	23	2.11496			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.8: The analysis of variance for total nitrogen (N) in the different management treatments **(**as shown in Appendix 4.1) at 10-20 cm soil depth.

Variate: N

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	0.44627	0.14876	2.32	
Treatments	5	0.32188	0.06438	1.00	**0.449**
Residual	15	0.96226	0.06415		
Total	23	1.73041			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.9: The analysis of variance for carbon to nitrogen ratio (C:N ratio) in the different management treatments **(**as shown in Appendix 4.1) at 0-10 cm soil depth.

Variate: C:N ratio					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability.**
Replicates	3	5.396	1.799	0.72	
Treatments	5	37.966	7.593	3.05	**0.043**
Residual	15	37.307	2.487		
Total	23	80.668			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.10: The analysis of variance for carbon to nitrogen ratio (C:N ratio) in the different management treatments **(**as shown in Appendix 4.1) at 10-20 cm soil depth.

Variate: C:N ratio					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	23.889	7.963	4.05	
Treatments	5	40.455	8.091	4.11	**0.015**
Residual	15	29.507	1.967		
Total	23	93.851			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.11: The probability value (*p*-value) for the total carbon (Ct) and total nitrogen (N) stored in different aggregate fractions under different management treatments **(**as shown in Appendix 4.1) at 0-10 and 10-20 cm soil depth.

Depth (cm)	**Variables**	**Treatments**					
		BRFO	**BSFO**	**MFO**	**BRF**	**BSF**	**MF**
0-10	Ct	< 0.001**	0.065	0.097	0.263	0.768	0.005*
	N	0.011*	0.065	0.033*	< 0.001**	0.773	0.008*
10-20	Ct	0.071	0.194	0.536	0.248	0.053	0.042*
	N	< 0.001**	0.002**	0.630	< 0.001**	0.023*	0.179

*, **significance at $p < 0.05$ and $p < 0.01$, respectively.

Appendix 4.12: The analysis of variance for calcium (Ca) in the different management treatments **(**as shown in Appendix 4.1) at 0-10 cm soil depth.

Variate: Ca					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	11.575	3.858	2.65	
Treatments	5	58.434	11.687	8.01	**< 0.001**
Residual	15	21.877	1.458		
Total	23	91.886			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.13: The analysis of variance for calcium (Ca) in the different management treatments **(**as shown in Appendix 4.1) at 10-20 cm soil depth.

Variate: Ca					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	4.902	1.634	0.57	
Treatments	5	24.000	4.800	1.68	**0.201**
Residual	15	42.954	2.864		
Total	23	71.856			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.14: The analysis of variance for magnesium (Mg) in the different management treatments **(**as shown in Appendix 4.1) at 0-10 cm soil depth.

Variate: Mg					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	1.0958	0.3653	1.50	
Treatments	5	26.2796	5.2559	21.52	< **0.001**
Residual	15	3.6631	0.2442		
Total	23	31.0384			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.15: The analysis of variance for magnesium (Mg) in the different management treatments **(**as shown in Appendix 4.1) at 10-20 cm soil depth.

Variate: Mg					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	0.6318	0.2106	0.59	
Treatments	5	20.8576	4.1715	11.77	< **0.001**
Residual	15	5.3142	0.3543		
Total	23	26.8035			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.16: The analysis of variance for potassium (K) in the different management treatments **(**as shown in Appendix 4.1) at 0-10 cm soil depth.

Variate: K					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	1.17119	0.39040	7.13	
Treatments	5	1.29940	0.25988	4.75	**0.008**
Residual	15	0.82099	0.05473		
Total	23	3.29158			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.17: The analysis of variance for potassium (K) in the different management treatments **(**as shown in Appendix 4.1) at 10-20 cm soil depth.

Variate: K					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	0.43468	0.14489	3.82	
Treatments	5	0.43975	0.08795	2.32	**0.095**
Residual	15	0.56869	0.03791		
Total	23	1.44312			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.18: The analysis of variance for sodium (Na) in the different management treatments **(**as shown in Appendix 4.1) at 0-10 cm soil depth.

Variate: Na					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	0.529260	0.176420	34.99	
Treatments	5	0.147243	0.029449	5.84	**0.003**
Residual	15	0.075630	0.005042		
Total	23	0.752132			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.19: The analysis of variance for sodium (Na) in the different management treatments (as shown in Appendix 4.1) at 10-20 cm soil depth.

Variate: Na					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	0.453877	0.151292	29.05	
Treatments	5	0.135092	0.027018	5.19	**0.006**
Residual	15	0.078112	0.005207		
Total	23	0.667081			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.20: The analysis of variance for exchangeable acidity in the different management treatments **(**as shown in Appendix 4.1) at 0-10 cm soil depth.

Variate: Exchangeable acidity					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	1.7432	0.5811	2.27	
Treatments	5	25.8985	5.1797	20.21	< **0.001**
Residual	15	3.8439	0.2563		
Total	23	31.4856			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.21: The analysis of variance for exchangeable acidity in the different management treatments (as shown in Appendix 4.1) at 10-20 cm soil depth.

Variate: Exchangeable acidity					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	2.4928	0.8309	2.48	
Treat	5	9.8321	1.9664	5.88	**0.003**
Residual	15	5.0202	0.3347		
Total	23	17.3451			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.22: The analysis of variance for aluminium (Al) in the different management treatments (as shown in Appendix 4.1) at 0-10 cm soil depth.

Variate: Al					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	0.18783	0.06261	1.11	
Treatments	5	2.43577	0.48715	8.64	**< 0.001**
Residual	15	0.84578	0.05639		
Total	23	3.46938			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.23: The analysis of variance for aluminium (Al) in the different management treatments (as shown in Appendix 4.1) at 10-20 cm soil depth.

Variate: Al					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	0.14494	0.04831	1.01	
Treatments	5	0.55230	0.11046	2.31	**0.096**
Residual	15	0.71864	0.04791		
Total	23	1.41588			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.24: The analysis of variance for acid saturation (AS) in the different management treatments (as shown in Appendix 4.1) at 0-10 cm soil depth.

Variate: AS					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	245.01	81.67	1.46	
Treatments	5	4160.76	832.15	14.83	**< 0.001**
Residual	15	841.66	56.11		
Total	23	5247.44			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.25: The analysis of variance for acid saturation (AS) in the different management treatments (as shown in Appendix 4.1) at 10-20 cm soil depth.

Variate: AS					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	285.13	95.04	2.11	
Treatments	5	1365.72	273.14	6.07	**0.003**
Residual	15	674.54	44.97		
Total	23	2325.39			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.26: The analysis of variance for pH measured with potassium chloride (pH (KCl)) in the different management treatments (as shown in Appendix 4.1) at 0-10 cm soil depth.

Variate: pH (KCl)					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	0.089806	0.029935	4.26	
Treatments	5	5.183845	1.036769	147.45	**< 0.001**
Residual	15	0.105469	0.007031		
Total	23	5.379120			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.27: The analysis of variance for pH measured with potassium chloride (pH (KCl)) in the different management treatments (as shown in Appendix 4.1) at 10-20 cm soil depth.

Variate: pH (KCl)					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	0.47015	0.15672	3.08	
Treatments	5	2.80544	0.56109	11.03	**< 0.001**
Residual	15	0.76299	0.05087		
Total	23	4.03857			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.28: The analysis of variance for cation exchange capacity (ECEC) in the different management treatments (as shown in Appendix 4.1) at 0-10 cm soil depth.

Variate: ECEC					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	16.264	5.421	2.09	
Treatments	5	53.386	10.677	4.12	**0.015**
Residual	15	38.842	2.589		
Total	23	108.491			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.29: The analysis of variance for cation exchange capacity (ECEC) in the different management treatments (as shown in Appendix 4.1) at 10-20 cm soil depth.

Variate: ECEC					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	5.430	1.810	0.44	
Treatments	5	38.024	7.605	1.86	**0.162**
Residual	15	61.430	4.095		
Total	23	104.884			

d.f.: degrees of freedom, **s.s.**: sum of squares, **m.s.**: mean sum of squares, **v.r.**: variance ratio.

Appendix 4.30: The analysis of variance for zinc (Zn) in the different management treatments (as shown in Appendix 4.1) at 0-10 cm soil depth.

Variate: Zn					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	0.7380	0.2460	0.59	
Treatments	5	23.1239	4.6248	11.01	**< 0.001**
Residual	15	6.3012	0.4201		
Total	23	30.1631			

d.f.: degrees of freedom, **s.s.**: sum of squares, **m.s.**: mean sum of squares, **v.r.**: variance ratio.

Appendix 4.31: The analysis of variance for zinc (Zn) in the different management treatments (as shown in Appendix 4.1) at 10-20 cm soil depth.

Variate: Zn					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	1.0164	0.3388	0.43	
Treatments	5	11.7501	2.3500	2.95	**0.047**
Residual	15	11.9345	0.7956		
Total	23	24.7011			

d.f.: degrees of freedom, **s.s.**: sum of squares, **m.s.**: mean sum of squares, **v.r.**: variance ratio.

Appendix 4.32: The analysis of variance for manganese (Mn) in the different management treatments (as shown in Appendix 4.1) at 0-10 cm soil depth.

Variate: Mn					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	276.2	92.1	0.62	
Treatments	5	2869.8	574.0	3.84	**0.019**
Residual	15	2243.9	149.6		
Total	23	5389.9			

d.f.: degrees of freedom, **s.s.**: sum of squares, **m.s.**: mean sum of squares, **v.r.**: variance ratio.

Appendix 4.33: The analysis of variance for manganese (Mn) in the different management treatments (as shown in Appendix 4.1) at 10-20 cm soil depth.

Variate: Mn					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	134.3	44.8	0.28	
Treatments	5	3258.8	651.8	4.02	**0.016**
Residual	15	2432.6	162.2		
Total	23	5825.7			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.34: The analysis of variance for copper (Cu) in the different management treatments (as shown in Appendix 4.1) at 0-10 cm soil depth.

Variate: Cu					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	247.26	82.42	1.78	
Treatments	5	2820.92	564.18	12.19	**< 0.001**
Residual	15	694.18	46.28		
Total	23	3762.36			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.35: The analysis of variance for copper (Cu) in the different management treatments (as shown in Appendix 4.1) at 10-20 cm soil depth.

Variate: Cu					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	82.65	27.55	0.71	
Treatments	5	746.28	149.26	3.84	**0.019**
Residual	15	583.01	38.87		
Total	23	1411.94			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.36: The analysis of variance for phosphorus (P) in the different management treatments (as shown in Appendix 4.1) at 0-10 cm soil depth.

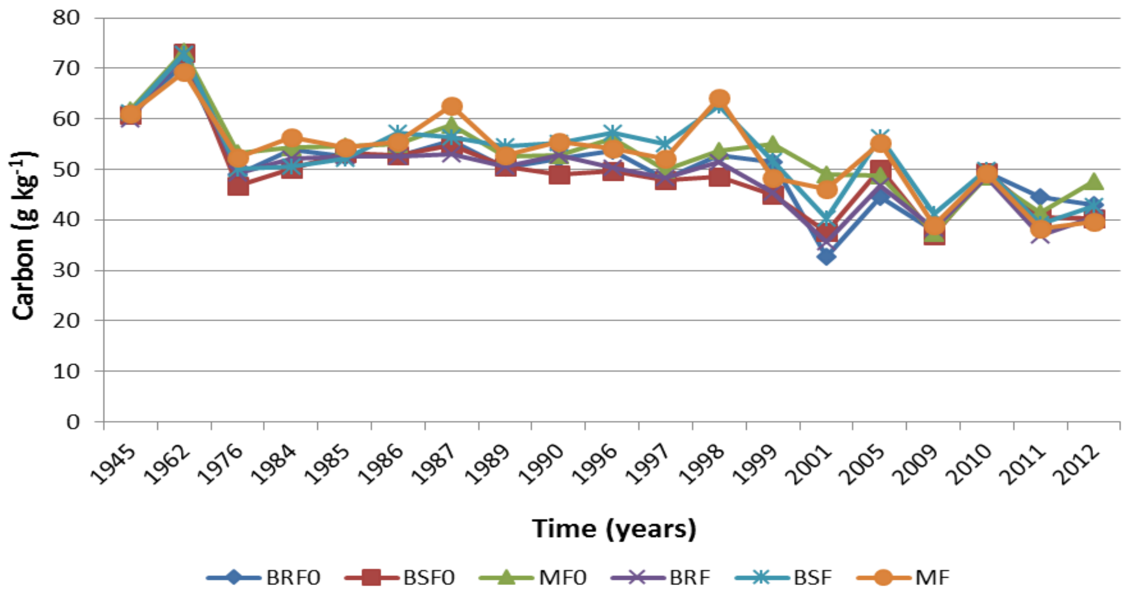
Variate: P					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	36.957	12.319	4.69	
Treatments	5	60.698	12.140	4.62	**0.009**
Residual	15	39.417	2.628		
Total	23	137.073			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 4.37: The analysis of variance for phosphorus (P) in the different management treatments (as shown in Appendix 4.1) at 10-20 cm soil depth.

Variate: P					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	38.322	12.774	1.39	
Treatments	5	112.575	22.515	2.46	**0.081**
Residual	15	137.385	9.159		
Total	23	288.282			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.



Appendix 4.38: The soil organic carbon (OC) measured since 1945 to 2012 of the BT1 trial. BRF0: burned with residues removed and not fertilized; BRF: burned with residues removed and fertilized; BSF0: burned with residues scattered and not fertilized; BSF: burned with residues scattered and fertilized; MF0: mulched and not fertilized; MF: mulched and fertilized at 0-20 cm soil depth.

Appendix 5.1: The analysis of variance for bacterial richness in the different management treatments: M: all mulched; B: all burnt; F: all fertilized.

Variate: Bacterial richness					
Treatment	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
M	1	1302.1	1302.1	27.696	**< 0.001**
B	1	12.2	12.2	0.261	**0.616**
F	1	570.4	570.4	12.132	**0.003**
Residuals	18	846.2	47.0		

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 5.2: The analysis of variance for bacterial evenness in the different management treatments: M: all mulched; B: all burnt; F: all fertilized.

Variate: Bacterial evenness					
Treatment	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
M	1	0.12935	0.12935	19.942	< **0.001**
B	1	0.00213	0.00213	0.328	**0.574**
F	1	0.14653	0.14653	22.591	< **0.001**
Residuals	18	0.11675	0.00649		

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 5.3: The analysis of variance for fungal richness in the different management treatments: M: all mulched; B: all burnt; F: all fertilized.

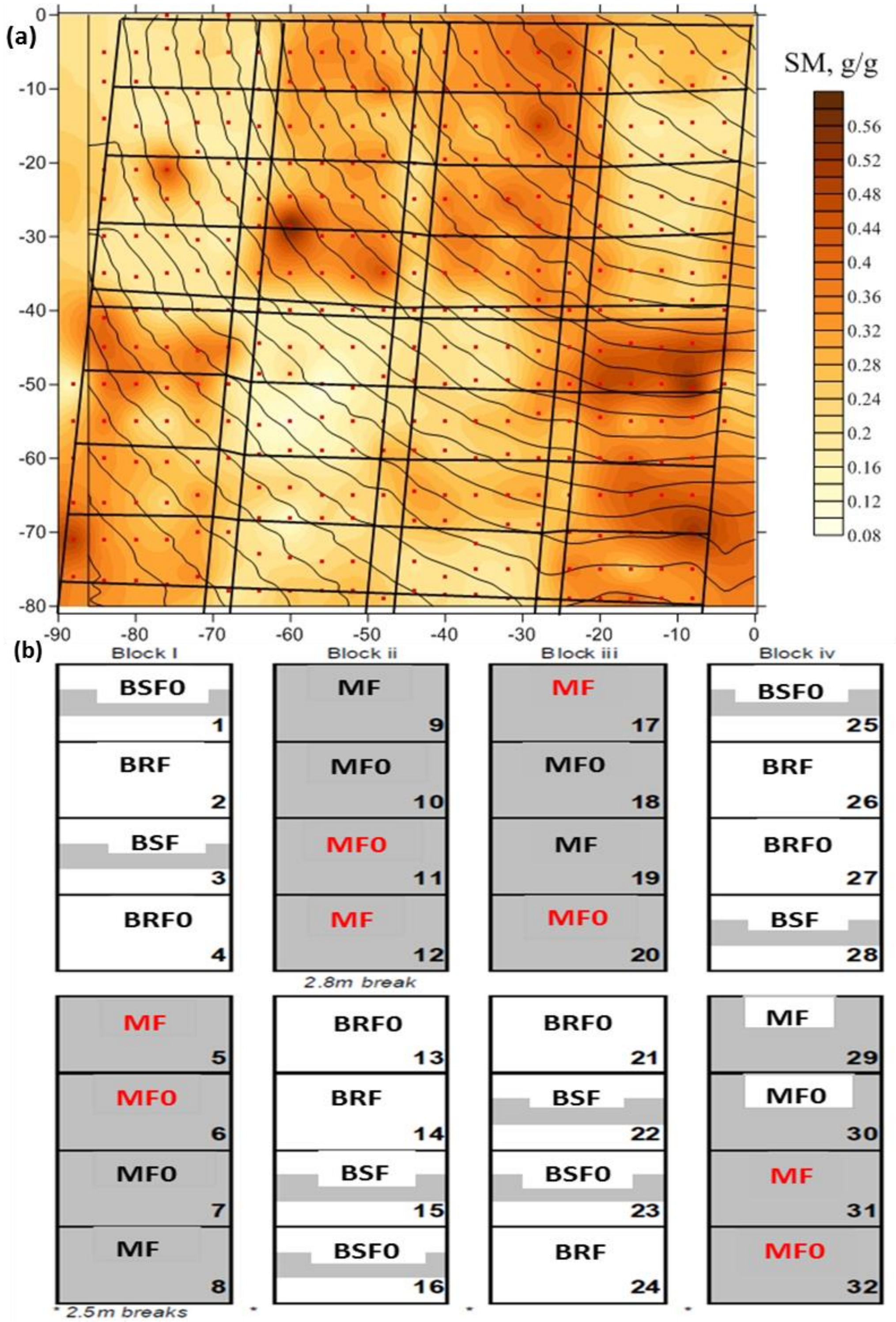
Variate: Fungal richness					
Treatment	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
M	1	9.190	9.19	1.141	**0.299**
B	1	60.06	60.06	7.456	**0.014**
F	1	181.50	181.50	22.531	< **0.001**
Residuals	18	145.00	8.06		

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 5.4: The analysis of variance for fungal evenness in the different management treatments: M: all mulched; B: all burnt; F: all fertilized.

Variate: Fungal evenness					
Treatment	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
M	1	0.00946	0.00946	1.794	**0.197**
B	1	0.00118	0.00118	0.223	**0.642**
F	1	0.11404	0.11404	21.63	< **0.001**
Residuals	18	0.0949	0.00527		

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.



Appendix 5.5: The (a) gravimetric soil moisture (SM) content and (b) the experimental site layout. BRF0: burned with residues removed and not fertilized; BRF: burned with residues removed and fertilized; BSF0: burned with residues scattered and not fertilized; BSF: burned with residues scattered and fertilized; MF0: mulched and not fertilized; MF: mulched and fertilized. Treatments in red were not sampled.

Appendix 6.1: The analysis of variance for mean weight diameter (MWD-Wt) measured with water treatment in the different management treatments: BRF0: burned with residues removed and not fertilized; BRF: burned with residues removed and fertilized; BSF0: burned with residues scattered and not fertilized; BSF: burned with residues scattered and fertilized; MF0: mulched and not fertilized; MF: mulched and fertilized at 0-10 cm soil depth.

Variate: MWD					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	0.3128	0.1043	0.88	
Treatments	5	6.0730	1.2146	10.23	< **0.001**
Residual	15	1.7815	0.1188		
Total	23	8.1672			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance

Appendix 6.2: The analysis of variance for mean weight diameter (MWD-Wt) measured with water treatment in the different management treatments **(**as shown in Appendix 6.1) at 10-20 cm soil depth.

Variate: MWD					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	0.13741	0.04580	0.82	
Treatments	5	3.02678	0.60536	10.78	< **0.001**
Residual	15	0.84228	0.05615		
Total	23	4.00647			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 6.3: The analysis of variance for mean weight diameter (MWD-Et) measured with ethanol treatment in the different management treatments **(**as shown in Appendix 6.1) at 0-10 cm soil depth.

Variate: MWD					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	0.01866	0.00622	0.07	
Treatments	5	1.70334	0.34067	4.01	**0.016**
Residual	15	1.27422	0.08495		
Total	23	2.99623			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 6.4: The analysis of variance for mean weight diameter (MWD-Et) measured with ethanol treatment in the different management treatments (as shown in Appendix 6.1) at 10-20 cm soil depth.

Variate: MWD					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability.**
Replicates	3	0.12261	0.04087	0.99	
Treatments	5	1.46107	0.29221	7.06	**0.001**
Residual	15	0.62106	0.04140		
Total	23	2.20474			

d.f.: degrees of freedom, **s.s.**: sum of squares, **m.s.**: mean sum of squares, **v.r.**: variance ratio.

Appendix 6.5: The analysis of variance for mean weight diameter (MWD-SCWEt) measured with slow capillary wetting ethanol treatment in the different management treatments (as shown in Appendix 6.1) at 0-10 cm soil depth.

Variate: MWD					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	1.7551	0.5850	2.78	
Treatments	5	1.3952	0.2790	1.33	**0.305**
Residual	15	3.1510	0.2101		
Total	23	6.3012			

d.f.: degrees of freedom, **s.s.**: sum of squares, **m.s.**: mean sum of squares, **v.r.**: variance ratio.

Appendix 6.6: The analysis of variance for mean weight diameter (MWD-SCWEt) measured with slow capillary wetting ethanol treatment in the different management treatments (as shown in Appendix 6.1) at 10-20 cm soil depth.

Variate: MWD					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	3	0.5484	0.1828	1.56	
Treatments	5	0.6702	0.1340	1.14	**0.382**
Residual	15	1.7620	0.1175		
Total	23	2.9807			

d.f.: degrees of freedom, **s.s.**: sum of squares, **m.s.**: mean sum of squares, **v.r.**: variance ratio.

Appendix 6.7: The analysis of variance for bulk density (ρ_b) measured in the different management treatments (as shown in Appendix 6.1) at 0-10 cm soil depth.

Variate: ρ_b					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	2	0.01733	0.00866	0.50	
Treatments	5	0.11804	0.02361	1.35	**0.319**
Residual	10	0.17448	0.01745		
Total	17	0.30984			

d.f.: degrees of freedom, **s.s.**: sum of squares, **m.s.**: mean sum of squares, **v.r.**: variance ratio.

Appendix 6.8: The analysis of variance for bulk density (ρ_b) measured in the different management treatments (as shown in Appendix 6.1) at 10-20 cm soil depth.

Variate: ρ_b					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	2	0.019808	0.009904	1.21	
Treatments	5	0.116291	0.023258	2.85	**0.074**
Residual	10	0.081540	0.008154		
Total	17	0.217640			

d.f.: degrees of freedom, **s.s.**: sum of squares, **m.s.**: mean sum of squares, **v.r.**: variance ratio.

Appendix 6.9: The analysis of variance for hydraulic conductivity (Ks) measured in the different management treatments (as shown in Appendix 6.1) at 0-10 cm soil depth.

Variate: Ks					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	2	0.6150	0.3075	0.63	
Treatments	5	14.2012	2.8402	5.82	**0.009**
Residual	10	4.8796	0.4880		
Total	17	19.6958			

d.f.: degrees of freedom, **s.s.**: sum of squares, **m.s.**: mean sum of squares, **v.r.**: variance ratio.

Appendix 6.10: The analysis of variance for hydraulic conductivity (Ks) measured in the different management treatments (as shown in Appendix 6.1) at 10-20 cm soil depth.

Variate: Ks					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	2	0.6370	0.3185	0.49	
Treatments	5	7.1492	1.4298	2.20	0.135
Residual	10	6.4858	0.6486		
Total	17	14.2719			

d.f.: degrees of freedom, **s.s.**: sum of squares, **m.s.**: mean sum of squares, **v.r.**: variance ratio.

Appendix 6.11: The analysis of variance for available water capacity (AWC) measured in the different management treatments (as shown in Appendix 6.1) at 0-10 cm soil depth.

Variate: AWC					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	2	16.917	8.458	1.98	
Treatments	5	30.021	6.004	1.41	**0.301**
Residual	10	42.656	4.266		
Total	17	89.594			

d.f.: degrees of freedom, **s.s.**: sum of squares, **m.s.**: mean sum of squares, **v.r.**: variance ratio.

Appendix 6.12: The analysis of variance for available water capacity (AWC) measured in the different management treatments **(**as shown in Appendix 6.1) at 10-20 cm soil depth.

Variate: AWC					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F probability**
Replicates	2	10.45	5.23	0.45	
Treatments	5	192.74	38.55	3.31	**0.051**
Residual	10	116.49	11.65		
Total	17	319.68			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 6.13: The Duncan multiple range-test for water content at different matric potentials measured in the different management treatments (as shown in Appendix 6.1) at 0-10 and 10-20 cm soil depth.

Matric potentials (kPa)	**0**	**2**	**4**	**6**	**8**	**10**	**33**	**100**	**1500**
At 0-10 cm depth									
Treatments				**Water content (%)**					
BRF0	44.87a	39.43a	37.65a	35.24a	34.54a	32.59a	31.63a	28.71a	21.07a
BSF0	61.12a	51.61ab	48.88a	46.76a	42.32ab	39.74ab	37.73ab	36.65ab	30.73ab
MF0	41.07a	40.70a	40.27a	39.63a	38.35a	36.95a	34.93a	34.21ab	25.36ab
BRF	59.47a	52.67ab	46.89a	41.82a	34.76a	31.71a	28.32a	25.43a	20.18a
BSF	57.47a	54.48b	51.59a	49.28a	47.59b	47.34b	45.04b	42.60b	35.99b
MF	60.50a	55.43b	49.04a	45.54a	42.42ab	39.13ab	37.93ab	35.90ab	27.99ab
At 10-20 cm depth									
BRF0	43.32ab	35.16ab	30.77ab	29.24ab	28.62ab	27.60ab	25.93ab	23.72ab	15.91ab
BSF0	56.79bc	49.68bc	47.75c	39.84bc	38.07bc	37.37bc	36.60bc	35.60bc	32.10cd
MF0	34.98a	23.60a	24.83a	23.68a	23.04a	22.22a	21.25a	19.13a	10.61a
BRF	58.23bc	56.04c	43.93bc	38.37bc	35.35abc	32.84abc	30.54abc	26.52ab	18.80abc
BSF	65.71c	62.85c	54.67c	46.84c	43.42c	42.95c	42.34c	41.52c	32.87d
MF	56.61bc	52.82bc	47.92c	44.74c	42.13bc	40.57bc	37.31bc	35.68bc	28.91bcd

Different small letters in a column within the same matric potential and depth indicate significantly different values ($p < 0.05$)

Appendix 6.14: The analysis of variance for penetrometer resistance (PR) measured in the different management treatments (as shown in Appendix 6.1) at 0-10 cm soil depth.

Variate: PR

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Rep	2	27775.	13887.	3.56	
Treatments	5	166690.	33338.	8.54	0.002
Residual	10	39020.	3902.		
Total	17	233485.			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.

Appendix 6.15: The analysis of variance for penetrometer resistance (PR) measured in the different management treatments (as shown in Appendix 6.1) at 10-20 cm soil depth.

Variate: PR

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Reps	2	45119.	22560.	2.67	
Treatments	5	98562.	19712.	2.33	0.119
Residual	10	84506.	8451.		
Total	17	228187.			

d.f.: degrees of freedom, **s.s**.: sum of squares, **m.s**.: mean sum of squares, **v.r**.: variance ratio.