

**SOIL CARBON AND PHOSPHORUS FRACTIONS AND
MICROBIAL ACTIVITY IN SANDY LOAM HUMIC SOIL
UNDER CONTRASTING SUGARCANE HARVEST SYSTEMS**

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PREFACE

The research contained in this thesis was completed by Nontokozo Mkhonza in KwaZulu-Natal, Pietermaritzburg Campus, Republic of South Africa. Experimental work was carried out while registered in the School of Agriculture, Earth and Environmental Sciences, University of KwaZulu-Natal, Pietermaritzburg, under the supervision of Professor Pardon Muchaonyerwa.

This thesis, submitted for the degree of Doctor of Philosophy in the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, School of Agriculture, Earth and Environmental Sciences, Pietermaritzburg campus, represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any other University. Where use has been made of the work of others, it is duly acknowledged in the text.



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November 2022

I certify that the above statement is correct and as the candidate's supervisor I have approved this thesis for submission.



.....

Professor Pardon Muchaonyerwa

Supervisor

November 2022

DECLARATION 1 - PLAGIARISM

I, Nontokozi Mkhonza, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
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DECLARATION 2 - PUBLICATIONS

The chapters of this thesis were written in different journal manuscript format. One manuscript has been published in the Journal of Soils and Sediments. Manuscripts from three other chapters are “under review”.

Chapter 4

NP Mkhonza & P Muchaonyerwa

Organic carbon and microbial activity in Umbric Rhodic Ferralsol soils under green cane relative to pre-harvest burning of sugarcane. Published in **Journal of Soils and Sediments**, <https://doi.org/10.1007/s11368-022-03358-x>

Author contributions:

NP Mkhonza conceived paper with P Muchaonyerwa, as the supervisor. NP Mkhonza collected and analysed data, and wrote the paper. P Muchaonyerwa contributed through supervision of the work and providing valuable comments to the manuscript.

Chapter 5

NP Mkhonza & P Muchaonyerwa

Aggregate stability and organic carbon fractions in loamy humic soils under green cane compared to pre-harvest burning (Submitted to the **Journal of Soil Science and Plant Nutrition**, Manuscript ID: JSSP-D-22-01280)

Author contributions:

NP Mkhonza conceived paper with P Muchaonyerwa, as the supervisor. NP Mkhonza collected and analysed data, and wrote the paper. P Muchaonyerwa contributed through supervision of the work and providing valuable comments to the manuscript.

Chapter 6

NP Mkhonza & P Muchaonyerwa

Charcoal- and iron/aluminium-bound carbon as affected by sugarcane cultivation on loamy humic soils (Submitted in **Environmental Monitoring and Assessment**)

Author contributions:

NP Mkhonza conceived paper with P Muchaonyerwa, as the supervisor. NP Mkhonza collected and analysed data, and wrote the paper. P Muchaonyerwa contributed through supervision of the work and providing valuable comments to the manuscript.

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November 2022

ABSTRACT

Soils have the potential to store large quantities of organic carbon, with benefits of mitigating climate change and improved crop/pasture productivity. Humic soils, which are only known to occur in South Africa, are highly weathered soils with high acidity, low base status, > 1.8% soil organic carbon (SOC) and good internal drainage and the main land uses on these soils include forestry, grassland, maize and sugarcane production. Where sugarcane is produced, pre-harvest burning is practiced with the aim of removing excess trash, for easy harvesting and improving sucrose recovery, while policy shifts appear to be developing globally towards green cane production, in view of climate change. There is a paucity of research findings on the effects of green cane relative to pre-harvest burning on SOC, phosphorus and microbial activity in these acidic and carbon-rich humic soils. Therefore, the objective of this study was to determine the effects of green cane relative to pre-harvest burning on concentrations of stocks and fractions of SOC, P fractions, soil microbial biomass and activities of enzymes associated with cycling of carbon and P in sandy loam humic soils. The soils were analysed for SOC and its fractions, soil aggregates stability, microbial biomass carbon (MBC), β -glucosidase enzyme, charcoal C, P fractions, MBP, acid and alkaline phospho-monoesterase and organo-mineral complexes. Soil C stocks and soil microbial quotient (SMQ) were calculated from SOC concentrations and MBC. Green cane retention resulted in higher SOC content and stocks, MBC, β -glucosidase activity and SMQ when compared to pre-harvest burning in these humic soils. The carbon content in the macro-aggregates fraction constituted > 60% of total SOC making it the primary C storage fraction for both green cane and burnt cane. The aggregate stability (only in the top 10 cm) and SOC in macro- and micro-aggregate were higher, while mineral associated C in $\mu\text{Silt} + \mu\text{Clay}$ was lower under green cane than under burnt cane. Additional analysis of effects of sugarcane production relative to forest showed that soils under sugarcane had lower charcoal-C than forest only at Wartburg but not Eshowe and Eston, while the SOC, Fe and Fe+Al in Al/Fe-OM complexes were significantly higher under sugarcane than forest only at Eshowe but not Eston and Wartburg. Soil charcoal-C was significantly higher under pre-harvest burning than green cane, with no differences in SOC, Al and Fe in Al/Fe-OM complexes, between the production systems. Green cane reduced pH and increased available P, P fixation through precipitation with Al and Fe and as CDB-P when compared to burnt cane. The MBP in the top 20 cm, and activity of acid phospho-monoesterase was significantly higher, while that of alkaline phospho-monoesterase was lower under green cane when compared to burnt cane. The findings of this study imply that green cane production on

sandy loam humic soils increase SOC storage, especially in macro-and micro-aggregates, microbial activity, and P availability, when compared to pre-harvest and that charcoal C and organo-mineral complexes, contribute to the high OC in these soils. The findings suggest that green cane production has a potential for sustainable sugarcane production when compared to burnt cane. Green cane production could contribute to lowering of greenhouse gas emissions when compared with the burnt cane.

Keywords: Acidic soils · Aggregates stability · Carbon fractions · Charcoal · Enzymes activities · Organo-mineral complexes· Phosphorus fractions

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TABLE OF CONTENTS

PREFACE.....	i
DECLARATION 1 - PLAGIARISM	ii
DECLARATION 2 - PUBLICATIONS	iii
ABSTRACT.....	v
ACKNOWLEDGEMENTS.....	vii
CHAPTER ONE.....	1
GENERAL INTRODUCTION.....	1
1.1 Rational for the research	1
1.2 Justification of the study	3
1.3 Research questions	4
1.4 Specific objectives.....	4
1.5 Hypothesis.....	5
CHAPTER 2	6
SOIL ORGANIC CARBON AND PHOSPHORUS AND THEIR FRACTIONS, AND MICROBIAL ACTIVITIES IN SOILS UNDER GREEN CANE AND PRE-HARVEST BURNING: A REVIEW.....	6
2.1 Introduction	6
2.2 Carbon dynamics in soils	8
2.3 Sugarcane harvesting systems, their benefits and challenges	9
2.4 Effects of burning crop residues on soil organic carbon mineralisation	10
2.5 Aggregates stability of soils under green cane relative to burnt cane.....	10
2.6 Fractionation of SOC in soils and effects of soil types	12
2.7 Effects of pre-harvest burning relative to green cane on soil organic carbon and its fractions.....	13
2.7.1 Carbon associated with macro- and microaggregates	15
2.7.2 Charcoal C fraction.....	16
2.7.3 Mineral associated C fraction	17

2.7.4 Microbial biomass carbon	17
2.8 Effects of burning and residues retention on phosphorus pools.....	18
2.8.1 Soil P fractions.....	18
2.8.2 Soil microbial biomass P	19
2.9 Effects of burning and green cane retention on soil enzymes activities	20
2.9.1 β -D-Glucosidase enzyme.....	20
2.9.2 Acid and Alkaline phospho-monoesterase	21
2.10 Distribution of humic soils in South Africa	22
2.11 Properties of humic soils	22
2.12 Conclusion.....	23
CHAPTER 3	25
Land use effects on soil organic carbon bound in aluminium/iron-organic matter complexes: A global meta-analysis	25
Abstract	25
3.1 Introduction	26
3.2 Methods and materials	27
3.2.1 Review setup.....	27
3.2.2 Definition of study variables, soil and environmental factors.....	30
3.2.3 Data analysis.....	32
3.3 Results	32
3.3.1 General statistics of soil and environmental conditions	32
3.3.2 Relationships among organo-mineral complexes, soil parameters, and environmental conditions.....	35
3.3.3 SOC, Al/Fe bound C and Al/Fe-organic matter complex under different climatic conditions.....	37
3.3.4 Distribution of SOC and Al, Fe and C in organo-mineral complexes under different land uses	41

3.3.5 Variation of organo-mineral associations with soil properties and effects of land use	42
3.4 Discussion	49
3.4.1 Climate effects on SOC, Al/Fe bound C, organo-mineral complexes.....	49
3.4.2 The impact of land use on SOC and organically bound Al and Fe	50
3.5 Conclusions	53
CHAPTER 4	54
Organic carbon and microbial activity in Umbric Rhodic Ferralsol soils under green cane relative to pre-harvest burning of sugarcane	54
Abstract	54
4.1 Introduction	55
4.2 Methods and material	57
4.2.1 Study sites and soils.....	57
4.2.2 Soil Sampling	59
4.2.3 Soil chemical analysis	59
4.2.4 Soil organic carbon.....	59
4.2.5 Determination of soil C stocks	60
4.2.6 Soil microbial biomass C.....	60
4.2.7 β -D-glucosidase enzyme assay	61
4.2.8 Scanning Electron Microscope analysis	61
4.2.9 Statistically analysis	61
4.3 Results	62
4.3.1 Selected soil physico-chemical properties.....	62
4.3.2 Soil organic carbon concentrations and stocks.....	64
4.3.3 Soil microbial biomass C and microbial biomass quotient	66
4.3.4 β -D-glucosidase enzyme assay	67
4.3.5 Relationship analysis of different soil quality indicators	68
4.3.6 Scanning Electron Microscope analysis of soil at selected depths.....	70

4.4 Discussion	71
4.4.1 Soil organic carbon content and C stocks.....	71
4.4.2 Soil microbial biomass carbon and β -D-glucosidase enzyme as affected by burning and soil depths	72
4.4.3 Scanning Electron Microscope	74
4.5 Conclusion.....	74
CHAPTER 5	75
Organic carbon fractions and aggregates stability in Umbric Rhodic Ferralsol soils under green cane compared to pre-harvest burning	75
Abstract	75
5.1 Introduction	76
5.2 Methods and materials	78
5.2.1 Study sites and soils.....	78
5.2.2 Soil structural stability.....	78
5.2.3 Soil organic carbon fractions	79
5.2.4 Soil particle morphology (Scanning Electron Microscopy)	82
5.2.5 Statistically analysis	82
5.3 Results	82
5.3.1 Aggregate stability.....	82
5.3.2 Soil organic carbon fractions associated with macro- and micro-aggregates and silt +clay	83
5.3.3 Carbon fractions from further fractionation of the macro-aggregates.....	84
5.3.4 Carbon fractions from further fractionation of the micro-aggregates	85
5.3.5 Overall soil carbon fractions.....	86
5.3.6 Correlation analysis	90
5.3.7 Principal Component Analysis	92
5.3.8 Soil morphology under pre-harvest burning and green cane harvest	93
5.4 Discussion	94

5.4.1 Effects of burning on soil aggregates stability	94
5.4.2 Response of soil organic carbon fractions to pre-harvest burning	94
5.4.3 Soil morphology in pre-harvest burning relative to green cane	97
5.5 Conclusion.....	97
CHAPTER 6	99
Charcoal- and iron/aluminium-bound carbon under contrasting sugarcane harvesting systems and to forest on loamy humic soils.....	99
Abstract	99
6.1 Introduction	100
6.2 Methods and materials	102
6.2.1 Soils (site description and characteristics).....	102
6.2.2 Soil sampling and processing	158
6.2.3 Charcoal-C method validation.....	159
6.2.4 Determination of Charcoal-C from humic soils	160
6.2.5 Carbon, aluminium (Al) and iron (Fe) in Al/Fe-organic matter complexes.....	160
6.2.6 Statistically analysis	161
6.3 Results	161
6.3.1 Effects of sugarcane production relative to forest in humic soils.....	161
6.3.2 Soil charcoal-C, non-charcoal POM-C and OC, Al and Fe in Al/Fe-organic matter complexes under green cane and burnt cane	164
6.3.3 Principal component analyses of management effects on different soil parameters	165
6.3.4 Relationship analysis of different soil quality indicators	166
6.4 Discussion	170
6.4.1 Distribution of charcoal-C	170
6.4.2 Effects of land use on Al/Fe-bound C and Alp- and Fep-organic complexes.....	171
6.5 Conclusion.....	172
CHAPTER 7	173

Green cane harvesting effects on fractions and availability of phosphorus and related enzymes relative to pre-harvest burning on a sandy loamy humic soil	173
Abstract	173
7.1 Introduction	174
7.2 Methods and material	176
7.2.1 Study sites (site and characteristics)	176
7.2.2 Soil P fractionation	176
7.2.3 Soil microbial biomass P	177
7.2.4 Acid and alkaline phospho-monoesterase assay	177
7.2.5 Statistical analysis.....	178
7.3 Results	178
7.3.1 Available phosphorus	178
7.3.2 Soil P Fractions.....	179
7.3.3 Microbial biomass P and activity of acid and alkaline phospho-monoesterase	181
7.3.4 Relationship between soil parameters and phosphorus fractions	184
7.4 Discussion	187
7.4.1 Soil phosphorus fractions	187
7.5 Conclusion.....	189
CHAPTER 8	191
GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS	191
8.1 General discussion.....	191
8.2 Conclusions	196
8.3 Recommendations for future research.....	196
REFERENCES	198

LIST OF TABLES

Table 2. 1 Average total organic carbon in sugarcane under different harvest systems (Souza et al. 2012)	14
Table 2. 2 Total C in native forest and in the burned and unburned sugarcane areas, 2, 6, 8 years after replanting, in the 0-10 and 10-20 cm depths (Galdos et al. 2009).	15
Table 2. 3 Average Soil microbial biomass C in sugarcane under different harvest systems (Souza et al. 2012)	18
Table 2. 4 Soil enzyme activity as affected by sugarcane management (Vuyyuru et al. 2020)	21
Table 3. 1 List of authors, countries, soil organic carbon, clay content, MAP (mean annual precipitation) and MAT (mean annual temperature) for the different experimental sites	29
Table 3. 2 List of classes defining the environmental factors and soil conditions.....	31
Table 3. 3 Descriptive statistics of soil and environmental parameters and organo-mineral complexes	34
Table 3. 4 Spearman rank correlation coefficients between selected soil and environmental conditions.....	36
Table 4. 1 Soil fertility status of soils in pre-harvest and green cane harvest systems	63
Table 4. 2 Effects of pre-harvest burning and green cane on Microbial Quotient.....	67
Table 4. 3 Spearman's rank correlation analysis of soil parameters response	69
Table 5. 2 Effects of pre-harvest burning and green cane retention on soil C fractions	84
Table 5. 3 Soil organic C fractions from further separation of macro-aggregates from the bulk soil C	85
Table 5. 4 Carbon fractions associated with micro-aggregates under in green cane relative to pre-harvest burning	86
Table 5. 5 Carbon associated with cPOM-C, fPOM-C, cPOM and μ Silt + μ Clay in the whole soil under green cane compared to pre-harvest burning	87
Table 5. 6 Pearson correlation analysis between soil parameters in the top 30 cm depth	91
Table 6. 1 Geographic coordinates and climate of the study sites (Malepfane et al. 2022)..	103

Table 6. 2 Effects of land use and site on total C concentration in soils under sugarcane and forest	158
Table 6. 3 Total, particulate and mineral associated soil organic carbon (g kg^{-1}) in humic soils under green cane and pre-harvest burning	159
Table 6. 4 Effects of land use and site on concentration (g kg^{-1}) of charcoal-C and Al/Fe bound C	162
Table 6. 5 Effects of land use and site on the concentration of Alp, Fep and Alp+Fep (g kg^{-1})	163
Table 6. 6 Charcoal-C, non-charcoal POM-C and Al/ Fe bound C concentration (g kg^{-1}) in green cane and pre-harvest burning	164
Table 6. 7 Effect of green cane relative to pre-harvest burning and depths on organically bound Al and Fe (g kg^{-1}).....	165
Table 6. 8 Spearman's rank correlation analysis of soil parameters response	169
Table 7. 1 Extractable P (mg kg^{-1}) in green cane retention relative to burnt cane.	179
Table 7. 2 Soil phosphorus fractions (mg kg^{-1})	180
Table 7. 3 Effects of burning and depth on Al-P (mg kg^{-1}).....	180
Table 7. 4 Pearson correlations between soil parameters and microbial response.....	185

LIST OF FIGURES

Figure 2. 1 Schematic diagram of soil organic carbon dynamics (Ramesh et al. 2019).....	9
Figure 2. 2 Physical fractionation scheme for SOC (Huang et al. 2010).....	12
Figure 2. 3 A map showing distribution of humic soils in South Africa (Fey 2010)	22
Figure 3. 1 Global distribution of the study sites shown by shaded circles on the map.	28
Figure 3. 2 Effect of climate conditions on soil carbon _a , Al/Fe bound C _b , organically bound Al _c and Fe _d	38
Figure 3. 3 Effect of temperature on soil organic carbon _a , Al/Fe bound C _b , organically bound Al _c and Fe _d	39
Figure 3. 4 Effect of precipitation on soil organic carbon _a , Al/Fe bound C _b , organically bound Al _c and Fe _d	40
Figure 3. 5 Effect land use (cropland, forestry and grassland) on soil organic carbon _a , Al/Fe bound C _b , organically bound Al _c and Fe _d	41
Figure 3. 6 Effect of soil pH on soil organic carbon _a , Al/Fe bound C _b , organically bound Al _c and Fe _d	43
Figure 3. 7 Effect of soil texture on soil organic carbon _a , Al/Fe bound C _b , organically bound Al _c and Fe _d	45
Figure 3. 8 Effects of Soil Texture on organically bound Al and Fe under different land uses. Figures a, c and e indicates organically bound Al in clay, loam and sand, respectively.	46
Figure 3. 9 Effects soil organic carbon content on organically bound Al and Fe concentration in different land use.	48
Figure 4. 1 Location of the study area within iLembe, KwaZulu-Natal, South Africa and the soils and geology information.....	58
Figure 4. 2 Effects of pre-harvest burning on soil organic carbon relative to green cane retention.	64
Figure 4. 3 Effects of pre-harvest burning on soil C stocks relative to green cane retention.	65
Figure 4. 4 Effects of pre-harvest burning on soil microbial biomass carbon relative to green cane retention.....	66
Figure 4. 5 Effects of pre-harvest burning on β -glucosidase enzyme activity relative to green cane retention.....	68

Figure 5. 1 Soil C fractionation scheme.....	81
Figure 5. 2 Mean weight diameter in green cane relative to pre-harvest burning.	83
Figure 5. 3 Relationship between MWD (mean weight diameter (mm)) and SOC (g kg^{-1}) concentrations in soils under green cane and pre-harvest burning.	88
Figure 5. 4 Relationship between the coarse soil organic carbon fractions and bulk soil organic carbon.....	89
Figure 5. 5 The first two axes of Principle Component Analysis are based on effects of pre-harvest burning and green cane on different soil properties.	92
Figure 5. 6 Scanning electron micro images of the $<53 \mu\text{m}$ fraction at 2000 times magnification zooming to $10 \mu\text{m}$, with depth increment, for pre-harvest burning at (a) 0-5 cm and (c) 80-100 cm depth and green cane at (b) 0-5 cm and (d) 80-100 cm depth.	93
Figure 6. 1 Charcoal-C retained at different application rates of charcoal after digestion. ..	160
Figure 6. 2 The first two axes of Principle Component Analysis are based on effects of land use on different soil properties.....	166
Figure 7. 1 Effects of green cane relative to pre-harvest burning and depths on soil microbial biomass phosphorus	181
Figure 7. 2 Effects of green cane relative to pre-harvest burning and sampling depth on acid phospho-monoesterase enzymes.....	182
Figure 7. 3 Effects of burning and sampling depth on alkaline phospho-monoesterase enzymes.	183
Figure 7. 4 The first two axes of Principle Component Analysis are based on the effect of sugarcane harvest system on different soil properties.	186

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Rational for the research

Storage of carbon (C) in soil contributes significantly to sustainability of agricultural production. This is achieved through improved water holding capacity and nutrient cycling, and the mitigation of climate change through decrease in soil organic C (SOC) losses as methane (CH₄) and carbon dioxide (CO₂) emissions. Globally, the terrestrial surface warming will likely to be elevated by 2.6 – 4.8°C at the end of this century (IPCC 2014). In the year 2015, at COP21, 192 different countries reached an agreement to reduce the rising global warming to <2% above pre-industrial levels by the end of the 21st century (Minasny et al. 2017). This agreement has been accompanied by a determined international research agenda targeting an increase in the overall soil C stocks by 0.4% per annum (Minasny et al. 2017). Soils could either be a sink or source of C depending on the management system (Lal 2019; Sahoo et al. 2019).

Humic soils (Umbric Rhodic Ferralsol), which are only known to occur in South Africa, are highly weathered and acidic, with > 1.8% organic C (Fey, 2010). Findings of Malepfane et al. (2022) demonstrated that some humic soils have > 10% C in the top 10 cm, while others have > 2% C to 100 cm depth. While national SOC stocks in humic soils are unknown, Malepfane et al. (2022) showed that some of these soils store > 400 Mg C ha⁻¹ compared to <100 Mg C ha⁻¹ for other soils, and if 1% of the C is mineralised, > 140 kg CO₂ ha⁻¹ will be emitted to the atmosphere. Poor management of humic soils could result to significant increase in CO₂ emissions and soil organic C loss, which is associated with decrease in soil fertility over time. The common land uses on humic soils include forest, grassland, and cropping, particularly sugarcane and maize production, where farmers management practices could significantly affect SOC sequestration/loss over time.

While protection of SOC in soil aggregates has often been used to explain organic matter stabilisation in soil environment (Zhong et al. 2017; Liu et al. 2021). A preliminary study showed that > 85% of C in some humic soils is in particulate form. This could possibly be a

result of protection in macro- and micro-aggregates (Fuentes et al. 2012) or due to acidity, which limits microbial activity, with lower SOC being associated with the mineral fraction. Soil pH is an important factor in decomposition of organic materials and mineralisation of elements in soils as it affects microbial communities (Sheng and Zhu 2018; Zhang et al. 2020). In addition to the effect of soil acidity, the SOC in particulate form in humic soils could also be a result of accumulation of charcoal, from the burning of biomass over the years, and from formation of Fe- and Al-organic matter complexes under acidic conditions in these soils. Considering that large portions of humic soils are under sugarcane production, total C stocks could be reduced by management, such as burning of trash in the long term, with such decline depending on the dominant fraction of SOC and its stability in the soils.

Globally, farmers practise pre-harvest burning where sugarcane trash is burnt, while others harvest without burning the trash (green cane). In South Africa, sugarcane is mostly produced in KwaZulu-Natal and Mpumalanga Provinces and approximately 18.0 million metric tons of sugarcane was produced in 2021/2022, which showed a decline, with several fluctuations over the years of production (Statista 2022). High biomass addition from green cane could increase SOC content (Ghimire et al. 2017). Decomposition of highly degradable sugarcane biomass could release labile C which can stimulate microbial activity and decomposition of inherent or native soil C (Positive priming effect). (Gao et al. 2017). Conversely, pre-harvest burning could produce charcoal that may remain in stable form, due to its recalcitrance, and may possibly translocate to deeper soil layers. Charcoal C can be stored in the soil for millions of years, and therefore contributes to SOC sequestration (Glaser et al. 2002; Lehmann et al. 2006). Unlike other C pools, which are highly degradable, charcoal is considered relatively more recalcitrant C sink. While addition of charcoal C could be resistant to microbial attack, there is evidence that this SOC fraction could take long time to decompose over time depending on management strategies (DeLuca and Aplet 2008). In addition, reduced soil aggregate stability through continuous burning could lead to erosion losses of the charcoal C from the soil surface (Abney and Berhe 2018; Girona-Garcia et al. 2018; Zavala et al. 2010). The effects of green cane relative to pre-harvest burning on bulk SOC concentration, SOC fractions in aggregates and soil C stocks in humic soils are not clearly understood. This understanding is useful in improving soil management policies, in view of sustainable crop production and climate change. The high acidity of these soils could limit microbial activities associated with cycling of SOC and P, which together with P fixation, could lead to P deficiencies in crops. Green cane could significantly increase substrate addition of large amounts of fresh residues resulting in

increased microbial and enzymes activities (Vuyyuru et al. 2020; Zainudin et al. 2022). Conversely, burning could reduce the effects of soil acidity, through the liming effects of ash as a results of calcium carbonates contained in the ash. The liming potential of ash would results to reduced P fixation. But then, this would result in a decrease in labile fractions of SOC due to increase microbial activities following the increase in soil pH. This would negatively affects the activities of microorganisms and enzymes associated with C and P cycling in soils. In addition, larger biomass removal through pre-harvest burning harvesting (tops not removed), may also export C and P from the agricultural land, with negative effects on total and available P, and other P fractions. The overall effects of green cane relative to pre-harvest burning on P fixation and activity of microorganisms and enzymes associated with P availability in humic soils are not clearly understood. These needs to be clearly understood in a view of reducing fertiliser P requirements for sustainable crop production. Therefore, there is a need to understand the effects of land use including pre-harvest burning relative to green cane on SOC stabilisation, P, microbial and enzymes activities associated with C and P cycling in humic soils.

1.2 Justification of the study

Soils are a key compartment for climate change regulation that can sequester large quantities of C (Bispo et al. 2017). Global warming and climate change have increased the interest in search on strategies of mitigation. The interest on understanding strategies to improve SOC storage in soils has rapidly increased following the declaration of “4 per thousand” program at COP21 in Paris (Lal 2019; Soussana et al. 2019). Umbric Rhodic Ferralsol, locally known as humic soils, which are known to occur mostly occur in parts of Eastern Cape, KwaZulu-Natal and Mpumalanga, South Africa, where they are generally under forest, grassland and cropping systems (Fey 2010), store large quantities of SOC (>1.8%) which is beneficial for crop productivity through improved soil fertility. Different management practices could lead to losses of the C in these soils contributing to climate change. Crop management systems, including residue retention and burning, could accelerate decomposition of resident SOC and also affect dynamics of soil P. Some areas covered by humic soils are under sugarcane production where retention of green residues or burning is practised. In addition, conversion of some areas under natural forest to sugarcane production could have significant effects on SOC in these soils, including accumulation of charcoal C as a result of burning of residues. However, continuous cultivation of humic soils could possibly results to significant losses of SOC

including the stable C in soils. Therefore, there is a need to understand the underlying stabilisation mechanisms of different SOC fractions and their contribution to SOC stocks, microbial activities and P dynamics in these soils as affected by green cane sugarcane production relative to pre-harvest burning in humic soils. The contribution of particulate SOC associated with macro- and micro-aggregate, charcoal-C and mineral associated fraction to overall SOC, in humic soils need to be clearly understood. The overall effects of green cane relative to pre-harvest burning on SOC concentration in the different fractions and C stock, P fixation and activity of microorganisms and enzymes associated with C and P cycling in humic soils are not clearly understood, and this understanding is useful in improving SOC storage and fertiliser P management. The findings from this study could make significant contributions on decision-making concerning the strategies for the appropriate management of humic soils, to ensure sustainability of production while mitigating climate change.

1.3 Research questions

- What are the effects of green cane relative to pre-harvest burning on SOC concentration and C stocks, MBC, and β -D-glucosidase enzyme activity in humic soils?
- How does pre-harvest burning relative to green cane affects aggregate stability and SOC fractions associated with aggregate sizes in humic soils?
- How do (i) sugarcane production relative to forest and (ii) green cane production relative to pre-harvest burning affect particulate SOC in charcoal (charcoal-C) form and in Al/Fe-OM complexes in humic soils?
- Could green cane production affect soil P fractions, microbial biomass phosphorus (MBP), activity of acid and alkaline phospho-monoesterase relative to pre-harvest burning in humic soils

1.4 Specific objectives

The general objective of the current study was to examine the effects of land use (sugarcane production and forest) on SOC and mechanisms of C stabilisation and microbial activities in selected humic soils of South Africa. The specific objectives were:

- Determine the effects of green cane relative to pre-harvest burning on SOC content and C stocks, MBC, and β -D-glucosidase enzyme activity in humic soils.

- Determine the effects of green cane relative to pre-harvest burning on aggregate stability and SOC fractions.
- Determine the effects of (i) sugarcane production relative to forest and (ii) green cane relative to burnt cane, on charcoal-C and Al/Fe-OM complexes in some humic soils, was also related. (This part of the thesis linked the work with other work in the research group which included humic soils of a variety of textures.)
- Determine the effects of green cane relative to pre-harvest burning on soil P fractions, MBP, activity of acid and alkaline phospho-monoesterase.

1.5 Hypothesis

- Green cane does not significantly increase bulk SOC and C stocks, but increase C sequestration and concentration of MBC and β -D-glucosidase enzymes activities in humic soils relative to pre-harvest burning.
- Green cane does not significantly reduce soil aggregates stability and SOC fractions compared to pre-harvest burning in humic soils.
- Sugarcane production relative to forest, and green cane relative pre-harvest burning reduce charcoal C and Al/Fe -OM complexes in humic soils.
- Green cane does not significantly increase P fixation, MBP and acid phosphor-monoesterase activity nor significantly reduce alkaline phosphor-monoesterase activity in humic soils relative to pre-harvest burning.

CHAPTER 2

SOIL ORGANIC CARBON AND PHOSPHORUS AND THEIR FRACTIONS, AND MICROBIAL ACTIVITIES IN SOILS UNDER GREEN CANE AND PRE-HARVEST BURNING: A REVIEW

2.1 Introduction

Globally, there have been a drastic increase in atmospheric CO₂ and climate change projections show rapidly changing climate conditions in Africa (Conway and Schipper 2011). Soils, as a major sink of C, could mitigate the effects of climate change through increased C sequestration (Lal 2004; Khan et al. 2021). Understanding the effects of different soil management systems on decomposition of soil organic matter (SOM) is essential for sustainable management of soils to reduce emissions of CO₂ and its contribution to global warming. The agricultural sector contributes approximately 10-12 % to the global greenhouse gas emissions (Zhang et al. 2014; Lynch et al. 2021). Sugarcane management could either be green cane harvest system, where large amount of trash is retained on the soil surface during harvesting, and pre-harvest system where the residues are burnt prior to harvesting (Cerri et al. 2011; Keeping et al. 2022). Pre-harvest burning of sugarcane could result to rapid increase in greenhouse gas emissions due to the increase in SOC depletion as CO₂ due to decomposition of SOM (Vera and Acreche 2018). Many areas under sugarcane production practice pre-harvest burning of the cane while others practice green cane production, where the cane is harvested without prior burning (Graham and Haynes 2002; Rasche and Sos Del Diego 2020).

Large quantities of C are lost to the atmosphere during burning of residues resulting in decrease C sequestration (Hobley et al. 2017; Merino et al. 2019). However, incorporation of burned crop residues may increase concentrations of SOC in the form of charcoal (charcoal-C), which is stable and highly resistant to decomposition and microbial attack, which may consequently improve SOC content and stocks over time. On the other hand, crop residues addition is reported to increase SOM content, but the response is highly dependent on the elemental composition of the residues returned (Lenka et al. 2019), which determine whether positive or negative priming effects would occur. For example, residues with low C: N would result to positive priming effects, where microbial activity rapidly increases and uses resident SOM as a substrate, thereby decreasing C sequestrations, while high C: N could result to negative

priming effects, resulting to accumulation of resident SOC (Liang et al. 2017; Ma et al. 2020). In addition to biochemical composition of residues added, SOC is often protected in soil aggregates and through formation of organo-mineral complexes which are stable against microbial decomposition. Long-term application of mineral fertilisers (including N and P) increase C sequestration in cropland system (Kirkby et al. 2016). This could be explained by the higher addition of C in residues, high roots growth especially, together with microbial biomass and net humification efficiency where the soil was fertilised with N and P (Kirkby et al. 2016).

The process of decomposition is mediated by microorganisms and associated enzymes (Singh et al. 2021). Enzymes plays a major role in nutrient cycling and in regulating the cycles of C and P (Eivazi et al. 2018), are sensitive to land use change, and are therefore, considered as sensitive soil quality indicators (Yu et al. 2019; Raiesi and Salek-Gilani 2020). The effects of burning on soil microbes and associated enzymes is indirect through changes in soil physicochemical properties and reduced substrate availability (Singh et al. 2021). Galdos et al. (2009) observed an increase in soil microbial biomass C under residues retention than burned residues. Soil above and below ground plant biomass is converted to charcoal during burning which is returned into the soil with time (Lewis et al. 2016). Addition of charcoal-C increases soil C sequestration due to high recalcitrant C, which is stable, therefore can be stored for millions of years (Glaser et al. 2002; Lehmann et al. 2006). Most of the charcoal-C mass stored in soils is highly resistant to decomposition as a result it can remain in soil for a longer period (Bartoli et al. 2021).

Addition of charcoal does not only increase soil C sequestration but also increases soil fertility by increasing capacity to retain water and nutrients, cation exchange capacity and reduces losses of nutrients through leaching (DeLuca et al. 2008; Hart and Luckai 2013; Jin et al. 2019). However, while charcoal is considered recalcitrant to microbial decomposition, burning residues also produces ash which increases soil pH that could enhance microbial decomposition. The interaction between C pools and CO₂ emissions rates is essential in understanding soils contribution to climate change (Dash et al. 2019) and managing soils for sustainable crop production under changing climatic conditions. The quantity of CO₂ released from the soils is highly dependent on the proportion of labile C fractions and their decomposition rate (Basile-Doelsch et al. 2020). Increase in decomposition of SOM and mineralisation could increase CO₂ emissions, available N, and fixation of released P in soils, particularly in acidic soils. Microbial biomass P could be essential in reducing P fixation,

through limiting contact time between inorganic P and soil minerals (Ayaga et al. 2006). The contribution of soil microbial P on P fertility needs to be understood for better fertiliser management. The contributions of sugarcane production to SOC sequestration, nutrient cycling and sustainability of the system may be affected by management of crop residues based on the type of harvest system used. The objective of this chapter was to review available literature on the effects of green cane relative to pre-harvest burning on SOC and its fractions, P fractions and microbial and enzymes activities.

2.2 Carbon dynamics in soils

Soil carbon dynamics are influenced by management practice, the soil chemical, biological and physical properties, and climatic conditions (Singh et al. 2018; Bai et al. 2019; Ramesh et al. 2019). Soil organic carbon is added into soils as plant residues and decomposed roots which is decomposed by microorganisms (Figure 2.1; Ramesh et al. 2019). Singh et al. (2018) reported that C stabilisation in soil is mainly through “(i) the biochemical recalcitrance, (ii) formation of organo-mineral complexes through chemical interactions; (iii) physical protection owing to occlusion within soil aggregates”. Microorganism affects C dynamics through degradation and decomposition of organic forms to inorganic forms in soil (Gougoulas et al. 2014; Swarup et al. 2019). Soil organic carbon is differentiated into several fractions depending on their degree of decomposition and stability (Tian et al. 2016; Ramesh et al. 2019).

The labile fractions of SOC are those which are sensitive and rapidly respond to environmental changes and addition of fresh biomass. On the other hand, the C in recalcitrant organic matter, resistant to decomposition, is high stable and not easily decomposed by microorganism as a result they accumulate for years in the soil (Singh et al. 2018; Ramesh et al. 2019). While dissolved organic carbon and particulate organic carbon are labile pools of C, their translocation, which vary with soil properties, may limit their decomposition rates in soils (Schluter et al. 2022). For instance, the C can be translocated from the surface to deeper layers in sandy soils and became more recalcitrant than when in the surface due to limited microbial activities as a result of low gas exchange (limited oxygen) (Singh et al. 2018). Stabilisation within micro and macro aggregates is one of the most important factors affecting organic matter stabilisation (Angst et al. 2021; Angst et al. 2022). Cultivation depletes C rich macro aggregates resulting in significant losses of C in soils (Benbi et al. 2015). In acidic soils, SOC could be protected and accumulates due to limited microbial activities to mediate the decomposition process, while liming increase microbial activity with potential effects on loss of SOC (Kunito

et al. 2016; Mkhonza et al. 2020). Understanding the effects of management on the accumulation/loss of these soil organic matter fractions is essential for the sustainability of the production system.

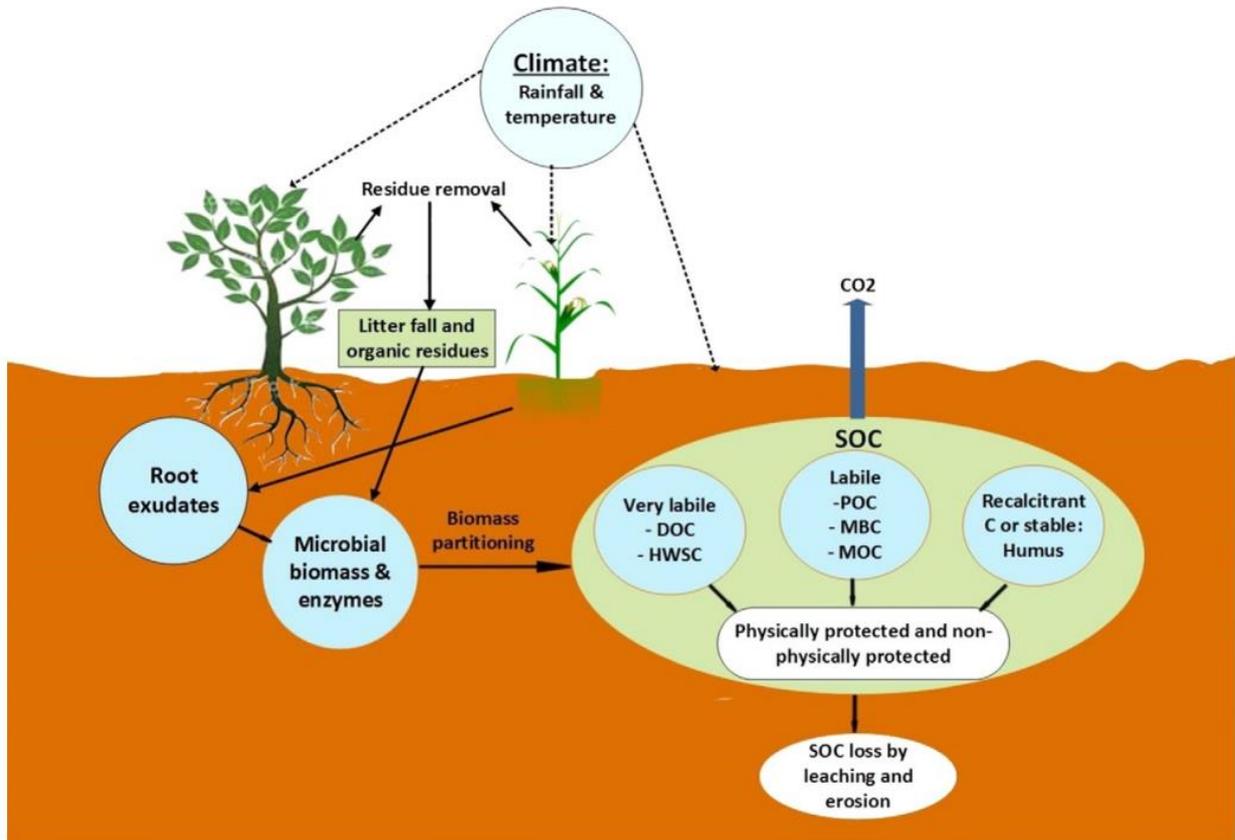


Figure 2. 1 Schematic diagram of soil organic carbon dynamics (Ramesh et al. 2019)

2.3 Sugarcane harvesting systems, their benefits and challenges

Production of sugarcane is practised at a global scale, including South Africa due to economic value. Sugarcane harvesting is either with burning prior to harvesting (pre-harvest burning) or green cane retention (mechanically harvest system) (Silva et al. 2019). Pre-harvest burning partially removes the trash before harvesting, while the green cane retention all the trash is retained on the soil surface after harvesting (Quassi de Castro et al. 2018; Silva et al. 2019). It is believed that pre-harvest burning of sugarcane results to increase sucrose content and easier process of harvesting when compared to green cane retention (Wood 1991). However, pre-harvest burning has been widely reported to negatively affects soil quality such as reducing soil aggregates stability, resulting to losses of SOC as CO₂ in the long-term, while green cane retention is reported to improve soil quality and productivity (Quassi de Castro et al. 2018; Mkhonza

and Muchaonyerwa 2022). In addition, pre-harvest burning contributes significantly to global warming through increase release of CO₂ (Mugica-Alvarez et al. 2018; Perillo et al. 2022).

2.4 Effects of burning crop residues on soil organic carbon mineralisation

Understanding the effects of burning on SOC mineralisation is critical for predicting SOC responses to climate change (Huang et al. 2019). Fire is used in the slash -and-burn to manage crop residues and ease the harvesting process in agricultural and forestry production (Mataix-Solera et al. 2011; Thomaz 2018). The effect of burning SOC is variable as it is affected by sterilisation, formation of ash, charcoal and also modification of structure of microorganisms responsible for mineralisation of C in soil environment (Gonzalez-Perez et al. 2004; Pandey and Singh 2010). Following fire event, there is evidence of an increase in nutrient availability in the top soil layers from the water-soluble component of ash, which become available for microorganisms (Gonzalez-Perez et al. 2004; Terzano et al. 2021). This change could also be explained by the increase in soil pH (Coates et al. 2018; Terzano et al. 2021).

The increase in available nutrient could increase microbial activity and increase rates decomposition of soil organic matter and mineralisation of elements in soils resulting in losses of C over time. Zhao et al. (2012) observed higher C mineralisation rates and losses of C as CO₂ emission in burned forest plot than unburned sites. In addition, a study conducted in South Africa, showed that burning of grassland residues resulted to higher losses of SOC as CO₂ emission due to increase in nitrogen availability from burning which enhance microbial activity (Abdalla et al. 2016). This high mineralisation rates could be explained by the increase in the labile SOC pool following burning which is more susceptible to losses as CO₂ (Wang et al. 2019). But then, the response of SOC mineralisation following fire event would vary depending on the fire severity, intensity and years (Jhariya and Singh 2021). Koster et al. (2018) observed that more mineralisation and losses of C as CO₂ were observed in fields where burning have been practised for years when compared to recently burnt fields. The effects of burning on CO₂ emission could depend on soil characteristics including stabilisation of the SOC in aggregates and formation of organo-mineral complexes.

2.5 Aggregates stability of soils under green cane relative to burnt cane

Soil aggregates stability and formation is significantly enhanced by soil organic matter (Zhou et al. 2020; Liu et al. 2021) especially up to a SOC content of 3-4 % (Mbanjwa et al. 2022).

Aggregation is largely affected by management systems and provides protection against microbial attack (Carter 2020). This explains low soil C observed in most cropland areas compared to vegetation (Liu et al. 2020) and grassland areas. Such effects in cropland are caused by tillage where soil C is exposed to microbial attack following disaggregation of soil aggregates (Liu et al. 2019). Scientific evidence shows that smaller aggregates tend to contribute more in soil C sequestration in most soils, while the decrease in aggregates size results to a decreases soil potential to sequester more C (Liu et al. 2020). Formation of organo-mineral complexes especially in acidic soils posing high Al and Fe the organic matter seems to be high due to the organo-mineral complexes. These organo-mineral complexes protect the organic matter from microbial attack (Singh et al. 2018). This explains the high organic matter in acidic soils which is recalcitrant, in addition to limited microbial activities in acidic soils, which further slows down decomposition process.

The effects of burning on soil aggregates have been extensively studied by several researchers (Garcia-Oliva et al. 1999; Mataix-Solera et al. 2002; Varela et al. 2010; Jordan et al. 2011). Burning crop residues/trash has been shown to results in the destruction of macro-aggregates to micro-aggregates, especially at high fire severity (Mataix-Solera et al. 2002a). Andreu et al. (2001) revealed that forest burning largely affected the aggregates with diameter > 2 mm, although Varela and Keizer (2010) observed no change and explained the observation using increased water repellence caused by fire, resulting to improved aggregates stability and countering the negative effects of reduced SOC. In addition, no change in aggregates stability was reported by Fernandez et al. (2019) where burned and unburned soils showed no differences. In a study conducted to assess the effects of long-term burning on soil aggregates stability, observations showed that burning reduced aggregates stability by 8% in the upper 30 cm depth (Abdalla et al. 2021). These differences in findings could be explained by the fire intensity, period of burning and soil characteristics, including degree of aggregation (Flanagan et al. 2020). In addition, the hydrophobic substances form a thin water film which protected the aggregates from the fire effects, thereby, increasing it resistance to slaking (Varela and Keizer 2010). The effect of burning on different SOC fractions needs to be clearly understood if fire is to be used as a tool for sustainable management of lands, considering the need to store SOC to mitigate climate change and ensure sustainable production under changing climatic conditions.

2.6 Fractionation of SOC in soils and effects of soil types

Fractionation of SOC to different fractions in soils is essential for understanding stabilisation of SOC and turnover rates in soil environments (Poeplau et al. 2018). In addition, this fractions aids in understanding of the management effects on SOC as some of them are more sensitive to environmental changes (Plaza-Bonilla et al. 2014; Li et al. 2018). The process involves physical and chemical separation of the soils. Soil OC is fractionated into particulate fraction and mineral associated C which differ in their turnover rates in soils (Figure 2.2). The concentration of the different fractions of SOC in soils is largely affected by the type of soil including soil texture and mineralogy (Feng et al. 2014; Cai et al. 2016). Soils with high clay content could have high concentration of mineral associated C fractions due to protection in microaggregates, while in sandy soils the POM fraction could be high depending on microbial activities. The mineral associated C is stabilised by fine clay and silt particles (Cai et al. 2016). Burning of crop residues could significantly affects the different SOC fractions.

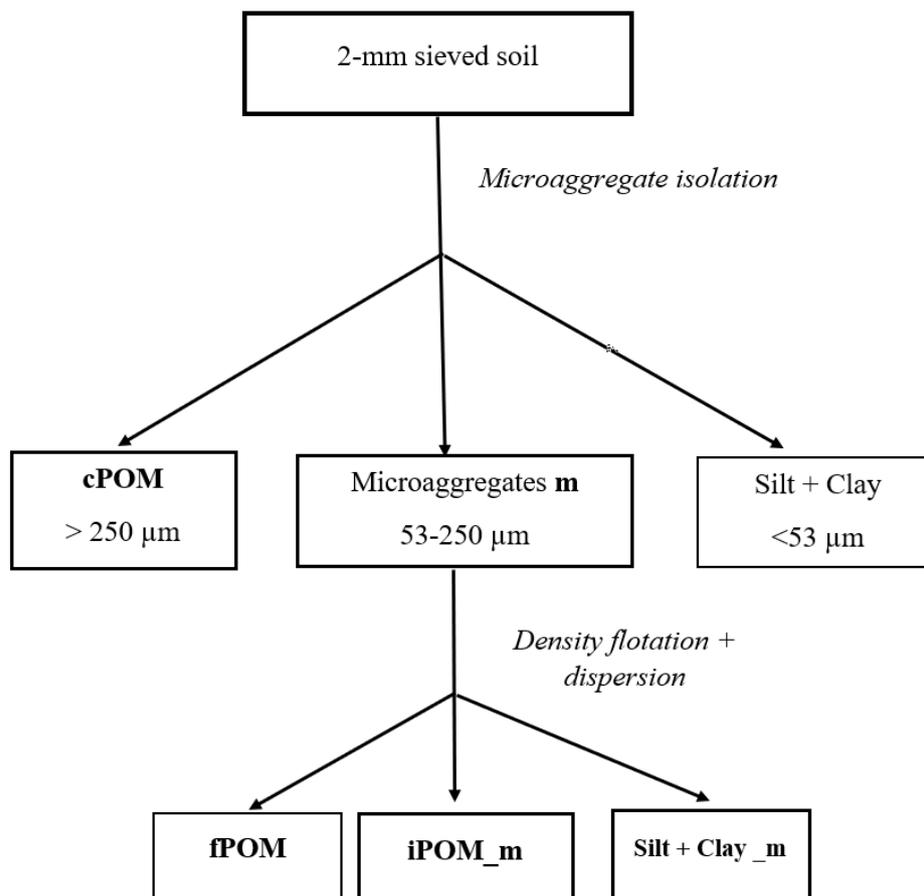


Figure 2. 2 Physical fractionation scheme for SOC (Huang et al. 2010)

2.7 Effects of pre-harvest burning relative to green cane on soil organic carbon and its fractions

The SOC content in soils is physical and chemical protected in soil aggregates or on soil mineral surfaces. The level of protection would vary depending on soil aggregates stability which is mainly affected by management practices (Abdalla et al. 2021). For instance, in pre-harvest burning system the decrease aggregates stability due to burning could expose protected SOC in aggregates to microbial attack (Mthimkhulu et al. 2016). Soil aggregates stability is essential for crop growth and SOC protection in soils and greatly influenced by soil structure (Merante et al. 2017). Physical fractionation of SOC is used as a tool to understand SOC dynamic in soil environment. Soil organic matter is physically fractionated into three broad fractions (i) coarse particulate organic matter which contains organic fragments $>250\ \mu\text{m}$, (ii) fine particulate organic matter, which consist of organic materials between $53 - 250\ \mu\text{m}$, and the mineral associated organic matter which is associated to the silt + clay $< 53\ \mu\text{m}$ (Torres-Sallan et al. 2018; Ramesh et al. 2019).

The particulate fraction of SOC comprises of 6-37% of the total C in agricultural soils and is believed to be easily accessible to microorganisms and easily mineralisable when compared to the mineral associated C (Samson et al. 2020). The SOC stored in both macro-aggregates and micro-aggregates is protected from microbial attack. The level of protection of SOC within the aggregates varies depending on aggregates stability. Destruction of soil aggregates by burning results to losses of soil organic matter due to reduced soil aggregates stability resulting to exposure of SOC for microbial attack (Zavala et al. 2010). Laskar et al. (2021) observed high concentration of SOC in the macro-aggregates than meso- and micro-aggregates and a positive relationship between SOC and aggregates stability. In addition, Garcia-Oliva et al. (1991) reported that about 80% of SOC was physical protected in macro-aggregates and slashing burning resulted to 32% decrease in SOC due to combustion. In sugarcane production, many farmers burn the cane before harvesting while others produce green cane (Graham and Haynes 2002). Green cane production increase addition of fresh biomass and trash blanket which protects the soil from erosion losses, hence increasing aggregation and further protection and storage of SOC.

Addition of residues either from forestry or sugarcane (green cane) is reported to increase C content and nutrients availability through decomposition. Graham and Haynes (2002) observed an increase in SOM and labile SOC concentration, which could benefits crops in the short-

term, under green residues retention than burning. However, the increase in labile C could result to positive priming effects resulting in loss of resident SOM. While burning of residues may increase charcoal C in soils, this may also be associated with increase in C losses as CO₂ emission (Galdos et al. 2009). On the other hand, several authors reported an increase in C sequestrations under green cane retention, consequently increasing SOC content. Since sugarcane residues are reported to be an important source of C (Tenelli et al. 2019), addition of residues into the soil will increase total soil carbon in the long-term. The addition of sugarcane residues under green cane increases SOC stocks, and microbial biomass and activity (Souza et al. 2012; Tenelli et al. 2019).

Losses of 40-50% of SOC in soil environment have been reported in literature in the top 5 cm depth, with the level of losses depending on fire intensity (Hobley et al. 2017). On the other hand, in fire adapted environments primary production may be elevated after fire resulting to addition of fresh biomass on soil surface increasing SOC again (Dai et al. 2005; Hobley et al. 2017). Zhao et al. (2012) observed an increase in SOC by 44% and 60% after autumn burning and spring burning. Several findings have shown that burning results in decrease in SOC over time in soil environment. These differences in observation could be because part of the SOC in burned plots could be in charcoal-C form. In addition, this could be explained by the fire severity which affects SOC differently. Souza et al. (2012) observed 54.1% higher total organic carbon in soils under sugarcane without burning (Table 2.1), while Galdos et al. (2009) reported that burning of sugarcane residues resulted to decrease in SOC when compared to unburned sugarcane and native forest in Brazil (Table 2.2). The no effects observed in the 10-20 cm depth could be explained by minimal disturbance in minimum tillage system which is reflected in the 0-5 cm depth.

Table 2. 1 Average total organic carbon in sugarcane under different harvest systems (Souza et al. 2012)

Management	Total organic carbon (g kg ⁻¹ soil)
Sugarcane with burn	8.10c
Sugarcane without burn	12.48b
Native forest	20.10a
CV (%)	4.14

Table 2. 2 Total C in native forest and in the burned and unburned sugarcane areas, 2, 6, 8 years after replanting, in the 0-10 and 10-20 cm depths (Galdos et al. 2009).

Years after replanting	Total C (g kg ⁻¹ soil)	
	0-10 cm	10-20 cm
Native Forest	30.12a	17.83ab
Burned sugarcane		
2 years	17.80cd	17.09ab
6 years	20.07bc	18.49ab
8 years	16.57d	15.40bc
Unburned sugarcane		
2 years	21.47bc	19.52a
6 years	19.84cd	16.78ab
8 years	23.30b	18.70ab

2.7.1 Carbon associated with macro- and microaggregates

The physical fractionation of SOC is essential for understanding the complexity of organic matter pools and assessing the dynamics of SOC in soils. This fractionation (physical fractionation) separates SOM into particulate organic matter (POM), which is made up of a fraction that is physically stabilised in aggregates and another fraction that is outside aggregates (free POM) (Anaya and Huber-Sannwald 2015; Yao et al. 2021). The distribution of SOC within and outside micro- and macroaggregates gives an indication of the SOC dynamics in different land use systems (Torres-Sallan et al. 2018; Traore et al. 2020). The level of sensitivity of these fractions differs based on physical and chemical protection (O'Brien and Jastrow 2013; Anaya and Huber-Sannwald 2014). The SOC in macroaggregates is easily accessible to microorganisms increasing the decomposition and are considered as POM (Six et al. 2002; Torres-Sallan et al. 2018). When this POM is decomposed the size of the macroaggregates is reduced to microaggregates and mineral associated C in silt and clay sized fraction (Torres-Sallan et al. 2018). But then, in forestry soils, this fraction could be protected due to high level of soil acidity limiting decomposition (Fuentes et al. 2012). The SOC contained inside microaggregates is not easily accessible to microorganism and the resident time of the C in this fraction is higher than that of C in macroaggregates (Torres-Sallan et al. 2018). Continuously burning degrades soil structure which expose the microaggregates within macroaggregates to

microbial attack, hence increasing decomposition. The effects of burning of residues may have variable effects on SOC in the form of POM within and outside aggregates. Green cane harvest systems favours formation of macroaggregates due to residue addition (Benbi and Senapati 2010; Liu et al. 2021) and less disturbance of soil aggregates which favours formation of macroaggregates, resulting in POM fraction of SOC. The particulate SOC fraction associated with macro- and micro-aggregate fractions could be in charcoal form, as a result of fire, but the effects of burning on this fraction may not be clear.

2.7.2 Charcoal C fraction

Burning of residues results to chemically altered residues known as pyrogenic matter, and the carbon within this material is referred to as recalcitrant pyrogenic carbon or charcoal (Hobley et al. 2017). Charcoal C in soils is produced from incomplete combustion of biomass (Skjempstad et al. 2002; Mastrolonardo et al. 2019; Parro et al. 2019). Due to its physical and biochemical properties, this pool is highly stable and resistant to decomposition (Zhang et al. 2018). However, there is evidence that charcoal decomposes over time and is not as recalcitrant as it is often reported in literature (Hobley et al. 2017). These conflicting results raise the need for more research on the effects of burning on carbon dynamics in soils and understanding the effects on charcoal in the long-term.

Charcoal stabilises soil C fluctuations by increasing the overall total soil C and considered the long-term C pool (Wang et al. 2016). High charcoal could increase C sequestration as a long-term carbon pool (Shah et al. 2021; Heinemeyer et al. 2018). Cultivation of the land could result in rapid decomposition of SOC including this C pool due to increase aeration increasing microbial activities and changes in soil moisture and temperature conditions (Wang et al. 2017; Krull et al. 2003). In addition, the amendments used in cultivated land use also enhance microbial activities (Chaudhry et al. 2012). However, in uncultivated soils this pool could persist for several years. The decomposition of charcoal pool and losses via erosion over time is reported in several literature (Ribeiro et al. 2021; Abney and Berhe 2018; Wang et al. 2016; Cusack et al. 2012; Czimczik et al. 2007). Wang et al. (2016) observed a decrease in recalcitrant charcoal C fraction due to land use change with cropland being lower than forestry sites, but then, the mineral associated C was higher in cropland than forestry soils. In acidic soils such as forestry this pool could even persist for many years due to limited activities of microorganisms. Depending on soil texture and other soil properties, charcoal C could be lost through translocation to deeper soil layer resulting to accumulation over time and the rate of

translocation would vary and maybe depth dependent (Hobley et al. 2016; Hobley et al. 2017). The deeper charcoal C could be protected more than the surface charcoal due to limited microbial activities in deeper soil layers than surface layers (Hobley et al. 2017).

2.7.3 Mineral associated C fraction

The mineral associated fraction of C is protected from microbial attack through its stabilisation through its interaction with clay particles in soils (Qiu et al. 2022; Xi et al. 2022; Carter 2020). The concentration of mineral associated C fraction varies depending on soil type and management. These minerals may reach their saturation level with C depending on soil silt and clay content (Samson et al. 2020). Continuous cultivation including tillage activities enhance the decomposition of this fraction over time decreasing the overall concentration in soils (Rahmati et al. 2020). Forestry and grassland soils have been found to have significant higher mineral associated C than the particulate C fraction (Cotrufo et al. 2019). The effects of burning on this fraction of SOC is not clearly understood. The distribution of POM, charcoal C and mineral associated C, as a proportion of SOC could have significant effects on soil microbial biomass.

2.7.4 Microbial biomass carbon

Soil microbial biomass C pool is the living fraction of SOC (Ramesh et al. 2019), and is highly labile and highly decomposable and contributes significantly to nutrient availability as it is a nutrient reservoir (Mukumbareza et al. 2016; Laik et al. 2021; Kumar et al. 2022). This pool sometimes results in temporarily unavailable nutrients (immobilisation by microorganisms), which could later be released back to the soil system. This pool is sensitive to management changes due to its turnover rate. This pool contributes 2-5% C of the total SOC (Bargali et al. 2018). The relationship between soil organic matter and soil MBC is controlled by soil aggregation, soil porosity, moisture, and soil aeration (Babur and Dindaroglu 2020). In acidic soils though MBC is reduced, it could reduce fixation of nutrients through immobilisation and slow release back to the soil solution when soil conditions are conducive. Due to its sensitivity it is considered as a good indicator of soil quality responsive to changes in management (Ramesh et al. 2019). Some management strategies could result in rapid decline or increase of MBC over time.

Soil MBC is reported to be highly correlated to SOM (Bargali et al. 2018) and normally forestry soils are characterised by high soil organic matter. A recent study by Lepcha and Devi (2020) demonstrated that MBC was highest in the forest (455 $\mu\text{g g}^{-1}$) followed by cardamom agroforestry (393 $\mu\text{g g}^{-1}$) and then paddy cropland (317 $\mu\text{g g}^{-1}$). The higher MBC under forestry could be explained by the high addition of plant litter contributing to the labile pool and C original in soil. A study conducted in South African agricultural soils to test the effects of tillage and residue retention on soil C pools showed that residue retention increases soil MBC compared to where residues are removed (Malobane et al. 2020).

Souza et al. (2012) observed a significantly higher MBC (by > 100%) in native forest and unburned management compared to burned area (Table 2.3), which could be explained by the contribution of trash (residues) both in forestry and sugarcane. The litter addition provided substrate for soil microorganisms (Souza et al. 2012). Conversely, Kaschuk et al. (2010) and Liu et al. (2010) observed significant increases in soil MBC in burned management which however decreased with time, due to depletion of readily available C in soil, and induced stress following burning as a result of rapid increase soil temperature and decrease soil moisture, hence, stressing microbial communities to a larger extend (Souza et al. 2012). The relationship of SOC and P availability suggests that any effects burning of crop residues may have on SOC fractions will also affect P and its fractions.

Table 2. 3 Average Soil microbial biomass C in sugarcane under different harvest systems (Souza et al. 2012)

Management	Microbial biomass C (g kg^{-1})
Sugarcane with burn	162.70c
Sugarcane without burn	328.45b
Native forest	523.79a
CV (%)	13.96

2.8 Effects of burning and residues retention on phosphorus pools

2.8.1 Soil P fractions

Phosphorus is one of the most limiting nutrients in soil productivity on 30-40% of the world's agricultural production land (Atere et al. 2019; Wang et al. 2022). In acidic and highly

weathered tropical soils P availability is limited due to high fixation capacity on oxides surfaces (Ahmed et al. 2019; Frazao et al. 2019). This is due to the high content of P fixation mineral such as hematite, goethite and gibbsite, which results to low P availability for plants (Frazao et al. 2019). Sugarcane straw addition enhance soil P availability through increase organic acids released from the mineralisation of residues, which may reduce P sorption sites in soils and increase P availability (Soltangheisi et al. 2021). Sugarcane straw addition enhance soil P availability through increase organic acids released from the mineralisation of residues, which may reduce P sorption sites in soils and increase P availability (Soltangheisi et al. 2021). However, sugarcane straw C: N ratio have been reported to ranging from 80:1 to 100:1, resulting in low decomposition of the straw, hence reducing nutrient release including P (de Castro et al. 2021). This straw may save as a long-term P source with slowly release overtime, but in the short term, intense immobilisation could be observed. Burning may reduce P fixation in soils, available P could be lost through erosion due to reduced soil aggregates stability. However, burning of sugarcane residues increases addition of ash which results to increase soil pH, and this could significantly reduce soil P fixation. For instance, in forestry soils, burnt forest resulted in 8.89% lower P associated with iron (Fe-P) than unburnt forest (Giardina and Rhoades 2001). A decrease in Fe-P fractions, from 35.4 mg kg⁻¹ to 4.30 mg kg⁻¹, with an increase in soil depth was reported by Resende et al. (2011). A study conducted by Schaller et al. (2015) showed that burning increase available P in soil solution. The effects of green cane relative to pre-harvest burning on P fractions, need to be clearly understood for purposes of sustainable sugarcane production.

2.8.2 Soil microbial biomass P

Soil microbial biomass P (MBP) is an essential labile pool of P which is slowly released over time to plants and can represent a substantial fraction of total soil P (Spohn and Widdig 2017). This P pool makes a large contribution to the plant available P pool in soil environments (Chen and He 2004). Brookes et al. (1984) showed that about 3% of the soil total P in cropped land was in the form of MBP while for grassland soils about 5 to 24 % was in the form of microbial biomass P. This differences could be explained by low P availability in croplands due to uptake by crop compared to grassland management. In acidic soil, this P fraction could be significantly reduced due to limited microbial activities. The effects of burning crop residues may reduce the substrate for microorganisms while the ash produced may have a liming effect which

supports microbial biomass and activity of associated enzymes, with effects on cycling of elements including C and P.

2.9 Effects of burning and green cane retention on soil enzymes activities

Enzymes in soils are essential in several processes such as organic matter decomposition and nutrients cycling, these makes them special in soil fertility (Prasad et al. 2021; Wade et al. 2021; Sharma 2022b). Soil enzymes are the catalyst that mediates the process of decomposition and release of nutrients from the SOM (Hoffland et al. 2020; Jing et al. 2020; Yadav et al. 2021; Sharma 2022b). Soil enzymes are protein substances which are essential in nutrient cycling and decomposition in soil systems and are sensitive to land management practices and are used as soil quality indicator (Mukumbareza et al. 2015).

2.9.1 β -D-Glucosidase enzyme

The β -D-glucosidase enzyme plays a significant role in the degradation of soil organic matter and related to the carbon cycle (Awad et al. 2012; de Almeida et al. 2015; Błonska et al. 2021). The β -D-glucosidase enzyme is sensitive to highly acidic soil conditions (Chen et al. 2021). Enzymes are indirectly and significantly affected by fire through changes in soil properties resulting in induced soil stress over time (Lombao et al. 2021). Armas-Herrera et al. (2016) observed a significant decrease in β -D-glucosidase enzyme in forestry soils in the top 3 cm depth by 49.0%. Vuyyuru et al. (2020) reported that pre-harvest burning decreases the β -D-glucosidase enzyme by 30.8% when compared to green cane (Table 2.4). Paddy straw burning resulted to a significant decrease in β -D-glucosidase enzyme (Kumar et al. 2019). Straw removal has been shown to reduce β -D-glucosidase enzyme activities by 46% in complete straw removal compared to no removal (Tenelli et al. 2019). The low β -D-glucosidase was due to low SOC content as substrates for enzymes. The addition of residues increases substrate availability required by the microorganisms (Adetunji et al. 2017). Fernandez-Garcia et al. (2019) observed a significant decrease in β -D-glucosidase enzymes activities with increase in fire severity. Geisseler et al. (2011) reported that the addition of plant residues significantly increased enzymes activities at different moisture level.

Table 2. 4 Soil enzyme activity as affected by sugarcane management (Vuyyuru et al. 2020)

Treatment	β -D-glucosidase ($\mu\text{g p-nitrophenyl released g}^{-1}$ moist soil h^{-1})
Sugarcane with Burnt Harvest	8.30b
Sugarcane with Green cane Harvest	10.86a
Sugarcane with Flooded Fallow	6.10b
Sugarcane with Sweetcorn Rotation	6.85b
Sugarcane with Rice Rotation	6.68b

2.9.2 Acid and Alkaline phospho-monoesterase

Alkaline and acid phospho-monoesterase are extracellular enzymes essential in the P cycle in soils, and release of P in available form from decomposition of organic matter (Jarosch et al. 2019; Wade et al. 2021). The activities of these enzymes vary depending on soil pH, with acid phospho-monoesterase dominating in the acidic conditions, while alkaline phospho-monoesterase being more dominant under alkaline conditions (Nannipieri et al. 2011; Jarosch et al. 2019). The activities of these enzymes depend on substrate availability, soil pH, temperature and presence or absence of inhibitors (Jarosch et al. 2019). Trujillo-Narcia et al. (2019) reported that acid phospho-monoesterase activity decreased from the first to second burning, and ranged from 5289 to 5515 $\mu\text{g p-nitrophenyl g}^{-1}$ moist soil h^{-1} . Similarly, Graham and Haynes (2005) observed high acid phospho-monoesterase in green cane and where residues were retained compared to pre-harvest burning. This decrease could be explained by the effects of fire on substrate availability in soils which are required by microorganisms. In addition, a portion of nutrients released from organic matter could be lost through volatilisation and as fly ash during burning subsequent reducing microbial activities (Saa et al. 1998). While most of the reviewed literature on the effects of burning on SOC and P fractions and microbial activity are mainly on soils with low SOC, large areas under sugarcane in South Africa are under humic soils, which are unique in that they are highly weathered and leached and yet they contain high concentrations of SOC. Most of the sugarcane is produced under the pre-burning harvest system while some farmers use green cane production system due to environmental concerns around burning of cane. Due to the high resident SOC, it is not clear whether soil quality benefits from green cane production compared to pre-harvest burning on humic soils.

2.10 Distribution of humic soils in South Africa

Humic soils occur in parts of South Africa, particularly in parts of Mpumalanga, Eastern Cape and KwaZulu-Natal provinces (Figure 2.3), are highly weathered soils characterised by high concentration of oxides of iron and aluminium oxides (Fey 2010). Small patches of humic soils also occur in Limpopo and Western Cape. The main soil forms include Kranskop (Humic / Yellow-Brown Apedal / Red Apedal), Magwa (Humic / Yellow-Brown Apedal) and Inanda (Humic / Red Apedal) (Soil Classification Working Group 2018). These soil forms could either have a thin and thick humic A horizon (Soil Classification Working Group 2018), which translate to Umbric Rhodic Ferralsol according to the world classification system (IUSS Working Group WRB 2015), and are considered highly productive, particularly for sugarcane, maize, forestry, pastures and vegetable crops.

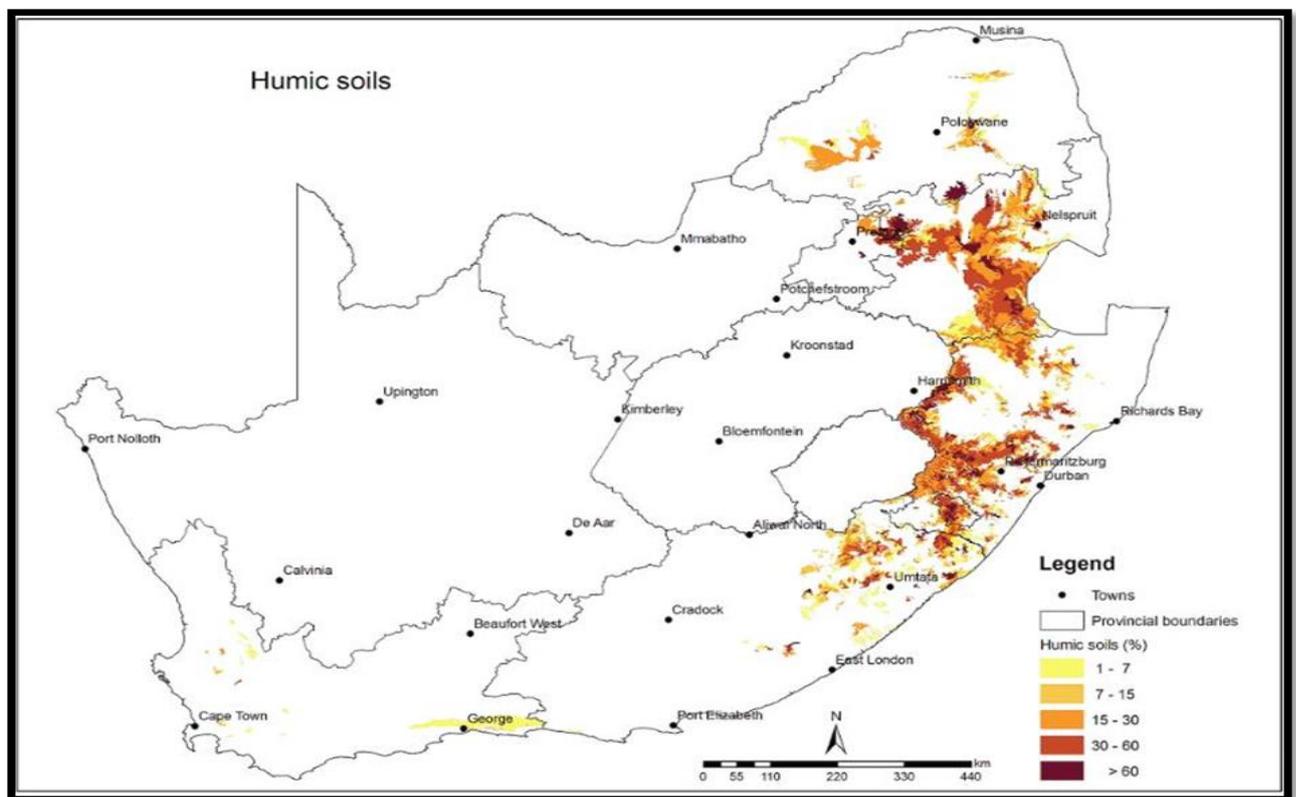


Figure 2. 3 A map showing distribution of humic soils in South Africa (Fey 2010)

2.11 Properties of humic soils

The high productivity of humic soil is attributed to the high SOM content and good drainage (Fey 2010). The thick layer of SOM in humic soils could be attributed to the high biomass production under the humid conditions (Olson and Gennadiev 2020), coupled with soil faunal

mixing by macro-organisms (Cesario et al. 2022) and slower rate of decomposition under the cool conditions, in acidic soils with low base status (Fey 2010). Furthermore, formation of organo-mineral complexes could increase the stabilisation of SOM in these soils (Feng et al. 2014; Nunez et al. 2022). The microbial activity in these soils could be limited by high acidity levels, hence increasing the accumulation of SOC. Conversion of these soils to agricultural use could result to significant losses of SOC depending on the land use management. For instance, in sugarcane production where burning is practised the SOC content could be reduced by 20 to 30% due to decrease in addition of fresh biomass (Dos Santos et al. 2020) and through microbial decomposition and SOC mineralisation resulting to losses of SOC as CO₂ emission (Mandal et al. 2020; Olson and Gennadiev 2020).

Humic soils are characterised by low base status, pH, and high buffering capacity with the high SOC resulting in low bulk density and good drainage (Fey 2010). The high acidity would increase P fixation as phosphates of Al and Fe (Al-P and Fe-P), limiting plant available P (Johan et al. 2021; Pierzynski and Hettiarachchi 2018). However, the high SOM content could reduce the effects of fixation due to its ability to complex formation with Al, limiting its ability to hydrolyse and produce H⁺ ions (Vermeiren et al. 2022). The competition between SOM and P for adsorption sites may make P more available in soil solution. Liming material is used to increase pH of those soils and addition of phosphate fertiliser are essential in humic soils (Fey 2010). A study conducted in humic soils showed that application of lime significantly increased mineral N concentration due to increased microbial activities (Mkhonza et al. 2020). The low bulk density of humic soils can be explained by the high SOM content and makes the soils conducive for root penetration and drainage, which consequently favour the growth of plants. The low bulk density, good drainage and reduced acidity due to liming of humic soils could enhance microbial activity, leading to rapid decomposition of SOM releasing P and other several essential nutrients. The rapid decomposition of SOM could possibly result in decrease in overall SOC content due to CO₂ emissions. While humic soils are known to be highly productive, effects of management on SOC and P and their fractions and microbial activity are not clearly understood.

2.12 Conclusion

Pre-harvest burning reduce soil aggregates stability, SOC in micro- and macroaggregates, β -D-glucosidase and acid phospho-monoesterase activity, and increase charcoal C concentration and alkaline phospho-monoesterase activity, when compared with green cane. Green cane

management increase soil microbial biomass C and P than pre-harvest burning, but then, pre-harvest burning reduced P fixation. Contrasting findings were found on charcoal and SOC which suggest that there is a need to understand charcoal-C and SOC losses over time. This is particularly important for humic soils, which naturally have high concentrations of SOC. Based on the findings from the review, the factors that govern the concentrations of SOC in different SOC fractions including that in organo-mineral complexes needs to be thoroughly reviewed based on available literature at a global scale, in a view of climate change mitigation. In addition, from the review, the effects of green cane relative to pre-harvest burning on SOC stocks, SOC fractions associated with aggregates, P availability, P fractions and microbial activities were reported from the top surface layers of mainly non-humic soils, and there is a need to evaluate these effects in humic soils to deeper soil layers.

CHAPTER 3

Land use effects on soil organic carbon bound in aluminium/iron-organic matter complexes: A global meta-analysis

Abstract

Purpose Organo-mineral complexes are essential in the protection of soil carbon (C) but land use effects may affect their stability with implications on C emissions. The objective of this paper was to review available literature on the effects of land use on SOC in Al/Fe-organic matter in soils at a global scale.

Methods The effects of land use on soil organic carbon bound in aluminium (Al)/ iron (Fe)-organic matter complexes were evaluated using 574 data points from research trials around the world. The land use were grouped into cropland, forest and grassland. In addition, the data was also grouped according to soil pH, texture, SOC and climate. The response of Al/Fe-organic matter under the different management was also related to soil properties and environmental conditions.

Results and discussion The concentration of SOC bound to Al and Fe (C_p) and organically bound aluminium (Al_p) and iron (Fe_p) were reduced by cultivation, with > 100% reduction in Al_p and Fe_p , when compared to uncultivated soils. On average, cultivated (cropland) and uncultivated (forest and grassland) had Al_p of 1.50 and 5.14 g kg⁻¹, respectively, and Fe_p of 1.08 and 5.34 g kg⁻¹, respectively. Cropland (1.50 g kg⁻¹) reduced Al_p significantly when compared to forest (4.48 g kg⁻¹) and grasslands (5.22 g kg⁻¹), with Fe_p having a similar trend (cropland, 1.08 g kg⁻¹; forest, 4.14 g kg⁻¹; grassland, 5.45 g kg⁻¹). In tropical climates conditions soil organic carbon SOC, Al_p and Fe_p concentrations were significantly low than subtropical and temperate. Soils in areas under higher precipitation and cool temperatures had higher SOC, Al_p and Fe_p concentration. For SOC bound to Al and Fe it was only higher in soils under high precipitation, but not affected by temperature conditions. The Al_p and Fe_p strongly positively correlated with SOC, highlighting the importance of Al/Fe-organic matter complex in stabilising SOC.

Conclusions On a global scale, these findings imply that cultivation, especially in tropical environments, decreases the stabilisation SOC in Al/Fe-organic matter complex, potentially contributing to CO₂ emissions.

Keywords: • Carbon sequestration • Climate • Land use • Organo-mineral complexes

3.1 Introduction

Globally, soils store about 2344 Pg C, with about 75% of this being SOC (Wen et al. 2019). A slight change in soil organic matter pool due to decomposition could have a significant contribution to climate change, through loss of CO₂ emission which increases atmospheric concentration of greenhouse gases (Wagai et al. 2020). In 1992, the Kyoto Protocol on climate change effects demands a fundamental understanding of the mechanism of C stabilisation in soils due to the large quantities of C stored in soil environments on a global scale (Lutzow et al. 2006). Land use effects on the stability of SOC in soils needs to be clearly understood at a global scale. Reports shows that agricultural land occupied 36.9% of the earth surface with several management strategies involved which strongly modify the total SOC stocks over time (The World Bank 2015). The SOC in organo-mineral complexes may comprise 25-98% of the total SOM (Doetterl et al. 2015) and is believed to be a more stable fraction than other SOC fractions in soils. This high stability in the SOC is because of the bonds between organic matter and the mineral surfaces reducing its susceptibility to microbial decomposition (Eusterhues et al. 2014; Takahashi and Dahlgren 2016; Tamrat et al. 2019), thereby protecting large quantities of C which could otherwise be lost to the atmosphere. Land-use change affects SOM (Don et al. 2011; Ramesh et al. 2019; Sahoo et al. 2019; Kirsten et al. 2021). Guimaraes et al. (2013) reported that cultivation resulted to 47.5% loss of SOM in the surface layer when compared to uncultivated site, and this could partly be explained by redistribution of SOM in deeper soil layers.

Song et al. (2018) reported that cropland results in moderate C loss when compared to grassland, but resulted in SOC increase in the subsoils. On average, about 67% of the total C in the topsoil was lost following conversion of natural forest to cropland (Llorente et al. 2010). On the other hand, Basile-Doelsch et al. (2009) observed an increase in organo-mineral complexes distribution and accumulation in deeper soil layers. Cultivation incorporates the use of liming material and fertilisation which increase microbial activities due to the modified soil environment. The effects of fertilisation depend largely on the type of fertiliser used either organic or inorganic fertiliser. While particulates organic matter and hot water-soluble organic matter, are more likely to be affected by management, including cultivation, there are indications that even the stable mineral-bound organic carbon pool may also be affected (Basile-Doelsch et al. 2009). A number of studies have shown that conversion from

uncultivated land to cropland resulted in significant destabilisation of organic matter, including the stabilised fraction of C (Basile-Doelsch et al. 2009; Beheshti et al. 2012; Durigan et al. 2017; Ahmed et al. 2022). Tillage systems increase the soil aeration, and with enough soil moisture these enhance microbial activities, which could increase decomposition of different soil organic matter pools regardless of the stability, including organo-mineral complexes. Other management strategies associated with different land uses, including fertiliser and lime application could also contribute.

Wang et al. (2016) reported that addition of organic and inorganic fertilisers in a rice fields had significant effects on organically bound Al, with organic fertiliser resulting in significantly higher concentrations by at least 46.9% than all the inorganic fertilisers. Application of inorganic fertilisers has also been found to significant increase SOC incorporated in organo-mineral complexes (Maillard et al. 2015). Land use (croplands, grasslands and forest) and different management strategies influence the composition of organic material added into the soil, including nutrients which are essential for microorganisms responsible for decomposition process in soil, thereby affecting SOM turnover (Shen et al. 2018). The addition of fertilisers increases the supply of nutrients in the soil, which also contributes to a rapid increase in microbial activities. Shen et al. (2018) reported a decrease in organo-Al complexes due to soil amendments resulting in changes in soil pH, with potential effects on SOC in organo-mineral complexes. Kryzevicius et al. (2019) observed a decrease in organic bound Al in limed soils compared to unlimed soils. The contradictions in the effects of fertiliser or lime application on organo-mineral complexes suggests that other factors such as soil type and climatic factors could also contribute. Therefore, there is an urgent need to understanding the effects of land use on organo-mineral complexes at a global scale in the view of climate change. The hypothesis for this study was that land use significantly affects the SOC bound in organo-mineral complexes in soils. Therefore, the objective of this paper was to review literature on the effects of land use on SOC in Al/Fe-organic matter in soils at a global scale

3.2 Methods and materials

3.2.1 Review setup

This study was a meta-analysis based on data collected from different land use (cropland, forestry, and grassland), which were differentiated into cultivated and uncultivated. Peer-reviewed articles from different journals published between 2005 and 2022 were used to create

the database. The journal articles were searched from academic search engines (Google Scholar, ResearchGate, Nature, SpringerLink, Science Direct, and Sabinet) to collect information on the effects of land use in organo-mineral complexes in soils under different land use. The search of the articles was done using several keywords “effects of land use on organic mineral complexes”, “cultivation decrease organo-mineral complexes”, “cropping effects on organo-mineral complexes”, “sodium pyrophosphate extractable aluminium and iron” and “Al/Fe bound C in soils”. The articles used were those which gave the effects of different land use on SOC bound in Al/Fe-organic matter complexes under named environmental conditions such as climate, soil properties, and SOC content. A total of 47 journal articles (Table 3.1) was used for this study with 574 data points. The studies used in this meta-analysis were from 18 countries (Brazil, Burkina Faso, Chile, China, Germany, Ghana, India, Japan, Malaysia, Mexico, New Zealand, Poland, Solomon Islands, Spain, Switzerland, Tanzania and USA) as shown on Figure 3.1. The main data point contributors were China (n = 137), USA (n = 47), Mexico (n = 88), India (n = 72), Germany (n = 61), Chile (n = 40) and Japan (n = 33).

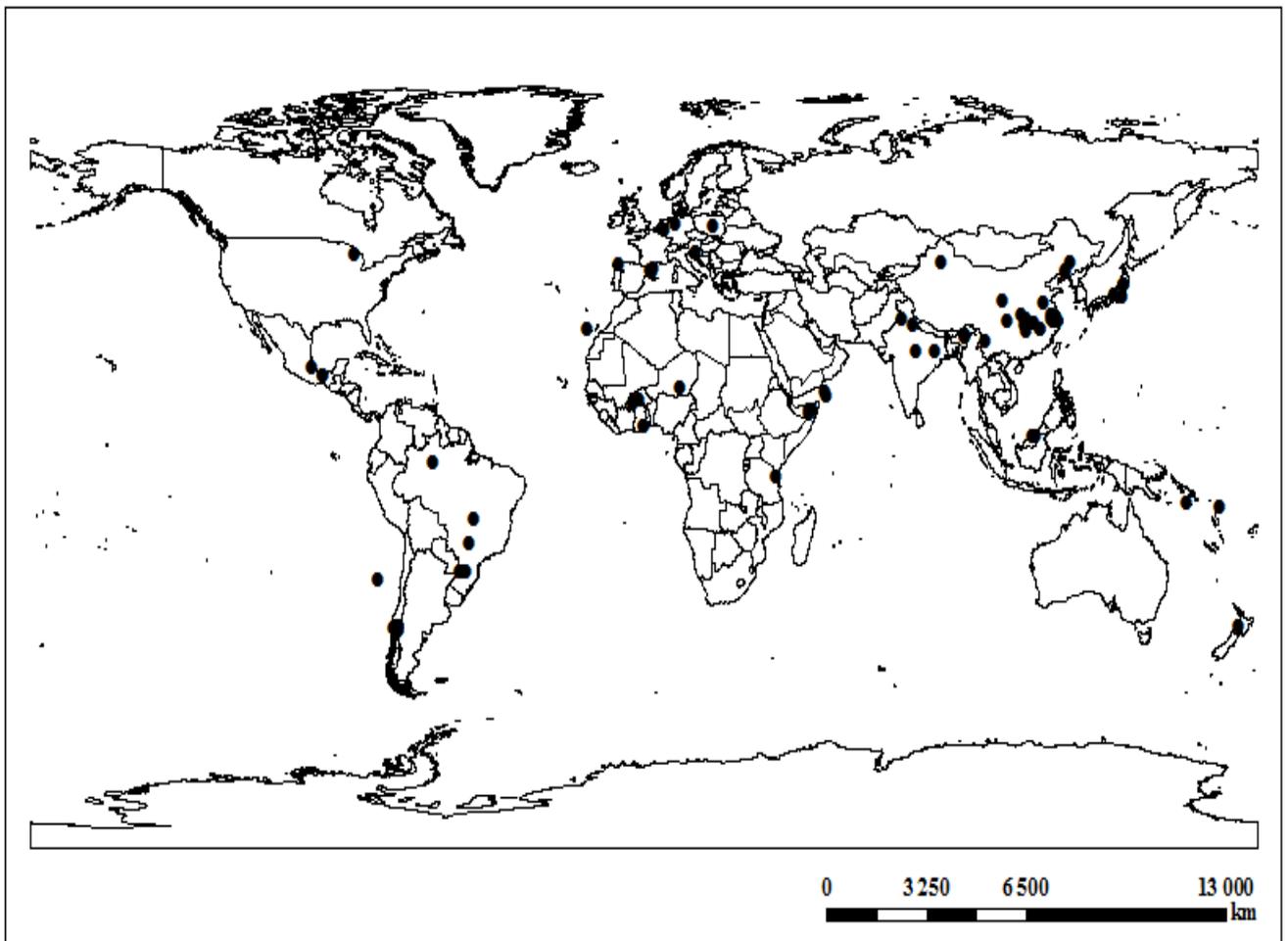


Figure 3. 1 Global distribution of the study sites shown by shaded circles on the map.

Table 3. 1 List of authors, countries, soil organic carbon, clay content, MAP (mean annual precipitation) and MAT (mean annual temperature) for the different experimental sites

No.	Author	Country	SOC	Clay	MAP	MAP	Land use
			g kg ⁻¹	%	mm	°C	
1	Anthonio et al. (2020)	China	-	-	1600	17.5	Cropland
2	Baumgarten et al. (2013)	Chile	73.3	16.8	4000	6.00	Forest
3	Bruun et al. (2010)	Ghana	43.8	29.5	1850	27.8	Forest
4	Cui et al. (2014)	China	-	11.0	1004	15.3	Cropland
5	Das et al. (2019)	India	17.5	30.8	1181	25.4	Cropland
6	Dick et al. (2005)	Brazil	17.2	52.0	1913	20.2	Forest
7	Eckmeier et al. (2010)	Switzerland	35.8	4.90	1800	12.0	Forest
8	Gamboa and Galicia (2012)	Mexico	34.1	-	932	9.40	Cropland
9	Garrido and Matus (2012)	Chile	77.1	19.4	1675	12.5	Cropland
10	Gartzia-Bengoetxea et al. (2020)	Spain	91.0	22.7	1200	9.00	Forest
11	Gruneberg et al. (2013)	Germany	-	34.3	675	6.80	Forest
12	Heinze et al. (2018)	Germany	27.0	2.50	718	8.70	Forest
13	Hernandez et al. (2012)	Spain	26.1	27.4	430	16.7	Cropland
14	Houunkpatin et al. (2018)	Burkina-Faso	9.10	29.0	956	28.6	Cropland
15	Huang et al. (2020)	China	18.5	25.0	1064	15.3	Cropland
16	Huang et al. (2021)	China	-	-	867	4.20	Cropland
17	Kaiser et al. (2012)	Germany	22.0	20.3	658	8.10	Forest
18	Kalita et al. (2019)	India	5.10	31.7	1739	24.2	Cropland
19	Kirsten et al. (2021)	Tanzania	42.6	24.7	1918	20.6	Cropland
20	Kov et al. (2018)	New Zealand	88.9	-	1500	16.0	Forest
21	Krettek et al. (2020)	Germany	14.6	0.10	750	9.00	Grassland
22	Kunito et al. (2016)	Japan	99.3	-	1658	11.3	Forest
23	Liu et al. (2019)	China	18.0	-	1325	16.3	Cropland
24	Lopez-Sangil and Rovira (2013)	Spain	27.7	22.5	732	13.8	Grassland
25	Maltoni et al. (2017)	Brazil	13.5	31.0	1370	23.5	Grassland
26	Miyazawa et al. (2013)	Japan	93.6	-	1661	10.3	Grassland
27	Neculman et al. (2013)	Chile	57.5	-	5000	5.00	Forest
28	Neculman et al. (2021)	Chile	57.5	36.7	5000	5.00	Forest
29	Panichini et al. (2012)	Chile	43.4	16.3	5000	4.50	Grassland
30	Panichini et al. (2017)	Chile	74.9	16.1	4500	9.00	Forest
31	Porrás et al. (2017)	USA	19.6	10.8	1195	9.00	Forest
32	Qi et al. (2021)	China	16.3	-	1360	16.8	Cropland
33	Simansky and Jonczak (2020)	Poland	6.70	5.10	560	8.00	Cropland
34	Traore et al. (2020)	Germany	4.70	23.5	950	30.0	Grassland
35	Wagai et al. (2013)	USA	69.9	-	1264	17.3	Cropland
36	Wagai et al. (2018)	Japan	52.8	-	1300	13.7	Cropland
37	Wang et al. (2016)	China	13.3	59.8	1122	16.2	Cropland
38	Wang et al. (2019)	China	19.3	12.0	800	13.5	Cropland
39	Wen et al. (2019)	China	14.6	-	824	8.60	Cropland
40	Wissing et al. (2013)	China	16.7	25.3	1325	16.3	Cropland
41	Xue et al. (2019)	China	15.3	45.1	853	17.9	Cropland
42	Yadav et al. (2017)	India	5.00	25.5	863	25.0	Cropland

43	Yan et al. (2016)	China	-	62.8	875	4.70	Cropland
44	Yang et al. (2021)	China	-	-	1300	16.0	Forest
45	Zhao et al. (2017)	China	12.3	37.4	1469	16.0	Forest
46	Zhou et al. (2009)	China	15.4	24.4	1150	18.3	Cropland
47	Zhuang et al. (2016)	China	40.9	-	1550	15.9	Cropland

3.2.2 Definition of study variables, soil and environmental factors

This study simplified the effect of land use on organo-mineral complexes into three land uses; namely cropland, forestry, and grassland. The data used were field-based, where all the samples collected from the field were analysed for organo-mineral complexes. Environmental conditions such as mean annual precipitation and mean annual temperature were categorised into arid, temperate, tropical, and subtropical depending on the amount of rainfall received (Table 3.2). For the soil conditions, soil pH was grouped into three classes acidic, neutral, and alkaline, as described by Halvin et al. (2013). Soil textural triangle was used for soil texture as described by Shirazi and Boersma (1984) to categorize texture into three classes depending on clay distribution percentage as clay, loam, and sand. Soil organic carbon content was classified into three classes; low, medium, and high (Table 3.2), as described by Lal (1994) . Mean annual temperature was categorized into three classes cool, warm and hot. Mean annual precipitation was categorized into three classes dry, arid-humid and wet.

Table 3. 2 List of classes defining the environmental factors and soil conditions

Environmental factors	Remarks	Class range	Name
Climate (MAP, mm year ⁻¹ ; MAT, °C year ⁻¹) ¹	Hot and dry	MAT > 25 MAP < 100	Arid
	Hot and wet	MAT > 20 MAP > 1500	Tropical
	Warm and arid-humid	MAT = 10-20 MAP > 100-1110	Subtropical
	Cool and arid-moist	MAT < 10 MAP = 120-1000	Temperate
MAP (mm year ⁻¹) ²	Dry	MAP < 100	Dry
	Arid-humid	MAP = 100-1000	Arid-humid
	Wet	MAP > 1000	Wet
MAT ³	Cool	MAT < 10	Cool
	Warm	MAT = 10-25	Warm
	Hot	MAT > 25	Hot
Clay (%)	Average clay content (%)	0-20	Low
		> 20-35	Medium
		> 35	High
Sand (%)	Average sand content (%)	0-25	Low
		> 25-50	Medium
		> 50	High
Silt (%)	Average silt content (%)	0-20	Low
		> 20-40	Medium
		> 40	High
Soil texture (%) ⁴	Clay content distribution (%)	> 32	Clay
		20-32	Loam
		< 20	Sand
Soil pH ⁵	Moderately acidic	< 6.6	Acidic
	Moderate carbonate	> 6.6-7.3	Neutral
	Soils with high carbonates	> 7.3	Alkaline
Soil organic carbon (g kg ⁻¹) ⁶	Low organic carbon	< 10	Low
	Medium organic carbon	10-20	Medium
	High organic carbon	>20	High

¹ Mathew et al. (2017)² Mathew et al. (2020)³ Mathew et al. (2020)⁴ Shirazi and Boersma, (1984)⁵ Halvin et al. (2013)⁶ Lal, 1994

3.2.3 Data analysis

Different statistical packages were used for data analysis. Summary statistics and correlation analyses were done using GenStat 20th Ed. The data set was tested for normality of the different variables using the R Statistical Software (R Core Team 2018) and outliers were removed before statistical analysis, using the non-parametric Kruskal-Wallis analysis. The outliers were determined using the 1st and 3rd quartile and the interquartile range from the dataset for each parameter. Statistically differences were tested at chi-square probability of $p < 0.0001$. Box plots were constructed using Sigma Plot 10.0 statistical software to show minimum, 25th quartile median, mean, 75th quartile and maximum values within the 5 and 95th percentile. Additionally, Pearson correlations coefficient at $p < 0.05$ were calculated among the different variables. Different descriptive stats were analyzed (mean, maximum, minimum, quartile 1 and 3 indicating 25th and 75th quartile, respectively, standard error mean, skewness, kurtosis, and coefficient of variation (CV%) for overall studies used in the database (Table 3.3). Because of low data points the effects of different soil pH classes, textural classes and SOC classes on SOC bound to Al/Fe-OM complexes was excluded, only the overall soil pH, bulk soil texture and SOC effects were analysed for SOC bound to Al/Fe-OM complexes.

3.3 Results

3.3.1 General statistics of soil and environmental conditions

The environmental and climate conditions from the current study varied widely, with MAP (mean = 1408 ± 39.5 mm yr⁻¹) ranging from 286 mm yr⁻¹ in Spain (Hernandez et al. 2012) to 5000 in Chile (Neculman et al. 2021; Neculman et al. 2012) (Table 3.3). Mean annual temperature (mean = 14.7 ± 0.30 °C; CV = 48.6%) varied from a minimum of -0.90 °C yr⁻¹ which was recorded in China (Huang et al. 2021) to a maximum of 30°C yr⁻¹ which was recorded in Germany (Traore et al. 2020). The SOC bound in Al/Fe-OM complexes varied widely (mean = 15.6 ± 1.79 ; CV = 71.6%) from 3.90 g kg⁻¹ in forest land use in Chile (Panichini et al. 2012) to 47.7 g kg⁻¹ also in forest land use in Chile (Panichini et al. 2017). The Fe in organo-mineral complexes (Fep) showed a wide variation (mean = 3.16 ± 0.34 g kg⁻¹; CV = 85.0%) ranging from 0.01 g kg⁻¹ under cropland in China (Qi et al. 2021) to 57.0 g kg⁻¹ under forest in USA (Wagai et al. 2013) (Table 3). Also, the Al in organo-mineral complexes (Alp) varied widely (mean = 3.59 ± 0.30 g kg⁻¹; CV = 65.2%) ranging from below detection 0.00 g kg⁻¹ in USA (Porras et al. 2017) and Malaysia (Wagai et al. 2013) under forest to 15.9 g kg⁻¹

under forest in Japan (Miyazawa et al. 2013). The clay (mean = $21.1 \pm 0.90\%$) exhibited the widest variation amongst the textural class (CV = 71.6%) ranging from 0.00 % in Germany (Krettek et al. 2020) to 67.4% in Brazil (Dick et al. 2005). Silt content (mean = 27.4 ± 0.92 ; CV = 54.1%) varied from 0.03% in Germany (Krettek et al. 2020) to 75.0% in China (Cui et al. 2014), while sand content (mean = 44.1 ± 1.66 ; CV = 60.5%) varied from 2.10% in Brazil (Dick et al. 2005) to 100% in Germany (Krettek et al. 2020). Soil organic C exhibited a wide variation (mean = $28.8 \pm 1.36 \text{ g kg}^{-1}$; CV = 75.2%) from 0.40 g kg^{-1} in Mexico (Gamboa and Galicia 2012) to 151 g kg^{-1} in Spain (Gartzia-Bengoetxea et al. 2020) (Table 3.3). The soil pH (mean = 5.52; CV = 24.8%) varied from 2.90 observed in Germany (Krettek et al. 2020) to 8.30 observed in China (Cui et al. 2014).

Table 3. 3 Descriptive statistics (Min: minimum, Max: maximum, Qt1 and Qt3: quartile 1 and quartile 3, respectively, SEM: standard error of mean, Skew: skewness, Kurt: kurtosis, CV%: coefficient of variation) of soil and environmental parameters and organo-mineral complexes

Variables	n	Mean	Median	Min	Max	Qt1	Qt3	SEM	CV%	Skew	Kurt
Alp ⁷	289	3.59	1.70	0.00	15.9	0.17	5.96	0.25	65.2	1.28	0.67
Al/Fe bound											
C ⁸	39.0	15.6	11.8	3.90	47.7	8.35	19.7	1.79	71.6	1.42	1.31
Clay	341	21.1	24.0	0.00	67.4	10.8	32.6	0.90	69.2	0.63	-0.09
Fep ⁹	217	3.16	1.30	0.01	57.0	0.40	4.33	0.34	85.0	6.19	60.3
Latitude	574	19.0	26.6	-40.8	116.6	11.2	35.0	1.20	151.1	-0.15	1.44
Longitude	574	22.5	10.5	-97.3	174.4	-72.1	112.3	3.55	377.9	-0.06	-1.48
MAP ¹⁰	574	1408	1175	286	5000	824	1661	39.5	67.1	2.45	6.22
MAT ¹¹	574	14.7	12.5	-0.90	30.0	8.60	20.0	0.30	48.6	0.51	-0.84
SOC ¹²	492	28.8	17.1	0.40	151.0	7.55	36.5	1.36	75.2	1.62	2.04
Sand	260	44.1	44.1	2.10	100.0	27.3	56.6	1.66	60.5	0.39	-0.42
Silt	258	27.4	25.2	0.03	75.0	19.0	34.5	0.92	54.1	0.42	0.50
Soil pH	384	5.52	5.30	2.70	8.30	4.50	8.30	0.07	24.8	0.27	-0.94

⁷ Organically bound Al

⁸ SOC bound to Al and Fe-Organic Matter

⁹ Organically bound Fe

¹⁰ Mean annual precipitation

¹¹ Mean annual temperature

¹² Soil organic carbon

3.3.2 Relationships among organo-mineral complexes, soil parameters, and environmental conditions

Table 3.4 shows the correlation among the environmental conditions and soil variables. The SOC bound in Al/Fe-OM complexes was significantly and strongly positively correlated to Alp, Fep, SOC, silt, MAT, and negatively correlated to MAP, soil pH and sand content. The Alp was significantly and strongly positively correlated to Fep, SOC, MAT, and negatively correlated to soil pH, sand and MAP. For organically bound Fe, the relationship was strongly positively correlated with MAT and SOC and negatively correlated to MAP. Clay was significantly and strongly positively correlated to silt content, and SOC and negatively correlated to soil pH and sand content. Soil organic carbon was positively correlated to silt, MAP, organically bound Al and Fe, Al/Fe bound C, clay content and negatively correlated to soil pH, MAT and sand.

Table 3. 4 Spearman rank correlation coefficients between selected soil and environmental conditions

	Al/Fe bound C	Alp	Clay	Fep	MAP	MAT	pH	Sand	Silt	SOC
Al/Fe bound C	1.000									
Alp	0.943**	1.000								
Clay	0.265	0.177	1.000							
Fep	0.754**	0.812**	-0.358	1.000						
MAP	-0.756**	-0.756**	0.232	-0.507*	1.000					
MAT	0.756**	0.756**	0.125	0.507**	-1.000**	1.000				
pH	-0.886**	-0.771**	-0.618**	-0.377	-0.756**	0.756**	1.000			
Sand	-0.667**	-0.522**	-0.806**	-0.015	0.760**	-0.760**	0.928**	1.000		
Silt	0.618**	0.441	0.773**	-0.045	-0.765**	0.765**	-0.883**	-0.985**	1.000	
SOC	0.943**	0.886**	0.530**	0.580**	0.697**	-0.697**	-0.943**	-0.783**	0.706**	1.000

* indicates significant difference at $p < 0.05$ and ** indicates significant differences at $p < 0.001$

3.3.3 SOC, Al/Fe bound C and Al/Fe-organic matter complex under different climatic conditions.

Figure 3.2 shows climate effects on SOC, Al/Fe bound C, and organically bound Al and Fe (Figures 3.2a, b, c, and d). Soil organic carbon was significantly lower under tropical (14.1 g kg^{-1}) when compared to subtropical (31.2 g kg^{-1}) and temperate (40.4 g kg^{-1}) climatic conditions (Figure 3.2a). Soil organic C in tropical climate conditions was 2.21 and 2.87 times lower than subtropical and temperate conditions, respectively (Figure 3.2a). The concentration of Al/Fe bound C was not affected climatic condition in subtropical (14.2 g kg^{-1}) and temperate (16.4 g kg^{-1}) (Figure 3.2b). Tropical (0.51 g kg^{-1}) climatic conditions showed lower organically bound Al when compared to subtropical (4.34 g kg^{-1}) and temperate (4.76 g kg^{-1}), which translated to 8.5 and 9.3 times lower, respectively (Figure 3.2c). Similarly, organically bound Fe was significantly lower in tropical (0.91 g kg^{-1}) conditions than subtropical (3.79 g kg^{-1}) and temperate (2.73 g kg^{-1}) conditions (Figure 3.2d). The Fep in a tropical climate was 4.16 and 3.00 times lower than subtropical and temperate climate conditions. Overall, SOC, Al/Fe bound C, Alp and Fep were significantly lower in tropical climatic zones compared to subtropical and temperate climates (Figures 3.2a, b, c and d).

Figure 3.3 shows the effects of temperature on SOC, Al/Fe bound C, organically bound Al and Fe (Figures 3.3 a, b, c and d). Soil organic carbon in cool (37.5 g kg^{-1}) temperatures was significantly higher than warm (28.6 g kg^{-1}) and hot (12.1 g kg^{-1}) temperatures, by 31.1% and > 100% higher, respectively (Figure 3.3a). For Al/Fe bound C it was not significantly different between different temperature conditions (Figure 3.3b). Similarly, Al/Fe-organic matter complexes were not significantly affected by temperature conditions (Figure 3.3c and d). Figures 3.4a, b, c and d, demonstrates the effects of precipitation on SOC, Al/Fe bound C, organically bound Al and Fe. Soil organic carbon was affected by precipitation, with wet (36.0 g kg^{-1}) areas having significantly higher SOC than arid-humid (16.7 g kg^{-1}), by > 100% (Figure 3.4a). The concentration of Al/Fe bound C was 86% significantly higher in wet (17.5 g kg^{-1}) conditions than arid-humid (9.39 g kg^{-1}) (Figure 3.4b). Organically bound Al was affected by precipitation, arid-humid (4.08 g kg^{-1}) was 2.26 times significantly lower than wet (1.80 g kg^{-1}) conditions (Figure 3.4c). While, the concentration of organically bound Fe was not affected by precipitation (Figure 3.4d).

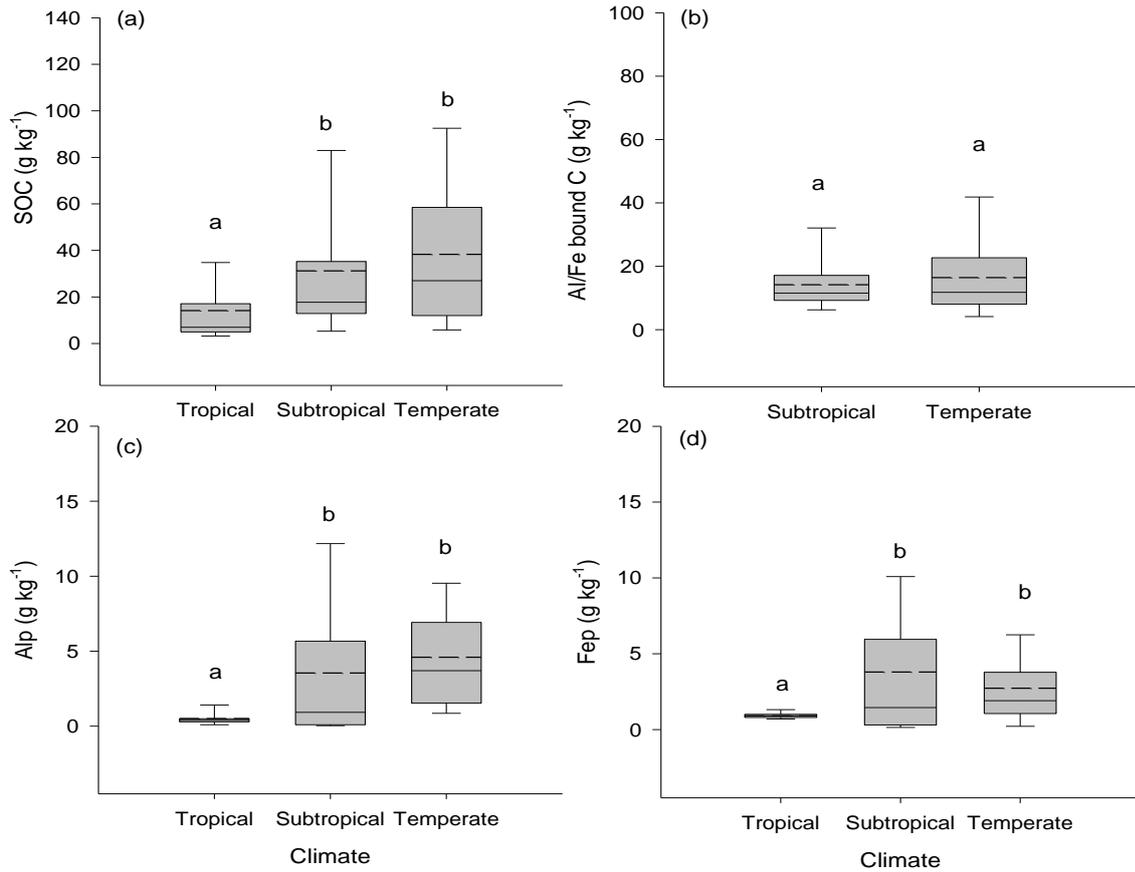


Figure 3. 2 Effect of climate conditions on soil carbon_a, Al/Fe bound C_b, organically bound Al_c and Fe_d. Each boxplot indicates the minimum, maximum, quartile 1 (25%) and quartile 3 (75%). Dotted lines indicate means and solid indicates a median. The different letters indicate a significant difference at $p < 0.05$. Where no data points were observed for the parameter the climate class was removed.

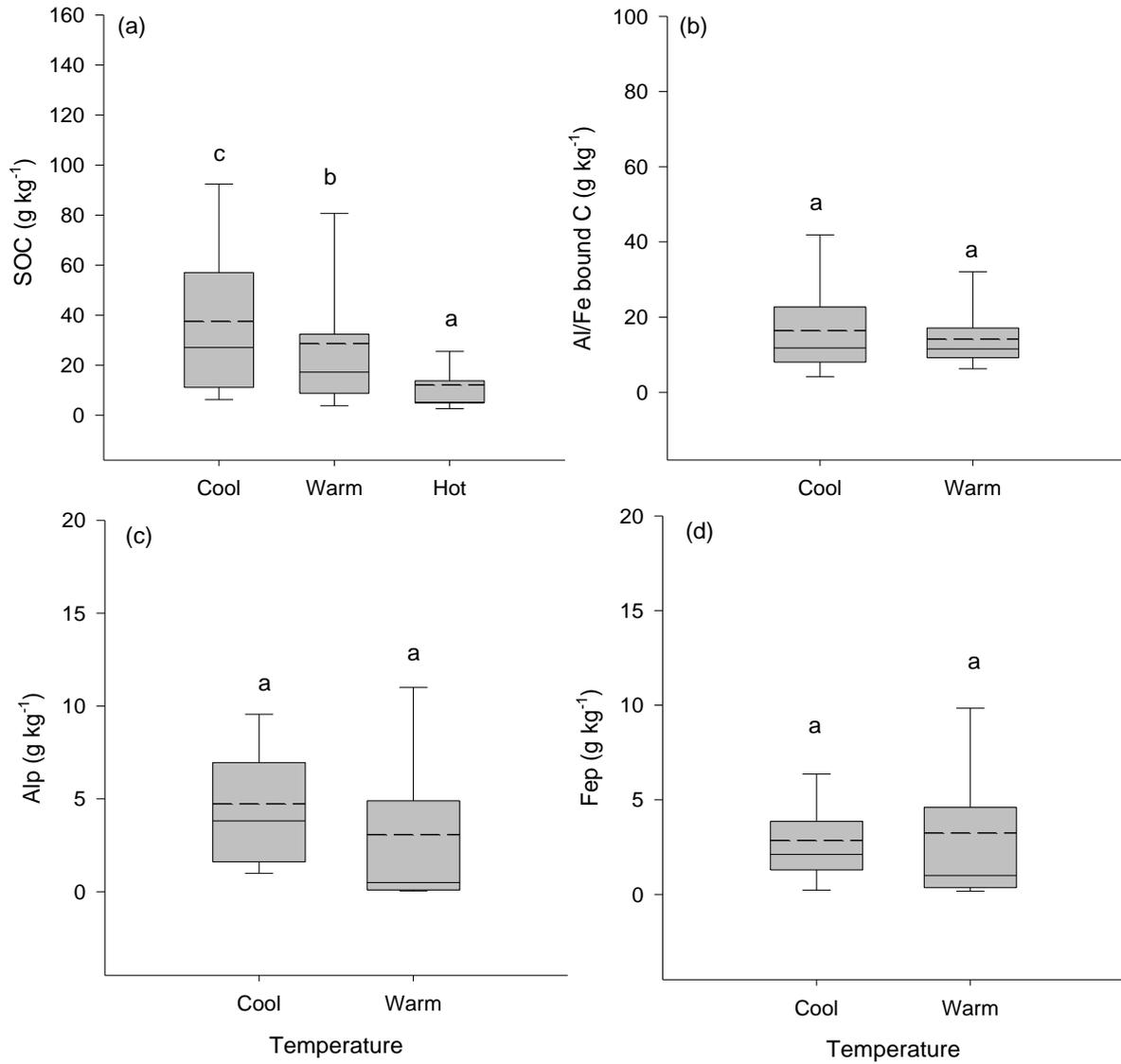


Figure 3. 3 Effect of temperature on soil organic carbon_a, Al/Fe bound C_b, organically bound Al_c and Fe_d. Each boxplot indicates the minimum, maximum, quartile 1 (25%) and quartile 3 (75%). Dotted lines indicate means and solid indicates a median. The different letters indicate a significant difference at $p < 0.05$. Where no data points were observed for the parameter the temperature class was removed.

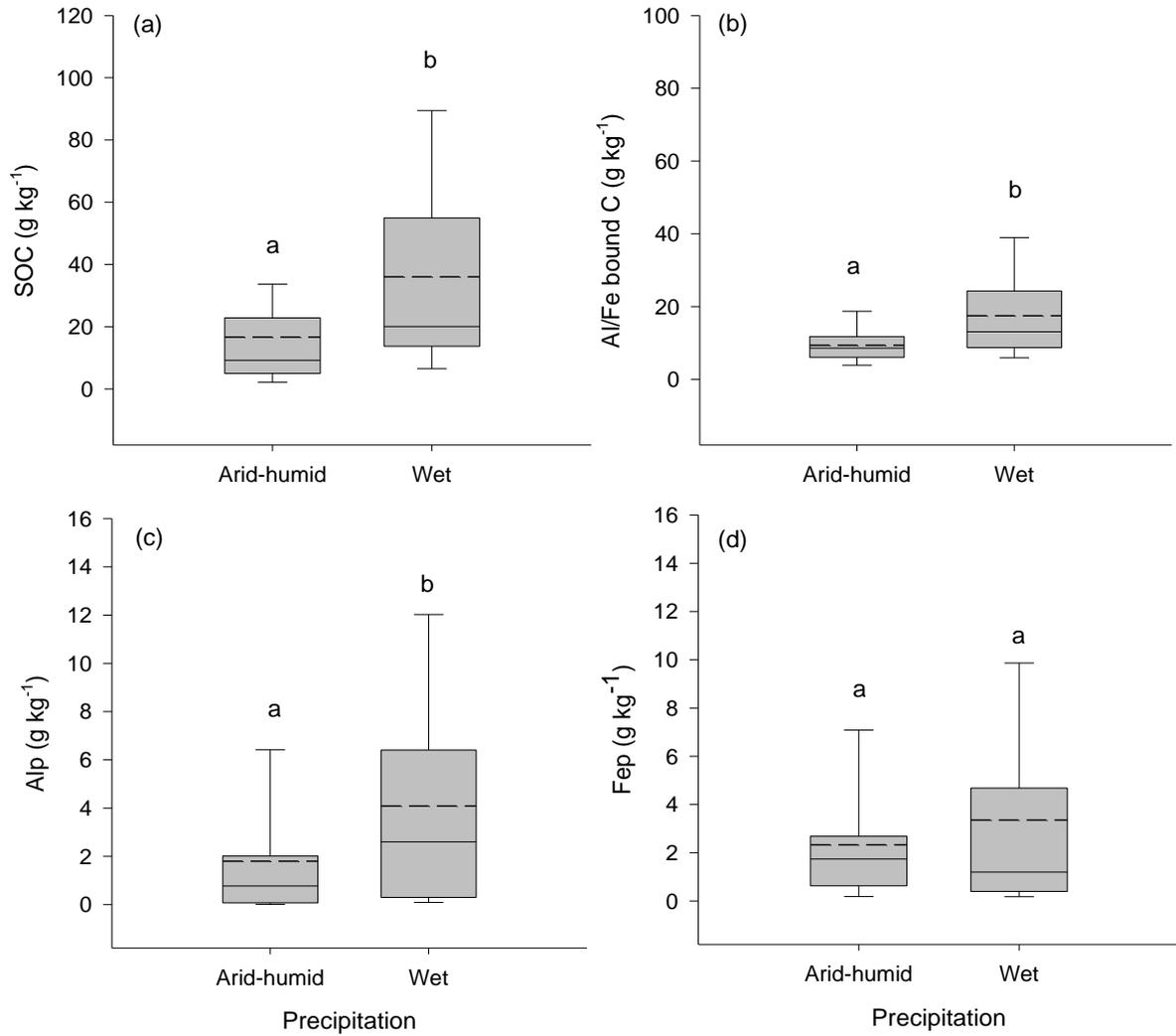


Figure 3. 4 Effect of precipitation on soil organic carbon_a, Al/Fe bound C_b, organically bound Al_c and Fe_d. Each boxplot indicates the minimum, maximum, quartile 1 (25%) and quartile 3 (75%). Dotted lines indicate means and solid indicates a median. The different letters indicate a significant difference at $p < 0.05$.

3.3.4 Distribution of SOC and Al, Fe and C in organo-mineral complexes under different land uses

Figures 3.5a, b, c, and d show the effects of land use on overall SOC content, Al/Fe bound C, Alp, and Fep. Cropland land use (17.5 g kg^{-1}) significantly decreased the overall SOC when compared to grassland (32.9 g kg^{-1}) and forest (40.8 g kg^{-1}) (Figure 3.5a). While it was not different from grassland (10.7 g kg^{-1}), cropland (14.2 g kg^{-1}) had significantly lower Al/Fe bound C when compared to forest (22.7 g kg^{-1}) (Figure 3.5b). Organically bound Al in cropland (1.50 g kg^{-1}) was significantly lower than the grassland (4.48 g kg^{-1}) and forest (5.22 g kg^{-1}) soils (Figure 3.5c), and the trend was similar to that of organically bound Fe (Fep) (Figure 3.5d).

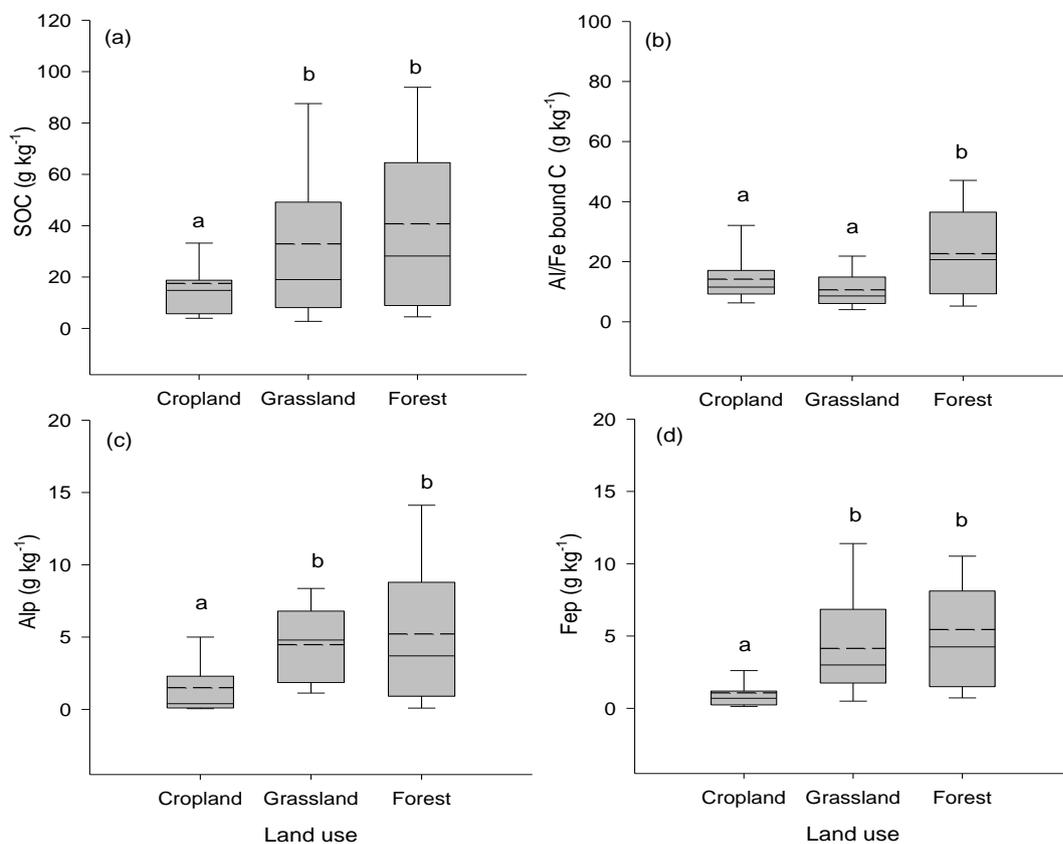


Figure 3. 5 Effect land use (cropland, forestry and grassland) on soil organic carbon_a, Al/Fe bound C_b, organically bound Al_c and Fe_d. Each boxplot indicates the minimum, maximum, quartile 1 (25%) and quartile 3 (75%). Dotted lines indicate means and solid indicates a median. The different letters indicate a significant difference at $p < 0.05$.

3.3.5 Variation of organo-mineral associations with soil properties and effects of land use

3.3.5.1 Soil pH and land use

Figure 3.6 shows the effects of soil pH on SOC, Al/Fe bound C, organically bound Al and Fe. Soil organic carbon content in acidic (35.0 g kg^{-1}) soil conditions was significantly higher than neutral (18.3 g kg^{-1}) and alkaline (12.5 g kg^{-1}) conditions, which translated to 91.3% and > 100% higher, respectively (Figure 3.6a). The concentration of Al/Fe bound C was 56% significantly higher in acidic (16.7 g kg^{-1}) soils than neutral (10.7 g kg^{-1}) soil pH. Similarly, organically bound Al and Fe concentrations were significantly higher in acidic soil conditions than neutral, which was higher than alkaline soil pH (Figures 3.6c and d). The Alp concentration ranged from 0.08 g kg^{-1} in alkaline conditions to 4.93 g kg^{-1} in acidic conditions (Figure 3.6c), while the Fep concentration ranged from 0.25 g kg^{-1} in alkaline conditions to 4.22 g kg^{-1} in acidic conditions (Figure 3.6d).

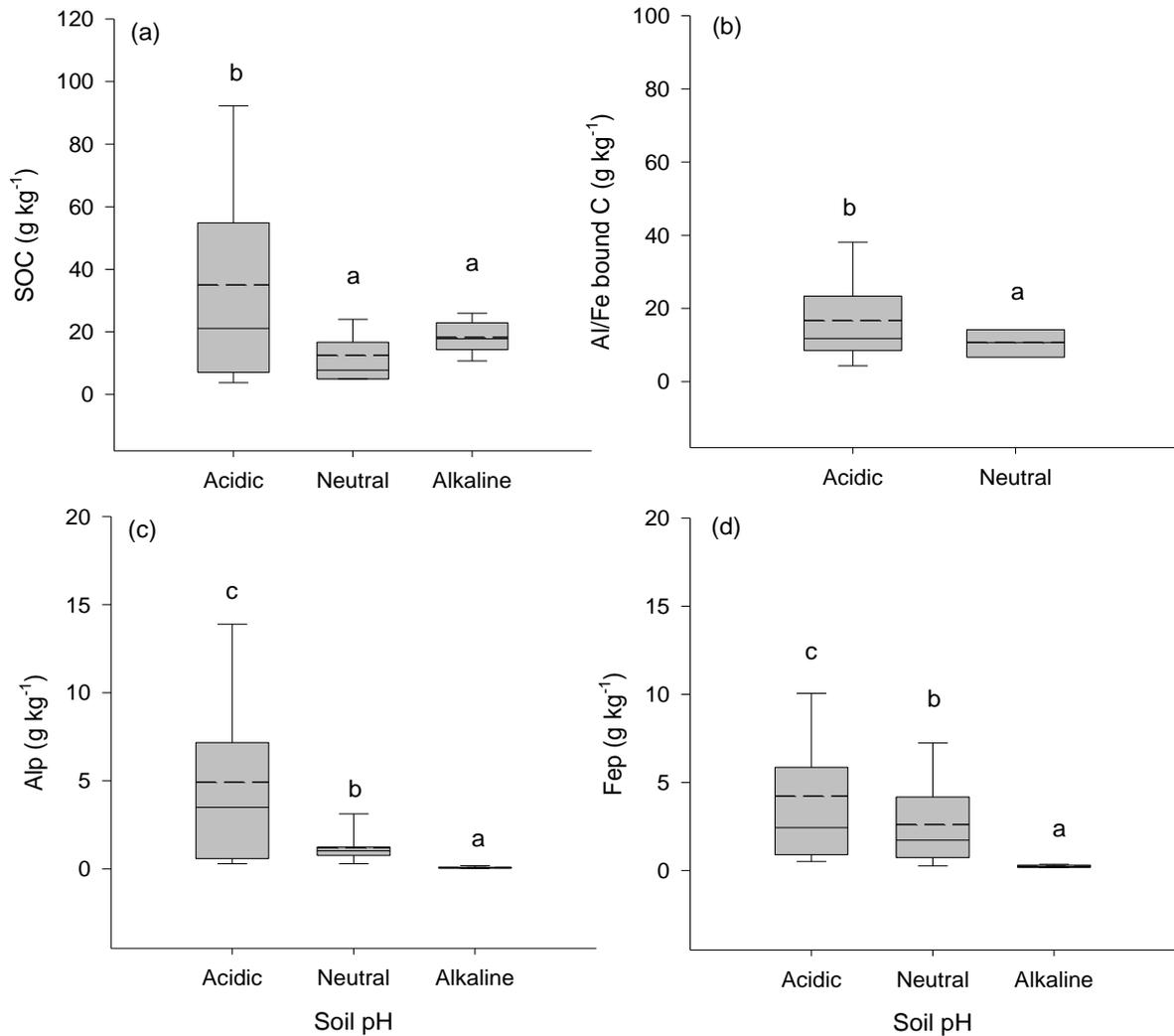


Figure 3. 6 Effect of soil pH on soil organic carbon_a, Al/Fe bound C_b, organically bound Al_c and Fe_d. Each boxplot indicates the minimum, maximum, quartile 1 (25%) and quartile 3 (75%). Dotted lines indicate means and solid indicates a median. The different letters indicate a significant difference at $p < 0.05$. Where no data points were observed for the parameter the soil pH class was removed.

3.3.5.2 Soil texture and land use

In the studies reviewed, soil organic carbon content was not affected by soil texture (Figure 3.7a). The Al/Fe bound C was significantly lower in clay (10.6 g kg^{-1}) than loam (17.4 g kg^{-1}) and sandy (17.2 g kg^{-1}) soil textures (Figure 3.7b). Similarly, the organically bound Al was significantly lower in clay (1.90 g kg^{-1}) soils than loam (3.47 g kg^{-1}) and sand (3.09 g kg^{-1}) soil textures (Figure 3.7c), while the organically bound Fe concentration was not affected by soil texture (Figure 3.7d). Land-use effects showed a significant effect under different soil textures (Figures 3.8a-f). For clay texture, Alp in forest (5.08 g kg^{-1}) was significantly higher than cropland (1.15 g kg^{-1}) by 4.41 times (Figure 3.8a) while Fep was not affected by land use. There was no significant difference between land uses on Alp and Fep concentrations in soils with loam texture (Figures 3.8c and d), with the Alp concentration being 3.49, 3.44, and 3.51 g kg^{-1} for cropland, forest and grassland, respectively (Figure 3.8c). In soils with sand texture, cropland had significantly lower Alp and Fep concentration in soils (by $> 100\%$) when compared to forest land use (Figure 3.8e; f), and the trend was the same for Fep (Figure 3.8f).

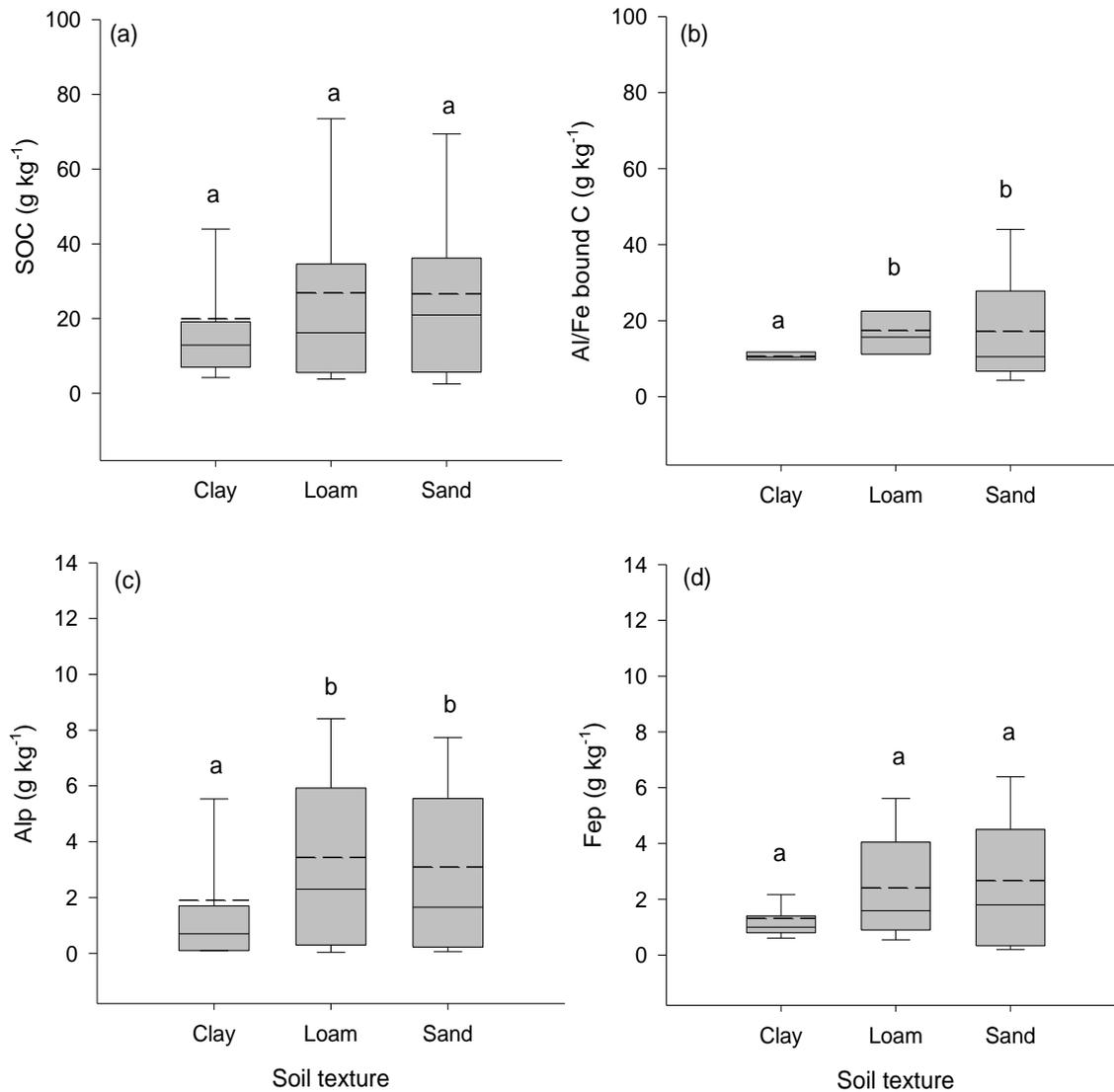


Figure 3. 7 Effect of soil texture on soil organic carbon_a, Al/Fe bound C_b, organically bound Al_c and Fe_d. Each boxplot indicates the minimum, maximum, quartile 1 (25%) and quartile 3 (75%). Dotted lines indicate means and solid indicates a median. The different letters indicate a significant difference at $p < 0.05$.

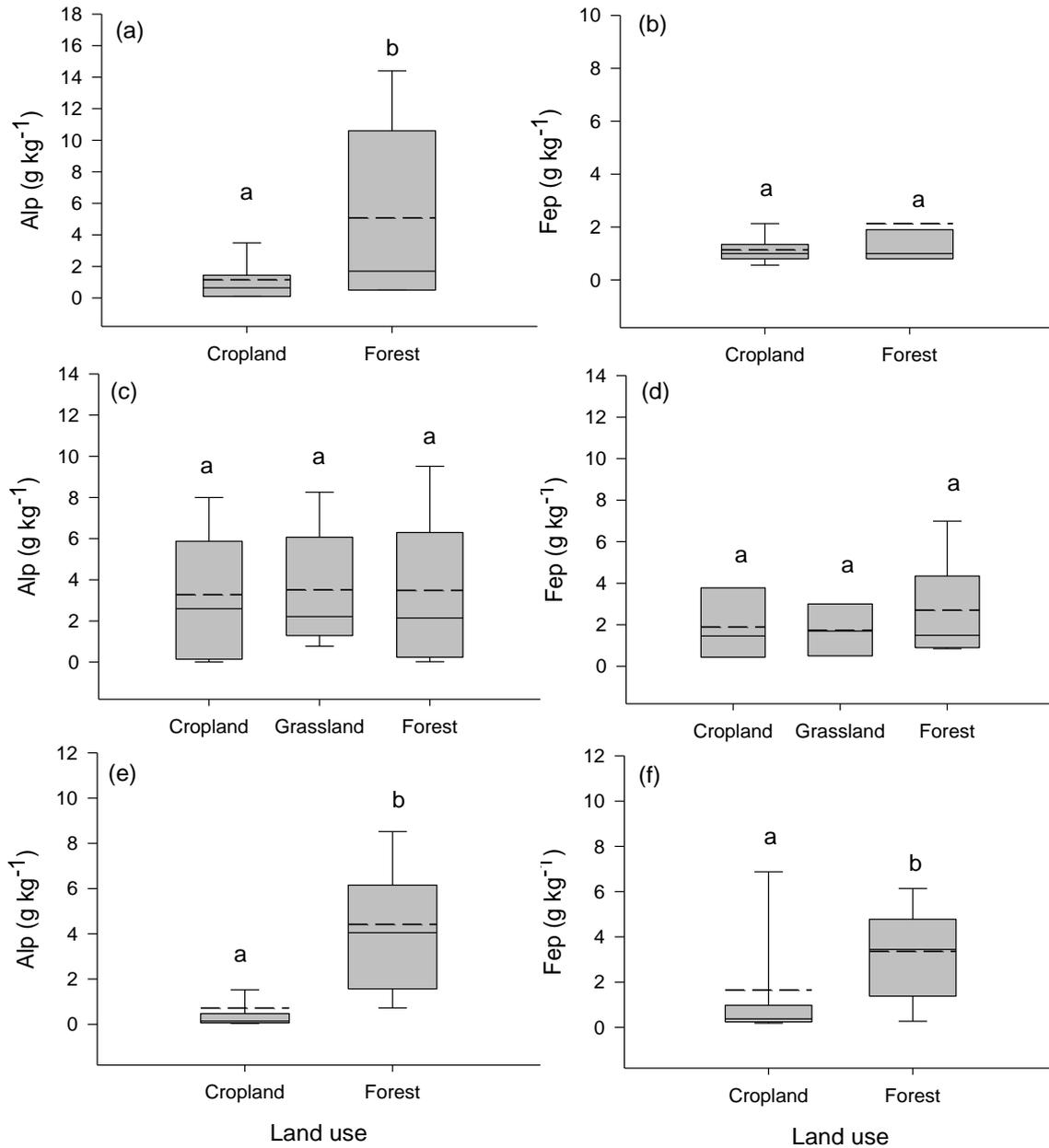


Figure 3. 8 Effects of Soil Texture on organically bound Al and Fe under different land uses. Figures a, c and e indicates organically bound Al in clay, loam and sand, respectively. Figures b, d and f indicates organically bound Fe in clay, loam and sand, respectively. Each boxplot indicates the minimum, maximum, quartile 1 (25%) and quartile 3 (75%). Dotted lines indicate means and solid indicates a median. The different letters indicate a significant difference at $p < 0.05$.

3.3.4.3 Soil organic carbon

Figures 3.9 a, b, c, d, e, and f show the effects of different soil organic carbon content on organically bound Al and Fe. Overall, organically bound Al ($r = 0.952$; $p < 0.001$) and Fe ($r = 0.707$; $p < 0.05$) exhibited a strongly significantly positively correlation with the soil organic carbon (Table 3.4). In soils with low SOC, there was no significant difference between land uses in organic bound Al and Fe (Figures 3.9a and b), while in soils with medium SOC content organically bound Al and Fe were significantly decreased by cropland (Figures 3.9c and d). In these soils, cropland (0.78 g kg^{-1}) showed $> 100\%$ lower Alp concentration than forest (2.40 g kg^{-1}) (Figure 3.9c), and the trend was similar to that of Fep concentration (Figure 3.9d). In soils with high SOC content, the concentration of Alp was not affected by land use (Figure 3.9e), while for Fep, cropland (2.02 g kg^{-1}) and grassland (4.14 g kg^{-1}) had lower concentration than forest (6.59 g kg^{-1}) (Figure 3.9f).

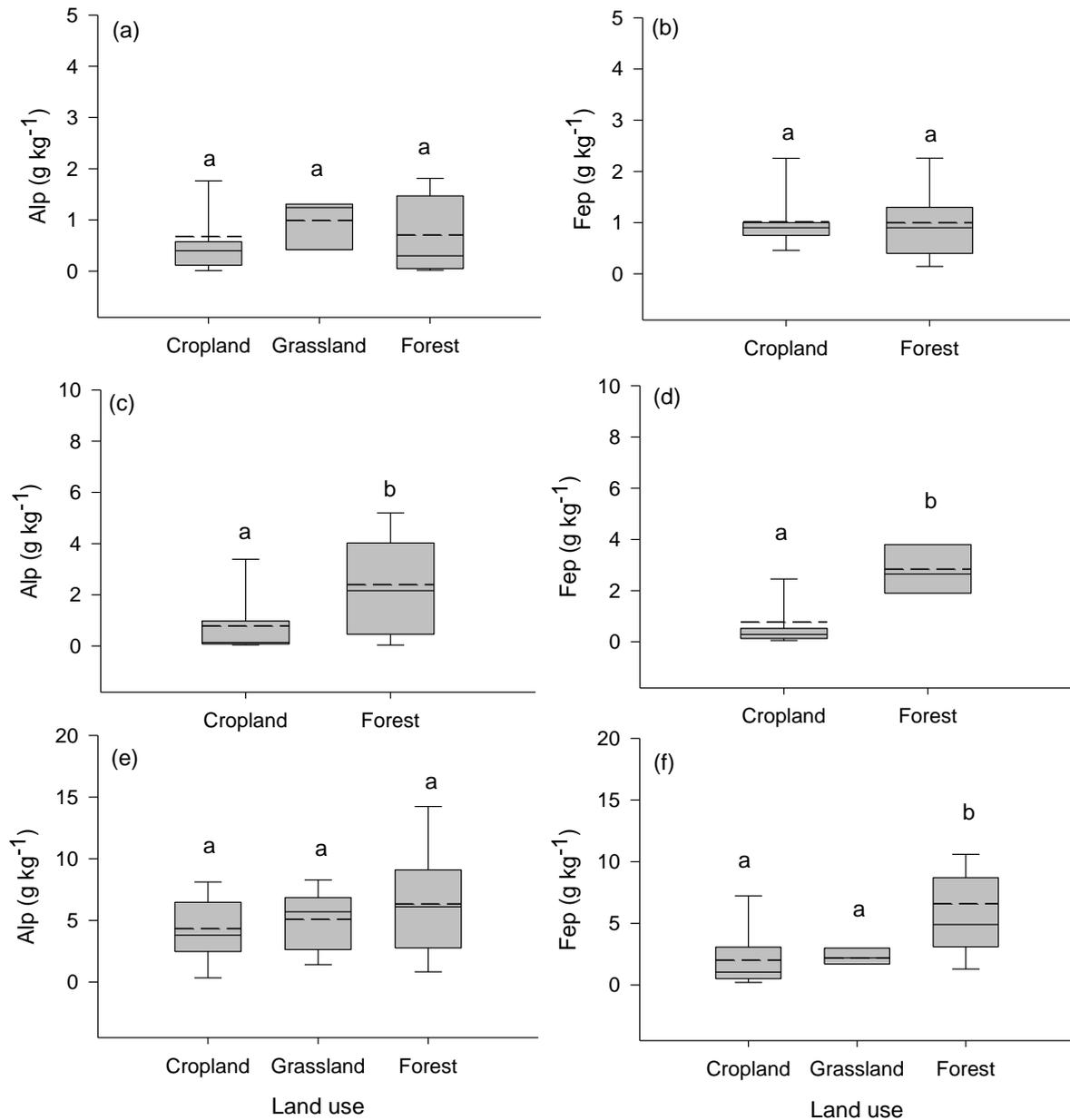


Figure 3. 9 Effects soil organic carbon content on organically bound Al and Fe concentration in different land use. Figures a, c and e indicates organically bound Al in low, medium and high SOC, respectively, Figures b, d and f indicates organically bound Fe in low, medium and high SOC, respectively. Each boxplot indicates the minimum, maximum, quartile 1 (25%) and quartile 3 (75%). Dotted lines indicate means and solid indicates a median. The different letters indicate a significant difference at $p < 0.05$. Where few data points were observed the land use was removed.

3.4 Discussion

3.4.1 Climate effects on SOC, Al/Fe bound C, organo-mineral complexes

The results from this review showed a significant effect of climate conditions on SOC, Al/Fe bound C, organically bound Al and Fe. The lower SOC in tropical climatic conditions is a result of high decomposition rates in tropical conditions due to high rainfall combined with high temperatures (Gmach et al. 2019; Ramesh et al. 2019). Dry and wetting cycles, especially in areas with high temperatures, could result in a significant flush in the decomposition process following wetting. This could result in rapid decomposition of C pools including those that are believed to be stable in soils, such as those in organo-mineral complexes. High temperatures and soil moisture are reported to enhance the decomposition process more resulting in the loss of SOC as carbon dioxide (Don et al. 2011; Kirsten et al. 2021). This rapid decomposition in SOC results in decrease in organo-mineral complexes concentrations in soils. The strongly positive correlation of SOC with Al and Fe in organo-mineral complexes (Table 3.4) emphasise the need for SOC in the formation of the complexes. The correlations of organically bound Al and Fe, and C bound to Al/Fe, which were strongly negatively correlated with MAP (Table 3.4) emphasise the effects of rainfall in facilitating the decomposition process reducing the SOC required for formation of Al/Fe-organic matter complexes.

In areas with hot temperatures ($> 25^{\circ}\text{C}$), SOC content was lowest (Figure 3.3a), due to high decomposition rates, especially when soil moisture is conducive (Blagodatskaya et al. 2016). However, in semi-arid and arid environments, the low SOC could be a result of low biomass production (Liu et al. 2014), which is required for the build-up of SOM. In temperate and subtropical regions microbial activities are limited due to lower temperatures causing minimal decomposition, coupled with high biomass additions, resulting in higher SOC content than tropical soil. This view agrees with the results of high SOC under cool temperatures ($<10^{\circ}\text{C}$) than warm and hot conditions (Figure 3.3a). The cool temperatures decrease the mineralisation of SOC (O'Donnell et al. 2016; Gutierrez-Giron et al. 2015) and increase accumulation due to the decrease in microbial activities, enzyme activities and biochemical processes (Hu et al. 2020; Xiao et al. 2018). The higher SOC in wet precipitation environments could be a result of limited oxygen, which limits activities of microorganism. In addition, the high SOC in areas with high rainfall could be explained by high biomass addition which increase the overall SOC over time.

3.4.2 The impact of land use on SOC and organically bound Al and Fe

The lower SOC in the cultivated sites is a result of aggregates breakdown due to conventional tillage activities during cultivation, exposing aggregate-protected SOM increasing decomposition by microorganisms (Hussain et al. 2021). The effects of tillage on aeration would vary depending on the extent of tillage. The lower SOC in cultivated land use was likely a result of increased mineralisation due to disruption of soil structure (Wairiu et al. 2003). Soil organic C stabilisation in soils is significantly controlled by soil pH (Matus et al. 2014). During cultivation different fertilisers including liming materials are applied which modified the soil conditions including soil pH and increase nutrients for microorganisms, hence decreasing the overall SOC over time. In forest and grassland, the soil acidity levels are general high, limiting microbial decomposition of SOM, coupled with accumulation of biomass, increasing the SOC content in the long term (Matus et al. 2014). Studies in the literature showed that cultivation results in a significant decrease in total SOC by 20–36% when compared to undisturbed land use (Bruun et al. 2013; Benbi et al. 2015). Porras et al. (2017) observed that organically complexed Al and Fe were the most predictors of SOC content in acidic soils. The soil amendments used during cultivation, such as organic and inorganic fertilisers, increase the decomposition of SOM pools, possibly including the organo-mineral complexes pool. The high organically bound Al and Fe in grassland and forest soils can be explained by the high SOC in those land use, which were strongly positively correlated (Table 3.4). However, Haddaway et al. (2017) argued that conventional tillage also redistributes SOC within the soil profile, potentially with no net increase/ gain in overall SOC content, and this effect may contribute in the observed lower SOC in cropped (cultivated) soils.

3.4.2.1 Soil pH and land use

Soil pH has a significant impact on the chemical and biological properties of soils and determines the rate of SOC decomposition (Paradelo et al. 2015). The higher SOC content and Al/Fe bound C in acidic soil conditions was expected due to the effects of soil acidity on microorganisms (Wang et al. 2017), which limits the processes of decomposition and mineralisation of SOC, resulting in accumulation of SOC compared to neutral and alkaline conditions. The Al/Fe bound C and SOC were negatively correlated with soil pH (Table 3.4). In addition to lower decomposition the lower availability of Fe and Al under neutral and alkaline condition also results in lower formation of the complexes with SOM, than under acidic conditions. Garrido and Matus (2012) also observed that the organo-mineral complexes

and SOC had an inverse relationship with soil pH (Garrido and Matus 2012), while Taboada et al. (2019) also observed higher organo-metallic complexes in acidic soils. Overall, SOC was negatively correlated to soil pH and this evidence that in lower soil pH high SOC will accumulate (Table 3.4). However, Wang et al. (2017) observed no significant differences in SOC content in soils with lower pH when compared to those with higher pH. The increase in soil pH to neutral and alkaline conditions results in a significant increase in microbial activities increasing the mineralisation of SOC in soils, hence reducing the overall concentrations (Liu et al. 2016; Zhou et al. 2017). In forest and grassland soils, the high level of soil acidity due to low pH limit microbial activities and results to accumulation of SOC instead of decomposition (Malik et al. 2018; Zhang et al. 2020). However, liming these soils could result in significant increase in microbial activities and losses of SOC as CO₂ reducing the overall concentration over time (Mkhonza et al. 2020). While in cropland where different liming materials are used for remediating soil acidity for crop production, the increase in soil pH could results to an increase in decomposition of SOC, hence reducing the overall concentration (Paradelo et al. 2015; Ghosh et al. 2016; Wang et al. 2016).

3.4.2.2 Soil texture and land use

The lack of effect of soil texture on SOC (Figure 3.6a) was not expected as clay soils are known to store more SOC content than loam and sand texture soils. The higher organically bound Al and Fe in loam and sandy soils than clay soils could be due to the reactivity of clay fractions, which could bind the Al and Fe in soil solution onto the surfaces with large surface area and high affinity for the metals (Alamgir 2016). This will reduce the overall concentration of available Al and Fe to bound with SOC in soil. As a result, sand and loam texture soils will have high concentration of solution Al and Fe, especially in acidic conditions, which binds with SOC, increasing the organically bound Al and Fe. Concentration of organically bound Al and Fe varies with the soil textural distribution in soils. From this meta-analysis, clay and sandy soils have high organically bound Al in uncultivated land use (forest) compared to cultivated land use (cropland). The high organically bound Al and Fe under uncultivated land use could be explained by high C content in the clay soils, which is bound with Al and Fe oxides in clay fraction, while cultivation exposes the SOC protected in organo-mineral complexes to microbial attack due to tillage and soil amendments. The higher organically bound Al and Fe in (uncultivated) forests soils was due to limited soil disturbance and lack of liming which facilitates microbial activities and decomposition and mineralisation of SOC. Cultivation

increases decomposition process, possibly including SOC in organo-mineral complexes, hence decreasing the overall concentrations regardless of soil texture. The high organically bound Al and Fe in forest compared to cropland in sandy soils could be explained by the high microbial activities in sandy croplands which increase soil aeration, hence increasing microbial decomposition of SOC (Chowdhury et al. 2021). In addition, the use of agricultural liming materials in cropland enhance soil microbial activities (Vazquez et al. 2019; Wang et al. 2021). While in forest soils with sandy textures the soil acidity and less soil disturbance could limit microbial activities, even though there is adequate aeration, hence resulting to accumulation of SOC instead of decomposition.

3.4.3.2 Soil organic matter and land use

In soils with low SOC content the lack differences in organically bound Al and Fe observed between land uses could be due to limited SOC to reacts with the Al and Fe in soils with low SOC. The lack of land use association on Alp and Fep in soils with low SOC could be because the organic matter was low such that the decrease in SOC due to cultivation is not significant. However, this was contrary to the findings of Porras et al. (2017), who reported that soils with low SOC content had lower concentration of organic bound Al and Fe than those with high SOC content in uncultivated forest soils. These differences could be due to other soil properties but not SOC concentration of the soils. The significantly higher organically bound Al and Fe in soils with medium and high SOC content than those with low contents, could be explained by the availability of SOC which reacted with Al and Fe forming organo-mineral complexes. In these soils, the lower organically bound Al and Fe in cropped soil than forest and grassland (uncultivated) could be explained by lower biomass addition coupled with rapid decomposition due to the effects of cultivation. The organically bound Al and Fe were strongly positively correlated with SOC content (Table 3.4), emphasising the need for SOC to form the complexes, especially in the presence of Al and Fe. The decrease in SOC concentration in cultivated land uses could have a significant effect on organo-mineral complexes formation through reduced available SOC to form complexes with Al and Fe (Xue et al. 2019). While it is clear that cropping reduces SOC, Fe and Al bound in organo-minerals complexes, when compared with uncultivated soils, effects of differences in management in cropped soils are not clearly understood. Management practices such as residue retention and liming increase biomass input in soils, which could increase the concentration of organo-minerals complexes, while liming enhances decomposition.

3.5 Conclusions

The following main conclusions can be drawn from the current study of 574 data points at a global scale, investigating the effects of land use (cultivated and uncultivated) on SOC bound to Al and Fe in soils:

- (i) Tropical climatic conditions significantly reduce the concentration of SOC, organically bound Al and Fe.
- (ii) The SOC and organically bound Al and Fe were higher in acidic soils than neutral or alkaline soils, and in soils with loam and sand texture than clay.
- (iii) Croplands have lower organically bound Al and Fe and overall SOC compared to forest and grassland soils.

Management decisions need to be made using these findings in terms of increasing C storage in soils, which will also benefit soil fertility, yields and food security. More research should focus on understanding the overall effects of increased biomass addition and liming in cropped soils on different organic carbon fractions, including the SOC, Fe and Al bound in organo-minerals complexes, especially in soils with high organic matter, such as humic soils. The overall effects of increased biomass addition and liming is not clearly understood, especially in soils with high organic matter, such as humic soils.

CHAPTER 4

Organic carbon and microbial activity in Umbric Rhodic Ferralsol soils under green cane relative to pre-harvest burning of sugarcane

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Abstract

Purpose Burning of sugarcane for easier harvesting and increase sucrose recovery due to trash removal, may reduce soil quality particularly for Umbric Rhodic Ferralsols that have naturally high concentrations of soil organic carbon (SOC). Harvesting without burning (green cane) retains large quantities of biomass on the surface, which may be an alternative to improve carbon (C) sequestration depending on soil (texture and soil pH) and environmental conditions (moisture and temperature), and microbial activity. In the current study, the effects of green cane relative to pre-harvest burning on SOC concentration and stocks, microbial biomass carbon (MBC), β -D-glucosidase enzyme activity were examined.

Materials and methods Soils under green cane were compared with adjacent pre-harvest burning fields. Green cane harvest management have been practiced for 73 years and the adjacent pre-harvest burning was practiced for a longer period (>73 years). The soils were sampled, in March 2021, from the 0-5, 5-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-80 and 80-100 cm depths, using micropits in the top 10 cm and using augers for deeper layers. The soils were analysed for SOC, MBC, β -D-glucosidase activity. In addition, soil microbial quotient (SMQ) was calculated.

Results and discussion The SOC concentration and stock, MBC, SMQ and β -D-glucosidase activity were significantly higher under green cane than under burnt cane. The overall effect of management up to 1 m depth on SOC showed that the SOC under green cane was 24% higher than for burnt cane. The MBC under green cane and pre-harvest burning constituted 3.52 and 1.25% of the total SOC, respectively, when averaged to 1 m depth. The concentration of all the parameters decreased with an increase in soil depth. The SMQ was not affected by soil profile depth. The β -D-glucosidase enzyme activity was higher under green cane than pre-harvest burning in the top 5 cm depth but not at deeper layers. The β -D-glucosidase activity was positively correlated to the bulk SOC and MBC.

Conclusions These findings imply that green cane production increases SOC storage, and microbial activity, when compared with the pre-harvest burning on the Umbric Rhodic Ferralsol in South Africa. The findings could be used to make informed decision in view of climate change.

Keywords β -glucosidase activities · Green cane retention · Microbial biomass carbon · Pre-harvest burning · Soil aggregation

4.1 Introduction

The contribution of soils in storing organic carbon (OC), which mitigates climate change effects, has drawn attention for researchers to study the management of soil OC, globally. Pre-harvest burning of sugarcane (burnt cane) for easier harvesting and increase sucrose recovery due to trash removal, may reduce SOC and overall soil quality and increase carbon dioxide (CO₂) concentration in the atmosphere, resulting in global warming and climate change. However, the effect of burning varies depending on the fire severity, soil texture and soil type (Pereira et al. 2018; Delijani et al. 2022). Pre-harvest burning of sugarcane only removes about 80% of the trash (the tops, green leaves and dry leaves) resulting in easier harvesting and reduced labour (Ripoli et al. 2000). Harvesting without burning (green cane) retains large quantities of biomass on the surface, which may be an alternative to improve carbon (C) sequestration depending on soil texture and soil pH, moisture and temperature, and microbial activity. Strategies that increase SOC storage, such as retention of residues, are essential for improving the fertility status of soils, their water retention capacity and nutrient cycling (Morais et al. 2020), which improve crop productivity.

The green cane harvest system has been proved to increase soil aggregation and SOC content over time, especially in top soil layers in other parts of the world (Graham and Haynes 2002; Razafimbelo et al. 2006; Cerri et al. 2011). While policies are being implemented to move from pre-harvest burning to green cane globally (Robertson and Thorburn 2007; Cerri et al. 2011; Galdos et al. 2013; Page et al. 2013) due to environmental concerns, there is no such management policy in the sugarcane industry of South Africa. In South Africa, sugarcane has been produced for more than 150 years and is mostly produced in KwaZulu-Natal and Mpumalanga provinces. In 2021/ 2022, South Africa produced 18.0 million tonnes of sugarcane, which showed a decreased from previous years (Statista 2022). Some of the sugarcane produced is used locally and export to other countries. Some of the soils on which

sugarcane is produced in South Africa, include those that are locally classified as humic soils (Soil Classification Working Group, 2018), and translate to Umbric Rhodic Ferralsols (IUSS Working Group WRB 2015)

These soils are well-drained, highly weathered, acidic and have high OC $> 18 \text{ g kg}^{-1}$, only known to occur in South Africa (Fey 2010). The effects of pre-harvest burning on SOC and microbial activity, in loamy Umbric Rhodic Ferralsols, with high concentrations of OC, is poorly understood. The high SOC, even with loamy texture, makes these soils unique and suggest that the mechanisms of SOC stabilisation and effects of management, including burning, on SOC may be significantly different from other soils. Galdos et al. (2009) observed an increase in soil C stocks and microbial biomass C under sugarcane residues retention than burned residues. In a long-term study high C concentration was reported in unburned sugarcane residues management compared to burned treatment (Canellas et al. 2003; Razafimbelo et al. 2006). The findings in Chapter 3 showed that effects of cropping on SOC bound on organo-mineral complexes are more pronounced in soils with high organic matter, including both clay and sandy soils. While these effects are clear for non-humic soils, no such studies have been done on these soils, which naturally have high concentrations of OC. Malepfane et al. (2022) showed that conversion of forests to sugarcane only lowered OC in the top 10 cm, with no significant effects on OC stocks in 1.0 m profiles of Umbric Rhodic Ferralsols. The effects of conversion of land under pre-harvest burning to green cane on soil OC concentrations and stocks and microbial activity, are not clearly understood.

Soil enzymes are the catalyst that mediate the process of decomposition and release of nutrients from the soil organic matter. It is well documented that any management practice increasing soil organic matter will consequently increase soil biological activities. Soil microbial biomass is essential for the formation of soil organic carbon in soil environment (Xu et al. 2020). This makes it special, in soil fertility, as a labile C pool and microorganisms are major drivers of C decomposition (Wei et al. 2022). Additionally, the activities of enzymes and microorganisms are highly governed by soil pH, which affects their concentration and distribution within the soil profile. Green cane and pre-harvest burning have been reported to significantly affect soil pH levels (Mthimkhulu et al. 2018) and SOC, which would consequently affect soil biological processes.

Burning sugarcane straw on soil surface has been shown to significantly increase soil pH levels than retention the residues (Oliveira Finho et al. 2021). While the reduction in acidity due to

pre-harvest burning could activate microbial activity, the loss of the labile OC, as CO₂, may reduce available substrate of the soil microorganisms. The overall effect could be more complex in Umbric Rhodic Ferralsols, which naturally have high levels of OC, even when converted to cropping. Conversely, residue retention in green cane increases the input of OC as added fresh biomass, which has been shown to significantly improve soil moisture storage and moderate soil temperature, also possibly improving microbial activity (Turmel et al. 2015). However, the effect of green cane on SOC concentration and stock, in the profile and microbial activity in Umbric Rhodic Ferralsols (acidic soils with high OC), locally known as humic soils in South Africa, are not understood.

While most research on soil OC has been focused on the topsoil layers, Malepfane et al. (2022) showed that large quantities of OC can be stored in deeper layers of some Umbric Rhodic Ferralsols with >100 g kg⁻¹ OC in the top 10 cm, and > 20 g kg⁻¹ to a depth of 1.0 m, resulting in higher OC stocks in the deeper layers than the topsoil. The authors also showed that sugarcane production reduced the concentration of OC only in the top 10 cm with no effects on profile OC stocks when compared with forest soils. There is a paucity of information on the effect of green cane, compared to burnt cane, on SOC and microbial activity, including in the subsoil, of Umbric Rhodic Ferralsols in South Africa. We hypothesise that (i) pre-harvest burning would result in a decrease in SOC concentration and stock (ii) pre-harvest burning would result to lower microbial biomass C and β-D-glucosidase enzyme activity, when compared to green cane retention. Therefore, in the current study, we examined the effects of green cane harvesting relative to pre-harvest burning on soil organic carbon concentration, microbial biomass carbon, β-D-glucosidase enzyme activity in a loamy Umbric Rhodic Ferralsols.

4.2 Methods and material

4.2.1 Study sites and soils

Two experimental sites were located in Stanger, KwaZulu-Natal, South Africa and all the fields were under sugarcane production (pre-harvest burning -29° 12' 58.86" S; 31° 9' 19.908" E and green cane -29° 12' 58.464" S; 31° 9' 20.448" E) (Figure 4.1). Soil samples were collected from green cane field adjacent to pre-harvest burning (burnt cane). The area had an annual rainfall of 1233 mm on average per annum and annual temperature with minimum of 19° C and maximum of 29° C. For the burnt cane, NPK fertiliser was applied once every year at 600

kg ha⁻¹ of 3:1:5 (26) or 500 kg ha⁻¹ of 4:1:6 (11) NPK, depending on season and soil test results, with no additional organic amendments. Lime application was done before planting, at about 1.0 t ha⁻¹ and gypsum at 3.0 t ha⁻¹ depending on acid saturation. Sugarcane was rotated with sunn hemp (*Crotalaria Juncea*) in summer and black oat (*Avena Strigosa*) in winter before replanting. The green cane farm was not burnt for over 73 years. Lime and gypsum were applied at a rate of 5 t ha⁻¹ and 2 t ha⁻¹, respectively, at replanting. Ploughing was only done when the lime and gypsum were to be applied. Minimum tillage system was used during replant which is normally about 17 years. The sugarcane was rotated with sweet potatoes, black or winter oats (*Avena strigosa*) and sunn hemp (*Crotalaria Juncea*) before replanting. Depending on soil tests analysis results, fertilisers were applied annual, the 2: 3: 4 (27) and 2: 3: 2 (22) NPK were applied in the furrow for plant cane at a rate of 500 kg ha⁻¹. For ratoon cane fertiliser 1: 0: 1 (36) and 5: 1: 5 (36) was applied in at a rate of 350 and 500 kg ha⁻¹, respectively. The soils were both underlain by Natal Group sandstone as a parent material. Both soils were classified as Inanda soil form with thick humic A horizon overlying red apedal B horizon over unspecified material (Soil Classification Working Group 2018), which was translated to Umbric Rhodic Ferralsols according to the World classification system (IUSS Working Group WRB 2015). The clay content of the soils was 45.6% under green cane and 48.1% under burnt cane.

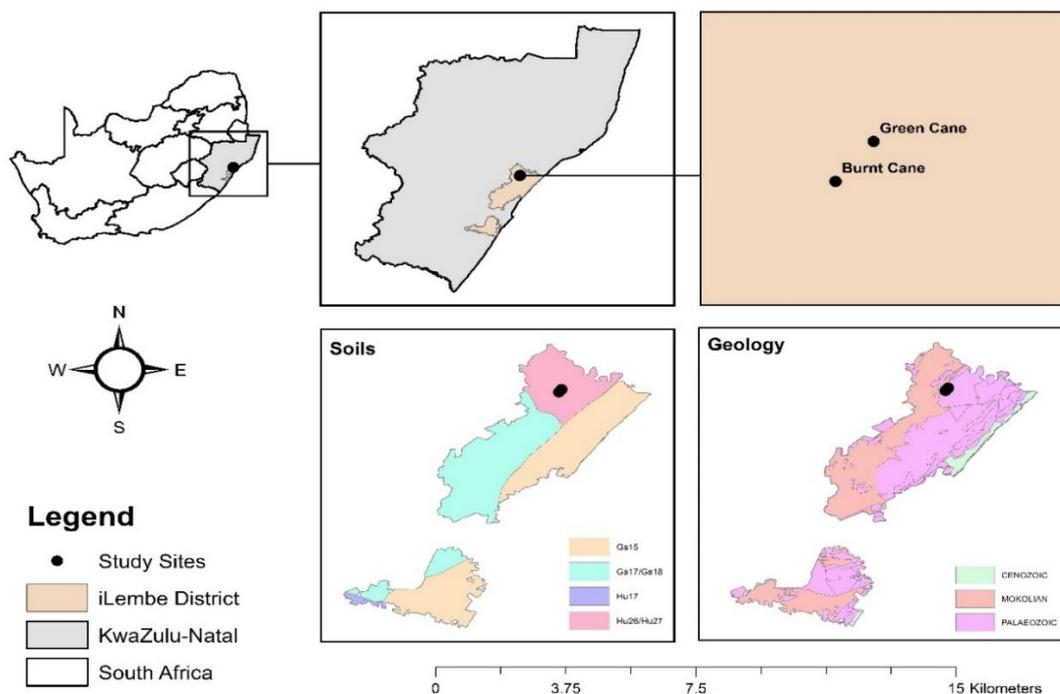


Figure 4. 1 Location of the study area within iLembe, KwaZulu-Natal, South Africa and the soils and geology information.

4.2.2 Soil Sampling

The soils were sampled, in March 2021, from the 0-5, 5-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-80 and 80-100 cm depths, with spades soil profile micropits in the top 10 cm and using augers for deeper layers. Four replicates were collected per sites. From each field, three soil sub-samples were collected and mixed to make a composite soil sampling for each soil depth. The soil samples were air dried, homogenised, and sieved (<2 mm) before analysis for some physicochemical properties, SOC, MBC, and β -D-glucosidase activity.

4.2.3 Soil chemical analysis

Soil pH, exchangeable bases and acidity were analysed using methods documented by the Non-Affiliated Soil Analysis Work Committee (1990). Briefly, soil pH was analysed in 1M KCl (1:2.5 soil to KCl suspension). Exchangeable bases (Ca^{2+} and Mg^{2+}) and acidity were extracted using 25 ml 1M KCl solution after shaking for 10 minutes in a shaker at a speed of 400 r.p.m. Thereafter, the concentration of Ca^{2+} and Mg^{2+} were then determined using the atomic absorption spectrophotometry (Varian 2600) (Van der Merwe et al. 1984). From the same extract exchangeable acidity was determined using the acid-base titration. Potassium, Zn, Mn and Cu were extracted using the Ambic-2 method, followed by quantification using the atomic adsorption spectrophotometry (Varian 2600) (Van der Merwe et al 1984). The clay content was estimated by the mid-infrared reflectance using the air dried, milled samples.

4.2.4 Soil organic carbon

Soil OC in soil was determined using the Walkley-Black dichromate acid wet oxidation method (Walkley and Black 1934). For this, 0.2 g soil sample (<0.5 mm) was weighed into 500 mL conical flask. Thereafter, 10 mL potassium dichromate was added to the sample while swirling the flask, followed by 20 mL of concentrated sulphuric acid under the fumed. Thereafter, the mixture was allowed to cool under the fume hood for 30 minutes before the addition of 170 mL deionised water in each flask followed by the addition of 10 mL phosphoric acid. Sodium fluoride (0.2g) and five drops of a ferroin indicator were added to the flask followed by titration using 0.5N ammonium ferrous sulphate, until the colour changed from dark green to dark brown. Two blank samples without soils were treated the same way as the samples. This was done to standardise the ferrous sulphate.

4.2.5 Determination of soil C stocks

The bulk density, for calculating OC stock, was estimated using the pedotransfer function (Abdelbaki 2016) on Equation 1:

$$BD = 1.66 - 0.308 (OC)^{0.5} \quad \text{Equation 1}$$

Where BD is the estimated soil bulk density; OC is organic carbon (%) of the soil.

The soil OC stock was calculated using Equation 2 (Malepfane et al. 2022):

$$\text{Soil C stock (Mg C ha}^{-1}\text{)} = C \times BD \times \text{profile depth} \times 10000 \times 0.001 \quad \text{Equation 2}$$

Where C is the soil carbon content (g kg^{-1}); BD is the soil bulk density (g m^{-3}); layer depth is the soil profile depth (cm); 10000 is a conversion factor for m^{-2} to ha^{-1} and 0.001 is a conversion factor for kg to Mg.

4.2.6 Soil microbial biomass C

The soil microbial biomass C (MBC) was determined using the chloroform-fumigation-extraction method (Vance et al. 1987). Moist sample (10 g) was fumigated in a vacuum desiccator with ethanol-free chloroform in a desiccator for 24 hours in a constant temperature room 25°C . Boiling chips were added to the chloroform followed by evacuation with vacuum pump for 5 minutes until the chloroform boiled, after which the tap was closed and incubated for 72 h. The same was done for the unfumigated samples without chloroform. After evacuation of the chloroform, soil MBC was extracted using 50 ml 0.5M K_2SO_4 solution from both fumigated and unfumigated samples. The mixture was shaken on a reciprocal shaker at 180 rpm for 30 minutes, thereafter, centrifuged and filtered using Whatman No. 1 filter paper into storage bottles. Organic C in the extracts was determined using the Walkley-Black dichromate oxidation method. Soil microbial biomass C was then determined as the difference in OC between fumigated and unfumigated samples. The OC was expressed in g kg^{-1} of soil corrected for moisture.

$$\text{MBC (g kg}^{-1}\text{)} = 2.64 * (\text{Extractable C in fumigated sample} - \text{extractable C in unfumigated sample})$$

Where: 2.64 is the Kc Factor (It is a factor used to correct the results for inefficiency of the extract.)

In addition to MBC, The SMQ was calculated as follows:

Soil Microbial Quotient = MBC / SOC (Lepcha and Devi 2020)

4.2.7 β -D-glucosidase enzyme assay

The activity of the β -D-glucosidase enzyme was assayed as described by Tabatabai (1994) using the substrate p-nitrophenol- β -D-glucopyranoside. Field moist soil (1.0 g) was placed in a 50 ml Erlenmeyer flask and treated with 0.25 mL toluene, 4 mL of modified universal buffer (MUB) and 1 mL of p-nitrophenyl- β -D- glucopyranoside prepared using the same MUB (pH = 6.0). The flask was then incubated at 37°C for 1 hour, after which the reaction was terminated by the addition of 1 ml of 0.5M calcium chloride and 4 mL of 0.1M Tris (hydroxymethyl) aminomethane adjusted to pH 12 using 0.5M sodium hydroxide solution, and filtered through Whatman No. 42 filter paper. The intensity of the yellow colour was read on the UV/VIS spectrophotometry at 410nm wavelength. All values for all treatments were corrected for a blank (using the concentration recorded from the controls where the substrate was added after the reaction was stopped using 1 mL calcium chloride and 4 ml 0.1M Tris (hydroxymethyl) aminomethane at pH 12). The p-nitrophenyl released was expressed as $\mu\text{g p-nitrophenyl released g}^{-1}$ moist soil h^{-1} .

4.2.8 Scanning Electron Microscope analysis

Selected soil samples from pre-harvest burning and green cane were used for scanning electron microscope (SEM) analysis. The samples were taken from the first 10 cm with depth increments of 0-5 and 5-10, and deeper layer were analysed 80-100 cm depths. The top two depths and deeper soil depths were chosen to see the variation in the surface, which had high SOC and deeper soil layers, which had significantly lower C levels under the sugarcane management systems. To examine the surface properties of the soils particles, the bulk soils were imaged with an SEM (EVO LS15, Carl Zeiss Microscopy, New York, USA).

4.2.9 Statistically analysis

The data of SOC, MBC, SMQ, C Stocks and β -D-glucosidase enzyme in Umbric Rhodic Ferralsols under green cane relative to pre-harvest burning were subjected to two-way ANOVA using GenStat Ed. 18. The least significant difference (LSD), at 5% level was used to compare treatments mean. The Tukey-Kramer test was also applied to separate treatment means at $p < 0.05$. Correlation analysis was done using spearman's rank correlation in GenStat.

4.3 Results

4.3.1 Selected soil physico-chemical properties

Although both were acidic ($\text{pH} < 5.00$), soil under pre-harvest burning ($\text{pH} = 4.03$) had significantly higher pH than green cane ($\text{pH} = 3.85$) (Table 4.1). Total bases were significantly affected by burning, with green cane ($5.08 \text{ cmol}_c \text{ kg}^{-1}$) having significantly higher than pre-harvest burning ($3.31 \text{ cmol}_c \text{ kg}^{-1}$). Exchangeable K was significantly higher in green cane fields (45.7 mg kg^{-1}) than pre-harvest burning (26.0 mg kg^{-1}). Similarly, to exchangeable K, exchangeable Mg was significantly higher under green cane than burnt cane, while exchangeable Ca was not affected by burning or depth. The soil pH, total bases, Mg, Ca concentrations were not affected by soil depth, except for exchangeable K which decreased with increase in soil depth, ranging from 20.2 mg kg^{-1} in the 20-30 cm depth to 65.4 mg kg^{-1} in the 20-30 cm depth. Copper, Mn, and Zn concentrations were higher under green cane than pre-harvest burning (Table 4.1).

Table 4. 1 Soil fertility status of soils in pre-harvest and green cane harvest systems

Factor	K	Ca	Mg	Cu	Zn	Mn	Soil pH	Total bases	Exch. Acidity
Burning	mg kg ⁻¹						cmol _c kg ⁻¹		
Green cane	45.7b	481	127b	5.74b	2.54b	7.23b	3.85a	5.08b	1.19
Pre-harvest burning	26.0a	462	38.0a	3.34a	0.74a	6.06a	4.03b	3.31a	1.39
LSD	9.90	160	8.08	1.19	0.516	1.18	0.231	1.05	0.252
Depth (cm)									
0-5	65.4c	386	87.7	4.01	2.03b	8.21b	3.89	4.01	1.33
5-10	43.6b	482	83.6	3.32	1.75ab	7.03ab	3.82	4.48	1.51
10-20	26.2ab	576	88.0	3.08	1.60ab	6.35ab	3.91	4.61	1.15
20-30	20.2a	456	71.6	3.46	1.18a	5.00a	3.91	3.69	1.18
LSD	14.0	227	11.4	1.37	0.365	1.67	0.11	1.48	0.356

Different lower-case letters within a column for each factor indicate significant differences ($p < 0.05$) among treatments. Where means do not have letters, within a column for each factor, they are not significantly different.

4.3.2 Soil organic carbon concentrations and stocks

Pre-harvest burning significantly decreased soil OC when compared to green cane retention, irrespective of depth (Figure 4.2a). The soil OC in green cane was 25% higher than that under burnt cane. For both green cane and pre-harvest burning, soil OC significantly decreased with the increase in soil depth. Even though SOC decreased with depths, the concentrations at deeper layers was still $> 14 \text{ g kg}^{-1}$ at 80-100 cm depth, with more OC in the first 30 cm depth ($> 60 \text{ g kg}^{-1}$) (Figure 4.2b). Soil OC ranged from 14.7 g kg^{-1} in the 80-100 cm depth to 68.6 g kg^{-1} in the 5-10 cm depth (Figure 4.2b). Soil C stocks were significantly lower with pre-harvest burning than green cane retention, with a reduction of 24.6% (Figure 4.3a). Soil organic C stocks increased with an increase in soil depth to a depth of 50-60 cm, with the stock ranging from 30.8 Mg ha^{-1} in the top 5 cm depths to 238 Mg ha^{-1} in the 50-60 cm depths (Figure 4.3b).

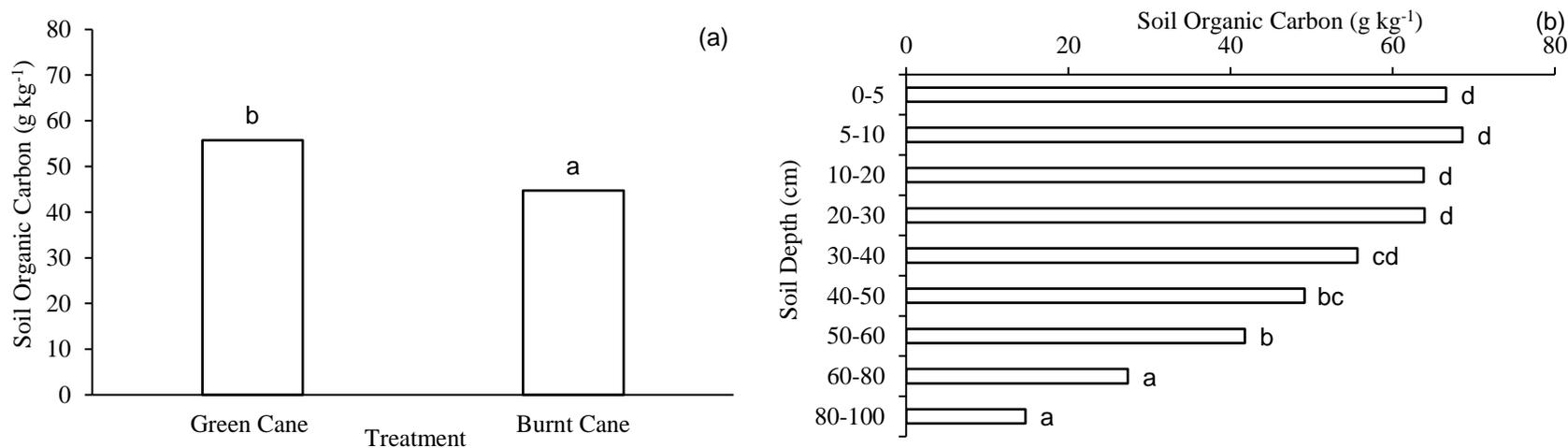


Figure 4. 2 Effects of pre-harvest burning on soil organic carbon relative to green cane retention. Figures a (LSD = 3.96) and b (LSD = 8.39) indicates the overall effects of burning relative to green and the effects soil profile depths, respectively. Different lower-case letters indicate significant differences ($p < 0.05$) among treatments.

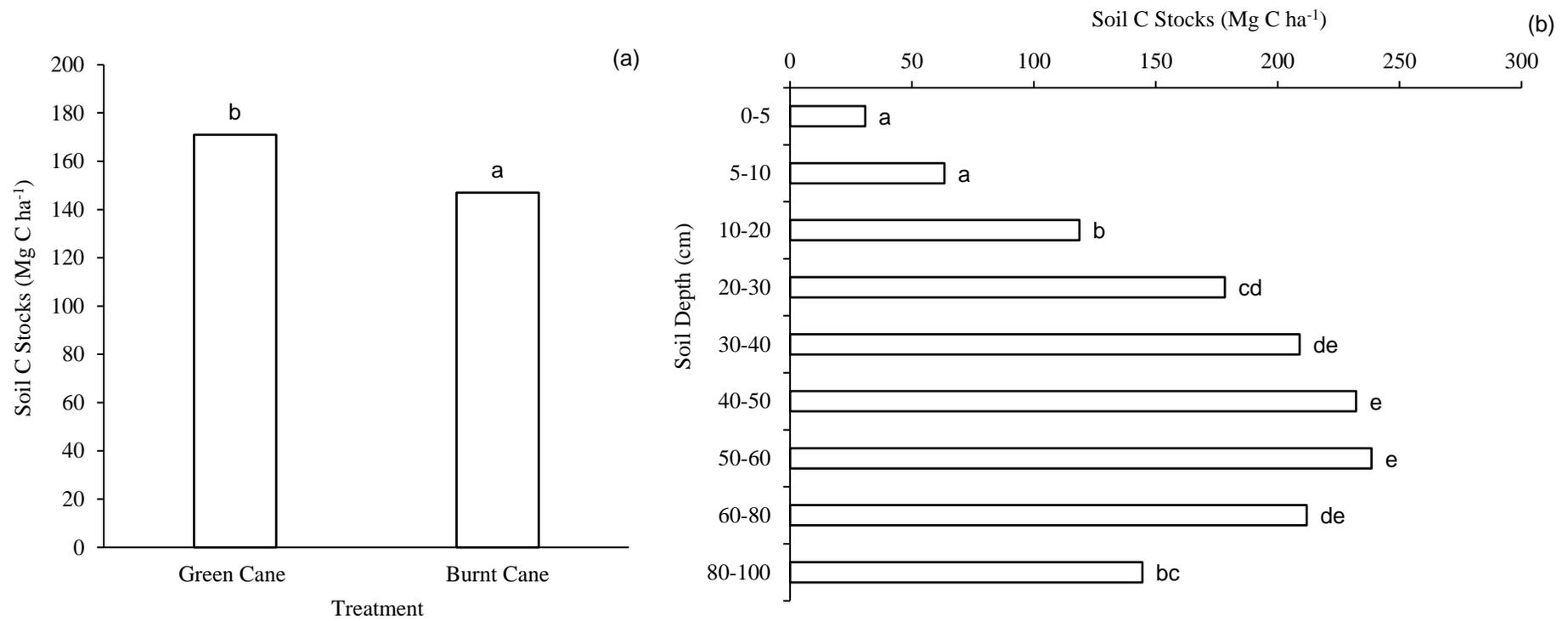


Figure 4. 3 Effects of pre-harvest burning on soil C stocks relative to green cane retention. Figures a (LSD = 15.7) and b (LSD = 33.3) indicates the overall effects of burning relative to green and the effects soil profile depths, respectively. Different lower-case letters indicate significant differences ($p < 0.05$) among treatments.

4.3.3 Soil microbial biomass C and microbial biomass quotient

Similar to soil OC concentration and stock, soil MBC under green cane (2880 mg kg⁻¹) was significantly higher than pre-harvest burning (820 mg kg⁻¹). The concentration of MBC in green cane was >100 % higher than the pre-harvest burning fields. The overall interaction between depth and treatment was significant. Soil MBC decreased with increase in soil depths (Figure 4.4). The MBC under green cane constituted 3.52% while that under pre-harvest burning constituted 1.25% C of total SOC. The top 20 cm depth in green cane had significantly higher than the 5-10 and 10-20 cm depths in pre-harvest burning (Figure 4.4). At 10-20 and 20-30 cm depth, MBC under green cane was significantly higher by >100% than that of pre-harvest burning in these depths for both depths. Similar to MBC, soil microbial quotient in green cane (0.040) was significantly higher than pre-harvest burning (0.011) (Table 4.2). The soil microbial quotient was not affected by soil profile depth (Table 4.2).

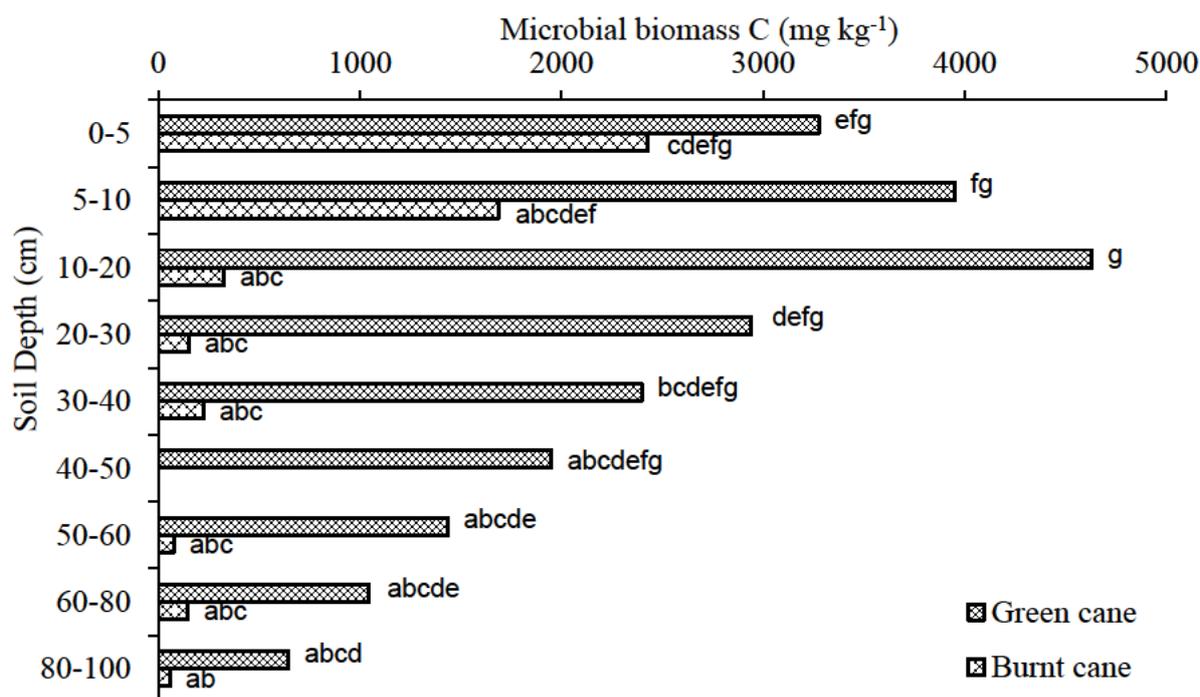


Figure 4. 4 Effects of pre-harvest burning on soil microbial biomass carbon relative to green cane retention (LSD = 1.28). Different lower-case letters indicate significant differences ($p < 0.05$) among treatments.

Table 4. 2 Effects of pre-harvest burning and green cane on Microbial Quotient

Factor	Microbial Quotient
Treatment	
Green Cane	0.040b
Pre-harvest burning	0.011a
LSD	0.010
Depth (cm)	
0-5	0.044
5-10	0.041
10-20	0.035
20-30	0.016
30-40	0.024
40-50	0.011
50-60	0.014
60-80	0.020
80-100	0.026
LSD	0.022

Different lower-case letters within a column for each factor indicate significant differences ($p < 0.05$) among treatments. Where means do not have letters, within a column for each factor, they are not significantly different.

4.3.4 β -D-glucosidase enzyme assay

Pre-harvest burning ($104 \mu\text{g p-nitrophenyl released g}^{-1} \text{ moist soil h}^{-1}$) decreased activity of β -D-glucosidase enzyme by 54.8% when compared to green cane ($161 \mu\text{g p-nitrophenyl released g}^{-1} \text{ moist soil h}^{-1}$) (Figure 4.5). The interaction effect of burning and depth on β -D-glucosidase enzyme activity was highly significant ($p < 0.001$). Burning significantly decreased activity of the enzyme only in the top 10 cm when compared to green cane management (Figure 4.5). In the top 5 cm depths, green cane the enzyme activity was > 100% higher than burnt cane. The activity of β -D-glucosidase decreased with an increase in soil depth ranging from 327 in the 0-5 cm (green cane) to $50.1 \mu\text{g p-nitrophenyl released g}^{-1} \text{ moist soil h}^{-1}$ in the 40-50 cm depth to (Figure 4.5).

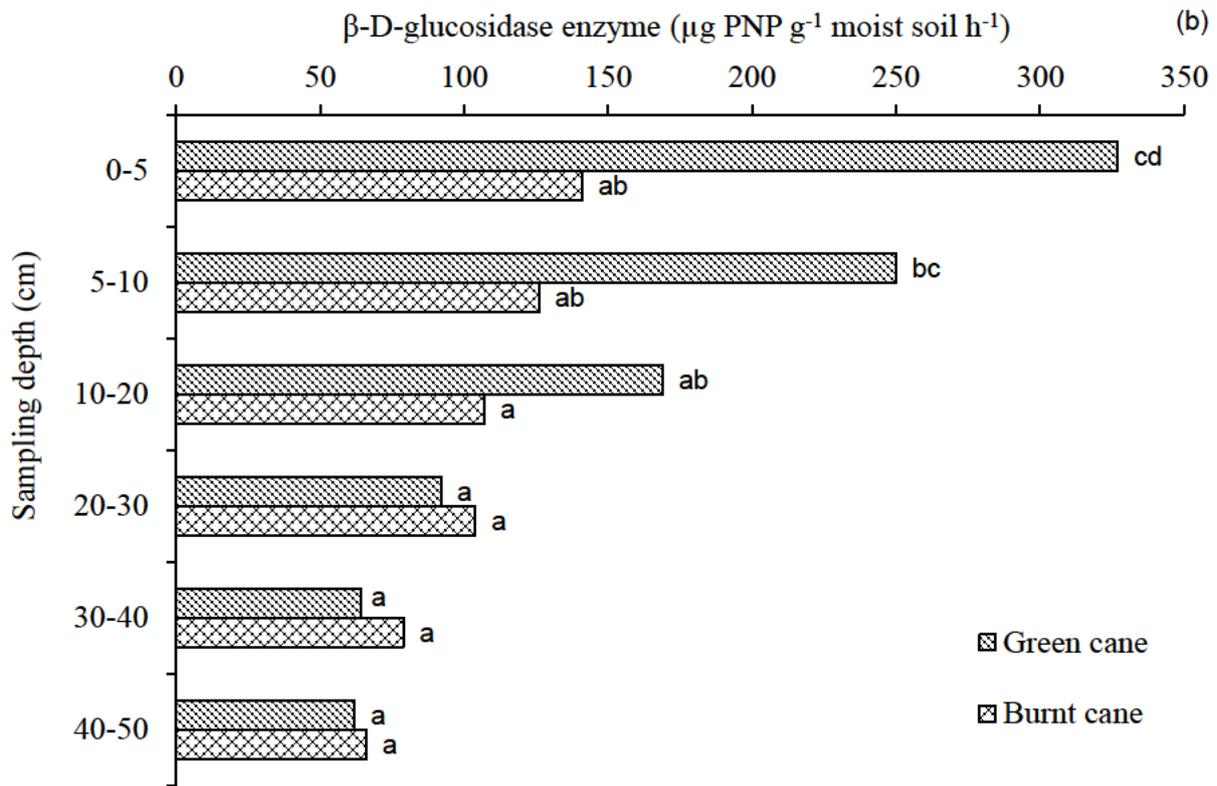


Figure 4. 5 Effects of pre-harvest burning on β -glucosidase enzyme activity relative to green cane retention (LSD = 76.9). Different lower-case letters indicate significant differences ($p < 0.05$) among treatments.

4.3.5 Relationship analysis of different soil quality indicators

The SOC was correlated negatively with pH and positively with all other parameters (C stocks, MBC, enzyme activity, exchangeable Ca) except for clay and exchangeable K and Mg (Table 4.3). The C stocks was correlated positively with clay, MBC, exchangeable Mg and SOC and correlated negatively with exchangeable K (Table 4.3). The MBC was positively with SMQ, Mg and SOC (Table 4.3). The β -D-glucosidase enzyme was correlated negatively with pH and positively with exchangeable K and SOC (Table 4.3). In addition to positive correlation with SOC, MBC and enzyme activity, exchangeable Ca was also positively correlated to clay and SOC and negatively correlated to SMQ. Clay content was negatively with enzyme activity and exchangeable K.

Table 4. 3 Spearman's rank correlation analysis of soil parameters response

	C Stocks	Ca	Clay	β -D-glucosidase	K	MBC	MQ	Mg	SOC	Soil pH
C Stocks	1.000									
Ca	0.336	1.000								
Clay	0.744**	0.341*	1.000							
β -D-glucosidase	-0.280	0.055	-0.567**	1.000						
K	-0.691**	-0.227	-0.679**	0.414*	1.000					
MBC	0.429*	-0.179	0.144	0.055	-0.022	1.000				
MQ	0.176	-0.443*	-0.011	-0.159	-0.133	0.786**	1.000			
Mg	0.514**	-0.052	0.208	0.022	-0.149	0.492*	0.198	1.000		
SOC	0.352*	0.503**	0.138	0.560**	0.133	0.401*	-0.110	0.270	1.000	
Soil pH	0.195	-0.273	-0.091	-0.440*	0.094	-0.481*	0.234	0.176	-0.413*	1.000

* Indicates significant different at $p < 0.05$; ** indicates significant different at $p < 0.001$. MQ indicates microbial quotient

4.3.6 Scanning Electron Microscope analysis of soil at selected depths

The SEM micro images showed granular structure with small porous particle grains, which was more pronounced in the surface depth with few blocky and irregular grain structures (Figures 4.6 a-f). The difference between the two sugarcane harvest systems were in terms of particle size distribution. The images showed organic matter and soil aggregates indicated laminar cemented aggregates. The micro-structures observed show colloid-sized “nanocomposite” and OM-metal oxides.

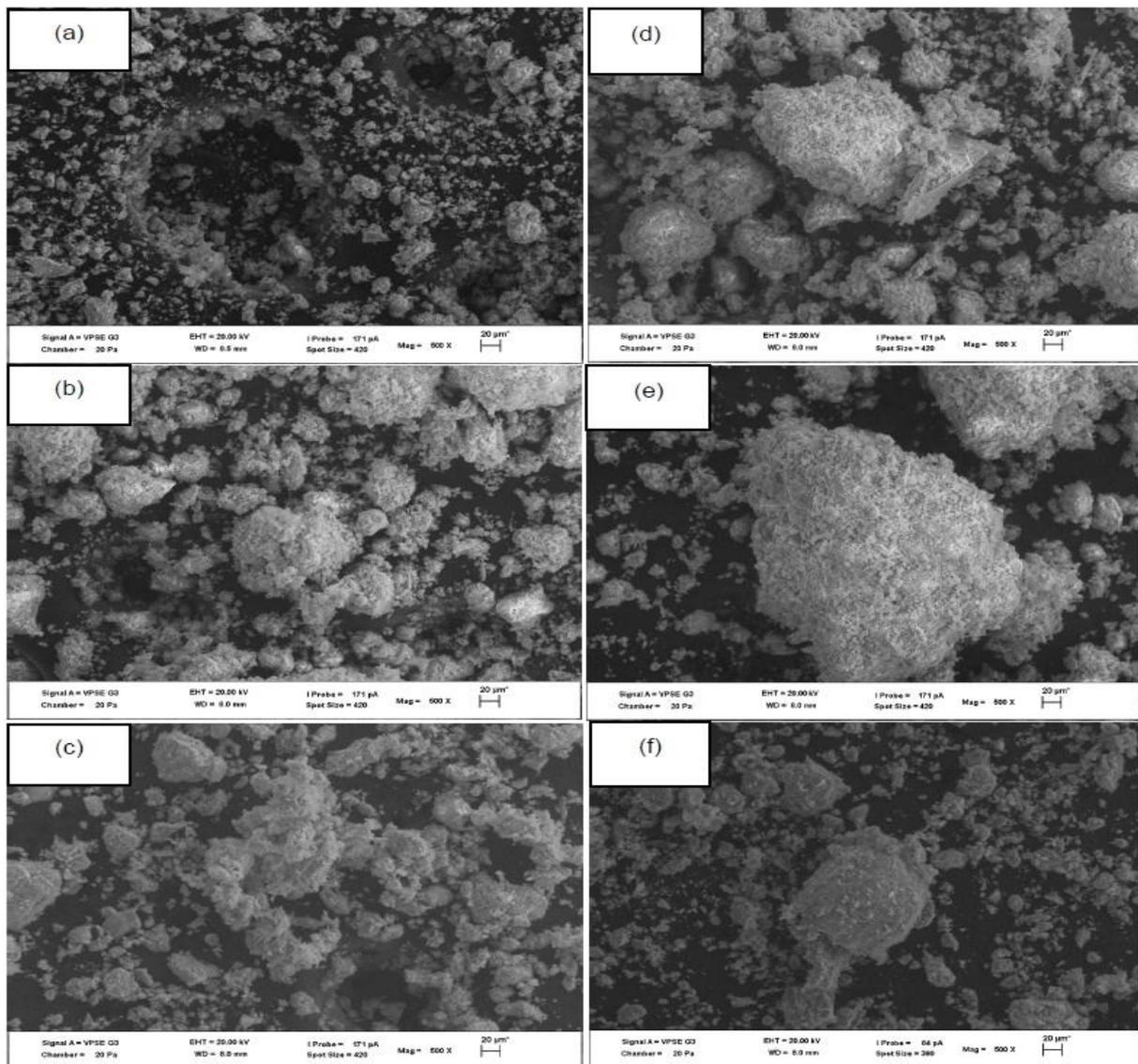


Figure 4. 6 Scanning electron microscope of the bulk soils at 500 times magnification zooming to 20 µm, for the top 10 cm and 80-100 cm depth. Figures 4.6 a-c indicates pre-harvest burning and Figures 4.6 d-f indicates green cane retention.

4.4 Discussion

4.4.1 Soil organic carbon content and C stocks

The higher SOC in the green cane harvest system than pre-harvest burning could be explained by the greater biomass addition, as sugarcane residues to the soil. The addition of fresh residues is reported to have significant positive effects on SOC (Morais et al. 2020; Weng et al. 2020). Also, the addition of the residues on the surface provides diverse C sources required by microorganisms (Vuyyuru et al. 2020). The lower SOC in the pre-harvest burning than green cane management could be explained by the decrease or lower amount of trash added as fresh biomass on the surface. Pre-harvest burning reduces biomass addition and could increase mineralisation and loss of SOC as CO₂ with time (Gonzalez-Perez et al. 2004). This could be explain by the increase in straw mineralisation due to The loss of SOC due to burning could have decreased soil aggregates stability, resulting in erosion of the soil, further reducing SOC (Liu et al. 2019). A study conducted in forestry soils, where burning was practiced, showed a significant decrease in SOC (41% decline on average) due to burning (Armas-Herrera et al. 2016). Also, the high acidity levels (pH < 5.00) in these soils could result in a decrease in decomposition due to inhibition of activity of most microorganism, which are essential in the mineralisation of SOC, resulting to the accumulation of SOC.

Retention of sugarcane residues resulted in stabilisation of SOC in acidic soil, which showed an increase in SOC content (Weng et al. 2020). Similarly, a study conducted in Brazil showed that green cane retention resulted in significantly higher SOC content when compared to pre-harvest burning (Galdos et al. 2009) with green cane having 30% higher OC, which was similar to the current study where green cane had 24% higher OC than burnt cane. Although not specifically on Umbric Rhodic Ferralsols, a study conducted by Graham and Haynes (2002) showed that green cane had significantly higher SOC than pre-harvest burning after 59 years of production in South African. Similarly, Trujillo-Narcia et al. (2019) reported a decrease in overall SOC following two consecutive burning periods in sugarcane areas compared to no burning. Wood (1991) observed a 20% decrease in SOC in the pre-harvest burning plot compared to the unburnt plot while Luca et al. (2018) reported that conversion from pre-harvest burning to green cane retention showed a significant increase in SOC with time.

The higher SOC higher under green cane than pre-harvest burning throughout the profile (the effect did not depend on the depth), was contrary to the generally observed effects of sugarcane production which are usually evident in the top soil but not to deeper soil layers. Luca et al.

(2018) reported that shifting from burning to green cane resulted in a significant increase in SOC with changes observed in the 0-5 cm depth to be 21% (from 11.9 g C kg⁻¹ to 14.5 g C kg⁻¹). While the higher SOC in the surface depth could be explained by the amount of plant litter addition (Lei et al. 2019), the higher concentration of C under at deeper layers under green cane suggests greater contribution of root biomass. The higher SOC even in a deeper layer in green cane is a result of the deep-rooted sugarcane resulting in root exfoliation and exudate (Lei et al. 2019) increasing biomass addition into deeper layer. The results of SOC stocks followed similar trends to those SOC concentrations with burning reducing SOC stocks. This observation indicated that the increase in SOC concentrations in Umbric Rhodic Ferralsols due to green cane, translated to higher amounts of C stored in the soil profile. This observation agreed with Luca et al. (2018) who reported an increase in SOC stocks in green cane than burnt cane. This high content of SOC in these soils could be explained by the acidic nature of these soils, irrespective of burning or green cane, which could have affected the microbial activity, even for the decomposition of labile pools of SOC, such as particulate organic matter. This view was supported by the negative correlation between soil pH with SOC, MBC and β -D-glucosidase activity. In addition, the high SOC could be in the stable form such as charcoal, aggregate protected C and C associated with Al/Fe-organic matter complexes. These organic matter fractions were not determined in the current study.

4.4.2 Soil microbial biomass carbon and β -D-glucosidase enzyme as affected by burning and soil depths

The microbial biomass represents the most essential reservoir of plant nutrients (Souza et al. 2012). The higher microbial biomass C in soil under green cane coincides with the higher SOC when compared with pre-harvest burning (Table 2). This relationship of MBC and SOC indicates that the microbes used the SOC as a substrate for their growth. This trend could be due to the addition of fresh biomass, more than burnt cane, increasing the SOC including the labile C pool. Similar results were reported in soils of the sugarcane industry in South Africa (Graham and Haynes 2002) where MBC was higher by 246% in fields under green cane (trash retention) than pre-harvest burning and removal of trash blanket. The abundant plant residues under green cane provides readily available substrates required by microorganisms to accumulate more biomass during decomposition (Souza et al. 2012). The added labile C, such as sugars, is essential for microbial growth (Buckeridge et al. 2020; Chuwongpanich et al. 2021). Galdos et al. (2009) observed 2.5 times increase in MBC in green cane than burnt cane, while Souza et al. (2012) observed higher MBC in harvesting without burning (328.45 mg kg⁻¹

¹), an increase of 102% when compared to burning practice (162.70 mg kg⁻¹) in acidic soils. Vuyyuru et al. (2020) also reported a significant decrease in MBC by 76.6% on average due to pre-harvest burning compared to green cane retention. Burning crop residues has been reported to drastically reduce microbial biomass (Gonzalez-Perez et al. 2004), including under sugarcane (Dominy et al. 2002; Fultz et al. 2016). The lower MBC in pre-harvest burning could be a result of stress-induced by burning in terms of changes in temperature and soil moisture (Souza et al. 2012). While a study on forest soils showed a significant decrease in MBC due to burning in the topsoil layers, with no differences in deeper layers (Armas-Herrera et al. 2016), the current study showed that green cane resulted in higher biomass than pre-harvest burning throughout the 1.0 m soil profile. This difference could be explained by SOC which followed the same trend. The decrease in MBC in both treatments with depths was a result of the decrease in substrates with an increase in soil depth. More plant biomass input is added to surface layers as litter together with roots, while at deeper layers only roots are the main contributors. The enzymes, β -D-glucosidase, is essential in degradation of organic matter and C cycling in soils. Soil SMQ was higher in green cane harvest system, which suggest that addition of fresh residues increase substrates availability for microorganisms resulting in increased microbial biomass. This is evidenced by the positive correlation between SMQ and MBC (Table 4.3). Similarly, Souza et al. (2012) observed higher SMQ in sugarcane fields without burning when compared to sugarcane with burning.

The strong correlations between the activity of this enzyme and SOC and MBC (Table 4.3), showed that soil microorganisms uses the soil organic carbon as a substrate and confirms that the β -D-glucosidase enzyme facilitates the transformations of OC by microorganisms in the loamy humic soils. These parameters were all significantly higher in green cane than burnt cane. From the current study, activity of β -D-glucosidase was significantly decreased by burning when compared to green cane retention, which was a result of decline in SOC and MBC associated with burning. There is limited scientific evidence in literature on the effects of sugarcane harvest systems on the activity of β -D-glucosidase, with none on humic soils. These results of the current study agreed with Vuyyuru et al. (2020), who reported that pre-harvest burning decreases the β -D-glucosidase enzyme by 30.8% when compared to green cane retention. Armas-Herrera et al. (2016) also observed a significant decrease in β -D-glucosidase activity of 49% in the top 3 cm depth in forestry soils due to burning. The decrease in β -D-glucosidase activity with soil depth was expected, following the same trend as MBC and SOC, which showed that labile C produced from organic matter mineralisation decreased with depth.

4.4.3 Scanning Electron Microscope

The porous and small particles structure observed in pre-harvest burning in the top 10 cm depth (Figures 4.6a and b) could be explained by burning reducing soil structural stability, hence decreasing aggregate stability. This explains the lower SOC in pre-harvest burning when compared to green cane system. The different laminar cemented aggregates and the porous structure were similar to what observed by Jimenez-Pinilla et al. (2016) and Guo et al. (2020). Addition of residues in green cane could increase aggregation in soils, hence increasing the protection of the SOC from microbial attack and increase accumulation of SOC. In addition, the porous structure observed could be explained by the high SOC content of these soils (Zaffar and Sheng-Gao 2015; Singh et al. 2021). The presence of colloid-sized “nanocomposites” observed were similar to what was observed by Wagai et al. (2020) indicating formation of organo-mineral complexes. This could have a significant effect on the stabilisation of the SOC in these soils over time, which could also explain the high SOC in these soils.

4.5 Conclusion

The study showed that green cane production, where trash is returned, resulted in higher soil OC storage in loamy Umbric Rhodic Ferralsols, when compared with burnt cane. The higher soil OC content under green cane, especially in the topsoil, supports higher microbial biomass (MBC) and activity (β -D-glucosidase enzyme), which are essential in C cycling in soil. Green cane harvest system improves soil quality in the long term when compared to burnt cane, which is essential for plant nutrition and growth. These findings suggested that green cane production improves OC and health of the loamy Umbric Rhodic Ferralsols, when compared with burnt cane, which could make a significant contribution sustainable sugarcane. There is need to understand the effects of green cane on soil aggregate stability and the different SOC fractions, which could affect the cycling of C and nutrients in these humic soils.

CHAPTER 5

Organic carbon fractions and aggregates stability in Umbric Rhodic Ferralsol soils under green cane compared to pre-harvest burning

A version of this chapter is under review for publication in **Journal of Soil and Sediments**

Abstract

Purpose Pre-harvest burning of sugarcane is a major management system globally and could have significant effect on soil organic carbon (SOC) fractions, aggregates stability and nutrient cycling, while green cane can be more beneficial on soil quality and environmental sustainability. The current study examined the effects of green cane relative to pre-harvest burning on aggregate stability, represented by mean weight diameter (MWD) and SOC fractions in a loamy Umbric Rhodic Ferralsol soils.

Methods Soil aggregates stability was determined using the wet-sieving method. The SOC fractions were determined using the physical and chemical separation method, followed by determination of SOC content in different fractions by the wet oxidation method.

Results and discussion Burning reduced soil aggregates stability by 42.8% when compared to green cane in the top 10 cm depths. The carbon in the macro-aggregates fraction constituted > 60% of total SOC, making it the primary C storage fraction for both green cane and burnt cane. Green cane had 20.4 and 45.2% higher SOC in macro- and micro-aggregate fractions, respectively, when compared to burnt cane. Burning increased mineral associated C from bulk soil fractionation by 23.7% when compared to green cane. Upon further dispersion of macro-aggregates, the SOC in the particulate organic matter (POM-C) of coarse and fine particle size were higher in green cane management. All the C fractions, except for the mineral associated C, decreased with increase in soil depth.

Conclusions These findings imply that green cane production increases aggregate stability and SOC storage in different soil fractions, except the mineral associated C, with potential reduction in greenhouse gas emission and sustainable sugarcane production when compared with the pre-harvest burning on sandy loam Umbric Rhodic Ferralsol soils.

Keywords · Acidic soils · Aggregates stability · Carbon sequestration · Green cane · Pre-harvest burning · Soil organic carbon

5.1 Introduction

Soils store the largest terrestrial carbon (C) pool (Giannetta et al. 2019; Tahir et al. 2022) and can either be a source of C significantly contributing to greenhouse gases and climate change, or C sink contributing to the mitigation of climate change, depending on management system (Lal 2004). While the labile forms of organic matter are more sensitive to management, the results of the meta-analysis in Chapter 3 showed that cropping even lowered SOC associated with organo-mineral complexes, particularly in soils with medium to high organic matter. In sugarcane production, pre-harvest burning has been the traditional harvest practice at a global scale, including in South Africa. This practice is done to remove excess trash to ease the harvesting process and concentrate the sucrose content compared to green cane retention system (Graham and Haynes 2002; Sandhu et al. 2017; Weng et al. 2020). Pre-harvest burning has been shown to have negative effects on soil quality indicators including soil aggregates stability, organic carbon fractions and nutrient cycling in other parts of the world (Pinheiro et al. 2010; Dos Santos et al. 2020). During burning C is lost to the atmosphere as carbon dioxide (CO₂) emission, reduces the overall SOC content and contributes negatively to climate change, in the long-term. The total SOC losses varies depending on the characteristics of the soil and the different SOC fractions. Fractions of SOC are highly diverse depending on the turnover rate and stability against decomposition by microorganisms in soil environments (Tivet et al. 2013).

Production of sugarcane, a crop of major agricultural importance in KwaZulu-Natal, could have significant effects on SOC fractions, especially in Umbric Rhodic Ferralsol soils, locally known as humic soils with high SOC content > 1.8%, and are well-drained, highly weathered and acidic (Fey 2010). Malepfane et al. (2022) reported that sugarcane production in these Umbric Rhodic Ferralsol (humic) soils, with pre-harvest burning, resulted in a decline in SOC particularly in the top soil, with no significant change in C stocks in a soil profile to a depth of 1.0 m. On the other hand, green cane retention has been reported to increase SOC content, water retention, soil biodiversity and essential nutrients due to an increase in fresh biomass addition and physical protection of the soil (Carvalho et al. 2017). Results in Chapter 4 showed that SOC (25% increase), microbial biomass carbon and β -glucosidase enzyme activity were increased by green cane production when compared to pre-harvest burning on sandy loam Umbric Rhodic Ferralsol soils of Northern KwaZulu-Natal (Mkhonza and Muchaonyerwa 2022). However, the distribution of SOC fractions in different soil aggregate sizes, as affected by green cane relative to pre-harvest burning on Umbric Rhodic Ferralsol in South Africa, is

not clearly understood. The SOC comprises of various fractions, which differ in their availability to microbial decomposition in soils, and their contribution to climate change and its mitigation (Lal 2004). The turnover of SOM largely depends on the distribution of the size of the fractions (Giannetta et al. 2019) and their associations with different aggregate size fractions (Giannetta et al. 2019).

Fractionation of SOC is essential for a better understanding of the C storage and cycling, which vary with the level of stability of the fractions (Cotrufo et al. 2015; Poeplau et al. 2018; Olayemi et al. 2022). These fractions of SOC are affected by several soil factors including soil type, climatic conditions and land use (Zhao et al. 2021). The particulate fraction of SOC accumulates more rapidly from crop residues and other inputs (Olayemi et al. 2022) and is an essential indicator of nutrient cycling and overall soil quality (Yang et al. 2012; Guimaraes et al. 2013). Conversely, the fraction of SOC that is associated with minerals is stabilised by clay minerals, against microbial decomposition, through strong bonds formed as part of the organo-mineral complexes (Olayemi et al. 2022). In Umbric Rhodic Ferralsols soils, both these fractions (particulate and mineral associated fractions of SOC) may be high. However, the effects of burning/fire on aggregates could expose this fraction to microbial attack resulting in a decrease in SOC over time (Nichols et al. 2021; Thomaz 2021). It is therefore essential to understand the effects of pre-harvest burning and green cane retention on different SOC fractions in a view of climate change.

The particulate C pool is essential for nutrients cycling and crop productivity as a labile pool (Graham and Haynes 2002), but in highly acidic humic soils this fraction may not be easily accessible due to limited activity of microorganisms by the high level of acidity. The higher MBC and β -glucosidase enzyme activity, especially in the top soil under green cane than pre-harvest burning (Chapter 4; Mkhonza and Muchaonyerwa 2022) suggests that green cane increases the labile SOC fractions, which are then used as substrates by soil microorganisms. Burning could result in a decrease in SOC content in different fractions due to reduced soil aggregates stability, hence, reducing the physical protection and increase decomposition of SOC (Abdalla et al. 2021). In addition, organic matter rapidly decomposes during burning, resulting in lower SOC, including the different fractions, especially the coarse particulate fraction. The reduced addition of fresh biomass coupled with burning could significantly reduce the coarse particulate soil organic matter fraction. This effect is not clearly understood in humic soils, which have high SOC, especially when pre-harvest burning is compared with the green cane harvest system. While green cane production increases biomass addition,

potentially increasing SOC fractions, including those in soil aggregates, the fresh residues could increase the C cycling in soils due to an increase in microbial activity. Therefore, there is a need to understand the effects of green cane production, relative to pre-harvest burning on soil C fractions to understand the level of protection of the C in the different fractions in Umbric Rhodic Ferralsol soils, and the risk of losses when microbial activity is increased by liming. The hypothesis of the study was that green cane production increases aggregate stability and associated particulate SOC fractions when compared to pre-harvest burning in sandy loam humic soils. The study examined the effects of green cane production on aggregate stability and associated particulate SOC fractions, relative to pre-harvest burning, in sandy loam humic soils.

5.2 Methods and materials

5.2.1 Study sites and soils

The same experimental sites used in Chapter 4 were used for this chapter. The details of the climatic conditions, soil types and management of the sugarcane, including cover crops and characterisation of the soils are detailed in Sections 4.2.1, 4.2.2 and 4.2.3 in Chapter 4. The characteristics of the soils are given on Table 4.1. The results of soil pH and SOC were detailed in table 4.1 and figure 4.2 in Chapter 4.

5.2.2 Soil structural stability

Samples from the top 20 cm depth were used for to study aggregate stability, using the wet sieving technique as described by Yoder (1936) and Marquez et al. (2004). For this, 80 g samples (8 mm) were physical fractionated by wet-sieving through mesh-sizes of 2 mm, 0.25 mm and 0.053 mm. The water-stable aggregates were separated into: (i) large macro-aggregates > 2 mm sieve in diameter, (ii) small macro-aggregate (SM) between 0.25- and 2-mm diameter, (iii) micro-aggregate between 0.053 and 0.25 mm, and (iv) the silt- and clay-sized aggregate < 0.053 mm in diameter. The aggregates in each sieve size were transferred into a pre-weighed aluminium pan and dried at 70 °C (Ran et al. 2020). Soil aggregates stability was calculated using mean weight diameter (MWD) using the equation below (Ran et al. 2020).

$$\text{MWD} = \frac{\sum_{i=1}^4 \bar{d}_i w_i}{\sum_{i=1}^4 w_i} \quad (1)$$

where d_i is the average diameter of the i^{th} fraction size of the different aggregates (mm); w_i is the mass of the i^{th} size aggregate (g).

5.2.3 Soil organic carbon fractions

Soil organic matter fractions to 1 m depth were determined using physical and chemical fractionation method as described by Stewart et al. (2008) (Figure 5.1). Firstly, the bulk soil sample was separated into three fractions using wet sieving method to obtain SOC in the macro-aggregates (> 250 μm), micro-aggregate (53-250 μm) and μSilt and μClay (< 53 μm) fractions. These fractions were oven dried overnight at 60°C and weighed. The SOC in these fractions was analysed as described by Walkley and Black (1934). The macro-aggregates and micro-aggregates fractions were further fractionated after dispersion with 5 g L⁻¹ sodium hexametaphosphate. For this, 10g of the aggregate fraction was treated with 50 ml sodium hexametaphosphate solution and shaken at 180 rpm over night for 16 hours. The suspension from the macro-aggregates was passed through 250 and 53 μm sieves to separate three fractions, while for micro-aggregates the suspension was passed through 53 μm sieve to separate two fractions, before drying overnight at 60°C, weighing and analysis of SOC. The SOC determined from materials >250, 53-250 and < 53 μm were termed coarse particulate organic matter carbon (cPOM-C), fine particulate organic matter carbon (fPOM-C) and mineral associated carbon ($\mu\text{Silt}+\mu\text{Clay-C}$). The fractions were dried. The results of C in each fraction were expressed as the percentage of the bulk soil. The SOC in macro- and micro-aggregates was calculated using below equations.

$$\text{mg } 10 \text{ g}^{-1} \text{ fraction} = \frac{\text{mg C oxidized} \times \text{mass of fraction retain}}{\text{mass of sample (g)}} \quad (2)$$

$$\text{mg in macroaggregate} = \frac{\text{mg } 10\text{g} \times \text{total mass of fraction}}{\text{mass of sample used for digestion(g)}} \quad (3)$$

$$\text{mg in soil} = \frac{\text{mg in macroaggregate} \times \text{total mass of fraction}}{\text{corrected mass}^{13}} \quad (4)$$

¹³ Mass of the fraction retained after sieving and corrected for moisture

$$mg\ kg^{-1}\ soil = \frac{mg\ in\ soil}{corrected\ mass} \times 1000 \quad (5)$$

$$g\ kg^{-1}\ soil = \frac{mg\ in\ kg\ soil}{1000} \quad (6)$$

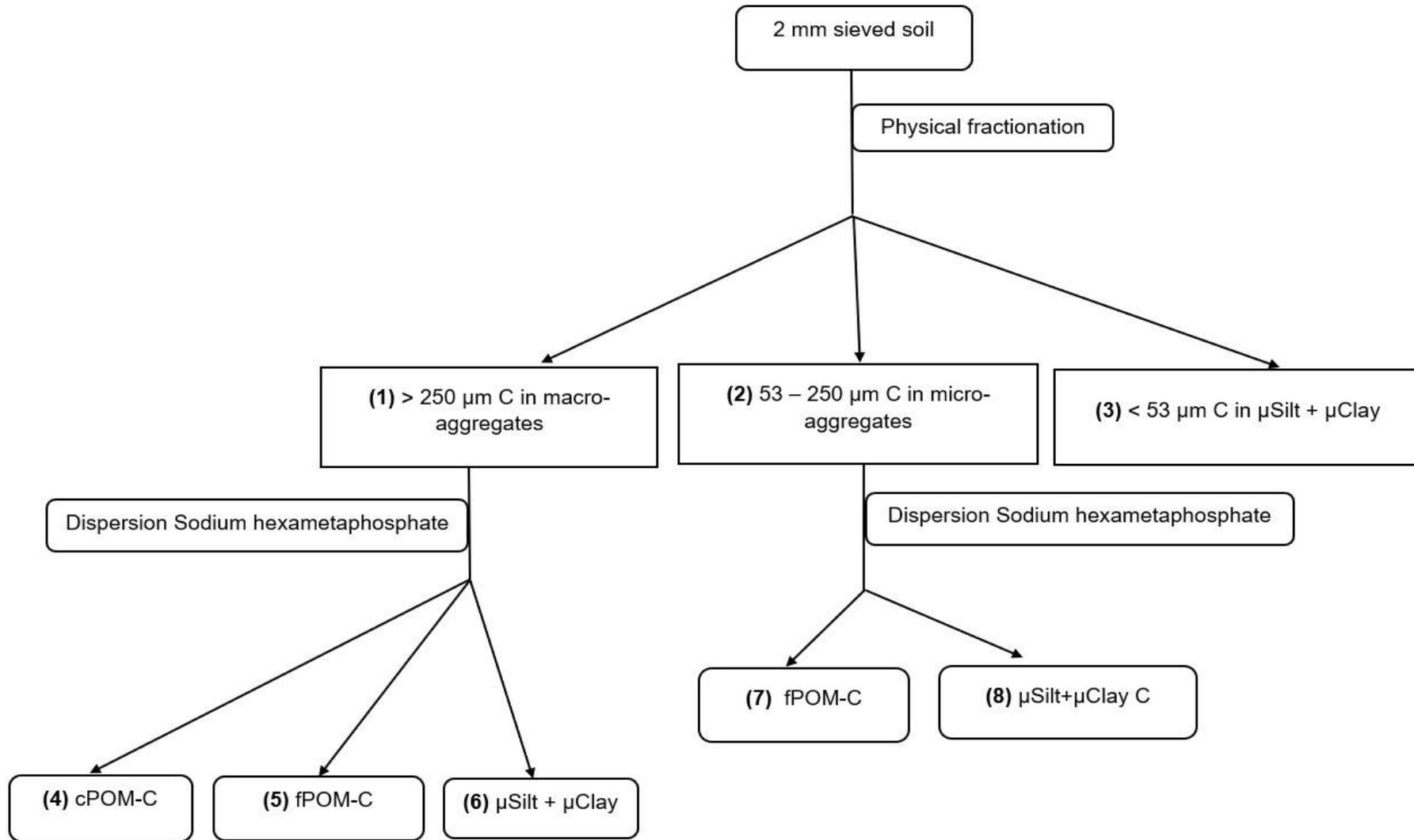


Figure 5. 1 Soil C fractionation scheme

The cPOM-C from further fractionation of macro-aggregates as the soil cPOM-C, while soil fPOM-C was calculated by addition of concentrations of this fraction from further fractionation of macro- and micro-aggregates. Soil $\mu\text{Silt}+\mu\text{Clay-C}$ was determined by addition of C in this fraction from initial fractionation of soil (aggregates) and from further fractionation of the macro- and micro-aggregates

5.2.4 Soil particle morphology (Scanning Electron Microscopy)

Selected soil samples for the fraction $< 53 \mu\text{m}$ from pre-harvest burning and green cane were used for scanning electron microscope (SEM) (EVO LS15, Carl Zeiss Microscopy, New York, USA) analysis. The samples were taken from the first 5 cm and the 80-100 cm depths to assess the effects on the surface soil and subsoil. To examine the surface properties of the soils, the bulk soils were imaged with an SEM (EVO LS15, Carl Zeiss Microscopy, New York, USA).

5.2.5 Statistically analysis

The data of aggregate stability and associated SOC fractions in the humic soils were subjected to two-way analysis of variance (ANOVA) with production system (green cane and pre-harvest burning) and soil depth as the main factors using GenStat Ed 18. The least significant difference (LSD), at 5% level was used to compare treatments means. The Tukey-Kramer test was also applied to separate treatment means at $p < 0.05$. Pearson's correlation analysis was done using IBM SPSS Statistics 27 up to the 30 cm depth for all parameters. In addition, to identify the effects of green cane and burning on different soil parameters, a Principal Component Analysis (PCA) was applied separately in R Statistical software 2018 for the top 30 cm depth .

5.3 Results

5.3.1 Aggregate stability

The interaction effect of sugarcane production system and soil depth on soil aggregate stability (MWD) was significant, with green cane having significantly higher stability than burnt cane, in the top 10 cm but not in the 10-20 cm depth (Figure 5.2). The MWD was 179% higher under green cane (3.38 mm) than pre-harvest burning (1.21 mm) in the top 5 cm, while in the 5-10 cm depth green cane (2.07 mm) had 63.9% higher than pre-harvest burning (1.26 mm) (Figure 5.2). Soil aggregates stability decreased with increase in soil depths only under green cane from 3.37 mm in the top 5 cm to 1.63 mm in the 10-20 cm depth (Figure 5.2).

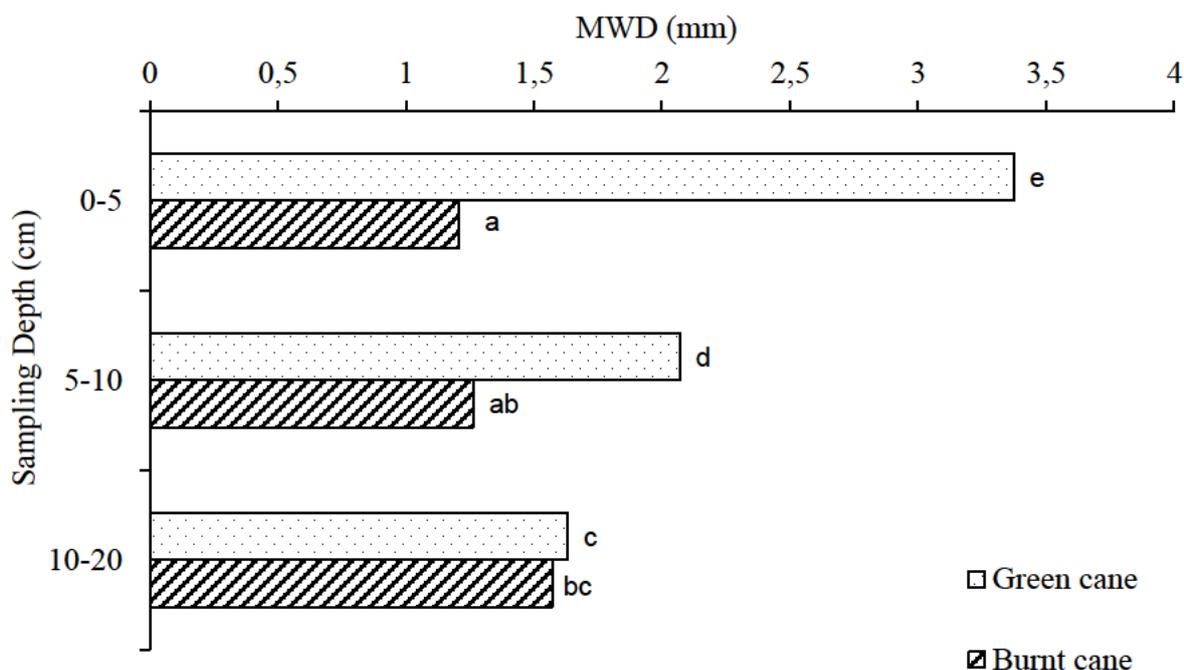


Figure 5. 2 Mean weight diameter in green cane relative to pre-harvest burning (LSD = 0.209). Different lower-case letters indicate significant differences ($p < 0.05$) among treatments.

5.3.2 Soil organic carbon fractions associated with macro- and micro-aggregates and silt + clay

The overall effects of sugarcane production system on SOC fractions were highly significant. The soil under green cane (33.6 g kg^{-1}) had 20.4% higher SOC in the macro-aggregates than pre-harvest burning (27.9 g kg^{-1}). The SOC in macro-aggregates fraction (Table 5.2) contributed $> 60\%$ of total SOC under both green cane and burnt cane. The SOC in the micro-aggregates fraction was significantly higher (45.2% higher) under green cane (12.2 g kg^{-1}) than under pre-harvest burning (8.40 g kg^{-1}), which constituted 21.9% and 18.8% of total SOC for green cane and burnt cane, respectively. The SOC associated with the silt and clay ($\mu\text{Silt} + \mu\text{Clay}$ protected fraction) was significantly lower (23.7% lower) under green cane than pre-harvest burning (Table 5.2). The SOC in this fraction constituted 4.85 and 7.47% of total SOC for green cane and pre-harvest burning, respectively. The overall effects of depth were significant (Table 5.2). The SOC in macro-aggregates was significantly higher in the top 5 cm depth when compared to the other depths, except the 5-10, 20-30 and 30-40 cm depth. The concentration decreased with increase in soil depth ranging from 41.0 g kg^{-1} in the top 5 cm to 13.3 g kg^{-1} in the 80-100 cm depth. For the micro-aggregates, the top 40 cm depth had significantly higher than all the other depths, except for the 20-30 and 30-40 cm depth, which

were similar to the deeper layers. This fraction ranged from 16.2 g kg⁻¹ in the 10-20 cm to 1.27 g kg⁻¹ in the 80-100 cm depth. The SOC in μ Silt + μ Clay fraction was significantly higher in the top 20 cm than the other depths, except for 20-30 cm, which was similar to the top 10 cm depths (Table 5.2).

Table 5. 1 Effects of pre-harvest burning and green cane retention on soil C fractions

Factor	Carbon in fractions (g kg ⁻¹)		
	macro-aggregates	micro-aggregates	μ Silt + μ Clay C
Burning			
Green cane	33.6b	12.2b	2.70 a
Pre-harvest burning	27.9a	8.40a	3.34 b
LSD	2.58	1.42	0.607
Depth (cm)			
0-5	41.0d	15.2ef	5.46de
5-10	38.1cd	14.7ef	5.38de
10-20	31.5bc	16.2f	6.45e
20-30	34.6cd	13.7def	4.05cd
30-40	34.2cd	11.3cde	2.38bc
40-50	30.3bc	8.89cd	1.69ab
50-60	30.8bc	7.46bc	1.05ab
60-80	23.2b	3.90ab	0.432ab
80-100	13.3a	1.27a	0.289a
LSD	5.48	3.00	1.29

Different lower-case letters within a column for each factor indicate significant differences ($p < 0.05$) among treatments.

5.3.3 Carbon fractions from further fractionation of the macro-aggregates

Burning decreased both the cPOM-C (by 14%) and fPOM-C fraction (by 71%), when compared to green cane (Table 5.3). In the different soil profile depths, C concentration decreased with increase in soil depth, ranging from 1.79 g kg⁻¹ in the 80-100 cm depth to 10.6 g kg⁻¹ in the 5-10 and 10-20 cm depth (Table 5.3). The top 20 cm depth was significantly higher than the other depths except for the 20-30 depth which was similar. The μ Silt + μ Clay C from the physical protected C fraction was not affected by burning but decreased with sampling depths (Table 5.3).

Table 5. 2 Soil organic C fractions from further separation of macro-aggregates from the bulk soil C

Factor	cPOM-C	fPOM-C	μ Silt + μ Clay
Burning	g kg ⁻¹		
Green cane	25.3b	4.20b	2.36
Pre-harvest burning	22.1a	2.46a	2.54
LSD	1.62	0.602	0.124
Depth (cm)			
0-5	24.6b	5.63d	3.79cd
5-10	24.9b	5.26cd	4.50d
10-20	22.2ab	5.26cd	3.41cd
20-30	25.9b	4.68bcd	3.90d
30-40	27.0b	3.06abc	1.49ab
40-50	26.5b	2.46ab	1.05a
50-60	26.5b	1.36a	1.64abc
60-80	22.2ab	0.977a	1.06a
80-100	13.6a	1.20a	1.23ab
LSD	3.43	1.28	0.262

Different lower-case letters within a column for each factor indicate significant differences ($p < 0.05$) among treatments. Where means do not have letters, within a column for each factor, they are not significantly different.

5.3.4 Carbon fractions from further fractionation of the micro-aggregates

Burning decreased the fPOM-C fraction in micro-aggregates by least 54%, when compared to green cane (Table 5.4). The fPOM-C concentration decreased with increase in soil depth, ranging from 1.79 g kg⁻¹ in the 80-100 cm depth to 10.6 g kg⁻¹ in the 5-10 and 10-20 cm depth (Table 5.4). The top 20 cm depth was significantly higher than the other depths except for the 20-30 depth which was similar. The μ Silt + μ Clay C from the physical protected C fraction was not affected by burning and/or sampling depths (Table 5.4).

Table 5. 3 Carbon fractions associated with micro-aggregates under in green cane relative to pre-harvest burning

Treatment	fPOM-C	μ Silt + μ Clay C
Burning	g kg^{-1}	
Green cane	8.07b	2.42
Pre-harvest burning	5.24a	2.55
LSD	0.839	0.915
Depth (cm)		
0-5	9.99e	2.65
5-10	10.6e	2.46
10-20	10.6e	2.31
20-30	8.96de	1.54
30-40	6.15cd	2.24
40-50	5.69bc	3.24
50-60	3.18ab	2.24
60-80	2.92ab	2.19
80-100	1.79a	3.48
LSD	1.78	1.94

Different lower-case letters within a column for each factor indicate significant differences ($p < 0.05$) among treatments. Where means do not have letters, within a column for each factor, they are not significantly different.

5.3.5 Overall soil carbon fractions

As indicated in Section 5.3.3, burning decreased the cPOM-C (by 14%), when compared to green cane (Table 5.5). The overall soil fPOM-C concentration under pre-harvest burning was 60% lower than under green cane (Table 5.5). The overall soil POM-C (cPOM-C+fPOM-C) was lower under pre-harvest burning than green cane. The soil μ Silt + μ Clay-C was not significantly different between pre-harvest burning and green cane (Table 5.5). While only the 80-100 cm depth had lower cPOM concentrations that the other depths except the 10-20 and 60-80 cm depth, the concentrations of soil fPOM-C, total POM-C and μ Silt + μ Clay-C declined with soil depth (Table 5.5). For these soils the relationship between MWD and SOC was very poor (Figures 5.3 a, b and c). However, there were positive relationships between the soil C fractions with the SOC concentration (Figures 5.4 a-c).

Table 5. 4 Carbon associated with cPOM-C, fPOM-C, cPOM and μ Silt + μ Clay in the whole soil under green cane compared to pre-harvest burning

Factor	cPOM-C	fPOM-C	POM-C	μ Silt + μ Clay
Burning	g kg^{-1}			
Green cane	25.3b	12.3b	38.6b	7.09
Pre-harvest burning	22.1a	7.71a	29.8a	7.80
LSD	2.86	1.13	3.23	1.25
Depth (cm)				
0-5	24.6b	15.6d	40.2c	11.9b
5-10	24.9b	16.0d	40.9c	12.1b
10-20	22.2ab	15.9d	38.1c	11.5b
20-30	25.9b	13.6d	39.6c	11.4b
30-40	27.0b	9.21c	36.2bc	5.98a
40-50	26.5 b	8.14bc	34.7bc	5.47a
50-60	26.5b	4.54ab	31.0bc	3.17a
60-80	22.2ab	3.90a	26.1ab	2.71a
80-100	13.6a	2.99a	16.6a	2.73a
LSD	6.07	2.39	6.85	2.65

Different lower-case letters within a column for each factor indicate significant differences ($p < 0.05$) among treatments. Where means do not have letters, within a column for each factor, they are not significantly different.

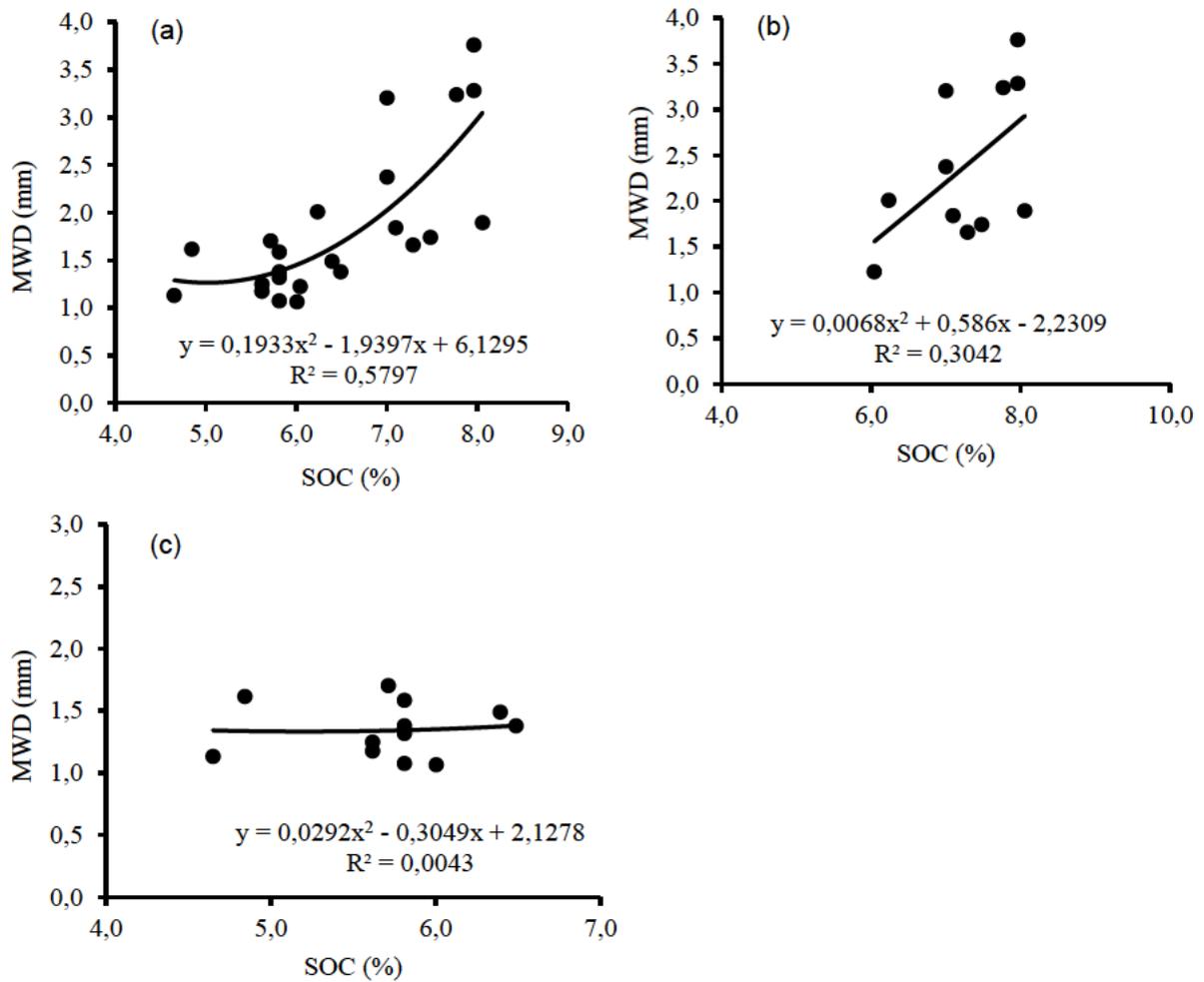


Figure 5. 3 Relationship between MWD (mean weight diameter (mm)) and SOC (g kg^{-1}) concentrations in soils under green cane and pre-harvest burning. Figures a, b and c represent all sites (both green cane and pre-harvest burning), green cane and pre-harvest burning, respectively.

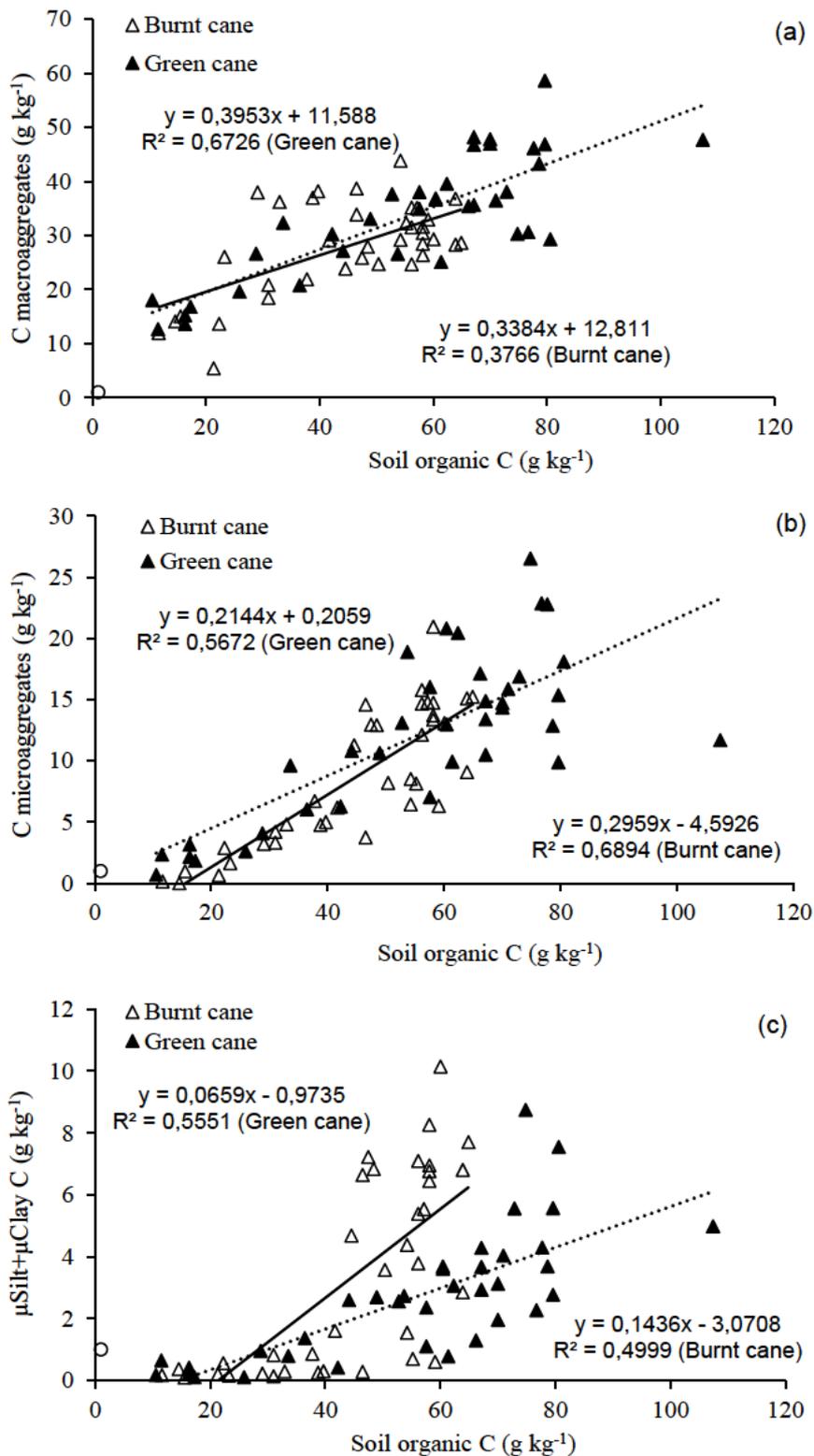


Figure 5. 4 Relationship between the coarse soil organic carbon fractions and bulk soil organic carbon. Figures a, b and c represent C associated with macroaggregates, microaggregates and μ Silt+ μ Clay fractions, respectively.

5.3.6 Correlation analysis

The C in fPOM-C was correlated negatively with $\mu\text{Silt}+\mu\text{Clay-C}$ and positively with C in macro-aggregate and $\text{fPOM}_{(\text{bulk soil})}$ (Table 5.6). The overall SOC correlated positively with fPOM-C, C in macro-aggregates and $\text{fPOM-C}_{(\text{bulk soils})}$, and negatively with soil pH. The C in $\mu\text{Silt}+\mu\text{Clay-C}$ from micro-aggregates was correlated positively to in $\mu\text{Silt}+\mu\text{Clay-C}$ from bulk soil and negatively with C in micro-aggregates and $\text{fPOM-C}_{(\text{bulk soil})}$. The C in macro-aggregates was negatively correlated to $\mu\text{Silt}+\mu\text{Clay-C}$ (macro-aggregates), while the C in micro-aggregates was positively correlated to $\text{fPOM-C}_{(\text{bulk soil})}$. The $\text{fPOM-C}_{(\text{bulk soil})}$ correlated negatively with C in $\mu\text{Silt}+\mu\text{Clay}_{(\text{bulk soil})}$. As for the exchangeable bases, Mg correlated positively with C in macro-aggregates and C in $\text{fPOM-C}_{(\text{bulk soil})}$.

Table 5. 5 Pearson correlation analysis between soil parameters in the top 30 cm depth

	SOC	Soil pH	fPOM-C	μ Silt+ μ Clay C(micro- aggregate)	C in macro- aggregates	C in micro- aggregates	μ Silt + μ Clay C(macro- aggregates)	fPOM(bulk soil)	μ Silt+ μ Clay C(bulk soil)	K	Ca	Mg	Clay
SOC	1.000												
Soil pH	-0.456**	1.000											
fPOM-C	0.662**	-0.373*	1.000										
μ Silt+ μ Clay C(micro-aggregate)	-0.023	0.119	-0.024	1.000									
C in macro-aggregates	0.565**	-0.449**	0.630**	0.209	1.000								
C in micro-aggregates	0.232	-0.441*	0.168	-0.474**	-0.046	1.000							
μ Silt + μ Clay C (macro-aggregates)	-0.283	0.329	-0.361*	-0.237	-0.577**	0.126	1.000						
fPOM(bulk soil)	0.508**	-0.532**	0.623**	-0.388*	0.272	0.876**	-0.077	1.000					
μ Silt+ μ Clay C(bulk soil)	-0.247	0.362*	-0.310	0.623**	-0.295	-0.285	0.612**	-0.378*	1.000				
K	0.245	-0.173	0.232	-0.117	0.313	0.186	0.041	0.261	-0.062	1.000			
Ca	0.194	-0.305	0.202	-0.252	0.044	0.264	-0.105	0.308	-0.290	0.148	1.000		
Mg	0.402*	-0.228	0.339	-0.274	0.096	0.437*	-0.124	0.512**	-0.323	0.420*	0.356*	1.000	
Clay	0.061	0.190	-0.012	-0.207	-0.253	0.211	-0.012	0.162	-0.178	0.151	0.632**	0.455**	1.000

* Indicates significant different at p 0.05; ** indicates significant different at p 0.01. The element K, Ca and Mg are exchangeable bases in soils

5.3.7 Principal Component Analysis

The first two component of pre-harvest burning relative to green cane harvest system explained 49.8% of the overall variation (Figure 5.5). The first axes (PC1) explained 29.8% of the variation while the second axes (PC2) explained 20.0%. The first axes (PC1) was associated with soil pH and mineral associated C (positive) and all the other soil parameters (negatively) (Figure 5.5). Pre-harvest burning was characterised by higher soil pH and mineral associated C, while green cane harvest system was characterised by higher aggregate stability and particulate SOC fractions.

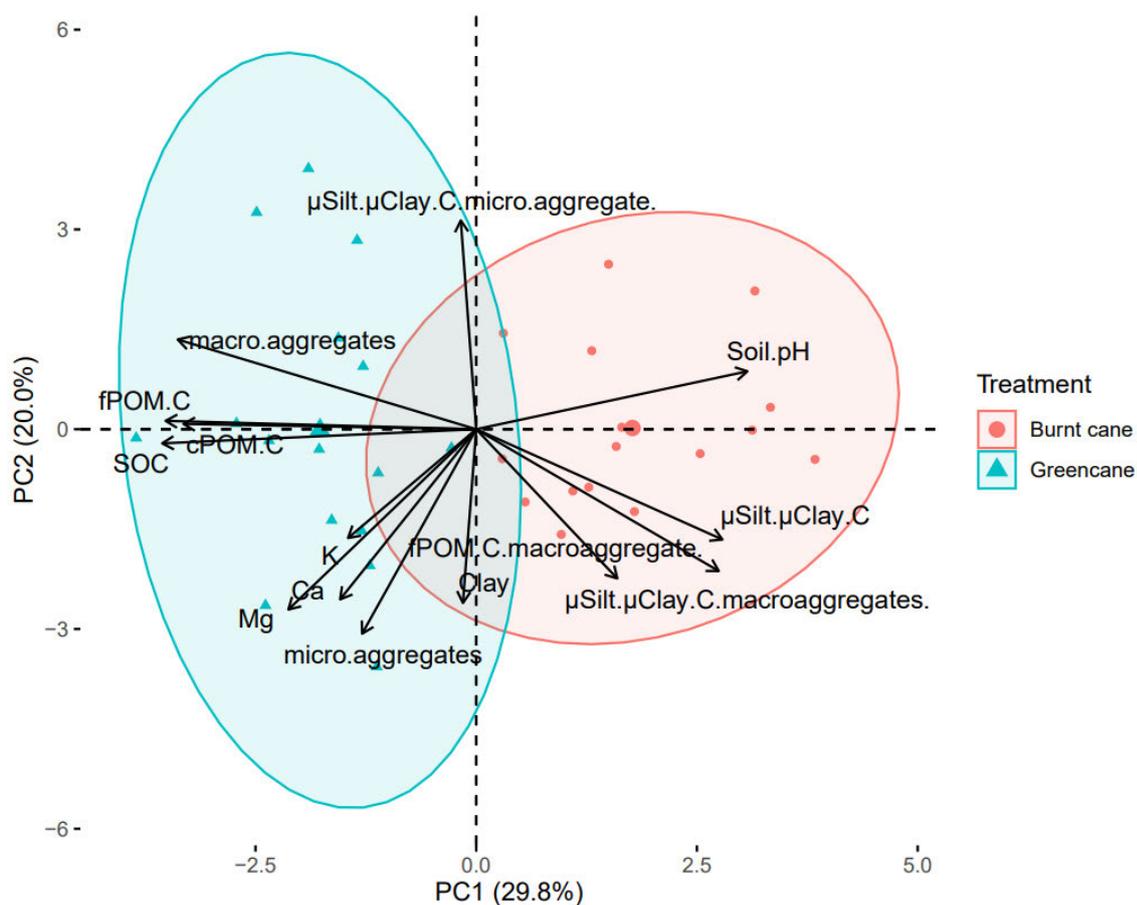


Figure 5. 5 The first two axes of Principle Component Analysis are based on effects of pre-harvest burning and green cane on different soil properties. The axes explained 49.8% variation using PC1 and PC2. The ellipse indicate the different treatments.

5.3.8 Soil morphology under pre-harvest burning and green cane harvest

Images from scanning electron microscope from $<53\ \mu\text{m}$ fractions of soils under pre-harvest burning and green cane are shown in Figure 5.6. The soil morphology from both pre-harvest burning and green cane showed a prismatic and granular structure. The soils have larger macro-aggregates and pores shown at $20\ \mu\text{m}$. Soils shows organic coatings on soil particles for both green cane and burnt cane. The soil particles showed a sponge architecture with a wide range of diameters with irregular structures (Figure 5.6a-d). Overall, there was no clear differences in observation between the residue management treatments.

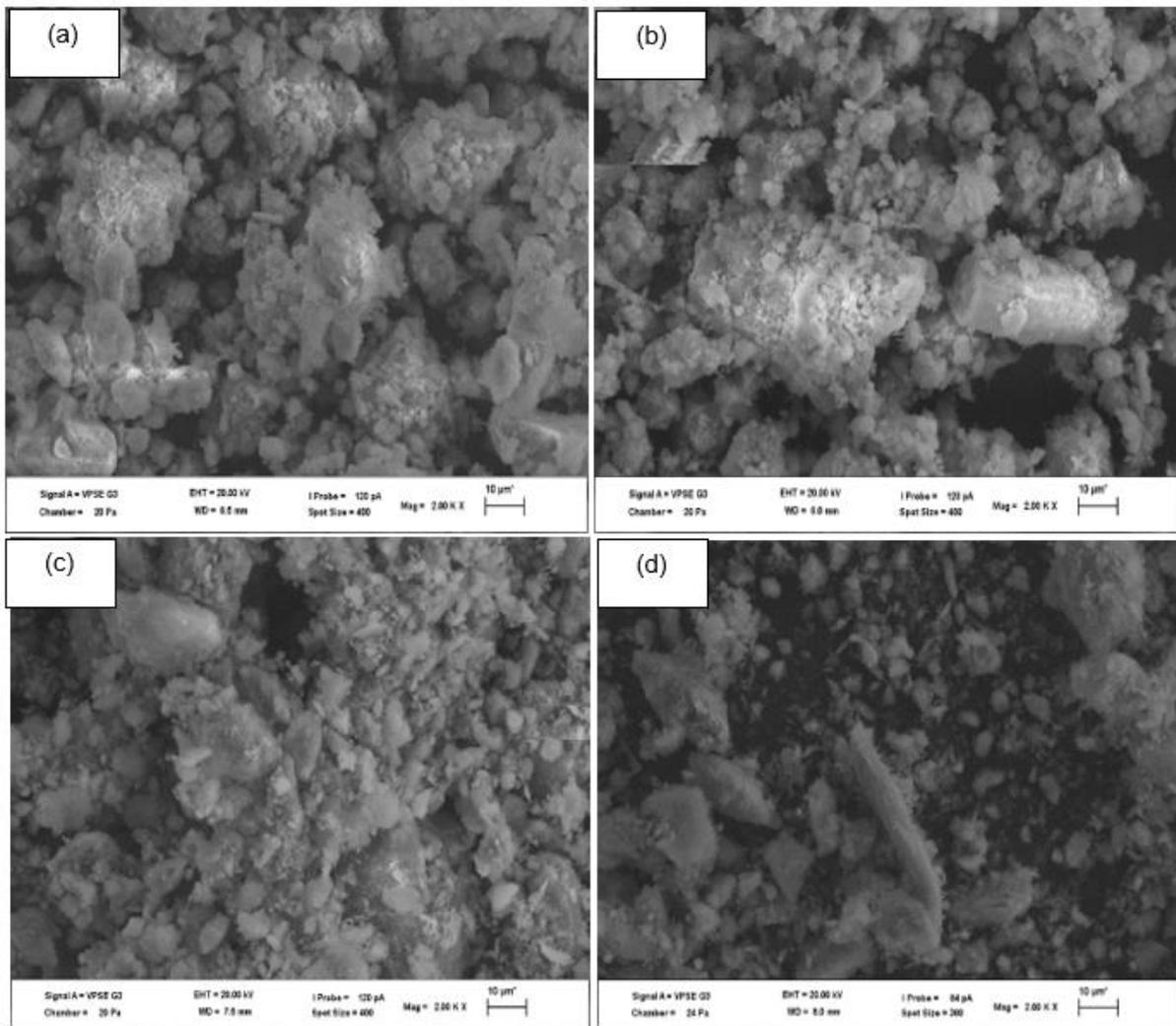


Figure 5. 6 Scanning electron micro images of the $<53\ \mu\text{m}$ fraction at 2000 times magnification zooming to $10\ \mu\text{m}$, with depth increment, for pre-harvest burning at (a) 0-5 cm and (c) 80-100 cm depth and green cane at (b) 0-5 cm and (d) 80-100 cm depth.

5.4 Discussion

5.4.1 Effects of burning on soil aggregates stability

The 42.8% lower aggregates stability of soil under pre-harvest burning than green cane, on average, could be explained by the effects of fire, which is reported to decrease the stability of aggregates in the surface soil depths (Bai et al. 2020). This may result in decreasing the concentration of SOC through combustion and erosion losses from the topsoil without cover. The addition of residues in green cane increases the physical protection of the soil surface (Silva et al. 2014) and increases SOC content and aggregates stability, and minimising soil erosion (Morais et al. 2020). Similar results were reported by Blair (2000), who observed that green trash retention resulted to 30% higher soil aggregate stability (MWD) compared to burnt treatment. While burning removes the fresh biomass, reducing SOC addition and aggregate stability, the relationship between MWD and SOC was very poor for these humic soils, which suggested that another factor contributes in the stabilisation of soil aggregates. Mthimkhulu et al. (2016) observed no increase in aggregates stability with retained residues than removal, and they reported that, under the conditions of their study, soil aggregate stability was controlled by the base status but not carbon. This view was supported by the positive correlations between MWD and exchangeable Ca, K and Mg in the current study (Table 5.6). Furthermore, recent findings have shown that aggregates stability increases with SOC to a maximum of about 3-4% C, and any additional increase in SOC would result in no changes in aggregates stability (Mbanjwa et al. 2022). The SOC in the soils of the current study was higher than this threshold with some layers having > 6.0% C (> 60 g kg⁻¹), which explains the poor relationship with MWD. The decrease in MWD with increase in soil depth could be explained by the decreased of SOC with increase in soil depth (Granged et al. 2011).

5.4.2 Response of soil organic carbon fractions to pre-harvest burning

The SOC content in macro-aggregates fraction, which constituted 60.3% and 68.0% of the total SOC concentration for pre-harvest burning and green cane retention, respectively, suggests that this fraction was the primary storage of SOC in these soils, irrespective of sugarcane management. The SOC in macro-aggregates and micro-aggregates are mainly comprised of the newly plant residue inputs (Six et al. 2002). Galdos et al. (2009) observed that SOC in macro-aggregates fraction was higher in the top 10 cm depth of unburned sugarcane (>100% higher) than fields under 2 to 8 years of burnt cane. This fraction of SOC constituted about 23.8% of

the total C, which was lower than the proportion observed in the current study (>60% of total SOC). In addition to being protected in aggregates and contributing to their stability, the negative correlation of the different POM-C fractions with soil pH confirms that acidic conditions in the humic soils limits microbial activity and decomposition of the SOC fractions even when not protected. The lower SOC in macro-aggregates fraction under pre-harvest burning could be explained by the effects of fire, which destroys POM-C the protected in aggregates decreasing its concentration (Torres-Sallan et al. 2018). The lack of significant differences in mineral associated C ($\mu\text{Silt}+\mu\text{Clay-C}$) between the two production systems, suggests that burning does not significantly affect this fraction. The POM-C fractions explain the results of aggregate stability, this was evident by the lower soil aggregates stability where POM-C fractions were lowered by burning the trash. The higher cPOM-C and fPOM-C in green cane further evidence the effects of burning cane on macro-aggregates, which reduces the physical protection of SOC in micro-aggregates within the macro-aggregate. The SOC associated with fPOM-C within macro-aggregates have been highlighted as the early indicator of management effects on soil quality (Torres-Sallan et al. 2018). A decrease in particulate organic C due to burning of sugarcane surface residues was observed by Dos Santos et al. (2020).

The higher coarse particulates carbon pool in green cane could be explained by the high input and maintenance of the fresh biomass and lower decomposition and mineralisation rates due to high soil acidity (Torres-Sallan et al. 2018; Xiao et al. 2018; Desrochers et al. 2020). The higher soil pH in pre-harvest burning could contribute significantly to decomposition of SOC in macro-aggregates fraction (cPOM-C and fPOM-C), which increase activity of microorganisms resulting to more rapid decomposition (Blair 2000; Wei et al. 2014). This was supported by the negative correlations of soil pH with all the soil C fractions. Also, the increase in nitrogen availability due to burning result to increase in microbial activities (Abdalla et al. 2016) which could enhance decomposition of particulate C fractions. The findings of Mkhonza et al. (2020), who reported a significant increase in mineralisation of nitrogen (N) in humic soils after treatment with lime, also confirm the importance of soil acidity in stabilising SOC in these soils. The lower the soil pH under green cane slowed decomposition process, and with the higher retention of residues over a long period, higher accumulation of SOC were recorded than burnt cane. In addition to the effects of soil pH, the higher particulate C fraction under green cane could be explained by the improved soil physical, chemical and biological properties due to residues retention, hence increasing the particulate C fraction (Turmel et al.

2015). The higher trash blanket in green cane than pre-harvest burning, which maintain the surface temperature and moisture, hence increasing accumulation of SOC as the residue is decomposed (Jennings et al. 2010). Green cane retention have been reported to reduce soil moisture losses, maintain cooler temperature and reduce erosion, these would protect the particulate C fraction, hence increase its concentration compared to pre-harvest burning (White and Webber 2018).

Like for macro-aggregates, reduced SOC in micro-aggregates fractions due to burning compared to green cane retention, could be a result of losses by the erosion and combustion/decomposition following breakdown of soil aggregates due to fire (Marcos et al. 2018; Fernandez et al. 2019). The concentration of C in micro-aggregates fractions, which constituted 18.8% and 21.9% of the total SOC for green cane and burnt cane, respectively, were lower than that in the macro-aggregates. Burning and lower retain of biomass to the soil reduced concentration of C in fPOM-C within micro-aggregates and this could be due to combustion by fire or decomposition by microorganisms, while the higher fPOM-C within micro-aggregates in green cane could be explained by the high level of acidity which limits microbial activities. Similar to fPOM-C within macro-aggregates, burning resulted to lower SOC concentration in this fraction when compared to green cane management. The higher cPOM in macro-aggregates and fPOM-C concentration within macro- and micro-aggregates and in the whole soil under green cane could be due to greater addition of fresh biomass, coupled with better protection in aggregates, in the more acidic conditions under green cane management. High SOC concentration as cPOM-C in macroaggregates regardless of management implies that cane management which affects aggregates may results to significant losses of C in these soils over time, especially if the soils are limed. However, the history of burning in both systems (>73 year before sampling under green cane) suggest that some of the POM-C fractions could be in the recalcitrant charcoal form, which may be resistant to decomposition even under conducive conditions (Doerr et al. 2018). The decrease in the concentrations of all the fractions, except $\mu\text{Silt}+\mu\text{Clay-C}$, with increase in soil depth could be explained by the decrease in fresh biomass addition as above ground litter and root biomass.

While the $\mu\text{Silt}+\mu\text{Clay-C}$ from the initial aggregate fractionation was significantly higher under burnt cane, the same fraction from further fractionation of macro- and micro-aggregates, and the sum of this fraction from the different stages (total soil $\mu\text{Silt}+\mu\text{Clay-C}$) were not significantly different between the production systems. This implies that pre-harvest burning

of sugarcane does not significantly affect SOC bound to the mineral fractions. The SOC in $\mu\text{Silt}+\mu\text{Clay}$ contributed between 4.85% and 7.61% of the overall SOC content in green cane and burnt cane, respectively. However, Sleutel et al. (2006) found that C in $\mu\text{Silt}+\mu\text{Clay}$ represented between 7% and 32% of the bulk SOC. The SOC associated with $\mu\text{Silt}+\mu\text{Clay}$ could be formed from surface interactions with minerals like kaolinite and oxides of Fe and Al, while a major portion could be in the form of Fe/Al-organic matter complexes. The contribution of these complexes in SOC stabilisation and the effects of burning cane, relative to green cane, needs to be clearly established.

5.4.3 Soil morphology in pre-harvest burning relative to green cane

The organic coating and cementing gel in the $<53\ \mu\text{m}$ fraction contributed to protection of SOC from microbial attack, especially in the green cane management. Addition of fresh biomass in green cane could have contributed to the formation of organic coating through increase in production of polysaccharides from microorganisms and roots, which then bound to the silt and clay fractions of the soil (Naveed et al. 2018). Polysaccharides mediates formation of POM fractions, and this may enhance the C concentration and physical protection in different fractions within the aggregates (Torres-Sallan et al. 2018). While in pre-harvest burning management the increase in soil pH could have resulted in increase in microbial activities which could produce exudates polysaccharides which acts as soil particle binding agent (Galloway et al. 2020). However, this did not result to higher SOC in the silt + clay fraction from the fractionation of the macro-and micro-aggregates, and the total fraction from the bulk soil under pre-harvest burning than green cane.

5.5 Conclusion

The study found that pre-harvest burning reduced soil aggregates stability, and carbon in macro- and micro-aggregates, when compared to green cane, in the sandy loam humic soils. The soil organic carbon in the form of cPOM-C in macro-aggregates and fPOM-C within both macro- and micro-aggregates are higher under green cane than burnt cane, while the C associated with silt and clay within soil aggregates is not affected by the harvest system used. These findings imply that burning could results to possible loses of soil organic carbon over time due to exposure of aggregates protected carbon. The high concentration of the different carbon fractions in humic soils, with higher proportion of POM-C fractions (cPOM-C and fPOM-C) suggest that recalcitrant charcoal carbon, which may be historical in nature, may be

present and needs to be studied. While the C associated with silt and clay, in soil aggregates is not affected by pre-harvest system, the potential variation in the form in which C occurs in this fraction suggests that pyrophosphate extractable carbon and the effect of harvest system on this fraction need to be explored in further studies. The effects of these production systems and their associated soil organic carbon fractions and soil pH on availability of nutrients, particularly phosphorus, also need to be explored in these soils.

CHAPTER 6

Charcoal- and iron/aluminium-bound carbon under contrasting sugarcane harvesting systems and to forest on loamy humic soils

A version of this chapter is under review for publication in **Soil Use and Management**

Abstract

Purpose Long-term sugarcane production with pre-harvest burning could add recalcitrant charcoal to OC in humic soils, while aluminium/iron-organic matter (Al/Fe-OM) complexes could dominate the mineral associated OC in these soils. The current study determined the effects of (i) sugarcane production relative to forest and (ii) green cane relative to burnt cane, on soil OC in charcoal (charcoal-C) and Al/Fe-OM complexes in selected humic soils.

Methods The soils were collected to a depth of 1 m from three sites (Eshowe, Eston, and Wartburg), under sugarcane and forest, and from Stanger, under sugarcane with pre-harvest burning compared with green cane production systems. Charcoal-C was determined by dry combustion after digestion with hydrogen peroxide and weak nitric acid, while Al/Fe and OC in Al/Fe-OM complexes were extracted with 0.1M sodium pyrophosphate.

Results and discussion Soils under sugarcane had lower charcoal-C concentration than forest only at Wartburg but not Eshowe and Eston and the concentration decreased with soil depth at all sites. Soil OC, Fe and Fe+Al in Al/Fe-OM complexes were significantly higher under sugarcane than forest only at Eshowe but not Eston and Wartburg.. The soil under green cane had significantly lower charcoal-C than under burnt cane, with no differences in OC, Al and Fe in Al/Fe-OM complexes, between the production systems.

Conclusions These findings imply that charcoal-C and Al/Fe-OM complexes contribute to the high OC in humic soils but the reduction of these fractions due to sugarcane production, relative to forest, varies between sites, while pre-harvest burning increases charcoal-C, but not OC in Al/Fe-OM complexes when compared to green cane.

Key words: • Charcoal-C • Green cane • Land use • Organic carbon • Organo-mineral complexes

6.1 Introduction

Soils store large quantities of organic carbon (OC) reducing the losses of OC from soil as carbon dioxide (CO₂). Conversion of natural forests to agricultural activities, that include tillage, increases decomposition of soil organic matter and emission of CO₂ (Che et al. 2021), which accounts for 60% greenhouse gases emissions (Rakhsh et al. 2020), and contributes to climate change. Increasing storage of OC in soil while minimising CO₂ emissions is a major strategy to reduce the contribution of agriculture to climate change and for mitigating its negative effects (Lasslop et al. 2019). The high OC storage capacity of soils has increased research interests in the mechanisms involved in stabilisation of soil organic carbon (SOC) in different environments (Sun et al. 2019). The main mechanisms of SOC stabilisation reported in literature are physical protection in water stable aggregates and formation of organo-mineral complexes (Kan et al. 2022; Kottakamp et al. 2022). Formation of Fe- and Al-organic matter complexes has been reported to reduce the accessibility of organic matter to microbial attack, and increases its residence time (Lawrence et al. 2015). For example, Lawrence et al. (2015) reported that SOC content was significantly correlated to Fe- and Al-organic matter complexes, in volcanic soils, emphasizing the importance of these complexes in stabilising SOC. In addition to mineral associated OC, for which organo-mineral complexes are a part, another stable fraction of OC is in the form of charcoal, which has received lesser attention in the literature.

Historical black C (charcoal) is reported to be highly resistant to microbial attack and decomposition (Lehmann et al. 2007), and is considered as an inert C pool in soils, which makes it an essential sink of global C (Jones et al. 2019). The high resistance of charcoal to decomposition could be due to the high C to nitrogen ratio (Schmidt et al. 2000) and the recalcitrant C and aromatic structure that may form (Nguyen et al. 2010). In addition, the hydrophobic substances, produced during burning, have been reported to increase SOC stability (Spaccini et al. 2006; Doerr et al. 1998). Briggs et al. (2012) reported that charcoal constitutes 5 to 30% of the total SOC content, depending on land use. Several authors investigated the concentration of charcoal-C under different land use such as forestry, grassland and cropping land (Eckmeier et al. 2007; Sedano et al. 2016; Hirsch et al. 2017; Wang et al. 2017; Mastrolonardo et al. 2018). In forestry and grassland soils charcoal C could accumulate for longer period due to limited soil disturbance with continuous burning resulting to addition of more charcoal. On the other hand, in cultivated crop land the concentration of charcoal could decrease due to increase microbial activities as a result of cultivation. While SOC stored as

charcoal is considered inert, it may increase soil pH (Hart and Luckai 2013) and cation exchange capacity and reduce losses of nutrients through leaching (DeLuca et al. 2008), with potential increase in microbial activity. Wardle et al. (2008) reported that charcoal inputs in forestry litter layer resulted in rapid decomposition of the litter. The conversion of natural forests to cropland with burning of crop residues could increase the concentration of charcoal but its stability in the cropped soil profile soils is poorly understood, considering the potential enhancement of its degradation due to cultivation, there is a need to understand such effects. Cultivation, including tillage and fertiliser and lime application, could alter the strength of bonds in the organo-mineral complexes, enhancing decomposition. The effects of site factors, land use and management on concentration of stable pools of SOC including charcoal and C in Al/Fe-organic matter complexes, need to be clearly understood, especially in humic soils, which are among the most productive soils of South Africa.

Large areas of humic soils in the KwaZulu-Natal Province are under commercial forest plantations and cropping, including sugarcane and maize. The high amounts of stored C (up to 400 Mg ha⁻¹ to a depth of 1.0 m), irrespective of site characteristics and land use (Malepfane et al. 2022) makes these soils essential in mitigating the effects of climate change. Although the mechanisms of OC stability in these soils are not clearly understood preliminary findings indicated that particulate organic matter (POM-C) formed >60% of total C at least in the top 30 cm both under green cane and pre-harvest burning (Chapter 5), suggesting that this potentially labile SOC is being protected from microbial decomposition. The POM-C dominated the SOC in the macro- and micro-aggregate size fractions (Chapter 5), suggesting that physical protection in water stable aggregates, is an important mechanism. While Malepfane et al. (2022) explained the high OC in humic soils using the low pH and concentrations of bases (also supported by negative correlation of the SOC fractions with pH (Chapter 5), which limit microbial activity, some of the POM-C could also be in the form of charcoal, while the OC associated with minerals (silt and clay) could be dominated by Al/Fe-organic matter complexes. The results in Chapter 5 also indicated that SOC associated with silt and clay (for which Al/Fe-organic matter complexes are a part) in the different aggregate size fractions was not affected by green cane production compared to burnt cane. However, the addition of lime and fertiliser and the occasional tillage operations on sugarcane farms could compromise the stability of these two stable OC fractions. Conversely, practicing pre-harvest burning of sugarcane may increase charcoal accumulation when compared to green cane production. The effects of conversion from forest to sugarcane production or from pre-harvest

burning to green cane on charcoal-C and OC in Al/Fe-organic matter complexes have never been studied in humic soils in South Africa. The understanding of concentrations of C associated with charcoal and Al/Fe-organo-mineral complexes, throughout the soil profile to at least 1 m depth is critical for agricultural production and climate change mitigation.

It was hypothesised that a (i) high proportions of SOC in humic soils is in the form of charcoal and Al- and Fe-organo-mineral complexes, including in the subsoil (ii) concentrations of charcoal-C and OC associated with Al/Fe-organic matter complexes are lower in cultivated soil relative to forest, and under green cane relative to burnt cane. Therefore, the objectives of the study were to determine the effects of (i) sugarcane production relative to forest and (ii) green cane relative to pre-harvest burning on charcoal-C and OC, Al and Fe in Al/Fe-organic matter complexes in whole profiles (1 m) in selected loamy humic soils.

6.2 Methods and materials

6.2.1 Soils (site description and characteristics)

The soils used in this study were clay loam classified as Inanda form from Wartburg and sandy clay loam classified as Magwa soil form from Eston and Eshowe and were among those used by Malepfane et al. (2022). For each site, the soils were cultivated with sugarcane and compared with adjacent ones under forest. The geographical coordinates and some climatic information area shown on Table 6.1. The Eston and Wartburg sites had commercial wattle (*Paraserianthes lophantha*) forest adjacent to the sugarcane (*Saccharum officinarum*) fields. For both sites (Eston and Wartburg) under sugarcane fertiliser nitrogen, phosphorus and potassium (NPK) were applied regularly, while poultry manure (10 t ha⁻¹) and dolomitic lime (1 to 10 t ha⁻¹), to reduce the soil acid saturation to 20%, were applied occasionally. The Eston site under forest had acid saturation of 80.6 and 83.8% in the 0-5 and 5-10 cm depth, respectively while the sugarcane had 0.523 and 17.4% in the 0-5 and 5-10 cm depth, respectively. Prior to re-planting sugarcane, kale (*Brassica oleracea* var. *sabellica*) was usually planted as a rotation crop at Wartburg.

At Eshowe natural forest was compared with adjacent sugarcane fields. The sugarcane was treated with fertiliser N, P and K, applied at 130, 20, and 140 kg ha⁻¹ respectively, and dolomitic lime, applied once every 10 years at, 1 to 10 t ha⁻¹ to reduce soil acid saturation to 20%. The forest had acid saturation levels of 2.84 and 28.4% in the top 0-5 and 5-10 cm depths,

respectively. The sugarcane had 18.5 and 26.8% acid saturation in the top 0-5 and 5-10 cm depth, respectively. Sunn hemp (*Crotalaria juncea*) or oats (*Avena sativa*) was usually planted as a rotation crop before re-planting sugarcane. At all sites, pre-harvest burning of sugarcane was practised, and trash and residues were removed from the soil surface.

To assess the effect of pre-harvest burning and green cane retention on different soil quality parameters, two adjacent farms located in Stanger (KwaDukuza). The site and characteristics are as described in section 4.2.1 of chapter 4. Soil sampling was done as described in section 4.2.2 of chapter 4. The samples were air-dried and sieved ($2 < \text{mm}$) before analysis of soil pH, P, SOC, Ca, Mg, K, Zn, Mn, total cation, exchangeable acidity and clay content as detailed in section 4.2.3 of chapter 4.

Table 6. 1 Geographic coordinates and climate of the study sites (Malepfane et al. 2022)

Study site	Coordinates	MAP	Maximum	Minimum	Elevatio
		mm	MAT	MAT	n
Wartburg	29°28'S; 30°37'E	798	24.5	11.1	933
Eshowe	28°52'S; 31°25 'E	1113	26.7	15.4	555
Eston	29°37'S; 30°23'E	805	24.2	12.4	928

MAP represents mean annual precipitation; MAT represents mean annual temperature.

6.2.2 Soil sampling and processing

Two experimental sites were used in the current study located in Stanger (-29° 12' 58.86" S; 31° 9' 19.908" E), KwaZulu-Natal, South Africa and all the fields were under sugarcane production with one field under pre-harvest burning and the adjacent one under green cane retention. The details of the climatic conditions, soil types and management of the sugarcane, including cover crops and characterisation of the soils are detailed in Sections 4.2.1, 4.2.2 and 4.2.3 in Chapter 4. The characteristics of the soils are given on Table 4.1. The determination of soil pH and clay content, and total SOC are detailed in chapter 4 section 4.2.3 and 4.2.4, respectively, and the results summarised on Table 6.2. For the green cane relative to pre-harvest burning total SOC concentration was presented in Chapter 5 in Table 5.1. The results POM-C and mineral associated C of the soils under green cane relative to pre-harvest burning are presented in Table 6.3. The clay content of the soils was 45.6% under green cane and 48.1% under burnt cane. For soil pH, green cane had a pH of 3.85 and pre-harvest burning 4.03.

Table 6. 2 Effects of land use and site on total C concentration in soils under sugarcane and forest

Site	Land use	Total C (g kg ⁻¹)	Soil pH	Clay content (%)
Eshowe	Sugarcane	31.7b	4.41b	22.9a
	Forest	28.4b	4.12a	26.4a
Eston	Sugarcane	22.2a	4.41b	33.9b
	Forest	29.8b	4.05a	24.3a
Wartburg	Sugarcane	19.4a	4.08a	37.6b
	Forest	37.6c	4.33b	43.8c
LSD		3.79	0.073	2.85
Depth				
	0-5	49.2f	4.46e	28.4ab
	5-10	46.8f	4.31cd	30.5abc
	10-15	32.2e	4.19abc	32.6abc
	15-20	30.2de	4.06a	32.8abc
	20-30	25.9cde	4.09a	32.4abc
	30-40	25.8cde	4.14ab	34.6c
	40-50	23.9bcd	4.14ab	33.9bc
	50-60	19.9abc	4.21abc	31.8abc
	60-80	16.0ab	4.28bcd	30.5abc
	80-100	12.0a	4.42de	27.5a
LSD		4.89	0.094	3.68

Different lower-case letters indicate significant differences ($p < 0.05$) among treatments.

Table 6. 3 Total, particulate and mineral associated soil organic carbon (g kg⁻¹) in humic soils under green cane and pre-harvest burning

Treatment	SOC	POM-C	Mineral associated C
Green cane	55.7b	37.6b	2.70a
Pre-harvest burning	44.7a	29.8a	3.34b
LSD	3.96	3.23	0.607
Depth (cm)			
0-5	66.6d	40.2c	5.46de
5-10	68.6d	40.9c	5.38de
10-15	63.8d	38.1c	6.45e
20-30	63.9d	39.6c	4.05cd
30-40	55.6cd	36.2bc	2.38bc
40-50	49.1bc	34.7bc	1.69ab
50-60	41.8b	31.0bc	1.05ab
60-80	27.3a	26.1ab	0.432ab
80-100	14.7a	16.6a	0.289a
LSD	8.39	6.85	1.29

Different lower-case letters indicate significant differences ($p < 0.05$) among treatments. SOC represents soils organic carbon; POM-C represents carbon in particulate organic matter; mineral associated C represents carbon bound to silt and clay

6.2.3 Charcoal-C method validation

A preliminary experiment was conducted with soils with low SOC from 80-100 cm depth of Eston (forest) and Wartburg (sugarcane), to determine the efficiency of recovery of charcoal from the method used in this study. Biochar (used to represent charcoal) was produced from pine bark (<2 mm) from Crammond Farm, and pyrolysed at 650°C in a furnace, with no oxygen to avoid oxidative combustion. The biochar was sieved to <500 µm sieve to increase contact with the soil particles, and applied to 20 g soil to represent charcoal-C at 0, 0.05, 0.1, 0.5, 1.0, 2.0 and 5.0% charcoal-C (w/w). The samples were then digested with 30% hydrogen peroxide (H₂O₂) and 10 ml of 1M nitric acid (HNO₃) on a hot plate for 16 hours, as described by Kurth et al. (2006), and the total C remaining in the sample was analysed using LECO Trumac CNS Auto-analyser version 1.1x (LECO Corporation, 3000, Lakeview, Ave, ST, Joseph, MI, USA). The results were used for checking the effectiveness of the method. Linear regression model was used to determine the relationship between added charcoal-C and measured concentrations after digestion using Microsoft Excel, as indicated in Figure 6.1. From the results, the added charcoal-C explained >99% of the variation of the measured charcoal-C, indicating high efficiency of the method. The method was then used for further analysis of charcoal-C in soil samples.

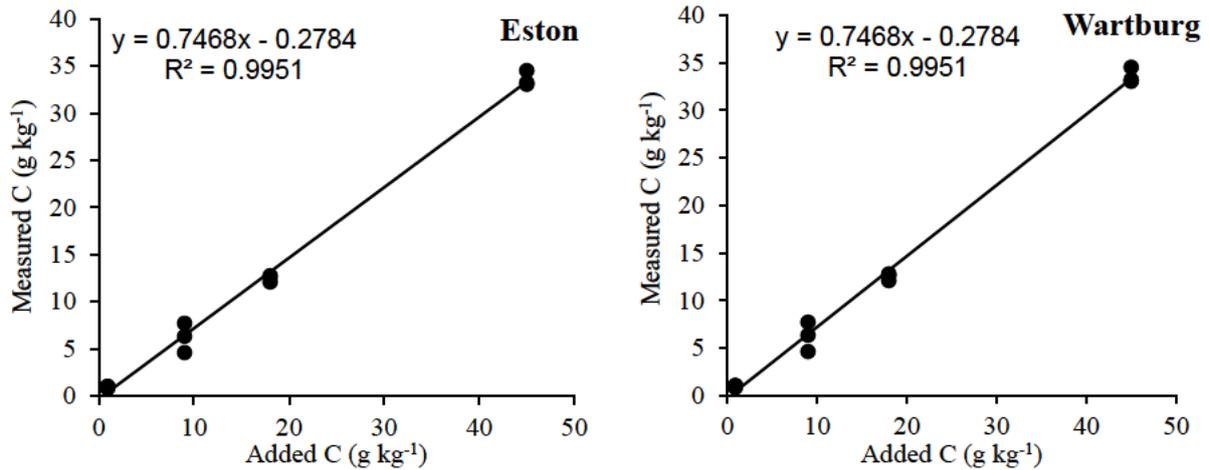


Figure 6. 1 Charcoal-C retained at different application rates of charcoal after digestion.

6.2.4 Determination of Charcoal-C from humic soils

The samples were analysed for charcoal-C concentration using the digestion method as described by Kurth et al. (2006), with three replicates for each depth. For this, 1.0 g dried sample (< 500 μm) in 250 ml Erlenmeyer flask, was treated with 20 ml of 30% hydrogen peroxide (H_2O_2) and 10 ml of 1M nitric acid (HNO_3). The flask was swirl while mixing at room temperature for over 30 minutes under the fume hood. Thereafter, the flask was then heated on a hot plate at 100°C for 16 hours, with occasional swirling to observe occurrence of effervescence. After 16 hours, flasks which showed effervescence were heated for an additional 4 hours. Thereafter, the samples were filtered into storage bottles through Whatman filter paper No. 1, and the residues on the filter paper were oven dried (60°C) and ground using a mortar and pestle. Total C remaining in the sample, which was considered as charcoal C, was then determined by dry combustion with LECO Trumac CNS Auto-analyser. Non-charcoal POM-C was calculated for the green cane and pre-harvest burning soils using below equation. Where POM-C indicates the SOC concentration in the bulk soils (fPOM-C + cPOM-C).

$$\text{Non-charcoal} = \text{POM-C} - \text{Charcoal C}$$

6.2.5 Carbon, aluminium (Al) and iron (Fe) in Al/Fe-organic matter complexes

The OC, Al and Fe associated with Al/Fe-organic matter complexes were extracted with 0.1 M Na-pyrophosphate as described by Blakemore et al. (1981). For this, 50 ml of 0.1M Na-pyrophosphate was added to 0.5g soil sample and shaken for 16 hours on a reciprocal shaker at 180 rpm. Thereafter, 5 drops of 0.4% superfloc were added while shaking vigorously before

centrifugation at 20000 rpm for 30 minutes. The supernatant was then filtered through Whatman No.1 filter paper and 1 ml of each extract was diluted 20 times with deionised water. The concentration of Al and Fe in the Na-pyrophosphate extract (Alp and Fep) was determined using an inductively coupled plasma atomic emission spectrometry (Varian 720-ES ICP-AES). The OC (Cp) in the same extract was analysed using Walkley-Black method as described by Garrido and Matus (2012).

6.2.6 Statistically analysis

The data of charcoal-C, total C, Cp, Alp and Fep were subjected to two-way analysis of variance (ANOVA) using GenStat Ed. 18, with site+land use combination and depth or sugarcane production system and depth as the main factors. The least significant difference (LSD), at 5% level was used to compare treatments means (site/land use combination and depth). The Tukey-Kramer test was also applied to separate treatments means at $p < 0.05$. The data were also subjected to Spearman's rank correlation analysis using GenStat Ed. 18. Furthermore, a Principal Component Analysis (PCA) was applied separately to show the effects of management and land use on different soil elements, in R Statistical software 2018.

6.3 Results

6.3.1 Effects of sugarcane production relative to forest in humic soils

6.3.1.1 Charcoal-C concentrations

The overall effects of land use at the different sites showed that sugarcane cultivation had significantly lowered charcoal-C concentrations (0.794 g kg^{-1}) relative to the uncultivated forests (6.38 g kg^{-1}) at Wartburg but not Eshowe and Eston (Table 6.4). There were no differences in the charcoal-C in soils under sugarcane between the three sites, while the concentrations in forest soils were in the order; Wartburg (6.38 g kg^{-1}) > Eston (3.52 g kg^{-1}) > Eshowe (1.68 g kg^{-1}) (Table 6.4), which translated to 14.2, 10.1 and 3.10% of total SOC. The charcoal-C in the soils under sugarcane constituted 3.30, 8.50 and 2.50% of total SOC at Wartburg, Eston and Eshowe, respectively. The overall concentration of charcoal-C decreased with increase in soil depth (Table 6.4), with the top 10 cm depth having significantly higher concentration (>100% higher) compared to the deeper layers (Table 6.4). The concentrations of charcoal-C ranged from 1.07 g kg^{-1} in the 80-100 cm to 7.21 g kg^{-1} in the 5-10 cm depth.

6.3.1.2 Organic carbon in Al/Fe-organic matter complexes

Sugarcane cultivation significantly increased OC in Al/Fe-organic matter complexes (Cp) (16.7 g kg⁻¹) relative to the forests (13.6 g kg⁻¹) at Eshowe but not Wartburg and Eston (Table 6.4). Between the three sites, the concentration of OC in Al/Fe-organic matter was significantly higher at Eshowe and Wartburg when compared to Eston. The concentration of OC in Al/Fe organic matter in forest soils was significantly higher at Wartburg (16.7) when compared to Eston (13.6 g kg⁻¹) and Eshowe (13.6 g kg⁻¹) (Table 6.4). The proportion of Cp translated to 37.8, 40.1 and 42.2 % of total SOC in the forest soils at Wartburg, Eston and Eshowe, respectively. The Cp in the soils under sugarcane constituted 58.4, 46.3 and 48.5% of total SOC at Wartburg, Eston and Eshowe, respectively. Overall, the Cp decreased with increased soil profile depths (Table 6.4), with the top 10 cm having significantly higher concentration than the layers below 15 cm depth (Table 6.4). The concentration of Cp ranged from 7.91 g kg⁻¹ in the 80-100 cm to 20.0 g kg⁻¹ in the top 5 cm depth.

Table 6. 4 Effects of land use and site on concentration (g kg⁻¹) of charcoal-C and Al/Fe bound C

Site	Land use	Charcoal C	Al/Fe bound C
Eshowe	Sugarcane	0.794a	16.7c
	Forest	1.68a	13.6ab
Eston	Sugarcane	1.87ab	10.8a
	Forest	3.90b	13.6ab
Wartburg	Sugarcane	0.791a	14.0bc
	Forest	6.38c	16.7c
LSD		0.747	2.64
Depth			
0-5		4.81bc	20.2e
5-10		7.21c	19.4e
10-15		2.30ab	17.1de
15-20		2.39ab	14.7bcd
20-30		2.21ab	12.8bc
30-40		1.58a	14.8cd
40-50		1.72ab	13.0bcd
50-60		1.25a	12.1abc
60-80		1.16a	10.4ab
80-100		1.07a	7.91a
LSD		0.964	2.64

Different lower-case letters indicate significant differences ($p < 0.05$) among treatments.

6.3.1.3 Concentrations of Al and Fe in Al/Fe-organic matter complexes

The overall effects of land use at different sites showed that sugarcane cultivation had significantly lowered Alp, Fep and Alp+Fep concentrations relative to the forests only at Eshowe (Table 6.5). The soil under sugarcane at Eshowe had higher Alp and Alp+Fep than those at Eston and Wartburg and higher Fep than that at Wartburg, while under forest, the soils from Eshowe had higher concentrations of all three parameters than the other two sites. The Alp concentration in the Eshowe forest soil was > 100% higher than the other forest soils (Table 6.5), and was lower in the 0-5 and 80-100 cm than the 60-80 cm depth for all sites. The Fep increased with soil depth, with the top 10 cm having significantly lower concentrations than layers below 30 cm depths, and the concentration ranged from 3.37 g kg⁻¹ in the top 5 cm to 7.35 g kg⁻¹ in the 80-100 cm depth (Table 6.5). The top 10 cm depths showed a significantly lower concentration of Alp+Fep than the soil layers below 30 cm, except for 80-100 cm depth, ranging from 8.45 g kg⁻¹ in the top 5 cm to 15.2 g kg⁻¹ in 60-100 cm depth. The Alp+Fep was higher in the 60-80 cm than the top 10 cm and the 80-100 cm depths.

Table 6. 5 Effects of land use and site on the concentration of Alp, Fep and Alp+Fep (g kg⁻¹)

Site	Land use	Alp	Fep	Alp+Fep
Eshowe	Sugarcane	7.50b	6.74c	14.3c
	Forest	11.7c	9.96d	21.7d
Eston	Sugarcane	4.23a	5.17abc	9.67ab
	Forest	5.18a	5.59bc	10.8b
Wartburg	Sugarcane	4.01a	3.63a	7.64a
	Forest	4.98a	4.88ab	9.86ab
LSD		0.926	1.17	2.10
Depth				
0-5		5.08a	3.37a	8.45a
5-10		5.16ab	3.78ab	8.94ab
10-15		6.58ab	5.85abc	12.4abcd
15-20		6.69ab	6.25bc	12.9bcd
20-30		6.33ab	5.96bc	12.3abcd
30-40		6.95ab	7.07c	14.0cd
40-50		6.91ab	7.35c	14.1cd
50-60		6.81ab	7.18c	14.2cd
60-80		7.06b	7.35c	15.2d
80-100		5.08a	7.53c	10.7abc
LSD		1.20	1.51	2.72

Different lower-case letters indicate significant differences ($p < 0.05$) among treatments.

6.3.2 Soil charcoal-C, non-charcoal POM-C and OC, Al and Fe in Al/Fe-organic matter complexes under green cane and burnt cane

Green cane (4.31 g kg⁻¹) resulted in significantly lower charcoal-C concentration than pre-harvest burning (5.48 g kg⁻¹) (Table 6.6). The charcoal-C under pre-harvest burning was 27% higher than under green cane (Table 6.6), and constituted 7.74 (green cane) and 12.3% (burnt cane) of the total SOC. The top 20 cm depth had significantly higher charcoal-C than the depths below 30 cm, and the concentration ranged from 9.16 g kg⁻¹ in the 5-10 cm to 1.85 g kg⁻¹ in the 80-100 cm depth (Table 6.6). The non-charcoal POM-C was significantly higher in green cane than pre-harvest burning by 36.5% and decreased with increase in soil depth (Table 6.6). The OC in Al/Fe-organic matter complexes (Cp) was not significantly different between green cane and pre-harvest burning (Table 6.6) and constituted 40.2 and 42.5% of the total SOC under green cane and burnt cane, respectively. The concentration of Cp decreased with soil depth, ranging from 7.72 g kg⁻¹ in the 80-10 cm to 25.4 in the top 5 cm depth, with the top 40 cm (>20 g kg⁻¹) having higher than the layers below 50 cm (Table 6.6). Similar to Cp, there were no significant differences in Alp and Fep between green cane and pre-harvest burning (Table 6.7). Only the 80-100 cm depth had significantly lower Fep and Alp than the top 60 cm, where the layers did not differ (Table 6.7).

Table 6. 6 Charcoal-C, non-charcoal POM-C and Al/ Fe bound C concentration (g kg⁻¹) in green cane and pre-harvest burning

Treatment	Charcoal C	Non-charcoal POM-C	Al/Fe bound C
Green cane	4.31a	33.3b	18.5
Pre-harvest burning	5.48b	24.4a	19.0
LSD	0.297	1.60	3.62
Depth			
0-5	7.76de	32.4b	25.4d
5-10	9.16e	31.8b	23.1d
10-15	7.04cd	31.0b	23.2d
20-30	5.33bc	34.2b	24.8d
30-40	3.71ab	32.5b	21.3cd
40-50	3.88ab	30.8b	16.2bc
50-60	2.82a	28.2b	15.1b
60-80	2.49a	23.6ab	12.5b
80-100	1.85a	14.7a	7.22a
LSD	0.630	3.40	3.62

Different lower-case letters indicate significant differences ($p < 0.05$) among treatments. Where means do not have letters, within a column for each factor, they are not significantly different. No letters in mean indicate no significant differences.

Table 6. 7 Effect of green cane relative to pre-harvest burning and depths on organically bound Al and Fe (g kg⁻¹)

Treatment	Alp	Fep	Alp+Fep
Green cane	13.0	15.0	28.0
Pre-harvest burning	13.0	14.0	26.0
LSD	1.50	1.30	4.00
Depth			
0-5	16.0c	15.5bc	32.5c
5-10	15.8c	16.7bc	32.5c
10-20	14.8c	15.8bc	30.6bc
20-30	14.9c	16.3bc	31.2bc
30-40	16.0c	19.4c	35.5c
40-50	14.8c	18.0bc	32.8c
50-60	11.7bc	15.1bc	26.8bc
60-80	7.74b	9.84ab	17.6ab
80-100	2.32a	2.84a	5.16a
LSD	3.20	2.70	8.50

Different lower-case letters indicate significant differences ($p < 0.05$) among treatments. Where means do not have letters, within a column for each factor, they are not significantly different. No letters in mean indicate no significant differences.

6.3.3 Principal component analyses of management effects on different soil parameters

Principal component analysis (PCA) showed that two PCAs accounted for 48.5% of the variability within the dataset, with the first two PC explaining 48.5% variation (Figure 6.2). The first PC2 explained 30.1% variability of the data (Figure 6.2). The second PC2 explained 18.4% variability of the data. The forest soils were characterised by high charcoal C, total C, Al/Fe-OM bound C and Mehlich 3 extractable Fe (Figure 6.2), while the soils under sugarcane cultivation were characterised by high silt, organically bound Alp, Fep+Alp and Fep (Figure 6.2) and lower clay content.

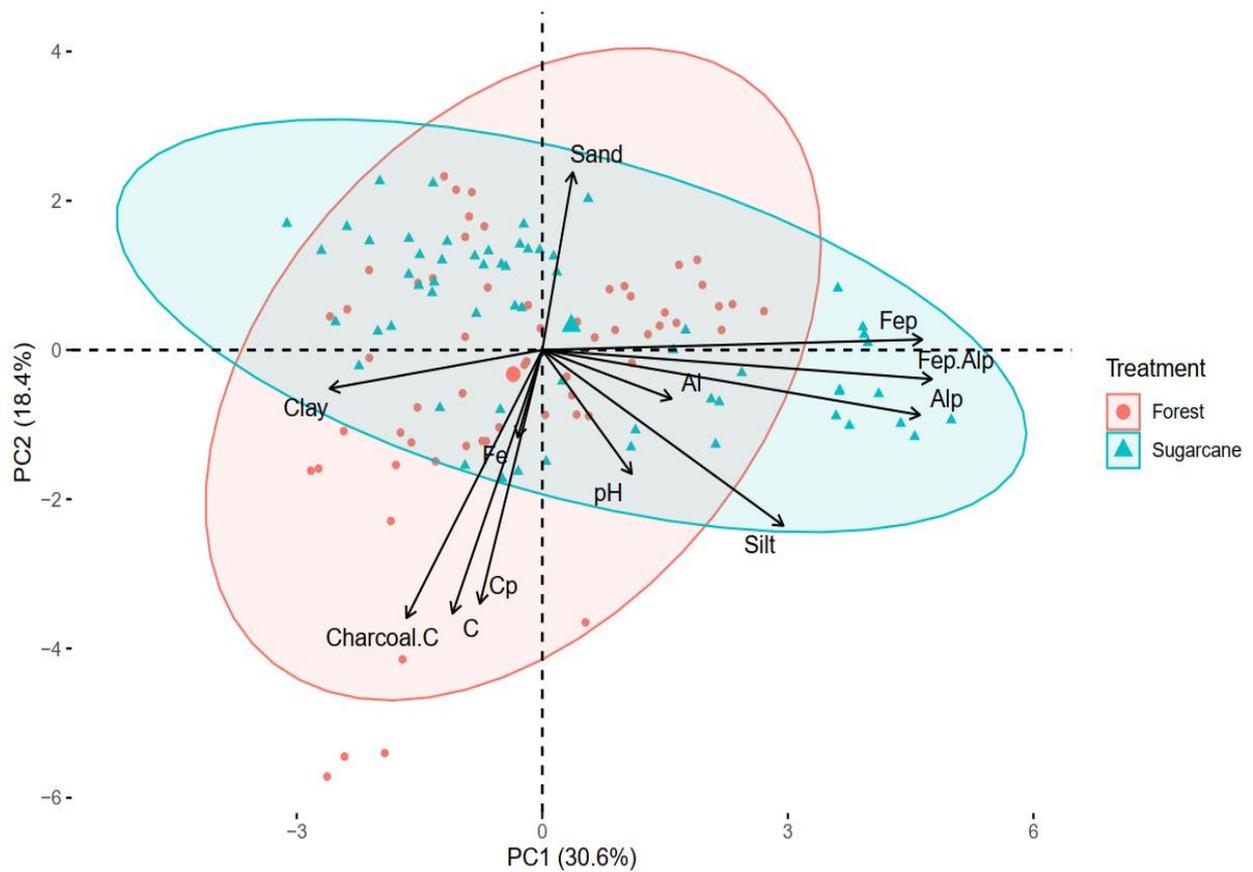


Figure 6. 2 The first two axes of Principle Component Analysis are based on effects of land use on different soil properties. The axes explained 48.5% variation using PC1 and PC2. Groups represents the different managements where forestry (uncultivated) sugarcane (cultivated) land use.

6.3.4 Relationship analysis of different soil quality indicators

In addition to charcoal-C and Cp, total C was correlated weakly and positively with silt and Mehlich 3 extractable Fe and Al negatively with sand content. Charcoal-C was correlated positively with total C, Cp, Mehlich 3-extractble Fe, pH and negatively correlated to organically bound Fe and Fep+Alp. In addition to charcoal-C, Cp was only positively correlated with total C but not the different fractions of Fe and Al, pH, clay, silt and sand. Organically bound Fe showed a highly positive correlation to organically bound Fep+Alp, and weak correlation to silt. The organically bound Fep+Alp was positively correlated to silt. Organically bound Al exhibited a strong positive correlation with organically bound Fe and Fep+Alp, and a weak negative correlation with clay (Table 6.8).

Table 6. 8 Spearman's rank correlation analysis of soil parameters response

	Al	Alp	C	Charcoal-C	Clay	Cp	Fe	Fep	Fep+Alp	Sand	Silt	pH
Al ¹⁴	1.000											
Alp ¹⁵	0.382**	1.000										
C ¹⁶	0.152	0.094	1.000									
Charcoal-C	-0.098	-0.188	0.463**	1.000								
Clay	-0.075	-0.253*	0.030	0.048	1.000							
Cp ¹⁷	0.024	0.120	0.622**	0.319*	0.104	1.000						
Fe ¹⁸	0.126	0.038	0.316*	0.279*	-0.307*	0.186	1.000					
Fep ¹⁹	0.332**	0.907**	-0.164	-0.232*	-0.291*	-0.169	-0.023	1.000				
Fep+Alp	0.370**	0.972**	-0.042	-0.223*	-0.294*	-0.040	0.000	0.974**	1.000			
Sand	-0.022	-0.070	-0.195*	-0.130	-0.628**	-0.102	0.391**	-0.009	-0.033	1.000		
Silt	0.170	0.397**	0.189*	0.169	-0.417**	0.008	-0.058	0.361**	0.397**	-0.385**	1.000	
pH	0.122	0.149	0.065	0.192*	-0.040	0.003	-0.092	0.127	0.129	-0.220*	0.295*	1.000

* Indicates significant different at $p < 0.05$; ** indicates significant different at $p < 0.001$.

¹⁴ Mehlich 3 extractable aluminium

¹⁵ Organically bound aluminium

¹⁶ Total carbon

¹⁷ Al/Fe bound C

¹⁸ Mehlich 3 extractable iron

¹⁹ Organically bound iron

6.4 Discussion

6.4.1 Distribution of charcoal-C

The higher charcoal-C content under forest than under sugarcane (Table 6.4) at Wartburg could be explained by burning of forest biomass resulting in charcoal-C accumulation into the soil as burnt biomass (Wang et al. 2017). Soil biomass C is converted to charcoal-C during burning of biomass, which then accumulate into the soil surface with time and is converted from labile biomass C to passive C fraction (Boot et al. 2015). Limited soil disturbance in uncultivated forest soils could possibly reduce decomposition of the charcoal-C over time. In addition, the higher clay content (33-52%) in this uncultivated soil (Malepfane et al. 2022) than under sugarcane could also contribute in explaining the higher charcoal-C (6.38 g kg^{-1}) through increase protection in the microaggregates. On the other hand, the addition of both organic and inorganic fertilisers, which increases activity of microorganism resulting to rapid decomposition of resident soil C in the soil including charcoal-C pool, could have contributed in the lower charcoal-C under sugarcane at Wartburg (Table 6.4). Knicker (2011) reported that charcoal-C is not as recalcitrant as commonly assumed. Hilscher and Knicker (2011) reported that pyrogenic soil organic matter derived from grass biomass can be exposed to attack by microorganisms. However, the decomposition of the charcoal occurs at a slow rate (Boot et al. 2015; Krishnaraj et al. 2016). In addition to microbial decomposition, more frequent burning resulting to higher combustion of charcoal and losses, as a result of erosion and leaching, could also have contributed to lower charcoal-C under sugarcane. However, these effects of land use on concentration of charcoal-C was not significant for soils from Eshowe and Eston. The evidence of charcoal C in deeper soil layers for all land use could be explained by the potential leaching/eluviation as confirmed by the accumulation of charcoal-C in deeper layers in the porous humic soils, with high drainage (Fey 2010), which could promote movement of charcoal-C down the soil profile. The decrease in charcoal-C with depth was also reported in several articles in the literature (Pingree et al. 2012; Zhan et al. 2013; Boot et al. 2015; Hobley et al. 2017; Huang et al. 2018; Sawyer et al. 2018; Wang et al. 2018).

The higher charcoal-C under pre-harvest burning is a result of regular burning, which increase addition of incomplete burnt biomass which accumulates in the soils as charcoal C (Singh et al. 2015; Goswami et al. 2020). Burning has been reported to increase charcoal-C concentration with time in agricultural areas (Hobley et al. 2017). Huang et al. (2018) observed an increase

from 0.03 to 36.91 g kg⁻¹ in charcoal concentration following burning of a forest. In the current study, the charcoal-C was 5.48 and 4.31 g kg⁻¹, which translated to constitute 12.3 and 7.74 % of the total SOC in soils under pre-harvest burning and green cane, respectively. These differences in charcoal-C could be explained by the higher labile C provided by green cane. Addition of labile organic matter increase the activities of microorganisms due to increase substrate availability hence increasing decomposition of resident organic matter, including the charcoal (Li et al. 2017; Zhang et al. 2019). The concentration of charcoal-C also translated to 16.3 and 10.0% of the carbon in the particulate organic matter (POM-C) fraction, suggesting that 83.7 and 90% of the POM-C in the soil under pre-harvest burning and green cane, respectively, were not in the form of recalcitrant charcoal-C. The higher litter addition increase C, particularly in no-charcoal form, including as POM-C and mineral- associated C. The non-charcoal-C in POM form could therefore have been stabilised by protection in aggregates and limited microbial activity under the acidic conditions of the humic soils.

6.4.2 Effects of land use on Al/Fe-bound C and Alp- and Fep-organic complexes

The higher OC bound to Fe/Al in soils under sugarcane from Wartburg and Eshowe than Eston could be explained by the high level of acidity in these soils (pH < 5.00), which limits microbial activities and the high concentrations of Mehlich 3 extractable Al and Fe which could bind with C in acidic humic soils (Malepfane et al. 2022). The OC bound to Al/Fe was > 40% of the total soil C in all the soil, which was similar to Zhao et al. (2016), who observed that on average, Fe-bound C only contributed for 37.8% to the total C in soil. However, this fraction of C was not correlated with Alp and Fep, which were higher under forest than under sugarcane only in the soils from Eshowe. The high Al/Fe bound C in sugarcane than forest in Eshowe could be explained by the high acid saturation under sugarcane compared to forest site. The higher concentrations of Alp and Fep at Eshowe than the other sites, particularly under forest, could be a result of higher oxides of Al and Fe due to the higher rainfall at this site than the other two. In addition, this could be to the contribution of soil pH to the formation of organo-mineral complexes (Shen et al. 2018; Tamrat et al. 2019). Low pH results in the high soluble Al and Fe in the soil environment, and in the presence of high SOC, it promotes the formation of organo-mineral complexes (Che et al. 2021). The Alp and Fep are poorly crystal minerals and have high surface area which affects C in soils (Fang et al. 2019). Verde et al. (2005) in Andisols showed significantly higher concentration of Alp in uncultivated forestry (10.2 g kg⁻¹) compared to cultivated agricultural areas (4.9 g kg⁻¹), which was >100% higher. Tilling during

sugarcane cultivation results in changes in soil conditions, including aeration resulting to increase in decomposition of protected soil organic matter, including in organo-mineral complexes over time (Veloso et al. 2019). The metal/ C_p ratio of these soils was > 0.12, which is the critical value for formation of allophane or SOM complexes (Panichini et al. 2012). For green cane relative to pre-harvest burning, Al/Fe bound C was not affected by burning, but decreased with increase in soil depths, following the same trends as for Alp and Fep. This similarity in the trends shows that green cane does not increase the C in the Al/Fe-organic matter complexes compared to pre-harvest burning. Based on this, green cane increases the non-charcoal POM-C fraction but not the C in Al/Fe-organic matter complexes and charcoal-C (lower under green cane). The high extractable Al and Fe concentrations in these soils (Malepfane et al. 2022) could have been extracted with sodium pyrophosphate resulting to higher concentration of Al and Fe masking the differences within managements. The differences in Al/Fe bound C in green cane relative to pre-harvest burning could be because of the method where the C concentration extracted was not that only bound to Al and Fe, but also from other SOC fractions.

6.5 Conclusion

Charcoal-C forms a low proportion of total OC in both cultivated and uncultivated humic soils, and the concentration is reduced by sugarcane production compared to forest in some sites, while pre-harvest burning increases the proportion of charcoal-C than green cane. Non-charcoal-C is the dominant portion of POM-C, is also reduced by pre-harvest burning relative to green cane and it generally occurs at higher concentration than the OC bound in Al/Fe-organic matter complexes in humic soils. The effects of conversion of natural forest to sugarcane production on Al/Fe bound C in humic soils vary with sites, with no effects on some sites and a decrease on others. The high C in humic soils is stabilised by formation of Fe/Al-organic matter complexes, which protect the OC. These findings imply that while charcoal-C is an important mechanism, the non-charcoal portion of POM-C and Fe/Al-organic matter complexes are more important fractions of the high OC in humic soils, and that both the charcoal- and non-charcoal-C portions are reduced by cultivation, depending on soil properties and site. Also, OC bound to Al/Fe-organic matter complexes are not affected by land use except for Eshowe. There is a need to understand the effects of the high concentrations of labile non-charcoal C, the higher SOC in green cane relative to burnt cane, and the high Al and Fe complexed with OC under acidic conditions on P availability particularly in humic soils.

CHAPTER 7

Green cane harvesting effects on fractions and availability of phosphorus and related enzymes relative to pre-harvest burning on a sandy loamy humic soil

A version of this chapter is under review for publication in **Biology and Fertility of Soils**

Abstract

Purpose The dominant organic carbon (OC) in humic soil is in the form of non-charcoal particulate organic matter, while significant amounts are bound in organo-mineral complexes. The effects of production system of sugarcane grown on these soils on soil phosphorus (P) fractions and activity of enzymes associated with P cycling need to be clearly understood. The objective of the current study was to determine the effects of green cane, relative to burnt cane, on soil P fractions, microbial biomass P (MBP), and activity of acid and alkaline phospho-monoesterase enzymes in sandy loam humic soils.

Methods The soil samples were collected to a depth 1 m. The soils were analysed for extractable P, P fractions, acid and alkaline phospho-monoesterase and MBP. Extractable P was determined using the Ambic-2 method. The acid and alkaline phospho-monoesterase enzyme activities were assayed using p-nitrophenyl-phosphate as a substrate. Soil microbial biomass phosphorus was determined using fumigation extraction method.

Results and discussion Green cane reduced pH and increased P availability, P precipitation with Al (Al-P) and Fe (Fe-P) and fixation on their oxides (CDB-P), when compared to burnt cane. The Al-P and CDB-P fractions were 38 and 41% higher, respectively, under green cane than burnt cane. Pre-harvest burning reduced Ca-P fraction when compared to green cane. The Fe-P and Ca-P decreased with depth in the top 30 cm with no changes at deeper layers. Green cane resulted significantly (>6 times) higher MBP, in the top 20 cm, activity of acid phospho-monoesterase by 82% in the top 10 cm, and lower alkaline phospho-monoesterase by 37%, when compared to burnt cane.

Conclusions These findings imply that green cane increases extractable P, P fixed, MBP and acid phospho-monoesterase activity in sandy loam humic soils.

Keywords: · Acid phospho-monoesterase · Alkaline phospho-monoesterase · Microbial biomass · Phosphorus fractions

7.1 Introduction

Phosphorus (P) is one of the essential nutrients required in large quantities for sugarcane production (Turrion et al. 2018) but its availability is limited due to precipitation with aluminium (Al) and iron (Fe) and fixation on oxide surfaces in acidic soils (Gonzalez-Rodriguez and Fernandez-Marcos 2018; Johan et al. 2021; Vermeiren et al. 2022). Phosphorus deficiency limits crop productivity on more than 40% of the world soils (Balemi and Negisho 2012). Scientific evidence shows that only 20-30% of the applied phosphate fertiliser is readily available for plant uptake (Oliveira Filho et al. 2017b). Sugarcane is considered as one of the most important crops as it is produced by more than 70 countries, globally (Val-Moraes et al. 2016), and where it is produced under dryland conditions, the soils are usually acidic. Understanding the distribution of P fractions in highly acidic soils is essential for better P fertiliser management strategies in sugarcane production systems (Oliveira Filho et al. 2021). The acidic humic soils are among the most important soils in the sugarcane industry of South Africa, and the common practice of pre-harvest burning of sugarcane may significantly affect soil P cycling and availability in soil environments (Santin et al. 2018).

The widely practised pre-harvest burning results in excessive removal of straw, with its constituent nutrients including P, these nutrient are lost through erosion of the ash from the soil surface (Lopez-Hernandez and Sequera 2012), and results in significant loss of SOC and soil biodiversity, causing poor nutrient cycling (Karlsson et al. 2017; Menandro et al. 2019; Tenelli et al. 2021). Under green cane harvesting the green tops of the sugarcane is cut at a lower level, which results in removal of the lower biomass as cane for the sugar mill than burnt cane. With pre-harvest burning the whole stalk is removed with the nutrient contained in the stalk. However, the carbonates and oxides produced as part of ash from pre-harvest burning of cane could increase soil pH, possibly decrease soil P fixation and increasing availability of the nutrient (Ballard 2000; Trujillo-Narcia et al. 2019). Trujillo-Narcia et al. (2019) observed an increase in soil pH following burning of sugarcane straw. The overall effect of the increase in soil pH coupled with high nutrient removal on concentrations of available P is not clearly understood, when compared with green cane production. Globally, some farmers are converting to green cane production with the aim of increasing soil carbon storage (SOC) and retention of nutrients with the residues (Cardoso et al. 2019; Ferreira et al. 2016; Trujillo-Narcia et al. 2019), with potential effects on P availability. The high organic matter in humic soils also contains a significant portion of organic P (Khan et al. 2016), which could be mineralised and become more readily available. Effects of green cane on soil P fractions,

microbial biomass and activity of enzymes associated with P cycling in humic soils are not clearly understood.

Most agricultural soils have high P stocks, but only 1% of it dissolves to available P (Damon et al. 2014; Yan et al. 2017; Zhu et al. 2018) with the rest remaining in organic form or being easily removed from the soil solution through precipitation, sorption, and microbial immobilisation (Barea and Richardson 2015; Zhu et al. 2018; Jilani et al. 2021). Phosphatase enzymes play a significant role in soil P cycle by speeding up mineralisation of soil organic P into inorganic P contributing to increase in P availability for crop productions (Bajouco et al. 2020). The change in management from pre-harvest burning to green cane could affect activity of microorganisms and associated enzymes that govern P dynamics, especially the conversion from organic forms to inorganic forms (Sahu et al. 2017; Prasad et al. 2021). The abundance of the enzymes will depend on soil conditions including soil organic matter, pH, exchangeable acidity, and total P available (DeForest et al. 2012; Ai et al. 2015). The application of lime is commonly used to ameliorate the effects of soil acidity, raises soil pH and increases enzymes activity in soils. In addition, the surface temperature of between 160 to 200°C during burning, oxidises significant amount of organic matter, causing loss of substrate (Val-Moraes et al. 2016). The addition of fresh substrates, which is used as energy source, during green cane production, could result in increase in enzymatic activities and increase microbial biomass phosphorus (MBP), which could limit P fixation, and increase P availability in soil environment (Graham and Haynes 2005; Vuyyuru et al. 2020). The temporarily immobilised P could be released back to the soil system reducing the contact time between the P and soil constituents responsible for its fixation. The contribution of MBP on P fertility needs to be understood for better fertiliser management in humic soils.

Large areas on sugarcane production in South Africa are on the acidic humic soils. The results in Chapter 6, showed high concentrations of non-charcoal POM-C, even under burnt cane, suggesting that the decomposition of this fraction and mineralisation could result in high available P. In addition, the high concentration of OC associated with Fe/Al-organic matter complexes (Chapter 6) could limit precipitation fixation of P through precipitation with Fe and Al. The higher SOC (Chapter 4), POM-C (Chapter 5), especially the non-charcoal fraction (Chapter 6) and MBC (Chapter 4) under green cane compared to pre-harvest burning on humic soils suggests that green cane may increase P availability and microbial activity in these soils. The decomposition of the organic matter fractions increases mineral P, which can remain available, and taken up microorganisms, forming microbial biomass P or is fixed by Fe and Al.

The higher microbial biomass and the associated MBP could aid in decreasing P fixation and increasing P availability to crops in these soils.

The cycling of organic P through microbial action is facilitated by enzymes which are secreted by soil organisms and plant roots (Wade et al. 2021). However, the results in Chapter 4 also showed that green cane lower soil pH, with potentially higher concentration of soil solution Al and Fe, which may result in the formation of Al-P and Fe-P decreasing P availability, while the slight increase in soil pH from ash under pre-harvest burning could decrease in P fixation by Al and Fe oxides. A study conducted in South Africa under sugarcane showed that phosphatase enzymes activities (acid and alkaline phosphor-monoesterase) was higher under green cane where trash was retained compared to where trashed was removed (Graham and Haynes 2005). However, no studies have been reported on the effects of sugarcane harvest system in humic soils on soil P fractions, MBP and activities of phospho-monoesterase enzyme, which are essential in soil P cycling in soil environments, in humic soils. We hypothesises that green cane could increase (i) available P and affect other fractions (ii) microbial biomass P and (iii) activity of phospho-monoesterase enzymes compared to pre-harvest burning of cane. Therefore, the objective of the current study was to determine the effects of green cane relative to pre-harvest burning on soil P fractions, microbial biomass P, acid and alkaline phospho-monoesterase.

7.2 Methods and material

7.2.1 Study sites (site and characteristics)

The soils used in this study are the same as those in Chapters 4. The site characteristics, soil sampling and analysis are as described in Sections 4.2.1, 4.2.2 and 4.2.3. The SOC was determined as described in Section 4.2.4 of chapter 4. The soil chemical properties and SOC content were detailed in table 4.1 and figure 4.2 in Chapter 4.

7.2.2 Soil P fractionation

Soil P fractionation was analysed as described by Zhang (2009) for non-calcareous soils, for samples from the whole soil profile (100 cm). The fractionations were done on 1.0 g soil sample firstly using 50 ml 1M ammonium chloride (1M NH_4Cl) for soluble P. The soil suspension was shaken for 30 minutes followed by centrifugation 4000 rpm then filtered using Whatman No.1 filter papers. Loosely bound P (Al bound P) was extracted from the residue using 50 ml of 0.5M ammonium fluoride (0.5M NH_4F), with shaking for one hour, followed by centrifugation

and filtration. After washing the soil residue twice with 25 ml saturated NaCl and centrifuged, Fe bound phosphate was extracted using 50 ml of 0.1M sodium hydroxide (0.1M NaOH), with shaking for 17 hours, followed by centrifugation at 4000 rpm and filtration using Whatman No.1 filter paper. The soil residue was washed twice with 25 ml NaCl solution before adding 40 ml of 0.3M sodium citrate dihydrate (0.3M Na₃C₃H₆O₇) and 5 ml of 1M sodium bicarbonate (1M NaHCO₃) and heated for 15 min in a water bath at 85°C. Thereafter, 1 g of sodium dithionite (Na₂S₂O₄) was added followed by rapid stirring. The suspension was heated again for 15 minutes and then filtered using Whatman No.1 filter paper, and the soil residue was washed twice using 25 ml NaCl solution, combining the washings with the CDB extracts (CDB-P). Calcium bound P (Ca-P) was determined using 0.25 M sulphuric acid (0.25 M H₂SO₄) before being shaken for one hour and centrifuged, and the residue was washed with NaCl solution. The P concentrations in various solutions were determined using phosphomolybdate method (Murphy and Riley 1962).

7.2.3 Soil microbial biomass P

Soil microbial biomass P (MBP) was determined as described by Brookes et al. (1984), only for samples collected from the top 40 cm. Briefly, moist sample (10 g) was fumigated in a vacuum desiccator with ethanol-free chloroform in a constant temperature room 25°C. Boiling chips were added to the chloroform followed by evacuation with vacuum pump for 5 minutes until the chloroform boiled, after which the tap was closed and incubated for 72 hrs. The same was done for the unfumigated samples without chloroform. After evacuation of the chloroform, soil MBP was extracted from both fumigated and unfumigated samples using 0.5M NaHCO₃ (pH = 8.5) by shaking for 30 minutes using a reciprocal shaker followed by filtering through the Whatman No.1 filter paper. The inorganic P in the extracts for both fumigated and unfumigated samples was then determined by the spectrophotometry at 882 nm wavelength using the ascorbic acid-molybdate method (Murphy and Riley 1962). The MBP concentration was then calculated with a K_{EP} conversion factor of 0.4, as described by Brookes et al. (1984). This was done without spiked P.

7.2.4 Acid and alkaline phospho-monoesterase assay

The acid and alkaline phospho-monoesterase enzyme activities were assayed as determined using p-nitrophenyl-phosphate as a substrate (Eivazi and Tabatabai 1977), only for samples collected from the top 40 cm. For this, a field moist soil sample was incubated at 37°C for 1

hour in 4 mL modified universal buffer (pH 6.5 and 11 for acid and alkaline phospho-monoesterase, respectively) and 1 mL of p-nitrophenyl-phosphate substrate prepared in the same buffer. After 1 hour, the reaction was stopped by addition of 1 mL 0.5M CaCl₂ and 4 ml of 0.5M NaOH. The flask was then swirled for few seconds the soil suspension was then filtered through Whatman. No. 1 filter paper. The intensity of the yellow colour was measured using the UV/VIS spectrophotometer at 410nm wavelength. The same procedure was done for the control, but the addition of the substrate was done after incubation and addition of CaCl₂ and NaOH followed by filtering. Thereafter, the concentration of phospho-monoesterase was calculated as the difference between the samples and the controls and expressed as µg p-nitrophenyl released g⁻¹ moist soil h⁻¹.

7.2.5 Statistical analysis

The data of P fractions (and available P), soil pH, MBP and phospho-monoesterase enzymes (acidic and alkaline) in the humic soils from different green cane relative to pre-harvest burning were subjected to two-way analysis of variance (ANOVA) using GenStat Ed. 18. The least significant difference (LSD) was used to compare treatments mean at 5% level. The Tukey-Kramer test was also applied to separate treatments means at $p < 0.05$. Correlation analysis was done using the Pearson's Correlation in IBM SPSS Statistics 27. In addition, to identify the effects of green cane and burning on different soil parameters, a Principal Component Analysis (PCA) was applied separately using R Statistical software 2018.

7.3 Results

7.3.1 Available phosphorus

The soil under green cane had significantly higher extractable (available) P concentration (28.1% higher) when compared to pre-harvest burning. The overall effects showed that extractable P concentrations significantly decreased with depth and ranged from 9.01 mg kg⁻¹ in the 80-100 cm to 13.6 mg kg⁻¹ in the 5-10 cm depth (Table 7.1).

Table 7. 1 Extractable P (mg kg⁻¹) in green cane retention relative to burnt cane.

Factor	Extractable P
Burning	
Green cane	7.93b
Pre-harvest burning	6.19a
LSD	0.834
Depth (cm)	
0-5	13.3d
5-10	13.6cd
10-20	12.6bc
20-30	10.6ab
30-40	9.44a
40-50	9.01a
50-60	9.93ab
60-80	8.90a
80-100	9.01a
LSD	1.77

Different lower-case letters indicate significant differences ($p < 0.05$) among treatments.

7.3.2 Soil P Fractions

The overall effects showed that soils under green cane burning had significantly higher soluble P (104% higher), Ca-P and CDB-P (41% higher) when compared to pre-harvest burning management (Table 7.2). Soil depth did not show any significant effects on soluble P and CDB-P but Ca-P decreased with increasing depth, with high concentrations in the top 30 cm depth and ranged from 142 mg kg⁻¹ in the top 5 cm to 82.2 mg P kg⁻¹ in the 40-50 cm (Table 7.2). Overall, green cane (149 mg kg⁻¹) resulted in higher Al-P concentration (higher fixation by Al) when compared to burning (108 mg kg⁻¹); a 38% reduction due to pre-harvest burning, with the significant differences being in the top 20 cm. Under green cane, the top 20 cm depth had significantly higher Al-P than depths below 30 cm, with concentration ranging from 118 mg kg⁻¹ in the 80-100 cm to 216 mg kg⁻¹ in the top 5 cm depth, while under pre-harvest burning the concentrations were not different between the depths (Table 7.3). There were no significant differences between green cane and pre-harvest burning in terms of Fe-P fraction, but the concentration significantly decreased with soil depth ranging from 343 mg kg⁻¹ in the 0-5 cm to 63.8 mg kg⁻¹ in the 80-100 cm depth (Table 7.2).

Table 7. 2 Soil phosphorus fractions (mg kg⁻¹)

Factor	Soluble-P	Fe-P	CDB-P	Ca-P
Burning				
Green cane	30.1b	167	251b	111b
Pre-harvest burning	14.7a	157	178a	103a
LSD	1.23	21.7	47.2	6.30
Depth (cm)				
0-5	22.8	343d	234	142d
5-10	22.6	282cd	220	135cd
10-20	22.5	247bc	210	129cd
20-30	22.9	187b	260	115bc
30-40	21.7	104a	159	91.1a
40-50	22.5	89.3a	208	86.4a
50-60	22.2	75.8a	189	84.8a
60-80	22.2	66.8a	228	84.2a
80-100	22.2	63.8a	220	96.2a
LSD	2.61	45.9	100	13.3

Different lower-case letters indicate significant differences ($p < 0.05$) among treatments. No letters in mean of a parameter indicate no significant differences.

Table 7. 3 Effects of burning and depth on Al-P (mg kg⁻¹)

Depth (cm)	Green cane	Pre-harvest burning
0-5	216e	128ab
5-10	195de	124ab
10-20	169cd	123ab
20-30	151bc	115ab
30-40	127ab	99.6a
40-50	121ab	97.1a
50-60	123ab	95.1a
60-80	121ab	94.1a
80-100	118ab	92.5a
LSD		20.8

Different lower-case letters indicate significant differences ($p < 0.05$) among treatments. Al-P represents a fraction of phosphorus associated with aluminium.

7.3.3 Microbial biomass P and activity of acid and alkaline phospho-monoesterase

The overall effect of management on MBP was highly significant with green cane (5.80 g kg^{-1}) had 6 times more MBP than pre-harvest burning (0.95 g kg^{-1}). The MBP was significantly higher under green cane (>100% higher) than under pre-harvest burning only in the top 20 cm depth (Figure 7.1) and the concentration ranged from 0.061 g kg^{-1} in the 40-50 (deepest measured) to 3.03 g kg^{-1} in the top 5 cm under burnt cane, and from below detection in the 40-50 cm to 14.2 g kg^{-1} in the top 5 cm depth under green cane.

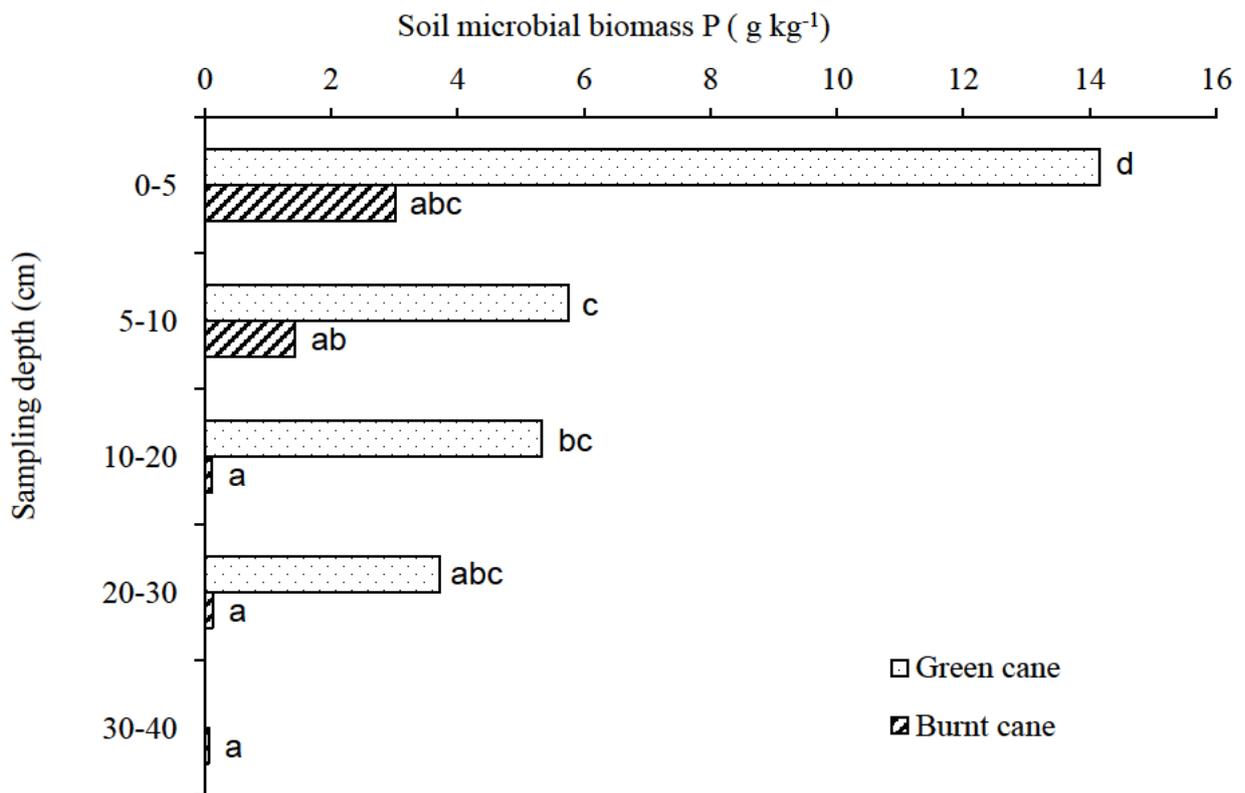


Figure 7. 1 Effects of green cane relative to pre-harvest burning and depths on soil microbial biomass phosphorus (LSD = 2.35).

Similar to MBP, overall, the acid phospho-monoesterase activity was higher (82% higher) under green cane (5.67 mg p-nitrophenyl g⁻¹ moist soil h⁻¹) compared to pre-harvest burning (3.11 mg p-nitrophenyl g⁻¹ moist soil h⁻¹), with the treatment differences being significant only in the top 10 cm depth (Figure 7.2). The activity of acid phospho-monoesterase enzyme decreased with increase in soil depths (Figure 7.2). Overall lower (37% lower) alkaline phospho-monoesterase activity was observed under green cane (479 μg p-nitrophenyl g⁻¹ moist soil h⁻¹) than pre-harvest burning (656 μg p-nitrophenyl g⁻¹ moist soil h⁻¹) (Figure 7.3a). Overall alkaline phospho-monoesterase enzyme activity significantly declined with soil depth (Figure 7.3b).

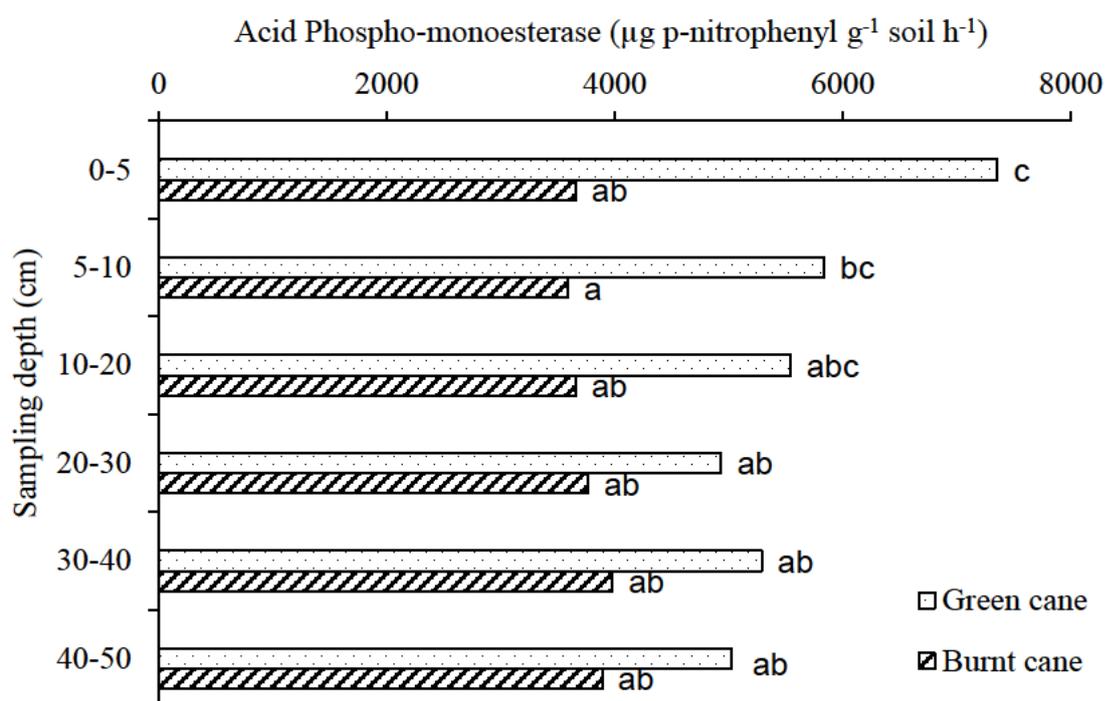


Figure 7. 2 Effects of green cane relative to pre-harvest burning and sampling depth on acid phospho-monoesterase enzymes (LSD = 938.3).

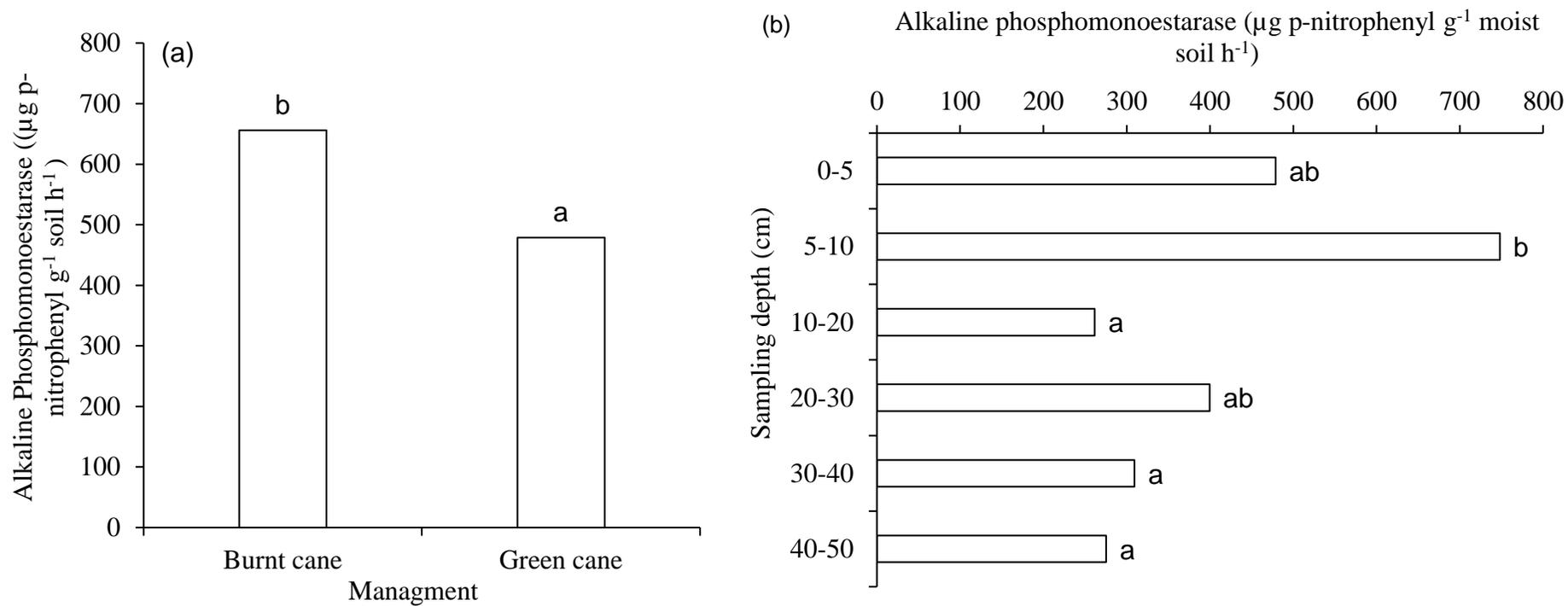


Figure 7. 3 Effects of burning and sampling depth on alkaline phospho-monoesterase enzymes. Figures a (LSD = 149.4) and b (LSD = 258.7) represent the cane management and depth effects, respectively.

7.3.4 Relationship between soil parameters and phosphorus fractions

7.3.4.1 Correlation analysis

Soil pH was negatively correlated with all parameters, including all P fractions and MBP, except CDB-P and exchangeable K (Table 7.4). Extractable P was positively correlated to all other P fractions, MBP, SOC, acid phospho-monoesterase activity, clay and all the exchangeable bases. Soluble P was positively to extractable P, Al-P, CDB-P, and SOC, acid phospho-monoesterase activity and negatively correlated to soil pH. The Al-P and Fe-P fractions were positively correlated with each other and with SOC, Ca-P, clay, extractable P and all the exchangeable bases (Table 7.4). Ca-P was positively correlated to extractable P, SOC, clay and all the exchangeable bases. The MBP and acid phospho-monoesterase were positively correlated with each other and with all other parameters, including all P fractions, and alkaline phospho-monoesterase, except CDB-P (Table 7.4). Alkaline phospho-monoesterase was positively correlated with all parameters, including all P fractions and MBP, except extractable P, soluble P and CDB-P (Table 7.4).

Table 7. 4 Pearson correlations between soil parameters and microbial response

	Acid _{phosp}	Alkal _{phosp}	MBP	Soluble P	Al-P	Fe-P	Ca-P	CDB-P	Soil pH	Extrac. P	SOC	Clay	Ca	K	Mg
Acid _{phosp}	1.000														
Alkal _{phosp}	0.508**	1.000													
MBP	0.587**	0.419**	1.000												
Soluble P	0.370**	-0.111	0.306**	1.000											
Al-P	0.667**	0.452**	0.770**	0.534**	1.000										
Fe-P	0.643**	0.614**	0.692**	0.007	0.768**	1.000									
Ca-P	0.638**	0.464**	0.540**	0.194	0.712**	0.825**	1.000								
CDB-P	0.142	0.084	0.119	0.438**	0.187	0.067	0.194	1.000							
Soil pH	-0.500**	-0.227*	-0.248*	-0.593**	-0.556**	-0.352**	-0.406**	-0.114	1.000						
Extrac. P	0.581**	0.159	0.607**	0.828**	0.796**	0.408**	0.517**	0.368**	-0.638**	1.000					
SOC	0.833**	0.570**	0.525**	0.301**	0.627**	0.651**	0.580**	0.122	-0.542**	0.506**	1.000				
Clay	0.499**	0.498**	0.393**	-0.005	0.521**	0.743**	0.684**	0.043	-0.262*	0.288**	0.526**	1.000			
Ca	0.425**	0.390**	0.451**	0.086	0.550**	0.645**	0.526**	0.067	-0.228*	0.280**	0.409**	0.778**	1.000		
K	0.398**	0.337**	0.554**	0.079	0.537**	0.603**	0.437**	-0.007	-0.167	0.326**	0.400**	0.405**	0.403**	1.000	
Mg	0.396**	0.342**	0.470**	0.109	0.604**	0.658**	0.517**	-0.045	-0.338**	0.357**	0.499**	0.481**	0.386**	0.559**	1.000

* Indicates significant different at $p < 0.05$; ** indicates significant different at $p < 0.01$. Acid-phosp represents acid phospho-monoesterase activity; Alkal-phosp represents alkaline phospho-monoesterase activity.

7.3.4.2 Principal Component Analysis

The first two axes of the PCA of the different sugarcane harvest systems explained 64.2% of the variation (Figure 7.4). The first axes (PC1) explained 49.0% while the second axes (PC2) explained 15.2% of the variation. The first axes (PC1) were positively related to clay, Al-P, Fe-P, Ca-P, soluble P, SOC, MBP, all exchangeable bases, acid and alkaline phospho-monoesterase activity. For the second axes (PC2) was only positively correlated to soil pH. Burning was characterised by high soil pH, calcium, clay and alkaline phospho-monoesterase enzyme (Figure 7.4). Green cane was characterised by higher SOC, extractable P, soluble P, Al-P, CDB-P, MBP, exchangeable bases, and acid phospho-monoesterase (Figure 7.4). Soil pH had negative relationship with extractable P, soluble P, Al-P, and CDB-P.

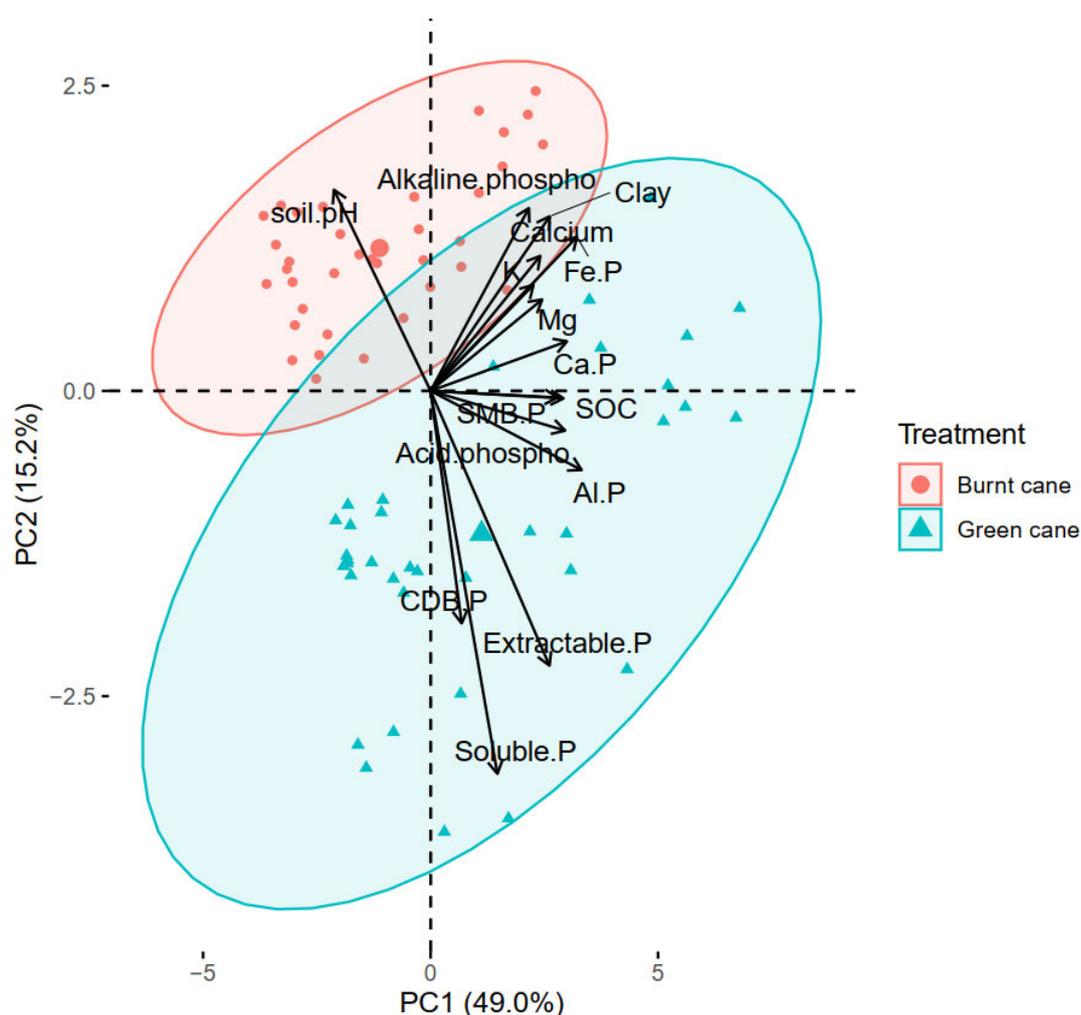


Figure 7. 4 The first two axes of Principle Component Analysis are based on the effect of sugarcane harvest system on different soil properties. The axes explained 64.2% variation using PC1 and PC2. Groups represents the different sugarcane harvest systems.

7.4 Discussion

7.4.1 Soil phosphorus fractions

The generally low Ambic-2 extractable P in all the treatment was due to high soil acidity associated with low soil pH ($\text{pH} < 5$), which is associated with high P fixation. However, the higher extractable P in green cane compared to pre-harvest burning shows that availability of P under the green cane management could meet P recommendations for sugarcane. For burnt cane, it was surprising that, while P fixation decreased, the available P would not meet the recommended P, which was 35 kg ha^{-1} , and there would be a need for external addition of P to maintain optimum yields. In addition, the soils used in this study have high P fixation capacity and acidity (Fey 2010), which is confirmed by the high levels of Al-P, Fe-P and CDB-P, irrespective of the cane production system, in this study. The higher concentration of all P fractions under green cane, indicates that total P was higher, and could be a result of higher biomass addition under green cane (Maltais-Landry and Frossard 2015; Soltangheisi et al. 2021), as opposed to removal, with the nutrients (e.g., P), resulting of higher labile soil organic matter (SOC) fractions than burnt cane. During green can harvesting cutting of the green tops of the cane is done lower, leaving more biomass on the land resulting in more nutrients retained, compared to pre-harvest burning which results in greater nutrient removal (Robertson and Thorburn 2007). The decomposition of the residues and mineralisation increases P availability. In addition, increases the soil pH due to ash addition under pre-harvest burning (Pereira et al. 2017; Fernandez-Garcia et al. 2019) could also have contributed to the lower P fixation as Al-P and on CDB-P under pre-harvest burning than green cane management. This view was supported by the higher pH under pre-harvest burning ($\text{pH } 4.29$) than under green cane ($\text{pH } 3.79$) (Table 7.1). While the pH difference is 0.8pH units, the one under green cane harvesting is below $\text{pH } 4-4.3$ (equivalent to $\text{pH } 5-5.3$ in water), which is the critical value for solubility of Al oxides), resulting in higher soluble Al which then precipitate with P. Burning produces ash with carbonates and their reaction with water will release CO_2 and OH^- , which neutralises acidity through reaction with H^+ and solution Al and Fe (lowering Al-P and Fe-P) and reduces pH-dependent positive charge of the oxides (lowering CDB-P). Similar results were reported in forestry soils by Giardina and Rhoades (2001), where burnt forest resulted in lower (8.89% lower) Fe-P fixation than when not burnt, although the effect of burning on Fe-P was not significant in this study. The decrease in Fe-P fractions from 35.4 mg kg^{-1} to 4.3 mg kg^{-1} with increase in soil depth was also reported by Resende et al. (2011).

The higher soil pH under pre-harvest burning as a result of ash addition could result in the dissolution of occluded P and desorption of fixed P into bioavailable forms (Santin et al. 2018). However, higher Ambic-2 extractable P, soluble P and Al-P fractions were observed under green cane, where pH was lower, than burnt cane, possibly as a result of higher P removals with the cane under burnt cane. This is supported by the positive correlation of available P with SOC, MBC and MBP. While burning of soil organic matter increases P availability (Schaller et al. 2015), through mineralisation of organic P, which is released into soil solution as available P (Schaller et al. 2015; Fernandez-Garcia et al. 2019), soluble P and Ambic 2 extractable P were lower under pre-harvest burning than green cane system. In addition to the higher nutrient removal of P as a constituent of biomass under burnt cane, another contribution to the lower concentrations of P fractions, including available P, under pre-harvest burning could be due to loss of the ash, with the mineral P, due to wind and water erosion (Resende et al. 2011; Santin et al. 2018).

Leaving residues on the soil surface has been reported to reduce the soil surface temperature by 2°C (Moitinho et al. 2013), which could reduce the losses of P from decomposition and erosion, resulting in an increase in availability of P in green cane more than burnt cane. The decrease in P fixation with an increase in soil depth could be explained by lower solution Al and lower positive charges on oxide surfaces due to the increase in soil pH. The decrease in extractable P with depths could be explained by the decrease in SOC and MBP biomass due to a decrease in fresh biomass addition and organic carbon. The positive correlation between extractable P and SOC suggested that P availability is increased by increase in concentration of SOC.

The higher SOC, with its constituent organic P, provided a substrate for microbial biomass, resulting in mineralisation of P and increased MBP, which were observed under green cane than burnt cane. The amount of straw retained on the surface has a significant effect on P dynamics (Soltangheisi et al. 2021). The addition of residues following the green cane harvest system is shown to increase labile organic matter on the surface (Panosso et al. 2011; Torres et al. 2013; Vuyyuru et al. 2020), which increases microbial biomass, as in this study, and is essential for P cycling (Khan et al. 2016). The MBP was positively correlated to SOC ($r = 0.525$; $p < 0.01$) indicating the importance of soil organic matter as a source of P for microorganisms. The MBP observed under green cane (22.8 mg kg⁻¹) was >93% higher than under pre-harvest burning (11.8 mg kg⁻¹), in agreement with Vuyyuru et al. (2020), and may be working as a temporal store of P thereby reducing the potential for its fixation and making

it more available. The positive correlation between MBP and acid phospho-monoesterase enzyme activity, emphasizes the importance of this enzyme in the cycling of P by microorganisms in these humic soils. Like SOC, the microbial biomass (MBP) and the associated enzymes responsible for P cycling decrease with an increase in depth, emphasizing the importance of SOM as a substrate of microorganisms and associated enzymes responsible for P cycling. While roots of the sugarcane crop and microorganisms can produce the acid phospho-monoesterase enzyme, the strong relationship of the activity of this enzyme with MBP, indicates that the microorganisms took up significant portions of the P mineralised by this enzyme from organic matter (Tian et al. 2020).

The higher activity of acid phospho-monoesterase, in green cane management suggested that P cycling in these sugarcane production systems is controlled by this enzyme and less so by alkaline phospho-monoesterase activity. The dependence of microorganism on acid phosphatase could be explained by the lower pH and higher level of exchangeable acidity, under green cane than under burnt cane. Graham and Haynes (2005) also reported higher acid phospho-monoesterase activity under green cane, where residues were retained, compared to pre-harvest burning. This enzyme is highly sensitive to soil pH and increases in soil pH would decrease its activity (Pereira et al. 2017; Wade et al. 2021), as shown under burnt can in this study, where ash produced increases pH and increases the activity of the alkaline phospho-monoesterase enzyme. This was supported by the strong negative correlation between acid phospho-monoesterase and soil pH (Figure 7.4). Docherty et al. (2012) and Noyce et al. (2016) reported that the addition of ash to the soil surface decreased activity of this enzyme. Similarly, Trujillo-Narcia et al. (2019) reported that acid phospho-monoesterase activity decreased from the first to second burning, from 5289 to 5515 $\mu\text{g p-nitrophenyl g}^{-1}$ moist soil h^{-1} . Ash addition is reported to increase soil pH due to an increase in carbonates additions, and ecosystems fires showed a decrease in acid phospho-monoesterase with increase in burning severity (Fernandez-Garcia et al. 2019). The higher alkaline phospho-monoesterase in the surface layer is a result of the high substrate concentration and roots (which are sources of enzymes) in the topsoil layers and decrease with an increase in soil depth.

7.5 Conclusion

The results of this study showed that green cane production on sandy loam humic soil results in higher P fractions, including plant available P, MBP and activity of the acid phospho-monoesterase enzyme, when compared with the commonly practiced burnt cane. The high

MBP in green cane could be beneficial to increasing phosphorus cycling in these highly acidic soils, which have high fixation capacity. Green cane production needs to be monitored to understand the seasonal variation of available P and MBP relative to burned cane, which contribute significantly to sustainability of the production system, in humic soils. Further studies should, therefore, focus on the dynamics of P availability, MBP and activity of acid and alkaline phospho-monoesterase enzyme, responsible for P cycling, together with P concentrations in sugarcane tissue, between and within seasons on humic soils.

CHAPTER 8

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

8.1 General discussion

Humic soils are intensely weathered and highly acidic characterised by high SOC content (> 1.8%), having high buffering capacity and good drainage, with low bulk density and base status (Fey 2010; Mkhonza et al. 2020), and they could be a major sink or source of C depending on land use. Soil organic carbon is a soil quality indicator that provides nutrients for crop growth and reduces losses of C as CO₂ emission (Guoju et al. 2020; Wang et al. 2019). The concentration of SOC varies within land use, this could either decrease or increase, especially in the topsoil (Malepfane et al. 2022; Sharma et al. 2020). Cultivation reduces the concentration of overall SOC mainly due to soil structural disturbance (Liu et al. 2006; Sul et al. 2013). Malepfane et al. (2022) reported that humic soils can store up to 400 Mg ha⁻¹ and that cropping did not have a significant effect on the soil OC stocks. The effects of land use on SOC concentration would vary depending on the period of cultivation and management (Wang et al. 2019). A laboratory incubation study by Mkhonza et al. (2020) showed that liming of some humic soils increased the mineralisation of nitrogen and P, with a reduction of P fixation in different fractions. While lime is applied every year for annual crops, the frequency of application for sugarcane in South Africa ranges from once every three years to once every 10 years (Malepfane et al. 2022), which may restrict the SOC lost through decomposition in the long term. Harvesting systems also vary in the sugarcane industry of South Africa, with some farmers practising pre-harvest burning while a smaller proportion practice green cane production. Retention of residues under green cane could have a significant effect on the concentration of SOC, microbial biomass, and enzymes activities in soils (Chalise et al. 2019; Novara et al. 2019), while burning could increase the incorporation of the highly stable charcoal C in soils (Bryanin et al. 2018; Carter et al. 2018; Jha et al. 2020; Ping et al. 2022; Su et al. 2022). The effects of land use on SOC and its fractions indirectly affect microbial biomass and enzyme activities (Min et al. 2022; Zhang et al. 2022). Soil enzyme activities are sensitive to environmental changes and are positively correlated to SOC (Xu et al. 2020). The effects of sugarcane harvest system on SOC, phosphorus and associated microbial activity in humic soils are not clearly understood. The overall objective of this study was to determine the effects of

sugarcane harvest system (pre-harvest burning and green cane) on SOC and its fractions, SOC stocks, P fractions and microbial activity in sandy loam humic soils.

The accumulation of SOC over time largely depends on the inputs and outputs (Prommer et al. 2020). The findings of this study showed that green cane production resulted in higher SOC concentrations, which could be explained by the large volume of fresh biomass retained on the surface that incorporated into the soils over time (Shahbaz et al. 2017). Continuously addition of sugarcane straw, with high C: N ratio of 80:1, results in slow decomposition, hence, increasing accumulation of the fresh biomass. Pre-harvest burning decreases the addition of fresh biomass, which is essential for SOC accumulation, resulting in an overall decrease in SOC content (Tenellie et al. 2021). The effects of the higher biomass addition were also shown on soil nutrients, including exchangeable Mg and K, available P, Mn, Zn and Cu, which were also higher under green cane than burnt cane. While Sandhu et al. (2013) reported that the higher nutrients added under green cane will take long to show the benefits through increased availability, the fields in this study were under green cane for >73 years, resulting in greater concentrations in available forms. The SOC stocks (to 1000 mm depth) both under green cane and pre-harvest burning were lower than those observed under sugarcane at Eshowe (Malepfane et al. 2022), a site with closely related climatic (>1000 mm rainfall per year) and soil factors (e.g., sandy loam texture) to those at Stanger. The lower SOC stocks in this study (Stanger) could be caused by warmer mean temperatures (19-29°C) and higher rainfall (1233 mm per year), causing more SOM decomposition than Eshowe with cooler mean temperatures (15.1-26.7°C) and 1113 mm rainfall per year (Malepfane et al. 2022). This was supported by the findings in Chapter 3, which showed that cropping resulted in lower SOC in warmer than cooler climate, due to enhanced decomposition. While Malepfane et al. (2022) reported no differences in SOC stocks between forest and sugarcane, green cane increased the stocks by 21% when compared to pre-harvest burning in this study. The SOC concentration and stocks were higher under green cane at all depths, with the greatest stocks in the depths between 30 and 80 cm, emphasising the contribution of root biomass of sugarcane.

The higher SOC under green cane enhanced soil aggregation while residues protected the soil aggregates from destabilisation (Dharmakeerthi et al. 2015; Gmach et al. 2021; Shahbaz et al. 2017). In this study, the aggregate stability was significantly higher under green cane than burnt cane, in agreement with the results of SOC, especially in the top 10 cm depth. Like SOC, the C in the macro-aggregates and micro-aggregates was also greater under green cane and decreased with an increase in soil depth, emphasising the importance of soil aggregates in

protecting soil organic matter. Further fractionation of the SOC in the macro- and micro-aggregates, after dispersion, also showed that the coarse particulate and fine particulate organic C fractions were higher under green cane while C associated with silt and clay, in all aggregate fractions, were not different between the harvest systems. This highlights that a larger proportion of the high concentration of SOC in humic soils is in particulate form. From the meta-analysis in Chapter 3 and results of Chapter 6, cultivation reduced the concentration of organo-mineral complexes when compared to uncultivated land use, and the high OC, Al and Fe in the Al/Fe-organic matter complexes under forest could be explained by the less soil disturbance and high soil acidity limiting the activities of microorganisms (Fey 2010; Zhang et al. 2020). In addition, this was evidenced by the higher OC, Al and Fe in Al/Fe-OM under acidic soils compared to alkaline conditions. Sugarcane production when compared to forest showed that higher OC bound to Al/Fe-OM complexes at Eshowe, while Alp, Fep and Alp+Fep were lower under sugarcane. These findings suggest that the results of OC bound to Al/Fe-OM complexes may have included soluble SOC, which was not complexed in the Al/Fe-OM complexes. It is well documented that SOC strongly correlates positively with Al and Fe oxides (Feng et al. 2014; Rakhsh et al. 2020; Sarkar et al. 2018; Xue et al. 2019). Therefore, the use of Fep and Alp may be more reliable in indicating the concentrations of Fe/Al-OM complexes in humic soils.

While the harvesting system does not affect the mineral associated C fraction, green cane resulted in higher particulate organic C fractions in the soils, which could be explained by the addition of fresh biomass in green cane resulting in an increase in particulate fraction (Liu et al. 2010). Li et al. (2016) observed more than 50% of the C in the fine particulate fraction was derived from crop residues while Sun et al. (2012) and Liu et al. (2016) reported that crop residue addition could alter the distribution of SOC in soil macroaggregates (>250 μm). Overall, the coarse particulate carbon fraction in the study soils constituted more than 50% of the total SOC concentration, and burning reduced the SOC concentration in this fraction. This could be explained by the effects of burning on soil aggregates, resulting to a decrease in the physical protection of C in coarse aggregates (Campo et al. 2022; Pellegrini et al. 2021). Additionally, this was evidenced by the soil aggregates stability in this study, which showed that burning reduced the aggregates stability, particularly in topsoil layers. The major mechanisms of SOC stabilisation observed in these soils include organo-mineral complexes and charcoal C, which is highly resistance to decomposition (Wen et al. 2019). In addition, the

high proportion of coarse particulate carbon in these soils indicate that the high level of acidity in these soils limit microbial activities (Fey 2010).

The concentration of POM-C in the macro- and micro aggregates was higher in green cane when compared to burnt cane, and decreased with an increase in soil depths. About 11-18% of POM-C was in the form of charcoal C, and this charcoal C fraction formed 8-12% of the overall SOC concentration, which was lower under green cane retention. The higher concentration of charcoal C could be explained by the effects of continuous burning in pre-harvest burning management that increase the incorporation of burnt biomass into the soil. On the other hand, charcoal C is not accessible to microorganisms (Kuzyakov et al. 2009; Lim et al. 2014; Nayak et al. 2014). Alternatively, the lower charcoal POM-C under green cane could also have been a dilution effect due to higher non-charcoal POM-C than burnt cane. In the Eshowe site, the concentration of charcoal-C was not different between sugarcane, where they practice continuous burning, when compared to soils under forest, possibly because the cultivation and modification of soil conditions with liming and fertilisers, could enhance microbial activity and decomposition of SOC including that in charcoal form (Aye et al. 2016; Whitehead et al. 2018). A larger portion of the POM-C fraction was in the form of non-charcoal POM-C (82-89% of POM-C fraction), which was higher under green cane harvest system than burnt cane.

The higher proportion of non-charcoal POM-C, as a proportion of SOC, under green cane provided a labile substrate for microorganisms (Witzgall et al. 2021), and the decomposition of this SOC fraction increases microbial biomass and enzymes activities essential for C and nutrient cycling in soils, including P. The mineralisation of the SOC pool and its fractions is governed by several enzymes that are essential in C cycling such β -D-glucosidase (Durenkamp et al. 2010; Prommer et al. 2020; Zheng et al. 2015; Zhao et al. 2019). Burning reduces the concentration of MBC due to the reduced addition of fresh biomass and reduced overall SOC concentration. The results of MBC responded similarly to the bulk SOC and β -D-glucosidase enzyme activity. The incorporation of residues following burning is reported to decrease the content of SOC in enzymes and MBC (Trujillo-Narcia et al. 2019). Vuyyuru et al. (2020) reported that continuous burning significantly reduced the concentration of MBC and β -D-glucosidase in soils. The concentration of biomass C and β -D-glucosidase activity in soils could be affected by the high organo-mineral complexes (Lim et al. 2014), due to the decrease in accessibility of the SOC to microorganism to mineralise and store some of the C as biomass (Rakhsh et al. 2020). This could explain the low MBC in pre-harvest burning due to less coarse particulate and higher charcoal C concentration, with no effect on mineral-associated C. The

higher MBC and activity of the enzyme responsible for C cycling under green cane could suggest that higher CO₂ could be emitted, although its effects on climate change could be balanced by the higher SOC stocks than under pre-harvest burning harvest system.

The higher concentration of labile form of SOC as non-charcoal POM-C and the increased microbial activity, as shown by the higher MBC and β -D-glucosidase enzyme activity, could result in rapid decomposition (Hartley et al. 2010; Liu et al. 2019), increasing mineralisation of nutrients such as P, increasing their availability. Phosphorus is a major limiting macronutrient, which is required in large quantities by crops (Katsalirou et al. 2016; Sun et al. 2018). It is mostly deficient in acidic soils, that include humic soils (Fey 2010), due to precipitation with Al (Sun et al. 2019). Approximately 30-60% of the total soil P is in organic form, which requires soil phosphatase hydrolysis to be converted to inorganic forms required by crops (Ye et al. 2017). Phospho-monoesterase enzymes facilitates the turnover of organic phosphorus in soils (Zheng et al. 2015; Zhu et al. 2017). The higher acid phospho-monoesterase activity under green cane retention when compared with burnt cane, could be explained by the high level of soil acidity, and suggested that higher P mineralisation occurs in green cane harvest system than burnt cane. This was confirmed by the higher soluble P and extractable P under green cane. The mineral P in these acidic soils is susceptible to fixation on Al/Fe oxides or precipitation as phosphates of Al and Fe. This was supported by the higher Al-P and Fe-P and CDB-C under green cane that had higher non-charcoal POM-C, higher microbial biomass (MBC) and enzyme activities together with lower pH than burnt cane. In addition to potential fixation, some of the mineral P was taken up by micro-organisms, as indicated by the higher MBP under green cane.

The higher fixation of P under green cane could be explained by the higher level of acidity in soil compared to that of burnt cane. Burning reduced P fixation on Al and Fe oxides, which could be explained by the increase in soil pH due to continuous burning resulting to decrease in soil acidity. The higher MBP could also reduce the fixation on oxides surfaces or precipitation with Al and Fe (Khan et al. 2012). In addition, to the effects of aggregates stability, this explains the lower extractable P in pre-harvest burning and possibilities are that a large proportion of the available P was converted to biomass P. The MBP pool is essential P pool, in that this P will be released in available form over time. This MBP will reduce the fixation of P in soils. However, in this study high MBP did not reduce P fixation in green cane when compared with burnt cane, but extractable P was also higher under green cane. The higher extractable P could be due to higher P added as larger biomass addition resulting to saturation

of fixation sites, hence increase extractable P. Higher MBP in green cane retention was similar to the higher MBC and SOC, which are essential in increasing the MBP. In addition to fixation and immobilisation, the mineral P is available for uptake by plants. The higher available P (extractable P) under green cane retention means that the subsequent crop, including as a ratoon crop of sugarcane, could benefit from this residual P. The extractable P under green cane was higher than the threshold for sugarcane, which is 35 mg kg⁻¹, indicating that only little P fertiliser will be required to be added in the soil, for maintenance, while larger amounts are required under burnt cane. The MBP which was also higher under green cane could also contribute to available P during the season as a slow nutrient release source.

8.2 Conclusions

Green cane retention increases the SOC concentration, but not the C stocks, in humic soils when compared to pre-harvest burning. While sugarcane production (with burning) decreases concentration of Al/Fe organo-mineral complexes, when compared to forest, this fraction is not affected by burning when compared to green cane in sandy loam humic soils. Soil aggregates stability (>100%) and particulate SOC in aggregates (20.4%) are increased while charcoal-C (27.1%) is reduced under green cane when compared to burnt cane. Green cane retention increases MBC (>100%), MBP (>100%) and activity of β -D-Glucosidase (54.8%) and acid phospho-monoesterase (82%) enzymes, reduces the activity of alkaline phospho-monoesterase (37%) when compared to pre-harvest burning on sandy loam humic soils. Also, when compared to burnt cane, green cane results in higher concentrations of P fixed in the different fractions (7.77 to 41%) as well as available P (28.1%). The high SOC concentration in green cane harvest systems increased the SOC stored and makes P more available and reduces the fertiliser P required, which are expensive compared to burnt cane, in the sandy loam humic soils. These findings could make a significant contribution in making an informed decision in sugarcane production systems, in view of climate change.

8.3 Recommendations for future research

In view of climate change, green cane harvest system is a better system than pre-harvest burning in terms of SOC storage, which would reduce losses of C as CO₂ emissions, and phosphorus availability in humic soils, which naturally have high SOC stored. The soils under green cane harvest system is recommended, to improve coarse particulate carbon fraction, especially the labile non-charcoal C. Future studies should focus on changes in SOC, MBC,

MBP, soil enzymes activities, soil moisture and P uptake during the growing season to observe the benefits during the growth stages of the crop in these humic soils. In addition, future work should also examine greenhouse gas emissions, microbial population and diversity and yield and sugarcane equality as affected by green cane or pre-harvest burning practices, should be examined.

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