EFFECTS OF MAIZE (*Zea mays* L.) AND SORGHUM (*Sorghum bicolor* (L.) Moench) RESIDUE MANAGEMENT ON SOIL CARBON SEQUESTRATION, NUTRIENT CYCLING AND SPINACH (*Spinacia oleracea*) YIELD

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Submitted in fulfilment of the academic requirements for the degree of Doctor of Philosophy

Discipline of Soil Science

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2025

DECLARATION

I, **Nozibusiso Odette Mbava,** declare that:

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I as candidate's supervisor have approved this thesis for submission

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Dr R. Zengeni (Supervisor)

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Prof P. Muchaonyerwa (Co-Supervisor)

ABSTRACT

The increasing threat of climate change and declining soil quality presents significant challenges to global food security and crop production. This necessitates the adoption of agricultural practices that mitigate climate change while improving soil quality and crop yields. This study aimed to investigate the effects of maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.) residue management, on soil carbon storage, nutrient cycling and spinach (*Spinacia oleracea*) yield. Contrasting cultivars of maize (SC701 and R201) and sorghum (AS8 and PAN8816) were grown at Ukulinga Research Farm of the University of KwaZulu-Natal. The aboveground biomass was harvested at maturity and ground. Half of the ground biomass was retained as raw feedstock, while the other half was pyrolyzed to produce biochar types at 350 and 650ºC in a muffle furnace and at ~400°C in a field kiln. The feedstocks and biochars were characterized for proximate and elemental composition, chemical properties, structural characteristics and functional groups. An incubation study was conducted to assess the effects of crop type, cultivar and conversion of the residues to biochar on CO_2 emissions, nitrogen (N) and phosphorus (P) mineralization and soil pH. Treatments included feedstock and biochar produced at 350°C and 650°C, applied at a rate equivalent to 10 t C ha^{-1} in 100 g of soil and were replicated three times with a control (soil only). The CO_2 emissions were periodically measured for 120 days. A separate incubation experiment with identical treatments was set up with enough replication to allow for destructive sampling to analyze N and P mineralization and soil pH periodically for 120 days. A field experiment was conducted during the summer and winter seasons of 2023 at the same research farm to evaluate the effects of crop type, cultivar and conversion to biochar of residues on CO_2 emissions, soil carbon pools, soil fertility and spinach yield. In the summer, biochar produced in the kiln was applied at 5 t C ha^{-1} to 15 m^2 plots. In winter, the plots were split into two, where one half received repeated applications to assess cumulative effects, while the other half was left untreated to evaluate residual effects. Spinach seedlings were planted and grown for 12 weeks, with weekly CO_2 emissions measured using a CO_2 auto-analyzer. At the end of each season, spinach was harvested and soil samples were collected to analyze soil carbon pools, chemical properties and enzyme activities. The characterization study revealed that biochars produced at 650ºC exhibited enhanced stability, with a reduction in labile functional groups and an increase in fixed carbon content, making them highly suitable for carbon sequestration. Additionally, biochars produced at this temperature demonstrated improved pH and ash content, making them effective for ameliorating acidic soils and enhancing nutrient retention. The incubation study showed that cultivar-specific differences and pyrolysis temperatures significantly influenced CO_2 emissions, N and P mineralization and soil pH than broad crop type. Feedstock treatments resulted in the highest CO_2 emissions, whereas biochars, particularly those produced at 650°C consistently emitted lower CO_2 emissions and increased nutrient availability compared to feedstock and biochars produced at 350°C. Biochars from cultivars R201 and AS8 were identified as more effective for carbon storage, while those from SC701 and PAN8816 enhanced N availability, making them ideal for improving soil fertility. Field experiments revealed that CO_2 emissions, soil carbon pools, soil chemical properties, enzyme activities and spinach biomass yield parameters were more strongly influenced by cultivar-specific differences than by crop type. Biochar consistently outperformed feedstock in improving soil carbon pools, chemical properties, enzyme activities (urease and alkaline phosphomonoesterase activity) and spinach biomass yields, particularly in the summer season. In contrast, feedstock applications were more effective in enhancing other enzyme activities (β-glucosidase and acid phosphomonoesterase activity). Biochars from cultivars SC701 and PAN8816 improved soil pH, nutrient availability and spinach biomass yields, while feedstocks increased micronutrient tissue concentrations. In the first season, biochar treatments significantly increased SOC pools compared to the control. Among maize cultivars, the application of biochar from R201 cultivar (M-R201-B) resulted in the highest SOC content (20.54 g kg^{-1}), giving a 276% increase over the control, while the application of SC701 feedstock (6.37 g kg^{-1}) yielded the lowest SOC content. Similarly in sorghum cultivars biochar from cultivar AS8 resulted in the highest (19.50 g kg^{-1}) while feedstock of cultivar AS8 gave the lowest SOC content (6.11 g kg^{-1}). Biochar applications also enhanced POC and POXC pools with biochars from R201 and PAN8816 cultivars showing the highest contents of these pools amongst the treatments. Additionally, biochar treatments enhanced MBC with SC701 and PAN8816 biochars exhibiting higher concentration while feedstock treatments resulted in lower MBC concentration. Cumulative biochar applications significantly increased soil carbon pools while reducing CO_2 emissions compared to the control. Moreover, the residual effects resulted in increased carbon pools compared to the control. These findings highlight the importance of crop cultivar selection and pyrolysis conditions in influencing the characteristics of biochar types and maximizing the benefits of applications of these materials from maize and sorghum. They provide valuable insights into sustainable residue management practices, demonstrating their potential to mitigate climate change through carbon storage while improving soil quality and crop yields.

Key words: Biochar, carbon sequestration, crop residues, nutrient recycling, soil quality

ACKNOWLEDGEMENTS

Firstly, I am deeply grateful to God Almighty for granting me the opportunity, strength, health and protection to complete this study. Without His guidance, this achievement would not have been possible.

I would like to sincerely thank the National Research Foundation (Doctoral Block Grant), the Water Research Commission (K5/2721/4) and Umngeni Resilience Project for their generous financial support, which made this research possible.

My heartfelt gratitude goes to my supervisors, Dr. Rebecca Zengeni and Prof. Pardon Muchaonyerwa, for their unwavering guidance, patience, academic support and encouragement throughout the duration of this project. Your mentorship is much appreciated.

I extend my thanks to Prof. Alistair Clulow for providing the weather data from Ukulinga Farm, which was instrumental during my field experiments. I am profoundly grateful to Prof. Seyoum Tilahun and his students for constructing the kiln used for biochar production. I also deeply appreciate the assistance of Mr. Ndumiso Mazibuko, the technician in the Agricultural Engineering Discipline, for his invaluable support during biochar production.

To the technical staff of the Soil Science Department (Mr. Nhlanhla Magagula, Mr. Joe Buthelezi and Miss Nosipho Dlamini) and to Mr. Khulekani Mkhonza and Mr. Thokozani Nkosi, thank you for your unwavering support and assistance with my laboratory work. I extend my sincere appreciation to Mr. Mangesh Deepa (Ukulinga Farm Manager), Mr. Gcina Mnyandu, the entire Ukulinga Farm staff and the CERU staff (Mr. Armstrong Gwebu, Mr. Gcwala Zuma and Mr. Syabonga Mlotshwa) for their assistance during my field trials and biochar production. Your dedication was greatly appreciated. I am deeply thankful to the Dlamini family (Mam Florence, Makhosi, Nkosi, Lindani, Bongeka, and Thandazani) for their assistance with planting and maintaining my field trials.

Special thanks to Dr. Nontokozo Mkhonza, Dr. Takudzwa Mandizvo, Dr. Isaac Sanusi, Dr. Bonginkosi Vilakazi, Miss Samukeliwe Vilakazi, Mr Sipho Ntonta, Mr. Sbonakaliso Zwane and Mr. Barnabas Oluoch for assisting me in various ways and ensuring the smooth running of this work. Your contributions have been invaluable.

To all my friends, I express my heartfelt gratitude for your unwavering support and care throughout my study. Ningadinwa yimi nangelinye ilanga.

Lastly, to my beloved parents (Mr. B.F. Mbava (Babazi) & Mrs. A.S. Mbava (MaMbhele)) and my siblings, ngyabonga ngothando nangakho konke eningenzela kona. UJehova anibusise MaBanda Amahle, niyisibusiso empilweni yami!!

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THESIS OUTLINE

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This chapter introduces the background, rationale of the study and the research objectives.

Chapter 2: Systematic Review

This chapter presents a systematic review of the global impacts of crop residues and biochar on CO_2 emissions, soil carbon dynamics, soil quality and crop yields, providing a comprehensive understanding of their potential as sustainable soil amendments.

Chapter 3: Characterization of Amendments

This chapter focuses on the biochemical and structural characterization of the feedstocks and biochars used in the study highlighting differences in their composition and properties.

Chapter 4: Incubation Study

This chapter examines the effects of maize and sorghum feedstocks and biochars on CO_2 emissions, as well as the mineralization of N and P under controlled conditions.

Chapter 5: Field Experiment – Carbon Pools and CO_2 Emissions

This chapter assesses the effects of maize and sorghum feedstocks and biochars on CO_2 emissions and soil carbon pools under field conditions. This chapter is under review in *Soil & Tillage Research* Journal.

Chapter 6: Field Experiment – Soil Fertility and Spinach Yield

This chapter evaluates the impacts of maize and sorghum feedstocks and biochars on soil fertility parameters and spinach yield parameters under field conditions

Chapter 7: General Discussion, Conclusions and Recommendations

The final chapter synthesizes the findings from all previous chapters, provides conclusions and recommendations for future research and practical applications.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

The world's population is expected to continue growing over the next 50 to 60 years, reaching a peak of approximately 10.3 billion in the mid-2080s, up from 8.2 billion in 2024 (United Nations, 2024). This continued growth further intensifies the demand for agricultural production. This rising demand is challenged by soil quality degradation caused by intensive agricultural practices, coupled with the adverse effects of climate change, which presents a significant threat to global food security (Al-Wabel et al., 2015; Morecroft et al., 2019). Among the major drivers of climate change is the excessive release of carbon dioxide (CO_2) and other greenhouse gases from terrestrial sources, particularly through fossil fuel combustion, deforestation, and land-use changes into the atmosphere (Yoro and Daramola, 2020). By 2020, the global atmospheric CO_2 concentration had reached an alarming 412 parts per million (ppm) and projections indicate that it could rise to between 500 and 600 ppm by 2030 if mitigation measures are not implemented (Friedlingstein et al., 2022; Mahato et al., 2022). Elevated atmospheric CO_2 concentrations not only exacerbate climate change but are also linked to substantial soil carbon (C) losses, which compromise soil quality and crop yields (Sun et al., 2020; Soong et al., 2021). Approximately 3.4 billion people live in rural areas globally, and many of them are highly exposed to the impacts of climate change (IPCC, 2019). Within this population there are smallholder farmers who play a critical role in food production, producing up to 80% of the region's food and managing extensive tracts of agricultural land. Furthermore, in South Africa and across sub-Saharan Africa, smallholder farmers are particularly vulnerable to soil degradation, climate change, and limited access to agricultural inputs (Mani et al., 2021). These farmers often rely on organic sources such as crop residues and, to a limited extent, synthetic inputs. However, a lack of knowledge or access to optimal residue management practices frequently results in inefficient nutrient cycling and a continued decline in soil fertility (Tofu and Wolka, 2023). Thus, implementing strategies to reduce carbon emissions is critical for mitigating climate change. When CO_2 emissions are reduced, more carbon is retained and sequestered in the soil, enhancing soil carbon pools, which are a primary indicator of soil quality (Lei et al., 2019). Increased soil carbon improves nutrient retention and supports microbial activity, ultimately contributing to better soil health and productivity (Wiesmeier et al., 2019).

While large quantities of synthetic fertilizers have been applied to agricultural systems to improve crop productivity (Gu et al., 2012; Lou et al., 2023), their excessive and continuous use has led to several negative effects on soil quality. Overuse of synthetic fertilizers contributes to soil acidification, nutrient imbalances, and a decline in soil organic matter, all of which reduce soil fertility and biological activity (Pahalvi et al., 2021). In addition, the leaching and runoff of excess nutrients, particularly nitrogen and phosphorus, can lead to environmental issues such as groundwater contamination and eutrophication of water bodies (Gu et al., 2021). Considering these challenges, the Food and Agriculture Organization (2017) emphasized the importance of environmentally friendly and sustainable agricultural practices to increase food production while preserving the environment. This strengthens the need for cost-effective practices that enhance soil quality, increase crop yields, mitigate climate change and maintain environmental sustainability. One promising approach is crop residue management, particularly the incorporation of crop residues (feedstock) into soils. Crop residues, which are plant parts such as leaves, stems, roots, chaff and husks that are left in the field after harvesting economic parts of the crop, and are regarded as waste, are a valuable source of organic matter (Lal, 2005). Globally, about 4 billion metric tons of crop residues are produced annually, with 74% originating from cereal crops, including maize and sorghum (Lal, 2005; Rahman et al., 2024). Returning crop residues to the soil not only promotes carbon sequestration, but also supplies essential nutrients (Hao et al., 2020). Crop residues are estimated to contain, on average, 40% C, 0.8% nitrogen (N), 0.1% phosphorus (P) and 1.3% potassium (K) (Adimassu et al., 2019). Incorporating these residues into soils can recycle nutrients and improve soil physical, chemical and biological properties (Kumar and Goh, 1999). However, the release of nutrients from residues varies among crops and is influenced by their biochemical composition, such as the C:N ratio, lignin and polyphenol contents (Torma et al., 2018). High quality residues (with high N, low lignin, narrow C:N ratio (<25:1)) decompose rapidly and release nutrients efficiently, while those of low quality (low N, high lignin, wide C:N ratio (>25:1)) may result in N immobilization (Partey et al., 2014). However, the direct incorporation of crop residues can lead to unintended consequences such as accelerating CO_2 emissions from microbial decomposition (Gong et al., 2012). To address these challenges, the conversion of crop residues into biochar has emerged as a promising alternative. Biochar, a carbon-rich material produced through pyrolysis of biomass such as crop residues under limited oxygen (Lehmann and Josephs, 2015), offers a stable reservoir of carbon due to its highly aromatic and condensed structure, which makes it resistant to microbial decomposition. This chemical stability allows biochar to persist in soil for hundreds to thousands of years, effectively sequestering carbon and reducing greenhouse gas emissions (Li and Chan, 2022; Tang et al., 2022). When applied to soil, it also enhances fertility, improves physical and biological properties and creates favorable conditions for crop growth resulting in increased crop yields (Pitello et al., 2018; Domingues et al., 2020).

The properties of biochar are influenced by both the feedstock type and pyrolysis conditions. Generally, an increase in pyrolysis temperature increases biochar total C, fixed C, specific surface area, stability, its pH and hydrophobic characteristics (Enders et al., 2012; Kloss et al., 2012; Chandra and Bhattacharya, 2019). Higher pyrolysis temperatures also increase biochar aromaticity and recalcitrance, making it more resistant to microbial degradation and a more stable carbon sink (Gai et al., 2014; Cheng et al., 2017). Given the abundance of residues from cereal crops like maize and sorghum, there is a critical need to understand the biochemical composition of residues from different cultivars of these crops, the effects of pyrolysis temperature and the potential of residues and their biochar to sequester carbon and improve soil quality. Such knowledge will enable effective management of crop residues in agricultural systems, supporting sustainable farming practices and environmental resilience.

1.2 Problem Statement and Justification

Smallholder farmers are important contributors to global food production, accounting for 51-77% of the world's food supply and 75% of food in Sub-Saharan Africa (SSA) is produced through smallholder systems (Herrero et al., 2017). Despite their critical role, these farmers face significant challenges due to declining soil fertility caused by continuous cropping. Additionally, the high costs of chemical fertilizers and their negative environmental impacts, have placed immense pressure on farmers in SSA to sustain and increase crop yields with limited agricultural inputs while simultaneously mitigating greenhouse gas emissions, to combat climate change (Benaffari et al., 2022; Shi et al., 2022). Addressing these challenges necessitates the adoption of eco-friendly, cost-effective alternatives to improve soil fertility and productivity while sequestering carbon. Maize and sorghum, cultivated extensively by both smallholder and commercial farmers in SSA, are central to the region's agricultural systems (Dunjana et al., 2022; Santpoort, 2022). Maize (*Zea mays* L.) is the third most important cereal crop globally serving as a primary source of energy and protein in human nutrition and as a staple food for millions of households (Naveed et al., 2014). Conversely sorghum (*Sorghum bicolor* (L.) Moench), the fifth most important cereal crop worldwide, is renowned for its exceptional resilience to biophysical stresses, including drought and poor soil conditions, making it essential for food security in SSA, especially in view of climate change and its effects. Sorghum is a source of food to over 500 million people, further highlighting its significance in global food systems (Abreha et al., 2021; Chakrabarty et al., 2021).

With climate change intensifying, breeding efforts have produced diverse cultivars of maize and sorghum with agronomic traits suitable for adaptation to shifting climatic conditions, thereby supporting food security for the growing global population (Li et al., 2024; Wang et al., 2024). Due to genetic differences, these cultivars vary in both yield potential and biomass production, resulting in the accumulation of substantial crop residues in agricultural fields (Parra and Ganibin, 2022; Sekhon et al., 2022). These residues offer a valuable resource for improving soil quality and mitigating greenhouse gas emissions. Despite this, there is still a critical knowledge gap regarding the specific biochemical composition of maize and sorghum residues from different cultivars, and how these characteristics are altered through pyrolysis at varying temperatures as well as their subsequent impacts on CO_2 emissions, carbon sequestration, soil fertility and crop yields. Therefore, there is a need to address these knowledge gaps by evaluating the composition of maize and sorghum residues from different cultivars, their transformation into biochar under varying pyrolysis conditions and their application effects on soil quality, carbon dynamics and crop productivity. The findings will contribute to developing sustainable residue management practices, offering smallholder and commercial farmers practical solutions to enhance agricultural resilience, productivity and environmental sustainability in the face of global climate challenges.

1.3 Research Questions

1. How do crop residue and biochar amendments from different maize and sorghum cultivars impact soil quality and crop productivity globally?

2. How do different cultivars of maize and sorghum influence the biochemical composition of their raw feedstock and biochar, and how are these characteristics affected by pyrolysis conditions?

3. What are the effects of raw feedstock and biochar derived from different maize and sorghum cultivars on CO_2 emissions, nitrogen and phosphorus mineralization, and soil pH under controlled laboratory conditions?

4. How does the application of feedstock and biochar from different maize and sorghum cultivars influence CO_2 emissions, soil organic carbon pools, soil quality, and spinach yield under field conditions?

1.4 Aim and Objectives

The aim of this study was to evaluate the potential of maize and sorghum residues (feedstock) and their biochar as sustainable amendments for improving soil quality, enhancing crop yields, mitigating greenhouse gas emissions particularly CO_2 and contributing to carbon sequestration. The specific objectives of this study were to:

1. Consolidate findings from various studies to provide a global perspective on the effects of crop residue and biochar amendments on soil quality and crop productivity, through a systematic review.
2. Determine effects of different cultivars of maize and sorghum and pyrolysis conditions on the characteristics of biochars relative to the feedstocks.
3. Determine the effects of feedstock and biochar from different cultivars of maize and sorghum on CO_2 emissions, nitrogen and phosphorus mineralization, as well as soil pH under controlled conditions through a laboratory incubation study.
4. Determine the effects of feedstock and biochar from different cultivars of maize and sorghum SOC pools, soil biological quality and spinach yield under field conditions.

1.5 Hypotheses

- The biochemical composition of maize and sorghum residues varies significantly across cultivars, influencing the stability and functional properties of their derived biochars under different pyrolysis conditions.
- Biochar and feedstock derived from different cultivars of maize and sorghum exhibit varying effects on soil properties due to differences in their biochemical composition.
- The mineralization of biochar and feedstock is influenced by both the pyrolysis temperature and the biochemical composition of the original feedstock.
- Biochar application reduces CO_2 emissions, enhances soil quality and improves spinach yields more effectively than feedstock application.
- The application of biochar derived from maize and sorghum residues improves soil quality, enhances soil carbon pools, and increases crop productivity more effectively than raw feedstock, with effects dependent on cultivar and pyrolysis temperature.

CHAPTER 2

Global impacts of crop residues and biochar on CO_2 emissions, soil carbon pools and quality and crop yields: A Systematic review

Abstract

Declining soil quality and increased carbon dioxide emissions in agriculture present significant challenges for sustainable food production and environmental conservation. Therefore, there is a need to understand the existing sustainable management practices to gain proper knowledge on possible ways to prevent further deterioration of soil quality and a decline in crop yields. This systematic review examines the global effects of raw crop residues and crop residue-derived biochar on soil carbon dioxide (CO_2) emissions, soil carbon pools, soil quality and crop yield across diverse field crops. Following PRISMA guidelines, 62 field studies from 18 countries were selected, focusing on parameters like soil organic carbon (SOC), microbial biomass carbon (MBC), enzyme activity and crop yields. Results reveal that raw crop residue application consistently increased CO_2 emissions in 100% of studies that assessed CO_2 emissions, whereas biochar had mixed effects. Both amendments positively influenced SOC (96% of studies), MBC and enzyme activity, compared to the control with biochar showing longer-term benefits due to its stable carbon structure. Enhanced nutrient uptake and crop yields were observed with biochar and residues, improving soil fertility and productivity across various crops. However, the effectiveness varied based on residue type, application rate, agricultural practice employed and environmental conditions, emphasizing the need for context-specific management practices. This review provides comprehensive insights into sustainable soil management, supporting residue and biochar application as practical strategies to enhance soil quality and mitigate impacts of climate change.

Keywords: Biochar, carbon, carbon dioxide, crop residues, feedstock, nutrient uptake

2.1 Introduction

Declining soil quality is a major constraint to crop production worldwide. This deterioration is primarily attributed to conventional agricultural practices such as monocropping, intensive tillage and excessive fertilizer application (Derpsch et al., 2024). While these practices increase crop yields to meet the demands of a growing global population, they also contribute to land degradation and exacerbate climate change by promoting CO_2 emissions through the accelerated breakdown of soil organic matter (Lal et al., 2013). Therefore, the agricultural sector should adopt sustainable soil management practices to prevent soil degradation caused by conventional agricultural practices while simultaneously improving soil quality and maintaining or enhancing crop yields. Incorporating crop residues into soil represents one such sustainable practice that is accessible and can positively impact soil quality. Crop residues, the biomass remaining after harvesting economic parts (grain, seed, fibre and fruits), are among the most abundant forms of organic materials available in agricultural fields (Turmel et al., 2015; Grzyb et al., 2020). These residues contain essential nutrients, including carbon (C), nitrogen (N), phosphorus (P), potassium (K) and micronutrients necessary for crop growth (Wang et al., 2020). Furthermore, crop residues contribute to climate change mitigation by reducing evaporation, sequestering C in the soil through addition of the organic materials (C bearing) coupled with reduction in CO_2 emissions, increasing organic matter and enhancing soil structure (Kareem et al., 2022). However, the benefits of crop residue incorporation are inconsistent, as their chemical composition controls the decomposition of these residues and their effects on soil quality (Grzyb et al., 2020). For instance, residues high in nitrogen, low in lignin and cellulose, with a low C: N ratio and a low lignin: N ratio typically decompose rapidly, leading to higher nutrient mineralization rates. Meanwhile, low-quality residues with slower mineralization rates can limit nutrient availability through immobilization (Manzoni et al., 2023).

Alternatively, crop residues can be applied as biochar, a carbon-rich, recalcitrant product produced by the pyrolysis of biomass at high temperatures without oxygen (Mukherjee et al., 2014). Biochar offers a low-cost, environmentally friendly approach to enhancing soil quality and increasing crop yields, although its effectiveness on crop yields can vary depending on factors such as soil type, crop species, biochar properties and environmental conditions. It improves soil quality, reduces greenhouse gas emissions, and is crucial in climate change mitigation (Lehmann et al., 2021). The biochar, which is recalcitrant, contributes to carbon sequestration as it supports the accumulation of soil organic matter, which is the key driver of improved soil quality. Like crop residues, biochar's effects on soil depend on its chemical and physical properties, which vary based on feedstock type, pyrolysis temperature and residence time (Yaashika et al., 2020). While many studies have reported the positive effects of crop residues and biochar on soil carbon pools, soil quality indicators and crop yields, the research is region-specific and knowledge gaps remain regarding the impact of these amendments across diverse geographical regions. This systematic review aims to bridge these gaps by consolidating findings from various studies to provide a global perspective on the effects of crop residue and biochar amendments on soil quality and crop productivity.

2.2 Materials and methods

2.2.1 Research question

This systematic review was conducted to address the research question: "How do the applications of raw crop residues and biochar derived from the residues influence soil CO_2 emissions, carbon pools, soil fertility, enzyme activity and crop yields in agricultural soils?" based on the framework as outlined by Koutsos et al. (2019) following the six steps as presented in Figure 2.1

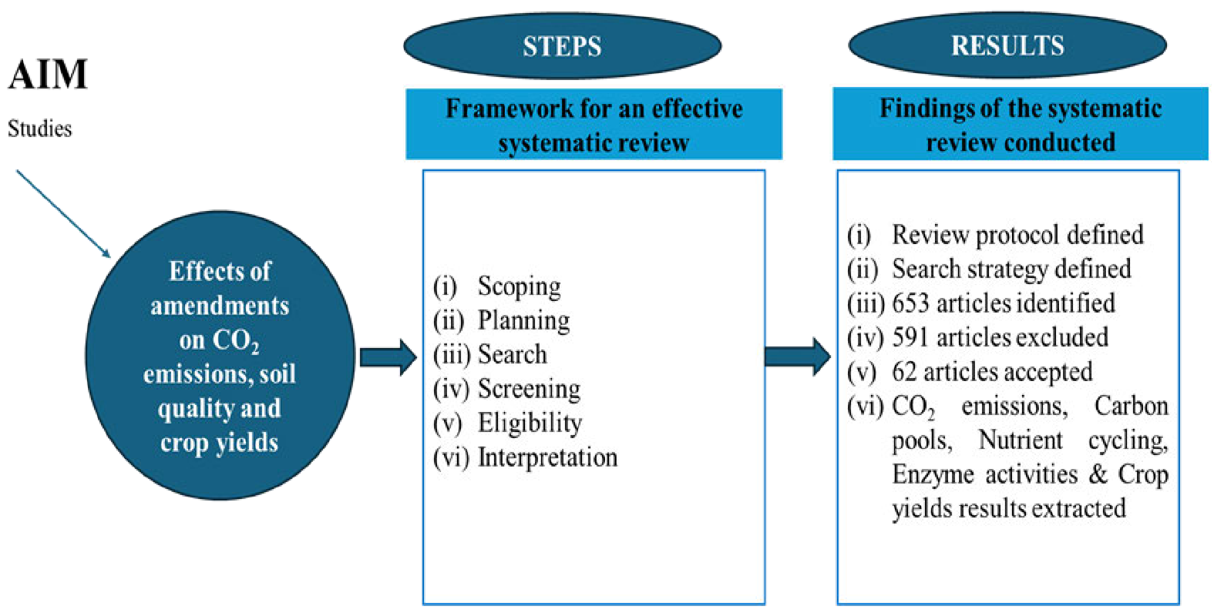

Figure 2.1: Framework for conducting a systematic review on the effects of crop residue amendments on CO_2 emissions, soil quality, and crop yields (adopted from Koutsos et al., 2019)

2.2.2 Protocol and registration

This review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. No prior registration was completed for this review.

2.2.3 Information sources

A comprehensive search was performed in five major electronic databases to ensure broad coverage of relevant literature. All searches were conducted in August 2024 in the following databases:

1. Scopus (www.scopus.com)
2. Web of Science (www.webofknowledge.com)
3. ScienceDirect (www.sciencedirect.com)
4. Science.gov (www.science.gov)
5. Google Scholar (www.scholar.google.com)

2.2.4 Search strategy

A Boolean search strategy was developed to identify studies on the research question. This search yielded a total of 653 publications. The search terms and Boolean operators are shown in Table 2.1.

Table 2.1: Boolean search strategy for identifying peer-reviewed articles on the effects of crop residue amendments on CO_2 emissions, soil carbon dynamics, soil quality, and crop yields across databases

Databases	Primary Terms	Expanded Terms	Explanation
Scopus Web of Science ScienceDirect Science.gov Google Scholar	Crop residues	"raw crop residues" OR "crop residues" OR "crop residue application" OR "agricultural residues" AND	Captures the aspect of raw crop residue application
	Biochar	"biochar" OR "biochar application" OR "biochar derived from crop residues" AND	Focuses on biochar derived from crop residues
	Soil carbon dynamics and CO_2 emissions	"soil carbon dynamics" OR "carbon sequestration" OR "carbon dioxide emissions" OR "carbon pools" AND	Covers the various terms related to carbon pools, carbon dynamics in soil and CO_2 emissions
	Soil fertility	"soil fertility" OR "soil health" OR "soil quality" OR "nutrient cycling" AND	Focuses on different soil fertility aspects
	Enzyme activity	"enzyme activity" OR "soil enzymes" OR "soil biochemical activity" AND	Covers different soil enzyme activity and its impact
	Crop yields	"crop yields" OR "agricultural productivity" OR "crop performance AND	Includes various terms for crop yields and productivity
	Agricultural soils	"agricultural soils" OR "farm soils" OR "cropland soils"	Restricts the search to studies conducted in soils only

2.2.5 Study selection process

The citations from each database were imported into DistillerSR (Evidence Partners Incorporated, Ottawa, ON, Canada) for initial management. Duplicate citations were removed using DistillerSR's duplicate removal function, resulting in 581 unique papers. These were then imported into EndNote 21 for further organization and screening.

The title and abstract of each study were screened manually to assess relevance, following the inclusion and exclusion criteria. Full-text screening was conducted for studies that met the initial criteria based on title and abstract. This process was repeated until only studies that clearly aligned with the research question remained. A total of **62** articles were selected for final inclusion in the review.

2.2.6 Eligibility criteria

The review included only studies that met specific inclusion and exclusion criteria (Table 2.2).

Table 2.2: Criteria for including and excluding studies in the review

Criteria	Description
Publication period	Studies published between 2009 and 2024 were selected to capture recent research advancements and trends in the field, reflecting substantial progress in agricultural soil amendment practices.
Study type(s)	Only field experiments were included to ensure findings are directly applicable to real-world agricultural conditions. Incubation studies, pot experiments, greenhouse experiments, literature reviews, and meta-analyses were excluded due to limited practical applicability.
Crops and residues	The review focused exclusively on field crops such as cereal crops, legumes, and fibre crops, representing the predominant sources of agricultural residues globally. Studies using other organic wastes or non-field crops were excluded for consistency with the review's agricultural focus.
Geographic scope	Studies from any country were included to provide a global perspective on the effects of these amendments, ensuring findings are relevant across diverse climatic and soil conditions.
Outcome measures	Eligible studies reported on at least one of the following outcomes: soil carbon dioxide emissions, soil carbon dynamics (carbon sequestration and carbon pools), soil fertility, enzyme activity, and crop yields, as these outcomes directly relate to soil health and agricultural productivity.

2.2.7 Data synthesis

A narrative synthesis approach was used to collate findings across studies. Results were grouped based on measured outcomes, such as effects on CO_2 emissions, carbon pools, enzyme activities, soil fertility and crop yields. Due to the diversity in experimental designs and reported outcomes, meta-analysis was not feasible.

2.3 Results

2.3.1 Overview of studies identified

This systematic review included only studies conducted under field conditions, as they best reflect natural environments where crops are subjected to varying soil and climate influences. These factors play a significant role in crop growth, nutrient cycling and carbon sequestration, which are central to this review. Field experiments offer realistic insights into long-term soil, crop and environmental interactions, surpassing the scope of short-term laboratory or greenhouse studies. A total of 62 articles were used, of which 50 articles used residues from a single crop, while 12 articles studied combined residues from multiple crops (Table 2.3). Only raw feedstock and biochar were considered, as they are the most common forms in crop residue management for improving soil quality, enhancing carbon sequestration, and mitigating climate change. These forms are widely researched and relevant to real-world agricultural applications. Seventeen articles focused solely on biochar, 21 on raw feedstock and 24 investigated both (Table 2.3). Most articles examined the effects of residue application across multiple parameters; 25 articles assessed CO_2 emissions, 41 investigated soil organic carbon (SOC), 51 examined various chemical properties, 14 analyzed other carbon pools, 38 studied crop yields, 13 focused on nutrient uptake, 14 looked at enzyme activity and 20 explored microbial biomass carbon (MBC) (Table 2.3).

Table 2.3: Summary of 62 studies on the effects of amendments on CO_2 emissions, soil carbon pool dynamics, nutrient cycling, soil enzyme activities and crop yields

						Parameters							
Authors	**Article title**	**Key findings**	**Country**	**Residue used**	**Crop type used**	**CO_2 emission**	**SOC**	**MBC**	**Enzyme activities**	**Chemical properties**	**Other carbon pools**	**Crop yields**	**Nutrient uptake**
Lu et al., 2020	Legacy of soil health improvement with carbon increase following one-time amendment of biochar in a paddy soil-A rice farm trial	One-time biochar amendment increased soil organic carbon (SOC) by 45%, improved soil microbial biomass and enzyme activities by approximately 30%, and resulted in a 10% increase in grain yield over several years. These changes demonstrated a legacy of soil health improvement, supporting the idea that biochar can enhance soil fertility and crop productivity in the long term.	China	Biochar	Wheat		✓	✓	✓	✓		✓	
Ma et al., 2021	Alterations of soil labile organic carbon fractions and biological properties under different residue-management methods with equivalent carbon input	Biochar application significantly increased soil organic carbon (SOC) and SOC stock compared to feedstock. Biochar primarily enhanced particulate organic carbon (POC), light fraction organic carbon (LFOC), and permanganate oxidizable carbon (POXC). Feedstock incorporation improved WEOC and MBC fractions.	China	Feedstock and Biochar	Wheat and Barley		✓		✓	✓	✓		
Zhang et al., 2017	Effects of straw and biochar amendments on aggregate stability, soil organic carbon, and enzyme activities in the Loess Plateau, China	Feedstock and biochar amendments significantly increased soil organic carbon (SOC) and microbial biomass carbon (MBC). Biochar amendment also enhanced urease activity, indicating improved nitrogen cycling in the soil.	China	Feedstock and Biochar	Wheat		✓	✓	✓	✓			
Akinrinade et al., 2023	Growth, yield, and proximate composition of plantain (Musa species) cultivated on soils amended with crop residues	The amendments enhanced the growth parameters, including plant height and leaf area, and improved yield components.	Nigeria	Feedstock and Biochar	Rice		✓			✓		✓	✓
Duan et al., 2020	Wheat straw and its biochar differently affect soil properties and field-based greenhouse gas emission in a Chernozemic soil	Biochar application significantly reduced CO_2 and N_2O emissions compared to feedstock, thus lowering the global warming potential of the soil. Biochar also improved barley yield by 9.9 to 20.4% over two years, whereas feedstock increased emissions. Applying biochar decreased the greenhouse gas emission intensity by up to 50.9%, making biochar a more sustainable option for mitigating environmental impacts than feedstock.	Canada	Feedstock and Biochar	Wheat	✓		✓		✓	✓	✓	
Dubey et al., 2022	Animal manures and plant residue-based amendments for sustainable rice-wheat production and soil fertility improvement in eastern Uttar Pradesh, North India	Rice husk biochar significantly improved soil quality, nutrient uptake, and crop yields in rice-wheat cropping systems. Notably, biochar enhanced soil organic carbon. The findings suggest amendments promote sustainable agriculture, soil fertility, and food security in low-input systems.	India	Feedstock and Biochar	Rice		✓	✓	✓	✓		✓	✓
Guo et al., 2023	Estimation of in-season nitrogen requirement for drip-irrigated spring maize without or with straw incorporation using critical nitrogen dilution curve	Feedstock and biochar incorporation increased nitrogen use efficiency, reduced nitrogen requirement during the reproductive stage, and enhanced crop yields compared to no amendment incorporation. The biochar application showed the highest benefits in terms of nitrogen recovery efficiency and yield improvement.	China	Feedstock and Biochar	Maize		✓			✓		✓	✓
Hansen et al., 2017	The effects of straw or straw-derived gasification biochar applications on soil quality and crop productivity: A farm case study	Biochar increased soil pH and exchangeable potassium (K) but did not significantly affect soil organic carbon (SOC) content or crop yields over three years. The results suggest that biochar can improve certain soil chemical properties, such as potassium bioavailability and liming potential. However, further studies are needed to assess long-term soil quality and crop productivity impacts.	Denmark	Feedstock and Biochar	Wheat	✓	✓			✓		✓	
Lu et al., 2015	Influence of biochar on the moisture of dark brown soil and yield of maize in Northern China	Biochar enhanced maize grain yield by 5% to 12% over two years. Combining biochar and crop residue improved soil moisture characteristics and reduced the negative impact of allelochemicals from decomposing maize residue.	China	Biochar	Maize		✓			✓		✓	
Ma et al., 2021	Responses of greenhouse gas emissions to different straw management methods with the same amount of carbon input in cotton field	Biochar application significantly reduced CO_2, CH_4, and N_2O emissions compared to feedstock. Cotton was highest under biochar treatment.	China	Feedstock and Biochar	Wheat	✓						✓	
Zhao et al., 2024	Effects of biochar application methods on greenhouse gas emission and nitrogen use efficiency in paddy fields	Whether applied once or over three consecutive years, biochar application improved rice yield. The study concluded that biochar effectively mitigates greenhouse gas emissions and enhances crop productivity in rice systems.	China	Biochar	Maize		✓	✓		✓		✓	✓

Mierzwa-Hersztek et al., 2017	Effect of wheat and miscanthus straw biochars on soil enzymatic activity, ecotoxicity, and plant yield	Wheat feedstock and biochar significantly increased plant biomass production compared to soil with mineral fertilization. However, biochars adversely affected soil enzymatic activity and ecotoxicity more than feedstock straw. Biochar application improved crop yields.	Poland	Feedstock and Biochar	Wheat				✓	✓	✓	✓	
Mosharrof et al., 2022	Influence of rice husk biochar and lime in reducing phosphorus application rate in acid soil: A field trial with maize	Applying rice husk biochar significantly increased soil nutrient availability (N and P) while reducing Al and Fe concentrations. Maize production increased by 62.38% compared to the control, with a 231.88% increase in phosphorus uptake. The combination of 75% lime and 50% phosphorus with RHB effectively sustained maize yield and reduced carbon loss through CO_2 emissions.	Malaysia	Biochar	Rice	✓				✓		✓	✓
Munera-Echeverri et al., 2020	Effect of conservation farming and biochar addition on soil organic carbon quality, nitrogen mineralization, and crop productivity in a light textured acrisol in the sub-humid tropics	Conservation farming practices, including residue retention and biochar addition, significantly increased soil organic carbon (SOC), nitrogen mineralization rates, and hot-water extractable carbon (HWEC). Biochar addition increased SOC stock by 90%, although it did not affect nitrogen mineralization rates.	Zambia	Biochar	Pigeon pea		✓				✓	✓	
Nyambo et al., 2020	Carbon input and maize productivity as influenced by tillage, crop rotation, residue management, and biochar in a semiarid region in South Africa	Feedstock and biochar application improved soil organic carbon (SOC) and cumulative carbon input. Biochar did not show significant yield improvements over feedstock application in the short term, but the maize-oat-maize (MOM) rotation showed the highest cumulative biomass production and SOC.	South Africa	Feedstock and Biochar	Maize		✓					✓	
Oladele, 2019	Changes in physicochemical properties and quality index of an alfisol after three years of rice husk biochar amendment in a rainfed rice-maize cropping sequence	Rice husk biochar significantly improved soil pH; however, the effects on soil organic carbon (SOC), total nitrogen (TN), and C: N ratio diminished after three years, suggesting a need for biochar reapplication.	Nigeria	Biochar	Rice		✓			✓			
Singh et al., 2021	Rice straw biochar application to soil irrigated with saline water in a cotton-wheat system improves crop performance and soil functionality in north-west India	Applying rice straw biochar to soils irrigated with saline water improved crop yields, soil pH, electrical conductivity, and microbial biomass carbon. Biochar applications increased seed cotton yield by 23% and wheat grain yield by 27% compared to unamended soils.	India	Biochar	Rice			✓		✓		✓	✓
Yang et al., 2017	Effects of maize stover and its derived biochar on greenhouse gases emissions and C-budget of brown earth in Northeast China	Biochar application reduced CO_2 and N_2O emissions compared to feedstock while increasing soil carbon sequestration. Additionally, biochar enhanced carbon gains in the soil, making it more effective in sequestering carbon than feedstock.	China	Feedstock and Biochar	Maize	✓				✓	✓	✓	
Zhang et al., 2023	Biochar return in the rice season and straw mulching in the oilseed rape season achieve high nitrogen fertilizer use efficiency and low greenhouse gas emissions in paddy-upland rotations	Biochar application increased rice and rapeseed yields compared to feedstock application.	China	Feedstock and Biochar	Rice and Rapeseed							✓	✓
Campos et al., 2020	Biochar amendment increases bacterial diversity and vegetation cover in trace element-polluted soils: A long-term field experiment	Biochar application enhanced soil properties such as total carbon content and pH. However, biochar did not significantly affect enzymatic activities and soil respiration rates.	Spain	Biochar	Rice	✓			✓	✓	✓		
Chen et al., 2021	Amendment of straw biochar increased molecular diversity and enhanced preservation of plant-derived organic matter in extracted fractions of a rice paddy	Compared to control, Biochar application significantly enhanced soil organic carbon (SOC) content by 36%. It also increased different carbon pools.	China	Feedstock and Biochar	Maize		✓	✓		✓			
Chen et al., 2024	Changes in aggregate-associated carbon pools and chemical composition of topsoil organic matter following crop residue amendment in forms of straw, manure, and biochar in a paddy soil	Biochar amendment significantly increased soil organic carbon (SOC) by 24% compared to feedstock amendment, which did not alter SOC. Biochar enhanced macro-aggregation and preserved maize-derived organic carbon (OC) within particulate organic matter (POM), contributing to long-term carbon sequestration.	China	Feedstock and Biochar	Maize			✓		✓			
Haefele et al., 2011	Effects and fate of biochar from rice residues in rice-based systems	In rice-based systems, Biochar from rice husks increased total organic carbon (TOC), total soil nitrogen, and the C/N ratio. Applying biochar improved soil fertility, particularly on poor soils, and led to yield increases ranging from 16% to 35%. However, biochar reduced nitrogen availability in fertile soils with high C/N ratios.	Thailand	Feedstock and Biochar	Rice		✓			✓		✓	✓

Hu et al., 2014	Wheat straw and its biochar had contrasting effects on soil C and N cycling two growing seasons after addition to a black chernozemic soil planted to barley	Wheat feedstock addition increased CO_2 and N_2O emissions by 40% and 17%, respectively, whereas biochar addition did not affect these emissions. Biochar doubled the gross nitrification rates compared to the control and increased soil NO_3^--N concentrations, indicating its potential to improve nitrogen cycling and reduce greenhouse gas emissions.	Canada	Feedstock and Biochar	Wheat	✓	✓			✓			
Mehmood et al., 2020	Biochar addition leads to more soil organic carbon sequestration under a maize-rice cropping system than continuous flooded rice	Biochar application significantly increased soil organic carbon (SOC) concentration and crop yields in both maize-rice (M-R) and rice-rice (R-R) systems. SOC accumulation was 9% higher in the M-R system compared to the R-R system. Biochar also improved soil aggregation, particularly increasing the proportion of small macro-aggregates contributing to higher SOC sequestration.	China	Feedstock and Biochar	Maize and Rice		✓			✓		✓	
Bilgili et al., 2019	The effects of biochars produced from the residues of locally grown crops on soil quality variables and indexes	Biochar from locally grown crop residues such as cotton feedstock and maize improved key soil quality parameters. These included total nitrogen and crop yields for soil. Biochar significantly increased the soil quality index (SQI) by up to 138.5%.	Turkey	Biochar	Maize and Cotton					✓		✓	
Cao et al., 2021	Mitigating the global warming potential of rice paddy fields by straw and straw-derived biochar amendments	Applying rice feedstock or biochar to rice paddies impacted greenhouse gas (GHG) emissions differently. Biochar application increased CO_2 uptake by 43.5% and slightly reduced N_2O emissions by 8.4%. Both feedstock and biochar amendments decreased the global warming potential by mitigating N_2O and enhancing CO_2 uptake.	China	Feedstock and Biochar	Rice	✓				✓			
Feng et al., 2022	Amendment of crop residue in different forms shifted micro-pore system structure and potential functionality of macroaggregates while changing their mass proportion and carbon storage of paddy topsoil	Amendments with crop residues in different forms, such as fresh, manured, and pyrolyzed (biochar) maize residue, significantly increased soil organic carbon (SOC) and particulate organic carbon (POC) storage in macroaggregates. Biochar had the most substantial impact, increasing SOC by 31.3% and POC by 63.3%. Biochar also enhanced the micro-pore system structure within macroaggregates, improving soil function and carbon sequestration.	China	Feedstock and Biochar	Maize		✓	✓		✓			
Mankaa'Abusi et al., 2024	Soil properties and agronomic effects of repeated biochar amendment and fertilization in an urban horticultural system of Burkina Faso	Repeated biochar applications in an urban vegetable production system significantly increased soil organic carbon (SOC) by 51%. However, mineral fertilization without biochar led to a 32% decrease in SOC over six months. Biochar addition improved nitrogen use efficiency (NUE), particularly when combined with organic and mineral fertilizers.	Burkina Faso	Biochar	Maize		✓			✓	✓	✓	✓
Sandhu et al., 2017	Analyzing the impacts of three types of biochar on soil carbon fractions and physiochemical properties in a corn-soybean rotation	Biochar derived from maize feedstock significantly increased the pH of acidic soil at an eroded landscape position. However, no significant change was observed in soil pH at the depositional landscape. The 10 Mg/ha application rate improved water-soluble carbon (WSC) in sandy loam soils.	USA	Biochar	Maize					✓			
Zhang et al., 2019	Effects of maize straw and its biochar application on organic and humic carbon in water-stable aggregates of a mollisol in Northeast China: A five-year field experiment	Both maize straw and its biochar significantly increased soil organic carbon (SOC) and humic carbon (HC) in water-stable aggregates. Applying biochar was more effective than maize feedstock in increasing the stability of soil aggregates and preserving organic carbon in larger aggregates.	China	Feedstock and Biochar	Maize		✓			✓			
Zhang et al., 2012	Effects of biochar amendment on soil quality, crop yield, and greenhouse gas emission in a Chinese rice paddy: A field study of 2 consecutive rice growing cycles	Biochar application increased rice productivity, soil pH, soil organic carbon (SOC), and total nitrogen while decreasing soil bulk density. Biochar reduced nitrous oxide (N_2O) emissions but increased methane (CH_4) emissions in both cycles. Over two rice cycles, biochar decreased the global warming potential (GWP) and greenhouse gas intensity (GHGI) by up to 34.8%.	China	Biochar	Wheat	✓	✓			✓	✓	✓	
Bhattacharyya et al., 2012	Effects of rice straw and nitrogen fertilization on greenhouse gas emissions and carbon storage in tropical flooded soil planted with rice	The study found that the combined application of rice straw and nitrogen fertilizer significantly increased greenhouse gas emissions (methane, CO_2, and nitrous oxide) compared to inorganic fertilizers alone. The rice feedstock + green manure improved soil carbon storage, microbial biomass carbon (MBC), and enzyme activities.	India	Feedstock	Rice	✓	✓		✓	✓		✓	
Iqbal et al., 2009	CO_2 emission in a subtropical red paddy soil (ultisol) as affected by straw and N-fertilizer applications: A case study in Southern China	Nitrogen fertilizer and rapeseed feedstock significantly increased soil CO_2 emissions in red paddy soil. The combination increased cumulative CO_2 fluxes from row soils by 18%, inter-row soils by 33%, and bare soils by 19%, compared to nitrogen fertilizer alone.	China	Feedstock	Rapeseed	✓	✓			✓		✓	

Li et al., 2022	Biochar incorporation increases winter wheat (*Triticum aestivum L.)* production with significantly improving soil enzyme activities at jointing stage	Biochar in soil improved winter wheat yield by enhancing soil water storage, soil carbon, and nitrogen content. Biochar also significantly increased soil enzyme activities, including invertase, urease, alkaline phosphatase, and catalase, particularly at the jointing stage of winter wheat growth.	China	Feedstock and Biochar	Maize		✓	✓	✓	✓		✓	
Urra et al., 2018	Effects of corn stover management on soil quality	Incorporation of maize feedstock into soil improved soil quality by increasing soil organic carbon (SOC) and microbial biomass. After six years of treatment, maize feedstock incorporation resulted in higher SOC and microbial activity than no feedstock application, which decreased SOC and microbial gene abundance.	Spain	Feedstock	Maize		✓		✓	✓		✓	
Biau et al., 2013	Stover management and nitrogen fertilization effects on corn production	Feedstock incorporation had a positive impact on SOC levels without penalizing corn yields. Higher nitrogen fertilization rates increased corn yields, while continuous feedstock removal caused SOC depletion.	Spain	Feedstock	Maize		✓			✓		✓	✓
Tan et al., 2021	Effects of biochar application with fertilizer on soil microbial biomass and greenhouse gas emissions in a peanut cropping system	The study found that applying biochar with either organic or mineral fertilizer significantly increased soil microbial biomass carbon (MBC) and decreased cumulative methane (CH_4) emissions by 68.67%. There was no significant change in microbial biomass nitrogen (MBN) or cumulative carbon dioxide (CO_2) emissions.	China	Biochar	Rice and Cotton	✓	✓	✓		✓		✓	
Ghimire et al., 2011	Soil organic carbon sequestration as affected by tillage, crop residue, and nitrogen application in a rice-wheat rotation system	The study found that no-tillage combined with crop residue application significantly increased soil organic carbon (SOC) sequestration, particularly in the upper 15 cm of soil. No-tillage systems with residue application stored 34% more SOC at 0-5 cm depth than conventional tillage.	Nepal	Feedstock	Wheat and Rice		✓			✓			
Zhang et al., 2012	Effect of biochar amendment on maize yield and greenhouse gas emissions from a soil organic carbon-poor calcareous loamy soil from Central China Plain	Biochar application significantly increased maize yield by 15.8% at 20 t/ha and 7.3% at 40 t/ha without nitrogen fertilization. With nitrogen fertilization, maize yield increased by 8.8% and 12.1% under 20 t/ha and 40 t/ha of biochar, respectively. Biochar also reduced nitrous oxide (N_2O) emissions by 10.7% and 41.8% at 20 t/ha and 40 t/ha, respectively.	China	Biochar	Wheat	✓	✓			✓		✓	
Abalos et al., 2013	Role of maize stover incorporation on nitrogen oxide emissions in a non-irrigated Mediterranean barley field	The study found that incorporating maize feedstock into Mediterranean barley fields increased CO_2 emissions, ammonium and nitrate concentration and crop yields.	Spain	Feedstock	Maize	✓						✓	✓
Maris et al., 2017	Effect of stover management and nitrogen fertilization on N_2O and CO_2 emissions from irrigated maize in a high nitrate Mediterranean soil	Maize feedstock incorporation increased carbon dioxide (CO_2) emissions. Nitrogen use efficiency decreased with higher fertilizer rates. The study highlights that efficient irrigation can reduce emissions of gases in high-nitrogen soils.	Spain	Feedstock	Maize	✓				✓		✓	
Muchelo & Akpa, 2024	Enhancing maize (*Zea mays* L.) yields in limited fertilizer farming systems through soil nutrient recycling in Sub-Saharan Africa	The study demonstrated that applying organic amendments, such as cattle manure, Tithonia compost, and maize residues, significantly increased maize grain yields and improved the nutrient content of maize residues in Sub-Saharan Africa. Over two years, maize yields increased by 87%, 27%, and 13% for cattle manure, Tithonia compost, and maize residues.	Uganda	Feedstock	Maize		✓		✓	✓		✓	✓
Storlien et al., 2014	Carbon dioxide and nitrous oxide emissions impacted by bioenergy sorghum management	The study examined the impacts of nitrogen fertilization, residue management, and crop sequence on CO_2 and N_2O emissions from bioenergy sorghum production. Nitrogen fertilization significantly increased cumulative N_2O emissions, while residue return increased cumulative CO_2 emissions. Crop sequence had little effect on emissions.	USA	Feedstock	Sorghum	✓	✓			✓		✓	
Yang et al., 2017	Effects of maize stover and its biochar on soil CO_2 emissions and labile organic carbon fractions in Northeast China	Both maize feedstock and biochar treatments significantly increased soil CO_2 emissions compared to the control, with maize feedstock leading to higher CO_2 emissions than biochar. Maize feedstock and biochar also enhanced soil organic carbon (SOC) and labile organic carbon fractions such as particulate organic carbon (POC), dissolved organic carbon (DOC), and microbial biomass carbon (MBC).	China	Feedstock and Biochar	Maize	✓	✓	✓			✓		
Hu et al., 2019	Comparative effects of crop residue incorporation and inorganic potassium fertilization on soil C and N characteristics and microbial activities in cotton field	Feedstock incorporation significantly increased soil water-soluble organic carbon (WSOC), microbial biomass carbon (MBC), and microbial biomass nitrogen (MBN), enhancing soil microbial activities. The incorporation of wheat feedstock had a stronger impact on soil carbon and nitrogen cycles than cotton feedstock.	China	Feedstock	Wheat and Cotton		✓	✓	✓	✓			

Malobane et al., 2020	Microbial biomass carbon and enzyme activities as influenced by tillage, crop rotation, and residue management in a sweet sorghum cropping system in marginal soils of South Africa	The study showed that no-till (NT) combined with a sorghum-vetch-sorghum rotation (S-V-S) and 30% residue incorporation resulted in the highest microbial biomass carbon (MBC) and enzyme activities (β-glucosidase, acid phosphatase, and urease) compared to conventional tillage (CT) and lower residue incorporation treatments.	South Africa	Feedstock	Sorghum			✓	✓			
Tejada & BenÃtez, 2014	Effects of crushed maize straw residues on soil biological properties and soil restoration	Applying maize feedstock significantly improved soil biological properties, including microbial biomass, soil respiration, and enzyme activities (dehydrogenase, urease, β-glucosidase, phosphatase, and arylsulphatase).	Spain	Feedstock	Maize	✓		✓	✓	✓		
Sandhu et al., 2017	Analyzing the impacts of three types of biochar on soil carbon fractions and physiochemical properties in a corn-soybean rotation	Biochar application at 10 Mg/ha significantly improved soil pH in acidic, eroded landscapes but had limited effects on soil carbon and nitrogen fractions.	USA	Biochar	Maize					✓		
Zhu et al., 2015	Short-term responses of soil organic carbon and carbon pool management index to different annual straw return rates in a rice-wheat cropping system	The study found that applying 50% of annual feedstock (50%S) in a rice-wheat cropping system significantly increased soil organic carbon (SOC), dissolved organic carbon (DOC), easily oxidizable carbon (EOC), and microbial biomass carbon (MBC) compared to no feedstock application.	China	Feedstock	Wheat and Rice		✓	✓				✓
Wang et al., 2023	Impacts of biochar amendment and straw incorporation on soil heterotrophic respiration and desorption of soil organic carbon	Biochar incorporation reduced CO_2 efflux compared to feedstock incorporation in the North China Plain. CO_2 efflux was significantly higher under feedstock incorporation during the summer, while biochar reduced soil organic carbon (SOC) desorption. Biochar showed greater potential for enhancing SOC stability and reducing carbon losses.	China	Feedstock and Biochar	Maize	✓		✓		✓		
Yang et al., 2018	Labile organic carbon fractions and carbon pool management index in a 3-year field study with biochar amendment	The study demonstrated that biochar incorporation significantly increased soil organic carbon (SOC) and labile organic carbon fractions, including particulate organic carbon (POC), easily oxidizable carbon (EOC), light fraction organic carbon (LFOC), and microbial biomass carbon (MBC).	China	Biochar	Maize			✓			✓	
Ndzelu et al., 2020	Corn straw return can increase labile soil organic carbon fractions and improve water-stable aggregates in Haplic Cambisol	Maize feedstock application into the soil significantly increased soil organic carbon (SOC) and labile SOC fractions such as water-extractable organic carbon (WEOC), permanganate oxidizable carbon (POXC), and light fraction organic carbon (LFOC).	South Africa	Feedstock	Maize		✓			✓		✓
Chang et al., 2023	Maize straw application as an interlayer improves organic carbon and total nitrogen concentrations in the soil profile: A four-year experiment in a saline soil	Maize feedstock application significantly improved soil organic carbon (SOC) and total nitrogen (TN) concentrations, particularly in the 20-60 cm soil layer. Feedstock application in 12 Mg/ha rates showed the greatest improvement in SOC, TN and C: N.	China	Feedstock	Maize		✓			✓		✓
Kim et al., 2022	Co-responses of soil organic carbon pool and biogeochemistry to different long-term fertilization practices in paddy fields	Long-term application of rice feedstock significantly improved soil organic carbon (SOC) fractions, microbial biomass carbon (MBC), and labile organic carbon pools in paddy fields.	Korea	Feedstock	Wheat and Rice					✓	✓	
Chen et al., 2016	The influence of the type of crop residue on soil organic carbon fractions: An 11-year field study of rice-based cropping systems in southeast China	Incorporation of different crop residues significantly influenced soil organic carbon (SOC) and its labile fractions, such as microbial biomass carbon (MBC), dissolved organic carbon (DOC), hot-water extractable carbon (HWC), and permanganate-oxidizable carbon (POXC).	China	Feedstock	Wheat, Rice and Rapeseed		✓			✓	✓	✓
Lu et al., 2021	Impacts of continuous biochar application on major carbon fractions in the soil profile of North China Plain's cropland: In comparison with straw incorporation	The study found that biochar incorporation at 9.0 Mg/ha significantly increased soil organic carbon (SOC) by 81% over a 0-20 cm depth compared to feedstock incorporation, which increased SOC by 15%. Biochar also reduces soil inorganic carbon content over the same depth.	China	Feedstock and Biochar	Maize and Wheat		✓			✓	✓	
Cong et al., 2020	Changes in soil organic carbon and microbial community under varying straw incorporation strategies	The study found that different forms of maize residue incorporation (whole straw, chopped straw, pelletized straw, straw biochar, and straw ash) significantly increased soil organic carbon (SOC) and dissolved organic carbon (DOC) in both topsoil and subsoil. Chopped straw showed the highest increase in SOC and microbial biomass carbon (MBC) compared to other treatments.	China	Feedstock and Biochar	Maize		✓					
Malobane et al., 2020	Tillage, crop rotation and crop residue management effects on nutrient availability in a sweet sorghum-based cropping system in marginal soils of South Africa	The study examined the effects of different tillage, crop rotation, and crop residue management practices on nutrient availability in a sweet sorghum-based cropping system in South Africa. A 30% crop feedstock incorporation increased NH_4^+-N, NO_3^--N, available phosphorus (Available P), cation exchange capacity (CEC), calcium (Ca), magnesium (Mg) and potassium (K) by 3.58% to 31.94% compared to no crop application.	South Africa	Feedstock	Sorghum					✓		

Gura & Mnkeni, 2019	Crop rotation and residue management effects under no till on the soil quality of a haplic cambisol in Alice, Eastern Cape, South Africa	Feedstock application significantly improved soil organic carbon (SOC), microbial biomass carbon (MBC), soil respiration, and nutrient availability (N, P, K, Ca, Mg, Zn).	South Africa	Feedstock	Maize	✓	✓	✓	✓		
Mirzaei et al., 2021	Preliminary effects of crop residue management on soil quality and crop production under different soil management regimes in corn-wheat rotation systems	The study revealed that feedstock application at different rates (100%, 75%, 50%, 25%, and 0%) improved soil organic carbon (SOC), soil physical properties (such as aggregate stability and water retention), and crop yields (wheat and corn) in both conventional tillage (CT) and no-till (NT) systems.	Iran	Feedstock	Maize and Wheat	✓	✓		✓	✓	
Arora et al., 2023	Effect of residue recycling on leaf area index and nutrient uptake pattern of wheat crop under different potassium management practices	Feedstock retention combined with the split application of 100% recommended dose of potassium (RDK) significantly improved the leaf area index (LAI) and nutrient uptake (N, P, K) in wheat.	India	Feedstock	Maize	✓			✓	✓	✓

SOC=Soil organic carbon; **MBC=** Microbial biomass carbon

The experiments reviewed were conducted across 18 different countries. The largest number of studies came from China (29 articles), followed by Spain (6) and South Africa (5). These were followed by studies from India (4), USA (3), and Canada and Nigeria with 2 each, while the rest had 1 each (Figure 2.2).

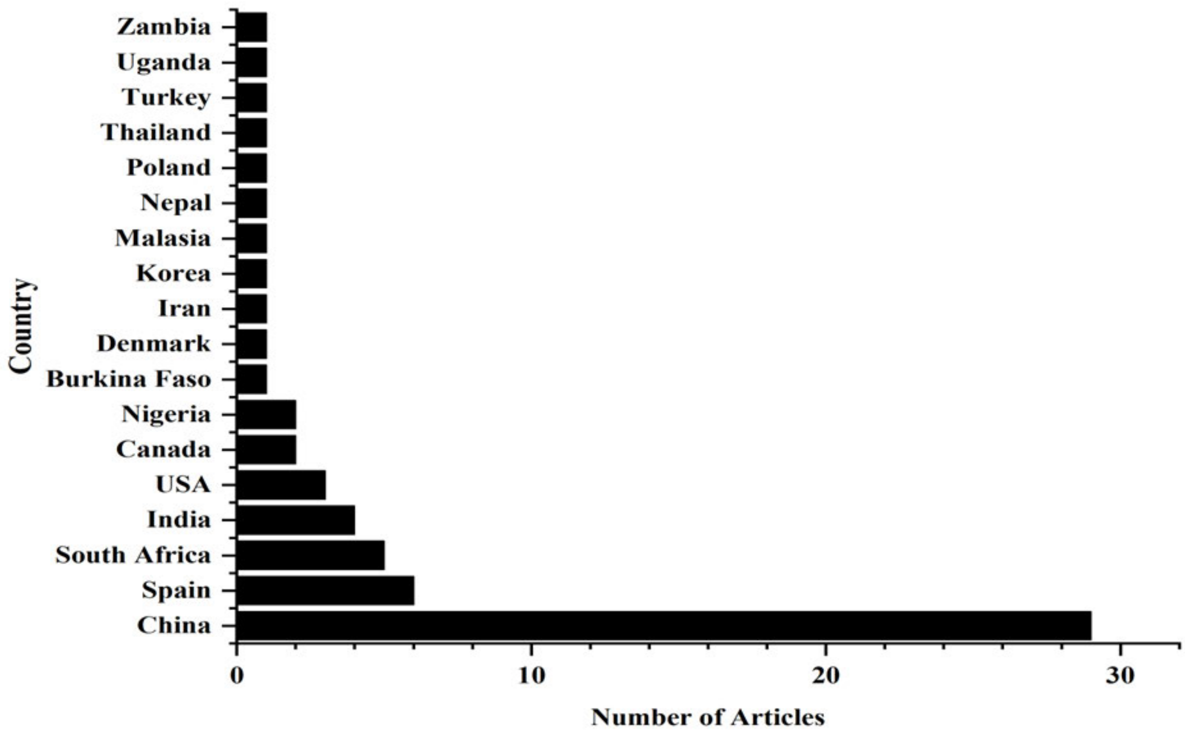

Figure 2.2: Number of publications by country of experiment

The review focused on residues from cereal, legume and fibre crops, excluding tuber and sugar crops. Tuber and sugar crops leave minimal residues, while cereals, legumes, oil and fibre produce substantial biomass often returned to the soil. As a result, the review covered eight crop types, with maize being the most frequently studied (31 articles), followed by wheat (17 articles) and rice (16 articles). Pigeon pea and barley residues were the least represented, with only one article each (Figure 2.3).

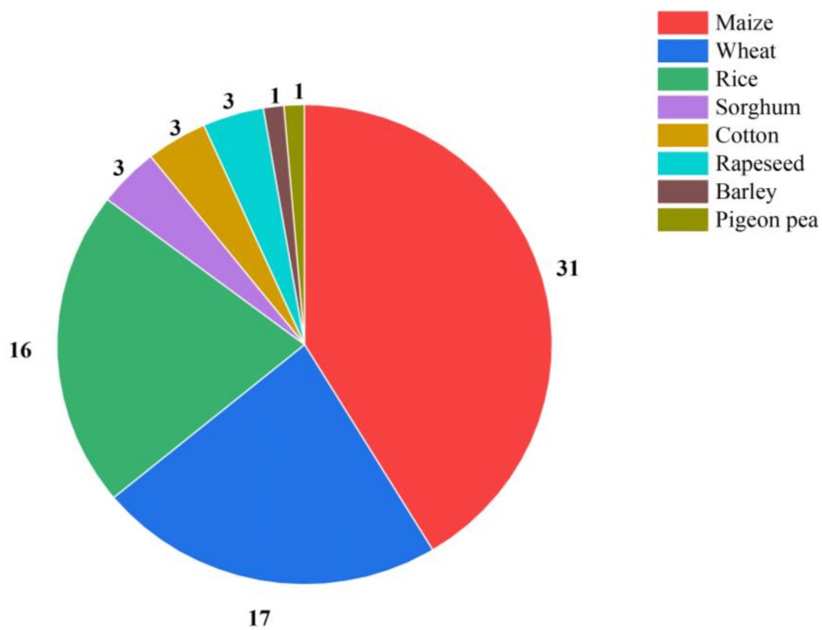

Figure 2.3: Different crop types used as residue amendments in the present study

2.3.2 Summary of amendment effects on CO_2 emissions, soil quality and crop yield

The effects of both raw crop residues (feedstock) and biochar on various soil quality indicators were assessed across 62 studies, with findings presented in Table 2.4.

2.3.2.1 CO_2 emissions

Feedstock application consistently resulted in increased CO_2 emissions, with 100% of studies indicating an increase in emissions compared to control treatments (Table 2.4). In contrast, biochar application showed more varied effects, with 46% of studies reporting a reduction in CO_2 emissions, 31% an increase and 23% found no significant impact.

2.3.2.2 Carbon pools (SOC, MBC and other pools) and enzyme activity

The effects of feedstock and biochar applications were positive for SOC and other carbon pools. Nearly all studies (96% for feedstock and 96% for biochar) indicated increased SOC, with no studies showing a decrease (Table 2.4). For MBC, feedstock application showed a stronger effect, with 93% of studies reporting an increase, whereas biochar application resulted in increase in 80% and a reduction in 20% of the studies. The application of both feedstock and biochar increased enzyme activity (urease, phosphatases, dehydrogenase & β-glucosidase) across all studies.

2.3.2.3 Soil chemical properties (Total Nitrogen, Nitrate-N, Ammonium-N, Available P, Exchangeable bases, CEC, pH, EC)

Feedstock and biochar applications generally enhanced soil chemical properties. Total nitrogen content increased in 97% of studies with feedstock application and 92% with biochar, with negligible reductions (Table 2.4). Applications of both amendments also consistently increased nitrate-N and available P, exchangeable bases and CEC (100% of studies in each case). Feedstock had a more substantial effect on ammonium-N, with 63% of studies showing an increase, whereas 67% of biochar studies reported a reduction. Both amendments also had favorable impacts on soil pH, with only minor variations in response.

2.3.2.4 Crop production (crop yields and nutrient uptake)

Crop yield improvements were reported in 84% of studies with feedstock and 85% with biochar, showing a positive trend for both applications (Table 2.4). Nutrient uptake results varied with 80% of the studies looking at nutrient uptake reporting increases with feedstock application while 20% reported no effects. On the other hand, 71% of the studies reported increased nutrient uptake and 29% reported no effects on nutrient uptake when biochar was applied.

Table 2.4: Effect of feedstock and biochar amendments on CO_2 emissions, soil quality indicators and crop yields across reviewed studies

		Feedstock Application			Biochar Application		
Broad Parameter	**Specific parameter**	**Studies increased (%)**	**Studies Reduced (%)**	**Studies with No Effects (%)**	**Studies Increased (%)**	**Studies Reduced (%)**	**Studies with No Effects (%)**
CO_2 emission	CO_2 emission	100	0	0	46	31	23
Carbon pool	SOC	96	0	4	96	0	4
	MBC	93	7	0	80	20	0
	TC	75	25	0	100	0	0
	Other pools	100	0	0	100	0	0
Soil chemical property	Total Nitrogen	97	0	3	92	4	4
	Nitrate-N	100	0	0	100	0	0
	Ammonium-N	67	0	33	33	67	0
	Available P	100	0	0	100	0	0
	Exchangeable Bases	100	0	0	100	0	0
	Soil CEC	100	0	0	100	0	0
	Soil pH	76	4	20	95	0	5
	Soil EC	57	0	43	50	25	25
Soil Biological Property	Enzyme activity	100	0	0	100	0	0
Crop Production	Crop yield	84	3	13	85	0	15
	Nutrient uptake	80	0	20	71	0	29

2.4. Discussion

2.4.1 Variations in chemical composition of raw crop residues (feedstock) and biochar derived from different crops

The biochemical composition of crop residues plays a key role in their decomposition and nutrient release in soil, with environmental factors also influencing these processes (Chen et al., 2016). While residues from different crops share basic components, their compositions vary significantly between crop species, cultivars of the same crop and even different parts of a single plant (Chen et al., 2016). Understanding this variability is essential before applying residues to soil as it directly affects their impact. For example, residues with a C:N ratio > 30 lead to net N immobilization, while those with a C:N ratio < 30 promote net N mineralization (Abalos et al., 2012; Muchelo & Akpa, 2024). In the reviewed studies, the biochemical composition of residues varied widely, even within the same crop type. For instance, rice feedstock showed total C content ranging from 107.3 g kg^{-1} (Akirinande et al., 2022) to 424.67 g kg^{-1} (Chen et al., 2016), total N content from 2.84 g kg^{-1} (Akirinande et al., 2022) to 14.43 g kg^{-1} (Chen et al., 2016) and a C:N ratio between 28.23 (Chen et al.,2016) and 67.3 (Abalos et al., 2023). These variations highlight that even residues from the same crop can influence soil properties differently depending on their biochemical composition.

Feedstock decomposition occurs relatively quickly providing only short-term effects. In contrast, biochar produced through the thermal conversion of organic materials under oxygen-limited conditions, is more stable due to its high recalcitrant carbon content (Zhang et al., 2019). Like feedstock, the biochemical composition of biochar depends on the type of feedstock, its initial composition and pyrolysis conditions, which affect its decomposition and effects in the soil (Ma et al., 2021). Biochar composition also varies significantly even within biochars derived from the same crop and pyrolysis temperature. For example, rice biochar showed total C contents ranging from 248.6 g kg^{-1} (Mosharrof et al.,2022) to 701.3 g kg^{-1} (Dubey et al., 2022), total N content from 1.6 g kg^{-1} (Campos et al., 2020) to 19.0 g kg^{-1} (Dubey et al., 2022), C:N ratios between 109.5 (Cao et al., 2021) and 112.7 (Tan et al., 2021), and pH values from 8.15 (Mosharrof et al., 2022) to 10.3 (Tan et al et al., 2021). These variations suggest that biochars from the same crop type can result in diverse soil responses depending on their specific properties. Biochar generally exhibits higher minimum carbon content and C:N ratios compared to feedstock reflecting its recalcitrant nature, slower decomposition rate and greater potential for carbon sequestration. These properties make biochar a valuable amendment for improving long-term soil carbon storage, while feedstock is more suited for short-term nutrient cycling.

2.4.2 Effects of feedstock and biochar application on CO_2 emissions

The observed variations in CO_2 emissions due to residue applications, including feedstock and biochar highlight the complex interactions between amendment types, amendment properties, soil properties and environmental factors. Feedstock incorporation consistently increases CO_2 emissions due to the rapid decomposition of labile carbon, which stimulates microbial activity and soil respiration. For instance, maize feedstock increased cumulative CO_2 emissions to 4937.41 kg CO_2-C ha^{-1} significantly higher than control treatments (Maris et al., 2017). Similar trends were observed across other feedstocks as easily decomposable carbon sources enhanced microbial respiration (Abalos et al., 2013). In contrast, biochar amendments generally reduce CO_2 emissions due to their recalcitrant carbon structure, which resists microbial degradation (Yang et al., 2017a; Campos et al., 2020). For example, in a study conducted by Ma et al. (2021), the application of wheat straw biochar reduced CO_2 emissions by 12.4% in 2017 and 8.1% in 2018 compared to the control. Biochar's ability to stabilize carbon makes it an effective strategy for carbon sequestration. However, variability exists as some studies reported no effects or increased emissions influenced by biochar properties, application rates and environmental factors (Hu et al., 2014; Tan et al., 2021). The variability in biochar effects on CO_2 emissions can be influenced by crop type, region and soil conditions. For instance, wheat derived biochar was associated with reductions in CO_2 emissions, particularly in humid regions with silty clay loam soils when pyrolyzed at temperatures around 450^{o}C (Hansen et al., 2017; Duan et al., 2020; Ma et al., 2021). In contrast, rice husk biochar showed no significant impact on CO_2 emissions in Mediterranean climates with sandy loam soils, especially when pyrolyzed at 500^{o}C (Campos et al., 2020). However, in tropical hot and humid regions rice husk biochar applications tended to increase CO_2 emissions, particularly in sandy clay soils (Mosharrof et al., 2022).

The interaction between biochar and nitrogen fertilization further influences CO_2 emissions. While combining biochar with nitrogen fertilizers stabilized soil carbon without increasing emissions

(Zhang et al., 2012), feedstock combined with fertilizers increased emissions due to enhanced organic matter decomposition (Bhattacharyya et al., 2012). Environmental conditions such as soil moisture and temperature also play a role in CO_2 emission, with irrigation and precipitation events caused emission peaks, especially in feedstock treatments, reflecting the dynamic interaction between residue decomposition and climatic factors (Storlien et al., 2014). These findings highlight biochar's potential as a sustainable strategy for reducing CO_2 emissions compared to feedstock. Its effectiveness depends on biochar characteristics, application rates, soil properties and environmental conditions, highlighting the need for customized management strategies to maximize its carbon sequestration potential.

2.4.3 Effects of feedstock and biochar application on carbon pools

Feedstock and biochar applications significantly influence soil carbon pools, including SOC, total carbon (TC), MBC, particulate organic carbon (POC) and permanganate oxidizable carbon (POXC). Their impacts depend on the biochemical composition of the amendments, application rates, environmental conditions and soil management practices. Feedstock provides immediate carbon inputs through rapid decomposition while biochar offers long term stability due to its recalcitrant structure and resistance to microbial degradation.

Biochar consistently outperforms feedstock in stabilizing long term carbon pools such as SOC and TC. For example, maize biochar increased SOC by 36% compared to a 12% increase with maize feedstock after 2 years of their application in soil (Chen et al., 2021). Similarly, wheat biochar applied at 40 t ha^{-1} increased SOC by 55.2%, which was attributed to its slow turnover and soil structure improvements (Zhang et al., 2012). Although feedstock decomposes quickly, it provides immediate improvements to SOC and TC as observed with maize feedstock which increased SOC by 17.2% when incorporated (Li et al., 2022). Combining biochar and feedstock increased their complementary effects resulting in a 32% increase in TC compared to individual applications (Duan et al., 2020). Labile carbon pools, such as POC and POXC benefit from both amendments. Biochar contributes to POC stabilization by protecting it within soil aggregates, enhancing its long-term persistence. In contrast, feedstock provides immediate increases in POC through rapid decomposition. For example, maize feedstock increased POC by 43.8% whereas maize biochar resulted in a 32.3% increase after 3 years of their application in soil (Yang et al., 2017b).

Similarly, POXC concentration increased to 853 mg kg^{-1} in a study combining cow manure with rice feedstock highlighting the role of organic inputs in nutrient cycling and soil aggregation (Kim et al., 2022). Microbial biomass carbon, a key indicator of soil biological activity, responded positively to both feedstock and corresponding biochar. Feedstock provided short term increases in MBC due to its labile carbon content as shown in a study where finely crushed maize feedstock increased MBC by 43-51% (Tejada & Benitez, 2014). On the other hand, biochar contributed to long term MBC stability by improving soil structure and moisture retention, enhancing microbial communities (Li et al., 2022). While the feedstock provides rapid, short-term increases in labile carbon fractions, biochar ensures long-term carbon stability, contributing to sustained soil quality and carbon sequestration. The applications of feedstock and biochar address both immediate and long-term soil health needs, enhancing soil fertility and mitigating climate change by increasing soil carbon stocks and reducing soil carbon depletion.

2.4.4 Effects of feedstock and biochar on soil enzyme activity

Enhancing soil enzyme activity, a critical driver of nutrient cycling and soil quality is also the benefits of feedstock and biochar applications. These amendments promote enzymes involved in organic matter decomposition and nutrient availability, such as dehydrogenase, β-glucosidase, urease and phosphatase. The effectiveness of these increases in enzyme activity depends on the type of amendment, its biochemical composition and application rates. Feedstock applications consistently increase enzyme activities due to their rapid decomposition and provision of labile carbon. For example, finely crushed maize feedstock combined with urea increased dehydrogenase, urease, β-glucosidase and phosphatase activities by 43-51% (Tejada & Benitez, 2014). Similarly, maize feedstock incorporation increased alkaline phosphatase, β-glucosidase and urease activities (Urra et al., 2018). Higher application rates further enhanced these effects as observed with 30% feedstock applications increasing β-glucosidase and acid phosphatase activities (Malobane et al., 2020).

Biochar also positively influences enzyme activity, though its effects vary depending on biochar type and application rate. For instance, wheat biochar increased β-glucosidase and phosphatase activity (Lu et al., 2020), while maize biochar improved urease and alkaline phosphatase activities during critical growth stages compared to feedstock and control treatments (Li et al., 2022). These benefits are attributed to biochar's porous structure, which stabilizes the soil environment, retains moisture and enhances microbial activity and contributes to liming effects that can improve soil pH and enzyme functioning. These findings support the view that feedstock and biochar enhance soil enzyme activity thereby improving nutrient cycling and soil quality.

2.4.5 Effects of feedstock and biochar on selected soil chemical properties

Feedstock and biochar applications significantly improved soil chemical properties including total nitrogen (TN), nitrate (NO_3^--N), ammonium (NH_4^+-N), available phosphorus (P), cation exchange capacity (CEC), exchangeable bases (Ca, Mg, and K) and soil pH. While feedstock offers immediate benefits through rapid decomposition and nutrient release, biochar delivers sustained improvements due to its stability and resistance to microbial degradation. Both amendments enhanced TN levels with feedstock providing rapid increases in the mineral form via organic matter decomposition and biochar ensuring long-term nitrogen retention by reducing leaching. For example, maize feedstock increased TN by 4% (Feng et al., 2022), while biochar applications over three years achieved sustained TN increases compared to single applications (Mierzwa-Hersztek et al., 2017). Combining these amendments with inorganic fertilizers further increased soil TN content as observed in rice husk biochar and fertilizer treatments (Haefele et al., 2011).

Feedstock and biochar also influenced soil NO_3^--N and NH_4^+-N concentrations. Feedstock increased NO_3^--N concentrations through N mineralization. For instance, maize feedstock significantly increased NO_3^--N concentrations (Urra et al., 2018), while wheat biochar combined with nitrogen fertilizer improved NO_3^--N retention (Zhang et al., 2012). Higher feedstock application rates further increased NO_3^--N concentrations (Malobane et al., 2020), supporting sustainable N management. However different patterns were observed with NH_4^+-N, as the application of feedstock or biochar had no significant effect on NH_4^+-N concentration (Hu et al., 2014; Urra et al., 2018). The NH_4^+-N concentrations increased with higher feedstock rates as Malobane et al. (2020) reported an increase in NH_4^+-N with 30% feedstock applications which was the highest feedstock application rate (Malobane et al., 2020). Biochar combined with organic amendments such as cow manure, further enhanced NH_4^+-N concentrations supporting microbial activity and nitrogen stabilization (Bhattacharyya et al., 2012; Kim et al., 2022).

Available P also improved with both amendments, with feedstock application contributing through decomposition and mineralisation (Gura & Mnkeni 2019; Chen et al., 2016), while biochar enhanced P bioavailability by displacing fixed phosphates and adjusting soil pH. For instance, rice husk biochar combined with lime and fertilizer increased available P by 291.53% (Mosharrof et al., 2022). Combining feedstock and biochar with fertilizers also increased P availability (Haefele et al., 2011).

Biochar's high surface area and porous structure improve soil properties by increasing the retention of nutrients and water, while its surface functional groups such as carboxyl and hydroxyl groups contribute to the negative charge that enhances soil CEC and exchangeable bases (Oladele, 2019). Similarly, feedstock contributes humic substances that enhance nutrient retention also resulting in an increase in CEC (Malobane et al., 2020). Combining biochar or feedstock with fertilizers or organic amendments further improved exchangeable base concentrations in the soil (Kim et al., 2022; Mosharrof et al., 2022).

Soil pH improvements were more pronounced with biochar due to its alkaline nature, particularly in acidic soils. For example, rice husk biochar increased pH from 4.90 to 6.84 over three years (Oladele, 2019), while wheat biochar increased pH from 5.4 to 6.0 (Duan et al., 2020). Feedstock slightly increased pH during decomposition, as observed with maize feedstock increasing pH from 7.40 to 7.70 (Yang et al., 2017a). However, in calcareous soils the effects of both amendments were minimal (Urra et al., 2018). Therefore, these findings show that feedstock provides immediate nutrient availability and fertility improvements, while biochar offers long-term benefits by stabilizing nutrients, increasing CEC and adjusting soil pH, with the effects depending on soil types. Applying these amendment strategies to different soil types and combining them with fertilizers or organic amendments can maximize their potential to improve soil quality and support sustainable agricultural systems.

2.4.6 Effects of feedstock and biochar on crop yields and nutrient uptake

Feedstock and biochar applications generally improve soil fertility and nutrient availability enhancing crop yields and nutrient uptake which are key factors that directly influence plant growth. However, this depends on the type of amendment, its biochemical composition, environmental conditions and the soil nutrient status. Feedstock provides immediate benefits through rapid decomposition and nutrient release, while biochar offers long-term advantages by retaining nutrients, improving soil structure and water holding and providing a stable environment for crops. Maize feedstock increased maize yields (Muchelo & Akpa, 2024), while barley feedstock improved cotton lint yields through enhancing soil fertility, microbial activity and nutrient cycling during critical growth stages (Zhao et al., 2024). However, N immobilization during microbial decomposition occasionally reduced yields, as seen with maize feedstock (Yang et al., 2017a). Combining feedstock with N sources, such as urea or green manure, mitigated these effects and improved yields particularly in rice systems (Bhattacharyya et al., 2012).

Biochar has also demonstrated significant yield improvements when applied in the soil. For instance, wheat biochar improved barley yields compared to the feedstock (Duan et al., 2020), and maize biochar applied at 80 Mg ha^{-1} resulted in the highest maize yields by enhancing soil moisture retention and nutrient availability (Lu et al., 2015). Combining biochar with chemical fertilizers further improved crop yields, as seen in rice systems where the combination of biochar and N fertilizer significantly increased rice yields (Zhao et al., 2024). However, limited yield responses were observed in some cases, such as with rice husk biochar application due to its low nutrient content, insufficient application rates or high initial soil fertility (Dubey et al., 2022). Both amendments significantly enhanced nutrient uptake, particularly of N, P and K. Biochar's porous structure minimized nutrient leaching, ensuring consistent availability over time, as demonstrated by increased N and P uptake in wheat and rice (Dubey et al., 2022). However, some studies reported no significant effects of these amendments, which was attributed to site-specific soil properties, the chemical composition of the amendments, and application rates that were insufficient to influence nutrient uptake (Haefele et al., 2011; Mank'Abusi et al., 2024).When combined with lime and fertilizers, biochar further improved uptake of N, P, K, Ca and Mg in maize systems (Mosharrof et al., 2022). Feedstock also improved nutrient uptake by releasing nutrients during decomposition, as observed in maize feedstock applications that enhanced N, P and K uptake in wheat (Arora et al., 2023). Combining either amendment with fertilizers also enhanced nutrient uptake, such as increased N, P and K uptake in rice systems with rice husk biochar or feedstock paired with fertilizers (Haefele et al., 2011).

The application of feedstock and biochar is an effective way to improve crop growth and nutrient uptake. Feedstock quickly provides nutrients that plants can use right away, while biochar helps keep nutrients in the soil in long-term. These results show that adjusting how these materials are used for specific crops and soil types can provide a long-term and sustainable method for managing nutrients and increasing crop yields.

2.5 Conclusion

This systematic review highlighted the potential of raw crop residues (feedstock) and crop residue-derived biochar as sustainable soil amendments that improve soil quality, increase crop productivity and reduce CO_2 emissions. Biochar application in most studies demonstrated reductions in CO_2 emissions, improved soil organic carbon (SOC) stability and promoted long-term carbon sequestration, making it a promising strategy for mitigating agricultural greenhouse gas emissions. Raw crop residues (feedstock), while beneficial in enhancing microbial biomass carbon (MBC) and soil enzyme activity, showed variable effects on CO_2 emissions due to rapid decomposition and microbial priming effects. Both amendments positively influenced soil fertility indicators, including N and P availability and cation exchange capacity, which are critical for crop productivity.

These findings, identify biochar as a superior amendment for long-term soil carbon improvement, whereas feedstock contributes to short-term fertility improvements but may require frequent reapplications. The variability observed in the effectiveness of these amendments can be attributed to differences in residue type, application rate, soil type, climate and management practices. This review highlights the importance of a customized approach in residue and biochar management to maximize benefits for soil health, yield improvement and environmental sustainability. One effective approach is the co-application of biochar with chemical and other organic fertilizers which can enhance nutrient availability and improve crop yields making biochar a valuable solution for soil sustainable fertility management.

2.7 Future research perspectives

This review provides strong evidence of the benefits of raw crop residues on improving soil quality, enhancing crop yield and reducing CO_2 emissions (biochar), another review on the performance of these amendments on different soil types and different environmental conditions globally should be conducted. This broader research base will enable farmers worldwide to make informed decisions about sustainable soil management practices suitable for their local soils, climate and cropping systems. Among the 62 studies included in this systematic review, studies examining residues from different cultivars of the same crop were limited and there was a lack of comparative studies on feedstock and biochar derived from different crop types, such as maize versus sorghum or wheat versus rice. These crops are among the most researched and globally significant staple foods however, their residues and biochar treatments remain underexplored in terms of comparative effects on CO_2 emissions, soil carbon pools, nutrient cycling, enzyme activities and crop yields. Most research focused on single crop types or compared feedstock and biochar within the same crop, often without specifying the cultivar used.

This gap is particularly critical given the distinct biochemical and structural properties of residues from different crop types, which are likely to influence biochar characteristics, decomposition rates and subsequent impacts on soil quality and greenhouse emissions. Future research should prioritize studies that assess the effects of feedstock and biochar from various cultivars of major field crops, with an emphasis on staple cereals like maize and sorghum. This approach will provide valuable insights into which types of residues and biochar treatments yield the most benefits for specific regions. Addressing this gap is a key focus of the experimental studies presented in subsequent chapters, which evaluate the effects of raw feedstock and biochar derived from different cultivars of maize and sorghum on CO_2 emissions, nutrient cycling, soil enzyme activities and crop yields. Such research will help guide farmers in effectively managing their residues to enhance soil carbon pools, reduce greenhouse gas emissions and sustainably improve crop yields.

CHAPTER 3

Cultivar variation is more important than crop type on characteristics of residues and biochar from maize and sorghum

Abstract

Conversion of crop residues to biochar can enhance soil carbon storage and reduce carbon dioxide (CO_2) emissions from agricultural soils, with these effects being influenced by crop type and cultivar, and pyrolysis conditions. This study aimed to characterize residues of different cultivars of maize (SC701 and R201) and sorghum (AS8 and PAN8816) and biochars derived from their pyrolysis in a muffle furnace at 350 and 650°C, and in a kiln (about 400°C). The feedstocks (residues) and biochars were analyzed for proximate composition (moisture content, volatile matter, ash content, and fixed carbon), elemental composition (total carbon, total hydrogen & total nitrogen), and chemical properties (pH, total phosphorus, lignin content, exchangeable bases & cation exchange capacity). Structural characteristics and functional groups were also analyzed using scanning electron microscopy (SEM) and Fourier transform infrared (FTIR) spectroscopy, respectively.

The results demonstrated significant differences in the chemical composition of feedstocks and biochars among different cultivars within each crop. Increasing pyrolysis temperature increased ash content, pH, and total carbon, while reducing yield, volatile matter, total nitrogen, H:C and O:C ratios, as well as hydroxyl and phenolic functional groups. Higher fixed C content was observed in R201-B650 (maize) with a value of 47.13%, and in AS8-B350 (36.47%) and AS8-BKiln (36.31%) (sorghum) biochars, whereas the lowest fixed C content was found in SC701-Feedstock (12.23%) and PAN8816-Feedstock (13.17%). The highest total N content was observed in kiln-produced biochars from SC701 (3.77%) and PAN8816 (4.55%), while the lowest total N content was observed in AS8 feedstock (0.48%).

This study highlights cultivar-specific differences, with minimal crop type effects on feedstock and biochar quality, revealing that biochar from some cultivars and pyrolysis conditions are more appropriate for specific applications in carbon sequestration and soil fertility improvement.

Key words: Biochar, carbon sequestration, functional groups, nutrients, pyrolysis, stability

3.1 Introduction

The need for greater food production with poor management may result in deteriorating soil quality. Conversely, the use of chemical fertilizers to address poor soil fertility may increase the generation of crop residues (Verhulst et al., 2010a). The crop residues produced are mainly from cereals (74%), sugar crops (10%), legumes (8%), tubers (5%) and oilseeds (3%) (Lal, 2005). In Sub-Saharan Africa (SSA), cereal crops are a primary source of energy in human diets and contribute immensely to food security in many households (Hadebe et al., 2017). Maize and sorghum are among the major cereal crops that generate large amounts of biomass (Blanco-Canqui and Lal, 2009). In South Africa, the annual production of maize is approximately 10.2 million tons (Mangani et al., 2019), while approximately 27.5 million tonnes of sorghum are produced across 27.3 million hectares in the Sub-Saharan Africa (SSA) (Bakari et al., 2023). Residues of these crops are used in various ways, including animal fodder, biofuel and surface mulch, while a significant portion is burned to clear fields before planting subsequent crops (Erenstein, 2002). The environmental pollution caused by burning crop residues (suspended particulate matter, smoke, greenhouse gases, soil erosion) calls for adopting their sustainable management. Retention of the residues as a mulch, may enhance soil physical, chemical and biological fertility (Wilhelm et al., 2007; Blanco-Canqui and Lal, 2009).

On average, the commonly grown cereal crops such as maize and sorghum are estimated to contain 40% carbon (C), 0.8% nitrogen (N), 0.1% phosphorus (P) and 1.3% potassium (K) (Adimassu et al., 2005). The incorporation of crop residues into the soil significantly changes the labile pool of soil organic matter, with significant effects on nutrient cycling (Wang et al., 2005). Crop residue retention is considered a cost-effective strategy to mitigate greenhouse gas (GHG) emissions from agriculture (Venkateswaran et al., 2021). However, their conversion into biochar could be even more beneficial due to its potential to resist microbial degradation, mitigating GHG emissions leading to long-term C sequestration. Production of biochar transforms agricultural waste into valuable assets and recovers up to 50% of biomass carbon compared to only 3% from open burning and less than 10-20% from 5-10 years of biomass decomposition (Baldock and Smernik, 2002).

Biochars are distinguished by their porous structures and high surface areas, which enhance their capacity for adsorbing and retaining moisture and nutrients in soil. There is considerable interest in applying biochar into the soil as a long-term carbon sink, thereby mitigating climate change

(Prayogo et al., 2014). The remarkable stability of biochar, potentially persisting for hundreds to thousands of years, makes it an ideal approach for carbon sequestration, thereby reducing emissions to the atmosphere (Lehmann and Joseph, 2024). The positive effects of biochar have been reported to depend on the physical and chemical properties of the resource (biochar), which depend on pyrolysis conditions and the type of feedstock used (Downie et al., 2009; Mimmo et al., 2014).

Biochar produced at higher pyrolysis temperatures is characterized by a greater degree of carbon aromaticity, being more depleted of hydrogen and oxygen (Heitkötter and Marschner, 2015), which enhances chemical recalcitrance and resistance to degradation in soil (Li and Chen, 2018). Moreover, higher pyrolysis temperature generally leads to increased biochar pH, electrical conductivity, ash content, exchangeable cations (Dai et al., 2014) and decreased biochar yields, acidic functional group concentrations, volatile matter content and cation exchange capacity (Novak et al., 2009). The differences in moisture content, lignin, cellulose, hemicellulose content, carbon content, macro-nutrients and micro-nutrients between feedstocks (Whitbread et al., 2003), could impact the composition of the resulting biochars.

The chemical composition of maize and sorghum, which are the most widely cultivated cereal crops, varies not only between the two crops but also among the cultivars of each crop (He et al., 2020; Hassan et al., 2019). These differences can significantly impact their suitability for biochar production. For example, Coulibally et al. (2020) reported that maize feedstock contained 47.6% total C, 0.55% total N, and a C:N ratio of 85.9 while sorghum feedstock contained 33.1% total C, 0.27% total N and a much higher C:N ratio of 122. In addition, significant variability exists within cultivars of the same crop. For instance, Sandhu et al. (2022) reported that maize residues contained 42.3% total C, 0.54% total N and a C:N ratio of 84, while Verachtert et al. (2009) reported 36.8% total C, 1.04% total N and a C:N ratio of 35.38. Similarly, Anguria et al. (2017) reported that sorghum residues exhibited total C and total N contents of 32.1 and 2.77%, respectively, with a C:N ratio of 11.5, while Jung et al. (2013) reported 42.8% total C, 1.32% total N and a C:N ratio of 31. These studies did not specify the cultivar names but referred only to the crop type. The chemical composition of maize and sorghum residues were different from those reported by Coulibally et al. (2020), with no clear trend between the two crops, but strongly suggests that these differences could be due to cultivar-specific characteristics. Understanding these variations is crucial for improving feedstock selection and adapting applications such as carbon sequestration, biochar production and soil fertility management.

The application of biochar has the potential to enhance soil fertility and promote crop growth through modifications to soil physicochemical properties (Glaser et al., 2002; Lehmann et al., 2006; Yamato et al., 2006). Most studies have focused only on the effect of pyrolysis temperature on a single crop or on different crop types (Purakayastha et al., 2015; Zhang et al., 2020). Studies comparing the effects of pyrolysis conditions and feedstock from different cultivars of the same crop are lacking, especially for maize and sorghum, which are the most important cereal crops in Sub-Saharan Africa. Therefore, the objective of this study was to investigate the effects of different pyrolysis temperatures on characteristics of biochar derived from different cultivars of maize and sorghum.

3.2 Materials and methods

3.2.1 Biomass and feedstock production

This study utilized two different cultivars for each crop between maize and sorghum (Table 3.1), which were planted on 436 m^2 plots (one plot per cultivar) at the Ukulinga Research Farm at the University of KwaZulu-Natal (located at 29.667° S latitude, 30.406° E longitude, and 811 m altitude). The region's long-term average temperature and rainfall are 18°C and 738 mm, respectively. The soil had a loamy texture (30% sand, 34.9% silt, 24.4% clay), classified as Westleigh soil form (Soil Classification Working Group, 1991), which translated to Luvisols (IUSS Working Group WRB, 2014), with 1.9% total C, 0.17% total N, 8.87 mg kg^{-1} available P, 1043 mg kg^{-1} Ca, 313.7 mg kg^{-1} Mg, 91.94 mg kg^{-1} K and a pH_{KCl} of 4.73. The field was ploughed and the crops grown with basal fertilizer (NPK 2:3:4) applied at the recommended rates during the summer season (December 2020 to April 2021). Upon reaching maturity, the above-ground biomass of each cultivar was harvested, chopped, dried and milled to pass through a 2 mm sieve.

Table 3.1: A list of maize and sorghum cultivars used in the study

Crop Type	Cultivar Name	Source	Attributes
Maize	SC701	McDONALDS Seeds, South Africa	Commercial
Maize	R201	Zimbabwe	Commercial
Sorghum	PAN8816	PANNA Seeds, South Africa	Commercial
Sorghum	AS8	ACCI	Experimental Hybrid

ACCI=African Centre for Crop Improvement

3.2.2 Biochar production

Biochars were produced from the milled feedstocks of the maize and sorghum cultivars using two different methods. Known masses of the milled feedstocks were pyrolyzed in a muffle furnace at either 350 or 650°C, with a heating rate of 10°C/min for 2 hours, to produce biochars (Vilakazi et al., 2023). Other biochars were produced at an average pyrolysis temperature was about 400°C (Kekana and Zungu, 2013), for 3 hours using an on-site kiln located at the Agricultural Engineering Section at Ukulinga Research Farm. After cooling, the biochars were weighed and their yields were calculated using Equation (3.1). The biochars were stored in airtight plastic containers and labelled according to their pyrolysis conditions: B350 (pyrolyzed at 350°C), B650 (pyrolyzed at 650°C) and BKiln (pyrolyzed with on-site kiln). All the products (treatments) used in the study (Table 3.2) were characterized as described in the subsequent sections.

$$\textbf{Yield (\%)} = \frac{Mass\ of\ biochar\ (g)}{Mass\ of\ raw\ feedstock\ (g)} \times 100 \qquad (3.1)$$

Table 3.2: Description of different treatments used in the study

Treatment	Description
AS8-R	Sorghum experimental hybrid cultivar AS8 feedstock/residues
PAN8816-R	Sorghum commercial cultivar PAN8816 feedstock/residues
R201-R	Maize commercial cultivar R201 feedstock/residues
SC701-R	Maize commercial cultivar SC701 feedstock/residues
AS8-B350	Sorghum experimental hybrid cultivar AS8 biochar produced 350ºC
PAN8816-B350	Sorghum commercial cultivar PAN8816 biochar produced 350ºC
R201-B350	Maize commercial cultivar R201 biochar produced 350ºC
SC701-B350	Maize commercial cultivar SC701 biochar produced 350ºC
AS8-B650	Sorghum experimental hybrid cultivar AS8 biochar produced 650ºC
PAN8816-B650	Sorghum commercial cultivar PAN8816 biochar produced 650ºC
R201-B650	Maize commercial cultivar R201 biochar produced 650ºC
SC701-B650	Maize commercial cultivar SC701 biochar produced 650ºC
AS8-BKiln	Sorghum experimental hybrid cultivar AS8 biochar produced in kiln
PAN8816-BKiln	Sorghum commercial cultivar PAN8816 biochar produced in kiln
R201-BKiln	Maize commercial cultivar R201 biochar produced in kiln
SC701-BKiln	Maize commercial cultivar SC701 biochar produced in kiln

3.2.3 Proximate analysis

Proximate analysis was conducted using the American Society for Testing and Materials (ASTM) standard 1762-84 protocol (Vilakazi et al., 2023). The analysis included determination of volatile matter, ash content, moisture content and fixed carbon. Moisture content was determined by oven-drying 1 g of air-dried feedstock and biochar samples at 105°C for 2 hours. The percentage moisture content was calculated using Equation 3.2. Volatile matter was determined by heating 1 g of oven-dried feedstock and biochar samples in a muffle furnace at 950°C for 6 minutes. The volatile matter content was calculated using Equation 3.3. Ash content was determined by heating 1 g of oven dried feedstock and biochar sample in a muffle furnace at 750ºC for 6 hours. Ash content (Equation 3.4) and Fixed C (Equation 3.5) were then calculated (Vilakazi et al. 2023).

$$\textbf{Moisture (\%)} = \frac{Mass\ of\ air\ dried\ sample\ (g) - Mass\ of\ oven\ dried\ sample\ (g)}{Mass\ of\ air\ dried\ sample\ (g)} \times 100 \quad (3.2)$$

$$\textbf{Volatile Matter (\%)} = \frac{Mass\ of\ oven\ dried\ sample\ (g) - Mass\ of\ sample\ heated\ at\ 950oC\ (g)}{Mass\ of\ oven\ dried\ sample\ (g)} \times 100 \quad (3.3)$$

$$\textbf{Ash content (\%)} = \frac{Mass\ of\ sample\ heated\ at\ 750oC\ (g)}{Mass\ of\ oven\ dried\ sample\ (g)} \times 100 \quad (3.4)$$

Fixed C (%) = 100 -Volatile matter content (%)-Ash content (%) (3.5)

3.2.4 Elemental and other chemical analyses of feedstock and biochar

Total C and N of the feedstocks and biochars were determined by dry combustion with the Leco Trumac (CNS) autoanalyser (LECO Corporation, 3000 Lakeview Ave, ST. Joseph, MI, USA), using 0.2 g of ground sample (< 250 µm). Total hydrogen (H) was analyzed using the Leco CHS elemental analyzer. Total oxygen (O) was calculated using Equation 3.6 (Vilakazi et al., 2023).

Total O (%) = 100 – [Total C (%) + Total H (%) + Total N (%) + Ash (%)] (3.6)

The pH was measured in 1 M KCl (1:10; feedstock/biochar: solution) using a pH meter (Enders et al., 2012). Total phosphorus (TP) was determined through digestion with sulphuric acid and hydrogen peroxide on a hot plate without pH adjustment, followed by analysis using ascorbic acid (Okalebo et al., 2002). Lignin content was determined for feedstock only using the Van Soest method (Van Soest et al., 1991). Exchangeable bases and cation exchange capacity (CEC) were determined using 1M ammonium acetate (NH_4OAC) at pH 7 (Okalebo et al., 2002; Anderson & Ingram, 1994).

3.2.5 Surface functional groups

Surface functional groups of the feedstock and biochar were determined using Fourier Transform Infrared (FTIR) spectroscopy, as described by Vilakazi et al. (2023). The FTIR spectra in the mid-infrared range of 4000 to 400 cm^{-1} were used to assign chemical functional groups to the corresponding wave numbers (Coates, 2000).

3.2.6 Surface morphology characteristics

The surface morphology of the feedstock and biochar was examined using Scanning Electron Microscopy (SEM), with the SEM images captured using an EVO LS15 microscope (Carl Zeiss Microscopy, New York, USA). To enhance conductivity, the feedstock and biochar samples were mounted on black conductive adhesive tape and coated with gold using a gold sputtering machine (Quorum Q150R ES, Quorum Technologies, East Sussex, UK). The magnified images were then viewed and recorded.

3.2.7 Statistical Analysis

The proximate, elemental and chemical characteristics of the feedstock and biochar from the cultivars of maize and sorghum were analyzed using GenStat 20th edition (Payne et al., 2012). A two-way analysis of variance (ANOVA) was conducted to determine significant differences among treatments, including type of feedstock (crop type x cultivar combinations), pyrolysis conditions and interactions between feedstock type and pyrolysis temperature. The combinations of crop type and cultivar were used as the feedstock factor. The means were compared and further separated using the least significant difference (LSD) at $p < 0.05$ by the Tukey-HS test. A principal component analysis (PCA) using OriginPro 2024 (OriginLab Corporation, Northampton, USA) was conducted to study relationships among the variables. The PCA biplots were used to visualize associations between treatments and measured parameters. Vectors positioned closely or parallel to each other indicated strong positive associations, while those in opposite directions (180^o) represented strong negative associations. Perpendicular vectors (90^o) represented weak or no correlation between variables.

3.3 Results

3.3.1 Effects of feedstock type and pyrolysis temperature on yield and moisture of biochar

There were significant effects of feedstock and pyrolysis temperature on biochar yield ($p<0.001$). Pyrolysis temperature was the primary determinant of biochar yield than crop type and cultivar (Figure 3.1A). For sorghum cultivar AS8, the highest biochar yield was obtained at B-350, while the yield at B-650 was lower but not significantly different from the yield produced in the B-Kiln. Similarly, sorghum cultivar PAN8816 produced its highest yield at B-350, though this yield did not significantly differ from that of B-Kiln and yield from B-Kiln did not significantly differ from B-650 yield. For maize cultivar R201, there were no significant differences in biochar yield among all treatments. Lastly the maize cultivar SC701 had the highest yield at B-350 while the yield at B-650 was lower but not significantly different from B-Kiln yield (Figure 3.1A). For each pyrolysis temperature/condition, there were no differences between the maize and sorghum cultivars.

Moisture content was significantly influenced by pyrolysis temperature and cultivar specific differences rather than crop type ($p<0.001$). It significantly decreased with increasing pyrolysis temperature across all the cultivars. The trend was: Feedstock > Biochar at 350°C > Biochar produced by Kiln > Biochar at 650°C, across all cultivars (Figure 3.1B). Among the maize cultivars, the treatment with feedstock of R201 had the highest moisture content (12.79%), while biochar of cultivar SC701 pyrolyzed at 650ºC was lowest (2.90%) (Figure 3.1B). In sorghum cultivars feedstock of PAN8816 (11.11%) had the highest while biochar of AS8 pyrolyzed at 650ºC had lowest moisture content (2.08%) (Figure 3.1B).

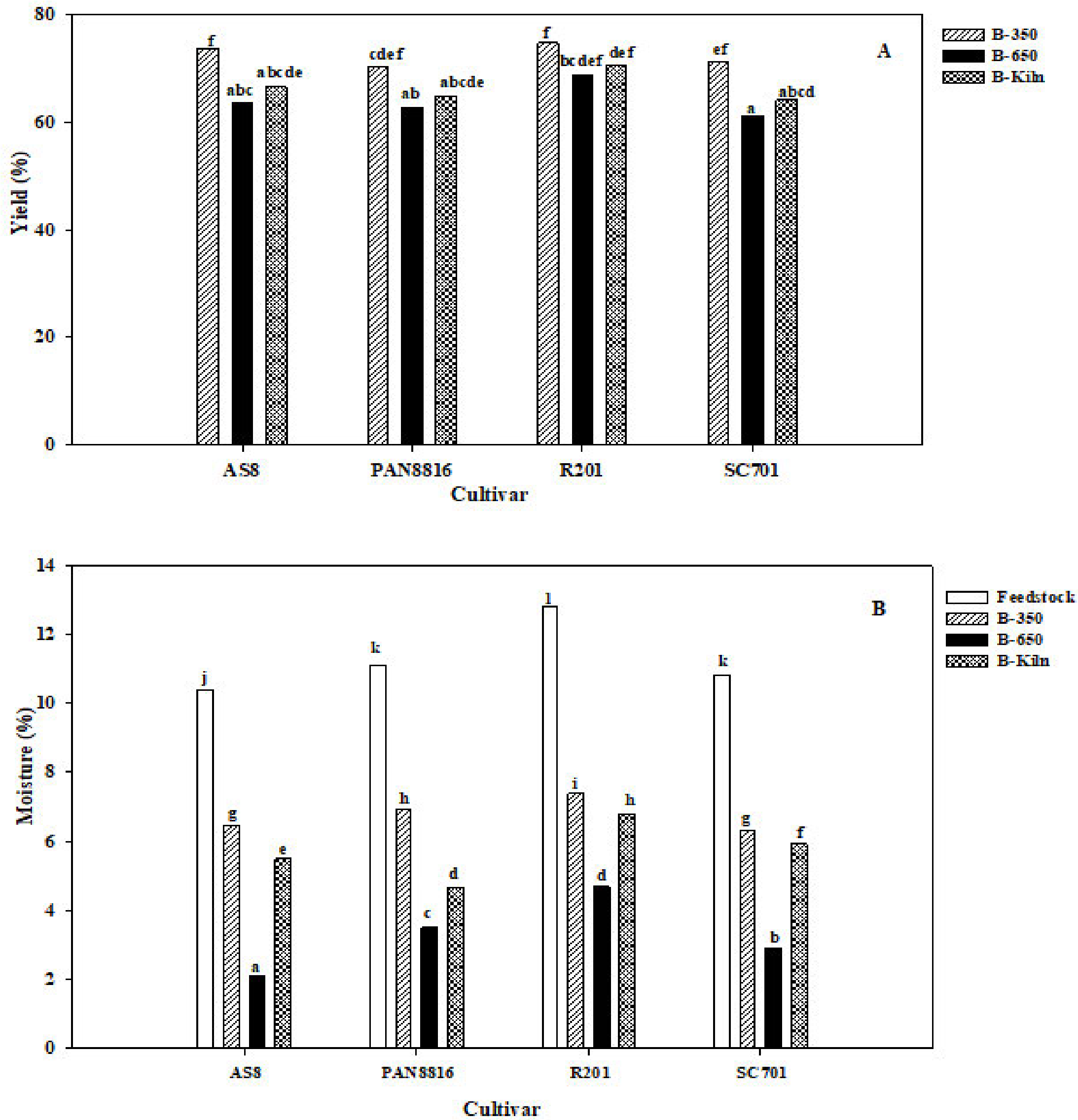

Figure 3.1: Effects of feedstock type and pyrolysis temperature on (A) yield and (B) moisture content. Bars within each sub-figure (A or B) that share the same letter are not significantly different at $p \leq 0.05$.

3.3.2 Effects of feedstock type and pyrolysis temperature on ash content, volatile matter and fixed carbon

The ash content, volatile matter, and fixed C of biochar were significantly influenced by cultivar and pyrolysis temperature but not crop type ($p<0.001$). Fixed C content was lower in feedstock treatments and increased in biochars with no consistent order across all cultivars. Amongst the maize cultivars, R201-B650 had highest fixed C (47.13%) while SC701 feedstock had the lowest (12.23%). For sorghum the AS8-B350 (36.47%) and AS8-BKiln (36.31%) treatments exhibited higher fixed C content while feedstock of both AS8 (14.10%) and PAN8816 (13.17%) had lowest fixed C content (Figure 3.2A). Ash content increased with increasing pyrolysis temperature and differed significantly among treatments ($p<0.001$). The order of ash content was consistent across cultivars with B650>B-Kiln>B350>Feedstock (Figure 3.2B). Among the maize cultivars ash content was lowest in feedstock of the R201 cultivar (6.50%), while the highest was for SC701-B650 (46.13%) (Figure 3.2B). For sorghum, the lowest ash content was observed in AS8 feedstock (11.48%) while the highest was in PAN8816-B650 (41.82%) (Figure 3.2B). In contrast to ash content, volatile matter exhibited an opposite trend, decreasing with increasing pyrolysis temperature and showing significant cultivar differences ($p<0.001$). It followed the order B650<B-Kiln<B350<Feedstock across cultivars (Figure 3.2C). Amongst the maize cultivars, volatile matter was highest in feedstock of R201 (76.27%) while SC701-B650 exhibited lowest (27.28%). For sorghum cultivars, volatile matter was highest in feedstock of AS8 (74.42%) while PAN8816-B650 (30.66%) exhibited the lowest (Figure 3.2C).

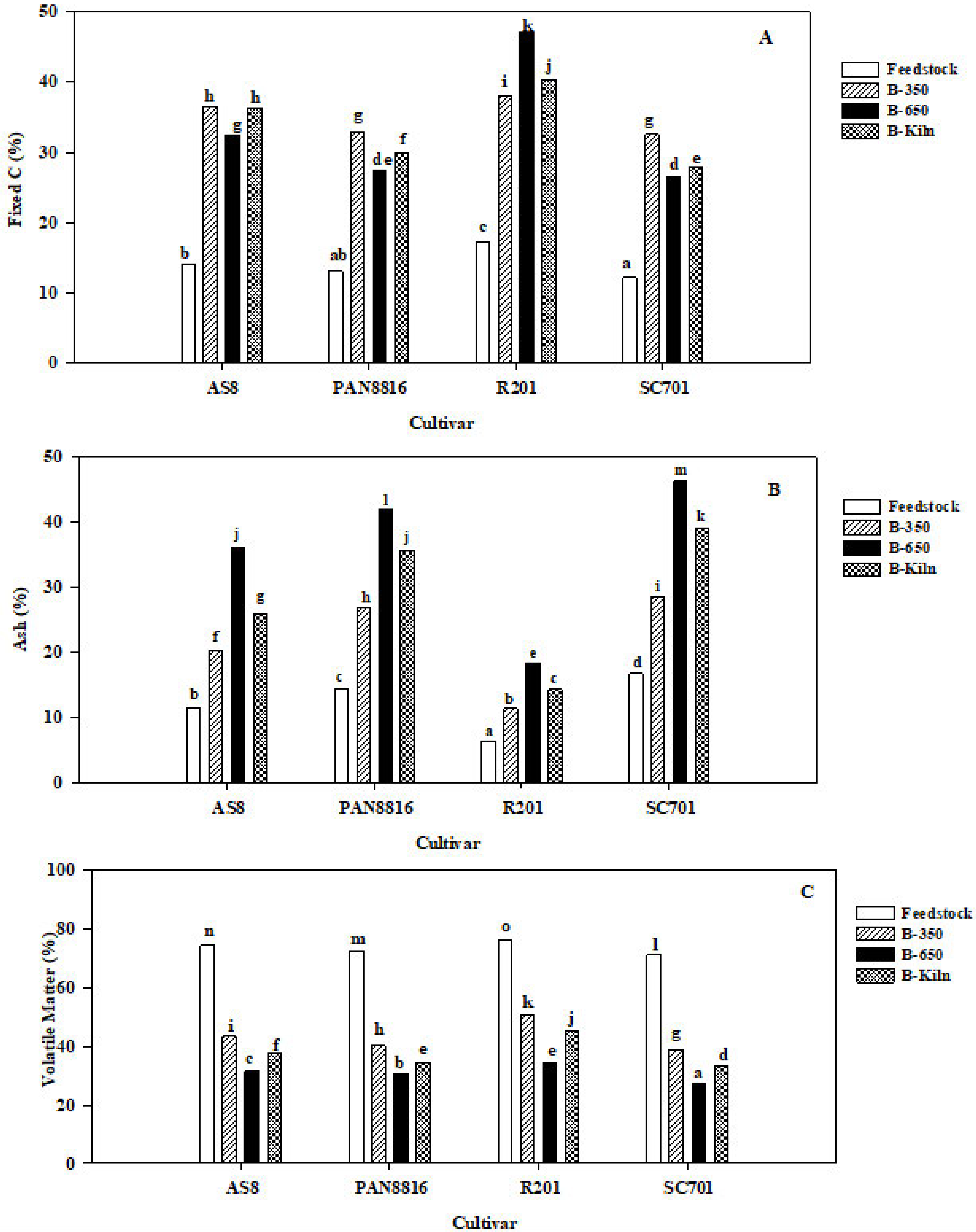

Figure 3.2: Effects of feedstock type and pyrolysis temperature on Fixed C (A), Ash content (B) and Volatile matter content (C). Bars within each sub-figure (A, B, or C) that share the same letter are not significantly different at $p \leq 0.05$.

3.3.3 Feedstock and pyrolysis temperature effects on total carbon, nitrogen and phosphorus

Total C, N and P were significantly affected by pyrolysis temperature and cultivar specific differences ($p<0.001$, Figure 3.3). The total C in all feedstock treatments did not significantly differ (Figure 3A) but was lower than in biochars of the different materials. The total C in the biochars increased with increasing pyrolysis temperature for sorghum cultivar AS8 but not the other biochars (Figure 3.3A). Total N content in feedstock and biochar pyrolyzed at 650ºC did not differ except for maize cultivar R201 and was lower than the other biochars. The biochars produced with the kiln had higher total N than the other biochars, except for that produced from R201. Amongst maize cultivars total N was highest in SC701-BKiln (3.77%) and was lowest in R201 feedstock (0.32%), while for sorghum cultivars it was highest in PAN8816-BKiln (4.55%) and lowest in AS8 feedstock (0.48%) and AS8-B650 (0.69%) (Figure 3.3B). In contrast, total P content increased with decreasing pyrolysis temperature, with biochar treatments having higher total P content than their corresponding feedstocks. The order of total P content was generally Feedstock<B650<B-Kiln<B350, except for cultivar R201, which showed no significant difference, across all pyrolysis temperatures. Among maize cultivars SC701 feedstock had lower total P content (0.38%) while all biochar from R201 did not significantly differ and had higher total P content. In sorghum cultivars AS8 (0.34%) and PAN8816 (0.33%) feedstocks had lower total P while both B350 sorghum treatments were higher (0.62% P) and did not significantly differ (Figure 3.3C).

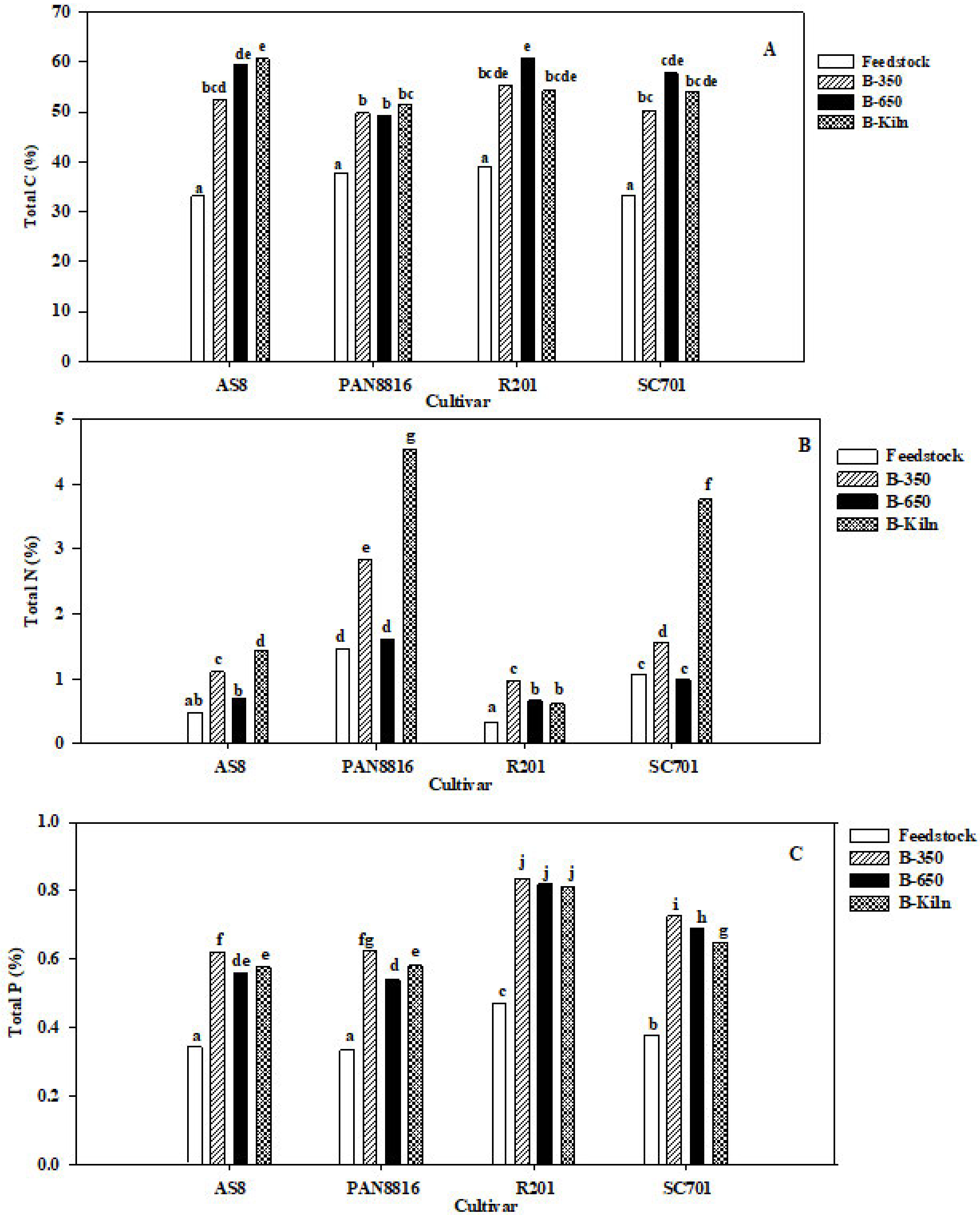

Figure 3.3: Effects of feedstock type and pyrolysis temperature on Total C (A), Total N (B) and Total P content (C). Bars within each sub-figure (A, B, or C) that share the same letter are not significantly different at $p \leq 0.05$.

3.3.4 Feedstock and pyrolysis temperature effects on elemental ratios, lignin and pH

The C: N, C: P, H: C and O: C ratios were influenced by cultivar specific differences and pyrolysis temperatures (Table 3.3). The C:N ratio showed a slight decrease from feedstock to biochar 350°C followed by a slight increase at 650°C. In maize cultivars it was higher in R201 feedstock (153.3) which did not significantly differ from R201-B650 (92.25) and R201-BKiln (88.82) while all other treatments (including those from sorghum) did not significantly differ. The C:P ratio did not significantly differ amongst maize treatments while for sorghum cultivars, it was higher in PAN8816 feedstock (113.05), which was not significantly different to AS8 feedstock (96.38), AS8-B650 (107.06), PAN8816-B650 (91.37) and AS8-BKiln (105.13) and all other treatments did not significantly differ (Table 3.3). The H:C ratio decreased with increasing pyrolysis temperature, in the order feedstock > B350 = B kiln > B650, without differences between crop types and cultivars for each pyrolysis condition. Similarly, the O:C ratio also decreased with increasing pyrolysis temperature, with higher values in feedstock treatments and lower values in biochar treatments for both maize and sorghum.

The pH of feedstock and biochar from different maize and sorghum cultivars was significantly influenced by cultivar specific differences and pyrolysis temperature than crop type ($p<0.001$, Table 3.3). It increased with increasing pyrolysis temperature. In most treatments, the pH followed the order of feedstock < B350 < BKiln < B650, ranging from 4.26 in AS8-R to 11.4 in SC701-B650 (Figure Table 3.3). In maize cultivars pH was highest in SC701-B650 and lowest in R201 feedstock (5.09). While for sorghum cultivars it was higher in AS8-B650 (10.58) and was lowest in AS8 feedstock (4.26) (Table 3.3).

Table 3.3: Elemental ratios, pH and lignin content of feedstock and biochar produced from different cultivars of maize and sorghum at different pyrolysis temperatures

Crop Type	Cultivar	Residue Type	Pyrolysis (°C)	C:N	C:P	H:C	O:C	pH	Lignin:N	Lignin (%)
Maize	R201	Feedstock	N/A	153.3^{e}	82.76^{abcd}	0.14^{d}	1.26^{d}	5.09^{b}	153.28^{b}	38.57^{d}
	SC701	Feedstock	N/A	31.08^{abcd}	88.42^{abcde}	0.17^{e}	1.30^{d}	6.75^{d}	3.9^{a}	4.18^{a}
	R201	Biochar	350	57.02^{abcd}	66.19^{a}	0.06^{bc}	0.52^{c}	9.46^{g}		
	SC701	Biochar	350	32.11^{abcd}	69.23^{ab}	0.08^{c}	0.31^{abc}	9.75^{i}		
	R201	Biochar	650	92.25^{de}	74^{abc}	0.02^{a}	0.32^{abc}	10.62^{n}		
	SC701	Biochar	650	58.42^{abcd}	83.74^{abcd}	0.03^{a}	0.00^{a}	11.4^{p}		
	R201	Biochar	Kiln	88.82^{cde}	66.88^{a}	0.06^{bc}	0.51^{c}	9.92^{j}		
	SC701	Biochar	Kiln	14.36^{ab}	83.08^{abcd}	0.07^{c}	0.00^{a}	10.86^{o}		
Sorghum	AS8	Feedstock	N/A	68.87^{abcd}	96.34^{cdef}	0.17^{e}	1.49^{d}	4.26^{a}	51.65^{ab}	24.79^{c}
	PAN8816	Feedstock	N/A	25.78^{abcd}	113.05^{f}	0.12^{d}	1.20^{d}	5.82^{c}	6.99^{a}	10.21^{b}
	AS8	Biochar	350	47.49^{abcd}	84.69^{abcde}	0.08^{c}	0.42^{bc}	9.57^{h}		
	PAN8816	Biochar	350	17.5^{abc}	79.81^{abc}	0.06^{bc}	0.35^{abc}	9.09^{f}		
	AS8	Biochar	650	86^{bcde}	107.06^{ef}	0.04^{ab}	0.02^{a}	10.58^{m}		
	PAN8816	Biochar	650	30.72^{abcd}	91.37^{bcdef}	0.03^{a}	0.12^{ab}	10.44^{l}		
	AS8	Biochar	Kiln	42.41^{abcd}	105.13^{def}	0.08^{c}	0.12^{ab}	8.04^{e}		
	PAN8816	Biochar	Kiln	11.34^{a}	88.77^{abcde}	0.07^{bc}	0.09^{ab}	10.10^{k}		
LSD(p<0.05)				39.83	12.43	0.014	0.21	0.02	72.83	1.89

Values on the same column with different letters are significantly different ($p<0.05$).

3.3.5 Effects of feedstock type and pyrolysis temperature on exchangeable bases and CEC

Exchangeable bases and CEC were more influenced by cultivar specific differences and pyrolysis temperature than crop type ($p<0.001$, Table 3.4). However, exchangeable Ca did not exhibit a consistent trend with increasing pyrolysis temperatures across the cultivars. Amongst the maize cultivars exchangeable Ca was highest in R201 feedstock (7.43 cmolc kg^{-1}) and lowest in R201-BKiln (1.61 cmolc kg^{-1}), while for sorghum it was highest for PAN8816-B650 (4.60 cmolc kg^{-1}) and lowest in AS8-BKiln (1.21 cmolc kg^{-1}). Exchangeable Mg decreased with increasing pyrolysis temperature for maize cultivars whereas in sorghum cultivars, B350 treatments exhibited higher exchangeable Mg while B650 and BKiln biochars showed no significant differences. In maize cultivars Mg concentration was highest in R201 feedstock (1.96 cmolc kg^{-1}) and lowest in SC701-BKiln (1.10 cmolc kg^{-1}), R201-B650 (1.15 cmolc kg^{-1}) and SC701-B650 (1.21 cmolc kg^{-1}). For sorghum cultivars exchangeable Mg was highest in AS8-B350 (2.02 cmolc kg^{-1}) and PAN8816-B350 (1.99 cmolc kg^{-1}) and lowest in AS8-BKiln (0.63 cmolc kg^{-1}). In maize cultivars, exchangeable K was higher in feedstocks than in biochars. In sorghum cultivars it was higher in PAN8816-B350 (3.73 cmolc kg^{-1}) and PAN8816 feedstock (3.60 cmolc kg^{-1}) and was lower in AS8 feedstock (0.87 cmolc kg^{-1}). The CEC for maize cultivars was highest in R201 feedstock (14.91 cmolc kg^{-1}) and was lowest in SC701-BKiln (5.47 cmolc kg^{-1}) while for sorghum cultivars it was higher in PAN8816-B650 (17.24 cmolc kg^{-1}) and was lowest in AS8-BKiln (3.98 cmolc kg^{-1}) (Table 3.4).

Table 3.4: Exchangeable bases and CEC of feedstock and biochar from different cultivars of maize and sorghum

Crop Type	Cultivar	Residue Type	Pyrolysis	Ca	Mg	K	CEC
			°C	**cmolc kg^{-1}**			
Maize	R201	Feedstock	N/A	7.43^{k}	1.96^{g}	4.70^{i}	14.91^{k}
	SC701	Feedstock	N/A	3.21^{h}	1.57^{e}	3.17^{f}	8.65^{i}
	R201	Biochar	350	2.71^{ef}	1.49^{e}	2.14^{d}	7.10^{f}
	SC701	Biochar	350	3.30^{hi}	1.75^{f}	2.29^{de}	8.21^{h}
	R201	Biochar	650	2.07^{c}	1.15^{cd}	2.19^{d}	6.42^{e}
	SC701	Biochar	650	2.49^{d}	1.21^{cd}	1.89^{cd}	6.17^{e}
	R201	Biochar	Kiln	1.61^{b}	1.51^{e}	2.23^{d}	7.72^{g}
	SC701	Biochar	Kiln	3.48^{i}	1.10^{cd}	2.21^{d}	5.47^{c}
Sorghum	AS8	Feedstock	N/A	2.60^{de}	0.91^{b}	0.87^{a}	5.11^{b}
	PAN8816	Feedstock	N/A	2.90^{fg}	1.04^{bc}	3.60^{gh}	8.08^{h}
	AS8	Biochar	350	3.28^{hi}	2.02^{g}	2.69^{e}	8.79^{i}
	PAN8816	Biochar	350	4.47^{j}	1.99^{g}	3.73^{h}	11.07^{j}
	AS8	Biochar	650	2.00^{c}	1.24^{d}	1.98^{cd}	5.85^{d}
	PAN8816	Biochar	650	4.60^{j}	1.04^{bc}	3.30^{fg}	17.24^{l}
	AS8	Biochar	Kiln	1.21^{a}	0.63^{a}	1.64^{bc}	3.98^{a}
	PAN8816	Biochar	Kiln	3.10^{gh}	1.20^{cd}	1.34^{b}	6.87^{f}
LSD($p<0.05$)				0.11	0.1	0.23	0.16

Values on the same column with different letters are significantly different ($p<0.05$)

3.3.6 Feedstock and pyrolysis temperature effects on functional groups and physical structures

The chemical functional groups of various cultivars and their corresponding biochar are presented in Tables 3.5 (maize) and 3.6 (sorghum). Both feedstock and biochar exhibited a range of functional groups, including carboxyl, aromatic, ether, phosphate and nitrogen-containing groups. The spectral absorption patterns for different maize and sorghum cultivars and their biochar were similar, with minimal cultivar-specific effects and differences. Instead, the pyrolysis temperature had a more pronounced impact on the functional groups present. The feedstock of various maize and sorghum cultivars exhibited a characteristic band at 3640-3530 cm^{-1} attributed to the O-H stretching of phenolic groups. Furthermore, the FTIR spectra of all maize and sorghum cultivars and their corresponding biochars, produced at 350°C and from the Kiln, displayed bands at 3570-3200 cm^{-1} and 3000-2500 cm^{-1}, which corresponded to the O-H stretching of hydroxyl groups and O-H stretching of carboxylic acid groups, respectively and another band between 1100-1000 cm^{-1}, indicative of the presence of phosphate ions.

Additionally, biochars produced at 650°C and by Kiln displayed a band at 3130-3070 cm^{-1}, corresponding to the aromatic C-H stretch. In contrast, biochars produced at 350°C and by Kiln showed a band at 2930-2850 cm^{-1}, attributed to the stretching of aliphatic groups. Biochars produced at 650°C exhibited absorption bands at 2000-1750 cm^{-1}, 1650-1550 cm^{-1}, and 680-610 cm^{-1}, which corresponded to aromatic C=C stretching, N-H bending of secondary amines, and alkyne bending, respectively. All biochars displayed a band at 900-670 cm^{-1}, attributed to the C-H bending of aromatic C. Organic nitrates (SC701-R), ammonium ion (residue & biochar from kiln for SC701), carbonate ions (all biochars from R201), phosphate ion (residues and biochars at 350°C for both maize cultivars), C-H stretching (all treatments except biochars at 650°C for both maize cultivars), O-H tertiary alcohol bending (residues and all biochars from R201) and C-O stretch alkyl-substituted ether (biochars at 650°C and from kiln) were only present in some of the products from maize residues but not those of sorghum.

Table 3.5: Functional groups observed in the FTIR spectra of feedstock and biochar of different cultivars of maize

Wave Number (cm^{-1})	Functional Group	R201 -R	R201 -B350	R201 -B650	R201 -Bkiln	SC701 -R	SC701 -B350	SC701 -B650	SC701 -Bkiln
3640-3530	O-H Stretching, Phenols	3638.7				3542.33			
3570-3200	O-H Stretching, Hydroxyl group	3267.54	3279.46		3258.76	3345.37	3447.5		3238.45
3130-3070	Aromatic C-H Stretch			3104.59	3087.28			3122.42	3091.91
3000-2500	O-H Stretching of Carboxylic Acid	2952.41	2997.63		2964.95	2920.26	2928.55		2995.23
2930-2850	C-H Stretching of Aliphatic group		2848.25		2842.31		2923.85		2910.57
2000-1750	Aromatic C=C Stretching			1906.93				1911.49	
1700-1500	C-C Bending of Aromatic C	1682.42	1649.56	1597.77	1553.22	1668.45	1632.66	1611.34	1579.67
1650-1550	N-H Bending of Secondary Amine			1632.42			1570.8	1614.59	
1640-1620	Organic Nitrates					1633.34			
1650-1580	Aromatic ring C=C Stretching		1631.42	1642.85	1598.17		1624.28	1639.62	1593.49
1490-1410	Carbonate ions		1421.15	1430.49	1425.82				
1490-1455	C-H Stretching	1482.35	1464.63		1458.56	1471.37	1456.44		1459.88
1430-1390	Ammonium ion					1417.81			1400.45
1410-1310	O-H Tertiary Alcohol Bending	1393.86	1380.2	1399.48	1400.01				
1380-1350	Nitrate ion					1375.24	1379.31		
1270-1230	Aromatic Ethers, Aryl-O Stretch					1248.38			
1150-1050	C-O Stretch Alkyl-Substituted Ether			1123.87	1074.21			1121.63	1089.45
1100-1000	Phosphate ion	1084.59	1042.28			1067.42	1028.91		
1050-990	Aliphatic Phosphate	1030.73				1030.66			
995-850	Aromatic Phosphates						989.69		
900-670	C-H bending of Aromatic C		789.06	878.25	789.47		794.07	878.68	795.43
680-610	Alkyne Bending			662.13				657.42	

Table 3.6: Functional groups observed in the FTIR spectra of feedstock and biochar of different cultivars of sorghum

Wave Number (cm^{-1})	Functional Group	AS8 -R	AS8 -B350	AS8 -B650	AS8 -Bkiln	PAN8816 -R	PAN8816 -B350	PAN8816 -B650	PAN8816 -Bkiln
3640-3530	O-H Stretching, Phenols	3629.17				3591.42			
3570-3200	O-H Stretching, Hydroxyl group	3534.92	3269.15		3261.93	3541.49	3256.17		3238.49
3130-3070	Aromatic C-H Stretch			3117.93	3089.15			3122.49	3097.82
3000-2500	O-H Stretching of Carboxylic Acid	2971.49	2634.17		2514.28	2889.62	2592.85		2481.19
2930-2850	C-H Stretching of the Aliphatic group	2919.28	2884.59			2905.49	2867.43		
2000-1750	Aromatic C=C Stretching			1928.49				1921.17	
1700-1500	C-C Bending of Aromatic C		1642.59	1578.28	1691.49		1532.82	1665.17	1589.93
1650-1550	N-H Bending of Secondary Amine			1621.49				1617.28	
1650-1580	Aromatic ring C=C Stretching			1624.19	1592.85			1641.46	1607.17
1510 – 1450	C=C-C ring stretching of aromatic C		1489.62	1503.85	1497.29		1462.17	1500.82	1475.49
1380-1350	Nitrate ion		1364.59				1372.28		
1270-1230	Aromatic Ethers, Aryl-O Stretch				1254.82				1267.41
1050-990	Aliphatic Phosphate	1018.38	1038.48			1019.74	1043.76		
995-850	Aromatic Phosphates								
900-670	C-H bending of Aromatic C		695.18	846.59	842.91		784.27	821.28	749.49
680-610	Alkyne Bending			623.49				611.93	

The SEM images of feedstock and biochar from different maize and sorghum cultivars, produced at different temperatures, are presented in Figures 3.4A-H (maize treatments) and Figures 3.5A-H (sorghum treatments). Structural changes were observed primarily as a result of increasing pyrolysis temperature, rather than cultivar differences. The feedstocks exhibited compact, smooth surfaces with minimal visible porosity. At 350°C, surfaces appeared slightly fragmented with the development of initial pores, while biochars produced at 650°C showed more pronounced cracking and well-developed porosity. Kiln-derived biochars exhibited intermediate characteristics. These temperature-induced changes were consistent across all cultivars, and no significant differences in surface structure were observed between cultivars at the same pyrolysis temperature.

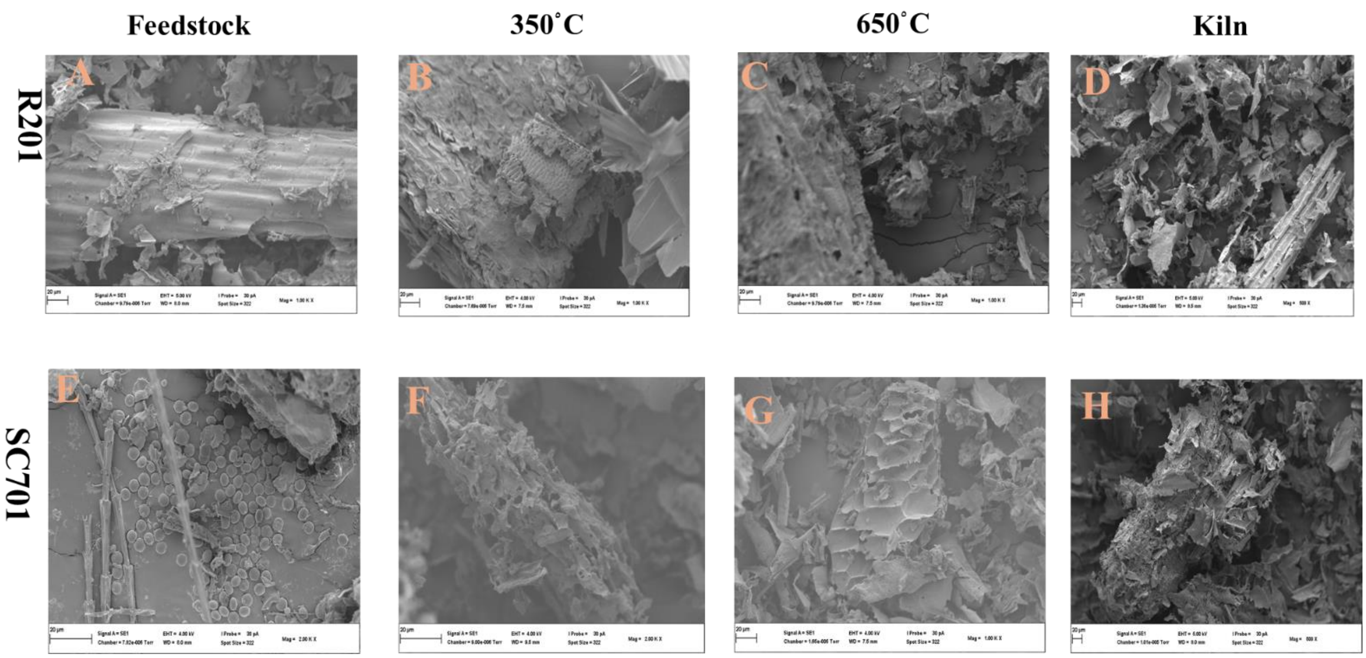

Figure 3.4: SEM images of maize feedstock and its biochar produced at different pyrolysis temperatures 350ºC, 650ºC and by kiln

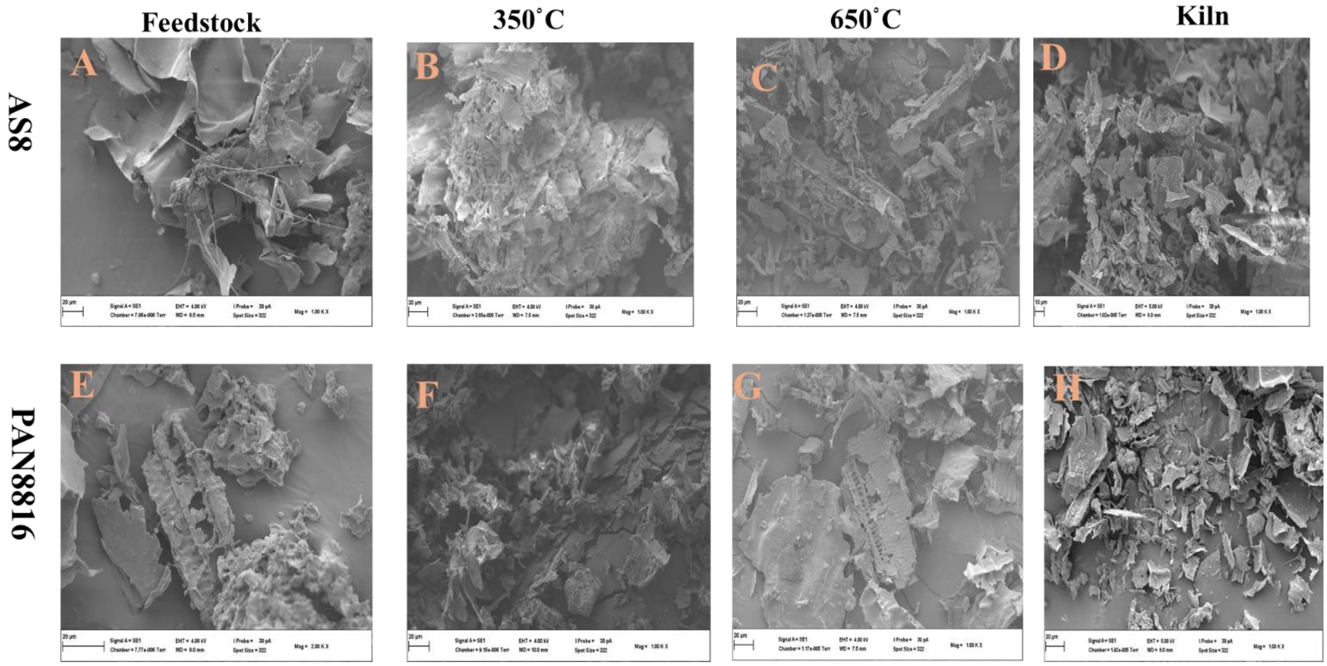

Figure 3.5: SEM images of sorghum feedstock and its biochar produced at different pyrolysis temperatures 350ºC, 650ºC and by kiln

3.3.8 Principal Component Analysis (PCA) of selected characterization variables for feedstock and biochar of different cultivars of maize and sorghum

The Principal Component Analysis (PCA) showed that the first two principal components (PC1 and PC2) together explained 76.01% of the total variability in the dataset, with PC1 accounting for 57.77% and PC2 contributing 18.24% of the variation. The PC1 had the highest eigenvalue (7.51), and its variability was predominantly driven by variables such as H:C ratio, O:C ratio, moisture content and volatile matter, which exhibited higher and positive eigenvector values while total C, fixed C and pH had higher negative values, compared to other variables. Conversely, PC2, with an eigenvalue of 2.37, was influenced positively by C:N ratio and total P and negatively by total N and ash content (Table 3.7).

Table 3.7: Summary of eigenvectors, eigenvalues and cumulative variation for selected characterization variables of feedstock and biochar from different cultivars of maize and sorghum

Variables	PC1	PC2
C:N	0.09	0.49
C:P	0.12	-0.4
Total C	-0.34	0.13
H:C	0.34	-0.08
Total N	-0.13	-0.43
O:C	0.35	0.11
pH	-0.35	-0.01
Total P	-0.28	0.36
Ash	-0.26	-0.38
Fixed C	-0.3	0.3
Moisture	0.34	0.1
Volatile Matter	0.36	0.09
CEC	0.06	0.02
Eigenvalue	7.51	2.37
Variability (%)	57.77	18.24
Cumulative (%)	57.77	76.01

The PCA biplots presented the relationships among the characterization variables, as well as between the variables and the treatments (biochar and feedstock) derived from different cultivars of maize and sorghum (Figure 3.6). The positioning of treatments in the PCA biplot highlighted distinct differences between feedstock and biochar. Feedstock treatments were located on the positive side of both PC1 and PC2, indicating strong associations with variables such as C:N ratio, H:C ratio, O:C ratio and C:P ratio. For example, R201 feedstock (R201-R), characterized by a high C:N ratio, was closer to the C:N vector, while PAN8816 feedstock (PAN8816-R), which had a higher C:P ratio was closely aligned with the C:P vector. In contrast, biochar treatments clustered towards the negative side of PC1 (i.e. all biochars) and PC2 (i.e. the sorghum biochars & SC701 biochars except SC701-B350), reflecting associations with variables like total P, fixed C, total C, pH, ash content and total N (Figure 3.6).

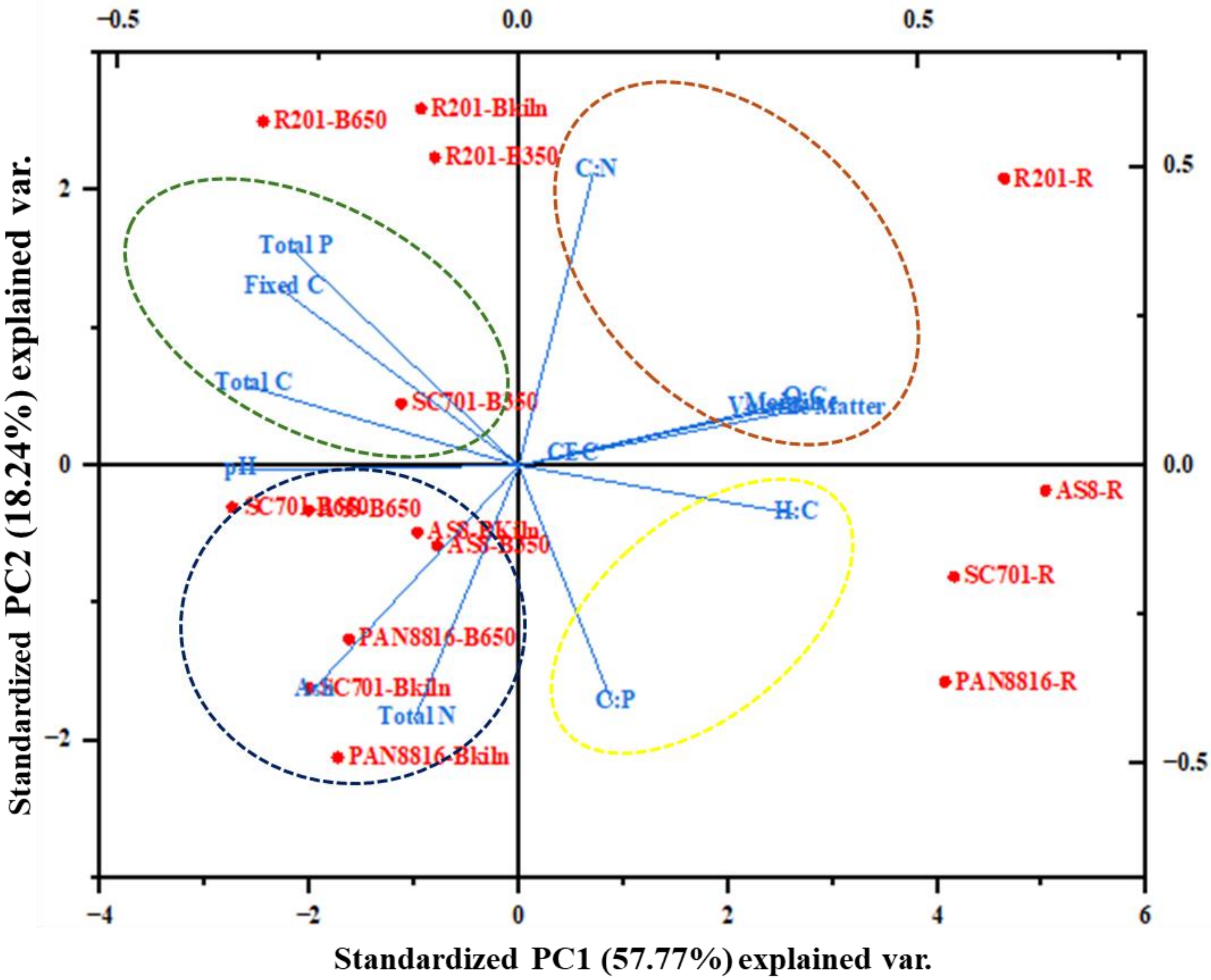

Figure 3.6: PCA biplot of selected characterization variables for feedstock (R) and biochar (B) of different cultivars of maize and sorghum

3.4 Discussion

The decrease in biochar yield with increasing pyrolysis temperature across feedstocks (higher at 350°C than 650°C) could be attributed to intensified thermal degradation of organic material (Bera et al., 2016) and dehydration of hydroxyl groups and decomposition of lignocellulose structures (Shen et al., 2019). These findings were consistent with Rafiq et al. (2016), who also observed a decrease in maize biochar yield with increasing pyrolysis temperature. The lack of significant differences in biochar yield among cultivars at each pyrolysis temperature suggests that feedstock composition may play a secondary role in this regard. The relatively higher yield observed for the R201 maize cultivar across all pyrolysis conditions highlighted the potential cultivar-specific resilience to pyrolysis due to specific characteristics, including the higher lignin content in the feedstock, which resisted thermal decomposition during pyrolysis.

Fixed C is an important indicator of biochar recalcitrance, reflecting its stability and resistance to decomposition (Crombie et al., 2013). While fixed C content is influenced by pyrolysis temperature, it is also strongly dependent on feedstock composition (Zhao et al., 2013). The consistently higher fixed C in biochar than in feedstock across all cultivars, reflected the conversion of labile organic components into stable carbon structures during pyrolysis. These findings were supported by PCA biplot results, where fixed C was strongly associated with biochar treatments (particularly R201 biochars) clustered on the negative side of PC1, emphasizing its role in biochar stability. It was not influenced by crop type, however, the magnitude of increase in fixed C content varied by cultivar and treatment. Fixed C is also associated with the degree of aromaticity and long-term stability of biochar (Joseph & Lehmann, 2015). Consequently, biochars such as R201-B650, AS8-B350 and AS8-BKiln, which exhibited higher fixed C content, demonstrate greater stability and recalcitrance compared to other biochars in this study. According to Enders et al. (2012), feedstocks with higher ash content inhibit the formation of aromatic carbon structures resulting in biochars with lower fixed C content. This study supports this view, as R201 and AS8 feedstocks, which had lower ash content compared to SC701 and PAN8816 feedstocks produced biochars with higher fixed C. These results highlight the critical influence of feedstock ash content on the carbon stability and composition of biochars.

Moisture content, volatile matter and ash content exhibited complementary trends influenced by both cultivar-specific differences and pyrolysis temperature. The decreased moisture content with increasing pyrolysis temperature was attributed to the removal of adsorbed water and the evaporation of aliphatic compounds resulting in biochars with reduced hygroscopicity and enhanced thermal stability (Demirbas, 2004). Similarly, volatile matter decreased due to devolatilization processes where low molecular weight hydrocarbons are broken down and stable aromatic structures are formed (Domingues et al., 2017). The higher labile fractions in the feedstocks made them more susceptible to rapid decomposition and short-term nutrient cycling. In contrast, the lower volatile matter content in biochars produced at higher pyrolysis temperatures (650ºC) enhanced their stability and suitability for long-term carbon sequestration. The increase in ash content with temperature indicated the accumulation of inorganic minerals as organic matter degraded (Cantrell et al., 2012). Cultivar specific differences were also visible with SC701-B650 and PAN8816-B650 biochars exhibiting the highest ash content among maize and sorghum cultivars as a result of generally higher ash content in their feedstock. The PCA biplot supported these observations, showing feedstocks clustering with moisture and volatile matter, while biochar treatments were associated with ash content and fixed carbon. Among the cultivars, R201 and AS8 feedstocks exhibited lower ash content and higher volatile matter producing biochars with enhanced stability and reduced hygroscopicity suitable for carbon sequestration.

Total C increased with pyrolysis temperature, with biochars exhibiting higher values than feedstocks. The strong correlation between total C and variables such as fixed C and ash content indicates enhanced stability at higher temperatures (Lehmann & Joseph, 2024). Total N content decreased at 650ºC probably due to thermal decomposition of nitrogen compounds. The findings of the current study are in line with the findings of Liu et al. (2018), who reported higher N content in rice straw biochar pyrolyzed at 400°C followed by a decline in N content at higher pyrolysis temperatures. The observed decrease in N content at higher pyrolysis temperatures can be attributed to increased N loss during pyrolysis. High pyrolysis temperatures facilitate the breakage of C-N bonds through mechanisms such as cleavage of amine bonds, thermal decomposition of amino acids or disruption of N-containing heterocyclic structures, leading to the formation of ammonia (Xu et al., 2021). This was further supported by the disappearance of N containing functional groups under high pyrolysis temperatures. Cultivar specific differences also played a role as feedstocks with higher initial nitrogen content, such as SC701 and PAN8816 produced biochars with higher nitrogen content. On the other hand, the increased total P content with decreasing pyrolysis temperature indicated that the retention of mineral components during the thermal degradation of feedstocks was influenced by feedstock composition, as cultivars with higher initial P content such as R201 among maize cultivars produced biochars with higher P content. In contrast, sorghum cultivars (AS8 and PAN8816) exhibited lower P content across feedstocks and biochars supported by their lower initial P composition. The strong association of total P with variables such as fixed C, ash content and total C as indicated by the PCA biplot highlights its important role in biochar stability and nutrient retention.

The O:C and H:C molar ratios are critical indicators of biochar aromaticity and stability. This study demonstrates that pyrolysis temperature plays a dominant role in reducing these ratios, as higher temperatures facilitate the loss of labile carbon compounds and the decomposition of cellulose and other volatile components. These transformations enhance the aromaticity of biochars while reducing their polarity aligning with findings by Novak et al. (2009). The low H:C and O:C ratios observed in biochars, particularly those produced at 650ºC falls within the stability thresholds (<0.6 for H:C and <0.4 for O:C) signifying a high level of aromaticity and maturity indicating their suitability for long-term carbon sequestration (Spokas, 2010). These biochars exhibit properties consistent with extended half-lives, ranging from 100 to 1000 years making them ideal candidates for mitigating atmospheric CO_2 concentrations. In contrast, biochars produced at lower temperatures (350ºC and by kiln) exhibited higher H:C and O:C ratios suggesting their suitability for short-term carbon cycling and nutrient release applications (Lehman et al., 2006; Spokas, 2010).

The PCA biplot further highlighted the relationship between the H:C and O:C ratio and amendment type with feedstock treatments clustering closer to H:C and O:C ratios on the positive side of PC1, indicating their higher content of labile fractions. The positioning of biochar treatments further from these ratios on the negative side, reflected their enhanced thermal stability and recalcitrance. This separation between feedstocks and biochars highlights the profound chemical changes induced by pyrolysis, driven by temperature and feedstock composition. Furthermore, the observed alignment of low H:C and O:C ratios with other stability enhancing characteristics, such as reduced volatile matter and higher fixed C content showed the overall impact of pyrolysis on biochar quality. This comprehensive understanding enables the strategic production of biochars designed for long-term stability or short-term nutrient availability. On the other hand, the C:N and C:P ratios of maize and sorghum biochars were directly related to the initial composition of their respective feedstocks. Specifically, biochars produced from feedstocks with higher C:N or C:P ratios also exhibited higher C:N and C:P ratios, indicating a consistent transfer of these elemental ratios from feedstock to biochar.

The increase in biochar pH with pyrolysis temperature indicates changes in chemical composition. Higher temperatures lead to the reduction in acidic surface functional groups, which makes the biochar more alkaline (Ding et al., 2014). The increase in ash content at higher temperatures is a key factor in increasing pH as ash contains carbonates. Enders et al. (2012) reported that biochars with high ash content were more alkaline therefore the high pH of SC701-B650 can be explained by its high ash content, which increases alkalinity. On the other hand, feedstocks with more volatile matter and moisture tend to have lower pH because they contain organic acids (Neem et al., 2016). This relationship was supported by PCA biplot results, which showed biochars grouping with ash content and pH, while feedstocks grouped with volatile matter and moisture. These findings show how pyrolysis temperature and feedstock composition affect biochar pH and could help to guide the production of biochars for specific uses such as improving soil pH or nutrient availability.

In this study, the effects of pyrolysis temperature on exchangeable bases (Ca, Mg, K) and cation exchange capacity (CEC) showed no consistent trend, which contrasts with the general expectation of an increase in these properties with higher pyrolysis temperatures as reported in previous studies (Wan et al., 2014; Khater et al., 2024). For maize cultivars, the exchangeable bases and CEC were highest in the feedstock of R201 but decreased in the biochars probably due to thermal transformation processes, as these processes involve the removal of surface functional groups and the formation of stable aromatic carbon structures as described by Josephs et al. (2010). The decline in CEC of maize cultivars at higher pyrolysis temperatures is consistent with the findings of Gaskin et al. (2008), who observed similar reductions in biochars produced from different feedstocks including poultry manure, eucalyptus, coffee husk and sugarcane bagasse. In contrast, sorghum biochars particularly those of PAN8816, exhibited higher exchangeable bases and CEC compared to their feedstocks. This could be due to that these elements were less affected by volatilization during pyrolysis. These results align with Khater et al. (2024), who reported increased exchangeable bases and CEC in biochars produced at higher pyrolysis temperatures.

These findings highlight that the effects of pyrolysis temperature on exchangeable bases and CEC can vary significantly depending on feedstock composition and crop type emphasizing the importance of considering these factors when producing biochars for specific applications.

The surface functional groups in biochars were primarily influenced by pyrolysis temperature with minimal differences observed between crop types. Both maize and sorghum cultivars exhibited absorption bands corresponding to similar functional groups at the same pyrolysis temperature. For instance, feedstocks and biochars produced at 350°C exhibited absorption bands between 3640-3530 cm^{-1} and 3570-3200 cm^{-1}, indicating the presence of phenolic and hydroxyl compounds (Putun et al., 2005; Cantrell et al., 2012). As the pyrolysis temperature increased the intensity of hydrogen-bonded hydroxyl groups decreased due to the loss of moisture and the decomposition of hydroxyl functional groups (Cantrell et al., 2012). Additionally, higher pyrolysis temperatures resulted in the degradation of phenolic compounds, such as lignin resulting in diminished O-H vibrations (Souza et al., 2009). At 650ºC pyrolysis temperature, the C-H stretching vibrations associated with cellulose and hemicellulose, evident in biochars produced at 350°C and by kiln disappeared indicating the degradation of aliphatic groups. This was accompanied by the development of dense aromatic structures as indicated by distinct peaks in the 1650-1580 cm^{-1} band corresponding to aromatic ring C=C stretching vibrations (Wang et al., 2013). Peaks in the 2000-1750 cm^{-1} band further supported the formation of aromatic structures, resulting from the degradation and depolymerization of lignocellulosic components (Major et al., 2018). These transformations align with the observed increase in fixed C at higher pyrolysis temperatures enhancing biochar stability (Azorgohar et al., 2014).

The distinct functional groups observed in maize cultivars, including organic nitrate, ammonium ion and carbonate ions, highlight the influence of feedstock composition on biochar characteristics. These groups were absent in sorghum residues suggesting that maize residues contain higher nitrogen and mineral content, which serve as precursors for these functional groups during pyrolysis. This aligns with the broader understanding that feedstock composition plays a critical role in determining the chemical properties of biochars (Cantrell et al., 2012). The unique presence of C-H stretching and C-O stretch alkyl-substituted ether in maize biochars further highlight the importance of structural carbohydrates which may be more abundant in maize residues than sorghum (Oyeleke et al.,2022). These findings provide valuable insights into how the interaction between feedstock composition and pyrolysis temperature shapes biochar functionality, advancing our understanding of residue-specific biochar production. For example, the presence of carbonate and phosphate ions in maize biochars suggests potential applications in soil pH regulation and phosphorus availability which are less pronounced in sorghum biochars.

The solid and smooth surfaces of the feedstocks can be attributed to the presence of volatile organic compounds, which blocked the pores and resulted in a more uniform surface (Pignatello et al., 2006). As the pyrolysis temperature increased from 350 to 650°C, the biochars exhibited increased formation of pores and cracks. This is likely attributed to the release of volatile matter during pyrolysis, which led to the visibility of pores in the biochar. Similar findings were reported by Méndez et al. (2013), who also observed the formation of pores in biochars as pyrolysis temperature increased, consistent with our results.

3.5 Conclusion

This study highlights the significant impact of crop type, cultivar and pyrolysis temperature on the chemical composition of feedstocks and biochars derived from maize and sorghum cultivars. Cultivar-specific differences were more noticeable than broad crop type variations, while pyrolysis temperature was another factor influencing biochar properties following cultivar specific differences with higher temperatures improving biochar stability. Biochars produced at higher pyrolysis temperatures (650ºC) exhibited enhanced stability as shown by the low H:C and O:C ratios, higher fixed C content, formation of stable aromatic structures and the reduced presence of labile functional groups, such as hydroxyl and carboxyl groups. These characteristics make biochars from cultivars such as R201-B650 and AS8-B650 ideal for long-term carbon sequestration. Feedstocks like R201 and AS8, with high C:N and C:P ratios demonstrated slower decomposition rates making them suitable for long-term carbon storage when biochar production is not feasible.

Biochars such as SC701-BKiln and PAN8816-BKiln exhibited higher total N content, making them suitable sources of N for long-term N improvements while their feedstocks are better suited for short-term N supply due to rapid decomposition. The presence of specific functional groups, such as carbonate and phosphate ions in R201 biochars, enhances their potential for retaining essential nutrients like calcium and phosphorus, contributing to improved soil fertility. Additionally, SC701-B650 and AS8-B650 with their higher pH levels are well-suited for ameliorating acidic soils. Biochars with higher ash content, such as SC701-B650 and PAN8816-B650 are particularly effective in improving soil structure and long-term fertility due to their concentrated mineral content. Biochars with highly porous structures such as those produced at 650^{o}C are better suited for improving soil water retention and nutrient availability.

This study highlights the need to consider feedstock composition, cultivar-specific differences and pyrolysis conditions to adjust biochar properties for specific agricultural applications. Biochars and feedstock from maize and sorghum cultivars demonstrate diverse potentials, from carbon sequestration to nutrient recycling and soil fertility improvement. Future research should focus on the long-term field performance of these feedstocks and biochars, particularly their impacts on soil nutrient recycling, soil fertility, carbon sequestration and crop productivity to validate their role in sustainable soil management and climate change mitigation.

CHAPTER 4

CO_2 Emissions, nitrogen and phosphorus mineralization in soils amended with feedstock and biochar produced from different cultivars of maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L. Moench)

Abstract

Combined effects of soil fertility decline and climate change pose a severe threat to crop productivity. The application of crop residues could be of benefit in nutrient recycling while biochar can be a strategy for carbon sequestration, improving nutrient availability, enhancing soil fertility and mitigating the effects of climate change. This study aimed to investigate the effects of raw crop residues (feedstock) and biochar of different cultivars of maize and sorghum on CO_2 emissions, nitrogen (N) and phosphorus (P) mineralization in soil. An incubation study was conducted by amending soils with feedstock (R) or biochar (B) produced at pyrolysis temperatures of 350ºC and 650ºC from two maize cultivars (R201 and SC701) and two sorghum cultivars (AS8 and PAN8816). Treatments were mixed with 100 g of soil at an application rate equivalent to 10 t C ha^{-1} replicated 3 times and compared to a control (soil only). CO_2 emissions were trapped in 1M NaOH and measured periodically over 120 days. A separate experiment, with identical treatments was set up for destructive sampling to analyze N and P mineralization and soil pH on days 0, 7, 14, 21, 28, 42, 56, 84, 112 and 120. CO_2-C emissions increased rapidly from day 0, peaking early and declined after day 42. CO_2 emissions were significantly influenced by cultivar specific differences and pyrolysis temperature depending on the cultivar. Among the maize cultivars, SC701 feedstock emitted the highest CO_2-C (308 mg CO_2-C kg^{-1} soil) which was 89% higher than the control (162.8 mg CO_2-C kg^{-1} soil) while for sorghum cultivars, PAN8816 feedstock emitted the highest emissions (496.8 mg CO_2-C kg^{-1} soil), 205% higher than the control. Nutrient mineralization (NH_4^+-N, NO_3^--N & extractable P) and soil pH were also significantly influenced by cultivar specific differences and pyrolysis temperature. In maize treatments, NH_4^+-N concentrations were generally reduced, while for sorghum, PAN8816-B350 increased NH_4^+-N concentrations to 17.14 mg N kg^{-1} soil. Nitrate-N concentrations increased by 17% with the application of SC701-B650 in maize cultivars and by 64% with PAN8816-B350 in sorghum cultivars. For extractable P, the highest concentrations were observed in R201-B650 for maize

cultivars and AS8-B650 for sorghum. These findings highlight that biochars produced from specific cultivars at different pyrolysis temperatures can simultaneously sequester carbon and enhance N and P availability. This dual role of biochar in reducing CO_2 emissions while improving soil nutrient availability highlights its potential for sustainable soil management and food production, particularly in addressing soil fertility decline and climate change.

Key words: Biochar, carbon sequestration, crop residues, mineral nutrients, soil fertility

4.1 Introduction

The decline in soil fertility due to poor agricultural practices such as monocropping, excessive chemical fertilizer use, extensive tillage and other anthropogenic activities is a major cause of reduced crop yields (Heerink, 2005; Nord et al., 2022). These practices accelerate the mineralization of soil carbon, leading to increased CO_2 emissions, depletion of soil organic carbon (SOC) and soil erosion. Sustainable agricultural practices that prevent soil degradation while enhancing soil fertility and crop productivity are therefore essential. The addition of organic materials, such as crop residues, animal manures and organic wastes, is widely recognized as an effective strategy for improving soil fertility (Powlson et al., 2012). Globally, crop residues are the most abundant organic materials with an annual production of 6 billion tons of which 74% comes from cereals, 10% from sugar crops, 8% from legumes, 5% from tuber crops and 3% from oilseed crops (Lal, 2005). These residues are rich in essential nutrients such as carbon (C), nitrogen (N), phosphorus (P), potassium (K) and micronutrients that are essential for crop growth. However, the extent to which crop residues improve soil fertility depends on their biochemical composition, such as C, N, P, lignin content and the C:N ratio (Kaleem Abbasi et al., 2015; Schmatz et al., 2017). High quality residues (C:N ratio <32) promote net mineralization while low quality residues (C:N ratio >32) often lead to nitrogen immobilization (Zavalloni et al., 2011). Thus, understanding residue quality is critical for improving their benefits in soil systems.

Residues from different crop types, crop parts and even cultivars of the same crop vary significantly in their biochemical composition and nutrient release patterns (Redin et al., 2014; Coulibaly et al., 2020). However, findings on residue decomposition and nutrient release are inconsistent. For example, Liu et al. (2022) found that soybean (*Glycine max*) leaves increased

CO_2 emissions and reduced SOC while maize stems and soybean roots enhanced SOC by 17% and 16%, respectively. Similarly, Zhang et al. (2016) reported increased soil available N, total N, available P, total P and SOC following the application of maize residues. In contrast, Meng et al. (2017) and Kaleem Abbasi et al. (2015) reported nitrogen immobilization after applying maize residues, soyabean roots, maize roots and shoots. These contradictions highlight the need for further studies focusing on commonly grown crop residues, such as maize and sorghum, to better understand their effects on nutrient cycling and CO_2 emissions.

To improve the residence time and recalcitrance of organic materials in soils, biochar production through pyrolysis has gained increasing attention. Biochar is a stable, carbonaceous material produced under limited oxygen conditions (Askeland et al., 2019). Similar to crop residues, biochar can supply essential nutrients such as N, P and K and improve soil properties including pH, cation exchange capacity (CEC), bulk density, water holding capacity and microbial activity (Purakayastha et al., 2019; Nguyen et al., 2020). Additionally, biochar plays a critical role in mitigating climate change by sequestering carbon and reducing greenhouse gas emissions (Lehmann, 2007). The composition and stability of biochar are influenced by the feedstock type and pyrolysis temperature. Biochars produced at higher pyrolysis temperatures (e.g. 650°C) exhibit greater carbon stability due to increased aromaticity whereas biochars produced at lower temperatures (e.g. 350°C) contain more labile fractions and volatile matter (Biederman and Harpole, 2013). Biochars from animal manures tend to have higher N and P content than those derived from plant-based feedstocks (Purakayastha et al., 2019). Despite these benefits, the effects of biochar on CO_2 emissions and nutrient cycling remain inconsistent. For example, Olaniyan et al. (2020) observed increased CO_2 emissions with increasing biochar application rates while Lin et al. (2015) reported no significant effects of biochar on greenhouse gas emissions.

Breeding efforts have led to the development of maize and sorghum cultivars with enhanced tolerance to biophysical stresses such as drought and poor soil fertility, alongside increased yield potential (Badu-Apraku et al., 2023; Kaliamoorthy et al., 2024). Despite these advancements, there is still limited understanding of how cultivar differences in biochemical composition influence CO_2 emissions and nutrient mineralization patterns, particularly when residues are converted to biochar. Preliminary findings from earlier work (MSc study) on wheat residues showed significant variation in C, N and P mineralization across cultivars (Mbava, 2019). However, this work was limited to feedstocks only and did not include biochar. This highlights the need to investigate the comparative effects of both feedstock and biochar amendments on CO_2 emissions and nutrient mineralization, particularly from residues of widely cultivated crops such as maize and sorghum.

Maize and sorghum are staple cereals extensively grown by commercial and smallholder farmers, particularly in Sub-Saharan Africa (SSA). Maize provides the primary source of calories for over 900 million people globally and is the third most important cereal crop after rice and wheat (McMillen et al., 2022). Sorghum, on the other hand, is widely cultivated in arid and semi-arid regions of SSA due to its drought tolerance (Prasad et al., 2021). Given the widespread cultivation of these crops and the abundant availability of their residues, it is essential to explore their variation in CO_2 emissions and nutrient cycling when their feedstocks and biochars are applied to soils. Therefore, this study aims to compare the effects of feedstock and biochar of different cultivars of maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L. Moench) on CO_2 emissions, soil pH nitrogen and phosphorus mineralization.

4.2 Materials and methods

4.2.1 Biomass and feedstock production

The biomass and feedstock production process were carried out as outlined in Section 3.2.1 of Chapter 3.

4.2.2 Biochar production and characterization

Biochars were produced from milled feedstock of maize or sorghum cultivars. These were pyrolyzed at temperatures of 350^{o}C and 650^{o}C at a rate of 10^{o}C/min for 2 hours (Vilakazi et al., 2023). Feedstock and biochars were characterized as outlined from section 3.2.2 to 3.2.6 Biochemical composition of feedstock and biochars of different cultivars of maize and sorghum used in this study are presented in Table 4.1.

Table 4.1: Biochemical composition of feedstock and biochars of different cultivars of maize and sorghum

Crop Type	Treatment	Total C	Total N	Total P	Fixed C	Ash	Lignin	pH	C:N	C:P
		-------------------	-------------------	-------------------	--------%	-------------------	-------------------			
Maize	R201 R	38.89^{a}	0.32^{a}	0.47^{c}	17.24^{c}	6.5^{a}	38.57^{d}	5.09^{b}	153.3^{b}	82.76^{abc}
	SC701 R	33.29^{a}	1.07^{c}	0.38^{b}	12.23^{a}	16.73^{d}	4.18^{a}	6.75^{d}	31.08^{a}	88.42^{abcd}
	R201 B-350	55.37^{bcd}	0.97^{c}	0.84^{h}	38.03^{b}	11.44^{b}		9.46^{f}	57.02^{a}	66.19^{a}
	SC701 B-350	50.3^{bc}	1.57^{d}	0.73^{g}	32.59^{e}	28.59^{h}		9.75^{h}	32.11^{a}	69.23^{a}
	R201 B-650	60.66^{d}	0.66^{b}	0.82^{h}	47.13^{h}	18.34^{e}		10.62^{k}	92.25^{ab}	74^{ab}
	SC701 B-650	57.78^{cd}	0.99^{c}	0.69^{f}	26.59^{d}	46.13^{k}		11.4^{l}	58.42^{a}	83.74^{abc}
Sorghum	AS8 R	33.09^{a}	0.48^{a}	0.34^{a}	14.1^{b}	11.48^{b}	24.79^{c}	4.26^{a}	68.87^{a}	96.34^{bcd}
	PAN8816 R	37.62^{a}	1.46^{d}	0.33^{a}	13.17^{ab}	14.32^{c}	10.21^{b}	5.82^{c}	25.78^{a}	113.05^{d}
	AS8 B-350	52.51^{bcd}	1.11^{c}	0.62^{e}	36.47^{f}	20.19^{f}		9.57^{g}	47.49^{a}	84.69^{abc}
	PAN8816 B-350	49.74^{bc}	2.84^{e}	0.62^{e}	32.81^{e}	26.88^{g}		9.09^{e}	17.5^{a}	79.81^{ab}
	AS8 B-650	59.59^{d}	0.69^{b}	0.56^{d}	32.48^{e}	36^{i}		10.58^{j}	86^{ab}	107.06^{cd}
	PAN8816 B-650	49.33^{d}	1.61^{d}	0.54^{d}	27.51^{d}	41.82^{j}		10.437^{i}	30.72^{a}	91.37^{abcd}
	LSD (p<0.05)	4.8	0.1	0.02	0.65	0.52	1.89	0.01	46.69	14.42

R-Feedstock, **B**-Biochar, M-Maize, S-Sorghum, Values on the same column with different letters are significantly different (p<0.05)

4.2.4 Soil collection and analysis

The soil used in this study was collected from an arable field located at Ukulinga the University of KwaZulu-Natal Research Farm. Soil samples were collected from the 0-20 cm depth at random points using an auger, then mixed thoroughly to form a composite sample. It was air dried, ground and passed through a 2 mm sieve then a subsample of 0.5 kg was taken for determination of soil physical and chemical properties (Table 4.2). Particle size analysis was determined using the hydrometer method (Okalebo et al., 2002). Soil pH was determined in 1M KCl (1: 2.5; Soil: KCl). Electrical conductivity (EC) was determined in H_2O (1: 2.5; Soil: H_2O) using an EC meter. Total C and N were determined using an automated LECO-CNS Trumac autoanalyzer (Leco Corporation, 2012). Extractable P was determined calorimetrically following AMBIC-2 extraction as described by Non-Affiliated Soil Analysis Working Committee (1990). Exchangeable bases were determined using 1M ammonium acetate (NH_4OAC) method as described by Ross and Kettering, (1995). Exchangeable acidity was determined in 1M KCl as described by Lourenzi et al. (2011).The soil was loam in texture classified as Chromic Luvisols (IUSS Working Group WRB, 2014).

Table 4.2: Selected physico-chemical properties of the soil used in the incubation study

Soil Property	Values
Bulk density (g cm^{-3})	1.24
Sand (%)	30.0
Silt (%)	34.9
Clay (%)	24.4
Texture	Loam
pH (KCl)	4.73
P (mg kg^{-1})	8.87
K (mg kg^{-1})	91.94
Ca (mg kg^{-1})	1043
Mg (mg kg^{-1})	313.71
Exch. Acidity (cmol kg^{-1})	0.038
Zn (mg kg^{-1})	2.90
Mn (mg kg^{-1})	12.10
Cu (mg kg^{-1})	5.81
TC %	1.9
TN%	0.17

4.2.5 CO_2-C emission under incubation conditions

The study was conducted as a completely randomized design with 13 treatments (Table 4.3), replicated three times. The treatments included feedstocks and biochars of two maize cultivars (R201 and SC701) and two sorghum cultivars (AS8 and PAN8816). Biochars were produced at two pyrolysis temperatures (350ºC (B350) and 650ºC (B650)). A control treatment (unamended soil) was also included. For each treatment, 100 g of soil was thoroughly mixed with the feedstock or biochar at an application rate equivalent to 10 t C ha^{-1}. The mixtures were placed into 100 ml plastic containers and were slowly wetted to fill up 50% pore space. Each of these containers was then placed inside a 500 ml airtight plastic container. A vial containing 25 ml of 1M NaOH solution was added to each plastic containers to trap the CO_2 produced during the incubation. The plastic containers were tightly sealed and incubated for 120 days in the dark at a constant temperature of 25ºC. CO_2-C trapped in the NaOH solution was measured periodically at 0, 3, 7, 14, 21, 28, 42, 56, 84, 112 and 120 days of incubation. The amount of CO_2-C trapped in the NaOH solution was determined by firstly precipitating the carbonates with $BaCl_2$ followed by back-titration with 0.5M HCl after adding 3 drops of phenolphthalein indicator. At each sampling day, the used NaOH solution was replaced with fresh NaOH to ensure saturation with CO_2 throughout the incubation period. The amount of CO_2-C emitted from the soil during the incubation period was calculated using the following formulae:

Moles of NaOH reacted with CO_2 (y) = Total moles of NaOH- moles of HCl added--------(4.1)

Mass of CO_2 = $(\frac{y}{2}) \times 44$ ---(4.2)

Mass of CO_2 in mg kg^{-1} soil = Mass of $CO_2 \times 10000$---(4.3)

Mass of C in mg kg^{-1} soil = (((Mass of CO_2 (mg kg^{-1})) ×12)/44) -------------------------------(4.4)

Where y is moles of NaOH reacted with CO_2, 12 and 44 are molar masses of C and CO_2 respectively.

4.2.6 Mineral nitrogen, extractable phosphorus and soil pH determination

This part of the study followed the same setup as the CO_2-C emission experiment, using identical treatments (Table 4.3). The key difference was the inclusion of sufficient replicates (39 pots per sampling time) to allow for destructive sampling at each time point. In this experiment, 100 g of soil was mixed with each treatment at a rate equivalent to 10 t C ha^{-1} and placed into 500 ml plastic containers. The containers were drilled with four holes near the top rim to facilitate airflow. The soils were slowly moistened to achieve field capacity and maintained at it throughout the experiment. The containers were incubated in the dark at a constant temperature of 25°C for 120 days. Destructive sampling was conducted at 0, 7, 14, 21, 28, 42, 56, 84, 112 and 120 days of incubation. During each sampling time mineral nitrogen (NH_4^+-N + NO_3^--N) was extracted using 2M KCl and analyzed with a Thermo Scientific Gallery Discrete Autoanalyser (Scientific Thermo Fischer, 2014). Extractable phosphorus was determined using the AMBIC-2 extraction method (Non-Affiliated Soil Analysis Work and Soil Science Society of South Africa, 1990) and soil pH was measured in a 1M KCl solution (1:2.5, soil:KCl) using a calibrated pH meter.

Table 4.3: Treatments used in the study, including the feedstocks and biochars of sorghum and maize

Treatment	Treatment Description
R201-R	Feedstock of maize cultivar R201
SC701-R	Feedstock of maize cultivar SC701
R201-B350	Biochar of maize cultivar R201 pyrolyzed at 350°C
SC701-B350	Biochar of maize cultivar SC701 pyrolyzed at 350°C
R201-B650	Biochar of maize cultivar R201 pyrolyzed at 650°C
SC701-B650	Biochar of maize cultivar SC701 pyrolyzed at 650°C
PAN8816-R	Feedstock of sorghum cultivar PAN8816
AS8-R	Feedstock of sorghum cultivar AS8
PAN8816-B350	Biochar of sorghum cultivar PAN8816 pyrolyzed at 350°C
AS8-B350	Biochar of sorghum cultivar AS8 pyrolyzed at 350°C
PAN8816-B650	Biochar of sorghum cultivar PAN8816 pyrolyzed at 650°C
AS8-B650	Biochar of sorghum cultivar AS8 pyrolyzed at 650°C
Control	Unamended soil

4.2.6 Statistical Analysis

A two-way analysis of variance (ANOVA) was conducted using GenStat 20th edition (Payne et al., 2011) to assess the effects of cultivar and amendment type on all measured parameters, including CO_2-C emissions, ammonium-N and nitrate-N, extractable phosphorus and soil pH. Mean separations were performed using the least significant difference (LSD) test at a significance level of $p = 0.05$. Bivariate correlations among CO_2-C emissions, NH_4^+-N, NO_3^--N**,** extractable phosphorus, pH and biochemical properties were determined following Pearson's correlation analysis through OriginPro 2024 (OriginLab Corporation, Northampton, USA).

4.3 Results

4.3.1 Effects of feedstock and biochar from different cultivars of maize and sorghum on CO_2-C emissions

CO_2-C emissions significantly differed among treatments throughout the incubation period, showing differences between feedstocks and biochars ($p < 0.001$). Emissions increased rapidly from the start of the incubation, with maximum peaks observed within 21 days and between 28 and 42 days for all maize and sorghum treatments (Figure 4.1). After 42 days CO_2-C emissions began to decline across all treatments. The control treatment initially emitted the lowest CO_2-C, however, after day 84, it exhibited higher emissions than all biochar treatments (Figure 4.1). Among all treatments, the feedstock from sorghum cultivar PAN8816 consistently emitted the highest CO_2-C emissions throughout the incubation period. The results showed that CO_2-C emissions were influenced more by cultivar specific differences and pyrolysis temperature than by crop type. Both maize and sorghum cultivars exhibited variability in emissions (Table 4.4). Generally, all amendments increased CO_2-C emissions compared to the control, with feedstock treatments producing significantly higher CO_2-C emissions compared to their biochars for both maize and sorghum cultivars. Among the maize cultivars, SC701 feedstock emitted the highest CO_2-C (308 mg CO_2-C kg^{-1} soil) which was 89% higher than the control (162.8 mg CO_2-C kg^{-1} soil), while emissions from R201 treatments showed no significant differences across feedstock and biochar. In sorghum cultivars, PAN8816 feedstock produced the highest CO_2-C emissions (496.8 mg CO_2-C kg^{-1} soil), a 205% increase compared to the control, whereas biochar treatments generally emitted less CO_2-C, with emissions decreasing as pyrolysis temperature increased (Table 4.4).

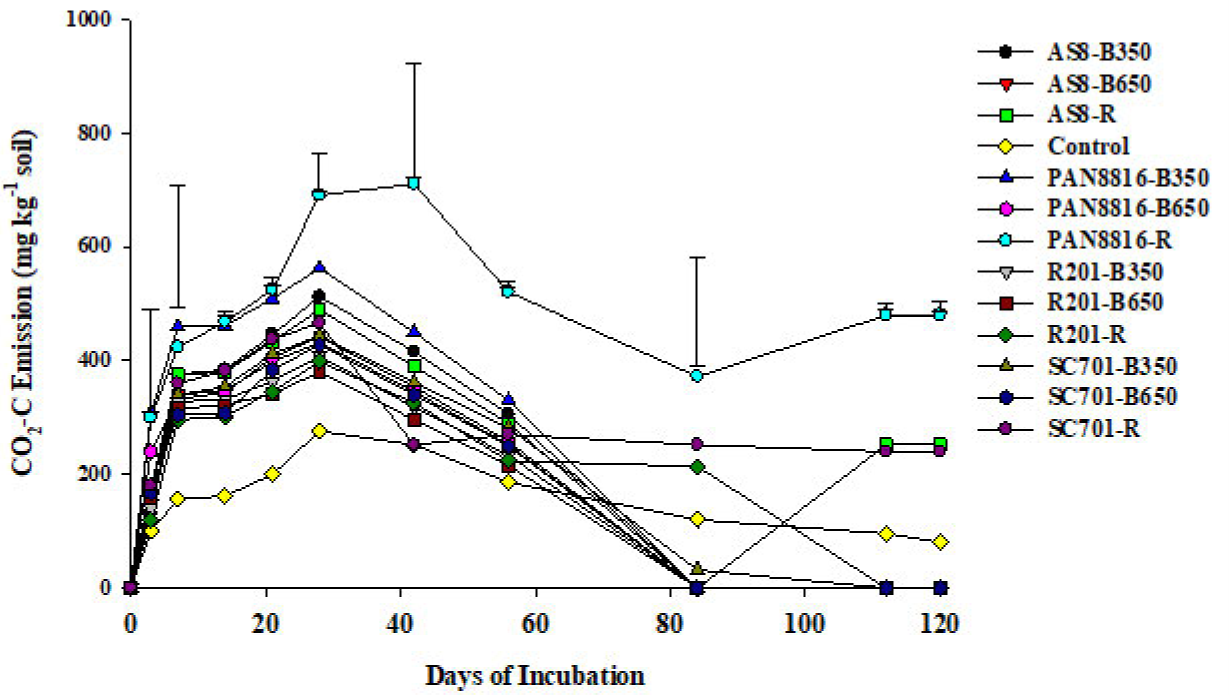

Figure 4.1: Trends in CO_2-C emissions during the 120-day incubation period for soils amended with feedstock (R) and biochar pyrolyzed at 350ºC (B350) and at 650ºC (B650) from different maize (R201 & SC701) and sorghum (AS8 & PAN8816) cultivars along with the control treatment. The vertical error bars indicate LSD ($p<0.05$).

Table 4.4: Average CO_2-C emissions, cumulative CO_2-C (mg CO_2-C kg^{-1} soil), nitrate-N (mg N kg^{-1} soil), ammonium-N (mg N kg^{-1} soil) pH and extractable phosphorus (mg P kg^{-1} soil) released from feedstock and biochar of different cultivars of maize and sorghum incubated for 120 days

Crop Type	Treatment	CO_2-C Emission	Cum. CO_2-C Emission	NH_4^+-N	NO_3^--N	Extractable P	pH
		---mg kg^{-1} soil---					
Maize	**R201 R**	222.6^{bc}	1426^{b}	6.72^{a}	8.1^{a}	7.34^{e}	4.99^{b}
	SC701 R	308^{e}	1777^{g}	12.57^{c}	11.35^{bc}	6.58^{d}	5.03^{bc}
	R201 B-350	212.4^{bc}	1453^{bc}	7.87^{a}	10.23^{b}	10.10^{g}	5.24^{d}
	SC701 B-350	240.2^{cd}	1623^{de}	12.66^{c}	13.4^{d}	8.08^{f}	5.26^{d}
	R201 B-650	203.2^{b}	1404^{b}	7.2^{a}	13.78^{d}	10.96^{h}	5.40^{f}
	SC701 B-650	218.2^{bc}	1484^{bc}	11.37^{bc}	19.29^{f}	7.99^{f}	5.36^{ef}
Sorghum	**AS8 R**	304^{e}	1803^{g}	12.02^{bc}	12.4^{cd}	6.11^{c}	5.12^{c}
	PAN8816 R	496.8^{f}	2655^{i}	16.82^{e}	24.92^{g}	5.07^{b}	4.82^{a}
	AS8 B-350	257.2^{d}	1727fg	12.36^{c}	16.4^{e}	7.46^{e}	5.29^{de}
	PAN8816 B-350	308^{e}	2141^{h}	17.14^{e}	27^{h}	6.82^{d}	5.36^{ef}
	AS8 B-650	226.4^{bcd}	1544^{cd}	10.52^{b}	17.37^{e}	7.36^{e}	5.43^{f}
	PAN8816 B-650	237.6^{cd}	1644^{ef}	12.04^{bc}	18.91^{f}	6.79^{d}	5.43^{f}
	Control	162.8^{a}	939^{a}	15.17^{d}	16.44^{e}	2.67^{a}	4.84^{a}
	LSD ($p<0.05$)	19.03	57.12	0.92	0.88	0.2	0.06

Values on the same column with different letters are significantly different ($p<0.05$)

4.3.2 Effects of feedstock and biochar from different cultivars of maize and sorghum on cumulative CO_2-C emissions

Cumulative CO_2-C emissions significantly differed among all treatments over the incubation period ($p < 0.001$). Emissions steadily increased throughout the incubation with clear differences observed between feedstock, biochar and the control treatments (Figure 4.2). The control treatment consistently exhibited the lowest cumulative CO_2-C emissions throughout the incubation period. Cumulative CO_2-C emissions were in the order of feedstock > B350 > B650 for all cultivars except for R201 which had no significant differences across all treatments (Figure 4.2). Cumulative CO_2-C emissions were influenced more by cultivar specific differences and pyrolysis temperature than by crop type, as each crop type had one high CO_2-C emitting and one relatively lower emitting cultivar. The effects of pyrolysis temperature varied by cultivar, with biochar produced at 650°C generally resulting in lower cumulative CO_2-C emissions compared to 350°C and feedstock treatments for most treatments (Table 4.4). Among the maize cultivars, SC701 feedstock exhibited the highest cumulative CO_2-C emissions (1777.4 mg CO_2-C kg^{-1} soil), while emissions from R201 feedstock and biochars did not significantly differ from SC701-B650 and resulted in lower cumulative emissions. For sorghum cultivars, PAN8816 feedstock produced the highest cumulative CO_2-C emissions (2655.4 mg CO_2-C kg^{-1} soil) while AS8-B650 was lowest (1544 mg CO_2-C kg^{-1} soil) (Table 4.4).

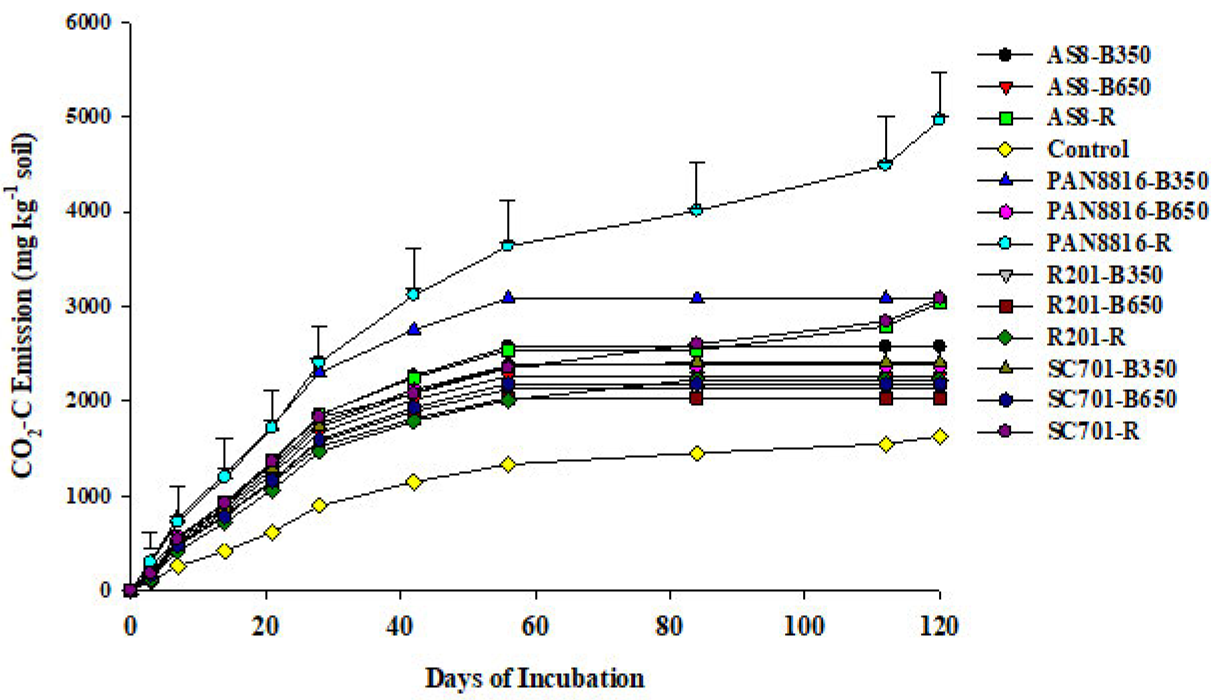

Figure 4.2: Trends in cumulative CO_2-C emissions during the 120-day incubation period for soils amended with feedstock (R) and biochar pyrolyzed at 350ºC (B350) and at 650ºC (B650) from different maize (R201 & SC701) and sorghum (AS8 & PAN8816) cultivars along with the control treatment. The vertical error bars indicate LSD ($p<0.05$).

4.3.3 Effects of feedstock and biochar from different cultivars of maize and sorghum on ammonium-N mineralization

Ammonium-N (NH_4^+-N) release significantly differed among all treatments during the incubation period ($p < 0.001$). It increased rapidly from the start of the incubation, reaching maximum peaks within 14 days, followed by a sharp decline for all treatments thereafter (Figure 3). Among the treatments, maize cultivar R201 feedstock and its biochars consistently exhibited the lowest NH_4^+-N concentrations within the first 21 days of the incubation compared to other treatments (Figure 4.3). Ammonium-N concentrations were influenced more by cultivar specific differences and pyrolysis temperature than by crop type. The effect of pyrolysis temperature on NH_4^+-N concentrations varied depending on the cultivar for instance, among the maize cultivars, SC701 treatments consistently exhibited higher NH4+-N concentrations than R201 treatments (Table 4.4).

However, NH_4^+-N concentrations for both SC701 and R201 treatments were lower than the control (15.17 mg N kg^{-1} soil). For the sorghum cultivars, PAN8816 feedstock (16.82 mg N kg^{-1} soil) and PAN8816-B350 (17.14 mg N kg^{-1} soil) exhibited the highest NH_4^+-N concentrations while all AS8 treatments exhibited lower NH_4^+-N concentrations, which were lower than that of the control (Table 4.4).

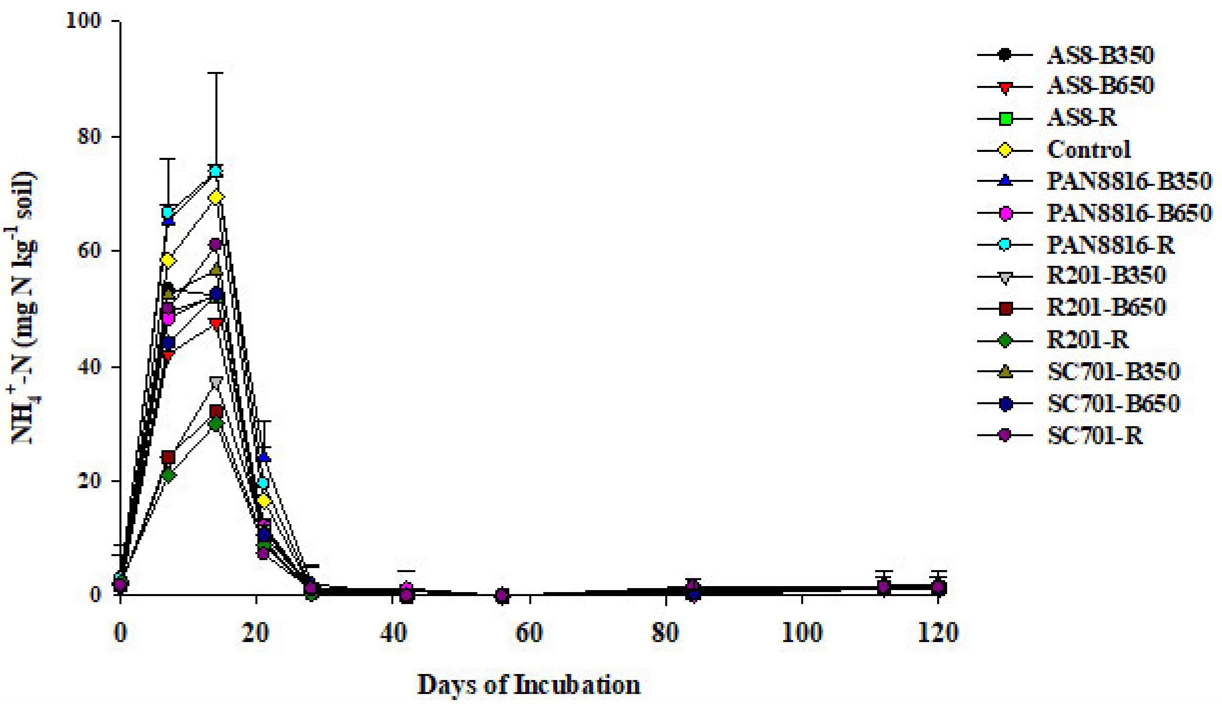

Figure 4.3: Trends in NH_4^+-N release during the 120-day incubation period for soils amended with feedstock (R) and biochar pyrolyzed at 350ºC (B350) and at 650ºC (B650) from different maize (R201 & SC701) and sorghum (AS8 & PAN8816) cultivars along with the control treatment. The vertical error bars indicate LSD ($p<0.05$).

4.3.4 Effects of feedstock and biochar from different cultivars of maize and sorghum on nitrate-N mineralization

Nitrate-N (NO_3^--N) concentrations significantly differed among the treatments during the incubation period ($p < 0.001$). Nitrate-N concentrations showed a slow release during the first 21 days of the incubation, followed by a steady increase from day 21 to 56 and a sharp increase at day 56 of incubation. After day 84, NO_3^--N concentrations stabilized for most treatments (Figure 4.4). Among the treatments, maize cultivar R201 feedstock exhibited the lowest NO_3^--N release throughout the incubation period while sorghum cultivar PAN8816-B350 and PAN8816 feedstock were highest (Figure 4.4). Nitrate-N concentrations were influenced by cultivar specific differences and varied with pyrolysis temperature depending on the cultivar (Table 4.4). For maize cultivars, they followed the order of feedstock < B350 < B650. The lowest NO_3^--N concentration was observed in R201 feedstock (8.1 mg N kg^{-1} soil), which was 103% lower than the control (16.44 mg N kg^{-1} soil) while the highest NO_3^--N concentration was observed in SC701-B650 (19.29 mg N kg^{-1} soil). For sorghum cultivars, AS8 feedstock exhibited the lowest NO_3^--N concentration (12.4 mg N kg^{-1} soil) which was 33% lower than the control while PAN8816-B350 exhibited the highest NO_3^--N concentration (27. mg N kg^{-1} soil), which was 64% higher than the control (Table 4.4).

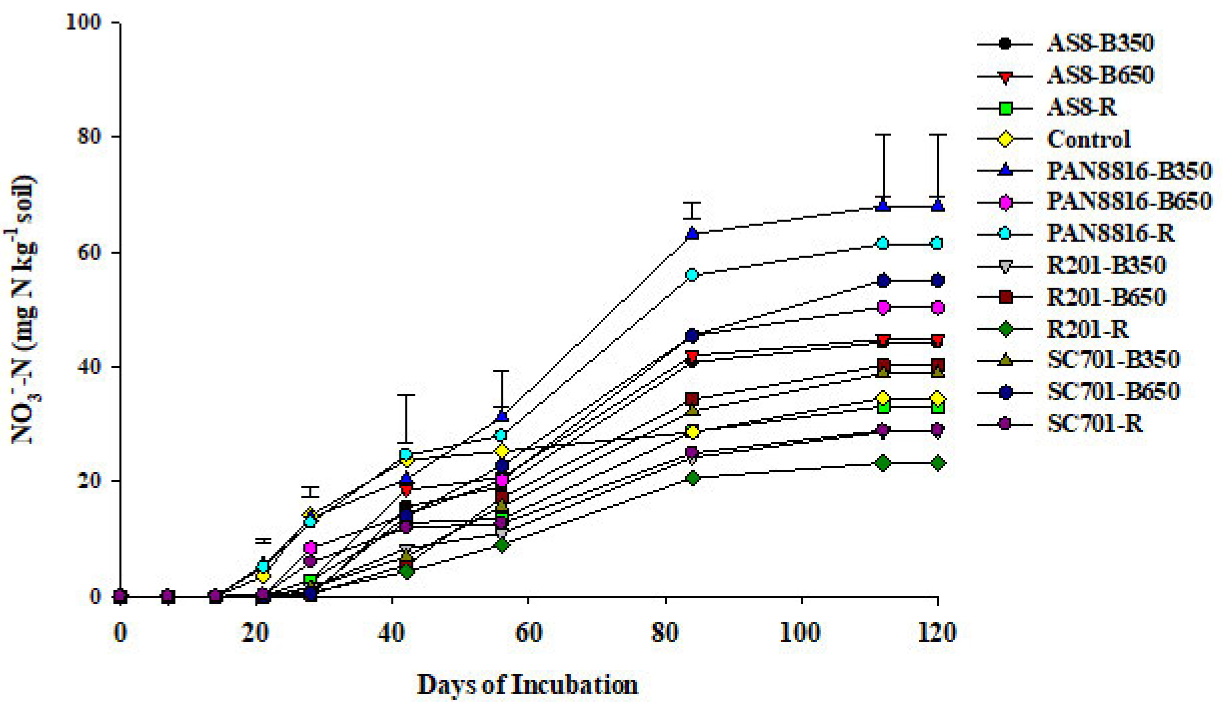

Figure 4.4: Trends in NO_3^--N release during the 120-day incubation period for soils amended with feedstock (R) and biochar pyrolyzed at 350ºC (B350) and at 650ºC (B650) from different maize (R201 & SC701) and sorghum (AS8 & PAN8816) cultivars along with the control treatment. The vertical error bars indicate LSD ($p<0.05$).

4.3.5 Effects of feedstock and biochar from different cultivars of maize and sorghum on extractable P

Extractable P concentrations significantly differed among all treatments at all incubation days ($p < 0.001$). It increased rapidly from the start of the incubation, with maximum peaks observed around day 28 for most treatments. The control treatment consistently released the lowest extractable P while R201-B650 and R201-B350 exhibited the highest extractable P concentrations throughout the incubation period (Figure 4.5). Extractable P concentrations were significantly influenced by cultivar specific differences and pyrolysis temperature depending on the cultivar (Table 4.4). In all cultivars, feedstock treatments exhibited lower extractable P than their respective biochars. The extractable P concentrations from biochars also did not significantly differ except for R201 where B650 resulted in higher extractable P than B350. Among the maize cultivars, SC701 feedstock had the lowest extractable P (6.58 mg P kg^{-1} soil), while R201-B650 was highest (10.96 mg P kg^{-1} soil). For the sorghum cultivars, PAN8816 feedstock exhibited the lowest extractable P (5.07 mg P kg^{-1} soil), while both AS8-B350 (7.46 mg P kg^{-1} soil) and AS8-B650 (7.36 mg P kg^{-1} soil) were highest (Table 4.4).

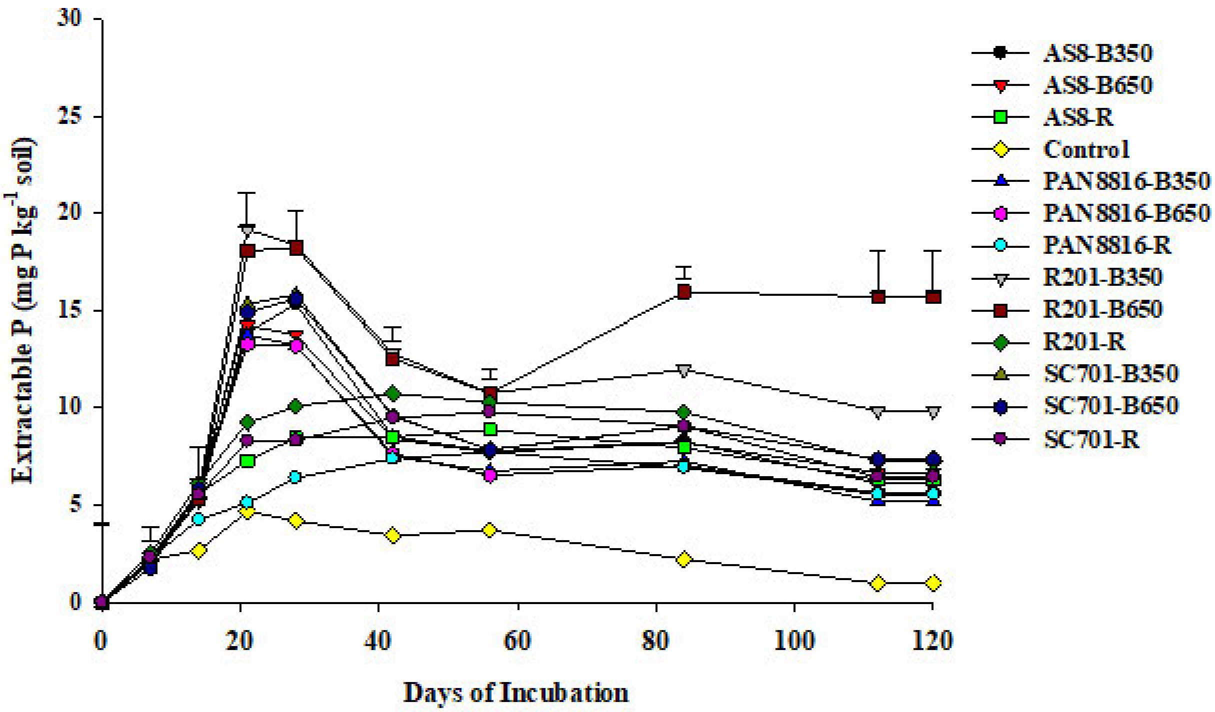

Figure 4.5: Trends in extractable P release during the 120-day incubation period for soils amended with feedstock (R) and biochar pyrolyzed at 350ºC (B350) and at 650ºC (B650) from different maize (R201 & SC701) and sorghum (AS8 & PAN8816) cultivars along with the control treatment. The vertical error bars indicate LSD ($p<0.05$).

4.3.6 Effects of feedstock and biochar from different cultivars of maize and sorghum on soil pH

Soil pH significantly differed among treatments throughout the incubation period ($p < 0.001$). It generally increased from day 0 with multiple peaks observed during the incubation period before stabilizing after 84 days (Figure 4.6). Soil pH was influenced more by cultivar specific differences and pyrolysis temperature than by crop type (Table 4.4). The control treatment recorded the lowest soil pH (4.84), which was not significantly different from that of PAN8816 feedstock (4.82). Soil pH was generally in the order feedstock < B350 < B650 for both maize and sorghum treatments (Table 4.4).

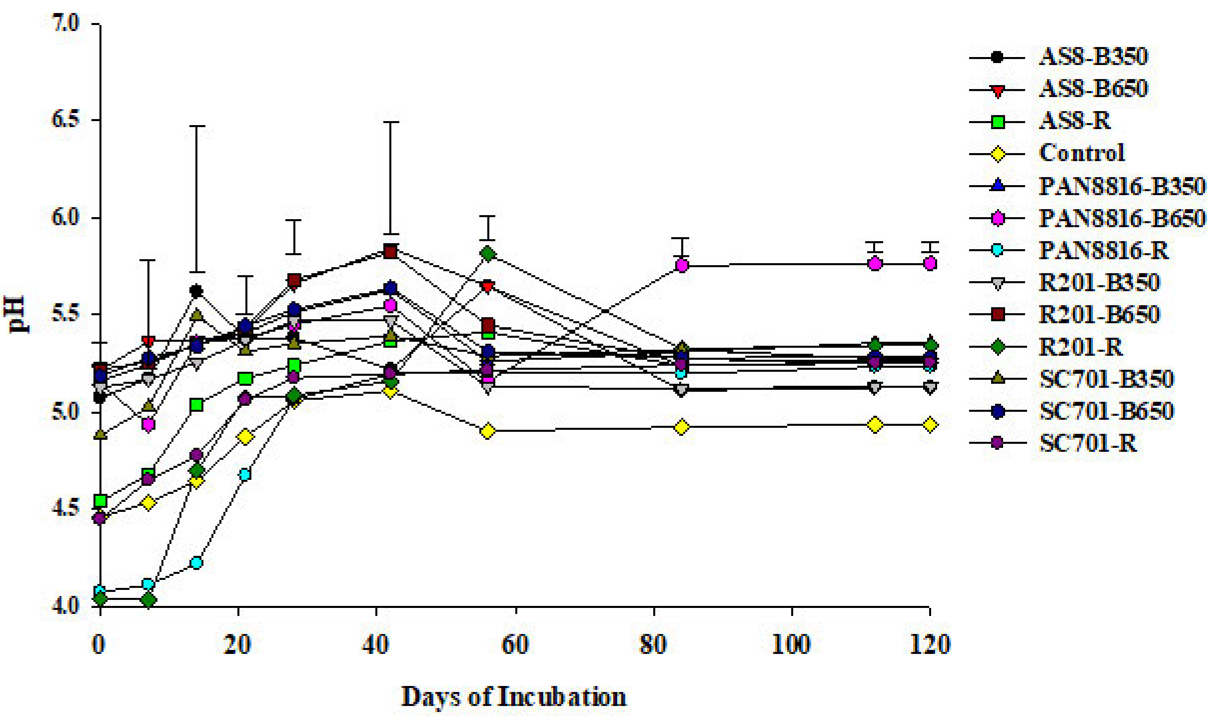

Figure 4.6: Trends in soil pH during the 120-day incubation period in soils amended with feedstock (R) and biochar pyrolyzed at 350ºC (B350) and at 650ºC (B650) from different maize (R201 & SC701) and sorghum (AS8 & PAN8816) cultivars along with the control treatment. The vertical error bars indicate LSD (p<0.05).

4.3.7 Relationship between CO_2-C emissions, ammonium-N, nitrate-N and phosphorus mineralization, soil pH and residue biochemical properties

The relationships between measured variables with initial residue biochemical properties are presented in Figure 4.7. CO_2-C emissions showed significant negative correlations ($p < 0.05$) with residue total P (r=-0.67), residue total C (r=-0.60), residue fixed C (r=-0.60), soil pH (r=-0.71) and soil extractable P (r=-0.72) while it significantly positively correlated ($p < 0.05$) with residue C:P ratio (r=0.64), NH_4^+-N (r=0.75) and cumulative CO_2-C (r=0.96). Cumulative CO_2-C emissions were significantly positively correlated ($p < 0.05$) with NO_3^--N (r=0.70) and NH_4^+-N (r=0.86) but negatively correlated ($p < 0.05$) with residue total P (r=-0.56). Extractable P exhibited significant positive correlations ($p < 0.05$) with residue total P (r=0.89), residue total C (r= 0.70), and residue fixed C (r= 0.81) while it significantly negatively correlated ($p < 0.05$) with residue C:P ratio (r=-0.75) and NH_4^+-N (r=-0.71).

Ammonium-N was significantly positively correlated ($p < 0.05$) with residue total N (r=0.76) and NO_3^--N (r=0.81) while it showed a negative correlation ($p < 0.05$) with residue C:N ratio (r=-0.80). Nitrate-N exhibited a significant positive correlation ($p < 0.05$) with residue total N (r=0.75) and a significant negative correlation with residue C:N ratio (r=-0.60). Soil pH significantly positively correlated ($p < 0.05$) with residue total P (r=0.67), residue total C (r= 0.82), residue fixed C (r=0.77) and residue pH (r=0.85). Residue ash content was positively correlated ($p < 0.05$) with residue pH (r= 0.75) while residue C:N ratio was significantly negatively correlated with residue total N (r=-0.76). Residue C:P ratio showed a negative correlation ($p < 0.05$) with residue total P (r=-0.78), while residue fixed C positively correlated with residue total P (r=0.91), residue total C (r=0.89), and residue pH (r=0.79). Residue pH significantly positively correlated ($p < 0.05$) with residue total P (r=0.77) and residue total C (r=0.92), while residue total C also correlated positively with residue total P (r = 0.85).

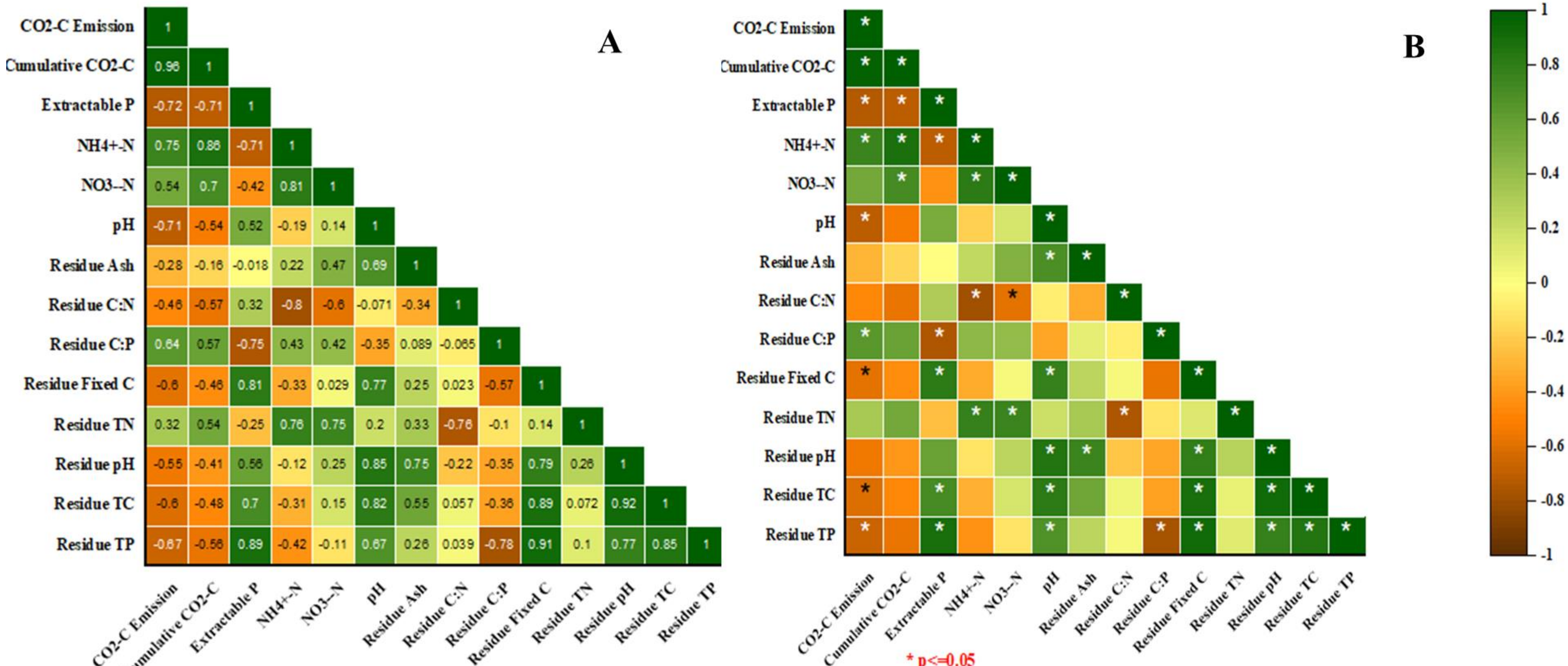

Figure 4.7: Pearson's linear correlation between CO_2 emissions, NH_4^+-N, NO_3^--N, extractable P and soil pH and the biochemical composition of feedstock and biochar from different cultivars of maize and sorghum. Figure A shows the correlation coefficients while B indicates the significance levels ($p < 0.05$), with significance denoted by *. Residue TC= Total carbon content in feedstock/biochar; Residue TC= total phosphorus in feedstock/biochar; Residue TN= total nitrogen content in feedstock/biochar; Residue pH= pH of feedstock/biochar; Residue Ash= ash content of feedstock/biochar; Residue C:N= C:N ratio of feedstock/biochar; Residue C:P= C:P ratio of feedstock/biochar

4.4 Discussion

The findings of this incubation study highlight that CO_2-C emissions and nutrient mineralization were driven more by cultivar specific differences than by crop type. Specifically, within each crop type one cultivar consistently exhibited higher CO_2-C emissions, NH_4^+-N, NO_3^--N and extractable P, while the other cultivar showed lower emissions and concentrations. This pattern suggests that the biochemical composition of each cultivar such as total C, total N, total P, C:N and C:P ratios played a more significant role than the broader crop classification. Supporting this observation, You et al. (2020) reported that the decomposition of residues varies depending on their chemical composition. This was evident in this study, as residues from different cultivars of maize and sorghum exhibited different biochemical compositions despite belonging to the same crop type (Table 4.1). For instance, the greater availability of labile organic carbon in certain cultivars likely stimulated microbial activity, leading to enhanced CO_2-C emissions and nutrient mineralization. Therefore, these findings deviate from the common expectation that crop type would dominate such differences and emphasize the importance of understanding cultivar specific traits when selecting crop residues for improving soil quality.

The effects of pyrolysis temperature were also influenced by cultivar specific differences, rather than being uniform across all cultivars. Some cultivars exhibited greater sensitivity to pyrolysis temperature, while others showed smaller differences, highlighting variability in the interaction between feedstock properties and pyrolysis temperature. These findings suggest that selecting the appropriate pyrolysis temperature and feedstock requires a thorough understanding of cultivar specific traits. This view is supported by Khan et al. (2024), who emphasized the importance of aligning biochar production to feedstock characteristics to make it more effective. The sharp increase in CO_2-C emissions observed in amended soils at the beginning of incubation can be attributed to the stimulation of microbial activity due to the addition of readily available carbon sources. This aligns with findings by Dumale Jr et al. (2009), who reported that organic material amendments rapidly enhance microbial respiration leading to an initial spike in CO_2-C emissions. The higher emissions associated with feedstock treatments suggest that these materials contain a greater proportion of labile carbon which is easily decomposed by microbes (Watanabe and Sato, 2015). In contrast, biochar amended soils exhibited lower emissions probably due to the higher stability and recalcitrance of biochar carbon as reported by Case et al. (2014). This supports previous studies demonstrating that biochar reduces the release of CO_2-C compared to feedstocks, due to its reduced proportion of labile carbon (Zavalloni et al., 2011).

The similar CO_2-C emissions observed across all biochars, regardless of pyrolysis temperature or cultivar can be attributed to the biochemical characteristics of biochar. The initial carbon content of the feedstocks in this study was not significantly different, which may have resulted in biochars with similar carbon structures and decomposition potential. This consistency coupled with uniform pyrolysis conditions, probably minimized variability in CO_2-C emissions between biochars from different cultivars or pyrolysis temperatures. Furthermore, significant negative correlation between CO_2-C emissions and residue properties (total C, fixed C and pH) further reinforces the role of carbon stability and residue characteristics in regulating microbial activity and carbon mineralization. This relationship highlights that residues with higher total C, fixed C and pH such as biochars are more recalcitrant and less prone to microbial decomposition resulting in lower CO_2-C emissions. Feedstocks which generally had lower total C, fixed C and pH provided a readily decomposable carbon source that stimulated higher microbial respiration and CO_2-C emissions. These findings are consistent with Hailegnaw et al. (2019) who reported that biochars are more stable and contain carbon that is less accessible to microbes resulting in slower decomposition of organic matter and lower CO_2-C emissions. This highlights the potential of biochar as an effective tool for mitigating CO_2-C emissions and enhancing the long-term stability of soil organic carbon.

The differences among maize and sorghum cultivars, as well as the effects of pyrolysis temperature, were more pronounced in cumulative CO_2-C emissions (Figure 4.2). Cumulative CO_2-C emissions were generally higher for feedstocks and biochars produced at 350°C. This could be due to the higher content of labile C fractions and volatile matter in these treatments, which are more readily utilized by microorganisms leading to increased mineralization rates (Whitman et al., 2012). In contrast biochars produced at 650°C generally resulted in the lowest cumulative CO_2-C emissions, supporting the view that biochars produced at higher pyrolysis temperatures are more stable in the soil due to their increased degree of aromaticity and aromatic condensation (Peng et al., 2011). These findings align with those of Deng et al. (2020) who reported decreased cumulative CO_2-C emissions with biochars produced at higher pyrolysis temperatures highlighting their higher stability and potential for long-term carbon sequestration. The lower emissions observed for biochars at 650°C further demonstrate their recalcitrance and resistance to microbial decomposition. Among maize cultivars, the SC701 feedstock resulted in the highest cumulative CO_2-C emissions while all R201 treatments and SC701-B650 resulted in lower emissions. For sorghum cultivars, cumulative CO_2-C emissions were highest with PAN8816 feedstock, while AS8-B650 resulted in the lowest emissions. These results suggest that applying R201 treatments, SC701-B650 and AS8-B650 could serve as effective strategies for reducing cumulative CO_2-C emissions and preventing soil carbon depletion as these treatments exhibit higher recalcitrance and greater stability in the soil.

The observed increase in NH_4^+-N concentrations during the initial phase of incubation with peaks reached within 14 days can be attributed to the process of ammonification. During this period soil microbes rapidly decomposed organic nitrogen compounds present in the added residues (feedstocks and biochars), converting them into NH_4^+-N due to the availability of labile nitrogen such as proteins and amino acids in the residues (van Midden et al., 2024). Similar patterns have been observed in previous studies, where the release of NH_4^+-N corresponded to the microbial decomposition of easily degradable nitrogen compounds in organic amendments (Liyanage et al., 2022). Following the peak, NH_4^+-N concentrations declined sharply for all treatments. This decline can be primarily explained by nitrification, a microbial process where NH_4^+-N is oxidized to NO_3^--N by autotrophic bacteria such as *Nitrosomonas* and *Nitrobacter (*Baskaran et al., 2020*)*. The decrease in NH_4^+-N suggests that these microbes efficiently utilized the available ammonium, converting it into nitrate as the incubation progressed. Similar findings were reported by Marouani et al. (2021) and Liyanage et al. (2022) who observed higher NH_4^+-N concentrations at the beginning of their incubation experiment of different organic amendments and higher NO_3^--N concentrations at later stages of incubation following the decline of NH_4^+-N.

The effects of feedstock and biochars on soil NH_4^+-N and NO_3^--N differ depending on feedstock type, composition and pyrolysis temperature. Biochar and feedstock applications can reduce NH_4^+-N and NO_3^--N concentrations (Yao et al., 2012; Zheng et al., 2013). However, this is not always the case as some studies report positive impacts of feedstock and biochar on soil NH_4^+-N and NO_3^--N concentrations. For instance, Pendergast-Miller et al. (2011) found that hardwood biochar can increase soil NO_3^--N. Similarly, Kaleem Abbasi et al. (2015) observed mixed effects of residues as the application of *Glycine max* and the shoots and roots of *Trifolium* repens resulted in continuous N mineralization, the roots of *Glycine max* and *Zea mays*, as well as the shoots of *Zea mays* resulted to net N immobilization. In the present study, mixed effects on NH_4^+-N and NO_3^--N were observed. The application of some treatments reduced NH_4^+-N and NO_3^--N concentrations, while others increased them compared to the control. Thus, all R201, SC701 and AS8 treatments as well as PAN8816-B650 reduced soil NH_4^+-N concentration in comparison with the control. While the application of PAN8816 feedstock and its B350 biochar increased it. Regarding NO_3^--N concentration, the application of all R201 treatments, SC701 (feedstock and B350) and AS8 feedstock reduced its concentration while AS8 biochars (B350 and B650) had no effect and all PAN8816 treatments increased it compared to the control. The reduction of NH_4^+-N and NO_3^--N in certain treatments implies a decrease in soil mineral N. This may be attributed to enhanced N immobilization driven by the presence of amino acids and other labile organic compounds in the feedstocks and biochars which are readily utilized by soil microbes (Wang et al., 2012). These findings align with those of Zavalloni et al. (2011), who reported reduced NO_3^--N and mineral N after the application of wheat residues and wood biochar produced at 500°C. Similarly, Novak et al. (2010) observed a decline in NH_4^+-N and NO_3^--N following the application of switchgrass residues and pecan shell biochar (700°C). Odugbenro et al. (2018) also noted reduced NH_4^+-N and NO_3^--N concentrations, leading to a decrease in mineral N after the application of maize biochar produced at 450ºC.

Nitrogen mineralization in soil is influenced by factors such as the C:N ratio, total N and labile N fractions present in the applied feedstocks or biochars (Zavalloni et al., 2011). Residues with a wide C:N ratio (C:N > 32) are known to cause slower mineral N release or immobilization (Zavalloni et al., 2011). In the present study, the C:N ratio was found to be significantly negatively correlated with NH_4^+-N and NO_3^--N concentrations (Figure 4.7) indicating that reduced NH_4^+-N and NO_3^--N concentrations were associated with higher C:N ratios. Some treatments such as R201-R, R201-B350 and AS8-B650 had higher C:N ratios of 153.3, 92.25 and 86 respectively, which likely contributed to N immobilization. However, the C:N ratios of R201-B350 and AS8-B650 did not significantly differ from others suggesting that C:N ratio alone was not a controlling factor for all treatments. The higher NH_4^+-N and NO_3^--N concentrations in PAN8816 treatments could instead be attributed to their higher residue total N content particularly in the feedstock and B350 which exhibited significantly higher total N. Residue total N was also found to be significantly positively correlated with NH_4^+-N and NO_3^--N concentrations in this study. These results suggest that total N content plays a critical role in determining nitrogen availability in soils amended with feedstocks or biochars. It is important to note that reductions in soil mineral N following feedstock and biochar application are often short-term. For example, Hossain Bai et al. (2014) reported no significant effects of pinewood biochar (550ºC) on soil NH_4^+-N and NO_3^--N at six months. However, after six months NH_4^+-N concentrations declined while NO_3^--N concentrations increased. This indicates that the impacts of biochar and feedstock on soil nitrogen dynamics evolve over time and are influenced by ongoing decomposition, microbial activity and biochar stability.

Similar to mineral N, extractable P mineralization was significantly influenced by cultivar specific differences and pyrolysis temperature depending on the cultivar. Extractable P increased from the beginning of the incubation period suggesting the presence of labile P fractions in both feedstock and biochar. The effects of feedstock and biochar application on soil extractable P can be either positive or negative, depending on factors such as feedstock type, chemical composition and the pyrolysis temperature used to produce the biochar (Amendola et al., 2017; Li et al., 2017; Liu et al., 2017). In the present study, the application of all amendments had a positive effect on soil extractable P compared to the control. According to Mafongoya et al. (2002), P release in soil is positively correlated with the initial P content of the organic material, with total P content greater than 0.2% in residues leading to P mineralization. In this study, all treatments had a total P content greater than 0.2% and residue total P was positively correlated with extractable P. This correlation explains the increase in extractable P observed with all treatments compared to the control. The biochars and feedstocks likely contributed to P mineralization by providing labile P fractions and enhancing soil P availability. The highest extractable P concentrations were observed in R201 and AS8 treatments particularly in their biochar amendments for maize and sorghum cultivars, respectively. This is attributable to the higher total P content in these residues which resulted in greater P mineralization and availability in the soil. These findings are consistent with those of Gupta et al. (2024), who reported increased soil available P following the application of rice residue biochar and acacia wood biochar. Similarly, Zhai et al. (2014) observed an increase in soil available P after applying maize residue biochar.

In this study, the application of the different amendments resulted in increases in soil pH by most amendments in comparison with the control. Pyrolysis temperature also influenced soil pH more than the specific cultivar differences and crop type, with pH increasing as pyrolysis temperature increased especially for the maize varieties. Soil pH was lower with feedstock applications compared to biochar treatments probably due to the acidic nature of feedstocks and the organic acids released during their decomposition. Conversely, biochars significantly increased soil pH due to their alkaline nature, a result of the pyrolysis process. This aligns with previous studies highlighting biochar's liming effect due to its ash content and alkaline properties (Chintala et al., 2013; Silva et al., 2017). The higher soil pH with biochar applications can also be attributed to their higher residue ash content and pH compared to feedstocks. Correlation analysis revealed a significant positive relationship between soil pH, residue ash content and residue pH. Pyrolysis concentrates mineral ash and produces alkaline compounds, enhancing biochar's ability to buffer soil acidity (Nelissen et al., 2012). This explains the higher soil pH in B-650 biochars than B-350 biochars for maize treatments. Interestingly, although biochars produced at 350^{o}C and 650^{o}C had significantly different residue pH and ash content, these differences did not result in significant differences in soil pH for sorghum treatments, biochars from different cultivars and same pyrolysis temperatures had similar effects on soil pH. This could be due to a saturation effect where additional increases in ash content or residue pH had no further impact on soil pH. Both 350^{o}C and 650^{o}C sorghum biochars provided sufficient alkalinity to neutralize soil acidity reducing the influence of differences in biochar properties. These findings deduce that biochar generally exhibit a strong liming effect on soil pH, regardless of feedstock or pyrolysis temperature emphasizing biochar's ability to neutralize soil acidity and its value as a sustainable soil amendment for pH regulation (Coumaravel et al., 2011; Shetty et al., 2020).

4.5 Conclusion

This study demonstrated that CO_2 emissions, nitrogen mineralization (NH_4^+-N and NO_3^--N), phosphorus mineralization (extractable P) and soil pH were influenced more by cultivar specific differences than by crop type and by pyrolysis temperature depending on the cultivar. This highlights the importance of selecting both the cultivar and pyrolysis temperature to improve soil fertility and achieve carbon sequestration. The application of feedstock and biochar from different cultivars of maize and sorghum demonstrated potential for carbon sequestration enhancing nutrient availability and improving soil properties, including correcting soil acidity. Biochars produced at higher pyrolysis temperatures (650ºC) showed higher stability and lower CO_2 emissions. Among maize cultivars, R201 feedstock and biochars consistently resulted in lower CO_2 emissions making them a good option for carbon sequestration, while SC701-B650 increased NO_3^--N concentration making it suitable for improving nitrogen availability. For sorghum cultivars, AS8-B650 resulted in lower CO_2 emissions making it a good option for carbon sequestration while PAN8816-B350 increased both NO_3^--N and NH_4^+-N concentrations showing potential for improving nitrogen availability. For improving phosphorus availability, R201-B650 and the AS8 biochars could be the most effective treatments as they resulted in higher extractable P. All amendments used in this study (except PAN8816 residues) can be used to correct soil acidity.

These findings suggest that biochar is a more sustainable option for long term carbon sequestration and nutrient improvement compared to feedstock. However, the benefits of certain amendments may take longer to show, as shown by the lower NH_4^+-N concentrations observed with maize feedstocks and biochars. While these findings are promising, practical adoption by farmers would require careful consideration of local soil conditions, climate suitability, cultivar adaptability, and access to biochar production facilities. Furthermore, the knowledge and resources available to farmers regarding biochar technologies may vary significantly. Farmers may need to prioritize growing specific cultivars such as R201 (maize) and AS8 (sorghum) for biochar production to enhance carbon sequestration as well as phosphorus then grow PAN8816 (sorghum) and SC701 (maize) for improving mineral N availability. Future research should focus on validating these findings under farmer field conditions to better understand the effects of these amendments on soil properties and crop yields under real environmental conditions. This will help establish the practicality of adopting this residue management strategy for sustainable agriculture.

CHAPTER 5

Soil carbon pools and carbon dioxide emission as affected by amendment with residues and biochar of maize (*Zea mays L.*) and sorghum (*Sorghum bicolor L.*) cultivars

A version of this chapter was submitted to the Soil & Tillage Research Journal for publication:

Mbava N, Zengeni R and Muchaonyerwa P (2025). *Soil carbon pools and carbon dioxide emission as affected by amendment with residues and biochar of maize (Zea mays L.) and sorghum (Sorghum bicolor L.) cultivars. Journal of Soil & Tillage Research (**under review**)*

Abstract

Organic amendments, including crop residues and biochar can increase soil organic carbon (SOC), reduce carbon dioxide (CO_2) emissions and mitigate climate change. Understanding effects of crop type, cultivar and associated biochar types on SOC storage and CO_2 emissions is crucial in soil quality enhancement. This study determined the impacts of application of feedstock/crop residues (R) and biochar (B) from contrasting maize (M) and sorghum (S) cultivars on soil carbon pools and CO_2 emissions. The field trial was conducted during the summer and winter seasons of 2023 at Ukulinga research farm, University of Kwa Zulu-Natal. Residues and biochar from maize (SC701 & R201) and sorghum (AS8 & PAN8816) cultivars were applied at rates equivalent to 5 t C ha^{-1} to 15 m^2 plots in summer. In winter, the plots were split with half receiving the same treatments to assess cumulative effects while the other half was left untreated to assess the residual effects. Spinach seedlings were planted and grown for 12 weeks with weekly CO_2 emissions measurements. At the end of each season, soil samples were analyzed for SOC, particulate organic carbon (POC), permanganate oxidizable carbon (POXC), microbial biomass carbon (MBC) and β-glucosidase activity. The SOC pools and CO_2 emissions were more strongly influenced by specific cultivars than by the crop type. Cumulative applications of both feedstock and biochar generally increased SOC pools relative to residual effects. Cumulative applications and residual biochar effects reduced CO_2 emissions relative to feedstock application. The cumulative application of residues from PAN8816 sorghum cultivar (33.73 µmol m^2 s^{-1}) and SC701 maize cultivar (33.69 μmol m^2 s^{-1}) resulted in higher CO_2 emissions than AS8 sorghum cultivar (20.92 μmol m^2 s^{-1}) and R201 maize cultivar (18.81 μmol m_2 s^{-1}), which had lowest. Biochar applications from all cultivars (maize and sorghum) reduced CO_2 emissions with no significant differences between them and the control. Treatments with lower CO_2 emissions were associated with higher SOC pools, with biochar treatments generally resulting in higher carbon pools than feedstock treatments. Feedstock enhanced β-glucosidase activity while biochar, particularly from maize cultivar R201 suppressed it. This study reveals that both residues from different cultivars of maize and sorghum have the potential to enhance SOC pools, especially when applied as biochar which reduces CO_2 emissions from the soil.

Keywords: β-glucosidase, biochar, carbon sequestration, crop residues, soil carbon

5.1 Introduction

Declining soil quality in agriculture is significantly influenced by agricultural activities, including monoculture, excessive use of chemical fertilizers, intensive cultivation, and poor management of pesticides and herbicides (Prashar and Shah, 2016). Globally, the agricultural sector contributed approximately 16 billion tonnes of CO_2 equivalent emissions as of 2020 (FAO, 2022) therefore the sector has the responsibility reduce these emissions. The growing need for food due to increasing human population necessitates the development of sustainable soil management practices to remediate degraded soils and improve crop yields. Retention of crop residue (stems, leaves, chaff and husks), which are readily available and accessible (Turmel et al., 2015), after harvesting grain, seed or fiber (Agrawal et al., 2023), is considered to be a sustainable, cost-effective and efficient practice. Cereals contribute 75% of the approximately 3.5 Mg of crop residues produced each year, globally (Bhattacharyya et al., 2018). The crop residues have the potential to improve soil fertility through their composition of carbon (40-45%), nitrogen (0.6-1%), phosphorus (0.45-2%), potassium (14-23%) and micronutrients essential for crop growth (Wang et al., 2020).

The retention of crop residues could also decrease evaporation, sequester carbon dioxide (CO_2), increase organic matter, enhance soil structure and improve crop yields (Badía et al., 2013; Hoang and Marschner, 2019). The decomposition rate and nutrient release of crop residues depends on their quality or chemical composition (i.e. C:N ratio, lignin content, cellulose, hemicellulose and polyphenols concentrations), soil conditions (pH, moisture, temperature, texture) and type of residue (i.e. feedstock or biochar) (Grzyb et al., 2020). A C:N ratio > 25:1 results in the immobilization of inorganic nitrogen while a C:N <25:1 leads to N mineralization. Additionally, warm temperatures and optimum soil moisture enhance decomposition (Singh et al., 2020). Numerous studies have also showed the benefits of crop residues in improving soil quality (Chatterjee et al., 2018; Yue et al., 2023).

Chatterjee et al. (2018) showed that retention of wheat residue mulch improved the stable and labile soil carbon pool. Yue et al. (2023) found that the use of organic fertilizer and retaining maize straw significantly enhanced soil water content, field capacity, macroaggregates, total C, total N and microbial biomass C and N. However, the effectiveness of application of crop residues may depend on the chemical composition of the residue, soil properties and the climate of the area (Grzyb et al., 2020). In some cases, residue decomposition can lead to accelerated CO_2 emissions into the atmosphere, contributing to climate change (Widowati et al., 2011). Therefore, efforts to reduce greenhouse gas emissions resulting from the decomposition of organic matter, while maintaining soil fertility, are essential. Conversion of crop residues to biochar, a product of pyrolysis of organic materials in the absence of oxygen and at high temperatures (Adekiya et al., 2019), could be beneficial.

Biochar applications have been reported to enhance the physical, chemical and biological properties of the soil, thereby improving crop yields (Das et al., 2017). The stable aromatic structure of biochar could be essential to minimize CO_2 emissions to the atmosphere and increase SOC storage (Colantoni et al., 2016; Purakaystha et al., 2016; Xu et al., 2016; Alazzaz et al., 2020). At an estimated decomposition rate of 0.03% per year, biochar-based SOC can remain in the soil for hundreds of years (Glaser et al., 2001; Keith et al., 2011), with dual benefits of climate change mitigation and soil quality improvement (Liu et al., 2014). Kuppusamy et al. (2016) highlighted that the use of biochar can potentially sequester approximately 7.6 t ha^{-1} of CO_2 annually, which may result in buildup of SOC and its pools, that are the determinants of soil quality (Liefeld, 2005; Smith, 2008). The SOC comprises labile and recalcitrant fractions with differing sensitivities to land use change and management (Guo et al., 2018; Wang et al., 2020). The labile carbon pool includes particulate organic carbon (POC), microbial biomass carbon (MBC) and permanganate oxidizable carbon (POXC) (Zou et al., 2008). The contribution of biochar to SOC pools and soil fertility may depend on crop type and variety and pyrolysis conditions.

Sustainable management of residues of maize and sorghum, which are staple foods and commercial crops in Sub-Saharan Africa (Apraku et al., 2017; Hadebe et al., 2017) could be beneficial for soil health. While many researchers have compared the potential of residues from these crops to enhance soil quality and crop yields, studies comparing the residues and biochar from different cultivars of these crops in soil quality improvement and carbon sequestration particularly under field conditions are lacking. Hence, the objective of the current study was to determine effects of residues and biochar from different cultivars of maize and sorghum on SOC pools and CO_2 emissions from soil under field conditions.

5.2 Materials and Methods

5.2.1 Study Site

A field study was initiated in the summer season of 2023 (17 January 2023) at Ukulinga Research Farm, University of KwaZulu-Natal (UKZN), Pietermaritzburg, South Africa (29°67'S, 30°.406'E, altitude of 811 m). Ukulinga exhibits a warm temperate climate with an average annual rainfall of 750 mm, with 23% occurring during the winter months. The wettest months are January and February, receiving average monthly totals of 108 mm and 103 mm, respectively. Ukulinga experiences warm to hot summers and mild winters with occasional frost (Mengistu et al., 2016). The mean annual temperature is 18.4°C. The soil properties for the field study were described in detail in Section 4.2.4 of Chapter 4. Daily rainfall and air temperature data for both seasons (January 2023 to January 2024) were provided by the UKZN Agrometeorology Discipline, extracted from their weather station located 5 km away from Ukulinga Farm (Figure 5.1).

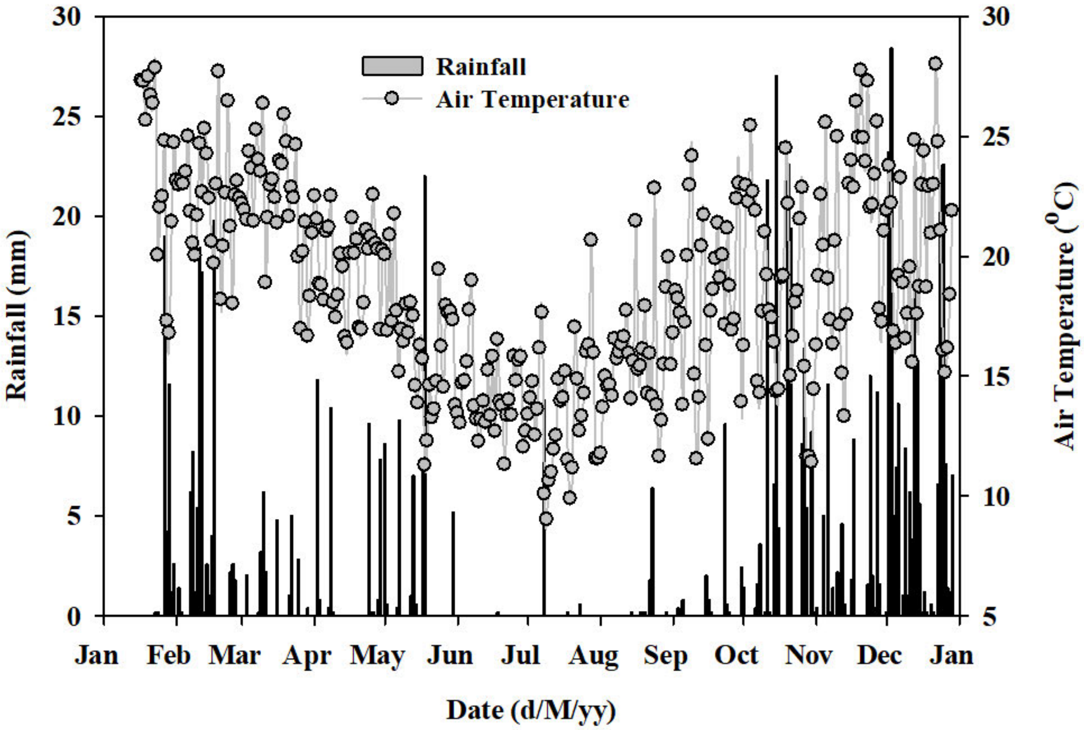

Figure 5.1: Air temperature and rainfall data for the duration of the experiments (summer and winter seasons) obtained from the Agmet station. (January 2023 to January 2024).

5.2.2 Biomass feedstock production

The biomass and feedstock production process were carried out as outlined in Section 3.2.1 of Chapter 3.

5.2.3 Biochar production

The biochar used in this study was produced from the above-ground biomass (feedstock) of the maize and sorghum cultivars using an on-site biochar kiln located at the Agricultural Engineering Discipline workshop at Ukulinga Research Farm. The pyrolysis temperature was approximately 400°C with a minimum residence time of 3 hours. The biochar was then pulverized and sieved using a 2 mm sieve before laboratory analysis in triplicate. Selected biochar properties are in Table 5.1.

Table 5.1: Composition of feedstock and biochar of different cultivars of maize and sorghum

Treatment	Total C (%)	Total N (%)	Total P (%)	Lignin (%)	C:N	C:P	Lignin: N	pH	Fixed C (%)	Ash (%)
M-R201-R	38.89[a]	0.324[a]	0.47[c]	38.57[e]	153.3[d]	82.76[ab]	153.28[b]	4.88[c]	17.24[d]	6.50[a]
M-SC701-R	33.29[a]	1.071[c]	0.377[b]	4.18[a]	31.08[a]	88.42[abc]	3.9[a]	5.75[d]	12.23[a]	16.73[d]
S-AS8-R	33.09[a]	0.481[ab]	0.343[a]	24.79[d]	68.87[b]	96.34[abc]	51.65[ab]	4.26[a]	14.10[c]	11.48[b]
S-PAN8816-R	37.62[a]	1.46[d]	0.333[a]	10.21[c]	25.78[a]	113.05[c]	6.99[a]	4.82[b]	13.17[b]	14.32[c]
M-R201-B	54.17[b]	0.61[b]	0.81[f]		88.82[c]	66.88[a]		9.923[f]	40.41[h]	14.30[c]
M-SC701-B	54[b]	3.765[e]	0.65[e]		14.36[a]	83.08[abc]		10.857[h]	27.90[e]	38.89[g]
S-AS8-B	60.62[b]	1.429[d]	0.577[d]		42.41[b]	105.13[bc]		8.04[e]	36.31[g]	25.95[e]
S-PAN8816-B	51.48[b]	4.545[f]	0.58[d]		11.34[a]	88.77[abc]		10.097[g]	29.82[f]	35.59[f]
LSD(p<0.05)	6.11	0.16	0.015	1.43	29.35	18.35	72.83	0.016	0.50	0.22

R-Feedstock, B-Biochar, M-Maize, S-Sorghum. Means within the same column followed by the same letter are not significantly different at $p \leq 0.05$

5.2.4 Experimental design

The experiment was conducted during both the summer (17 January 2023-17 April 2023) and winter (21 June 2023-16 September 2023) seasons of 2023. It was arranged as a randomized complete block design with three blocks based on terrain position (upper, middle & lower slope). Treatments consisted of 8 combinations of feedstock and biochar from 2 cultivars of maize and 2 cultivars of sorghum plus a control resulting in 9 treatments in total, replicated three times in summer, (Table 5.2). Each treatment was randomly allocated to one plot in each block and this resulted in a total of 27 plots which were 15 m^2 in size (5×3 m) with a 1 m buffer between the plots in summer (Figure 5.2). Prior to the application of treatments, NPK fertilizers were applied at recommended rates in all plots, including the control plot. The chemical fertilizer NPK 2:3:2 (22) was used to provide N, P and K of 100, 138 and 278 kg ha^{-1}, while Urea (46%) and KCl (52%) fertilizers were used to supplement N and K to meet the recommended rates for spinach growth (specifically, 2.20 kg of NPK 2:3:2 (22) fertilizer was applied per 15 m^2 plot to meet the phosphorus requirement. This application also supplied 0.14 kg of nitrogen and 0.14 kg of potassium, which were below the recommended rates. An additional 0.027 kg of nitrogen was supplied using urea (46%) and 0.54 kg of potassium using KCl (52%) to meet the full nutrient requirements. After the application of chemical fertilizers, feedstocks or biochar were applied at a rate equivalent to 5 t C ha^{-1} in each appropriate plot and incorporated to approximately 20 cm depth using a hand hoe, rake, and fork. This application rate was chosen because it represents a practical and agronomically feasible quantity that is commonly used in field studies and can realistically be adopted by smallholder farmers, considering both economic and logistical constraints. Fifty-four (54) 5-week-old spinach *(Spinacia oleracea)* seedlings purchased from Sunshine Seedlings were planted on 17 January 2023 in each plot. The field was irrigated after planting, with subsequent rainfed conditions throughout the season. Eight weeks after planting the spinach leaves were harvested, leaving two leaves per plant, to regrow. At 12 weeks after planting, the entire biomass (shoots and roots) was harvested. Following the spinach harvest, four soil samples (0-20 cm) were collected and combined to form a composite sample from each treatment plot. Fresh soil samples were thoroughly mixed and sieved through a 2 mm sieve. Half of the fresh soil samples, for the analysis of β-glucosidase activity and microbial biomass carbon (MBC), were stored in the fridge and analyzed within two weeks of sampling. The remaining half of the sample was air-dried for the analysis of soil organic carbon (SOC), particulate organic carbon (POC) and permanganate oxidizable carbon (POXC).

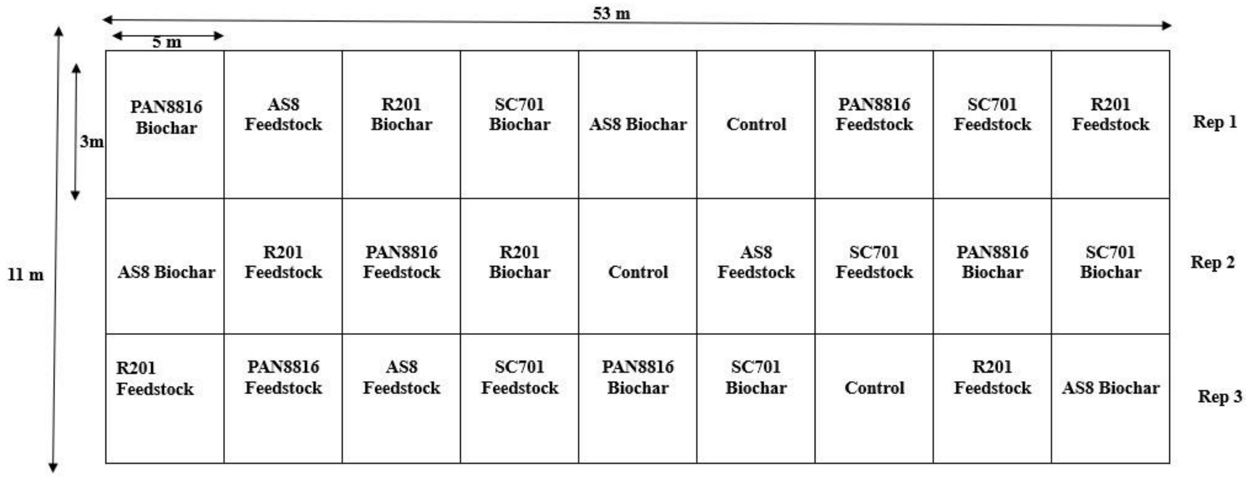

Figure 5.2: Field layout of the summer season field experiment

In the winter season, each of the 27 plots from the summer season were divided into two equal halves (2 × 3 m) with a 1 m buffer between the plots. In one half of each plot, treatments were applied for the cumulative additions of treatments, while in the other half, no treatment was applied to study the residual effects of summer treatments. Therefore, there were 54 plots of 6 m^2 and the treatments are detailed in Table 5.2, while the field layout is depicted in Figure 5.3. After the treatment applications (at 5 t C ha^{-1}), the soil in all plots (including control and residual) was homogenized using a hand hoe, fork, and rake. Twenty-seven spinach plants were planted per plot, and as it was the winter season, supplemental irrigation was provided to ensure water was not a limiting factor. After 8 weeks, the spinach leaves were harvested, leaving two leaves per plant, to regrow and after 12 weeks of planting, the entire biomass (both shoots and roots) was harvested. Four soil samples (0-20 cm) were then collected and combined to form a composite sample from each treatment plot. The fresh soil samples were thoroughly homogenized and sieved through a 2 mm sieve. The fresh soil samples for the analysis of β-Glucosidase activity and MBC were stored in the fridge and analyzed within two weeks of sampling. The remaining half of the sample was air-dried for the analysis of POC, SOC, and POXC.

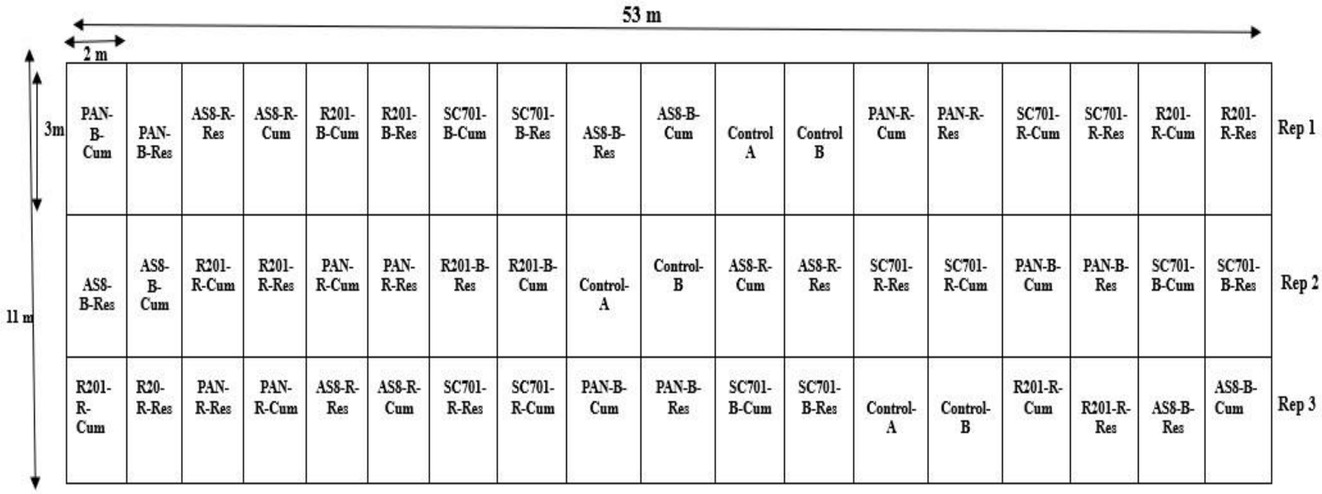

Figure 5.3: Winter season experiment field layout

Table 5.2: Treatments used in the study

Season		Treatment	Description
Summer		M-R201-R	Maize Cultivar R201 Feedstock
		M-SC701-R	Maize Cultivar SC701 Feedstock
		S-AS8-R	Sorghum Cultivar AS8 Feedstock
		S-PAN8816-R	Sorghum Cultivar PAN8816 Feedstock
		M-R201-B	Maize Cultivar R201 Biochar
		M-SC701-B	Maize Cultivar SC701 Biochar
		S-AS8-B	Sorghum Cultivar AS8 Biochar
		S-PAN8816-B	Sorghum Cultivar PAN8816 Biochar
		Control	No amendments applied
Winter	Residual effects	M-R201-R-Res	Maize Cultivar R201 Feedstock Residual Effects
		M-SC701-R-Res	Maize Cultivar SC701 Feedstock Residual Effects
		S-AS8-R-Res	Sorghum Cultivar AS8 Feedstock Residual Effects
		S-PAN8816-R-Res	Sorghum Cultivar PAN8816 Feedstock Residual Effects
		M-R201-B-Res	Maize Cultivar R201 Biochar Residual Effects
		M-SC701-B-Res	Maize Cultivar SC701 Biochar Residual Effects
		S-AS8-B-Res	Sorghum Cultivar AS8 Biochar Residual Effects
		S-PAN8816-B-Res	Sorghum Cultivar PAN8816 Biochar Effects
	Cumulative effects	M-R201-R-Cum-Cum	Maize Cultivar R201 Feedstock Cumulative Addition
		M-SC701-R-Cum	Maize Cultivar SC701 Feedstock Cumulative Addition
		S-AS8-R-Cum	Sorghum Cultivar AS8 Feedstock Cumulative Addition
		S-PAN8816-R-Cum	Sorghum Cultivar PAN8816 Feedstock Cumulative Addition
		M-R201-B-Cum	Maize Cultivar R201 Biochar Cumulative Addition
		M-SC701-B-Cum	Maize Cultivar SC701 Biochar Cumulative Addition
		S-AS8-B-Cum	Sorghum Cultivar AS8 Biochar Cumulative Addition
		S-PAN8816-B-Cum	Sorghum Cultivar PAN8816 Biochar Cumulative Addition
		Control	No amendments applied

5.2.5 Carbon dioxide emissions measurements

Carbon dioxide emissions were measured weekly in the 2nd season from each plot using an LCi-SD Soil Respiration Analysis System (ADC BioScientific Ltd., UK) from August to December 2023. The instrument automatically calculated soil respiration rates (CO_2 flux) by monitoring CO_2 concentrations and airflow, with results expressed in μmol m^{-2} s^{-1} and displayed in real time (NCI, 2022)

5.2.6 Soil analysis

5.2.6.1 Soil organic carbon (SOC)

The SOC was determined using the Walkey-Black method (Van Reeuwijk, 2002). Finely ground soil 0.5 g was weighed into a 500 mL Erlenmeyer flask, and 10 mL of 1N $K_2Cr_2O_7$ solution was added. The flask was gently swirled, then 20 mL of H_2SO_4 was added rapidly with continuous swirling and allowed to cool for 30 minutes. De-ionized water (170 mL), 10 mL of concentrated phosphoric acid and 5 drops of ferroin indicator were also added. The dichromate was back-titrated with 0.5 N ferrous ammonium sulfate solution, to a green endpoint (Van Reeuwijk, 2002). The formula by Van Reeuwijk (2002) below was used to calculate organic C.

Soil Organic C (%) $= N \times \frac{(V1-V2)}{s} \times 0.399$ (Equation 5.1)

Where:

N = Normality of ferrous sulphate solution

V1 = Volume of FAS required to titrate the blank (mL)

V2 = Volume of FAS required to titrate the sample (mL)

s = air-dry sample weight (g)

0.399= Equivalent weight of C (g) (0.003) × Correction factor for the incomplete oxidation (1.33) × conversion to percentage (100)

5.2.6.2 Particulate organic carbon (POC)

The POC was quantified in accordance with Cambardella & Elliot (1992). Twenty (20) grams of soil were dispersed by shaking for 15 hours on an end-over-end shaker in 60 ml of a 5 g L^{-1} sodium hexametaphosphate solution in a 100 ml tightly closed centrifuge tube. The dispersed soil suspension was sieved through a 53 µm sieve, and after multiple rinses with distilled water, the material retained on the sieve was transferred to a 100 mL beaker and oven-dried at 105°C. Once the soil was dry, it was ground using a mortar and pestle. The total organic C in the samples was determined following the Walkey Black Method (Van Reeuwijk, 2002). The POC was calculated following the method of Elliot et al. (1992) as outlined below:

POC (%) = $(DW) \times (\%\ C))/\text{mass of soil}$ (Equation 5.2)

Where:

DW=Dry weight of the soil retained on the sieve (g)

%C= Total organic C in the soil sample

Mass of soil= weight of the fresh soil used (20 g)

5.2.6.3 Permanganate oxidizable carbon (POXC)

POXC was analyzed following the method of Weil et al. (2003). About 2.5 g of air-dried soil was weighed into polypropylene 50-mL screw-top centrifuge tubes. Then 18 ml of deionized water and 2 ml of 0.2 M $KMnO_4$ stock solution were added to each tube, followed by shaking for 2 minutes at 240 oscillations per minute on an oscillating shaker. After shaking, the tubes were allowed to settle for exactly 10 minutes. Following this, 0.5 mL of the supernatant was transferred into a second 50 mL centrifuge tube and combined with 49.5 mL of deionized water. This transfer was conducted quickly, considering that the permanganate's reactivity with the soil persists as long as contact is maintained. The centrifuge tube was then capped and inverted to ensure thorough mixing. The standard concentrations of 0, 0.005, 0.01, 0.015 and 0.02 M were prepared from the KMnO4 stock solution, and a standard curve was generated using the concentration values of standards and the absorbance readings from the spectrophotometer. The absorbance of the sample solution was measured using a spectrophotometer set at 550 nm. As explained by Weil et al. (2003), the quantity of carbon oxidized corresponds to the amount of permanganate reduced.

Consequently, higher POXC values correlate with lower absorbance levels. The following equation derived from Weil et al. (2003) was utilized to calculate POXC:

POXC (mg kg^{-1} soil) = [0.02 mol/ L - (*a* + *b* × Abs)] × (9000 mg C/ mol) × (0.02 L solution/Wt) (Equation 5.3)

Where:

0.02 mol/L = initial solution concentration

a = intercept of the standard curve; b = slope of the standard curve

Abs = absorbance of sample

9000 = milligrams of carbon oxidized by 1 mole of MnO_4 changing from $Mn^{7+} \rightarrow Mn^{4+}$

0.02 L = volume of stock solution reacted

Wt = weight of air-dried soil sample in kg

5.2.6.4 Microbial biomass carbon (MBC)

The microbial biomass carbon (MBC) was determined by the fumigation incubations procedure (Alef & Nannipieri, 1995). Two 50 g moist soil samples (one fumigated and the other an unfumigated control), passed through a 2 mm sieve, were weighed into 100 mL plastic vials. Each sample was adjusted to 40% water holding capacity with distilled water. The fumigation process involved placing the designated sample with a beaker containing 25 mL of ethanol-free chloroform in a desiccator. The desiccator was evacuated until the chloroform boiled vigorously for 2 minutes and then left to incubate in the dark for 24 hours. The following day, the chloroform was removed through repeated evacuations approximately six times. The fumigated and unfumigated plastic vials were then placed separately into 1 L jars, each containing 20 mL of 1M NaOH in a 50 mL beaker, with 20 mL of water at the bottom of the jar. The jars were tightly closed. Then these samples were incubated in the dark at 25°C for 10 days. The CO_2 evolved during incubation was adsorbed by NaOH to produce Na_2CO_3 (Alef & Nannipieri, 1995). A 5 mL aliquot of the 1M NaOH from each incubation jar was transferred to a conical flask, after which 1 mL of 1.5 M $BaCl_2$ solution was added to precipitate the carbonate as insoluble $BaCO_3$. A few drops of phenolphthalein indicator were then added. The unreacted NaOH was titrated slowly with 0.1 M HCl under a magnetic stirrer until the color changed from pink to colorless. According to Alef &

Nannipieri (1995), acid should be added slowly at this stage to prevent possible dissolution of the precipitated $BaCO_3$. The calculation of evolved CO_2 followed thereafter.

CO_2-C (μg g^{-1} soil) = (B-S)/ [(M×E×A)/ (DW) (Equation 5.4)

Where:

B = Volume of acid needed to titrate the blank; S= Volume of acid needed to titrate the soil sample
M= Molarity of HCl; E= Equivalent weight to express the data as carbon
A= The ratio volume of NaOH to the NaOH aliquot.
DW= Dry weight of the soil sample (g) after moisture correction.

This was done for both fumigated and unfumigated samples.

Furthermore, Biomass C was calculated according to Alef & Nannipieri (1995) using the formula below:

Biomass C= F_C/K_C (Equation 5.5)

Where:

F_C= [CO_2- C evolved from fumigated] - [CO_2- C evolved from unfumigated soil]
K_C= 0.45 - A constant representing the fraction of the killed biomass mineralized to CO_2 over the incubation period

5.2.6.5 Beta-glucosidase activity

Soil β-glucosidase activity was determined using the colorimetric method outlined by Eivazi and Tabatabai (1988), by incubating 1 g of soil with 0.25 ml toluene, 4 ml modified universal buffer (pH 6), and 1 ml PNG solution for 1 hour at 37 °C. Subsequently, the suspension was filtered through Whatman filter paper no. 42, followed by the addition of 1 ml $CaCl_2$ solution and 4 ml Tris buffer (pH 12). Absorbance readings were taken at a wavelength of 400 nm using a spectrophotometer. The β-glucosidase activity was quantified as μg PNG g^{-1} dwt h^{-1} at 37 °C.

5.2.7 Statistical analysis

Treatment differences for the studied parameters were analyzed using one-way analysis of variance (ANOVA) in GenStat, 20th Edition. Data for each season were analyzed separately. Mean separation was performed using the Least Significant Difference (LSD) test at $p < 0.05$, and the Tukey-Kramer test was applied for further treatment mean comparisons at $p < 0.05$. Pearson's correlations among CO_2-C emissions, carbon pools and amendment biochemical properties were determined using GenStat 20th edition (Payne et al., 2011).

5.3 Results

5.3.1 Variations of CO_2 fluxes amongst feedstocks and biochar of different cultivars of maize and sorghum

Variations in CO_2 fluxes among feedstocks and biochar from different maize and sorghum cultivars are shown in Figure 5.4. In August, CO_2 emissions were initially low across all treatments but progressively increased throughout the season, reaching peak emissions in December. Feedstock treatments generally showed a more rapid increase in CO_2 emissions compared to biochar, both in cumulative applications and in their residual effects. Cumulative applications demonstrated higher CO_2 emissions than residual effects. Additionally, significant differences in emissions were observed between cultivars, with feedstock from SC701 (maize) and PAN8816 (sorghum) exhibiting the highest CO_2 fluxes.

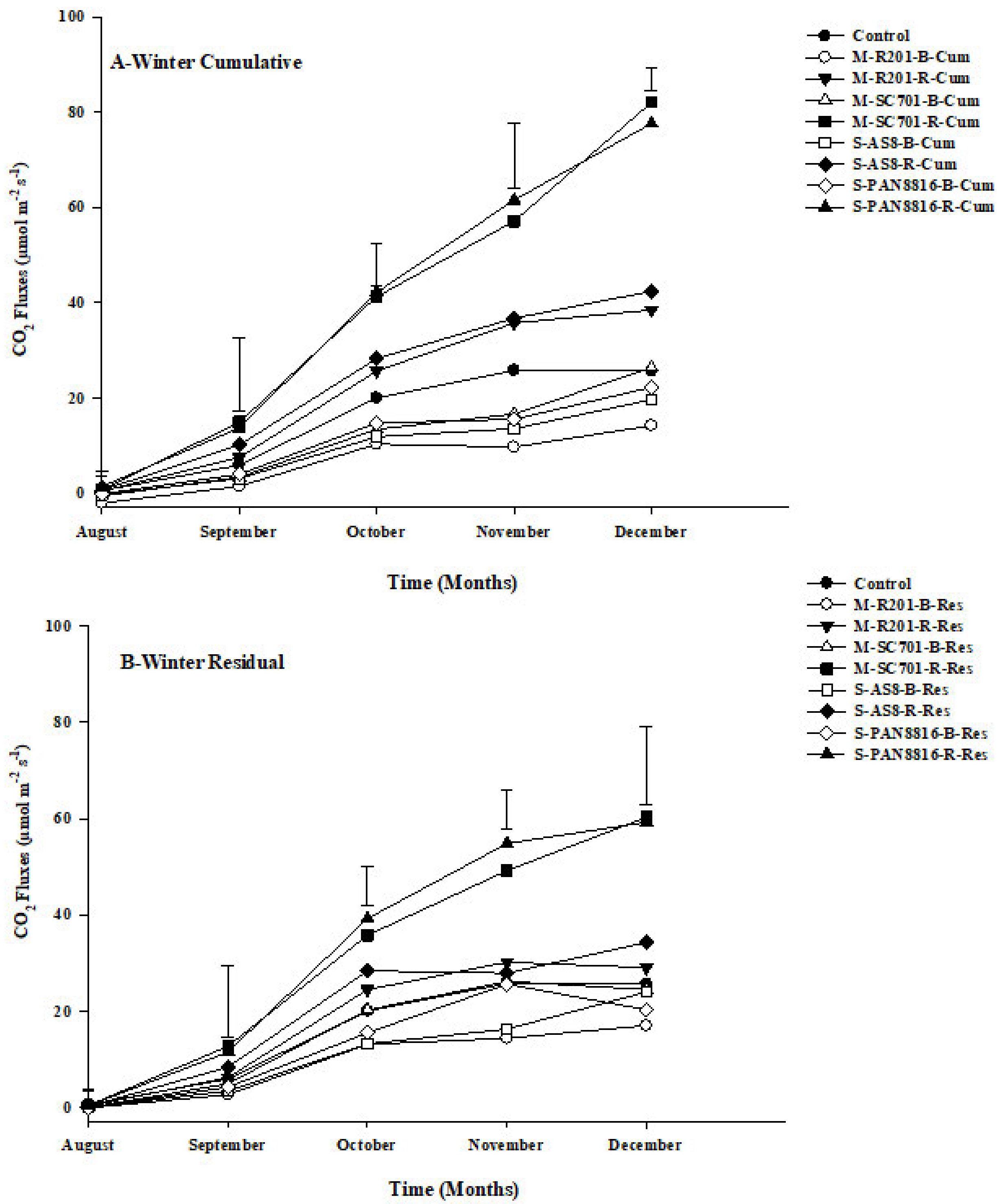

Figure 5.4: The effects of feedstock (R) and biochar (B) from different cultivars of maize (M) and sorghum (S) on CO_2 emissions (µmmol m^{-2} s^{-1}) in winter and summer season of 2023. Figure (A)

represents cumulative (Cum) application and (B) residual effects. Vertical error bars represent Lsd at $p<0.05$

There were significant variations in CO_2 emissions among the treatments ($p<0.001$). Overall, biochar generally exhibited significantly lower CO_2 emissions compared to feedstocks with no significant differences with that of the control treatment (Figure 5.5). Cumulative applications (Figure 5.5A) of feedstock from all cultivars resulted in generally higher average CO_2 emissions than residual effects while cumulative application of biochar resulted in lower CO_2 emissions than residual effects (Figure 5.5B). There was no significant trend suggesting that either maize or sorghum cultivars emitted more CO_2 than the other. Instead, the cultivar specific differences had a stronger influence on CO_2 emissions (Figure 5.5). Among the maize treatments, the cumulative application of feedstock from SC701 cultivar (M-SC701-R-Cum) resulted in highest CO_2 emissions (33.69 µmol m^2 s^{-1}), which was 137% higher than that of the control (14.19 µmol m^2 s^{-1}). Conversely, the cumulative application of biochar from the R201 cultivar (M-R201-B-Cum) resulted in the lowest CO_2 emission (5.88 µmol m^2 s^{-1}), which was not significantly different from that of the control (14.19 µmol m^2 s^{-1}) and that of M-SC701-B-Cum (10.09 µmol m^2 s^{-1}). Similarly, for sorghum, the cumulative applications of cultivar S-PAN8816 feedstock (S-PAN8816-R-Cum) resulted in the highest (33.73 µmol m^2 s^{-1}) while S-AS8 biochar (S-AS8-B-Cum) resulted in the lowest CO_2 emissions (8.33 µmol m^2 s^{-1}) which did not significantly differ from the control (14.19 µmol m^2 s^{-1}) and S-PAN8816-B (9.98 µmol m^2 s^{-1}). On the other hand, the CO_2 emissions from the cumulative applications of biochar from all cultivars of both maize and sorghum did not statistically differ (Figure 5.5A). The CO_2 emissions for residual effects exhibited the same trend as CO_2 emission from cumulative application of treatments (Figure 5.5B).

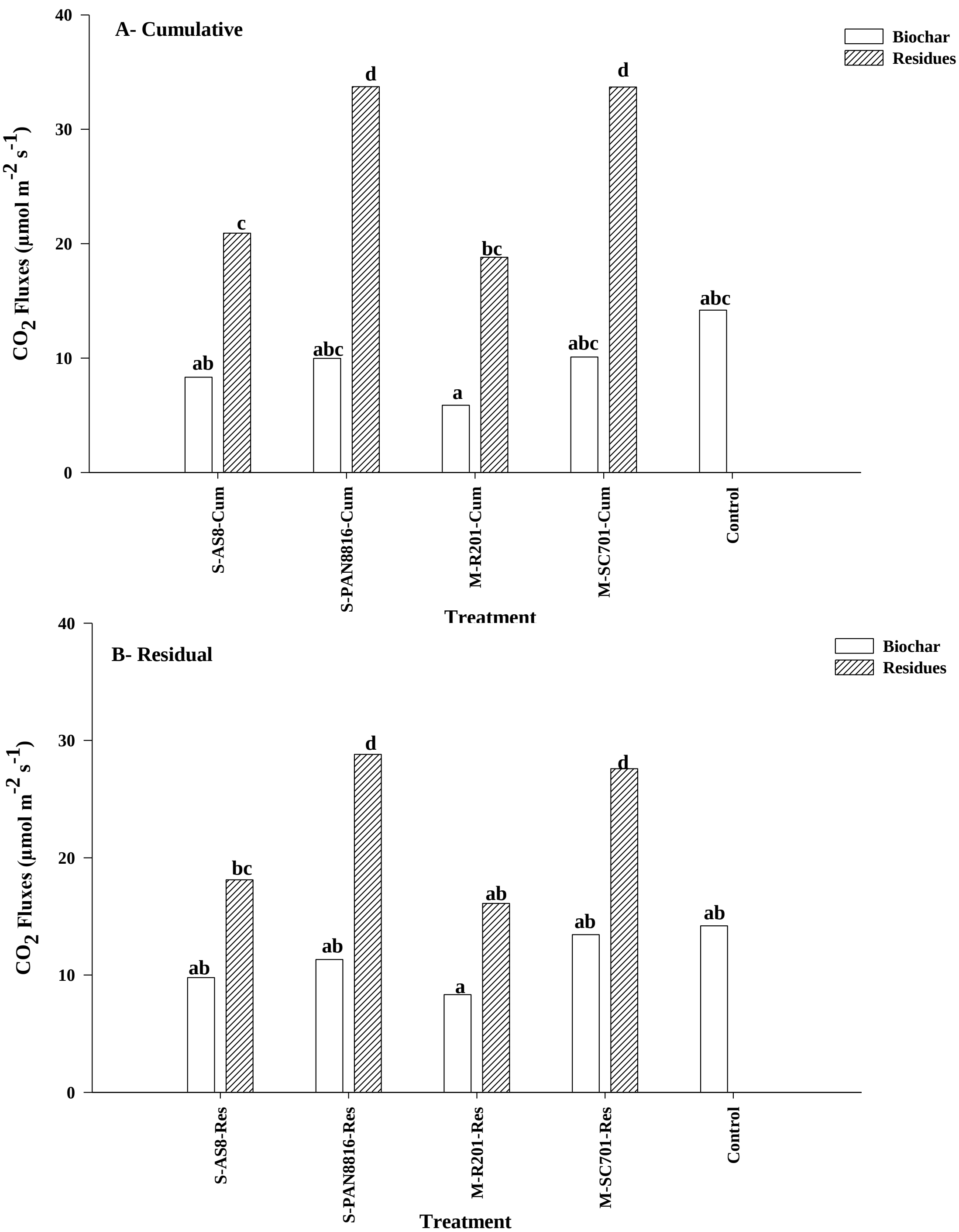

Figure 5.5: Mean CO_2 emissions (µmmol m^{-2} s^{-1}) measured after application of feedstock (R) and biochar (B) from different cultivars of maize (M) and sorghum (S). Figure (A) represents cumulative (Cum) application and (B) residual effects. Bars within each sub-figure (Aor B) that share the same letter are not significantly different at $p \leq 0.05$.

5.3.2 Variations of SOC amongst feedstocks and biochar of different cultivars of maize and sorghum

Significant differences in SOC were observed across seasons and treatments ($p<0.001$) with the applications of feedstock and biochar increasing the SOC content (Figure 5.6). The application of both feedstock and biochar consistently increased SOC content in all seasons (Figure 5.6) with biochar application resulting in higher SOC than feedstock application. The effects of feedstock and biochar application on SOC content did not depend on the crop type but rather the specific cultivar (Figure 5.6). In summer, the application of biochar from R201 cultivar (M-R201-B) resulted in the highest SOC content (20.54 g kg^{-1}), giving a 276% increase over the control, while M-SC701-R (6.37 g kg^{-1}) yielded the lowest SOC content. For sorghum cultivars S-AS8-B resulted in the highest (19.50 g kg^{-1}) and S-AS8-R- the lowest SOC content (6.11 g kg^{-1}) (Figure 5.6A). This same trend in SOC content was observed in the winter cumulative applications (Figure 5.6B) and residual effects (Figure 5.6C) of treatments.

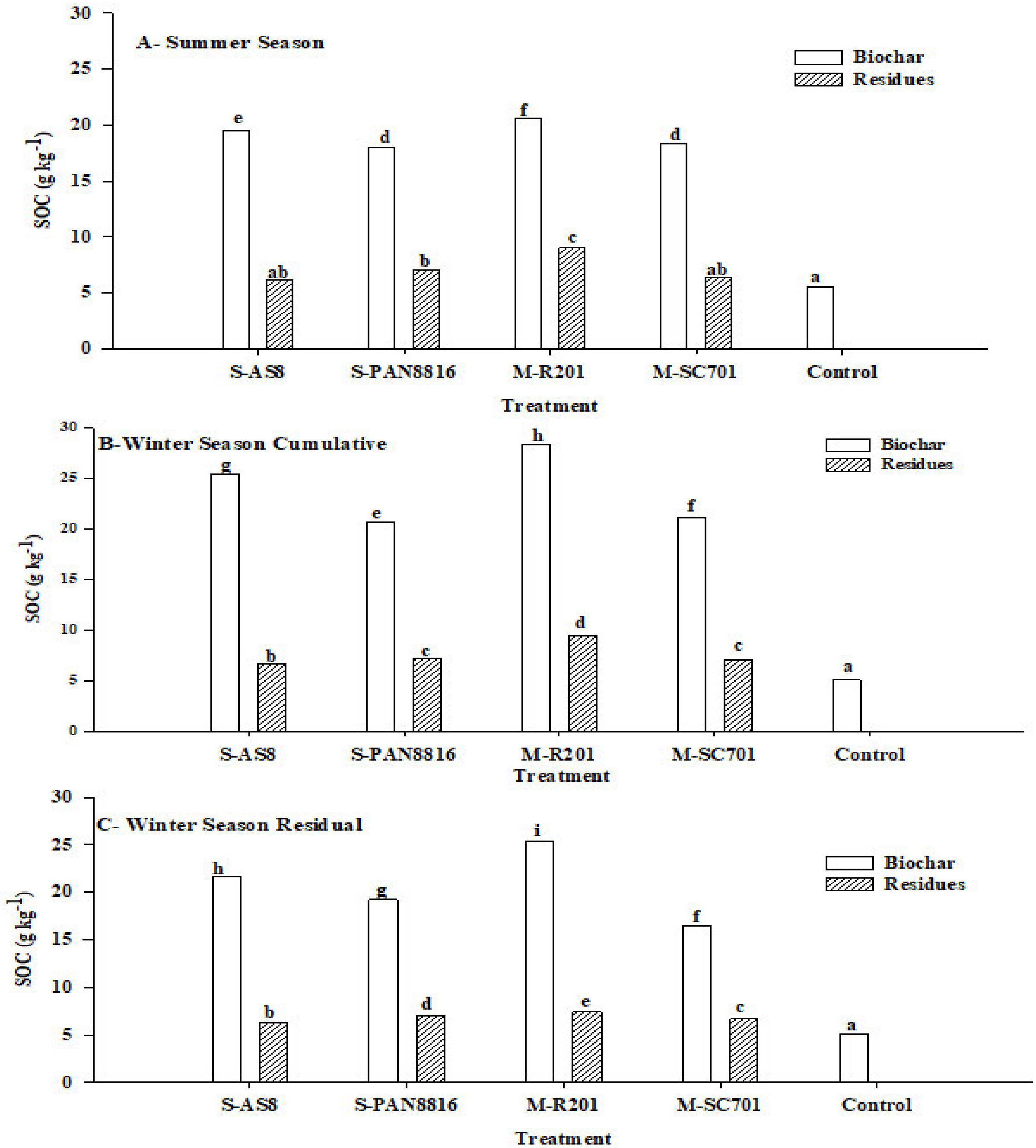

Figure 5.6: The effects of feedstock (R) and biochar (B) from different cultivars of maize (M) and sorghum (S) application on SOC (g kg $^{-1}$) in summer and winter season of 2023. Figure (A) represents first summer application, Figure (B) cumulative (Cum) application and Figure (C) residual (Res) effects from the summer season application. Bars within each sub-figure (A, B, or C) that share the same letter are not significantly different at $p \leq 0.05$.

5.3.3 Variations of POC amongst feedstocks and biochar of different cultivars of maize and sorghum

Variability in particulate organic carbon (POC) among treatments was significant ($p<0.001$) for the different seasons, with the application of biochar resulting in higher POC content compared to feedstock applications (Figure 5.7). For each treatment, the cumulative application of both biochar and feedstock in winter resulted in generally higher POC content, than the summer season treatments while residual effects had lower contents than in the summer (Figure 5.7). The POC content was influenced more by the cultivar type than by crop type, with both feedstock and biochar increasing POC relative to the control. Among the maize cultivars, in summer, the application of M-R201-B yielded the highest POC (10.08 g kg^{-1}), which was a 479% increase over the control (1.74 g kg-^{1}), while M-R201-R (2.13 g kg^{-1}) had the lowest POC levels, but with a 22% increase compared to the control. However, with sorghum, S-PAN8816-B (8.89 g kg^{-1}) resulted in highest and S-AS8-R (3.63 g kg^{-1}) gave lowest POC content (Figure 5.7A). Among the maize cultivars in the winter season, the cumulative application of M-R201-B (14.98 g kg^{-1}) again gave the highest POC resulting in a 12-fold increase relative to the control (1.14 g kg^{-1}), while the cumulative application M-SC701-R (3.38 g kg^{-1}) had the lowest POC content. On the other hand, the cumulative application of the sorghum cultivar S-PAN8816-B (12.89 g kg^{-1}) resulted in the highest while S-AS8-R (2.71 g kg^{-1}) exhibited the lowest POC, which was still 1.4 folds higher than the control (Figure 5.7B). Residual effects in winter generally followed the same trend as in the summer and cumulative winter applications (Figure 5.7C). Among all treatments across seasons, the cumulative application of M-R201-B during the winter season resulted in the highest (14.98 g kg^{-1}), while the residual effects of M-R201-R (1.01 g kg^{-1}) gave the lowest POC content (Figure 5.7).

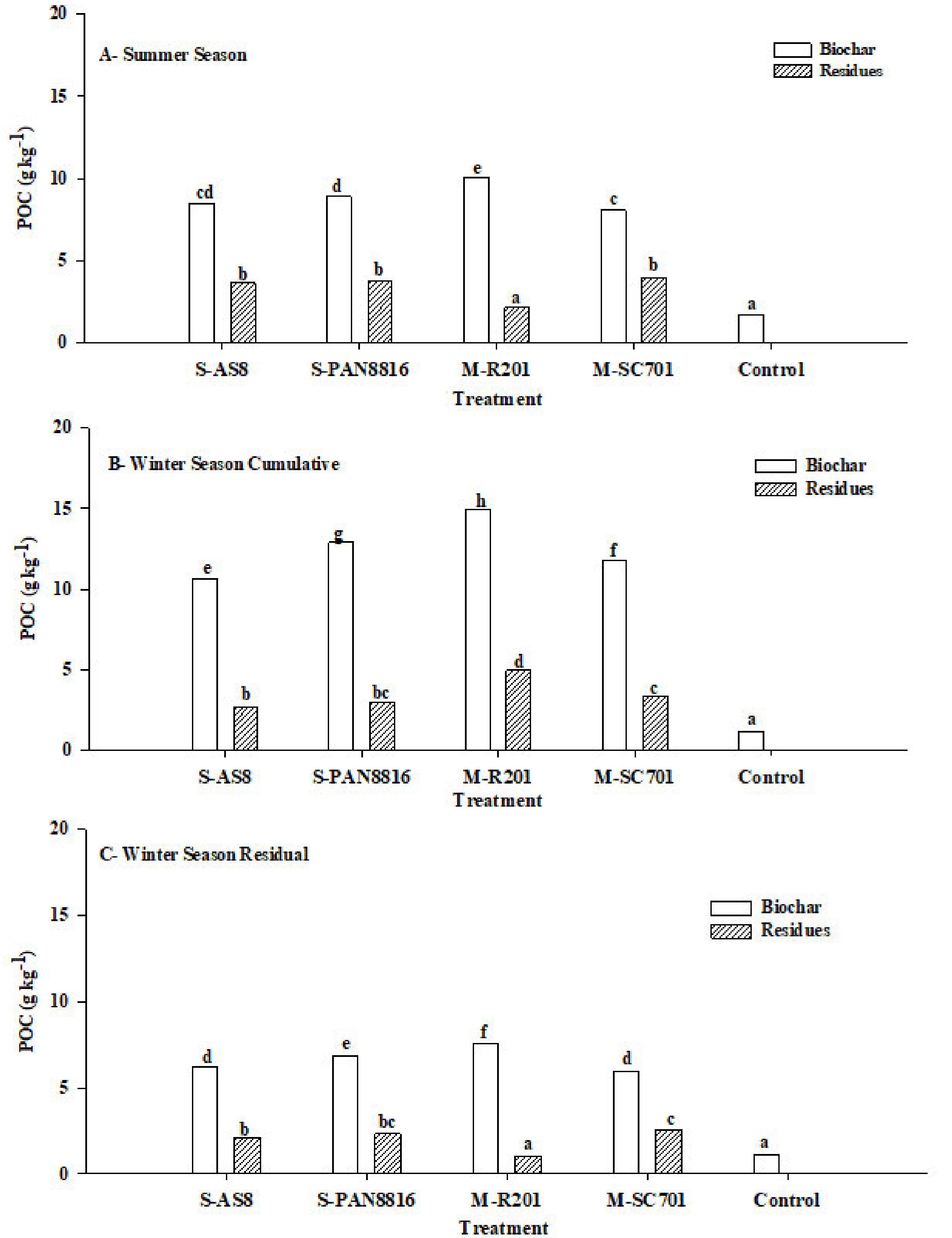

Figure 5.7: The effects of feedstock (R) and biochar (B) from different cultivars of maize (M) and sorghum (S) application on POC (g kg^{-1}) in summer and winter season of 2023. Figure represents (A) first summer application, (B) cumulative (Cum) application in both summer and winter and

(C) residual (Res) effects in winter from the summer season application. Bars within each sub-figure (A, B, or C) that share the same letter are not significantly different at $p \leq 0.05$.

3.4 Variations of POXC amongst feedstocks and biochar of different cultivars of maize and sorghum

A significant difference was observed among different treatments ($p<0.001$) across seasons, with the application of biochar in all seasons resulting in higher permanganate oxidizable carbon (POXC) content compared to feedstock applications (Figure 5.8). Similar to the other carbon fractions, the POXC content was influenced more by the cultivar type than by crop type, with both feedstock (except in the winter residual effects) and biochar increasing POXC relative to the control. During the summer season, the POXC for biochar treatments was in the order M-R201-B > S-AS8-B > S-PAN8816-B > M-SC701-B, while that of the feedstocks was in the order M-R201-R > S-AS8-R = M-SC701-R > S-PAN8816-R (Figure 5.8A). Similarly, in winter, the cumulative application of biochar resulted in higher POXC than the control and was in the order; M-R201-B > S-AS8-B > S-PAN8816-B = M-SC701-B; while there were no significant differences between the cultivar feedstocks/residues (Figure 5.8B). The residual effects of biochar application resulted in significantly higher POXC than the control and was in the order, M-R201-B > S-AS8-B = S-PAN8816-B > M-SC701-B while POXC did not differ among the residual feedstock treatments as well as the control.

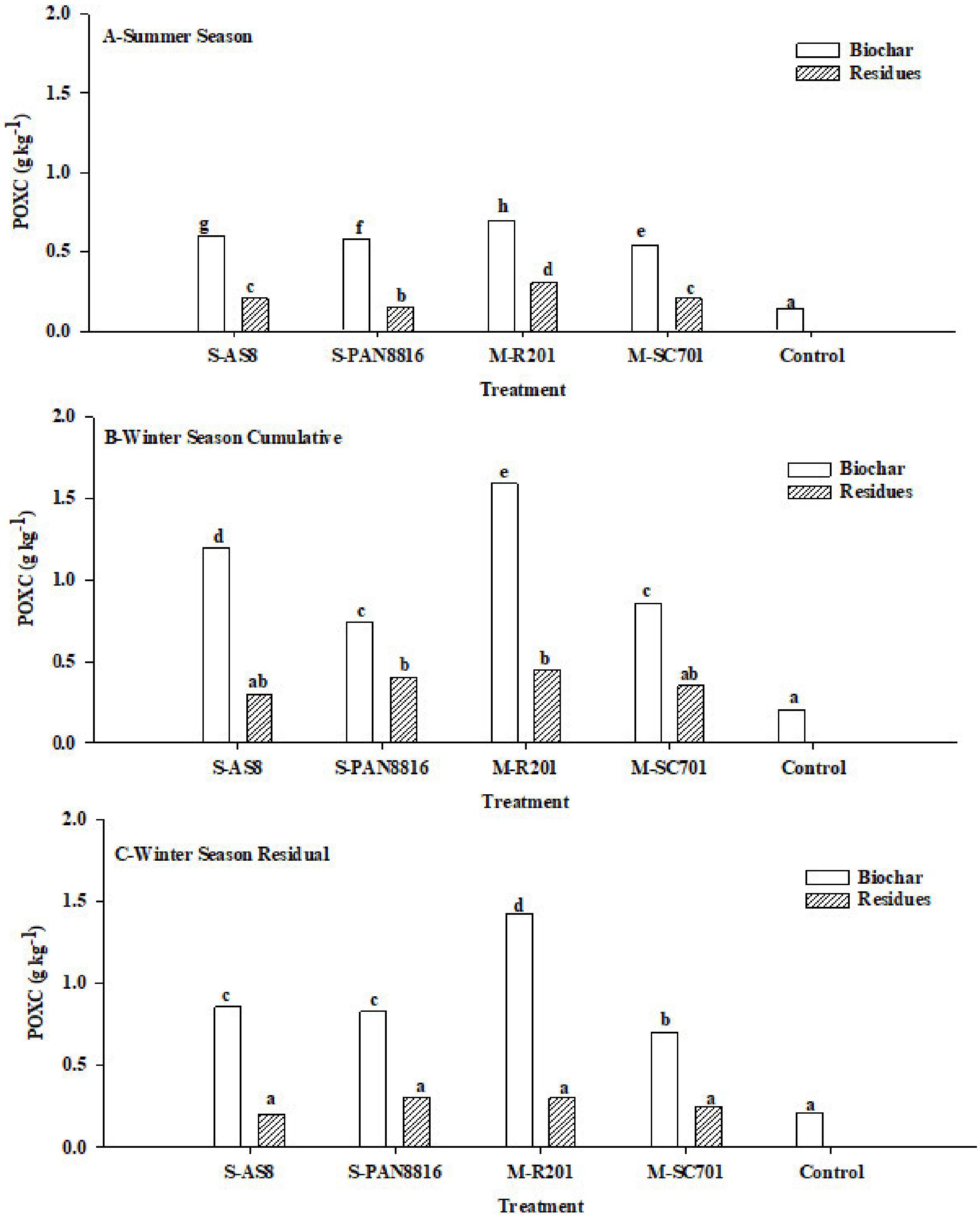

Figure 5.8: The effects of feedstock (R) and biochar (B) from different cultivars of maize (M) and sorghum (S) application on POXC (g kg ^{1}) in summer and winter season of 2023. Figure (A) represents first summer application, Figure (B) cumulative (Cum) application and Figure (C) residual (Res) effects from the summer season application. Bars within each sub-figure (A, B, or C) that share the same letter are not significantly different at $p \leq 0.05$.

5.3.5 Variations of MBC amongst feedstocks and biochar of different cultivars of maize and sorghum

The MBC content varied significantly across treatments and seasons ($p<0.001$) with differences influenced more by cultivar type than crop type (Figure 5.9). The application of feedstock resulted in lower MBC than their respective biochar in both summer and winter seasons (Figure 5.9). The application of feedstock of both maize and sorghum cultivars in summer resulted in lower MBC than that of the control (Figure 5.9A). Amongst the maize cultivars, summer application of M-SC701-B (0.82 g kg^{-1}) resulted in highest while M-R201-R (0.40 g kg^{-1}) gave the lowest MBC which was 35% lower than that of the control (0.54 g kg^{-1}). Whilst in sorghum cultivars, S-PAN8816-B (0.89 g kg^{-1}) resulted in highest while S-AS8-R (0.44 g kg-1) had the lowest MBC which was 23% lower than that of the control treatment. In winter, the cumulative application of feedstock and biochar yielded the results with the same trend as in the summer season with M-SC701-B (0.74 g kg^{-1}) and S-PAN8816-B (0.82 g kg^{-1}) yielding the highest while M-R201-R (0.33 g kg^{-1}) and S-AS8-R (0.34 g kg^{-1}) resulted in the lowest MBC content, which were 14% and 17% higher than that of the control (0.29 g kg^{-1}) (Figure 5.9B). The residual effects of M-SC701-B (0.58 g kg^{-1}) and S-AS8-B (0.56 g kg^{-1}) resulted in higher MBC content while those of M-R201-R (0.19 g kg^{-1}) and S-AS8-R (0.31 g kg^{-1}) were lower (Figure 5.9C).

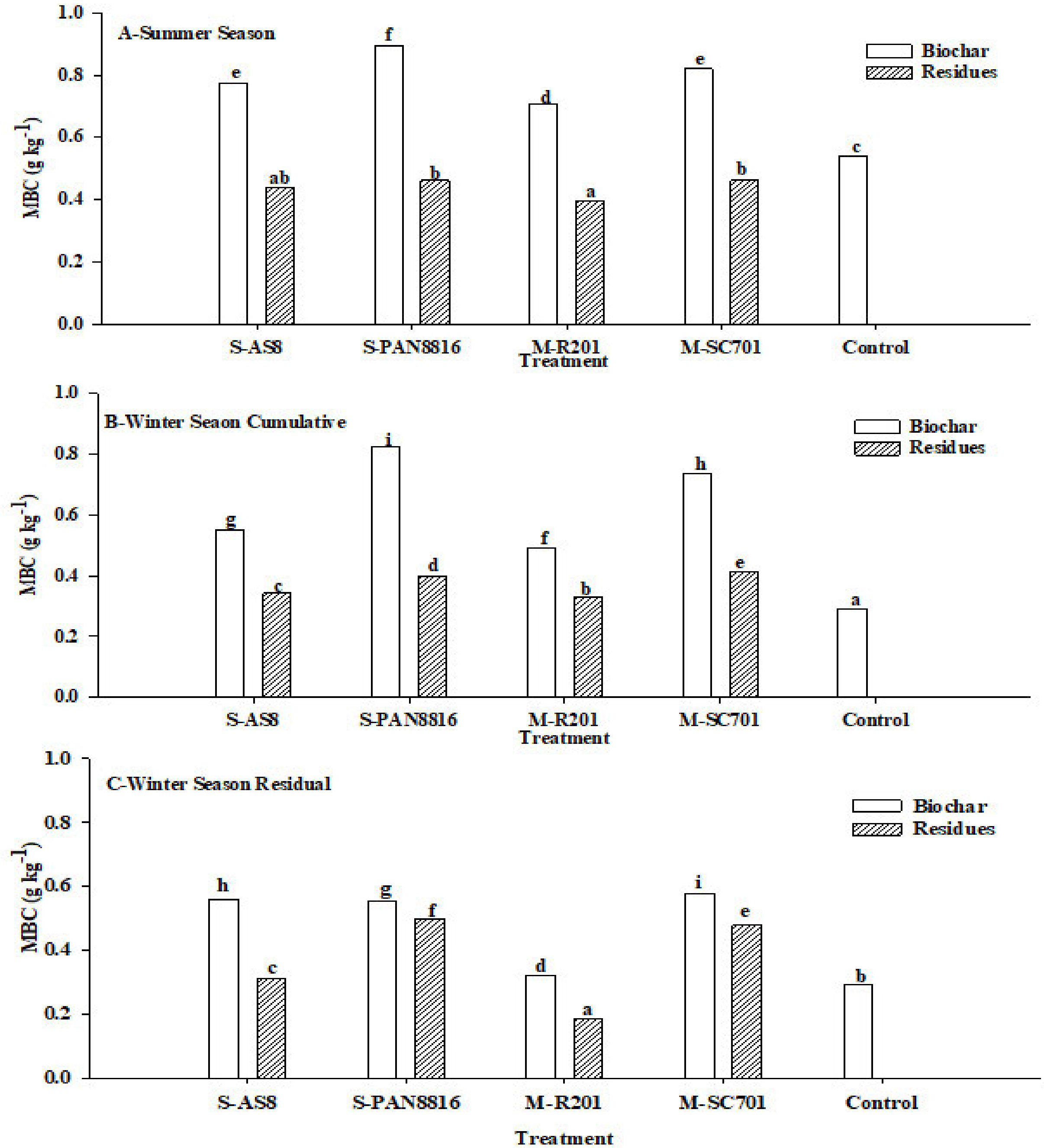

Figure 5.9: The effects of feedstock (R) and biochar (B) from different cultivars of maize (M) and sorghum (S) application on MBC (g kg ^{1}) in summer and winter season of 2023. Figure (A) represents first summer application, Figure (B) cumulative (Cum) application and Figure (C) residual (Res) effects from the summer season application. Bars within each sub-figure (A, B, or C) that share the same letter are not significantly different at $p \leq 0.05$.

5.3.6 Variations of β-Glucosidase activity amongst feedstocks and biochar of different cultivars of maize and sorghum

The β-glucosidase enzyme activity showed significant variation across treatments ($p<0.001$) for the different seasons. It was again influenced more by the specific cultivar differences than the crop type (Figure 5.10). Generally, the application of feedstock treatments led to higher β-glucosidase activity compared to their respective biochars across all seasons (Figure 5.10). During the summer season, the application of feedstock from all cultivars except M-R201-R increased β-glucosidase activity while the application of biochar from all cultivars suppressed it, when compared with the control (Figure 5.10A). Among the maize cultivars, the application of M-SC701-R (54.06 µg g^{-1} Dwt hr^{-1}) resulted in higher β-glucosidase activity than the other treatments, with M-R201-B (16.22 µg g^{-1} Dwt hr^{-1}) having the lowest activity. For the sorghum treatments the application of S-PAN8816-R (65.42 µg g^{-1} Dwt hr^{-1}) resulted in highest β-glucosidase activity than the other treatments, with S-AS8-B (30.55 µg g^{-1} Dwt hr^{-1}) resulting in the lowest activity (Figure 5.10A). In winter, the cumulative (Figures 5.10B) and residual (Figures 5.10C) effects of all treatments (except M-R201-B) increased β-glucosidase activity, relative to the control. The cumulative application of M-SC701-R (47.61 µg g^{-1} Dwt hr^{-1}) and S-PAN881-R (56.97 µg g^{-1} Dwt hr^{-1}) resulted in higher while M-R201-B (13.16 µg g^{-1} Dwt hr^{-1}) and S-AS8-B (26.36 µg g^{-1} Dwt hr^{-1}) gave lower β-glucosidase activity (Figure 5.10B). The same trend was observed in the winter residual effects (Figure 5.10C).

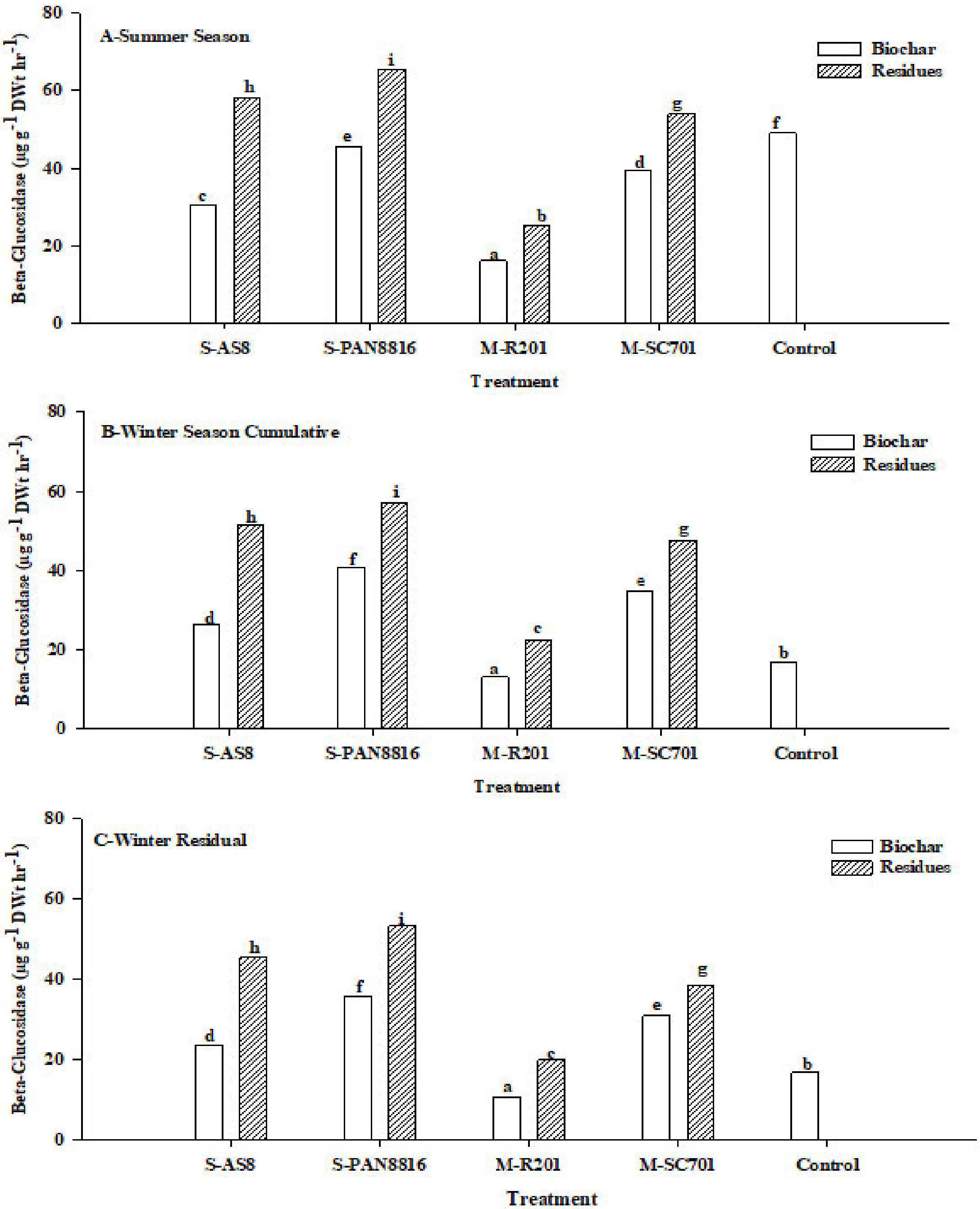

Figure 5.10: The effects of feedstock (R) and biochar (B) from different cultivars of maize (M) and sorghum (S) application on β-Glucosidase activity (µgg ^{1}DWt h^{-1}) in summer and winter season of 2023. Figure (A) represents first summer application, Figure (B) cumulative (Cum) application and Figure (C) residual (Res) effects from the summer season application. Bars within each sub-figure (A, B, or C) that share the same letter are not significantly different at $p \leq 0.05$.

5.3.8 The relationship between studied parameters with feedstock and biochar biochemical properties

Correlations between the studied parameters and the chemical composition of feedstock and biochar are presented in Table 5.3. In this study, β-glucosidase activity correlated positively ($p < 0.05$) with CO_2 flux (r=0.7566) and correlated negatively with pH (r=-0.7926), POXC (r=-0.7565) and fixed C (r=-0.7435). Carbon dioxide emission (CO_2 Flux) correlated negatively ($p <0.05$) with POC (r=-0.8308), POXC (r=-0.8397), fixed C (r=-0.8962), pH (r=-0.7444, p<0.001), total C (r=-0.8401, p<0.001) and SOC (r=-0.8773, p<0.001) while it correlated positively with β-glucosidase activity (r=0.7566, p<0.001). Microbial biomass carbon (MBC) correlated positively ($p < 0.05$) with POC (r=0.7847), ash content (r=0.9645, p<0.001), pH (r=0.8616), total C (r=0.7551), total N (r=0.8916) and SOC (r= 0.9743) while it negatively correlated with C:N ratio (r=-0.7269). Particulate organic carbon (POC) strongly positively correlated with POXC (r=0.9378, p<0.001), fixed C (r=0.9454, p<0.001), total C (r=0.9046, p<0.05) and SOC (r=0.9751, p<0.05). Permanganate oxidizable carbon (POXC) strongly positively correlated ($p < 0.05$) with fixed C (r=0.98), pH (r=0.812) total C (r=0.8784) and SOC (r=0.9702, p<0.001) while it weakly correlated with total N (r=0.2099).Ash content had a negative correlation ($p < 0.05$) with C:N ratio (r=-0.7391) and a positive correlation with pH (r=-.798) and total N (r=0.9276). Fixed C had a strong positive correlation with pH (r=0.8214, p<0.05), total C (r=0.9387, p<0.001) and SOC (r=0.991). pH had a positive correlation ($p < 0.05$) with total C (r=0.8417) and SOC (r=0.8854) while total C had a strong positive correlation with SOC (r=0.9572, p<0.001).

Table 5.3: Pearson's linear correlation between selected biochemical properties of maize and sorghum residues, carbon pools and CO_2 emissions

	BG	**CO_2 Flux**	**MBC**	**POC**	**POXC**	**Ash**	**C:N**	**Fixed C**	**pH**	**Total C**	**Total N**	**SOC**
BG												
CO_2 Flux	0.7566*											
MBC	-0.0887	-0.5239										
POC	-0.5846	-0.8308*	0.7847*									
POXC	-0.7565*	-0.8297*	0.5448	0.9378**								
Ash	0.0096	-0.4419	0.9645**	0.652	0.3752							
C:N	-0.5473	-0.1281	-0.7269*	-0.2911	-0.03	-0.7391*						
Fixed C	-0.7435*	-0.8962*	0.614	0.9463**	0.98*	0.459	-0.0579					
pH	-0.7926*	-0.7444**	0.8616*	0.9454**	0.812*	0.798*	-0.3921	0.8214*				
Total C	-0.6225	-0.8401**	0.7551*	0.9046*	0.8784*	0.6459	-0.2208	0.9387**	0.8417*			
Total N	0.1516	-0.3034	0.8916*	0.5235	0.2099*	0.9276**	-0.7003	0.2823	0.695	0.4581		
SOC	-0.6942	-0.8773**	0.9743*	0.9751*	0.9702**	0.5625	-0.1605	0.991**	0.8854*	0.9572**	0.3937	
BG	1**	0.7566**	-0.0887	-0.5846	-0.7565	0.0096	-0.5473	-0.7435	-0.4926	-0.6225	0.1516	-0.6942

**significant at ($p<0.001$), *significant at ($p<0.05$). Ash= ash content of residues (%), BG=β-glucosidase activity (µg g^{-1} Dwt hr^{-1}), Total C= Residue Total Carbon (%), C:N= Residue C:N ratio, CO_2 Flux= CO2 emission (µmol m^2 s^{-1}),C:P= Residue C:P ratio, Fixed C= Residue fixed carbon content (%),Lignin= Residue lignin content (%),Lignin: N=Residue Lignin: N ratio, MBC= Microbial biomass carbon (g kg^{-1}), Total N= Residue total nitrogen (%),SOC= Soil organic carbon (g kg^{-1}), Total P= Residue total phosphorus (%), pH= Residue pH, POC= Particulate organic carbon (g kg^{-1}),POXC= Permanganate oxidizable organic carbon (g kg^{-1})

5.4 Discussion

Soil management practices such as feedstock and biochar applications significantly impact soil respiration by altering soil physico-chemical and biological properties (Oertel et al., 2016; Battaglia et al., 2021). An important observation from the present study was that the effects of feedstock and biochar application from different cultivars of maize and sorghum on CO_2 emissions and carbon pools were more dependent on the specific cultivar than crop type (maize or sorghum). These findings indicate that the biochemical composition and decomposition of specific cultivars have a greater impact on CO_2 emissions and carbon cycling than the broader crop classification. Therefore, selecting the appropriate cultivar is crucial than focusing on the crop type in soil carbon dynamics and carbon sequestration. This insight is critical because it highlights that even within a crop type differences in cultivars can result in significant outcomes in carbon sequestration and CO_2 emissions. Therefore, by focusing on specific traits, farmers can make targeted decisions on improving carbon accumulation and minimizing CO_2 emissions from the application of residues.

In the present study CO_2 emissions displayed distinct seasonal variations with a consistent pattern observed across all treatments (Figure 5.4). The increase in CO_2 emissions, which were slow from August 2023 and more rapid from October to December 2023 across all treatments, particularly the feedstock treatments (Figure 5.4), could be attributed to the regulation of CO_2 emissions in soil by environmental factors such as soil temperature and moisture content (Delgado-Baquerrio et al., 2017; Rojas et al., 2017; Gray et al., 2019). Higher temperatures are known to stimulate microbial activity, thereby increasing soil respiration and subsequent CO_2 emissions (La Scala et al., 2010). Additionally, CO_2 emissions tend to rise with higher soil moisture levels (Moonis et al., 2021). In this study, the lower soil temperatures and limited rainfall in August could be the cause of low CO_2 efflux across all treatments during this month. The subsequent rise in CO_2 emissions from September, with a highest emission observed in December (Figure 5.4), could be linked to increased rainfall during this period together with higher soil temperatures, fostering conditions conducive to microbial activity and subsequent CO_2 emissions.

The increased CO_2 emissions due to application of feedstock, particularly cumulative applications, (Figure 5.5), with decreased emissions after biochar addition (Figure 5.5) could be because crop residues/feedstocks typically contain labile carbon, for microorganisms, thereby enhancing microbial activity and increasing SOC decomposition and subsequent CO_2 efflux (Singh et al., 2007; Wang et al., 2015). On the other hand, biochar has aromatic structure, which resists decomposition, subsequently reducing the mineralization rate of added carbon and lowering CO_2 efflux (Han et al., 2020). Furthermore, the lower volatile matter content of biochar could explain the consistent reduction in soil CO_2 emissions post-biochar application (Ameloot et al., 2013). These observations align with previous studies (Lentz et al., 2014; Zhang et al., 2017). Lentz et al. (2014) noted a 20% reduction in CO_2 emissions with wood biochar addition compared to manure addition. Similarly, Zhang et al. (2017) reported lower CO_2 fluxes in plots amended with wheat straw-derived biochar compared to wheat straw-amended plots.

The current study's results suggest that biochar exhibits stability in soil and has the potential to sequester carbon, emitting minimal quantities of CO_2 (Pal, 2015). The highest CO_2 emissions due to cumulative application of feedstock of M-SC701-R and S-PAN8816-R cultivars in winter could also be attributed to the biochemical composition of these cultivars. Carbon dioxide emissions correlated negatively with residue fixed C ($r = -0.8962$, $p < 0.05$), total C ($r = -0.8401$, $p < 0.001$), and pH ($r = -0.7444$, $p < 0.001$), which likely contributed to the higher CO_2 emissions observed for M-SC701-R and S-PAN8816-R, with fixed carbon contents of 12.23% and 13.17%, respectively. In contrast, the lower CO_2 emissions from biochars can be attributed to their higher fixed C content, greater total C and higher pH compared to feedstocks, for instance, M-R201-B and S-AS8-B had fixed C contents of 40.41% and 36.31%, respectively which is relatively higher than those of M-SC701-R and S-PAN8816-R. Furthermore, the lower CO_2 emissions observed in the residual treatments, could be because the most labile fractions of SOC could have mineralized in the summer, while the lower rate of decomposition of biochar treatments could have contributed particularly from cultivars M-R201 and S-AS8. These results suggest that continuous addition of biochar can cause sustained carbon sequestration with minimal carbon loss through respiration. This view was supported by the results of SOC and its different pools.

The significant increases in SOC content following the application of both feedstock and biochar (Figure 5.6) could be explained by the large amounts of carbon added compared to the control, together with slower decompositions of biochars and residues of some cultivars and lower CO_2. These results were in line with those by Medina et al. (2015) and Zheng et al. (2016). When incorporated into soils, organic amendments serve as carbon sources for microbes, depending on their initial carbon quantity and other nutrient availability (Ndzelu et al., 2020). The higher SOC content after biochar applications than feedstock treatments (Figure 5.6) could be due to the chemical recalcitrance of biochar. Biochar contains aromatic compounds which make it chemically recalcitrant and resistant to microbial degradation which accounts for its durability in soils than feedstock (Downie et al., 2009; Sohi et al., 2010; Das et al., 2022). The significant increase in SOC content due to cumulative application than residual effects (Figure 5.6B) suggests that cumulative applications provide a sustained input of stable carbon, promoting long-term carbon storage. The continuous carbon supply enhanced microbial activity, facilitating SOC decomposition and consequent enhancement of SOC content (Dungait et al., 2012). Among the treatments, the application of M-R201-B and S-AS8-B consistently yielded the highest SOC levels across all seasons. This outcome can be linked to their higher fixed C content compared to other biochar treatments, promoting a slower turnover rate and ultimately leading to increased SOC accumulation (Murtaza et al., 2024).

The application of biochar also increased the POC and POXC contents compared to feedstock applications (Figures 5.7 & 5.8). This was attributed to its high fixed C content and recalcitrant nature, which enhanced carbon stabilization. The strong positive correlations of fixed C with POC ($r = 0.9454$, $p < 0.001$) and POXC ($r = 0.98$, $p < 0.05$) reveal the central role of biochar in building stable soil carbon pools. Additionally, among the treatments, the application of M-R201-B and S-PAN8816-B resulted in the highest POC content across all seasons while the application of M-R201-B and S-AS8-B resulted to the highest POXC content across all seasons. This was attributed to the higher fixed C content for M-R201-B and AS8-B, which supported long-term stabilization of carbon resulting in increased higher POC and POXC after their application, as well as the higher pH of S-PAN8816-B, which created favorable conditions for carbon retention and microbial activity resulting in increased POC after it was applied. Moreover, POC exhibited a strong and positive correlation with SOC ($r=0.9751$; Table 5.3). This pool primarily consists of plant-derived residues along with microbial and micro-faunal debris, acting as an energy source for microorganisms and a repository of relatively labile SOC and plant nutrients (Purakayastha et al., 2008). Additionally, the porous structure of biochar may stimulate root exudates and its nutrients can promote root development, aiding in biochar agglomeration with soil to improve soil POC content (Shang et al., 2015). Research indicates that biochar is effective in improving soil aggregate formation and stability, which helps protect POC from rapid decomposition (Burrell et al., 2016), potentially explaining its increased content observed in this study. POXC content in the soil also exhibited a significant positive correlation with SOC ($r=0.9702$) (Table 5.3). This fraction is sensitive to environmental changes and susceptible to soil microbial decomposition, influencing soil nutrients, physicochemical properties, plant growth and climate change (Yang et al., 2017). Studies suggest that biochar enhances root biomass, improves root structure, leading to increased root exudates and microbial activities, thereby increasing POXC content in the soil. Furthermore, the sorghum and maize biochar used in this study were produced through slow pyrolysis (pyrolysis temperature ~400^{o}C), resulting in incomplete oxidation, potentially contributing to the increase in POXC content in the soil.

The significant increase in MBC with biochar compared to feedstock application (Figure 5.9), indicated accelerated microbial growth with biochar application, possibly due to the potential liming effect of the biochars (pH 8.0 to 10.9) compared to the residues/feedstocks (pH 4.3 to 5.8). However, literature presents inconsistencies regarding biochar's impact on soil MBC, with some studies reporting MBC reduction after biochar application, while others note MBC increase (Kolb et al., 2009; Dempster et al., 2012). Biochar type is considered a primary parameter influencing soil microorganisms (Steinbeiss et al., 2009). In this study, the increased soil MBC after biochar addition may be attributed to the large specific surface area and porous structure of biochar from cereal crop residues, providing a conducive habitat for microorganisms by maintaining water and air (Hale et al., 2015). Unlike in other carbon pools, the application of M-SC701-B and S-PAN8816-B resulted in higher MBC content. MBC correlated positively with ash content ($r = 0.9645$, $p < 0.001$), pH ($r = 0.8616$, $p < 0.05$), total C ($r = 0.7551$, $p < 0.05$) and total N ($r = 0.8916$, $p < 0.05$) while it negatively with the C:N ratio ($r = -0.7269$, $p < 0.05$) (Table 5.3). These treatments exhibited higher pH and total N content, along with lower C:N ratios, creating favorable conditions for microbial proliferation. The combination of nutrient availability (total N), improved soil conditions (higher pH) and a balanced C:N ratio contributed to the increased microbial biomass carbon observed in these treatments. Moreover, the higher MBC in these treatments could be attributed to the presence of labile organic compounds in these biochars, which may contain higher concentrations of easily accessible carbon fractions or microbial-available substrates that stimulate microbial activity and increase biomass, contributing to the observed increase in MBC after their application (Zhu et al., 2019). Again, the increased POC, POXC and MBC pool across all treatments due to the cumulative applications of biochar than residual effects treatments, suggest that cumulative applications lead to sustained increases in soil carbon pools and microbial activity due to the continuous input of organic material, which provides a steady source of carbon for microbial processes (Chavan et al., 2023). For farmers, this implies that repeated applications would be a better option than relying only residual effects from single application to maintain or improve soil quality over time.

The application of organic materials to soil influences soil enzymatic activity by stimulating microbial activity (Tejada et al., 2006). Beta-glucosidase activity plays a crucial role in carbon cycling, catalyzing the hydrolysis of cellobiose to provide an essential energy source for soil biological processes (Adetunji et al., 2017). In this study, the increase in β-glucosidase activity due to application of maize or sorghum feedstock and reduced β-glucosidase activity with biochar application, especially M-R201-B, was observed compared to the control (Figure 5.10), could be explained by the concentrations of labile organic matter. Feedstock contains higher concentration of labile organic matter that serve as readily available substrates for microbial activity, promoting β-glucosidase production. In contrast, biochar has a lower proportion of labile organic matter due to its stability and recalcitrant structure, resulting in reduced β-glucosidase activity (Zhou et al.,2024) These results were in agreement with the findings of other authors after incorporation of crop residues (Mukumbareza et al., 2006) and biochar (Luo and Gu, 2016). Beta-Glucosidase activity is known to be highly sensitive to soil pH, with activity diminishing as pH levels rise from 4.5 to 8.5 (Eivazi and Tabatabai, 1990).

In the present study, the pH of residues exhibited a negative correlation with β-glucosidase activity ($r=-0.7926$; Table 5.3) and the pH values of the biochar used in this experiment ranged from 8.04 to 10.86, which likely resulted in an increase in soil pH upon their application, resulting in reduced β-glucosidase activity compared to when residues (with pH 4.26 –5.75) were applied. Furthermore, the inorganic carbonate and organic anion contributions of biochar contribute to soil pH increase (Yuan et al., 2011). Given that all biochars were alkaline, the observed lower β-glucosidase activity in biochar treatments may be linked to the liming effects of biochar. Among the treatments M-SC701-R and S-PAN8816-R exhibited highest β-glucosidase activity of the 2 crops across all seasons possibly due to their lower pH and lower fixed C resulting to rapid decomposition when incorporated into the soil. In contrast, biochar from M-R201 and S-AS8 suppressed β-glucosidase activity possibly due to the more recalcitrant nature of biochar from these treatments due to their higher fixed C content which results in less readily available carbon for microbial degradation.

The positive correlation between β-glucosidase activity and CO_2 flux ($r=0.7566$, $p<0.05$) shows the enzyme's critical role in driving microbial decomposition. However, its negative correlation with POXC ($r=-0.7565$, $p<0.05$) suggests that higher enzymatic activity depletes labile carbon pools.

Despite initial expectations that biochemical properties such as lignin, ash content, fixed C, total C and C:N ratio would strongly influence CO_2 emissions and carbon pools, the correlation analysis revealed that carbon pools (SOC, POC MBC and POXC) were also closely related to each other, for instance, MBC showed a positive correlation with SOC ($r = 0.9743$, $p < 0.05$) and POC ($r = 0.7847$, $p < 0.05$), suggesting that these parameters were important substrates for the soil microbial biomass. Recent studies have demonstrated that microbial biomass plays a crucial role in regulating carbon cycling and sequestration, acting as the primary driver of soil organic matter decomposition and stabilization (Lepcha and Devi, 2020). The soil microbial community not only controls carbon stocks through decomposition but also contributes to carbon stabilization (Miltner et al., 2012). Furthermore, microbial biomass carbon is closely linked with stable soil carbon pools, as microbial activity regulates the turnover and delivery of nutrients (Liang et al., 2019). These findings suggest that microbial activity may have a stronger influence on carbon sequestration than biochemical composition, as microbial communities drive the formation and stabilization of soil organic carbon pools (Schimel and Schaeffer, 2012). The composition data still provides valuable insights. The higher fixed C content in biochar from M-R201 (40.41%) and S-AS8 (36.31%) likely contributed to the greater stability and accumulation of carbon in the soil compared to the lower fixed biochar carbon in M-SC701 (27.90%) and S-PAN8816 (29.82%). This reinforces the importance of biochar's stability in long-term carbon sequestration.

5.5 Conclusion

This study showed that carbon pools and CO_2 emissions were more influenced by specific cultivars than by crop type between maize and sorghum. Therefore, cultivar selection is more important in improving soil quality and enhancing carbon sequestration. Among the maize cultivar treatments, M-R201-B consistently resulted in lower CO_2 emissions and high SOC, POC and POXC across all seasons while M-SC701-B resulted in higher MBC across all seasons. Meanwhile, for sorghum cultivars S-AS8-B gave higher SOC and POXC, while S-PAN8816-B resulted in higher POC and MBC. The application of biochar and to some extent, feedstock of different maize and sorghum cultivars enhances soil quality by significantly increasing SOC pools (POC, POXC and MBC) and reducing CO_2 emissions. Cumulative applications, particularly of biochar were more effective in enhancing soil carbon pools and reducing CO_2 emissions compared to residual effects. Therefore, biochar is a more sustainable option for long term carbon sequestration and soil quality improvement for the different cultivars used. However, when crop residues are to be used, then the maize cultivar R201 and sorghum cultivar AS8, would be ideal for reducing CO_2 emissions and increasing SOC pools. As such, re-application of biochar should be considered for improving soil carbon pools and enhancing carbon sequestration. Therefore, farmers may need to consider growing cultivars such as R201 (maize) and AS8 (sorghum) so that they can use the residues to produce biochar which improve soil carbon pools and reduce CO_2 emissions. However, the benefits of lower CO_2 and higher SOC pools due to some cultivars of maize and sorghum may need to also relate with high crop yields if farmers are to adopt this approach, and this needs to be established with further studies.

CHAPTER 6

Effects of maize (*Zea mays L.*) and sorghum (*Sorghum bicolor L.*) feedstock and their biochar on selected soil fertility parameters and crop yield

Abstract

The application of crop residues to agricultural soils is a common practice used for enhancing soil organic matter, recycling essential nutrients and increasing crop yields. However, the impact of crop residues from different cultivars of the same crop on soil properties and crop yields remains unclear. This study investigated the effects of feedstock and biochar from different cultivars of maize and sorghum on selected soil chemical properties (pH, exchangeable acidity, total C and N, available P and exchangeable K), soil enzyme activities (urease, acid and alkaline phosphomonoesterase) and spinach biomass yield through field experimentation. The findings of this study revealed that cultivar differences in biochemical composition significantly influenced their impact on soil properties and spinach biomass yield. The application of biochar (B) and feedstock (R) improved soil chemical properties, enzyme activity and spinach biomass yield, with a more pronounced effect observed in the summer compared to the winter season. Biochar consistently enhanced soil properties, enzymatic activities (alkaline phosphomonoesterase and urease activity) and spinach biomass yield to a greater extent than the corresponding feedstock. In contrast, feedstock application generally resulted in higher micronutrient tissue concentration compared to biochar application. Among maize treatments, M-SC701-B improved soil pH, available P, exchangeable K, enzyme activities, nutrient uptake and spinach shoot dry weight while reducing exchangeable acidity. M-R201-B enhanced soil total carbon and M-R201-R increased acid phosphomonoesterase activity. Whereas for sorghum, S-AS8-B improved soil pH, available P, enzyme activities and total carbon, while S-PAN8816-B improved available P, exchangeable K, nutrient uptake and shoot dry weight. Our findings indicate that the application of both feedstock and biochar from different maize and sorghum cultivars is a promising strategy for enhancing soil fertility and crop yields, offering a potential for sustainable soil management and improved agricultural productivity.

Keywords: Biomass yield, enzyme activity, exchangeable bases, nutrient uptake, total carbon

6.1 Introduction

Soil fertility decline is a significant challenge facing smallholder farmers in Sub-Saharan Africa (SSA), as it leads to reduced crop yields (Lal, 2011). Retaining of crop residues /biomass (e.g., stems, leaves, seed pods) in the field after harvesting economically valuable parts such as grains and fruits (Khrisha and Mkondiwa, 2023), could be beneficial. Globally, approximately 5 billion metric tons of crop residues are generated annually (Shinde et al., 2023). While the residues are often left in the field for livestock grazing (Rusinamhodzi et al., 2013), they can also be incorporated into the soil, to improve organic matter and enhance overall soil quality (Lal et al., 2014). The organic matter improves soil physical, chemical and biological properties, including stimulating soil microbes, which decompose organic matter and mineralize nutrients in the soil, reducing the need for inorganic fertilizers (Fazal et al., 2022). However, predicting nutrient release from crop residues can be challenging, as the decomposition rate is significantly influenced by factors such as quality of the residues and prevailing climate and soil conditions (Dutta et al., 2022).

The application of crop residues has been shown to have several beneficial effects on soil properties and crop yields. It can increase soil pH, total nitrogen (N), available phosphorus (P), exchangeable potassium (K), calcium (Ca) and magnesium (Mg) and increase crop yields (Ogbodo, 2011). Ogbodo (2011) found that application of rice straw, burnt rice husk and legume residues led to significant increase in soil pH, soil organic C, total N, available P, exchangeable bases and rice yield. Similarly, Sabelina and Sepehya (2012) observed similar effects with wheat straw on availability and uptake of iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn). Tian et al. (2020) found that incorporating maize residue into the soil led to a significant increase in urease and acid phosphatase activity, indicating enhanced soil enzymatic activity. While these studies demonstrate the potential of crop residues to improve soil fertility and crop productivity, the practice may sometimes not yield significant benefits, depending on biochemical composition of the residues, soil properties and climatic conditions.

Significant decreases in soil pH (Cao et al. 2021) and grain yields (Soon and Lupwayi, 2012) have been observed following the application of maize residues. Thuy et al. (2008) reported that N uptake was increased by 14 kg N ha^{-1} in rice crops at Yixing and decreased by 3 kg N ha^{-1} in wheat crops at Ludhiana as a result of rice residue incorporation. These studies highlight the complexity of crop residue effects, emphasizing the need for careful consideration of local conditions and residue biochemical composition. Decomposition and nutrients release is facilitated under warm and moist soil conditions (Singh et al., 2020). High-quality residues (C:N ratio < 25, low lignin content, and high N and P content) undergo more rapid mineralization than those of low-quality, which may lead to N immobilization (Kumar and Goh, 1999). In addition to direct application of crop residues, biochar, carbonaceous material produced through the pyrolysis of biomass under limited oxygen (Kimani et al., 2021), can be applied to enhance carbon sequestration and nutrient availability (Han et al., 2023). Biochar has been reported to enhance soil fertility (Uzoma et al., 2011) including soil physical, chemical and biological properties (Ahmad Bhat et al., 2022; Kang et al., 2022).

A study by Major et al. (2010) demonstrated that applying wood biochar increased soil pH, maize nutrient uptake and exchangeable calcium and magnesium, while decreasing exchangeable acidity. Biochar has also been shown to create a favorable environment for soil microorganisms to thrive, thereby altering the microbial community structure, (Nielsen et al., 2018). Islami et al. (2017) found that biochars from farmyard manure and cassava significantly increased yields of maize and cassava and enhanced soil organic C and N, available P, cation exchange capacity (CEC) and exchangeable K. Application of eucalyptus biochar was also found to increase urease and acid phosphatase activity (Lopez et al. 2021). While biochar application has shown promising results in improving soil properties and crop yields, some studies have reported neutral or even negative effects. For example, Jones et al. (2012) found that applying biochar produced from trunks and large branches of *Fraxinus excelsior* L., *Fagus sylvatica* L. and *Quercus robur* L. had no significant impact on dissolved organic C and N and mineral N pools in soil. Additionally, Sanger et al. (2016) found that maize and wood biochar had no significant impact on the yields of winter wheat, winter rye and maize. These contrasting findings highlight the importance of careful consideration for biochar production and use. The effectiveness of biochar in improving soil quality and crop yields could depend on the feedstock, pyrolysis temperature, soil and climate conditions (Bhattarai et al., 2015; Ahmad Bhat et al., 2022).

Maize and sorghum are two of the most widely grown cereal crops globally, with maize being a staple food for many households, while sorghum is a vital crop in arid regions, particularly in Africa, due to its exceptional drought tolerance (Schober and Bean, 2008). The abundance of residues from these crops presents an opportunity for using them as livestock feed and conversion into biochar, with the potential to improve soil quality. While previous research has investigated the effects of crop residues and biochar from single crops (Lopez et al., 2012; Subehia and Sepehya, 2012; Tian et al., 2020;), a significant knowledge gap exists regarding the comparative impacts of crop residues and biochar derived from maize and sorghum residues and those from different cultivars of each crop. The current study investigated effects of feedstock and biochar from cultivars of maize and sorghum on selected soil properties and spinach yield.

6.2 Materials and Methods

6.2.1 Study site

The study site for this study is as described in section 5.2.1 of Chapter 5

6.2.2 Biomass and feedstock production

Biomass and feedstock production process was as outlined in section 3.2.1 of Chapter 3

6.2.3 Biochar production

Biochar production was as described in section 5.2.3. Laboratory analysis was performed in triplicates on the biochar samples (as described from section 3.2.2 to 3.2.6 of Chapter 3) and the selected properties are presented in Tables 6.1a and 6.1b.

Table 6.1a: Biochemical composition (macronutrients, lignin and pH) of feedstock and biochar of different maize and sorghum cultivars

Treatment	Total C (%)	Total N (%)	Total P (%)	Lignin (%)	C:N	C:P	Lignin: N	pH
M-R201-R	38.89^{a}	0.324^{a}	0.47^{c}	38.57^{e}	153.3^{d}	82.76^{ab}	153.28^{b}	4.88^{c}
M-SC701-R	33.29^{a}	1.071^{c}	0.377^{b}	4.18^{a}	31.08^{a}	88.42^{abc}	3.9^{a}	5.75^{d}
S-AS8-R	33.09^{a}	0.481^{ab}	0.343^{a}	24.79^{d}	68.87^{b}	96.34^{abc}	51.65^{ab}	4.26^{a}
S-PAN8816-R	37.62^{a}	1.46^{d}	0.333^{a}	10.21^{c}	25.78^{a}	113.05^{c}	6.99^{a}	4.82^{b}
M-R201-B	54.17^{b}	0.61^{b}	0.81^{f}		88.82^{c}	66.88^{a}		9.923^{f}
M-SC701-B	54^{b}	3.765^{e}	0.65^{e}		14.36^{a}	83.08^{abc}		10.857^{h}
S-AS8-B	60.62^{b}	1.429^{d}	0.577^{d}		42.41^{b}	105.13^{bc}		8.04^{e}
S-PAN8816-B	51.48^{b}	4.545^{f}	0.58^{d}		11.34^{a}	88.77^{abc}		10.097^{g}
LSD($p<0.05$)	6.113	0.1609	0.01455	1.429	29.35	18.35	72.83	0.0158

R-Feedstock, B-Biochar, M-Maize, S-Sorghum, Values on the same column with different letters are significantly different ($p<0.05$)

Table 6.1b: Biochemical composition (Exchangeable bases) of feedstock and biochar of different maize and sorghum cultivars

Treatment	Ca (cmolc kg^{-1})	K (cmolc kg^{-1})	Mg (cmolc kg^{-1})
M-R201-R	7.425^{f}	4.696^{d}	1.958^{d}
M-SC701-R	8.164^{g}	6.762^{e}	1.75^{bcd}
S-AS8-R	3.303^{c}	2.292^{a}	1.753^{bcd}
S-PAN8816-R	5.13^{e}	5.033^{d}	1.817^{cd}
M-R201-B	3.104^{b}	2.23^{a}	1.505^{ab}
M-SC701-B	3.214^{bc}	3.166^{b}	1.567^{abc}
S-AS8-B	2.706^{a}	2.588^{a}	1.463^{a}
S-PAN8816-B	4.471^{d}	3.734^{c}	1.991^{d}
LSD(p<0.05)	0.101	0.2746	0.1617

Values on the same column with different letters are significantly different ($p<0.05$)

6.2.4 Field experimental design

The experimental design of this study was as described in section 5.2.4 of Chapter 5 except for that the soils were analyzed for enzyme activities, including urease, acid phosphomonoesterase and alkaline phosphomonoesterase and for selected soil chemical properties namely pH, exchangeable acidity, total N, total C, available P, and exchangeable K.

6.2.5 Selected soil properties analyzed

6.2.5.1 Soil pH

About 10 g of soil was weighed into a 50 mL beaker and 1M KCl solution was added at a soil to solution ratio of 1: 2.5, following the method described by Okalebo et al. (2002). Samples were allowed to stand for 30 minutes with occasional stirring using glass rods. The pH of the supernatant was then measured using a PHM 210 electrode pH meter.

6.2.5.2 Total N and Carbon

Total N and C were analyzed using LECO TruMac CNS/NS Carbon/Nitrogen/Sulfur Determinator (LECO Corporation, 3000 Lakeview Ave, ST. Joseph, MI, USA). Air-dried soil was passed through a 0.5 mm sieve, then a 0.2 g sample was put into the LECO for analysis of C and N. The procedure was based on dry combustion of air-dried samples in crucibles, subjected to a 1450 ^{o}C furnace temperature for about 6 minutes.

6.2.5.3 Exchangeable acidity

About 10 g of soil was weighed into a 100 mL plastic centrifuge tube, then 50 mL of 1M KCI was added before shaking for 4 minutes (Lourenzi et al., 2011). The sample was centrifuged for 2 minutes at 400 rpm, then filtered with Whatman No. 41 filter paper into a glass bottle. A 25 mL aliquot was extracted into a 100 mL conical flask, while another 25 mL of 1M KCl only served as a blank. About 5 drops of phenolphthalein indicator were added to each sample, then titrated with 0.01M NaOH until it remained pink for at least 30 seconds. The blank was titrated first, following steps by Ndayegamiye and Cote (1989).

6.2.5.4 Analysis of Available-P

Available P was determined calorimetrically following AMBIC-2 extraction. Exactly 2.5 g soil was weighed into a 100 cm^3 centrifuge tube, and 25 ml of AMBIC-2 solution was added. The suspension was shaken at 180 cycles per minute for 30 minutes using a reciprocal shaker (Model E5850 Thomas Scientific, Swedesboro, NJ, USA). The supernatant was filtered using Whatman No. 41 filter paper into storage bottles. Two milliliter aliquot of the extract was diluted with 8 ml of distilled water, followed by addition of 10 ml of color reagent. The mixture was allowed to stand for 45 minutes and analyzed calorimetrically using UV/VIS spectrophotometer at a wavelength of 670 nm (Murphy and Riley, 1962).

6.2.5.5 Exchangeable bases

Five grams of soil was weighed into a 100 ml centrifuge tube, and 50 ml of 1M ammonium acetate (pH 7) was added to determine exchangeable K. The soil suspension was shaken for 30 minutes and left for 2 minutes before filtration into storage bottles using a Whatman No. 41 filter paper (Okalebo et al., 2002). Five ml of Caesium (1200 mg/L) solution was added to the extract as an ionization suppressant to reduce interference from other elements. The extracts were then analyzed for exchangeable K using an atomic absorption spectrophotometer (Varian AA 240) (Okalebo et al., 2002; Anderson & Ingram, 1993).

6.2.5.6 Acid and alkaline phosphomonoesterase enzyme assay

Acid and alkaline phosphatase activity was determined by incubation of soil with p-nitrophenol phosphatase (Tabatabai, 1994). One gram of moist soil was placed into a 50 mL Erlenmeyer flask, then 0.2 mL of toluene, 4.0 mL of MUB (adjusted to pH 6.5 for acid phosphatase and pH 11 for alkaline phosphatase), and 1.0 mL of 0.05 M p-nitrophenyl phosphate solution made was added. The contents were stoppered, swirled for a few seconds, and then incubated for 1 hour at 37°C, after which 1.0 mL of 0.5 M $CaCl_2$ and 4.0 mL of 0.5 M NaOH were added. The flask was swirled for a few seconds then the suspension filtered through Whatman No. 42 filter paper to produce a yellow-coloured filtrate (Tabatabai, 1994). All the steps done on the samples were also done on the blanks and standards. The absorbance of the filtrate was measured using a spectrophotometer (SPECTRO UV-11) set at a wavelength of 400 nm. The results were corrected for the blank, and p-nitrophenol per 1.0 mL of the filtrate was then calculated by reference to the calibration curve as follows (Alef & Nannipieri, 1995):

p-nitrophenol ($\mu g\ g^{-1}$ dwt hr^{-1}) = $(C \times V) / (dwt \times SW \times t)$ (Equation 6.1)

Where:

C = measured concentration of sample in µg g-1 mL filtrate

Dwt = dry weight of 1.0 g soil

V = total volume of soil suspension in mL

SW = weight of soil sample used (i.e., 1.0 g)

T = incubation time (i.e., 1 hour)

6.2.5.7 Urease enzyme assay

Fresh soil samples were ground and sieved to pass through a 2 mm sieve. Five grams of soil was then placed in 100 mL Erlenmeyer flasks and wetted with 2.5 mL of 0.08M aqueous urea solution (Kandeler & Gerber, 1988). The flasks were stoppered and placed in an incubator at 37 ºC for 2 hours. After which 30 mL of 1M KCl and 20 mL of 0.01M HCl were added to the flasks then shaken for 30 minutes. After centrifuging, the contents were filtered using Whatman No. 42 filter paper, before diluting 1ml of the filtrate with 10 mL of distilled water, then 5.0 mL of sodium salicylate solution and 2.0 mL of 0.1% sodium dichlorisocyanurate were added. The diluted filtrate was then analyzed for ammonium after 30 minutes using a SPECTRO UV-11 spectrophotometer set at 690 nm (Kandeler & Gerber, 1988). Standards and blanks containing 0, 0.5, 1.0, 1.5, and 2.0 $\mu g\ mL^{-1}$ NH_4^+-N were also analyzed as above. Then, urease activity was expressed as $\mu g\ NH_4^+\ g^{-1}$ Dwt $2hr^{-1}$ (Kandeler & Gerber, 1988).

6.2.6 Spinach biomass parameters measurements

6.2.6.1 Leaf area index (LAI)

The Leaf Area Index was determined at each harvest. The length and width of leaves from two out of six spinach plants per line in each plot were measured using a measuring tape. The leaf width was measured from one end of the leaf to the other, at the midpoint of the leaf length. The average leaf area was then calculated by summing the measurements and dividing by the number of plants measured for each treatment, following the method described by Msibi et al. (2014). The number of leaves was counted in 2 spinach plants per line in each plot before harvest. To get the average number of leaves, the total number of leaves was divided by the total number of plants whose leaves were counted (Msibi et al., 2014). To calculate the LAI, the following equation was used:

LAI= $(Y \times N \times AL)/AP$ (Equation 6.2)

Where:

Y= Number of plants per plot

N= Average number of leaves

AL= Average area per leaf (m^2)

AP= Area of plot

6.2.6.2 Shoot and roots biomass yield

At harvest, all shoots were cut approximately 1 cm above the soil surface, weighed for fresh weight, and oven-dried at ± 70 ºC for 72 h. The roots were dug with a spade, washed with tap water to remove soil particles, and were spread on paper towel to remove excess water, before they were weighed for fresh weight and then oven-dried at ± 70 ºC for 72 h. After oven drying, the samples were weighed for dry matter. The shoots were ground using a pestle and mortar to pass through a 1 mm sieve then analyzed for total S, C and N in triplicate using a Leco TruMac CNS/NS Carbon/Nitrogen/Sulfur Determinator (LECO Corporation, 2012). Tissue Zn, Cu, Fe, and Mn were determined, in triplicate, by ICP-MS after digestion of 0.5 g with 12 ml of 5:1 HNO3:H2O2 acid mixture (v/v) followed by dilution to 50 ml with deionized water (Okalebo et al., 2002).

The biomass yield of spinach (kg ha^{-1}) was calculated using the following formula:

Biomass yield (kg ha^{-1}) = (Total Biomass of each plot (kg)) / (Plot Area (ha)) (Equation 6.3)

Nutrient uptake (kg ha^{-1}) was calculated by multiplying dry matter yield of each treatment with concentration of nutrients in shoots using the following equation:

Nutrient uptake (kg ha^{-1}) = (Nutrient concentration in shoots (%) × Dry matter yield (kg ha^{-1})) / 100 (Equation 6.4)

6.2.7 Statistical analysis

Treatment differences for the studied parameters were analyzed using one-way analysis of variance (ANOVA) in GenStat, 20th Edition. Data for each season were analyzed separately. Mean separation was performed using the Least Significant Difference (LSD) test at $p < 0.05$, and the Tukey-Kramer test was applied for further treatment mean comparisons at $p < 0.05$ (Payne et al.,2011). A principal component analysis (PCA) was using OriginPro 2024 (OriginLab Corporation, Northampton, USA) was conducted to study relationships among the variables. The PCA biplots were used to visualize associations between treatments and measured parameters. Vectors positioned closely or parallel to each other indicated strong positive associations, while those in opposite directions (180º) represented strong negative associations. Perpendicular vectors (90º) represented weak or no correlation between variables.

6.3 Results

6.3.1 Feedstock and biochar application effect on selected soil chemical properties

6.3.1.1 Soil pH and exchangeable acidity

The application of feedstock and biochar amendments significantly impacted soil pH ($p<0.001$), with biochar treatments generally increasing soil pH compared to feedstock across seasons (Table 6.2). In summer for sorghum cultivars, S-AS8-B (6.33) resulted in higher while S-AS8-R (4.37) gave lower soil pH. While for maize, M-SC701-B resulted in the highest (7.43), while M-R201-R (4.23) had the lowest soil pH, which was not significantly different from M-SC701-R (4.48). This same trend was observed with cumulative and residual treatments in winter (Table 6.2).

Exchangeable acidity was also significantly affected by application of the amendments with feedstock applications consistently giving higher values than their biochars (Table 6.2). The differences were cultivar specific rather than crop dependent. In summer, the sorghum cultivar S-AS8-R (0.31 cmolc kg^{-1}) resulted in higher while S-AS8-B (0.13 cmolc kg^{-1}) had the lowest exchangeable acidity. While the maize cultivars M-R201-R (0.36 cmolc kg^{-1}) and M-SC701-R (0.35 cmolc kg^{-1}) resulted in higher exchangeable acidity and M-SC701-B was lowest (0.13 cmolc kg^{-1}). A more or less similar trend in exchangeable acidity was followed in the winter cumulative and residual applications (Table 6.2).

Table 6.2: Soil pH and Exchangeable acidity after the application of biochar (B) and feedstock (R) of different cultivars of maize (M) and sorghum (S)

Season	Treatment	pH(KCl)	Exch.Acid (cmolc kg^{-1})
Summer	S-AS8-B	6.33^{e}	0.14^{a}
	S-AS8-R	4.37^{ab}	0.31^{e}
	S-PAN8816-B	6.17^{e}	0.16^{b}
	S-PAN8816-R	4.69^{bc}	0.25^{d}
	M-R201-B	5.37^{d}	0.21^{c}
	M-R201-R	4.23^{a}	0.36^{f}
	M-SC701-B	7.43^{f}	0.13^{a}
	M-SC701-R	4.48^{ab}	0.35^{f}
	Control	4.85^{c}	0.21^{c}
LSD (p<0.05)		0.21	0.02
Winter Cumulative	S-AS8-B	6.93^{h}	0.13^{a}
	S-AS8-R	4.53^{c}	0.36^{e}
	S-PAN8816-B	6.56^{g}	0.12^{a}
	S-PAN8816-R	5.05^{e}	0.26^{c}
	M-R201-B	5.73^{f}	0.21^{b}
	M-R201-R	4.48^{b}	0.38^{e}
	M-SC701-B	7.85^{i}	0.10^{a}
	M-SC701-R	4.74^{d}	0.31^{d}
	Control	4.03^{a}	0.21^{b}
LSD (p<0.05)		0.02	34.66
Winter Residual	S-AS8-B	5.26^{f}	0.13^{a}
	S-AS8-R	4.15^{bc}	0.34^{d}
	S-PAN8816-B	4.97^{e}	0.13^{a}
	S-PAN8816-R	4.28^{cd}	0.27^{c}
	M-R201-B	4.47^{d}	0.2^{b}
	M-R201-R	3.95^{a}	0.42^{e}
	M-SC701-B	5.43^{f}	0.13^{a}
	M-SC701-R	4.18^{bc}	0.4^{e}
	Control	4.03^{ab}	0.21^{b}
LSD (p<0.05)		0.12	0.03

Values on the same column with different letters are significantly different (p<0.05)

6.3.1.2 Total C and total N

The application of biochar treatments consistently resulted in higher total Carbon (TC) ($p<0.001$) compared to their feedstocks (Table 6.3). The effects of the treatments were cultivar dependent than crop type as there were specific cultivars within each crop that resulted to higher or lower total C content in soil. The summer sorghum application of S-AS8-B (2.27%) resulted in higher while AS8-R (0.63%) which was not significantly different from S-PAN8816-R (0.83%) and the control (0.80%) resulted in lower TC. Among maize cultivars, M-R201-B (2.63%) resulted in higher while M-SC701-R (0.67%) which was not significantly different from the control (0.80%) resulted in lower TC. In winter, sorghum cumulative applications of both S-AS8-B (1.9%) and S-PAN8816-B (1.57%) resulted in higher, while S-AS8-R (0.57%) and S-PAN8816-R (0.70%) which were not significantly different from the control (0.60%) resulted in lower TC. While maize cumulative applications of M-R201-B (2.33%) resulted in higher and cumulative M-SC701-R (0.63%) which was not significantly different to M-R201-R (0.87%) and the control (0.60%) resulted in lower TC. The winter residual TC effects followed the same trend as for cumulative applications across treatments (Table 6.3)

Total soil N was also significantly affected by the application of different amendments ($p<0.001$) with biochar treatments generally having higher TN than their feedstock though this was not consistent across all seasons (Table 6.3). In summer, the sorghum cultivar S-PAN8816-B (0.23%) which was not significantly different to S-PAN8816-R (0.18%) resulted in higher while S-AS8-R (0.14%) and S-AS8-B (0.16%) which were not significantly different from the control (0.15%) resulted in lower TN. While for maize, M-SC701-B (0.23%) resulted in higher while M-R201-R (0.10%) which was not significantly different from the control (0.15%) gave lower TN. A more or less similar trend was followed in the winter residual applications while no significant differences among treatments was observed in the cumulative applications (Table 6.3).

6.3.1.3 Available P and exchangeable K

Available P content was significantly affected the application of different amendments in different seasons with biochar treatments resulting in higher available P compared to their feedstocks ($p<0.001$; Table 6.3). In the summer, sorghum application of both S-PAN8816-B (153.25 mg kg^{-1}) and SAS8-B (152.9 mg kg^{-1}) resulted in higher while S-PAN8816-R (36.83 mg kg^{-1}) gave lower available P. In maize, application of M-R201-B (147.06 mg kg^{-1}) resulted in higher while M-R201-R (66.48 mg kg^{-1}) had lower available P. With winter sorghum treatments, cumulative applications of S-AS8-B (91.29 mg kg^{-1}) resulted in higher while S-PAN8816-R (41.24 mg kg^{-1}) gave lower available P. While for maize, cumulative application of M-SC701-B (123.32 mg kg^{-1}) resulted in higher, while M-SC701-R (56.73 mg kg^{-1}) which was not significantly different form M-R201-R (61.58 mg kg^{-1}) resulted in lower available P. The residual winter effects of available P trends followed a similar trend to that of cumulative applications (Table 6.3).

Exchangeable K was higher in the summer compared to winter, with biochar treatments resulting in higher exchangeable K content than their feedstocks in all seasons ($p<0.001$) and the differences in exchangeable K were cultivar specific than crop type (Table 6.3). In summer, sorghum application of S-PAN8816-B (330.2 mg kg^{-1}) resulted in higher while S-PAN8816-R (122.9 mg kg^{-1}) had lower exchangeable K. For maize cultivars in summer, the application of M-SC701-B (409.2 mg kg^{-1}) resulted in higher while M-R201-R (146.7 mg kg^{-1}) had lower exchangeable K. This same trend was followed in both the winter cumulative and residual applications (Table 6.3).

Table 6.3: Soil Total C, Total N, Available P and Exchangeable K after the application of biochar (B) and feedstock (R) of different cultivars of maize (M) and sorghum (S)

Season	Treatment	Total C (%)	Total N (%)	Available P(mg kg^{-1})	K (mg kg^{-1})
	S-AS8-B	2.27^{d}	0.16^{bc}	152.98^{g}	239.8^{f}
	S-AS8-R	0.63^{a}	0.14^{abc}	42.90^{c}	127^{b}
	S-PAN8816-B	1.8^{c}	0.23^{d}	153.25^{g}	330.2^{g}
	S-PAN8816-R	0.83^{ab}	0.18^{cd}	36.83^{b}	122.9^{b}
Summer	**M-R201-B**	2.63^{e}	0.12^{ab}	147.06^{f}	217.1^{e}
	M-R201-R	1.07^{b}	0.10^{a}	66.48^{d}	146.7^{c}
	M-SC701-B	1.90^{c}	0.23^{d}	162.46^{h}	409.2^{h}
	M-SC701-R	0.67^{a}	0.17^{c}	72.80^{e}	165.4^{d}
	Control	0.80^{a}	0.15^{abc}	27.79^{a}	103.9^{a}
LSD (p<0.05)		0.14	0.03	2.02	4.13
	S-AS8-B	1.90^{b}	0.13^{a}	91.29^{f}	196^{f}
	S-AS8-R	0.57^{a}	0.13^{a}	49.13^{c}	130.5^{b}
	S-PAN8816-B	1.57^{b}	0.24^{a}	73.92^{e}	278.3^{g}
	S-PAN8816-R	0.70^{a}	0.15^{a}	41.24^{b}	125.1^{b}
Winter Cumulative	**M-R201-B**	2.33^{c}	0.11^{a}	96.64^{f}	179^{e}
	M-R201-R	0.87^{a}	0.33^{a}	61.58^{d}	148^{c}
	M-SC701-B	1.83^{b}	0.22^{a}	123.32^{g}	290.6^{h}
	M-SC701-R	0.63^{a}	0.16^{a}	56.73^{d}	163.7^{d}
	Control	0.60^{a}	0.09^{a}	29.11^{a}	97.7^{a}
LSD (p<0.05)		0.23	0.23	3.75	4.45
	S-AS8-B	1.90^{cd}	0.12^{cde}	65.14^{e}	169^{e}
	S-AS8-R	0.61^{a}	0.11^{bcd}	38.65^{b}	127.1^{b}
	S-PAN8816-B	1.37^{b}	0.18^{f}	55.36^{d}	195.7^{f}
	S-PAN8816-R	0.63^{a}	0.14^{e}	35.67^{b}	120.3^{b}
Winter Residual	**M-R201-B**	2.03^{d}	0.10^{abc}	76.32^{f}	162.3^{d}
	M-R201-R	0.73^{a}	0.07^{a}	48.41^{c}	141.5^{c}
	M-SC701-B	1.70^{c}	0.21^{f}	83.33^{g}	219^{g}
	M-SC701-R	0.53^{a}	0.14^{de}	49.10^{c}	161^{d}
	Control	0.60^{a}	0.09^{ab}	29.11^{a}	97.7^{a}
LSD (p<0.05)		0.15	0.03	2.75	3.83

Values on the same column with different letters are significantly different (p<0.05)

6.3.2 Feedstock and biochar application effects on selected soil enzyme activities

6.3.2.1 Acid phosphomonoesterase activity

Acid phosphomonoesterase activity was significantly affected by different amendments ($p<0.001$) with feedstock treatments resulting in higher activity compared to their biochars in both seasons (Figure 6.1). The differences in acid phosphomonoesterase activity were primarily cultivar specific rather than crop type based (Figure 6.1). In summer, application of the sorghum cultivar S-AS8-R (367.28 µg g^{-1} Dwt hr^{-1}) which was 76% higher than the control but not significantly different from S-PAN8816-R (251.29 µg g^{-1} Dwt hr^{-1}) resulted in higher while all other sorghum treatments had lower acid phosphomonoesterase activity (Figure 6.1A). While for maize summer treatments, M-R201-R (576.2 µg g^{-1} Dwt hr^{-1}), which was 176% higher than the control (208.5 µg g^{-1} Dwt hr^{-1}) resulted in higher while all other maize treatments had lower acid phosphomonoesterase activity (Figure 6.1A). A more or less similar trend was followed in the winter cumulative and residual applications (Figures 6.1B & C).

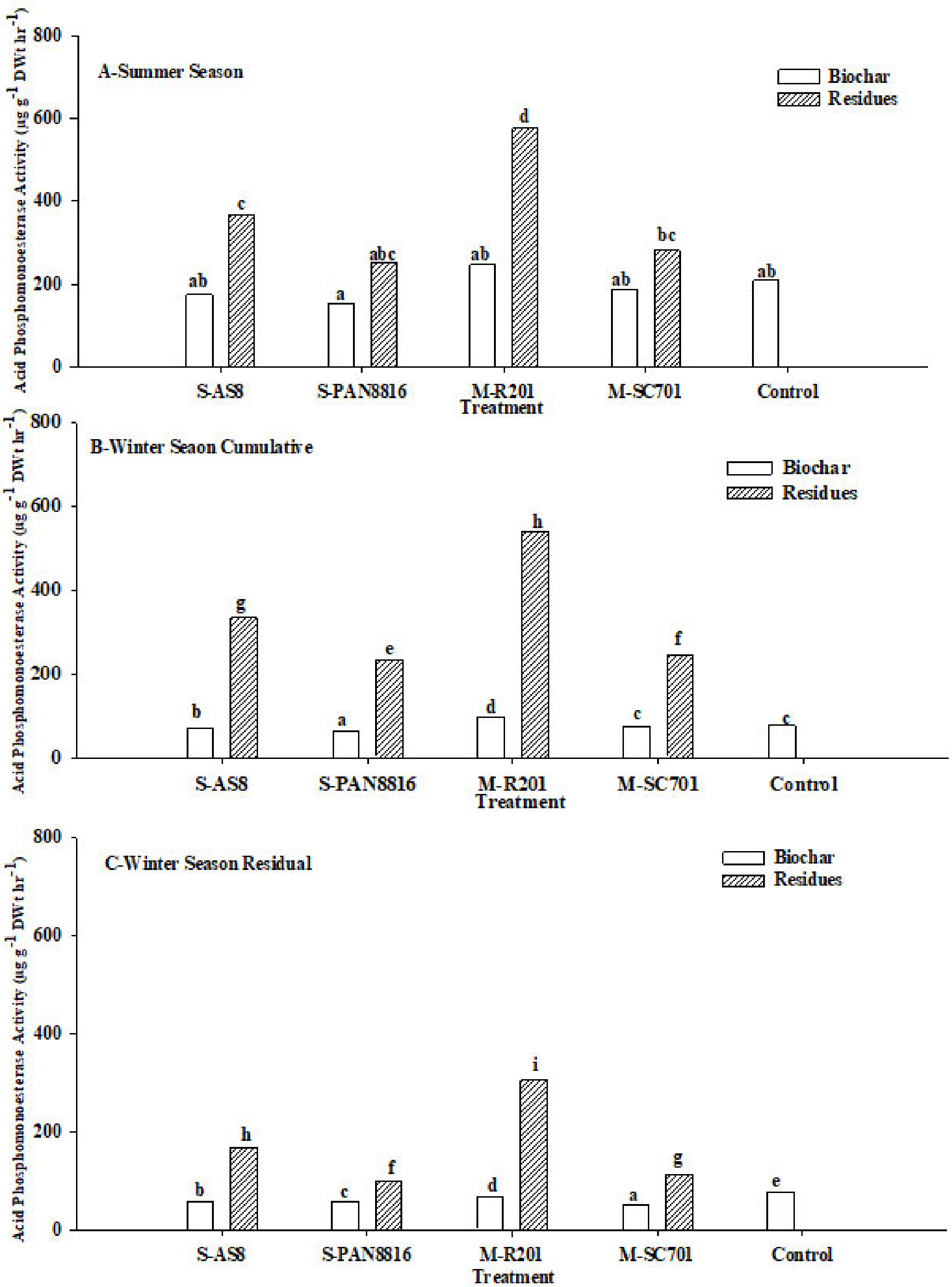

Figure 6.1: The effects of feedstock (R) and biochar (B) from different cultivars of maize (M) and sorghum (S) application on Acidic phosphomonoesterase activity (µg g^{-1} Dwt hr^{-1}) in summer and winter season of 2023. Figure (A) represents first summer application, Figure (B) cumulative (Cum) application and Figure (C) residual (Res) effects from the summer season application. Bars within each sub-figure (A, B, or C) that share the same letter are not significantly different at $p \leq 0.05$.

6.3.2.2 Alkaline phosphomonoesterase activity

Alkaline phosphomonoesterase activity was also significantly influenced by the application of different amendments ($p < 0.001$), with biochar applications being consistently higher than their feedstocks across all seasons (Figure 6.2). The differences were cultivar specific than crop type based (Figure 6.2). The sorghum summer application of S-AS8-B (385.74 µg g^{-1} Dwt hr^{-1}) which was 149% higher than the control (154.89 µg g^{-1} Dwt hr^{-1}) resulted in higher while S-AS8-R (105.20 µg g^{-1} Dwt hr^{-1}) gave lower alkaline phosphomonoesterase activity (Figure 6.2A). With maize, M-SC701-B (386.82 µg g^{-1} Dwt hr^{-1}), which was 268% higher than the control (208.5 µg g^{-1} Dwt hr^{-1}) resulted in higher and M-R201-R (72.39 µg g^{-1} Dwt hr^{-1}) which was not significantly different from M-SC701-R (90.69 µg g^{-1} Dwt hr^{-1}) had lower alkaline phosphomonoesterase activity (Figure 6.2A). A more or less similar trend was also followed in the winter cumulative and residual applications (Figures 6.2B & C).

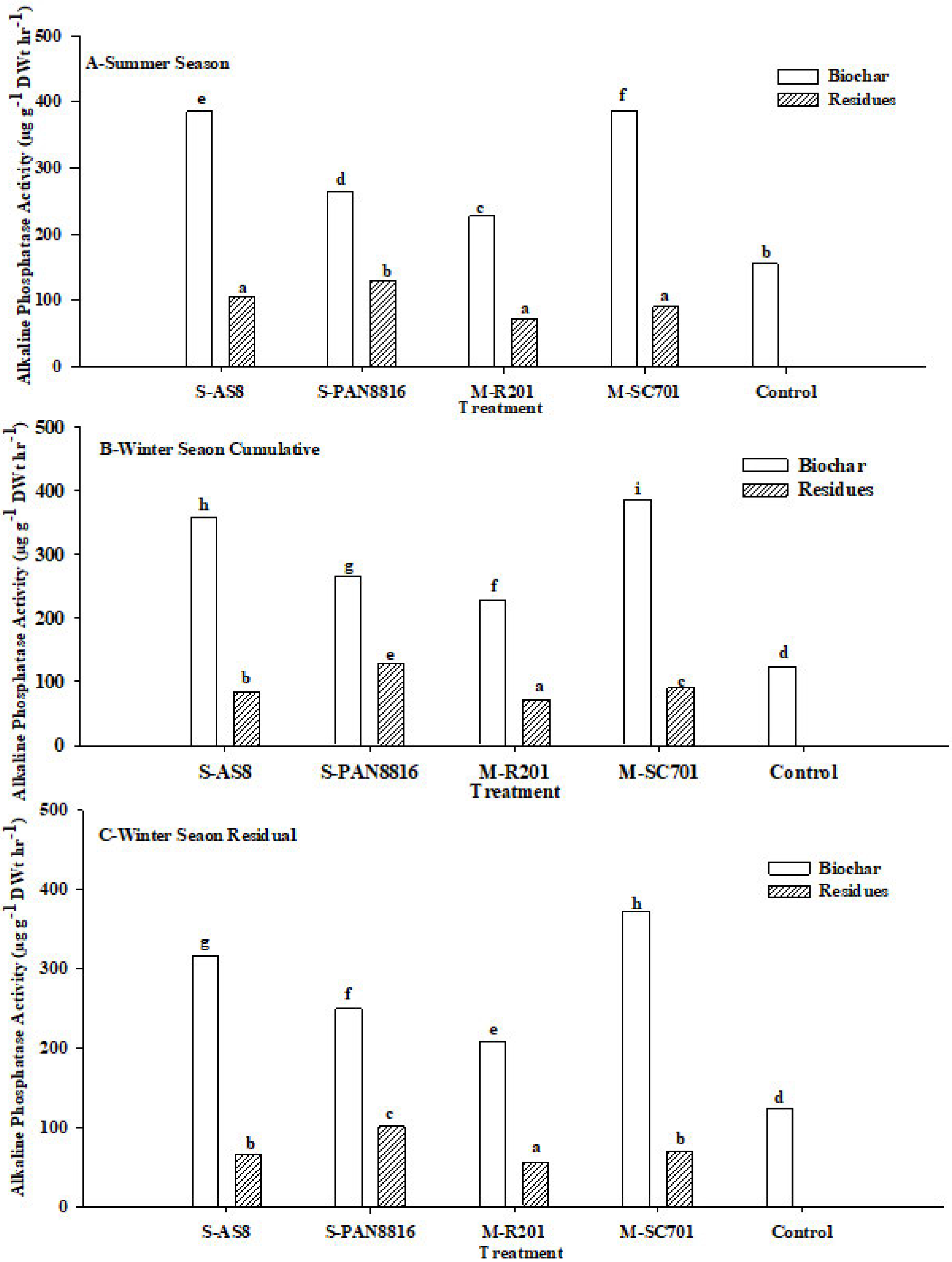

Figure 6.2: The effects of feedstock (R) and biochar (B) from different cultivars of maize (M) and sorghum (S) application on Alkaline phosphomonoesterase activity (µg g^{-1} Dwt hr^{-1}) in summer and winter season of 2023. Figure (A) represents first summer application, Figure (B) cumulative (Cum) application and Figure (C) residual (Res) effects from the summer season application. Bars within each sub-figure (A, B, or C) that share the same letter are not significantly different at $p \leq 0.05$.

6.3.2.3 Urease activity

Urease activity peaked in summer and was lowest in winter ($p < 0.001$), with biochar applications consistently resulting in higher activity compared to their feedstocks across seasons (Figure 6.3). The differences in urease activity were primarily cultivar specific rather than crop type based (Figure 6.3). In summer, the sorghum cultivar S-S-PAN8816-B (56.58 µg g^{-1} Dwt $2hr^{-1}$) which was 218% higher than the control (17.80 µg g^{-1} Dwt $2hr^{-1}$) but not significantly different from S-PAN8816-R (43.14 µg g^{-1} Dwt $2hr^{-1}$) and S-AS8-B (46.09 µg g^{-1} Dwt $2hr^{-1}$) resulted in higher urease activity than the other sorghum treatments (Figure 6.3A). While summer maize application of M-SC701-B (50.72 µg g^{-1} Dwt $2hr^{-1}$), which was 185% higher than the control resulted in higher urease activity while all other maize treatments did not significantly differ with each other and the control (Figure 6.3A). This same pattern was followed in the winter cumulative and residual treatments (Figures 6.3B & C).

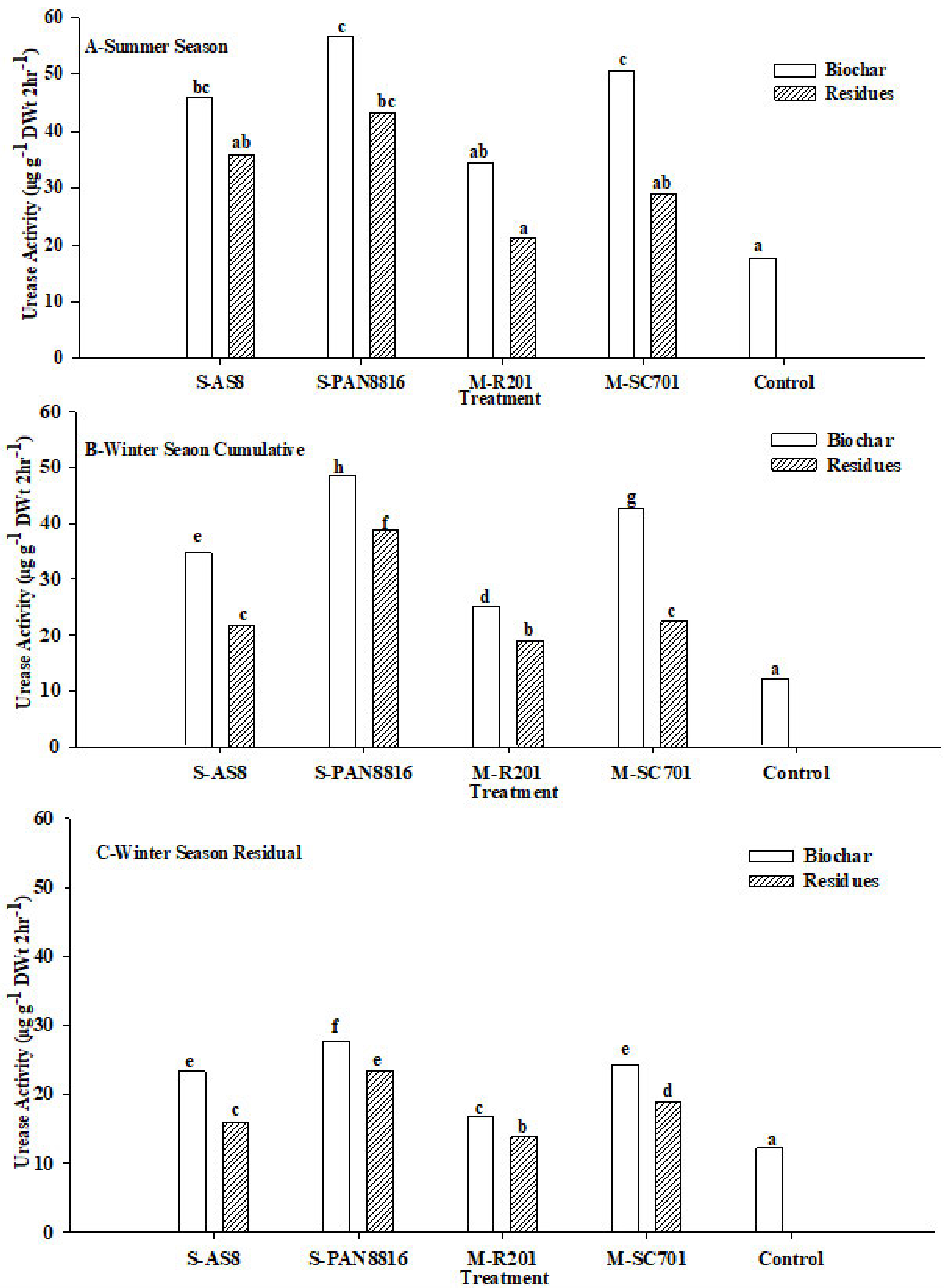

Figure 6.3: The effects of feedstock (R) and biochar (B) from different cultivars of maize (M) and sorghum (S) application on urease activity (µg g^{-1} Dwt $2hr^{-1}$) in summer and winter season of 2023. Figure (A) represents first summer application, Figure (B) cumulative (Cum) application and Figure (C) residual (Res) effects. Bars within each sub-figure (A, B, or C) that share the same letter are not significantly different at $p \leq 0.05$.

6.3.3 Effects of feedstock and biochar application on spinach growth parameters

6.3.3.1 Spinach shoot dry weight

Significant differences in shoot dry weight were observed amongst different treatments and seasons ($p < 0.001$, Figure 6.4), with differences being more cultivar than crop type specific across all seasons. The summer application of S-PAN8816-B (1743 kg ha^{-1}) and M-SC701-B (1528 kg ha^{-1}) resulted in the highest yield, which was 339% and 285% higher respectively than the control, while the rest of the treatments were lower and not different from the control (Figure 6.4A). Winter cumulative sorghum applications had the same trend as in summer season while maize cumulative application of biochar and feedstock of SC701 resulted in higher shoot dry weight (Figure 6.4B). A more or less similar trend was followed in the residual effects as in the winter cumulative applications (Figure 6.4C).

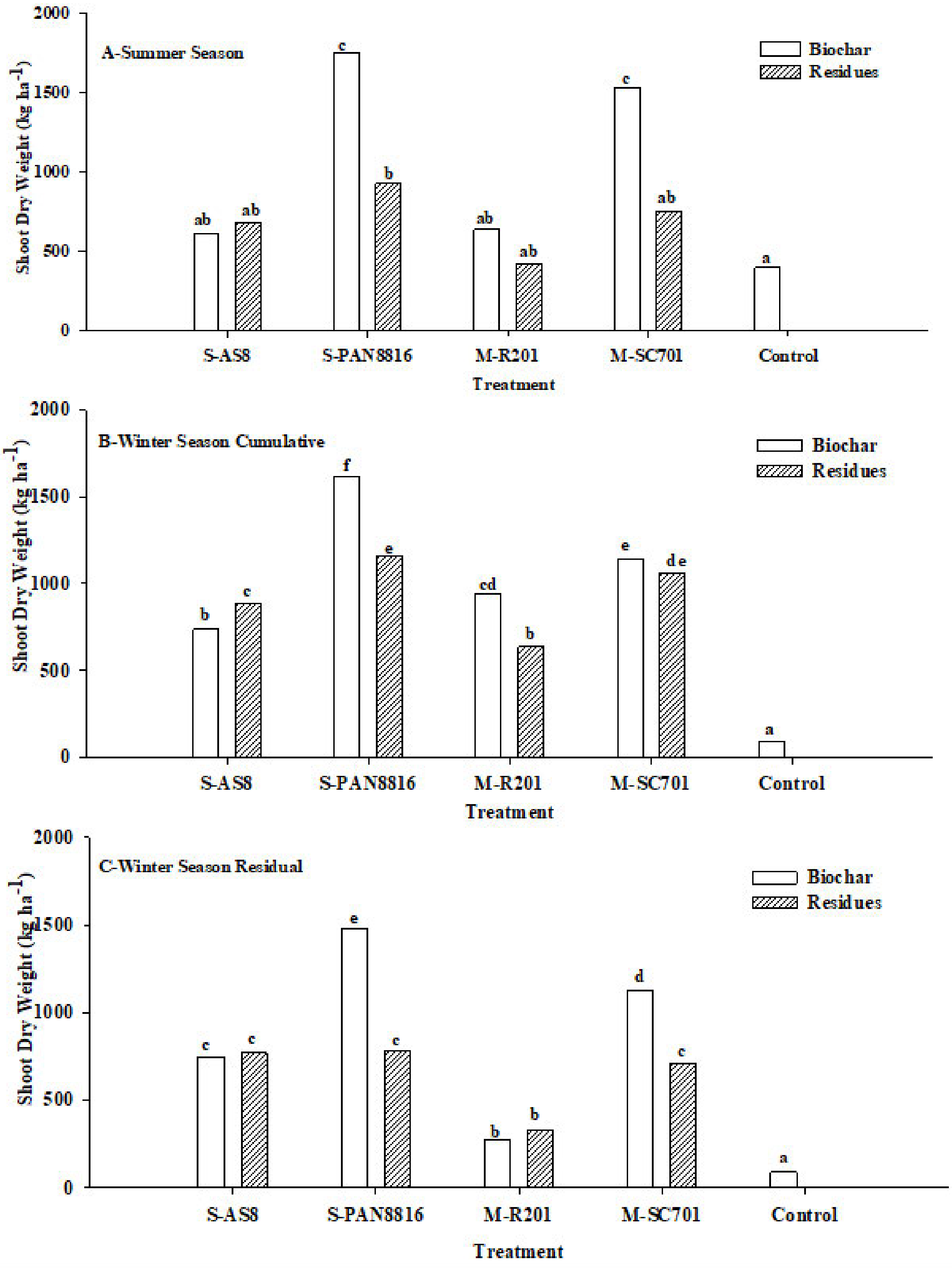

Figure 6.4: The effects of feedstock (R) and biochar (B) from different cultivars of maize (M) and sorghum (S) application on Spinach shoot dry matter (kg ha ^{1}) in summer and winter season of 2023. Figure (A) represents first summer application, Figure (B) cumulative (Cum) application and Figure (C) residual (Res)

summer season application. Bars within each sub-figure (A, B, or C) that share the same letter are not significantly different at $p \leq 0.05$.

6.3.3.2 Spinach root dry weight

The application of feedstock and biochar from different maize and sorghum cultivars significantly impacted spinach root dry weight ($p < 0.001$). In summer, M-SC701-B (563.7 kg ha^{-1}) and S-PAN8816-B (502.5 kg ha^{-1}) resulted in higher root dry weights compared to the control (291.7 kg ha^{-1}) but were not significantly different from most of the other treatments (Figure 6.5A). While in winter, both the cumulative applications and residual effects of all treatments were not significantly different from the control treatment, with M-R201-R mostly giving low root dry weight in each case (Figure 6.5B & C).

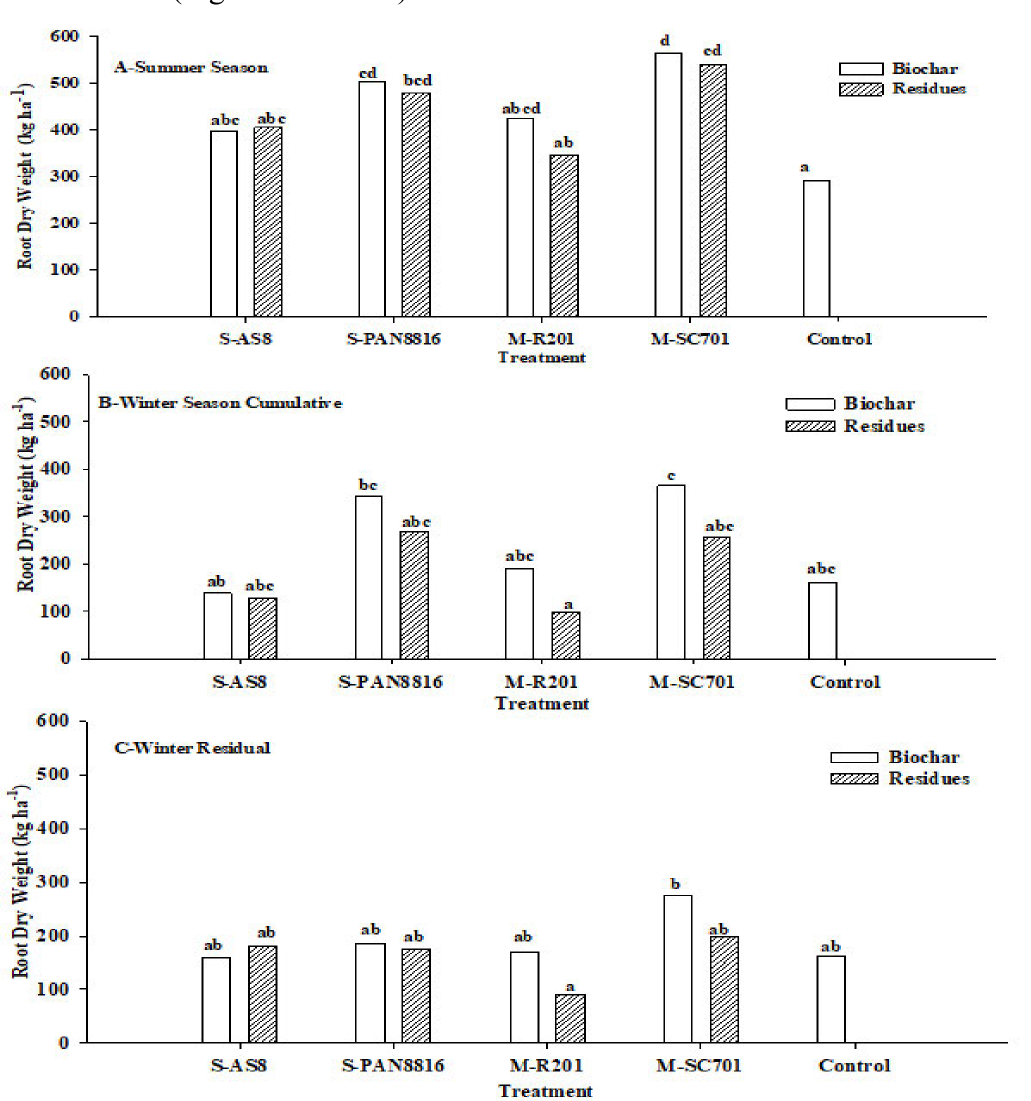

Figure 6.5: The effects of feedstock (R) and biochar (B) from different cultivars of maize (M) and sorghum (S) application on Spinach root dry weight (kg ha ^{1}) in summer and winter season of 2023. Figure (A) represents first summer application, Figure (B) cumulative (Cum) application and Figure (C) residual effects from the summer season application. Bars within each sub-figure (A, B, or C) that share the same letter are not significantly different at $p \leq 0.05$.

6.3.3.3 Spinach leaf area index (LAI)

The application of feedstock and biochar from different maize and sorghum cultivars significantly impacted LAI ($p < 0.001$). In the summer season, M-SC701-B (2.12) and M-SC701-R (1.83) resulted in higher LAI values compared to the control (0.16) and all other treatments (Figure 6.6A). A more or less similar trend to the summer season was observed in the winter cumulative and residual effects (Figures 6.6B & C).

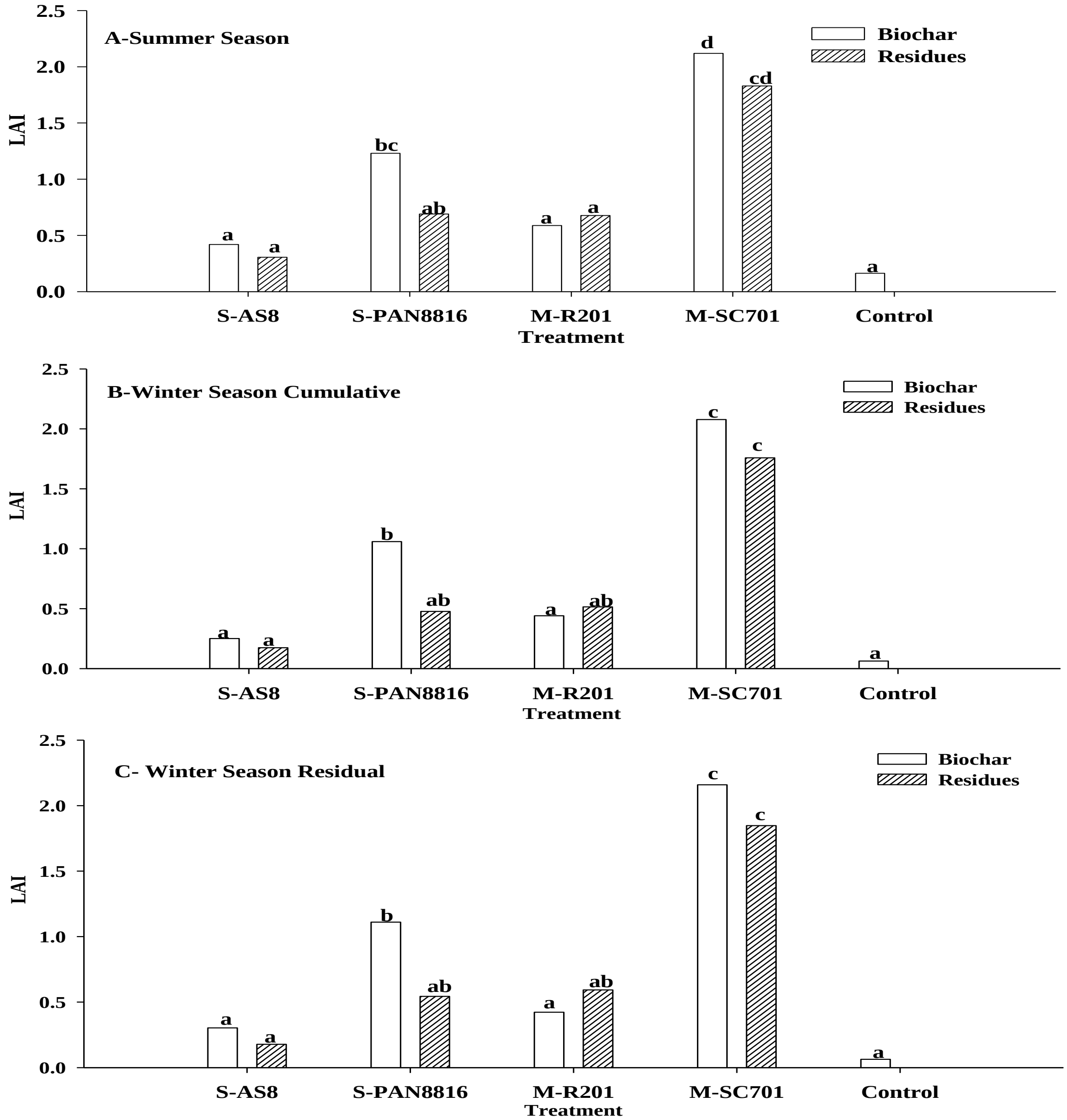

Figure 6.6: The effects of feedstock (R) and biochar (B) from different cultivars of maize (M) and sorghum (S) application on Spinach Leaf area index (LAI) in summer and winter season of 2023. Figure (A) represents first summer application, Figure (B) cumulative (Cum) application and Figure (C) residual (Res) effects from the summer season application. Bars within each sub-figure (A, B, or C) that share the same letter are not significantly different at $p \leq 0.05$.

6.3.4 Impact of application of feedstock and biochar amendments on spinach nutrient uptake and tissue nutrient concentration

3.4.1 N, P and K uptake

The application of feedstock and biochar amendments had a significant impact on nitrogen, phosphorus and potassium uptake in spinach shoots (Table 6.4; $p < 0.001$), with effects being cultivar specific than crop type across all seasons. Biochar treatments also generally resulted in higher nutrient uptake across seasons than their corresponding feedstock. The summer sorghum application of S-PAN8816-B (102.4 kg ha^{-1}) resulted in higher N uptake while all the other sorghum treatments were lower and not significantly different from the control. Conversely, maize application of M-SC701-B (85.11 kg ha^{-1}) gave highest N uptake than all other maize treatments that also did not differ from the control. In winter sorghum cumulative applications, S-PAN8816-B (65.03 kg ha^{-1}) resulted in higher while S-AS8-R (23.88 kg ha^{-1}) which was not significantly different to S-AS8-B (24.77 kg ha^{-1}) gave lowest N uptake. With maize, the cumulative application of M-SC701-B (53.95 kg ha^{-1}) resulted in higher while M-R201-R (15.14 kg ha^{-1}) had lower N uptake followed by the control (2.28 kg ha^{-1}). The winter residual effects followed a similar N uptake as for the cumulative applications. Overall comparison of all treatments showed that S-PAN8816-B resulted in the highest N uptake across all seasons while M-R201-R had the lowest (Table 6.4). The phosphorus uptake followed a similar trend as for N uptake (Table 6.4).

In the case of K uptake, summer sorghum application of S-PAN8816-B (110.38 kg ha^{-1}) resulted in higher K uptake than all the other sorghum treatments and the control. While maize application of M-SC701-B (84.89 kg ha^{-1}) resulted in higher K uptake than all the other maize treatments and the control. The winter cumulative sorghum application of S-PAN8816-B (28.3 kg ha^{-1}) also resulted in higher K uptake while S-AS8-R (9.18 kg ha^{-1}) which was not significantly different from S-AS8-B (9.82 kg ha^{-1}) were lowest. In maize, cumulative application of M-SC701-R (15.64 kg ha^{-1}) resulted to the highest K uptake while M-R201-R (6.16 kg ha^{-1}) was lower, and the control (0.58 kg ha^{-1}) being lower than all amendments. For the residual effects, most treatments did not statistically differ in K uptake (Table 6.4).

Table 6.4: Nitrogen, phosphorus and potassium uptake as affected by the application of biochar (B) and feedstock (R) of different cultivars of maize (M) and sorghum (S)

Season	Treatment	N Uptake (kg ha^{-1})	P Uptake (kg ha^{-1})	K Uptake (kg ha^{-1})
Summer	**S-AS8-B**	19.98^{ab}	2.00^{abc}	25.05^{ab}
	S-AS8-R	24.29^{ab}	2.65^{ab}	36.42^{a}
	S-PAN8816-B	102.4^{c}	9.90^{e}	110.38^{d}
	S-PAN8816-R	37.31^{b}	4.36^{cd}	51.61^{bc}
	M-R201-B	22.55^{ab}	5.41^{d}	26.65^{ab}
	M-R201-R	9.25^{a}	1.17^{a}	14.83^{a}
	M-SC701-B	85.11^{c}	8.36^{e}	84.89^{cd}
	M-SC701-R	31.52^{ab}	3.31^{bcd}	$42.6e^{ab}$
	Control	12.4^{ab}	1.33^{ab}	11.08^{a}
LSD (p<0.05)		15.44	1.26	21.74
Winter Cumulative	**S-AS8-B**	24.77^{c}	11.39^{c}	9.82^{c}
	S-AS8-R	23.88^{c}	7.88^{bc}	9.18^{c}
	S-PAN8816-B	65.03^{f}	20.33^{d}	28.3^{f}
	S-PAN8816-R	45.37^{d}	10.60^{c}	13.19^{d}
	M-R201-B	29.83^{c}	10.92^{c}	9.79^{c}
	M-R201-R	15.14^{b}	5.55^{b}	6.16^{b}
	M-SC701-B	53.95^{e}	11.18^{c}	13.46^{d}
	M-SC701-R	43.54^{d}	9.98^{c}	15.64^{e}
	Control	2.28^{a}	0.47^{a}	0.58^{a}
LSD (p<0.05)		4.49	2.22	1.19
Winter Residual	**S-AS8-B**	21.73^{c}	7.28^{c}	9.23^{ab}
	S-AS8-R	23.64^{c}	6.43^{c}	7.31^{ab}
	S-PAN8816-B	58.32^{f}	14.80^{e}	20.55^{b}
	S-PAN8816-R	26.24^{d}	6.57^{c}	6.48^{ab}
	M-R201-B	8.71^{c}	2.52^{b}	10.82^{ab}
	M-R201-R	6.82^{b}	2.67^{b}	2.41^{a}
	M-SC701-B	43.66^{e}	10.53^{d}	11.7^{ab}
	M-SC701-R	26.48^{d}	6.28^{c}	8.41^{ab}
	Control	2.28^{a}	0.47^{a}	0.58^{a}
LSD (p<0.05)		1.66	0.81	8.57

Values on the same column with different letters are significantly different (p<0.05)

6.3.4.2 Ca, Mg and S uptake

Calcium, Mg and S uptake by spinach significantly differed among treatments (Table 6.5, $p<0.001$), again with differences being more cultivar than crop type specific. For most cultivars, biochar resulted in higher Ca, Mg and S uptake than their corresponding feedstocks. The summer sorghum application of S-PAN8816-B (31.22 kg ha^{-1}) resulted in higher Ca uptake than all the other sorghum treatments and the control (2.55 kg ha^{-1}). Conversely for maize cultivars, the application of M-SC701-B (16.85 kg ha^{-1}) resulted in higher Ca uptake than all the other maize treatments that did not differ from the control. Generally, the cumulative applications of treatments in winter resulted in higher Ca uptake than in the summer season and winter residual effects. Winter sorghum cumulative application of S-PAN8816-B (88.32kg ha^{-1}) also resulted in highest while S-AS8-B (24.99 kg ha^{-1}) which was not significantly different to S-AS8-R (30.28 kg ha^{-1}) had lowest Ca uptake. In maize, the cumulative application of M-SC701-B (51.31 kg ha^{-1}) resulted in the highest while the control (2.07 kg ha^{-1}) followed by M-R201-R (16.26 kg ha^{-1}) had the lowest Ca uptake. The winter residual effects followed a more or less similar trend of Ca uptake as in the cumulative applications. Overall comparison of all treatments saw the application of S-PAN8816-B resulting in the highest Ca uptake across all seasons while M-R201-R was lowest (Table 6.5). The Mg and S uptakes had similar trends to the Ca uptake across seasons (Table 6.5).

Table 6.5: Calcium, Magnesium and Sulfur uptake as affected by the application of biochar (B) and feedstock (R) of different cultivars of maize (M) and sorghum (S)

Season	Treatment	Ca Uptake (kg ha^{-1})	Mg Uptake (kg ha^{-1})	S Uptake (kg ha^{-1})
Summer	S-AS8-B	9.94^{b}	9.72^{ab}	3.29^{ab}
	S-AS8-R	7.52^{ab}	8.72^{ab}	2.26^{ab}
	S-PAN8816-B	31.22^{d}	43.99^{d}	10.00^{c}
	S-PAN8816-R	10.22^{b}	14.09^{b}	4.21^{b}
	M-R201-B	7.32^{ab}	9.14^{ab}	3.10^{ab}
	M-R201-R	3.92^{ab}	5.46^{a}	1.86^{a}
	M-SC701-B	16.85^{c}	22.08^{c}	8.38^{c}
	M-SC701-R	8.04^{ab}	13.35^{b}	3.11^{ab}
	Control	2.55^{a}	4.24^{a}	1.68^{a}
LSD (p<0.05)		3.94	3.63	1.35
Winter Cumulative	S-AS8-B	24.99^{c}	2.07^{b}	2.89^{b}
	S-AS8-R	30.28^{cd}	3.23^{c}	2.69^{b}
	S-PAN8816-B	88.32^{g}	7.62^{e}	8.48^{e}
	S-PAN8816-R	60.68^{f}	3.93^{c}	4.36^{c}
	M-R201-B	30.27^{cd}	5.28^{d}	4.50^{c}
	M-R201-R	16.26^{b}	1.49^{b}	2.34^{b}
	M-SC701-B	51.31^{e}	4.95^{d}	5.42^{d}
	M-SC701-R	36.98^{d}	3.85^{c}	3.83^{c}
	Control	2.07^{a}	0.22^{a}	0.38^{a}
LSD (p<0.05)		4.68	0.46	0.42
Winter Residual	S-AS8-B	21.22^{b}	1.75^{cd}	3.22^{e}
	S-AS8-R	19.77^{b}	2.49^{e}	2.42^{cd}
	S-PAN8816-B	54.81^{d}	6.29^{g}	6.38^{g}
	S-PAN8816-R	19.55^{b}	1.98^{de}	2.77^{d}
	M-R201-B	7.84^{a}	1.31^{bc}	0.99^{b}
	M-R201-R	7.1^{a}	0.79^{b}	1.00^{b}
	M-SC701-B	37.28^{c}	4.23^{f}	4.94^{f}
	M-SC701-R	16.57^{b}	2.24^{de}	2.30^{c}
	Control	2.07^{a}	0.22^{a}	0.38^{a}
LSD (p<0.05)		3.85	0.33	0.26

Values on the same column with different letters are significantly different (p<0.05)

6.3.4.3 Spinach tissue micronutrient (Cu, Fe, Mn and Zn) concentration

Tissue micronutrient (Cu, Fe, Mn and Zn) concentration in spinach shoots significantly differed among treatments ($p<0.001$). It was also cultivar specific and generally higher in feedstock treatments compared to their corresponding biochars (Table 6.6). It was also generally higher in summer than both winter treatments. Cu concentration in the summer was highest for the sorghum cultivar S-AS8-R (31.12 mg kg^{-1}) and lowest in S-PAN8816-B (23.49 mg kg^{-1}) which was not significantly different from S-AS8-B (24.84 mg kg^{-1}). In the case of maize, M-R201-R (30.56 mg kg^{-1}) resulted in the highest while M-R201-B (22.11 mg kg^{-1}gave lowest Cu concentration. Winter cumulative application of the sorghum cultivar S-AS8-R (17.86 mg kg^{-1}) which was not significantly different from the control (17.28 mg kg^{-1}) resulted in highest while S-AS8-B (12.6 mg kg^{-1}) had lowest Cu concentration. While cumulative application of the maize cultivar M-R201-R (19.19 mg kg^{-1}) gave higher and M-R201-B (10.81 mg kg-1) resulted in lowest Cu concentration. The residual effects followed the same trend in Cu concentration as that of cumulative applications (Table 6.6).

Fe concentration in the summer sorghum cultivars was higher with S-AS8-R (913.7 mg kg^{-1}) which was not significantly different from S-PAN8816-R (892.3 mg kg^{-1}) and the control (820.9 mg kg^{-1}) and lowest in S-AS8-B (689.7 mg kg^{-1}) which was not significantly different to S-PAN8816-B (712.5 mg kg^{-1}). While for maize, application of M-SC701-R (860.5 mg kg^{-1}) which was not significantly different from the control (820.9 mg kg^{-1}) resulted in higher tissue Fe concentration than the other treatments. In winter sorghum cumulative applications, S-AS8-R (772 mg kg^{-1}) resulted in higher while S-PAN8816-B (529.4 mg kg^{-1}) had lower tissue Fe concentration. While for maize, the cumulative application of M-R201-R (656.9 mg kg^{-1}) which was not significantly different from M-SC701-R (654.4 mg kg^{-1}) and the control (655.8 mg kg^{-1}) resulted in higher while the M-SC701-B (474.6 mg kg^{-1}) had lower Fe concentration. The residual effects followed the same trend in tissue Fe concentration as for that of cumulative applications (Table 6.6).

Tissue Mn concentration in summer sorghum applications was higher with S-PAN881-R (1292.5 mg kg^{-1}) than all the other sorghum treatments which did not significantly differ (Table 6.6). While for maize, the application of M-R201-R (1232 mg kg^{-1}) which was not significantly different from M-SC701-R (1190.4 mg kg^{-1}) resulted in the highest while M-R201-B (1019.9 mg kg^{-1}) which was not significantly different from M-SC701-B (1050.4 mg kg^{-1}) gave lowest Mn concentration. A more or less similar trend was followed with winter cumulative applications and residual effects as for that of summer treatments (Table 6.6).

Zn concentration in summer sorghum treatments was higher with S-AS8-R (350.4 mg kg^{-1}) and lower with S-PAN8816-B (232.5 mg kg^{-1}) which did not significantly differ from the control (232.8 mg kg^{-1}). While for maize, the application of M-SC701-R (360.4 mg kg^{-1}) resulted in highest, and M-R201-B (228.4 mg kg^{-1}) which was not significantly different from the control (232.8 mg kg^{-1}) had lowest Zn concentration. This same trend in tissue Zn concentration was followed in the winter cumulative applications and residual effects (Table 6.6).

Table 6.6: Tissue micronutrient (Cu, Fe, Mn and Zn) concentration as affected by the application of biochar (B) and feedstock (R) of different cultivars of maize (M) and sorghum (S)

Season	Treatment	Tissue Cu (mg kg^{-1})	Tissue Fe (mg kg^{-1})	Tissue Mn (mg kg^{1})	Tissue Zn (mg kg^{-1})
Summer	S-AS8-B	24.84^{bc}	689.7^{a}	1075.7^{bcd}	260.6^{b}
	S-AS8-R	31.12^{g}	913.7^{c}	1120.9^{bcd}	350.4^{d}
	S-PAN8816-B	23.49^{ab}	712.5^{ab}	1049^{bc}	232.5^{a}
	S-PAN8816-R	27.44^{de}	892.3^{c}	1292.5^{e}	303.3^{c}
	M-R201-B	22.11^{a}	669.6^{a}	1019.9^{b}	228.4^{a}
	M-R201-R	30.56^{fg}	738^{ab}	1232^{de}	297.4^{c}
	M-SC701-B	25.65^{cd}	662.7^{a}	1050.4^{bc}	284.5^{c}
	M-SC701-R	28.75^{ef}	860.5^{c}	1190.4^{cde}	360.4^{d}
	Control	27.14^{de}	820.9^{bc}	781.6^{a}	232.8^{a}
LSD (p<0.05)		1.107	64.81	97.7	12.37
Winter Cumulative	S-AS8-B	12.6^{bc}	572.2^{c}	595.8^{b}	226.3^{c}
	S-AS8-R	17.86^{ef}	772^{f}	648.2^{c}	305.9^{e}
	S-PAN8816-B	11.36^{ab}	529.4^{b}	567.2^{b}	182.4^{b}
	S-PAN8816-R	13.67^{cd}	679.5^{e}	743.6^{d}	255^{d}
	M-R201-B	10.81^{a}	566.6^{c}	453.4^{a}	166.3^{ab}
	M-R201-R	19.19^{f}	656.9^{d}	796.4^{e}	219.7^{c}
	M-SC701-B	13.06^{bc}	474.6^{a}	444.1^{a}	218.3^{c}
	M-SC701-R	15.37^{d}	654.4^{d}	651.4^{c}	323.1^{e}
	Control	17.28^{e}	655.8^{d}	435.1^{a}	160.3^{a}
LSD (p<0.05)		1.048	9.97	21.40	10.84
Winter Residual	S-AS8-B	9.22^{ab}	465.2^{bc}	449.6^{b}	222.3^{de}
	S-AS8-R	13.71^{cd}	613.2^{f}	542.2^{c}	263.0^{e}
	S-PAN8816-B	11.63^{bc}	464.2^{b}	424.4^{b}	133.2^{ab}
	S-PAN8816-R	11.8^{cd}	579.2^{e}	664.8^{e}	198.2^{cd}
	M-R201-B	8.22^{a}	477.2^{c}	339.6^{a}	126.1^{a}
	M-R201-R	14.15^{d}	631.7^{g}	612.3^{d}	153.6^{abc}
	M-SC701-B	8.44^{a}	439^{a}	358.1^{a}	186.7^{bcd}
	M-SC701-R	12.29^{cd}	531.4^{d}	556.3^{c}	277.1^{e}
	Control	17.28^{e}	655.8^{h}	435.1^{b}	160.3^{abc}
LSD (p<0.05)		1.463	7.342	21.53	34.38

Values on the same column with different letters are significantly different (p<0.05)

6.3.5 Principal component analysis (PCA) of selected soil properties influenced by feedstock and biochar composition from different cultivars of maize and sorghum

The PCA revealed that the effects of biochar and feedstock on soil properties were driven by differences in their composition (Table 6.7). The first two principal components (PC1 and PC2) together explained 87.82% of the total variability in the dataset, with PC1 accounting for 69.99% and PC2 contributing 17.83% of the variation. PC1 had the highest eigenvalue (9.8) and was predominantly driven by variables such as alkaline phosphomonoesterase activity, soil pH, available P & exchangeable K, total C and residue pH, which exhibited higher and positive eigenvector values while variables such as acid phosphomonoesterase activity and exchangeable acidity had higher negative eigenvector values compared to other variables. Conversely, PC2, with an eigenvalue of 2.5, was influenced positively influenced by variables such as C: N ratio, total carbon, and residue P while negatively influenced by urease activity, total N and residue N (Table 6.7).

Table 6.7: Summary of eigenvectors, eigenvalues and cumulative variation for selected soil fertility variables as influenced by the application of feedstock and biochar from different cultivars of maize and sorghum

Variables	**PC1**	**PC2**
Acid Phosphomonoesterase	-0.27	0.09
Alkaline Phosphomonoesterase	0.31	0.05
Urease Activity	0.26	-0.32
Total C	0.26	0.36
Total N	0.15	-0.41
Available P	0.29	0.22
Exchangeable K	0.29	-0.09
pH	0.31	-0.03
Exchangeable Acidity	-0.31	0.003
Residue C	0.28	0.25
Residue N	0.25	-0.35
Residue P	0.23	0.4
Residue C:N	-0.18	0.42
Residue pH	0.3	0.11
Eigenvalue	9.8	2.5
Variance (%)	69.99	17.83
Cumulative (%)	69.99	87.82

The PCA biplots illustrated the relationships among soil variables and between these variables and the treatment amendments (biochar and feedstock) (Figure 6.7). Variables represented by vectors that were parallel or close to each other indicated strong positive associations, while those oriented in nearly opposite directions (180°) showed strong negative associations. Vectors positioned at perpendicular angles represented weak or no relationships. Biochar treatments clustered on the positive side of PC1, indicating their strong influence on soil properties such as pH, available P, and total carbon. Feedstock of maize cultivar R201 on the other hand was located on the positive side of PC2 and aligned with variables such as residue C:N ratio, exchangeable acidity and acid phosphomonoesterase activity (Figure 6.7).

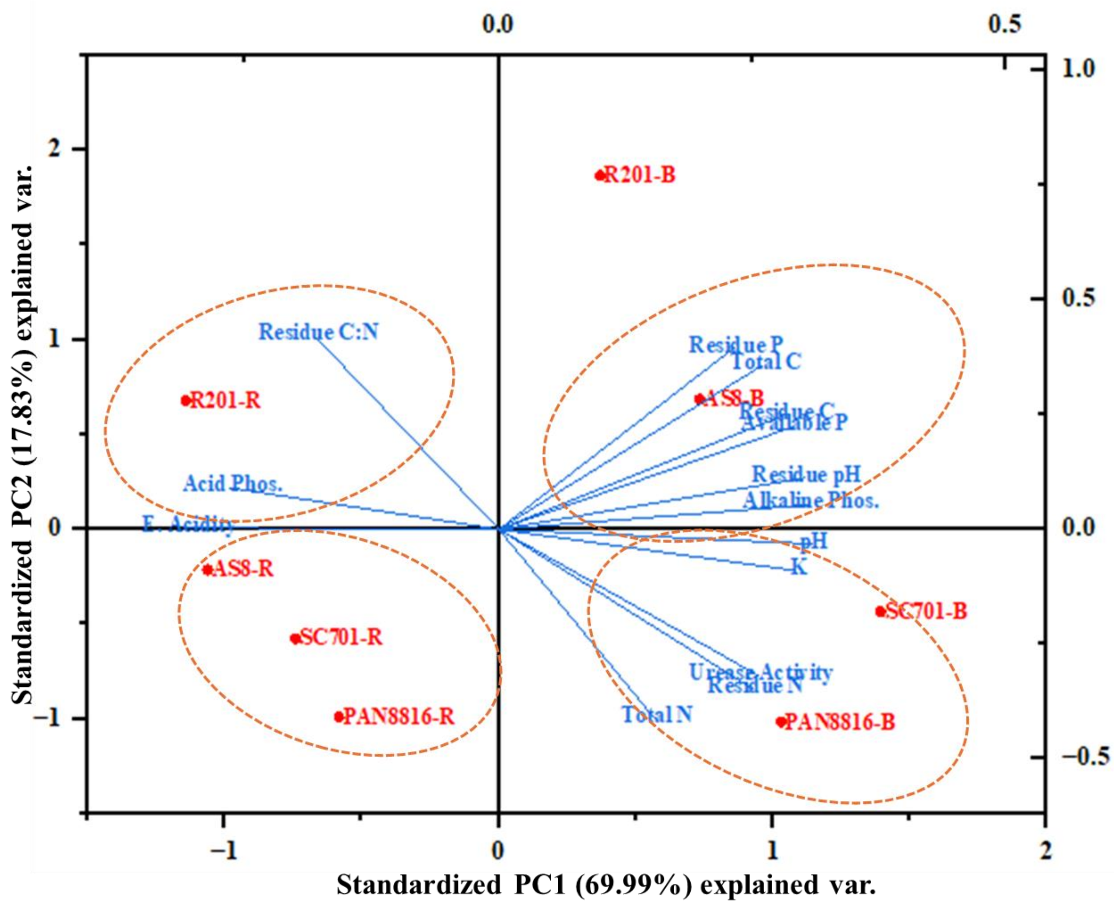

Figure 6.7: PCA biplot of selected soil fertility parameters as influenced by feedstock (R) and biochar (B) of different cultivars of maize and sorghum. Residue C:N= C:N ratio of feedstock and biochar, Acid Phos.= Soil acid phosphomonoesterase activity, E. Acidity= Soil exchangeable acidity, Residue P= Total phosphorus of feedstock and biochar, Total C= Soil total carbon, Residue C= Total carbon of feedstock and biochar, Available P= Soil available phosphorus, Residue pH= pH of feedstock and biochar, Alkaline Phosp.= Soil alkaline phosphomonoesterase activity, pH= Soil pH, K= Soil exchangeable K, Urease Activity= Soil urease activity, Residue N= Total N of feedstock and biochar, Total N= Soil total nitrogen

6.3.6 Principal component analysis (PCA) of selected spinach yield parameters influenced by feedstock and biochar composition from different cultivars of maize and sorghum

The PCA revealed distinct relationships between spinach yield parameters and the composition of feedstock and biochar amendments (Table 6.8). The first two principal components (PC1 and PC2) together explained 88.46% of the total variability in the dataset, with PC1 accounting for 64.83% and PC2 contributing 23.63% of the variation. PC1, with the highest eigenvalue (11.02), was positively associated with biomass-related parameters such as shoot and root dry weights, nutrient uptake, and residue nitrogen while it was negatively associated with biochemical composition variables such as residue C:N and spinach shoot micronutrients (Mn, Zn, Cu & Fe). In contrast, PC2, with an eigenvalue of 4.02, was positively influenced by shoot micronutrient concentrations (Zn, Fe, Mn & Cu) while negatively influenced by residue C, residue P, residue C:N and residue pH (Table 6.8).

Table 6.8: Summary of eigenvectors, eigenvalues and cumulative variation for selected spinach yield parameters as influenced by the application of feedstock and biochar from different cultivars of maize and sorghum

Variables	PC1	PC2
Root Dry Weight	0.23	0.16
Shoot Dry Weight	0.27	0.21
K Uptake	0.28	0.18
Mg Uptake	0.27	0.15
N Uptake	0.28	0.18
P Uptake	0.29	0.11
S Uptake	0.29	0.11
Ca Uptake	0.27	0.2
Residue C	0.18	-0.36
Residue N	0.29	0.1
Residue P	0.15	-0.42
Residue C:N	-0.22	-0.2
Residue pH	0.25	-0.26
Mn Concentration	-0.2	0.29
Zn Concentration	-0.15	0.32
Cu Concentration	-0.21	0.28
Fe Concentration	-0.22	0.31
Eigenvalue	11.02	4.02
Variance (%)	64.83	23.63
Cumulative (%)	64.83	88.46

The PCA biplots showed that biochar treatments clustered on the positive side of PC1, highlighting their influence on biomass production and nutrient uptake. Feedstock treatments were aligned along PC2, reflecting their contribution to improved micronutrient concentrations in spinach. Residue C:N ratio negatively correlated with biomass parameters, indicating slower nutrient release from treatments with high C:N ratios (e.g., R201-R). Overall, biochar treatments such as PAN8816-B and SC701-B enhanced spinach growth, while feedstock treatments (e.g., AS8-R, PAN8816-R, SC701-R) increased shoot micronutrient concentrations (Figure 6.8).

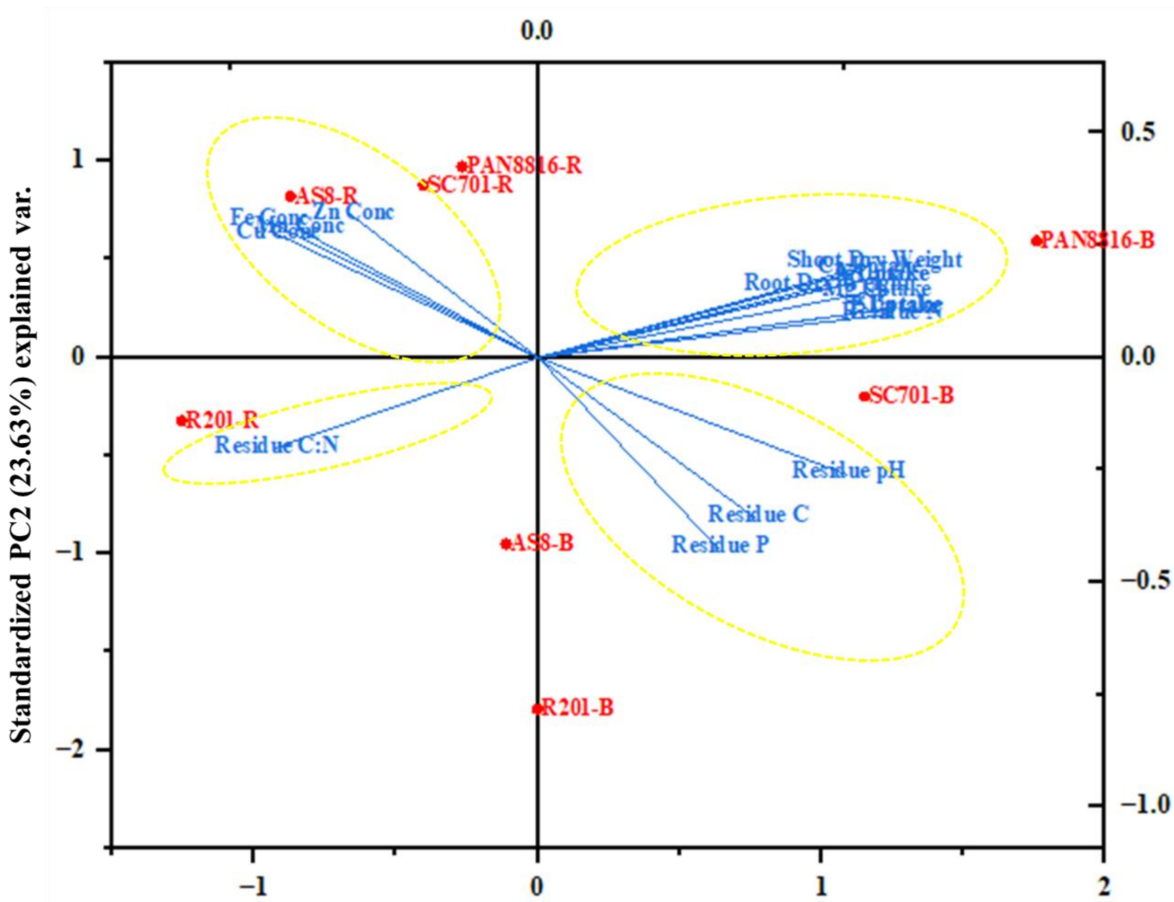

Figure 6.8: PCA biplot of selected soil fertility parameters as influenced by feedstock (R) and biochar (B) of different cultivars of maize and sorghum. Residue C:N= C:N ratio of feedstock and biochar,Fe Conc.=Fe concentration in spinach tissues, Cu Conc= Cu concentration in spinach tissues, Zn conc= Zn concentration in spinach tissues, N uptake= Nitrogen uptake by spinach, P uptake= Phosphorus uptake by spinach, K uptake= Potassium uptake by spinach, S uptake= Sulphur uptake by spinach, Ca uptake= Calcium uptake by spinach, Mg uptake= Magnesium uptake by spinach, Residue C:N= C:N ratio of feedstock and biochar, Residue P= Total phosphorus of feedstock and biochar, Residue C= Total carbon of feedstock and biochar, Residue pH= pH of feedstock and biochar, Residue N= Total N of feedstock and biochar

6.4 Discussion

Feedstock and biochar amendments significantly influenced the different soil fertility parameters. The effects of these amendments were cultivar specific rather than crop type dependent across all seasons, with each crop having one cultivar that exhibited the greatest impact and another that showed the least impact on soil properties. Biochar treated plots exhibited higher pH and lower exchangeable acidity compared to the feedstock-treated plots, with the most pronounced effects observed in plots receiving M-SC701-B, S-PAN8816-B and S-AS8-B biochars. According to Schulz et al. (2013), the alkaline bases present in biochar release significant amounts of cations into the soil, which increases proton consumption processes, thereby reducing soil acidity and increasing soil pH. Additionally, the increase in soil pH following biochar application may also be attributed to the decarboxylation of organic anions, releasing hydroxide ions or the high concentrations of basic cations (Ca, Mg and K) released during biochar decomposition. Generally, all feedstock treatments had lower initial residue pH (Table 6.1a) compared to their corresponding biochars, which likely contributed to the decreased soil pH observed after their application. In contrast, biochar applications resulted in higher pH values, suggesting that the alkaline nature of biochar can counteract soil acidity and increase soil pH. These changes in soil pH have important implications for nutrient availability, including P and S, nitrogen mineralization and the supply of basic cations like Ca^{2+}, Mg^{2+}, and K^{+} (Ding et al., 2016; Cely et al., 2015). These findings are supported by the PCA results, the biochar treatments were clustered closer to pH indicating the positive influence on their application to soil pH and also residue pH was positively associated with soil pH resulting in increased soil pH with application of biochar which had higher pH than feedstock (Figure 6.7).

The application of biochar also led to an increase in soil total C, with the M-R201-B and S-AS8-B treatments consistently resulting in highest total C across all seasons. In contrast, the feedstock treatments did not have a significant impact on total carbon content in the soil compared to the control. Biochar, being rich in carbon and primarily composed of aromatic compounds, is ideal for sequestering carbon in agricultural soils (Lehman and Joseph, 2024). The relatively stable C in biochar can be readily added to the soil, augmenting various carbon pools and thereby enhancing total soil carbon content (Khan et al., 2023). This stable C can persist in the soil for extended periods, contributing to long-term carbon sequestration and soil fertility improvements. Numerous studies have consistently reported a significant increase in soil C content both immediately and several years after biochar application (Chan et al., 2007; Biederman and Harpole, 2013; Liu et al., 2014; Nelissen et al., 2015). In the present study residue total C was positively associated with total soil C and also the biochar treatments were positioned in the positive side of PC1 along with soil total C indicating their positive influence on soil total C when applied (Figure 6.7). This sustained increase in soil C highlights the long-term potential of biochar to sequester carbon in agricultural soils, improving soil fertility and contributing to climate change mitigation.

The application of biochar and feedstock, however, did not result in significant differences in soil total N content in either season for most treatments. This may be attributed to the fact that both amendments initially supply nutrients for plant growth, but the impact is eventually masked by plant uptake, as plants utilize the available N for their growth and development (Cong et al., 2023). As a result, the initial increase in soil N is depleted over time, leading to no significant differences in soil total N between treatments. Another possible explanation for the lack of significant increase in soil total N could be the relatively short duration of the experiment. This is supported by the findings of Li et al. (2018), who conducted an 11-year study and observed a significant increase in total N only from the fifth year onwards. This suggests that the effects of biochar and feedstock on soil N dynamics may require a longer period to manifest, with a short-term study not capturing the full extent of these effects.

Application of both feedstock and biochar increased available P and exchangeable K in the soil in all seasons. However, biochar resulted in higher concentration of these parameters compared to feedstock treatments. These findings are consistent with those of previous researchers who also reported that biochar application in soil increase available P, CEC and exchangeable bases (Chan et al., 2007; Rondon et al., 2007; Alburquerque et al., 2014). This increase can also be attributed to the presence of ash in the biochar, which is rich in oxides, hydroxides and alkali metals, which are readily soluble leading to an increased soil pH (Steenari et al., 1999) and the release of free bases and other ions into the soil solution (Glaser et al., 2002). This increase could also be attributed to the increase in soil pH which is the main variable which controls the availability of nutrients in the soil. The application of biochar has been shown to increase soil pH, leading to improved nutrient availability, although the extent of this effect may vary depending on the type of biochar used (Khodadad et al., 2011; Gul et al., 2015).

In the present study the higher available soil P after biochar application can be primarily attributed to the higher initial P concentration in biochar compared to feedstock (Table 6.1a). As noted by Troeh and Thompson (2005), when soil pH is below 5.7, phosphorus exists mainly as $H_2PO_4^-$ ions, which readily react with iron (Fe) or aluminum (Al) compounds to form insoluble compounds. Therefore, when the feedstock treatments were applied in soil the soil pH was below 5.7 across all seasons which then resulted in lower available P than when biochars were applied. According to Solangi et al. (2023) P is more available in soil when the soil pH is between 6-7. Therefore, application of S-AS8-B and M-SC701-B improved the soil pH up to this range and thus resulted to higher available P across all seasons. These findings were further supported by the PCA analysis (Figure 6.7), as biochar treatments were clustered around available soil P and exchangeable K indicating their positive influence on these variables.

Soil enzyme activity plays a vital role in nutrient cycling by catalyzing the breakdown of soil organic matter (He et al., 2021). In the present study enzyme activities were significantly higher in the summer compared to the winter season, likely due to the favorable conditions for microbial activity during the summer months. Warm temperatures and high rainfall patterns in summer increased soil moisture, which accelerated organic matter decomposition and microbial activity (Fekete et al., 2007). In contrast, the winter season's colder temperatures and reduced rainfall resulted in decreased soil moisture, leading to reduced microbial activity and slowed organic matter decomposition. The unfavorable conditions during the winter season can even lead to the death of microbes, further contributing to the decrease in enzyme activity.

The application of feedstock treatments resulted in a significant increase in acid phosphomonoesterase activity compared to biochar application. This can be attributed to the lower initial feedstock pH (Table 6.1a) which likely enhanced the activity of acid phosphomonoesterase, an enzyme that thrives in acidic conditions, thereby promoting the release of nutrients from organic matter. Enzymatic activities are highly sensitive to soil pH, as it influences the concentration of inhibitors and activators in the soil solution and the effective concentration of the substrate (Dick et al., 2000). The lower initial pH of the feedstock contributed to the decrease in soil pH after its application, creating an environment that favors the activity of microbes responsible for breaking down acidic substrates. In contrast, the higher pH of the biochar may have reduced the activity of these microbes, resulting in lower acid phosphophomonoesterase enzyme activity. According to

Dick et al. (2000), acidic phosphatase is predominant in acid soils, whereas alkaline phosphatase is predominant in alkaline soils. The results of the current study align with these findings and are supported by the PCA analysis (Figure 6.7). The feedstock treatments were clustered closer to acidic phosphomonoestrase activity while the biochar treatments were clustered closer to the alkaline phosphomonoesterase indicating their positive influence towards these enzyme activities. Furthermore, acidic phosphomonoesterase was negatively associated with soil pH while alkaline phosphomonoesterase activity was positively associated with soil pH. This indicate that the application of feedstock treatments resulted in lower soil pH enhancing acidic phosphomonoesterase activity while the biochar treatments caused higher soil pH enhancing alkaline phosphomonesterase activity while reducing the acidic phosphomonoesterase activity. Acidic phosphomonoesterase activity and alkaline phosphomonoesterase activity were negatively correlated (Figure 6.7). Therefore, the treatments that exhibited higher acidic phosphomonoesterase had lower alkaline phosphomonoesterase activity. The consistently higher acidic phosphomonoesterase with M-R201-R and S-AS8-R across all seasons as well as the consistently higher alkaline phosphomonoesterase with S-AS8-B and M-SC701-B were attributed to their initial pH and the resultant soil pH after their application which favoured the activity of these enzymes. This increased phosphomonesterase activity plays a crucial role in mineralizing P for plants and soil microbes by catalyzing the hydrolysis of oxide and ester phosphate bonds (Shahzard et al., 2014).

Biochar also resulted in higher urease activity compared to feedstock application. This is consistent with the findings of Rousk et al. (2010), who reported that soil pH controls the functionality of soil enzymes. The increase in soil pH following biochar application created a favorable environment for urease activity, as urease is optimally active at neutral pH (6.5-7) (Miśkowiec and Olech, 2020). The biochars used in this study had higher pH and residue total N content compared to the feedstock, which created a favorable environment for urease activity. This is consistent with previous findings that biochar application accelerates N mineralization, leading to increased N cycling enzyme activity (Bailey et al., 2011). Amongst the cultivars, M-SC701-B and S-PAN8816-B resulted in higher urease activity across all seasons. This is attributed to the higher initial residue N in these biochars which induced this enzyme activity upon application and also was attributable to the optimum soil pH reached for urease activity when these biochars were applied. This view is supported by the PCA analysis (Figure 6.7). Biochars were clustered in PC1 along with urease activity suggesting that their application positively influence urease activity. Furthermore, urease activity positively correlated with initial residue total N and soil pH implying that the residues with higher initial total N and higher pH such as M-SC701-B and S-PAN8816-B increase urease activity.

Crop residues serve as a vital nutrient source, as their recycling can release essential soil nutrients, enhance soil fertility, leading to increased yields and reduced reliance on chemical fertilizers (Dhaliwal et al., 2019). The incorporation of feedstock and biochar can have varying effects on biomass yield, ranging from positive to mixed or even negative outcomes (Hossain et al., 2020; Mirzaei et al., 2021; Knoublach et al., 2021). Consistent with these findings, the present study revealed mixed effects of residue application on spinach yield, with most treatments resulting in increased spinach shoot dry weight, while others showed no significant impact. S-PAN8816-B and M-SC701-B resulted in the highest shoot dry weight across all seasons, suggesting that these treatments had a profound impact on soil fertility and crop productivity. The spinach shoots dry weight results align with the soil properties results observed, as the biochars derived from S-PAN8816-B and M-SC701-B cultivars also yielded the highest concentrations of available P, exchangeable K as well as increased soil pH across seasons. These biochars also had higher initial residue N content and lower C: N ratios, which facilitated rapid decomposition and mineralization of nutrients, thereby enhancing efficient nutrient uptake (Abbasi et al., 2015). Therefore, the soil conditions were conducive for optimal plant growth, as these biochars provided essential nutrients and a favorable pH range for spinach growth. These results are consistent with the findings of Knoblauch et al. (2021), who observed increased crop yields in both summer and winter seasons following biochar application to the soil. PCA results confirmed that biomass related parameters (shoot and root dry weights, nutrient uptake) along with biochar treatments were strongly associated with PC1, which accounted for 64.83% of the total variation (Figure 6.8). This suggests that biochar's ability to enhance soil properties, such as pH and nutrient availability, likely contributed to the observed improvements in spinach growth. The negative correlation between residue initial C:N ratio and biomass parameters further supports this, indicating that feedstock treatments, particularly those with higher C:N ratios (e.g., R201-R), released nutrients more slowly, limiting their immediate benefits for growth. Several field experiments have consistently demonstrated that biochar application leads to increased plant-available nutrients and nutrient uptake, resulting in enhanced crop productivity in agricultural soils (Major et al., 2010; Quilliam et al., 2012; Farkas et al., 2020). Moreover, the application of M-SC701-R, M-SC701-B and S-PAN8816-B resulted in higher LAI, aligning with the observed increase in shoot biomass under these treatments (M-S701-B & S-PAN8816-B). LAI was consistently higher with these amendments compared to the control and other treatments, indicating their positive impact on spinach growth. These results suggest that the application of these amendments enhanced the development of spinach leaf biomass and canopy structure by improving soil conditions, which promoted vegetative growth and leaf expansion. These findings are consistent with the work of Arora et al. (2023), who reported increased LAI in wheat following the application of crop residues, further supporting the role of residue amendments in improving crop productivity.

The application of M-SC701-B and S-PAN8816-B resulted in higher root dry weight compared to the control during the summer season. However, in the winter season, roots dry weight did not significantly differ among treatments, including the control. This variation can be attributed to the plant's resource allocation strategies, which depend on growth stage and environmental conditions. During summer, optimal conditions for photosynthesis enable plants to allocate resources to both root and shoot growth, promoting balanced development. In contrast, in winter with the reduced photosynthetic activity plants may prioritize maintaining shoot biomass, which is critical for photosynthesis and survival. Therefore, this explains the non-significant differences in root dry weight among treatments during the winter season (Zhou et al., 2023).

Micronutrient concentrations (Zn, Fe, Cu and Mn) in spinach shoots were significantly higher under feedstock treatments, as indicated by their clustering along PC2 in the PCA analysis (Figure 6.8). This suggests that feedstock amendments provided a sustained release of micronutrients, enhancing their availability even under residual conditions. The positive alignment of shoot micronutrient concentrations with PC2 highlights feedstock's critical role in supplying essential trace elements, whereas biochar primarily enhanced macronutrient availability. Leafy vegetables such as spinach are recognized as a rich source of dietary nutrients, particularly micronutrients, which are vital for human health and proper growth (Odhav et al., 2007). These micronutrients (Zn, Fe, Mn and Cu) play essential roles in physiological functions, forming integral components of blood, teeth, muscles and bones. Their adequate concentrations in plant tissues are thus crucial, as deficiencies or excesses can adversely impact human health. According to Thapa et al. (2020), the sufficiency ranges for micronutrients in plant tissues are 20-600 ppm for Fe, 10-600 ppm for

Mn, 10-250 ppm for Zn and 2-50 ppm for Cu, with optimal concentrations of 100 ppm, 50 ppm, 20 ppm, and 6 ppm, respectively. In this study, feedstock treatments frequently resulted in tissue micronutrient concentrations exceeding these sufficiency ranges, particularly for Mn and Fe, indicating a risk of over enrichment.

The higher micronutrient concentrations observed under feedstock treatments have significant implications for soil and crop management. While feedstocks effectively enhanced spinach nutritional quality, their excessive application poses risks of micronutrient toxicity for plants and consumers. For instance, Mn concentrations exceeding 1,000 ppm, as seen under all treatments in summer season except for the control treatment, could disrupt plant metabolic processes and present health risks to people. Similarly, Zn concentrations above 250 ppm, observed in some feedstock treatments, exceed the sufficiency threshold for spinach, potentially affecting plant growth and nutrient balance. To mitigate these risks, maybe combining feedstocks with biochar could be a practical strategy. Biochar's nutrient retention properties can regulate micronutrient release, preventing over enrichment while maintaining soil fertility. Also avoiding the application of the studied treatments in summer to prevent Mn toxicity could aid in mitigating these risks. These approaches would optimize the benefits of feedstock amendments while ensuring safe micronutrient levels in crop tissues, thereby balancing soil health, crop productivity and food safety.

6.5 Conclusion

This study found that the effects of soil amendments on soil fertility and spinach yield were more influenced by specific cultivars of maize and sorghum than by the crop type. This finding emphasized the importance of selecting suitable cultivars when aiming to improve soil quality and enhance crop productivity. Across both seasons, biochar amendments consistently outperformed feedstocks in improving soil pH, available phosphorus (P), exchangeable potassium (K), enzyme activities, nutrient uptake, and spinach shoot dry weight, particularly under summer conditions. Cumulative biochar applications were more effective than residual effects, highlighting the benefits of repeated application for sustained soil fertility.

Among the maize cultivars, M-SC701-B significantly improved soil pH, available P, exchangeable K, alkaline phosphomonoesterase and urease activity, macronutrient uptake, and spinach shoot biomass while lowering exchangeable acidity. M-R201-B, on the other hand, enhanced total soil carbon, and M-R201-R increased acid phosphomonoesterase activity across both seasons. While for sorghum, S-AS8-B enhanced soil pH, available P, total carbon and alkaline phosphomonoesterase activity, whereas S-PAN8816-B improved available P, exchangeable K, urease activity, nutrient uptake, and spinach yield.

While feedstock amendments increased acid phosphomonoesterase activity and spinach micronutrient concentrations, some micronutrient levels exceeded recommended thresholds for human consumption, raising concerns about potential toxicity. To mitigate this, combining feedstocks with biochar is recommended, as biochar can regulate nutrient release and help prevent over accumulation of micronutrients in spinach tissues.

CHAPTER 7

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 General Discussion

This study provides a comprehensive exploration of the potential of crop residues, applied either as feedstock or biochar, to improve soil quality, soil fertility, enhance crop productivity or mitigate greenhouse gas emissions. By integrating findings from a systematic review, laboratory incubation and field experiments, it highlights the critical influence of feedstock composition, cultivar-specific differences and pyrolysis conditions in determining the effectiveness of these soil amendments. Crop residues have long been recognized as valuable amendments for improving soil fertility, carbon sequestration and crop yields. The systematic review revealed the roles of feedstock and biochar in soil management with feedstock providing immediate benefits such as increased MBC and enhanced enzyme activities due to the rapid decomposition of labile carbon fractions (Tejada & Benitez, 2014). However, these benefits result in increased CO_2 emissions driven by microbial priming effects (Maris et al., 2017). While biochar, with its recalcitrant nature stabilizes SOC pools, reduces CO_2 emissions and enhances long-term carbon sequestration making it a more sustainable option (Campos et al., 2020). This was evident in the incubation study where feedstock treatments exhibited significantly higher CO_2 emissions while biochars produced at 650ºC consistently resulted in lower CO_2 emissions. These trends were further observed in the field study where the application of feedstock resulted in higher CO_2 emissions and enhanced β-glucosidase activity. In contrast the application of biochars particularly from cultivars R201 and AS8 enhanced SOC, POC and POXC while reducing CO_2 emissions, highlighting biochar's role in climate change mitigation. Therefore, these contrasting effects highlight the necessity of selecting the appropriate amendment depending on the desired soil management goals (Gong et al., 2012; Tang et al., 2022).

The characterization of feedstocks and biochars highlighted the profound influence of biochemical composition and pyrolysis conditions. Feedstock composition was highly cultivar dependent, with significant variations even within cultivars of the same crop type. Maize cultivar R201 and sorghum cultivar AS8 feedstocks exhibited higher lignin content and fixed C and they produced biochars with superior stability and recalcitrance. These biochars were characterized by lower H:C and O:C ratios, higher fixed C and aromatic structures, which contribute to their resistance to microbial decomposition and their potential for long-term carbon storage (Cantrell et al., 2012; Cantrell et al., 2012 Hassan et al., 2017). In contrast, feedstocks from SC701 and PAN8816, with higher ash content and nitrogen contents, produced biochars better suited for nutrient cycling due to their higher pH and mineral N availability (Schulz et al., 2013). The relationship between feedstock composition and biochar properties was further explained by the effects of pyrolysis temperature. Higher pyrolysis temperatures (650°C) reduced biochar yield but increased fixed C and ash content, enhancing biochar's stability and suitability for carbon sequestration. This was attributed to the greater volatilization of organic compounds leading to the loss of more volatile matter, increasing fixed C and ash content by promoting the breakdown of labile carbon fractions facilitating the formation of stable aromatic carbon structures, while concentrating mineral components in biochar (Hassan et al., 2017; Chatterjee et al., 2020). Interestingly, the biochar yield of R201 remained consistent across pyrolysis temperatures probably due to its higher lignin content. Lignin's aromatic structure provides thermal stability resisting decomposition during pyrolysis, which has important implications for selecting feedstocks to improve biochar production (Wang et al., 2017).

These chemical properties of biochar directly influenced the incubation study particularly CO_2 emissions and nutrient mineralization (NH_4^+-N, NO_3^--N and extractable P). Feedstock amendments resulted to higher CO_2 emissions due to their readily decomposable carbon content, while biochars reduced CO_2 emissions by providing a stable carbon source, consistent with findings by Hailegnaw et al. (2019). Biochars, particularly those produced at 650°C exhibited lower cumulative CO_2 emissions emphasizing their higher stability and carbon sequestration potential. This aligns with the observed increase in fixed C content at higher pyrolysis temperatures which enhances biochar recalcitrance by reducing the availability of labile C fractions. Furthermore, the H:C and O:C ratios which are indicators of biochar stability also support these findings. Biochars produced at 650ºC generally exhibited lower H:C and O:C ratios indicating the increased aromaticity and reduced oxygenated functional groups making them more resistant to microbial degradation (Lehman et al., 2006; Novak et al., 2009). Cultivar-specific differences were observed, among the maize cultivars, biochars from R201 characterized by higher fixed C content and aromaticity demonstrated superior stability corroborating to their lower CO_2 emissions during the incubation. Similarly, in sorghum cultivars the greater stability of AS8-B650 which exhibited higher fixed C content resulted in lower CO_2 emissions during the incubation study, these findings support the view that biochars with superior stability and aromaticity or biochars produced from feedstocks with high fixed C and low ash content are suitable for long-term carbon sequestration (Enders et al., 2012). Moreover, treatments from R201 particularly, R201-B650 which exhibited higher total P also resulted in higher extractable P concentrations. Similarly, the AS8-B350 and AS8-B650 treatments exhibited highest extractable P concentrations supporting the findings of Mafongoya et al. (2002) that P mineralization is positively correlated with the initial concentration of the residue (amendment) therefore the higher the total P in the amendment, the higher the amount of P will be mineralized. Also, the composition of feedstock biochar influence mineral N mineralization. Among maize treatments SC701-B650 which exhibited the highest ash content and lowest volatile matter resulted in highest NO_3^--N concentrations. This suggests that ash which contains mineral bound nutrients contributed to increased nitrogen mineralization. While R201 feedstock which had the lowest ash content and the highest volatile matter exhibited lowest NO_3^--N probably due to its higher proportion of organic compounds that undergo microbial immobilization rather than immediate mineralization. Similarly for sorghum cultivars, AS8 feedstock with lower ash and higher volatile matter content exhibited lowest NO_3^--N concentration while PAN8816-B350 which had higher total N content than B650 and feedstock treatments resulted to enhanced nitrogen availability (NH_4^+-N and NO_3^--N) supporting the view that nutrient release patters are influenced by amendment composition (Zavalloni et al.,2011). These findings further aligned with the results from the field experiments, where biochar application resulted to lower CO_2 emissions, enhanced soil carbon pools, improved nutrient availability and enhanced spinach biomass yields supporting the trends observed in the incubation study. The higher nutrient mineralization in biochar treatments observed in the incubation study explain the higher total N and available P in biochar treated soils in the field which further resulted to increased nutrient uptake by spinach. Furthermore, the higher CO_2 emissions observed from feedstock treatments during the incubation period were also observed in the field study where feedstock treatments application resulted to faster decomposition leading to higher CO_2 emissions.

Field experiments confirmed the sustained benefits of biochar in enhancing SOC pools (POC and POXC) and reducing CO_2 emissions particularly with cumulative biochar applications. Biochars derived from R201 and AS8 consistently outperformed those from SC701 and PAN8816 in stabilizing soil organic carbon pools and reducing emissions, further emphasizing the role of feedstock composition. The stability and recalcitrance of biochars from R201 and AS8, attributed to their high fixed C and low ash content, make them ideal for long-term carbon sequestration. Although the biochars used in the field studies were produced from the kiln rather than controlled temperatures (350ºC & 650ºC) as the biochars used in the incubation study, similar trends were observed across both experiments. The incubation study demonstrated that biochar consistently resulted to lower CO_2 emissions compared to feedstock which was also observed in the field where biochar treated soil had lower CO_2 emissions than feedstock treated soil. The field results further support the incubation findings that feedstock applications resulted in higher CO_2 emissions due to increased microbial decomposition of labile organic compounds as shown by significantly higher emissions in SC701-R and PAN8816-R treatments. These findings align with the global systematic review findings that feedstock incorporation increases CO_2 emissions due to rapid decomposition of labile carbon which stimulates microbial activity and soil respiration (Maris et al., 2017; Abalos et al., 2023). While biochar application results to lower CO_2 emissions due to its recalcitrant carbon structure which resist microbial degradation (Campos et al., 2020; Yang et al., 2017a). The results of the SOC, POC and POXC pools further align with characterization study findings which showed that R201-BKiln and AS8-BKiln contained higher fixed C, volatile matter and lower ash content than SC701-BKiln and PAN8816-BKiln and the significant increases in SOC content following their application across seasons confirm that biochars from these cultivars enhance long-term soil carbon storage, supporting their role in climate change mitigation (Duan et al., 2020). Feedstock application promoted higher microbial activity and β-glucosidase activity due to its readily available organic compounds (Tejada & Benitez, 2014; Urra et al., 2018) while biochar applications suppressed these activities. This suggests that while feedstocks provide short-term microbial activity and rapid decomposition of organic matter, biochar lead to longer term carbon stabilization in the soil with reduced microbial decomposition rates (Li et al., 2022: Kim et al.,2022).

On the other hand, biochars from SC701 and PAN8816 were more effective in improving soil fertility and crop yields. With P mineralization, the field experiment results did not fully align with those of the incubation study for maize treatments. In the incubation study R201-B650, which is biochar produced from maize cultivar R201, resulted in highest extractable P attributed to its total P content. However, in the field experiment it was biochar produced from cultivar SC701 for maize and from AS8 for sorghum cultivars which resulted to highest available P concentrations. This was attributed to the neutral soil pH observed with these treatments which enhanced alkaline phosphomonesterase activity, thereby increasing soil available P. These findings suggest that under field conditions, multiple soil properties and environmental factors interact to influence P availability, rather than total P of the amendment alone (Shahzard et al., 2014; Solangi et al., 2023). Nitrogen mineralization trends observed in the incubation study were consistent with those observed from the field study. The application of biochars from maize cultivars SC701 and sorghum cultivar PAN8816 which had higher initial total N, pH and ash content increase N mineralization. Their higher ash content, pH and nitrogen content neutralized soil acidity, enhanced urease activity, improved CEC and enhanced macronutrient availability, resulting in higher macronutrient uptake and higher spinach biomass. These findings align with previous studies highlighting the liming effect and nutrient enhancement capabilities of biochar (Khodadad et al., 2011; Gul et al., 2015). These findings further align with the findings of the global systematic review emphasizing the immediate nutrient mineralization potential of feedstock and long-term benefits of biochar. The higher NO_3^--N and available P concentrations observed in biochar amended soils are consistent with the review which indicated that biochar enhances N and P retention by reducing nutrients leaching and improving soil pH (Zhang et al.,2012; Mierzwa-Hersztek et al., 2017; Mosharoff et al., 2022).

Furthermore, the observed improved spinach biomass yield and nutrient uptake in soils aligns with review findings which reported that biochar enhances nutrient retention, induce microbial activities and minimize nutrient losses thereby supporting sustained crop productivity (Dubey et al., 2022; Zhao et al., 2024). Therefore, these findings highlight the importance of customizing biochar production to specific agricultural goals, whether it be carbon sequestration or fertility improvement. The findings of this study further reveals that the effects of feedstock and biochar on soil properties are mainly governed by biochemical composition rather than crop type. This challenges the assumption that crop type plays a dominant role and shifts the focus to cultivar-specific traits. The higher fixed C and lignin content of R201 and AS8 feedstocks made their biochars ideal for long-term carbon sequestration. While the higher ash and N content of SC701 and PAN8816 feedstocks made their biochars more effective for nutrient recycling and soil fertility improvement (Enders et al., 2012; Lehmann et al., 2015). Moreover, the consistent performance of R201 across pyrolysis temperatures suggests that feedstocks with high lignin content should be prioritized for biochar production, particularly when thermal stability and yield are critical factors

(Wang et al., 2017). This highlights the importance of understanding the relationship between feedstock composition and pyrolysis conditions for optimizing biochar production processes.

7.2 Conclusions and Recommendations

7.2.1 Conclusions

Raw crop residues (feedstock) and biochar have a potential for enhancing soil quality, mitigating climate change and increasing crop yields. Biochar is a superior option for long-term carbon sequestration and sustained soil quality improvement, while feedstock provides short-term benefits but requires frequent reapplications. The effectiveness of biochar and feedstock varies depending on residue type, residue chemical composition, application rate, environmental conditions and management practices. The chemical composition of feedstock and biochar, CO_2 emissions, nutrient mineralization, soil carbon pools, soil chemical properties, enzyme activities and crop productivity parameters were more strongly influenced by cultivar-specific differences than by broad crop type differences. Higher pyrolysis temperatures improved biochar stability by reducing labile functional groups and promoting the formation of stable aromatic structures. Overall, biochars demonstrated greater stability than feedstocks, with biochars produced at 650°C significantly reducing CO_2 emissions. Among the cultivars and treatments studied in the incubation study, R201 feedstock and biochars as well as AS8-B650 biochar consistently resulted in lower CO_2 emissions, making them suitable options for carbon sequestration. SC701-B650 and PAN8816-B350 biochars enhanced nitrogen availability, with SC701-B650 increasing NO_3^--N concentrations and PAN8816-B350 increasing both NO_3^--N and NH_4^+-N concentrations. For improving phosphorus availability, R201-B650 and AS8 biochars were the most effective treatments. In the field experiments, the application of kiln-produced biochar and to some extent feedstock, significantly enhanced soil quality by increasing soil organic carbon pools and reducing CO_2 emissions. Cumulative biochar applications were particularly effective in improving SOC pools, soil fertility parameters, crop yield parameters and enzyme activities. Feedstock amendments increased acid phosphomonoesterase and β-glucosidase activities as well as micronutrient shoot concentrations, while biochar amendments enhanced alkaline phosphomonoesterase and urease activities. Among the maize cultivar treatments, M-R201-B reduced CO_2 emissions while improving SOC, POC and POXC pools across seasons. M-SC701-

B consistently improved soil pH, available P, exchangeable K, alkaline phosphomonoesterase activity, macronutrient uptake and spinach shoot dry weight, while also reducing exchangeable acidity. In contrast, M-R201-R increased acid phosphomonoesterase activity and suppressed β-glucosidase activity and enhanced soil total carbon. For sorghum cultivars, S-AS8-B improved soil pH, available P, alkaline phosphomonoesterase activity and total carbon, while S-PAN8816-B increased available P, exchangeable K, urease activity, macronutrient uptake and spinach shoot dry weight.

7.2.2 Recommendations

- Feedstock composition, cultivar-specific differences and pyrolysis conditions should be carefully considered to enhance biochar properties for specific agricultural applications. This customized approach will ensure biochar's effectiveness in enhancing soil quality, carbon sequestration, nutrient retention and crop yields.
- Re-application of biochar and feedstock should be considered to enhance soil carbon pools, carbon sequestration, soil fertility and crop yields more as cumulative additions can sustain long-term benefits.
- Farmers may need to prioritize growing cultivars such as R201 (maize) and AS8 (sorghum) for biochar production, as their residues are effective in reducing CO_2 emissions, improving carbon pools and enhancing phosphorus availability. Additionally, PAN8816 (sorghum) and SC701 (maize) should be grown as their residues are suitable for improving mineral nitrogen availability. However, these benefits must be balanced with high crop yields to encourage farmer adoption, while further research is needed to validate this approach.
- Biochars with higher pH and ash content, such as those from cultivars SC701 and PAN8816 should be applied to acidic soils to ameliorate soil acidity and improve soil fertility.
- For immediate mineral nitrogen supply, residues such as PAN8816 and SC701 feedstocks should be applied though regular reapplications may be necessary. While for immediate carbon sequestration and phosphorus supply, feedstocks from cultivars R201 and AS8 are recommended as effective options.

- To mitigate the risk of micronutrient toxicity in crops, feedstocks should be mixed with biochar to regulate nutrient release and prevent over-enrichment.
- Long-term field studies are needed to validate the findings of this thesis under diverse environmental conditions, focusing on the impacts of these amendments on soil properties, greenhouse gas emissions and crop yields.
- Research should prioritize the effects of biochar and feedstock from different cultivars of major crops, particularly maize, sorghum, wheat and rice to establish cultivar-specific recommendations for sustainable residue management.
- The interaction between biochar amendments and soil microbial communities should be explored to better understand their role in nutrient cycling and carbon sequestration.
- Governments and agricultural extension services should promote the adoption of biochar as a sustainable soil amendment through subsidies and farmer education programs and regional guidelines should be developed to help farmers select appropriate residues and biochars based on local soil and crop requirements.

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LIST OF APPENDICES

Appendix A: ANOVA results for biochar yield from different maize and sorghum cultivars under different pyrolysis temperatures.

Variate: Yield_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	8	89.75	11.22	0.59	
Rep.*Units* stratum					
Cultivar	3	575.22	191.74	10.12	<.001
Pyrolysis_Temperature	2	1354.97	677.49	35.76	<.001
Cultivar.Pyrolysis_Temperature					
	6	57.84	9.64	0.51	0.800
Residual	88	1667.12	18.94		

Appendix B: ANOVA results for moisture content of feedstock and biochar from different maize and sorghum cultivars under different pyrolysis temperatures.

Variate: Moisture_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.02123	0.01062	1.06	
Rep.*Units* stratum					
Cultivar	3	22.65784	7.55261	754.25	<.001
Pyrolysis_Temp	3	401.64926	133.88309	13370.44	<.001
Cultivar.Pyrolysis_Temp	9	7.41762	0.82418	82.31	<.001
Residual	30	0.30040	0.01001		

Appendix C: ANOVA results for fixed C of feedstock and biochar different maize and sorghum cultivars under different pyrolysis temperatures.

Variate: Fixed_C_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.0132	0.0066	0.06	
Rep.*Units* stratum					
Cultivar	3	877.0795	292.3598	2521.26	<.001
Pyrolysis_Temp	3	3552.1897	1184.0632	10211.14	<.001
Cultivar.Pyrolysis_Temp	9	343.9232	38.2137	329.55	<.001
Residual	30	3.4787	0.1160		

Appendix D: ANOVA results for ash content of feedstock and biochar from different maize and sorghum cultivars under different pyrolysis temperatures.

Variate: Ash_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.03218	0.01609	0.22	

Rep.*Units* stratum					
Cultivar	3	2803.35670	934.45223	12967.11	<.001
Pyrolysis_Temp	3	3568.40050	1189.46683	16505.86	<.001
Cultivar.Pyrolysis_Temp	9	357.07804	39.67534	550.56	<.001
Residual	30	2.16190	0.07206		

Appendix E: ANOVA results for volatile matter of feedstock and biochar from different maize and sorghum cultivars under different pyrolysis temperatures

Variate: Volatile_Matter_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	8.636E-02	4.318E-02	0.55	
Rep.*Units* stratum					
Cultivar	3	5.493E+02	1.831E+02	2329.36	<.001
Pyrolysis_Temp	3	1.272E+04	4.241E+03	53951.94	<.001
Cultivar.Pyrolysis_Temp	9	8.293E+01	9.214E+00	117.23	<.001
Residual	30	2.358E+00	7.860E-02		

Appendix F: ANOVA results for total C content of feedstock and biochar from different maize and sorghum cultivars under different pyrolysis temperatures.

Variate: Carbon_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	10.659	5.329	0.87	
Rep.*Units* stratum					
Cultivar	3	207.904	69.301	11.31	<.001
Pyrolysis_Temp	3	3362.326	1120.775	182.97	<.001
Cultivar.Pyrolysis_Temp	9	305.770	33.974	5.55	<.001
Residual	30	183.767	6.126		

Appendix G: ANOVA results for total N content of feedstock and biochar from different maize and sorghum cultivars under different pyrolysis temperatures

Variate: Nitrogen_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.037051	0.018525	3.48	
Rep.*Units* stratum					
Cultivar	3	29.113137	9.704379	1823.54	<.001
Pyrolysis_Temp	3	22.851196	7.617065	1431.32	<.001
Cultivar.Pyrolysis_Temp	9	13.077523	1.453058	273.04	<.001
Residual	30	0.159652	0.005322		

Appendix H: ANOVA results for total P of feedstock and biochar from different maize and sorghum cultivars under different pyrolysis temperatures.

Variate: Total_P_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.00027919	0.00013960	1.71	
Rep.*Units* stratum					
Cultivar	3	0.36442558	0.12147519	1484.89	<.001
Pyrolysis_Temp	3	0.76727385	0.25575795	3126.34	<.001
Cultivar.Pyrolysis_Temp	9	0.02675875	0.00297319	36.34	<.001
Residual	30	0.00245422	0.00008181		

Appendix I: ANOVA results for C:N ratio of feedstock and biochar from different maize and sorghum cultivars under different pyrolysis temperatures.

Variate: C_N

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1254.1	627.1	1.10	
Rep.*Units* stratum					
Cultivar	3	41294.2	13764.7	24.12	<.001
Pyrolysis_Temp	3	10441.4	3480.5	6.10	0.002
Cultivar.Pyrolysis_Temp	9	11418.6	1268.7	2.22	0.049
Residual	30	17118.3	570.6		

Appendix J: ANOVA results for C:P ratio of feedstock and biochar from different maize and sorghum cultivars under different pyrolysis temperatures.

Variate: C_P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	168.21	84.11	1.51	
Rep.*Units* stratum					
Cultivar	3	4930.76	1643.59	29.60	<.001
Pyrolysis_Temp	3	2567.21	855.74	15.41	<.001
Cultivar.Pyrolysis_Temp	9	1311.10	145.68	2.62	0.023
Residual	30	1665.89	55.53		

Appendix K: ANOVA results for H:C ratio of feedstock and biochar from different maize and sorghum cultivars under different pyrolysis temperatures.

Variate: H_C

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.00022663	0.00011332	1.58	
Rep.*Units* stratum					
Cultivar	3	0.00540018	0.00180006	25.09	<.001
Pyrolysis_Temp	3	0.09133893	0.03044631	424.34	<.001
Cultivar.Pyrolysis_Temp	9	0.00207151	0.00023017	3.21	0.008
Residual	30	0.00215252	0.00007175		

Appendix L: ANOVA results for O:C ratio for feedstock and biochar from different maize and sorghum cultivars under different pyrolysis temperatures.

Variate: O_C

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.02864	0.01432	0.87	
Rep.*Units* stratum					
Cultivar	3	0.43159	0.14386	8.73	<.001
Pyrolysis_Temp	3	11.08467	3.69489	224.31	<.001
Cultivar.Pyrolysis_Temp	9	0.43282	0.04809	2.92	0.013
Residual	30	0.49418	0.01647		

Appendix M: ANOVA results for pH of feedstock and biochar from different maize and sorghum cultivars under different pyrolysis temperatures.

Variate: pH

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.017E-03	5.083E-04	6.22	
Rep.*Units* stratum					
Cultivar	3	1.505E+01	5.015E+00	61409.69	<.001
Pyrolysis_Temp	3	1.939E+02	6.462E+01	7.913E+05	<.001
Cultivar.Pyrolysis_Temp	9	1.035E+01	1.150E+00	14082.41	<.001
Residual	30	2.450E-03	8.167E-05		

Appendix N: ANOVA results for Lignin:N for feedstock and biochar from different maize and sorghum cultivars under different pyrolysis temperatures.

Variate: Lignin_N

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	4642.	2321.	0.96	
Rep.*Units* stratum					
Cultivar	3	43749.	14583.	6.06	0.030
Residual	6	14443.	2407.		

Appendix O: ANOVA results for lignin content of feedstock biochar different maize and sorghum cultivars

Variate: Lignin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.5533	0.7767	0.87	
Rep.*Units* stratum					
Cultivar	3	2137.8140	712.6047	798.49	<.001
Residual	6	5.3546	0.8924		

Appendix P: ANOVA results for Ca concentration of feedstock and biochar from different maize and sorghum cultivars under different pyrolysis temperatures.

Variate: Ca_cmolc_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.005335	0.002667	0.57	
Rep.*Units* stratum					
Cultivar	3	23.783248	7.927749	1698.55	<.001
Pyrolysis_Temp	3	19.643372	6.547791	1402.89	<.001
Cultivar.Pyrolysis_Temp	9	52.288795	5.809866	1244.78	<.001
Residual	30	0.140021	0.004667		

Appendix Q: ANOVA results for Mg concentration of feedstock and biochar from different maize and sorghum cultivars under different pyrolysis temperatures.

Variate: Mg_cmolc_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.001401	0.000700	0.20	
Rep.*Units* stratum					
Cultivar	3	0.680245	0.226748	66.22	<.001
Pyrolysis_Temp	3	3.702857	1.234286	360.44	<.001
Cultivar.Pyrolysis_Temp	9	3.217081	0.357453	104.38	<.001
Residual	30	0.102732	0.003424		

Appendix R: ANOVA results for K concentration of feedstock and biochar from different maize and sorghum cultivars under different pyrolysis temperatures

Variate: K_cmolc_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr
Rep stratum	2	0.08155	0.04077	2.15	
Rep.*Units* stratum					
Cultivar	3	10.22489	3.40830	179.94	<.001
Pyrolysis_Temp	3	9.95177	3.31726	175.13	<.001
Cultivar.Pyrolysis_Temp	9	23.23824	2.58203	136.31	<.001
Residual	30	0.56825	0.01894		

Appendix S: ANOVA results CEC of feedstock and biochar yield from different maize and sorghum cultivars under different pyrolysis temperatures

Variate: CEC_cmolc_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.015495	0.007748	0.87	
Rep.*Units* stratum					
Cultivar	3	165.851648	55.283883	6186.68	<.001
Pyrolysis_Temp	3	79.759069	26.586356	2975.21	<.001
Cultivar.Pyrolysis_Temp	9	313.741075	34.860119	3901.11	<.001
Residual	30	0.268078	0.008936		

Appendix T: ANOVA results of CO_2 emissions during a 120-day incubation of feedstock and biochar derived from different cultivars of maize and sorghum produced at varying pyrolysis temperatures.

Variate: Mass_C_in_mg_kg_soil

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	5007.	2503.	1.79	
Rep.*Units* stratum					
Treatment	12	2481342.	206778.	147.65	<.001
Day	9	7356759.	817418.	583.66	<.001
Treatment.Day	108	1589821.	14721.	10.51	<.001
Residual	258	361329.	1401.		

Appendix U: ANOVA results of cumulative CO_2 emissions during a 120-day incubation of feedstock and biochar derived from different cultivars of maize and sorghum produced at varying pyrolysis temperatures.

Variate: Mass_C_in_mg_kg_soil

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	274890.	137445.	10.89	
Rep.*Units* stratum					
Treatment	12	59706829.	4975569.	394.20	<.001
Day	9	283333525.	31481503.	2494.18	<.001
Treatment.Day	108	24982628.	231321.	18.33	<.001
Residual	258	3256470.	12622.		

Appendix V: ANOVA results of ammonium-N mineralization during a 120-day incubation of feedstock and biochar derived from different cultivars of maize and sorghum produced at varying pyrolysis temperatures.

Variate: NH4_N_mg_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	20.511	10.256	3.12	
Rep.*Units* stratum					
Treatment	12	3928.890	327.408	99.49	<.001
Day	9	144342.796	16038.088	4873.55	<.001
Treatment.Day	108	12345.116	114.307	34.73	<.001
Residual	258	849.037	3.291		

Appendix W: ANOVA results of nitrate-N mineralization during a 120-day incubation of feedstock and biochar derived from different cultivars of maize and sorghum produced at varying pyrolysis temperatures

Variate: NO3-N_mg_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	11.473	5.737	1.93	
Rep.*Units* stratum					
Treatment	12	10917.584	909.799	305.45	<.001
Day	9	116262.716	12918.080	4337.06	<.001
Treatment.Day	108	12334.448	114.208	38.34	<.001
Residual	258	768.461	2.979		

Appendix X: ANOVA results of extractable-P mineralization during a 120-day incubation of feedstock and biochar derived from different cultivars of maize and sorghum produced at varying pyrolysis temperatures.

Variate: P_mg_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.1571	0.0786	0.55	
Rep.*Units* stratum					
Treatment	12	1376.5231	114.7103	798.58	<.001
Day	8	5385.2906	673.1613	4686.37	<.001
Treatment.Day	96	1262.0543	13.1464	91.52	<.001
Residual	232	33.3251	0.1436		

Appendix Y: ANOVA results of soil pH during a 120-day incubation of feedstock and biochar derived from different cultivars of maize and sorghum produced at varying pyrolysis temperatures.

Variate: pH

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.01205	0.00603	0.51	
Rep.*Units* stratum					
Treatment	12	16.77944	1.39829	118.16	<.001
Day	9	14.41231	1.60137	135.32	<.001
Treatment.Day	108	15.51050	0.14362	12.14	<.001
Residual	258	3.05308	0.01183		

Appendix Z: ANOVA results for CO_2 emissions under field conditions during the winter season of 2023 following the cumulative application of feedstock and biochar produced by kiln.

Variate: CO2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.

Rep stratum	2	3.77	1.88	0.05	
Rep.*Units* stratum					
Treatment	8	39603.36	4950.42	132.49	<.001
Month	4	73184.73	18296.18	489.67	<.001
Treatment.Month	32	24131.64	754.11	20.18	<.001
Residual	358	13376.31	37.36		

Appendix AA: ANOVA results for CO_2 emissions under field conditions during the winter season of 2023, assessing the residual effects of feedstock and kiln-produced biochar.

Variate: CO2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.85	0.43	0.01	
Rep.*Units* stratum					
Treatment	8	19445.52	2430.69	65.62	<.001
Month	4	61139.17	15284.79	412.65	<.001
Treatment.Month	32	11775.33	367.98	9.93	<.001
Residual	358	13260.59	37.04		

Appendix AB: ANOVA results for SOC under field conditions during the summer season of 2023, following the application of feedstock and biochar produced using a kiln.

Variate: Organic_C_g_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.0789	0.0394	0.36	
Rep.*Units* stratum					
Treatment	8	1041.3555	130.1694	1192.62	<.001
Residual	16	1.7463	0.1091		

Appendix AC: ANOVA results for SOC under field conditions during the winter season of 2023, following the cumulative application of feedstock and kiln-produced biochar.

Variate: Organic_C_g_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.03380	0.01690	1.60	
Rep.*Units* stratum					
Treatment	8	2032.86720	254.10840	24057.60	<.001
Residual	16	0.16900	0.01056		

Appendix AD: ANOVA results for SOC under field conditions during the winter season of 2023, evaluating the residual effects of feedstock and kiln-produced biochar.

Variate: Organic_C_g_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.03380	0.01690	1.00	
Rep.*Units* stratum					

Treatment	8	1479.42600	184.92825	10942.50	<.001
Residual	16	0.27040	0.01690		

Appendix AE: ANOVA results for POC under field conditions during the summer season of 2023, following the application of feedstock and biochar produced using a kiln.

Variate: POC_g_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.001352	0.000676	0.18	
Rep.*Units* stratum					
Treatment	8	275.816112	34.477014	9273.00	<.001
Residual	16	0.059488	0.003718		

Appendix AF: ANOVA results for POC under field conditions during the winter season of 2023, following the cumulative application of feedstock and kiln-produced biochar.

Variate: POC_g_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr
Rep stratum	2	0.001803	0.000901	0.13	
Rep.*Units* stratum					
Treatment	8	711.739219	88.967402	13215.92	<.001
Residual	16	0.107709	0.006732		

Appendix AG: ANOVA results for POC under field conditions during the winter season of 2023, evaluating the residual effects of feedstock and kiln-produced biochar.

Variate: POC_g_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.012619	0.006309	2.80	
Rep.*Units* stratum					
Treatment	8	178.427947	22.303493	9898.00	<.001
Residual	16	0.036053	0.002253		

Appendix AH: ANOVA results for POXC under field conditions during the summer season of 2023, following the application of feedstock and biochar produced using a kiln

Variate: POXC_g_kg_soil

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.00002176	0.00001088	0.57	
Rep.*Units* stratum					
Treatment	8	1.17474856	0.14684357	7713.86	<.001
Residual	16	0.00030458	0.00001904		

Appendix AI: ANOVA results for POXC under field conditions during the winter season of 2023, following the cumulative application of feedstock and kiln-produced biochar.

Variate: POXC_g_kg_soil

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.006810	0.003405	0.89	
Rep.*Units* stratum					
Treatment	8	5.217936	0.652242	170.71	<.001
Residual	16	0.061134	0.003821		

Appendix AJ: ANOVA results for POXC under field conditions during the winter season of 2023, evaluating the residual effects of feedstock and kiln-produced biochar.

Variate: POXC_g_kg_soil

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.001226	0.000613	0.34	
Rep.*Units* stratum					
Treatment	8	4.235276	0.529410	297.51	<.001
Residual	16	0.028471	0.001779		

Appendix AK: ANOVA results for MBC under field conditions during the summer season of 2023, following the application of feedstock and biochar produced using a kiln.

Variate: MBC_g_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.0001497	0.0000748	0.27	
Rep.*Units* stratum					
Treatment	8	0.8588947	0.1073618	391.44	<.001
Residual	16	0.0043884	0.0002743		

Appendix AL: ANOVA results for MBC under field conditions during the winter season of 2023, following the cumulative application of feedstock and kiln-produced biochar.

Variate: MBC_g_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	3.271E-05	1.635E-05	2.19	
Rep.*Units* stratum					
Treatment	8	8.343E-01	1.043E-01	13965.80	<.001
Residual	16	1.195E-04	7.468E-06		

Appendix AM: ANOVA results for MBC under field conditions during the winter season of 2023, evaluating the residual effects of feedstock and kiln-produced biochar.

Variate: MBC_g_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	2.605E-06	1.302E-06	0.31	
Rep.*Units* stratum					

Treatment	8	5.693E-01	7.116E-02	16908.93	<.001
Residual	16	6.733E-05	4.208E-06		

Appendix AN: ANOVA results for Beta-glucosidase activity under field conditions during the summer season of 2023, following the application of feedstock and biochar produced using a kiln

Variate: BetaGlucosidase_Activity_ug_g_DW

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	6.050	3.025	2.98	
Rep.*Units* stratum					
Treatment	8	6320.917	790.115	779.41	<.001
Residual	16	16.220	1.014		

Appendix AO: ANOVA results for Beta-glucosidase activity under field conditions during the winter season of 2023, following the cumulative application of feedstock and kiln-produced biochar.

Variate: BetaGlucosidase_Activity_ug_g_DW

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.7160	0.8580	7.24	
Rep.*Units* stratum					
Treatment	8	5928.3237	741.0405	6252.53	<.001
Residual	16	1.8963	0.1185		

Appendix AP: ANOVA results for Beta-glucosidase activity under field conditions during the winter season of 2023, evaluating the residual effects of feedstock and kiln-produced biochar.

Variate: BetaGlucosidase_Activity_ug_g_DW

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.0153	0.0077	0.03	
Rep.*Units* stratum					
Treatment	8	4717.3099	589.6637	2287.62	<.001
Residual	16	4.1242	0.2578		

Appendix AQ: ANOVA results for exchangeable acidity under field conditions during the summer season of 2023, following the application of feedstock and biochar produced using a kiln.

Variate: Exch_Acidity_cmolc_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.00018962	0.00009481	1.10	
Rep.*Units* stratum					
Treatment	8	0.19491443	0.02436430	282.92	<.001
Residual	16	0.00137787	0.00008612		

Appendix AR: ANOVA results for exchangeable acidity under field conditions during the winter season of 2023, following the cumulative application of feedstock and kiln-produced biochar.

Variate: Exch_Acidity_cmolc_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.00005832	0.00002916	0.39	
Rep.*Units* stratum					
Treatment	8	0.26549315	0.03318664	445.22	<.001
Residual	16	0.00119264	0.00007454		

Appendix AS: ANOVA results for exchangeable acidity under field conditions during the winter season of 2023, evaluating the residual effects of feedstock and kiln-produced biochar.

Variate: Exch_Acidity_cmolc_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.0000222	0.0000111	0.05	
Rep.*Units* stratum					
Treatment	8	0.3127143	0.0390893	186.01	<.001
Residual	16	0.0033623	0.0002101		

Appendix AT: ANOVA results for exchangeable K under field conditions during the summer season of 2023, following the application of feedstock and biochar produced using a kiln.

Variate: K_mg_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.334	0.167	0.03	
Rep.*Units* stratum					
Treatment	8	260184.412	32523.052	5707.92	<.001
Residual	16	91.166	5.698		

Appendix AU: ANOVA results for exchangeable K under field conditions during the winter season of 2023, following the cumulative application of feedstock and kiln-produced biochar.

Variate: K_mg_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	51.075	25.538	3.87	
Rep.*Units* stratum					
Treatment	8	106954.310	13369.289	2027.13	<.001
Residual	16	105.523	6.595		

Appendix AV: ANOVA results for exchangeable K under field conditions during the winter season of 2023, evaluating the residual effects of feedstock and kiln-produced biochar.

Variate: K_mg_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	62.387	31.194	6.37	
Rep.*Units* stratum					
Treatment	8	33514.182	4189.273	854.99	<.001
Residual	16	78.397	4.900		

Appendix AW: ANOVA results for available P under field conditions during the summer season of 2023, following the application of feedstock and biochar produced using a kiln.

Variate: Available P_mg_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	6.333	3.167	2.32	
Rep.*Units* stratum					
Treatment	8	77794.869	9724.359	7111.80	<.001
Residual	16	21.878	1.367		

Appendix AX: ANOVA results for available P under field conditions during the winter season of 2023, following the cumulative application of feedstock and kiln-produced biochar.

Variate:Available P_mg_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.936	0.968	0.21	
Rep.*Units* stratum					
Treatment	8	21592.001	2699.000	575.90	<.001
Residual	16	74.986	4.687		

Appendix AY: ANOVA results for available P under field conditions during the winter season of 2023, evaluating the residual effects of feedstock and kiln-produced biochar.

Variate: Available P_mg_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.121	0.060	0.02	
Rep.*Units* stratum					
Treatment	8	8185.606	1023.201	406.13	<.001
Residual	16	40.311	2.519		

Appendix AZ: ANOVA results for soil pH under field conditions during the summer season of 2023, following the application of feedstock and biochar produced using a kiln.

Variate: pH_KCl

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.10776	0.05388	3.60	
Rep.*Units* stratum					
Treatment	8	28.82827	3.60353	240.59	<.001
Residual	16	0.23964	0.01498		

Appendix BA: ANOVA results for soil pH under field conditions during the winter season of 2023, following the cumulative application of feedstock and kiln-produced biochar.

Variate: pH_KCl

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.0003185	0.0001593	1.06	
Rep.*Units* stratum					
Treatment	8	41.0135407	5.1266926	33968.27	<.001
Residual	16	0.0024148	0.0001509		

Appendix BB: ANOVA results for soil pH under field conditions during the winter season of 2023, evaluating the residual effects of feedstock and kiln-produced biochar.

Variate: pH_KCl

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.006896	0.003448	0.74	
Rep.*Units* stratum					
Treatment	8	7.391030	0.923879	198.94	<.001
Residual	16	0.074304	0.004644		

Appendix BC: ANOVA results for soil total C under field conditions during the summer season of 2023, following the application of feedstock and biochar produced using a kiln.

Variate: Total_C_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.008452	0.004226	0.61	
Rep.*Units* stratum					
Treatment	8	13.793363	1.724170	250.15	<.001
Residual	16	0.110281	0.006893		

Appendix BD: ANOVA results for soil total C under field conditions during the winter season of 2023, following the cumulative application of feedstock and kiln-produced biochar.

Variate: Total_C_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.01556	0.00778	0.44	
Rep.*Units* stratum					
Treatment	8	11.24667	1.40583	79.08	<.001
Residual	16	0.28444	0.01778		

Appendix BE: ANOVA results for soil total C under field conditions during the winter season of 2023, evaluating the residual effects of feedstock and kiln-produced biochar.

Variate: Total_C_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.015556	0.007778	1.06	
Rep.*Units* stratum					
Treatment	8	9.333333	1.166667	158.49	<.001
Residual	16	0.117778	0.007361		

Appendix BF: ANOVA results for soil total N under field conditions during the summer season of 2023, following the application of feedstock and biochar produced using a kiln.

Variate: Total_N_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.0002889	0.0001444	0.46	
Rep.*Units* stratum					
Treatment	8	0.0515333	0.0064417	20.43	<.001
Residual	16	0.0050444	0.0003153		

Appendix BG: ANOVA results for soil total N under field conditions during the winter season of 2023, following the cumulative application of feedstock and kiln-produced biochar.

Variate: Total_N_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.03950	0.01975	1.09	
Rep.*Units* stratum					
Treatment	8	0.14390	0.01799	1.00	0.475
Residual	16	0.28904	0.01806		

Appendix BH: ANOVA results for soil total N under field conditions during the winter season of 2023, evaluating the residual effects of feedstock and kiln-produced biochar.

Variate: Total_N_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.00014074	0.00007037	0.71	
Rep.*Units* stratum					
Treatment	8	0.04682963	0.00585370	58.81	<.001
Residual	16	0.00159259	0.00009954		

Appendix BI: ANOVA results for acid phosphomonoesterase activity under field conditions during the summer season of 2023, following the application of feedstock and biochar produced using a kiln.

Variate: Acid_Phosphomonoesterase_Activity_ug_g_D

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	5.	2.	0.00	
Rep.*Units* stratum					
Treatment	8	1110690.	138836.	21.65	<.001
Residual	70	448836.	6412.		

Appendix BJ: ANOVA results for acid phosphomoesterase activity under field conditions during the winter season of 2023, following the cumulative application of feedstock and kiln-produced biochar.

Variate: Acid_Phosphomonoesterase_Activity_ug_g_D

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	6.980	3.490	1.54	
Rep.*Units* stratum					
Treatment	8	636368.367	79546.046	35000.32	<.001
Residual	16	36.364	2.273		

Appendix BK: ANOVA results for acid phosphomonoesterase activity under field conditions during the winter season of 2023, evaluating the residual effects of feedstock and kiln-produced biochar.

Variate: Acid_Phosphomonoesterase_Activity_ug_g_D

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.436E-01	7.178E-02	0.29	
Rep.*Units* stratum					
Treatment	8	1.608E+05	2.010E+04	82032.03	<.001
Residual	16	3.920E+00	2.450E-01		

Appendix BL: ANOVA results for alkaline phosphomonoesterase activity under field conditions during the summer season of 2023, following the application of feedstock and biochar produced using a kiln.

Variate: Alkaline_Phosphomonoesterase_Activity_ug

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	20.3	10.1	0.03	
Rep.*Units* stratum					
Treatment	8	1012404.2	126550.5	334.65	<.001
Residual	70	26470.8	378.2		

Appendix BM: ANOVA results for alkaline phosphomonoesterase activity under field conditions during the winter season of 2023, following the cumulative application of feedstock and kiln-produced biochar.

Variate: Alkaline_Phosphomoesterase_Activity_ug

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	8.154E-01	4.077E-01	0.45	
Rep.*Units* stratum					
Treatment	8	3.498E+05	4.373E+04	48364.78	<.001
Residual	16	1.447E+01	9.041E-01		

Appendix BN: ANOVA results for alkaline phosphomonoesterase activity under field conditions during the winter season of 2023, evaluating the residual effects of feedstock and kiln-produced biochar.

Variate: Alkaline_Phosphomonesterase_Activity_ug

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	9.844	4.922	2.52	
Rep.*Units* stratum					
Treatment	8	331801.036	41475.130	21213.79	<.001
Residual	16	31.282	1.955		

Appendix BO: ANOVA results for urease activity under field conditions during the summer season of 2023, following the application of feedstock and biochar produced using a kiln.

Variate: Urease_Activity_ug_g_DWt_2hr

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	2.24	1.12	0.01	
Rep.*Units* stratum					
Treatment	8	7357.20	919.65	12.15	<.001
Residual	70	5300.39	75.72		

Appendix BP: ANOVA results for urease activity under field conditions during the winter season of 2023, following the cumulative application of feedstock and kiln-produced biochar.

Variate: Urease_Activity_ug_g_DWt_2hr

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.4739	0.2369	1.31	
Rep.*Units* stratum					
Treatment	8	3592.5845	449.0731	2475.40	<.001
Residual	16	2.9026	0.1814		

Appendix BQ: ANOVA results for urease activity under field conditions during the winter season of 2023, evaluating the residual effects of feedstock and kiln-produced biochar.

Variate: Urease_Activity_ug_g_DWt_2hr

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.4666	0.7333	3.10	
Rep.*Units* stratum					
Treatment	8	679.0945	84.8868	358.37	<.001
Residual	16	3.7899	0.2369		

Appendix BR: ANOVA results for spinach LAI under field conditions during the summer season of 2023, following the application of feedstock and biochar produced using a kiln.

Variate: LAI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.06538	0.03269	0.76	
Rep.*Units* stratum					
Treatment	8	11.33628	1.41703	33.03	<.001
Residual	16	0.68643	0.04290		

Appendix BS: ANOVA results for spinach LAI under field conditions during the winter season of 2023, following the cumulative application of feedstock and kiln-produced biochar.

Variate: LAI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.09951	0.04976	1.20	
Rep.*Units* stratum					
Treatment	8	12.45033	1.55629	37.54	<.001
Residual	16	0.66333	0.04146		

Appendix BT: ANOVA results for spinach LAI under field conditions during the winter season of 2023, evaluating the residual effects of feedstock and kiln-produced biochar.

Variate: LAI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.11324	0.05662	1.21	
Rep.*Units* stratum					
Treatment	8	13.22332	1.65291	35.25	<.001
Residual	16	0.75019	0.04689		

Appendix BU: ANOVA results for spinach root dry weight under field conditions during the summer season of 2023, following the application of feedstock and biochar produced using a kiln.

Variate: Root_Dry_weight_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	4431.	2216.	0.83	
Rep.*Units* stratum					
Treatment	8	194126.	24266.	9.14	<.001
Residual	16	42499.	2656.		

Appendix BV: ANOVA results for spinach root dry weight under field conditions during the winter season of 2023, following the cumulative application of feedstock and kiln-produced biochar.

Variate: Root_Dry_weight_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	3577.	1789.	0.36	
Rep.*Units* stratum					
Treatment	8	191379.	23922.	4.81	0.004
Residual	16	79618.	4976.		

Appendix BW: ANOVA results for spinach root dry weight under field conditions during the winter season of 2023, evaluating the residual effects of feedstock and kiln-produced biochar.

Variate: Root_Dry_weight_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	5766.	2883.	0.88	
Rep.*Units* stratum					
Treatment	8	63585.	7948.	2.43	0.063
Residual	16	52427.	3277.		

Appendix BX: ANOVA results for spinach shoot dry weight under field conditions during the summer season of 2023, following the application of feedstock and biochar produced using a kiln.

Variate: Shoot_Dry_matter_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	78157.	39078.	1.22	
Rep.*Units* stratum					
Treatment	8	5393112.	674139.	21.01	<.001
Residual	16	513479.	32092.		

Appendix BY: ANOVA results for spinach shoot dry weight under field conditions during the winter season of 2023, following the cumulative application of feedstock and kiln-produced biochar.

Variate: Shoot_Dry_matter_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	965.	482.	0.28	
Rep.*Units* stratum					
Treatment	8	4257524.	532191.	309.67	<.001
Residual	16	27497.	1719.		

Appendix BZ: ANOVA results for spinach shoot dry weight under field conditions during the winter season of 2023, evaluating the residual effects of feedstock and kiln-produced biochar

Variate: Shoot_Dry_matter_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	3783.	1891.	1.81	
Rep.*Units* stratum					
Treatment	8	4457026.	557128.	534.30	<.001
Residual	16	16684.	1043.		

Appendix CA: ANOVA results for spinach Ca uptake under field conditions during the summer season of 2023, following the application of feedstock and biochar produced using a kiln.

Variate: Ca_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	16.276	8.138	1.57	
Rep.*Units* stratum					
Treatment	8	1802.095	225.262	43.55	<.001
Residual	16	82.753	5.172		

Appendix CB: ANOVA results for spinach Ca uptake under field conditions during the winter season of 2023, following the cumulative application of feedstock and kiln-produced biochar.

Variate: Ca_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	14.432	7.216	0.99	
Rep.*Units* stratum					
Treatment	8	15830.275	1978.784	270.19	<.001
Residual	16	117.177	7.324		

Appendix CC: ANOVA results for spinach Ca uptake under field conditions during the winter season of 2023, evaluating the residual effects of feedstock and kiln-produced biochar

Variate: Ca_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	7.878	3.939	0.80	
Rep.*Units* stratum					
Treatment	8	6464.627	808.078	163.53	<.001
Residual	16	79.063	4.941		

Appendix CD: ANOVA results for spinach K uptake under field conditions during the summer season of 2023, following the application of feedstock and biochar produced using a kiln.

Variate: K_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	356.8	178.4	1.13	
Rep.*Units* stratum					
Treatment	8	26354.8	3294.4	20.89	<.001
Residual	16	2523.5	157.7		

Appendix CE: ANOVA results for spinach K uptake under field conditions during the winter season of 2023, following the cumulative application of feedstock and kiln-produced biochar.

Variate: K_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.3528	0.1764	0.37	
Rep.*Units* stratum					
Treatment	8	1392.9823	174.1228	368.39	<.001
Residual	16	7.5626	0.4727		

Appendix CF: ANOVA results for spinach K uptake under field conditions during the winter season of 2023, evaluating the residual effects of feedstock and kiln-produced biochar.

Variate: K_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	34.90	17.45	0.71	
Rep.*Units* stratum					
Treatment	8	800.27	100.03	4.08	0.008
Residual	16	391.81	24.49		

Appendix CG: ANOVA results for spinach Mg uptake under field conditions during the summer season of 2023, following the application of feedstock and biochar produced using a kiln.

Variate: Mg_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	8.947	4.473	1.02	
Rep.*Units* stratum					
Treatment	8	3601.465	450.183	102.31	<.001
Residual	16	70.402	4.400		

Appendix CH: ANOVA results for spinach Mg uptake under field conditions during the winter season of 2023, following the cumulative application of feedstock and kiln-produced biochar.

Variate: Mg_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.10747	0.05374	0.75	
Rep.*Units* stratum					
Treatment	8	117.79614	14.72452	205.87	<.001
Residual	16	1.14436	0.07152		

Appendix CI: ANOVA results for spinach Mg uptake under field conditions during the winter season of 2023, evaluating the residual effects of feedstock and kiln-produced biochar.

Variate: Mg_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.13407	0.06703	1.87	
Rep.*Units* stratum					
Treatment	8	82.95615	10.36952	288.50	<.001
Residual	16	0.57509	0.03594		

Appendix CJ: ANOVA results for spinach N uptake under field conditions during the summer season of 2023, following the application of feedstock and biochar produced using a kiln.

Variate: N_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	145.37	72.68	0.91	
Rep.*Units* stratum					
Treatment	8	25924.35	3240.54	40.72	<.001
Residual	16	1273.44	79.59		

Appendix CK: ANOVA results for spinach K uptake under field conditions during the winter season of 2023, following the cumulative application of feedstock and kiln-produced biochar.

Variate: N_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	5.707	2.854	0.43	
Rep.*Units* stratum					
Treatment	8	9440.460	1180.058	175.78	<.001
Residual	16	107.413	6.713		

Appendix CL: ANOVA results for spinach N uptake under field conditions during the winter season of 2023, evaluating the residual effects of feedstock and kiln-produced biochar

Variate: N_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.0879	0.5439	0.59	
Rep.*Units* stratum					
Treatment	8	7742.7655	967.8457	1048.26	<.001
Residual	16	14.7726	0.9233		

Appendix CM: ANOVA results for spinach P uptake under field conditions during the summer season of 2023, following the application of feedstock and biochar produced using a kiln.

Variate: P_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.4899	0.2450	0.46	
Rep.*Units* stratum					
Treatment	8	229.9596	28.7450	54.16	<.001
Residual	16	8.4917	0.5307		

Appendix CN: ANOVA results for spinach P uptake under field conditions during the winter season of 2023, following the cumulative application of feedstock and kiln-produced biochar.

Variate: P_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	3.315	1.657	1.01	
Rep.*Units* stratum					
Treatment	8	677.891	84.736	51.56	<.001
Residual	16	26.295	1.643		

Appendix CO: ANOVA results for spinach P uptake under field conditions during the winter season of 2023, evaluating the residual effects of feedstock and kiln-produced biochar

Variate: P_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.1294	0.0647	0.29	
Rep.*Units* stratum					
Treatment	8	457.2126	57.1516	258.85	<.001
Residual	16	3.5327	0.2208		

Appendix CP: ANOVA results for spinach S uptake under field conditions during the summer season of 2023, following the application of feedstock and biochar produced using a kiln.

Variate: S_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.3603	0.1802	0.30	
Rep.*Units* stratum					
Treatment	8	210.0056	26.2507	43.25	<.001
Residual	16	9.7107	0.6069		

Appendix CQ: ANOVA results for spinach shoot uptake under field conditions during the winter season of 2023, following the cumulative application of feedstock and kiln-produced biochar.

Variate: S_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.04315	0.02158	0.37	
Rep.*Units* stratum					
Treatment	8	123.58770	15.44846	264.93	<.001
Residual	16	0.93298	0.05831		

Appendix CR: ANOVA results for spinach S uptake under field conditions during the winter season of 2023, evaluating the residual effects of feedstock and kiln-produced biochar.

Variate: S_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.08111	0.04055	1.86	
Rep.*Units* stratum					
Treatment	8	90.65246	11.33156	519.65	<.001
Residual	16	0.34889	0.02181		

Appendix CS: ANOVA results for spinach Cu tissue concentration under field conditions during the summer season of 2023, following the application of feedstock and biochar produced using a kiln.

Variate: Cu_mg_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.1157	0.0579	0.14	
Rep.*Units* stratum					
Treatment	8	225.6832	28.2104	69.01	<.001
Residual	16	6.5404	0.4088		

Appendix CT: ANOVA results for spinach Cu tissue concentration under field conditions during the winter season of 2023, following the cumulative application of feedstock and kiln-produced biochar.

Variate: Cu_mg_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	4.0064	2.0032	5.46	
Rep.*Units* stratum					
Treatment	8	214.6556	26.8320	73.20	<.001
Residual	16	5.8650	0.3666		

Appendix CU: ANOVA results for spinach Cu tissue concentration under field conditions during the winter season of 2023, evaluating the residual effects of feedstock and kiln-produced biochar.

Variate: Cu_mg_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.8996	0.9498	1.33	
Rep.*Units* stratum					
Treatment	8	210.5050	26.3131	36.83	<.001
Residual	16	11.4311	0.7144		

Appendix CV: ANOVA results for spinach Fe tissue concentration under field conditions during the summer season of 2023, following the application of feedstock and biochar produced using a kiln.

Variate: Fe_mg_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	242.	121.	0.09	
Rep.*Units* stratum					
Treatment	8	235962.	29495.	21.04	<.001
Residual	16	22429.	1402.		

Appendix CW: ANOVA results for spinach Fe tissue concentration under field conditions during the winter season of 2023, following the cumulative application of feedstock and kiln-produced biochar.

Variate: Fe_mg_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	483.75	241.88	7.28	
Rep.*Units* stratum					
Treatment	8	194670.76	24333.85	732.79	<.001
Residual	16	531.31	33.21		

Appendix CX: ANOVA results for spinach Fe tissue concentration under field conditions during the winter season of 2023, evaluating the residual effects of feedstock and kiln-produced biochar.

Variate: Fe_mg_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	13.25	6.63	0.37	
Rep.*Units* stratum					
Treatment	8	162733.80	20341.72	1130.52	<.001
Residual	16	287.89	17.99		

Appendix CY: ANOVA results for spinach Mn concentration under field conditions during the summer season of 2023, following the application of feedstock and biochar produced using a kiln.

Variate: Mn_mg_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	7458.	3729.	1.17	
Rep.*Units* stratum					
Treatment	8	527002.	65875.	20.69	<.001
Residual	16	50947.	3184.		

Appendix CZ: ANOVA results for spinach Mn tissue concentration under field conditions during the winter season of 2023, following the cumulative application of feedstock and kiln-produced biochar.

Variate: Mn_mg_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	880.2	440.1	2.88	
Rep.*Units* stratum					
Treatment	8	413269.7	51658.7	337.96	<.001
Residual	16	2445.7	152.9		

Appendix DA: ANOVA results for spinach Mn tissue under field conditions during the winter season of 2023, evaluating the residual effects of feedstock and kiln-produced biochar.

Variate: Mn_mg_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1579.5	789.7	5.10	
Rep.*Units* stratum					
Treatment	8	304532.8	38066.6	246.05	<.001
Residual	16	2475.4	154.7		

Appendix DB: ANOVA results for spinach Zn tissue concentration under field conditions during the summer season of 2023, following the application of feedstock and biochar produced using a kiln.

Variate: Zn_mg_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	138.60	69.30	1.36	
Rep.*Units* stratum					
Treatment	8	59103.87	7387.98	144.61	<.001
Residual	16	817.45	51.09		

Appendix DC: ANOVA results for spinach Zn tissue concentration under field conditions during the winter season of 2023, following the cumulative application of feedstock and kiln-produced biochar.

Variate: Zn_mg_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	90.80	45.40	1.16	
Rep.*Units* stratum					
Treatment	8	79431.94	9928.99	253.32	<.001
Residual	16	627.12	39.19		

Appendix DD: ANOVA results for spinach Zn tissue concentration under field conditions during the winter season of 2023, evaluating the residual effects of feedstock and kiln-produced biochar.

Variate: Zn_mg_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	302.7	151.4	0.38	
Rep.*Units* stratum					
Treatment	8	70618.8	8827.4	22.38	<.001
Residual	16	6312.1	394.5		