ASSESSING DECOMPOSITION AND SOIL CARBON SEQUESTRATION POTENTIAL OF SORGHUM RESIDUES FROM DIFFERENT CULTIVARS

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PREFACE

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ABSTRACT

Decomposition of crop residues may affect soil organic carbon (C) stocks, which are key for soil fertility improvement and mitigation of climate change. Numerous studies focus on decomposition from a single crop type and have given little attention to the difference between crop cultivars and/or plant parts. This opens a gap to conduct a comprehensive analysis of the impact of crop types, cultivars, and their plant parts on residues decomposition in soils. Data from 394 trials from across the world were used to assess cumulative CO2 emissions from residues of 17 crops during 0-30, 30-90, and 90-120 days (i.e., CR30, CR90, and CR120; 1-(CR30/120) ratio as C stability index of emission) of decomposition in a meta-study. In a separate incubation study, ground (<2mm) root and shoot residues (0.25g; n=3) from five sorghum cultivars (AS8, KZ5246, LP4403, OS-POTCH, and MAMOLOKWANE) were mixed with 100 g of soil in a 500 ml sealed plastic container and incubated at 25°C and 50% soil field capacity. The emitted CO₂-C was trapped in NaOH, and measurements were made at days 0, 7, 14, 28, 42, 56, 84, and 120. The amount of NH_4^+ -N and NO_3^- -N release were also analyzed using a Thermo Scientific Gallery Discrete Autoanalyzer. The decomposition potential of these sorghum cultivars was further assessed based on the percentage weight of residues remaining (DM) from buried litterbags, after 0, 14, 28, 42, 56, 84, 112, and 168 days in a field study. These were then analyzed for total C and N concentrations, using the LECO Trumac auto-analyzer, and lignin concentration using the ANKOM-200 method. Residue quality (i.e., C, N, and lignin concentrations) and/or selected soil properties (texture, pH, soil organic carbon concentration), and climatic conditions (temperature and precipitation) were considered as influencing factors of residue decomposition. Results of the meta-study showed that at all times, leguminous crops exhibited the highest CO₂ emissions per gram of C added (1003 mg CO₂-C g⁻¹C after 120 days) followed by grasses (947), oil crops (944), and cereals (846), with the legumes and grasses showing the lowest temporal C stability index of emissions. Maize residues however, emitted the least CO₂ (86 at day 30, 275 at 90, and 495 mg CO₂-C g⁻¹C at day 120), followed by two other lignin-rich crops (cotton and sunflower), while the highest emissions were from alfalfa residues. In the incubation experiment, emissions varied between 7.8 and 45 mg CO₂-C kg⁻¹ of the initial added C in roots and between 5.9 and $49.9 \text{ mg CO}_2\text{-C kg}^{-1}$ added C in shoots for OS-POTCH and KZ5246 residues, respectively (120 days). While over time, N mineralization decreased, with consistently higher N releases in shoots compared to root residues. In the litterbag experiment, the DM remaining of all five sorghum residue treatments decreased with time. By day 42, >50% of the initial DM had

decomposed in high-quality residues (i.e., OS-POTCH and MAMOLOKWANE); while at the end of the incubation (Day 168), less than 12.5% of residue DM remained in all treatments. The global trend pointed to decreased CO₂ emissions with increasing residue lignin. Contrary to what is generally believed, providing the soil with high lignin and high N concentration residues may foster C stabilization into soils by soil microbes. The shoot residues (i.e., OS-POTCH) tend to have more available N for plants uptake, while root residues (i.e., KZ5246) can be more commendable for N and C stocks to the soil. Overall, maize and root parts of KZ5246, and AS8 sorghum residues would be useful for retaining C and N balance in soils.

Keywords: Crop residue decomposition, Residue quality, C mineralization, N mineralization, Soil C stocks, C sequestration, C emissions, Soil carbon stabilisation.

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THESIS OUTLINE

This thesis consists of five chapters. Chapter one is the general introduction, which introduces the reader to the background and justification of the study. Chapter two is a meta-analysis conducted to evaluate the decomposition dynamics and soil carbon building potential from different crop residues, using results from available studies across the world. Chapter three is based on a laboratory incubation study (under controlled conditions), conducted to determine CO₂ emission and nitrogen mineralization from the root and shoot residues of different sorghum cultivars in soil. Chapter four is based on a field litterbag study, which focused on assessing the decomposition of root and shoot residues of different sorghum cultivars in a wheat field in Kwazulu-Natal, in South Africa. Lastly, chapter five is the general discussion and conclusion of the study, which includes the recommendations based on the findings.

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1. CHAPTER ONE:

GENERAL INTRODUCTION

1.1. Background and Justification

Global climate change has attracted unprecedented attention in recent years, due to concerns over the increase in mean temperature, and higher variability in precipitation and soil degradation, with an effect of declining crop productivity. High atmospheric carbon dioxide (CO₂) concentration is recognized as a major contributor to climate change. While the CO₂ comes from many sources, decomposition of agricultural wastes and soil organic matter makes a major contribution to the atmospheric carbon (C) (Anwar et al., 2019). On the other hand, the loss of a substantial proportion of soil organic carbon (SOC) results in soil degradation causing a reduction to ecosystem functions and services (i.e., loss of aggregate stability, declining soil fertility, poor water retention capacity, loss of soil biodiversity and poor productivity) (Lal, 2015). Thus, there is need to store more SOC to minimize its contribution to greenhouse gas emissions (GHG) and soil degradation. Crop residue retention either on the surface or below ground has been used for improving SOC.

Previous studies have shown that about 3.8 billion tons of crop residues are produced annually on a global scale, with a potential total amount of 0.6-1.2 Pg of C that can be sequestered through crop residue retention into the soil (Xu et al., 2017; Thangarajan et al., 2013; Lal, 2009). Crop residues can be retained in soils after harvest through surface litter or incorporation through tillage and root turnover and/or exudation (Mota et al., 2010; Menichetti et al., 2015). Plants direct between 40 to 60% of photosynthetically fixed C to roots and associated microorganisms (Keiluweit et al., 2015). Hence, Lynch et al. (2016) observed a 68 % average rate of residue C loss for surface-placed residue, with 86 % for buried residues. Higher C loss in buried residues could be due to greater contact with soil microbial biomass (Lynch et al., 2016). Moreover, straw retention under no-till was found to sequester more SOC (57 \pm 14 g Cm⁻² year⁻¹) than conventional intensive tillage (West and Post, 2002). However, the C and N dynamics in the soil can be influenced by crop type (i.e., cultivars) and crop parts

decomposability, which are significantly varied with residue biomass production and chemical composition (Ma and Petersen, 2003).

Tropical C4 cereal crops (i.e., sorghum, maize, and rice) have been observed to accumulate high biomass production due to their higher photosynthetic ability and water stress tolerance than temperate crops (Wright and Hons, 2005; and Brauteseth, 2009; Sheahan, 2014; Mathew et al., 2017). In support, Zengeni et al. (2021) observed high biomass accumulation (both shoot and root) in sorghum under drought stress conditions compared to the other crops which can be attributed to the inherent drought tolerance capability of sorghum rendered by an extensive root system (Anderson-Teixeira et al., 2013). Whereas the range of sorghum root biomass found in Zengeni et al. (2021) study differed from the range of 97 to 1176 g m⁻² reported by Waines et al. (2012) under greenhouse conditions but confirmed the existence of genotypic variation. Differences in performance among genotypes reflects genetic diversity (Bhutta et al., 2006), which is a consequence of variable genetic background of the genotypes. This agreed with Akman et al. (2017), who reported significant genotypic variation among 47 wheat genotypes evaluated under field conditions in Turkey.

On the other hand, Poorter et al. (2012) indicated that significant C concentration is determined at species level rather than genotype. Hence, the lack of significant variations in rate of decomposition between the residues of BT-maize and near-isogenic non-Bt-maize observed by Daudu et al. (2009); and Al-Kaisi, and Guzman (2013) was attributed to lack of significant differences in the chemical composition of residues. However, Murungu et al. (2011) found cereal cover crops, such as oats and rye grass to produce high biomass with large C and N concentrations and have potential to improve soil organic matter (SOM). In support, Mathew et al. (2017) found maize to have the highest capability to capture plant C stocks (6.3+/- 0.34 Mg C/ha⁻¹ per year) amongst the summer crops, with wheat having higher plant C stocks (2.2+/- 0.35 Mg C/ha⁻¹ per year) amongst winter crops. Moreover, crop residues can differ in the ways they use and/or circulate C and N in soils, due to their biochemical variation. Residues with a higher initial N concentration and lower C: N ratio can accelerate the initial C mineralization in comparison to residues with low N concentrations and high C/N ratio (Li et al., 2013). Murungu et al. (2011), observed that a largest proportion of significant difference in degradation rate constants of the residues was explained by the material quality parameters,

precisely the C: N ratio. The C: N ratio of residues is commonly acknowledged as the main factor affecting microbial mineralization of N in crop residues (Ernst et al., 2009), while recalcitrant lignin tissue can shield N from mineralization by chemical binding or physical isolation (Xu et al., 2017). Hence, Li et al. (2013) found soyabean residues to decompose more easily, with high C mineralization due to their high N concentration, and low C: N ratio compared to maize residues.

At the end of the experiment, Xu et al. (2017) found soyabean residues to have retained less C (37%) of the initial C added than maize (40%) and wheat (43% at P < 0.001). The N remaining was 69%, 73%, and 78% of the initial concentration for maize, soyabean, and wheat residues, respectively, after 12 months of decomposition, Cellulose and hemicellulose linked to lignin were found to be the major biopolymers encrusting carbohydrates from microbial degradation and providing resistance to residue decomposition (Xu et al., 2017). Pascault et al. (2010) explained the rapidly induced peak of C-CO₂ production (4.02±1.14 and 4.85±2.34 µg CO₂-C g⁻¹ soil h⁻¹) after the addition of alfalfa and rape residues to their low C: N ratio compared to lower C-CO₂ emitted by wheat straw (0.083 g⁻¹ soil h⁻¹) with higher C: N ratio, after a day of residues incorporation. Muhammad (2011) observed higher CO₂ emissions for sugarcane, followed by maize, and sorghum (despite their high C: N ratios, ranging 57-140) compared to cotton residues. This was attributed to a higher concentration of lignin (21%) and fiber (60%) in cotton, compared to a lower lignin (5.8–8.3%) and moderate fiber contents (48-52%) in the other materials (Muhammad, 2011). Overall, crop residues with great amounts of easily degradable C or N can increase microbial activity, leading to enhanced SOC losses (Xu et al., 2017). These observations were attributed to variation in the biochemical composition of different crops. In the same way, the variations between crop parts could be explained by differences in biochemical make-up between shoots and root tissues.

In a 112 day litterbag experiment by Daudu et al. (2009), significant variations in decomposition rate (across all parameters monitored) were observed between leaf and stem residues of BT-maize cultivars and of near-isogenic non-Bt maize cultivars. The higher proportion of faster decomposition observed in leaf residues of BT-maize was attributed to lower lignin: N (3.9) and C: N (18.1) ratios as opposed to stem residues (lignin: N, 7.2; C: N,

37.9). Roots are known to have a higher concentration of phenolic and ligneous compounds, and also the guaiacyl units, which form recalcitrant polymers of condensed aryl-aryl lignin linkages, as a result, they are resistant to decomposition (Bahri et al., 2006; Lu, et al., 2003). Moreover, their interaction with soils (i.e., aggregate formation) provides physical protection of the organic C from biodegradation (Puget and Drinkwater, 2001). Cereal crops were found to have heavier and longer root systems than the leguminous crops, making them better at amassing more C in root parts (Gregory et al., 1995). Shahbaz et al. (2018) observed a higher cumulative amount of primed SOM-C under root residue addition (365±22 μg C g⁻¹) than the shoots (261±10 μg C g⁻¹ 328). Moreover, the increase in substrate complexities (i.e., decreasing labile C availability), may lead to microbes initiating SOM mining, resulting in a positive priming effect (Shahbaz et al., 2018).

Moreover, but not surpassing the biochemical composition factor of influence in decomposability of plant materials, the rate of decomposition can also integrate the effect of the environment, such as soil temperature and precipitation (Murungu et al., 2011; Ruffo and Bollero 2003). Hu et al. (1999), presented 45.3% of mass loss in oats residues under buried litterbag study in 19 days, which was more rapid in decomposition as opposed to the surface placed oats material (20% mass loss) by Murungu et al (2011), after 20 days. The high decomposition in soil contact residues can be explained by soil micro-and macro-fauna activity, resulting in higher rate of decomposition (Hu, 1999). Whereas the early degradation of maize residues observed by Daudu et al. (2009) was attributed to micro-fauna activity, excluding the macro-faunal effect which might have limited accessibility to nylon litterbags pores, since the residues were not in direct contact with soil. Also, the temperature and moisture differences (including drying and wetting cycles) between the studies could be responsible for contrasting results (Daudu et al., 2009). In addition, soils with high clay concentration may enable the formation of micro-aggregates to further protect SOC, with the stability of SOC being attributed to the adsorption of C to clay and/or silt particles (Lemus and Lal, 2005).

Sub-Saharan Africa is dominated by arid and semi-arid areas, where communal farmers rely upon rain-fed agriculture but cannot afford the expensive mineral fertilizers for sustainable crop production and soil fertility management (Wang et al., 2018). Sorghum is considered as an important strategic crop amongst others (i.e., wheat, rice, barley, and maize), due to its lower

agronomic input requirements (i.e., irrigation and fertilizers supply). Its C4 photosynthesis system and drought tolerance (narrower stomata opening) can also help in SOM accumulation. These subsequently result in great SOC stock potential, owing to its biochemical properties and biomass production (Prakasham et al., 2014). Hence, different sorghum cultivars have been bred for different objectives including biomass production (i.e., source of food, fodder, feedstock, and SOM), wide adaptability i.e., drought resistance, nutrient, and water use efficiency and also, for economic development (renewable source of energy) among other traits (Prakasham et al., 2014). Thus, genetic manipulation of the morphologically diverse sorghum (grain sorghum, dual-purpose sorghum, and forage sorghum) has resulted in the recent development of improved cultivars such as sweet sorghum and high-biomass, and low lignin sorghum (brown midrib sorghum) (Prakasham et al., 2014). Despite the existing literature on the above and below-ground litter decomposition of different crops, the impacts of sorghum cultivars and their parts on SOC storage and nutrient availability in soils are not well understood. Hence, the current study is aiming at characterizing the root and shoot residues of different sorghum cultivars, based on their biomass production and biochemical properties.

Overally, this study seeks to determine the impacts of crop biochemical composition, and environmental factors on decomposition potential, which might have a great influence on carbon loss or carbon stock in the soil. This was done by a collection of available global data from published studies (meta-analysis study). Moreover, carbon (C) and nitrogen (N) release patterns, through decomposition and mineralization from roots and shoot residues of different sorghum cultivars, were used as a proxy for evaluating soil C stock. These were conducted under a controlled laboratory incubation study (confound environmental factors) and also, in a growing-wheat field litterbag study. The aim of these studies was to select a crop type, and the superior cultivar and crop part (i.e., roots or shoots) of sorghum that has greater positive contributions to carbon sequestration and available N.

1.1. Research questions

- a) Do different crop types vary in ability to influence residue decomposition potential?
- b) Do different sorghum cultivars vary in ability to decompose and sequester soil C stocks?
- c) Do shoots and roots of sorghum cultivars vary in ability to decompose and sequester soil C stocks and release nutrients?

1.2. Specific Objectives

- a) To evaluate the impact of crop type, selected soil properties, and environmental factors on residue decomposition potential, using available global data from published studies.
- **b)** To determine CO₂ emission and nitrogen mineralization through decomposition potential of root and shoot residues from different sorghum cultivars in the soil, under controlled conditions.
- c) To assess the decomposition patterns of shoot versus root residues of different sorghum cultivars, under field conditions.

1.3. Hypotheses

- i) Different crop types, soil and environmental factors will have a significant impact on decomposition and CO₂ emissions.
- ii) Root and shoot residues of different sorghum cultivars will differ in CO₂ emission and nitrogen mineralization, under controlled conditions.
- iii) Root and shoot residues of different sorghum cultivars will differ in decomposition patterns under field conditions.

2. CHAPTER TWO:

CROP RESIDUES DIFFER IN THEIR DECOMPOSITION DYNAMICS:

META-ANALYSIS OF AVAILABLE DATA FROM WORLD

LITERATURE

Abstract

Decomposition of crop residues may affect soil organic carbon (C) stocks, which are key for soil fertility and mitigation of climate change. Numerous independent studies across the world point to different parameters and results but their existence provides an opportunity to conduct a comprehensive analysis of the impact of crop type on residue decomposition. In the present study, data from 394 trials from across the world were used to assess cumulative CO₂ emissions from residues of 17 crops during 0-30, 0-90 and 0-120 days (i.e., C_R 30, C_R90 and C_R120, 1-[C_R30/120] ratio as a stability index of C emission) and to relate the results with residues quality (C, N and lignin concentration) and selected soil properties (texture, pH, soi organic carbon concentration). At all durations, legumes exhibited the highest CO₂ emission per gram of C added (1003 mg CO₂-C g⁻¹C after 120 days) followed by grasses (947), oilseed crops (944) and cereals (846), with the legumes and grasses showing the lowest temporal stability of C emission as pointed out by a 1-[C_R30/120] of 0.78 and 0.79, respectively, versus 0.82 and 083 for cereals and oilseed crops. At all durations, maize residues emitted the least C-CO₂-C (86, 275 and 495 mg CO₂-C g⁻¹C), followed by two other lignin rich crops (cotton and sunflower), while the highest emissions were from alfalfa residues that produced about 4 times more CO₂ (i.e., 359 at C_R30 and 1319 at C_R120) than maize. Overall, CO₂ emissions were positively correlated with soil clay concentration (r > 0.22), residues C concentration (e.g., r = 0.46 at $C_R 90$ and r =0.37 with emission stability, P<0.05) but negatively to residue N concentration (r = -0.26 at C_R120, P<0.05). The global trend pointed to decreased CO₂ emissions with increasing residue lignin. Contrary to what is generally believed, providing the soil with high lignin and high N concentration may limit priming and foster C stabilization into soils by microbes.

Keywords: Crop residue decomposition, C emissions, Soil carbon stabilisation, Soil C stocks

2.1. Introduction

The carbon (C) cycle has received considerable attention in recent years, due to concerns over the continued increase in atmospheric carbon dioxide (CO₂) concentration. Annual increases of CO₂ concentration in the atmosphere were observed on a global scale for the years 2018 (2.7%) and 2019 (0.6%) (Ritchie and Roser, 2020). Since the 10,000 BCE, South Africa is thought to have lost 2 Pg C (going from 15.8 to 13.8 Pg C), largely due to land use changes (Venter et al., 2021). While sub-Saharan Africa's (SSA) CO₂ emissions were 0.822 gigatons (GT) compared to the 36.5 GT for the world in the year 2014. The forecasted trends of SSA's CO₂ emissions showed emissions of 0.925 GT in 2020, which is expected to further increase in 2027 (0.930 GT) (Ritchie and Roser, 2020; Lin and Agyeman, 2020; WDI and EIA database). Global warming, as a result of high atmospheric CO₂ causes climate change, characterized by increase in mean temperature and higher variability in precipitation. In SSA, both low rainfall and high temperatures curtail plant productivity and biomass production and reduce the amount of residue retained in soils. Consequently, the need to sequester carbon in agricultural soils has been identified as a sustainable strategy to mitigate climate change and promote agricultural sustainability.

Soil is the second largest C reservoir with 11% (4655 Pg C), after the 87% of global carbon stocks being oceanic (38,000 Pg C), and has a higher C than the 2% found in the atmosphere (860 Pg C) (Xiao, 2015; Venter et al., 2021). Carbon transfer from the atmosphere to soil is achieved by plants through photosynthesis, leading to exudation of C compounds from roots during the growth cycle, and by the retention of plant root and shoot residues. Some of the C from plant residues or exudates can be mineralized and emitted back to the atmosphere as CO₂ while a significant proportion can be stabilized as soil organic matter (Ontl and Schulte, 2012; Dignac, 2017). Therefore, the rate of residue decomposition and associated CO₂ emissions is often used as a proxy for evaluating the potential of plant residues to become soil organic matter, (Johnson et al., 2007).

The potential to increase soil C stocks is mostly on managed agricultural land where numerous studies exist on the fate of C from different crop residues. Curtin et al. (2008) for instance, observed that barley straw emitted significantly higher (p< 0.05) amount of CO₂ (55 g CO₂-C

m⁻²) compared to wheat straw (47 g CO₂-C m⁻²) after 158 days. Ajwa and Tabatabai (1994) found 58% of organic C evolved as CO2-C from alfalfa-treated soils in 30 days which was higher compared to maize residues (30%), which was attributed to the higher C: N of maize residues. Zeng et al. (2010) attributed the higher CO₂ emissions from peanut root (maximum of 60 %) compared to maize root residues (35%) to biological nitrogen fixation, which increases N in leguminous plants, and thus lower their C: N ratio, and overall quality of the crop residues. Not only does crop residue decomposition depend on the type and quality of crop residues but also on the internal soil conditions (Mathew, et al., 2017; Stewart et al., 2015). Mathew et al. (2017) concluded that higher plant C stocks and C transfer to soils occurred in carbon rich clayey soils of tropical humid areas due to higher biomass production potential compared to sandy soils. Clayey soils also support high C stocks through their aggregation and ability to provide physical protection as well as mineral adsorption of C constituents (Elliott, 1986; Ajwa and Tabatabai, 1994; Martens, 2000; Clark, 2007; Mathew, et al., 2017). Several studies have also reported disparities in CO₂ emissions from residues of different crops under different soil types and climates, which has led to a lack of consensus on the impact of these factors on CO₂ emissions from soils (Li et al., 1994; Paustian et al., 2000; Gregorich et al., 2001; Alvarez, 2005; Abdalla et al., 2016; McClelland et al., 2012; Shakoor et al., 2021). However, the existence of multiple individual studies across the world provides an opportunity to conduct a comprehensive analysis of the main factors that control crop residue decomposition and CO₂ emissions and the consequences for the building of soil carbon stocks. Therefore, the objective of the current study was to evaluate the impact of crop type, soil and environmental factors on CO₂ emissions from residues of selected crops using available global data from published studies.

2.2. Materials and methods

2.2.1. Study setup: Database preparation

Data on CO₂ emissions from crop residues incubated in soil at laboratories were collated from studies across the world, published in peer reviewed journals accessible from public domains such as Google scholar, Ref-seek, Science Direct, Sci-Finder, Scopus, Springer Link,

Research-Gate and Web of Science. Key words such as "litter decomposition", "residue decomposition", "C mineralized", "crop residue CO₂ emissions", "C gases", "carbon dynamics" and "decomposition rate" were used to search for relevant journal articles. The available papers had to report on CO₂ emissions from crops (sorghum, wheat, and maize among others) and on crop quality factors affecting residue decomposition (e.g., total C and N, C: N ratio, lignin, cellulose or hemicellulose). Furthermore, environmental factors such as climatic information (mean annual precipitation and mean annual temperature), geographic variables (latitude, longitude) as well as soil variables (physicochemical properties) measured during laboratory experiments were considered as controlling variables. The studies had to strictly compare CO₂ emissions between soils incubated with and without (considered as the control) residues under the same conditions. Moreover, the mass of residues used must have been clearly stated or able to be deduced. The data were used to compile a database with quantitative and qualitative data on plant litter quality.

2.2.2. Variables of CO₂ emission

The effect of adding crop residues on soil CO_2 emissions was calculated as the difference between CO_2 emitted from the soil containing residues and CO_2 evolved from the control. The values were converted from their original units and normalized to common units (mg CO_2 -C/g C added of soil over 30, 90 and 120 day periods of incubation; Table 2.2). The amounts of total CO_2 emissions were recorded over 0-30; 30-90 and 90-120 day periods and were cumulatively representing lability of residue decomposition. The studies that did not cover the 0-30, 30-90, and 90-120 periods were estimated by use of a linear trendline equation. In addition, a ratio between CO_2 emissions at 30 relative to 120 days was calculated, which was used as a stability index to deduce the changes in CO_2 emissions between these two time periods, with the use of the equation: C stability index = (1- $(C_R30/120)$), terms used in the equation are defined in Table 2.1.

Table 2.1: Definition of CO₂ emission variables used in the database.

| Variable | Units | Definition |
|-------------------|--|--|
| C _R 30 | mgCO ₂ -C g ⁻¹ C | Cumulative CO ₂ emitted from crop residues in 0-30 days |
| C _R 90 | mgCO ₂ -C g ⁻¹ C | Cumulative CO ₂ emitted from crop residues in 30-90 days |
| C_R120 | mgCO ₂ -C g ⁻¹ C | Cumulative CO ₂ emitted from crop residues in 90-120 days |
| $1-(C_R30/120)$ | | Carbon emitted at day 30 as a fraction of carbon emitted at day 120 of the same experiment |

2.2.3. Crop quality, soil and environmental factors

Crops were categorized into cereals (i.e., barley, maize, oat, rice, rye, sorghum, wheat); legumes (namely alfalfa, bean, clover, pea, soyabean, vetch); grasses (i.e., grass), and oilseeds (e.g. canola, cotton, and sunflower), (Table 2.3). Cereals and grasses were separated due to their functional differences, with cereals having evolved and undergone selection by farmers, making them different from natural or forage grasses. In this study, grasses refer to natural or forage grass that is not used for human consumption while cereal refers to monocot grasses that are used primarily for grain production for human consumption. Legumes are defined as any crop that has a natural ability to fix nitrogen, while oilseed crops are those that are primarily used for the extraction of oil from their seed. Furthermore, crop quality was defined by residue chemical composition, such as initial C (TC), and lignin concentrations, C: N ratio, total nitrogen (TN), dissolved carbon (DC), and total phosphorus (TP); cellulose, polyphenols, and lignin: N ratio. In addition, soil properties such as texture, pH (CaCl₂), and organic carbon (SOC) were considered and classified into different categories (Table 2.3), following Abdalla et al. (2016); Mutema et al. (2015) and Mathew et al. (2017). Water-based pH was converted to CaCl₂ pH following the equation of Lierop (1981): (y = 0.53 + 0.98x). Where y is pH on the $CaCl_2$ scale and x is the water-based pH.

Climatic factors included a 30-year average rainfall and temperature (Mean annual precipitation: MAP) and (Mean annual temperature: MAT). The climate was further classified as tropical (hot and wet), sub-tropical (warm and arid to humid), or temperate (cool to cold and mild to warm). In cases where climatic characteristics were not present in a particular study,

appropriate data such as annual precipitation and temperature were obtained using the location coordinates or surrogate data for nearby prominent features (e.g., town) through Google search. In addition, the geographical positioning system (GPS) using latitude and longitude coordinates was used to depict the global distribution of the studies used in the meta-analysis (Fig. 2.1).

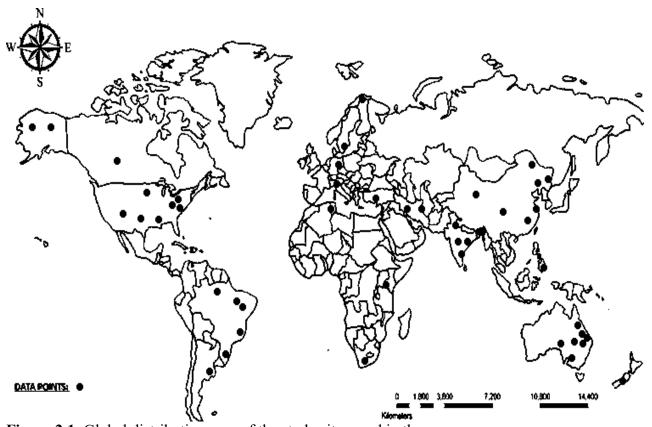


Figure 2.1: Global distribution map of the study sites used in the paper.

Table 2.2: Crop quality, soils and environmental variables classification used in the study.

| Variable | Variable remarks | Category | Symbol | Class | Ref. | | |
|---------------------------------------|---|----------------------|--------------|-----------------|----------------------------|--|--|
| | | Wheat, sorghum, | | Cereal, | | | |
| | All different crop | grass, | | Grass, | Mathew, et al. | | |
| Crop class | types categorised into four classes | vetch bean, soyabean | Crop class | Legume and | (2017) | | |
| | | Canola, cotton | | Oilseed | | | |
| Residue lignin | Lignin concentration | <10 | Lignin | Low | | | |
| concentration (%) | in crop residues | >10 | Ligiiii | High | | | |
| Residue lignin: N | Crop residue lignin | >10 | lignin: N | High | | | |
| ratio | to nitrogen ratio | <10 | | Low | | | |
| | Crop residue carbon | <20 | | Low | Puyuelo, (2011) | | |
| Residue C:N ratio | to nitrogen ratio | 20-30 | C: N | Medium | and Probert | | |
| | to merogen ratio | >30 | | High | (2005) | | |
| Residue N | Initial concentration of nitrogen found in | >10 | TN | High | | | |
| concentration (g N kg ⁻¹) | residues of different crops | <10 | - 11N | Low | | | |
| Residue total C | Initial total concentration of | >0.5 | TC | High | Abdalla et al., | | |
| added (g/kg soil) | carbon found in residues of different crops | <0.5 | TC | Low | (2016) | | |
| | | >32 | | Clayey | Mathew et al., | | |
| Clay concentration (%) | Soil Texture based on clay fraction | 20-32 | Soil texture | Silt | (2017) and Abdalla et al., | | |
| , , | | <20 | | Sandy | (2016) | | |
| Soil pH | | <5.4 | | Strongly acidic | Davies, 1971; | | |
| concentration | Soil pH | 5.5-6.4 | рH | Slightly acidic | Mathew et al. | | |
| (CaCl ₂) | Son pri | 6.5-7.4 | pii | Neutral | (2017) | | |
| (CaC12) | | >7.5 | | Alkaline | (2017) | | |
| Soil bulk density (g | Average bulk density in soil | <1.3 | BD | Low BD | Mathew et al., | | |
| cm ⁻³) | profile | >1.3 | . ББ | High BD | (2017) | | |
| CEC (11) | Soil cation exchange | >20 | CEC | Low CEC | | | |
| CEC (cmol _c kg) | capacity | <20 | CEC | High CEC | | | |
| | Based on the mean | >20 °C | MAT | Tropical | | | |
| | annual temperature | >1500 mm | MAP | Tropical | Mutema et al., | | |
| Climate | (MAT) and mean | 10-20 °C | MAT | Sub-tropical | (2015) and | | |
| Cillian | annual precipitation | 100-1110 mm | MAP | 540-Hopical | Mathew et al., | | |
| | (MAP) of the study | <10 °C | MAT | Temperate | (2017) | | |
| | site | 120-1000 mm | MAP | limporato | | | |

A total of 58 journal articles (Appendix E.1) were used, detailing different studies across the world, which provided 394 observations. The name of authors, year of paper publication, country and geographical location of experimental site, nature of experiment, experimental duration (time periods), crop(s) or crop types used in the experiments, quantitative information on plant quality, soil properties as well as C variables and environmental conditions were captured onto a database. The definitions and acronyms adopted in this paper are used to simplify the terms and definitions of variables for purposes of this analysis.

2.2.4. Data analyses

The data were compiled into a database and tested for normality of variables, absence of outliers, linearity, and homoscedasticity prior to statistical analyses. Descriptive summary statistics (minimum, maximum, median, mean, SEM: standard error of mean, 25th and 75th percentiles, skewness (Skew), kurtosis (Kurt), and coefficient of variation (CV%)) were calculated for all variables (Table 2.6). Furthermore, box plots were constructed to depict the distribution of the data showing minimum, 25th quartile median, mean, 75th quartile and maximum values within the 5 and 95th percentiles after removing the outliers (Fig. 2-9). Mean CO₂ emissions were computed for different crops, with different crop residue quality, soil properties, and environmental factor classes. Significant differences between factor classes were analyzed by use of non-parametric analysis (Kruskal-Wallis), at chi-square probability of < 0.001. In addition, bivariate Pearson correlations coefficients at p<0.05 were calculated among the variables, to determine the strength of associations between variables using Statistica 10.0 software (Table 2.7). Subsequently, principal component analysis (PCA), which converts non-linear factors and variables into linear combinations for visualization (Jambu, 1991), was conducted to investigate the multiple correlations between the variables (Fig. 2.10 and 2.11). Finally, because of low number of data points the variable of residue dissolved carbon, total residue phosphorus, cellulose and polyphenols were discarded.

2.3. Results

2.3.1. CO₂ emissions from crop residues

The summary statistics (Table 2.3) which were computed from all study sites showed that after C_R 30-days, cumulative CO_2 emissions (C_R 30) ranged between 0.3 and 920.1 mg CO_2 -C g^{-1} C, with a mean value at 196.5 mg CO_2 -C g^{-1} C. At C_R 120 days of incubation however, CO_2 emissions were 3.2-3640 mg CO_2 -C g^{-1} C and the mean was 914.2 mg CO_2 -C g^{-1} C (Table 2.3).

Table 2.3: Summary Statistics of plant, soil and environmental variables along with residue CO₂ emission in different time period.

| STATISTI CS | Lignin | C: N | TC | TN | Clay | Sand | Silt | pН | SOC | MAP | MAT | C _R 30 | C _R 90 | C _R 120 | 1- (C _R 30/1 20) |
|----------------|--------|---------|---------|--------|--------|---------|--------|---|-------|-----------|-------|-------------------|-------------------|--------------------|-----------------------------------|
| | % | | g/kg | | | % mm °C | | mg CO ₂ -C g ⁻¹ C | | | -, | | | | |
| N | 192 | 304 | 394 | 365 | 295 | 259 | 243 | 333 | 293 | 386 | 386 | 394 | 394 | 394 | 394 |
| Mean | 9,55 | 58,45 | 13,24 | 9,38 | 29,53 | 37,81 | 32,40 | 6,73 | 13,13 | 906,16 | 17,01 | 196,5 | 519,0 | 914,2 | 0,80 |
| Median | 7,70 | 39,16 | 1,15 | 4,00 | 25,30 | 32,00 | 28,00 | 6,80 | 9,50 | 656,00 | 17,00 | 138,0 | 415,6 | 795,0 | 0,80 |
| Min. | 0,02 | 7,80 | 0,04 | 0,01 | 2,00 | 1,00 | 1,00 | 3,87 | 0,50 | 89,40 | 3,90 | 0,3 | 1,1 | 3,2 | 0,49 |
| Max. | 29,40 | 409,00 | 165,76 | 407,00 | 77,30 | 96,00 | 83,00 | 8,60 | 39,00 | 2500,00 | 30,00 | 920,1 | 3205,0 | 3640,0 | 0,98 |
| Q1 | 5,35 | 20,30 | 0,40 | 0,90 | 14,30 | 17,90 | 16,30 | 5,60 | 8,41 | 551,00 | 10,00 | 31,0 | 118,4 | 200,4 | 0,75 |
| Q3 | 12,55 | 75,40 | 4,12 | 11,00 | 40,00 | 60,00 | 47,00 | 7,66 | 17,40 | 1095,00 | 24,00 | 304,9 | 858,9 | 1431,0 | 0,86 |
| Variance | 36,07 | 3257,25 | 1164,81 | 592,74 | 370,64 | 737,14 | 483,34 | 1,34 | 65,33 | 263814,21 | 46,19 | 37353,5 | 213397,5 | 585331, 4 | 0,01 |
| SD. | 6,01 | 57,07 | 34,13 | 24,35 | 19,25 | 27,15 | 21,99 | 1,16 | 8,08 | 513,63 | 6,80 | 193,3 | 461,9 | 765,1 | 0,07 |
| SE. | 0,43 | 3,27 | 1,72 | 1,27 | 1,12 | 1,69 | 1,41 | 0,06 | 0,47 | 26,14 | 0,35 | 7,7 | 18,4 | 30,5 | 0,00 |
| CV | 63,30 | 98,10 | 13,70 | 262,70 | 64,90 | 75,40 | 67,60 | 17,30 | 61,60 | 56,50 | 40,30 | 98,4 | 89,0 | 83,7 | 9.11 |
| Skewness | 1,06 | 2,52 | 2,97 | 12,47 | 0,92 | 0,51 | 0,68 | -0,30 | 1,04 | 1,15 | 0,10 | 1,07 | 1,10 | 0,73 | -0.25 |
| Kurtosis | 0,83 | 8,30 | 7,69 | 197,12 | 0,13 | -0,79 | -0,32 | -1,03 | 0,69 | 0,51 | -1,12 | 0,58 | 2,11 | -0,15 | -0.23 |

Statistics: Min and Max =minimum and maximum, respectively, Q1 and Q3= first and third quartile, SD = standard deviation, C emission variables: C_R 30; 90; 120 &1-(30/120)= residue CO_2 emission at day C_R 30; 90; 120 and 1-(30/120) ratio. Crop quality: lignin, C: N ratio; TC= total residue carbon; TN= total residue nitrogen; DC= residue dissolved carbon; TP= total residue phosphorus. Soil variables: Clay concentration; sand concentration; silt concentration; soil pH (CaCl), SOC= soil organic carbon concentration; Climatic variables: MAP= mean annual precipitation; MAT= mean annual temperature. "†" values are not zero, but rounded off to one decimal place.

Cumulative emissions also varied among different crops, with legumes exhibiting the highest emissions of 228 mg CO₂-C g⁻¹ C at C_R30, 586.7 mg CO₂-C g⁻¹ C at C_R90 and 1003 mg CO₂-C g⁻¹ C at C_R120. Grasses were second with, 217, 529.7 and 946.8 mg CO₂-C g⁻¹ C respectively, while oilseeds were third and cereals fourth. The average difference between legumes and cereals was 25% at C_R 30 days, 19% at C_R 90 days (Figure 2.2 A & B). On average, legumes

had the lowest stability (0.78) followed by grass (0.79), cereals (0.82), and oilseed (0.83) (Figure 2.2 D, Appendices B.1).

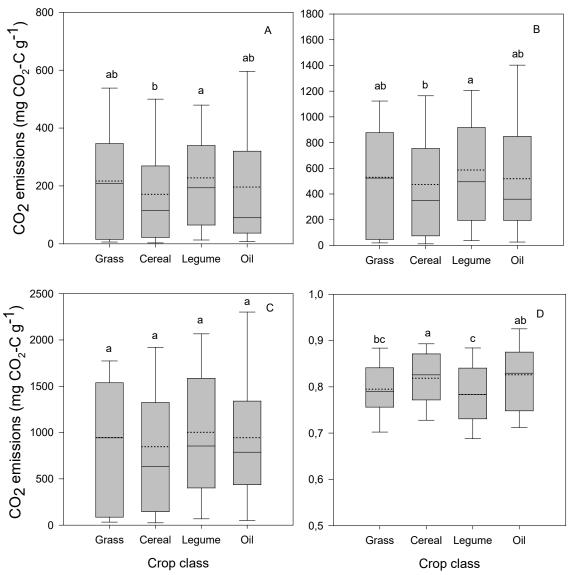


Figure 2.2: CO₂ emissions from residues of different crop classes incubated at different time periods (A: cumulative CO₂ emitted from crop residues at 30 days- C_R30 ; B: at 90 days- C_R90 ; C: at 120 days- C_R120 ; and D: at day 30 as a fraction of carbon emitted at day 120- (1- $C_R30/120$)). Plots with the same lower-case letters are not significantly different at Chi-square probability of < 0.001 (Chi and H values= 0.16, 5.24 at A; 0.08, 6.72 at B; 0.00, 13.79 at C and 0.00, 21005 at D, respectively)

Figure 2.3, Appendices C. 1., shows variations in CO₂ emissions between different crop types, with alfalfa having the highest emissions of 359 mg CO₂-C g⁻¹C amongst legumes (48% higher than clover and pea), while canola (293.5) had the highest amongst oilseed crops, and sorghum (261.1) had the highest amongst cereals (67% more than maize) at C_R30 days. Maize emitted the least cumulative CO₂ at C_R 30 (85.8 mg CO₂-C g⁻¹C), C_R 90 (275.1) and C_R 120 (495) days and exhibited the second highest 1-(C_R30/120) ratio of (0.84) after cotton, which was indicative of the highest C stability index, i.e., the lowest emission over time. In contrast, alfalfa exhibited the lowest C stability index from 1-(C_R30/120) of 0.76, followed by rye (0.77), clover and pea (0.78), canola, grass, and oat (0.79) (Figure 2.3 D).

2.3.2. The influence of crop quality on CO_2 emissions

Figure 2.4 and Appendices B.1. show that CO_2 emissions were not significantly different (p<0.05) between high and low residue C level from C_R30 to C_R120 days, however, low residue C concentration showed high C stability index i.e., 1-($C_R30/120$) ratio.

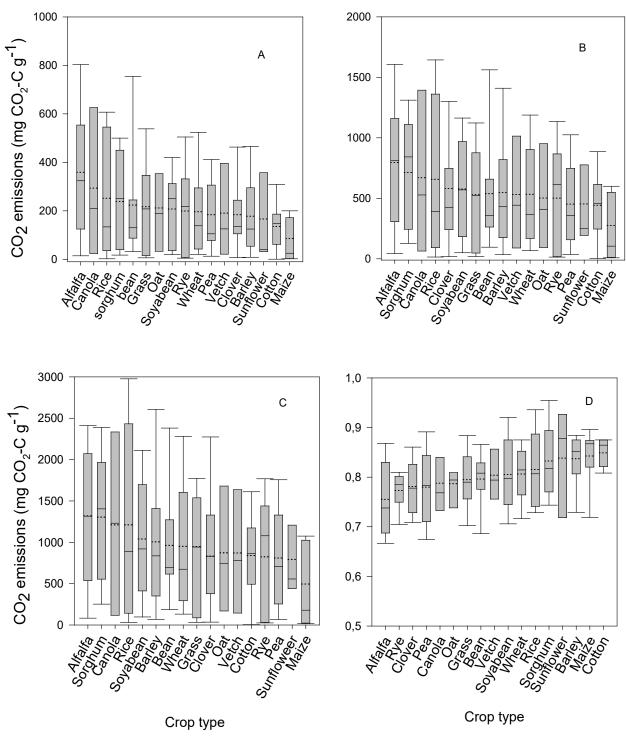


Figure 2.3: CO_2 emissions from residues of different crop type incubated at different time periods (A: cumulative CO_2 emitted from crop residues at 30 days- C_R30 ; B: at 90 days- C_R90 ; C: at 120 days- C_R120 ; and D: at day 30 as a fraction of carbon emitted at day 120- (1- $C_R30/120$)). Sorted by mean (dotted line)

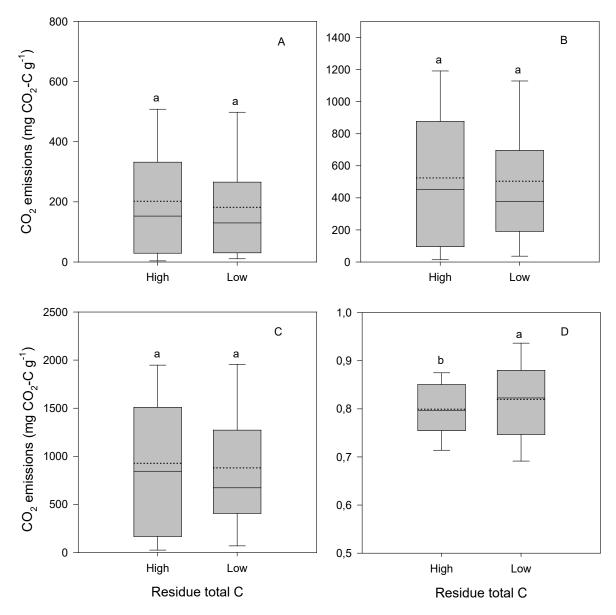


Figure 2.4: CO₂ emissions from crop residues of different initial C concentration incubated at different time periods (A: cumulative CO₂ emitted from crop residues at 30 days- C_R30 ; B: at 90 days- C_R90 ; C: at 120 days- C_R120 ; and D: at day 30 as a fraction of carbon emitted at day 120- (1- $C_R30/120$)). High (>0.5g/kg soil), low (<0.5g/kg soil) TC. Plots with the same lower-case letters are not significantly different at Chi-square probability of < 0.001 (Chi and H values= 0.00, 9.16 at A; 0.01, 7.18 at B; 0.64, 0.22 at C and 0.47, 0.52 at D, respectively)

Appendices B 1, shows the changes in CO₂ emissions from crop residues as a function of lignin concentration, being significant only at C_R90 and C_R120 (Figure 2.5 B & C). There was a

decrease in CO_2 emissions with increase in lignin concentration from 602 to 457 at C_R90 , and from 1055 to 817 mg CO_2 -C g^{-1} C at C_R120 . Moreover, the lignin concentration of the residues did not impact the C stability index of the emissions, 1-($C_R30/120$) (Figure 2.5 D, Appendices B 1).

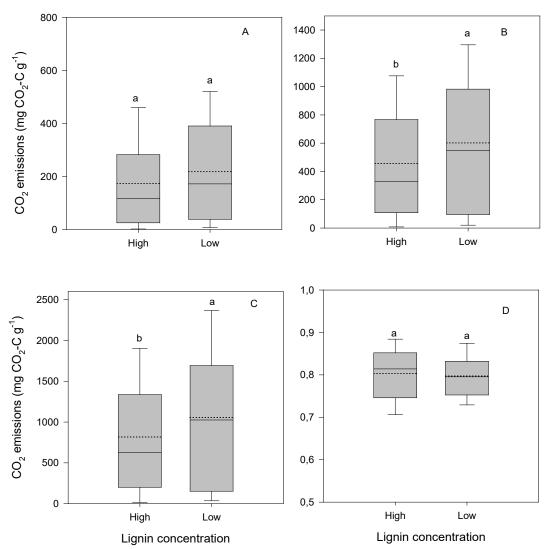


Figure 2.5: CO₂ emissions from crop residues of different lignin concentration incubated at different time periods (A: cumulative CO₂ emitted from crop residues at 30 days- C_R30 ; B: at 90 days- C_R90 ; C: at 120 days- C_R120 ; and D: at day 30 as a fraction of carbon emitted at day 120- (1- $C_R30/120$)). High (>10%), low (<10%) lignin. Plots with the same lower-case letters are not significantly different at Chi-square probability of < 0.001 (Chi and H values= 0.00, 12.06 at A; 0.00,13.84 at B; 0.00, 22.09 at C, and 0.50, 0.46 at D, respectively)

Cumulative CO_2 emissions significantly decreased with the increase in the C: N ratio of residues (Figure 2.6, Appendices B.1). On day C_R 30, residues with low C: N emitted an average 273.7 mg CO_2 -C g^{-1} C compared to the 201.1 mg CO_2 -C g^{-1} C emitted by the medium and 144.9 mg CO_2 -C g^{-1} C by the high C: N ratio, (P<0.05). A similar behavior was also observed at C_R90 and C_R120 (Figures 2.6 B & C). However, while the emission differences were significant between all C: N classes at C_R30 , there were no significant differences at C_R90 and C_R120 between medium and high C: N ratios. Finally, the stability index of the emissions increased with the increase in the C: N ratio of the residues (Figure 2.6).

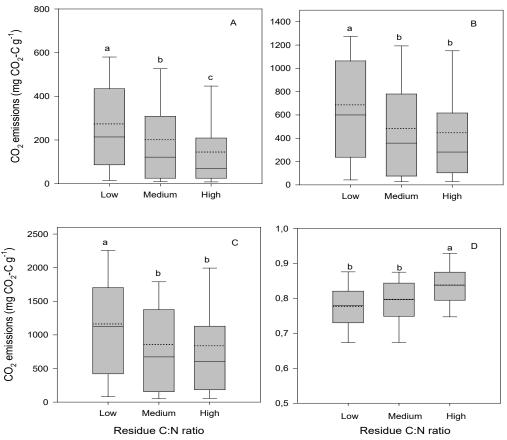


Figure 2.6: CO_2 emissions from crop residues of different C: N ratio incubated at different time periods (A: cumulative CO_2 emitted from crop residues at 30 days- C_R30 ; B: at 90 days- C_R90 ; C: at 120 days- C_R120 ; and D: at day 30 as a fraction of carbon emitted at day 120- (1- $C_R30/120$)). High (>30), medium (20-30), low (<20) C: N ratio. Plots with the same lower-case letters are not significantly different at Chi-square probability of < 0.001 (Chi and H values= 0.01, 9.72 at A; 0.28, 2.53 at B; 0.03, 7.22 at C and 0.00, 45.35 at D, respectively)

2.3.3. The impact of soil and environmental properties on CO_2 emission from different crop residues

There were significant variations in cumulative CO₂ emissions among the three different soil textural classes throughout the incubation periods (Figure 2.7). For instance, clayey soils exhibited significantly higher cumulative CO₂ emissions (224.4 after 30 days, 587.5 after 90 and 1060.6 mg CO₂-C g⁻¹ C after 120 days) as compared to sandy soils (178 after 30 days, 454.7 after 90 and 789.9 mg CO₂-C g-1 C after 120 days). Sandy soils showed significantly lower stability index (0.79) over time of emissions (1-(CR 30/120)).

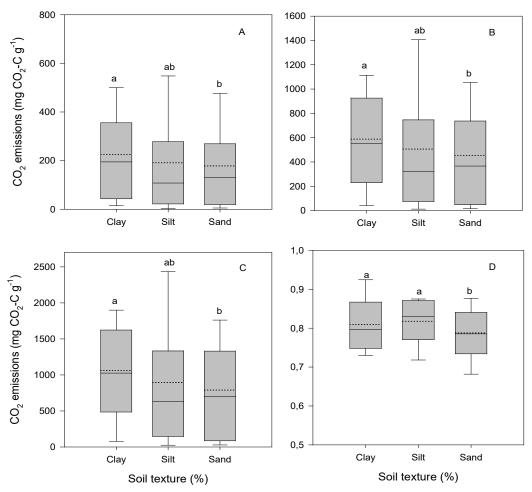


Figure 2.7: CO₂ emissions from soils of different texture ((%) -clayey, silt and sandy soil) at different day time periods (A: cumulative CO₂ emitted from crop residues at 30 days- C_R30 ; B: at 90 days- C_R90 ; C: at 120 days- C_R120 ; and D: at day 30 as a fraction of carbon emitted at day 120- (1- $C_R30/120$)). Plots with the same lower-case letters are not significantly different at Chi-square probability of < 0.001 (Chi and H values= 0.26, 2.67 at A; 0.63, 0.92 at B; 0.10, 4.70 at C; and 0.00, 14.07 at D, respectively)

Cumulative CO₂ emissions also varied due to soil pH (Figure 2.8). For all incubation durations, residues incubated under slightly acidic soils emitted significantly higher cumulative CO₂ emissions, followed by neutral and alkaline, then strongly acidic soils, which was lowest in all cases (Figures 2.8 A - C). Finally, emissions from alkaline soils were more stable (i.e., higher stability index) over time as compared to the other soil pH classes (Figure 2.8 D).

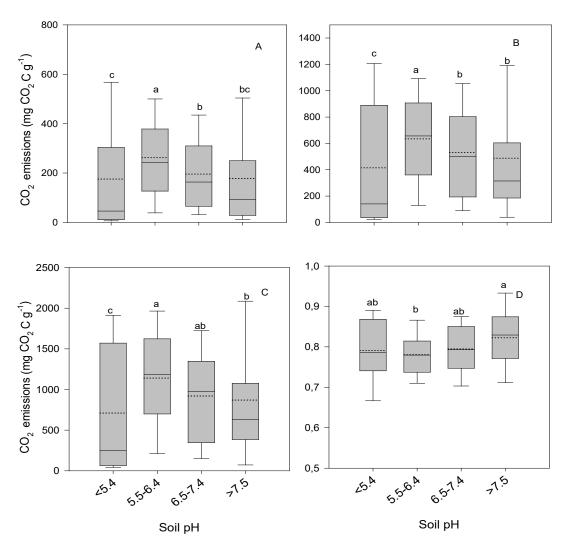


Figure 2.8: CO₂ emissions from soils of different pH level (highly basic, neutral, slightly acidic and highly acidic) at different day time periods (A: cumulative CO₂ emitted from crop residues at 30 days- C_R30 ; B: at 90 days- C_R90 ; C: at 120 days- C_R120 ; and D: at day 30 as a fraction of carbon emitted at day 120- (1- $C_R30/120$)). Strongly acidic (<5.4), slightly acidic (5.5-6.4), neutral (6.5-7.4), alkaline (>7.5) soil pH. Plots with the same lower-case letters are not significantly different at Chi-square probability of < 0.001 (Chi and H values= 0.01, 10.99 at A; 0.01, 12.64 at B, 0.25, 4.09 at C, and 0.00, 20.92 at D, respectively)

Significantly higher emissions as a result of the residues were found under tropical climates (252 mg CO₂-C g⁻¹ C), followed by subtropical (193 mg CO₂-C g⁻¹C) and temperate (150 mg CO₂-C gC⁻¹) at CR30 (Figure 2.9A). Similar trends were observed after C_R 90 and C_R120 days. Hence, tropical climate showed lower stability index over time of the emissions (0.78) as compared to subtropical and temperate climates (0.81 and 0.82 respectively, Figure 2.9D).

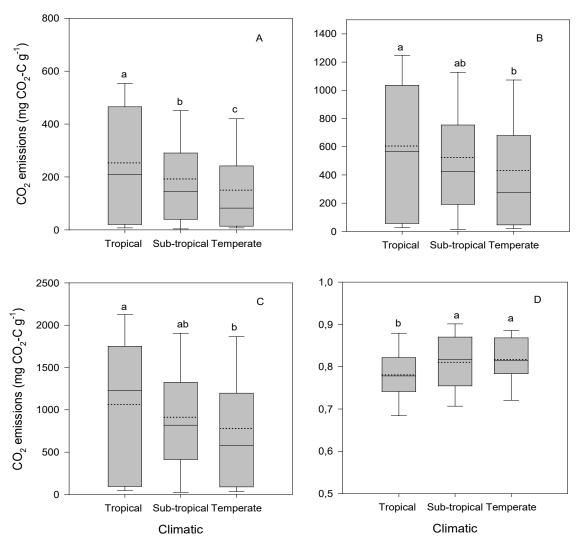


Figure 2.9: CO₂ emissions from climate of different rainfall and temperature level (as in tropical, sub-tropical and temperate (MAT, MAP) at different day time periods (A: cumulative CO₂ emitted from crop residues at 30 days- C_R30 ; B: at 90 days- C_R90 ; C: at 120 days- C_R120 ; and D: at day 30 as a fraction of carbon emitted at day 120- (1- $C_R30/120$)). Plots with the same lower-case letters are not significantly different at Chi-square probability of < 0.001 (Chi and H values= 0.84, 0.34 at A; 0.11, 4.40 at B; 0.68, 0.77 at C, and 0.00, 18.13 at D, respectively)

Overall, cumulative CO₂ emissions due to the residues exhibited significant negative correlations with lignin concentration; from day C_R30 (r= -0.08, p<0.05) to C_R 120 (r= -0.12, p<0.05). The stability of the emissions showed a positive but non-significant correlation with lignin concentration (r= 0.15, p>0.05). Similarly, negative significant correlations were observed between C: N and CO₂ emissions at day C_R 30 to C_R 120, as well as stability of C (r= -0.07, p<0.05). Additionally, similar negative correlations were observed with residue initial N, however, only significant at day C_R 90 (r= -0.28, p<0.05), and day C_R 120 (r= -0.26, p<0.05). Contrary, CO₂ emissions were positively correlated with the initial C concentration in residues with an r value between 0.40 and 0.59 across the study periods, and 0.37 for C stability index. Positive correlations of SOC with CO₂ emissions were only significant for C stability index (r=0.27, p<0.05). The soil clay concentration also had a significant positive correlation with the residue CO₂ emissions at the C_R 30 to 90 incubation period. While soil pH had a significant positive correlation with residue CO₂ emissions (r= 0.38 at C_R 30, r= 0.44 at C_R 90 and r= 0.42 at C_R 120 days, p<0.05). The MAP showed negative correlations with residue CO₂ emissions from C_R30 , C_R90 to C_R120 of r = -0.31, r = -0.32 and r = -0.31 respectively, (p<0.05), with insignificant impact on the 1-(CR 30/120) ratio, while MAT had non-significant correlations with CO₂ emissions (Table 2).

Table 2.4: Correlation matrix statistic table of plant, soil and climatic influence on residue CO₂ emission.

| | Lignin | C: N | TC | TN | Clay | Sand | Silt | рН | SOC | MAP | MAT | CR30 | CR90 | CR120 | 1-(CR30/ 120) |
|----------------|--------|--------|-------|--------|--------|--------|-------|--------|-------|--------|------|-------|-------|-------|------------------|
| Lignin | 1.00 | | | | | | | | | | | | | | |
| C: N | 0,15 | 1,00 | | | | | | | | | | | | | |
| TC | -0,06 | -0,06 | 1,00 | | | | | | | | | | | | |
| TN | 0,03 | -0,19 | -0,02 | 1,00 | | | | | | | | | | | |
| Clay | -0,08 | -0,11 | 0,51* | -0,19 | 1,00 | | | | | | | | | | |
| Sand | -0,02 | -0,15 | -0,42 | 0,32* | -0,64* | 1,00 | | | | | | | | | |
| Silt | 0,10 | 0,30* | -0,02 | -0,19 | -0,26* | -0,57* | 1,00 | | | | | | | | |
| pН | 0,02 | 0,15 | 0,16 | -0,63* | 0,26* | -0,45* | 0,29* | 1,00 | | | | | | | |
| SOC | -0,03 | 0,03 | 0,23* | -0,10 | 0,31* | -0,37* | 0,14 | -0,08 | 1,00 | | | | | | |
| MAP | -0,02 | -0,03 | -0,16 | 0,19 | -0,41* | 0,4* | -0,07 | -0,58* | -0,20 | 1,00 | | | | | |
| MAT | 0,00 | -0,20 | -0,21 | 0,16 | -0,40* | 0,41* | -0,08 | -0,40* | 0,03 | 0,43* | 1,00 | | | | |
| CR30 | -0,08* | -0,14* | 0,59* | -0,22 | 0,34* | -0,38* | 0,12 | 0,38* | 0,15 | -0,31* | 0,07 | 1,00 | | | |
| CR90 | -0,11* | -0,10* | 0,46* | -0,28* | 0,26* | -0,32* | 0,13 | 0,44* | 0,09 | -0,32* | 0,10 | 0,96* | 1,00 | | |
| CR120 | -0,12* | -0,09* | 0,40* | -0,26* | 0,22 | -0,29* | 0,14 | 0,42* | 0,07 | -0,31* | 0,10 | 0,93* | 0,99* | 1,00 | |
| 1-(CR30/CR120) | 0,15 | -0,07* | 0,37* | 0,11 | 0,10 | -0,12 | 0,05 | -0,19 | 0,27* | 0,19 | 0,21 | 0,27* | 0,07 | -0,03 | 1,00 |

Residue CO₂ emission at different time periods: - C_R 30= at day 30; C_R 90=at day 90; C_R 120= at day 120; C_R 120= at 1-(30/120)-day ratio. Lignin: residue lignin concentration; C: N: residue carbon to nitrogen ratio; TC: Initial residue carbon concentration; Clay, sand & silt: soil texture based on clay fraction (%clay); pH: soil pH (KCl); SOC: soil organic carbon concentration; MAP& MAT: climatic factors-mean annual precipitation and mean annual temperature. * Significant at p<0.05.

Multivariate correlations among the soil and residue properties and soil organic carbon concentration are presented in Figure 2.10. The two principal components of this PCA, which was generated using the CO₂ emission variables, explained 99% of the total variation in the data with the first principal component (PC1) accounting for 79% of the variance in the data and PC2 accounting for 20% (Figure 2.10). The emissions at C_R 30 to 120 days showed a positive correlation to PC1 and this axis can be thus interpreted as an axis of CO₂ emissions. The 1-(C_R 30/120) days ratio showed a positive correlation to PC2, meaning that PC2 is an axis of decreased stability over time of CO₂ emissions. Lignin, N, and C concentration of the residues showed negative coordinates on PC1 while the C: N ratio showed a positive coordinate. There is thus a tendency for emissions to increase as C: N increases but C, N, and lignin concentration decreases. Finally, the residue quality variables had only a little impact on the stability of the emissions, which appeared to decrease as soil organic concentration increased (Figure 2.10). Alfalfa correlates to the high emissions and low stability pole, while maize to the low emissions' high stability pole.

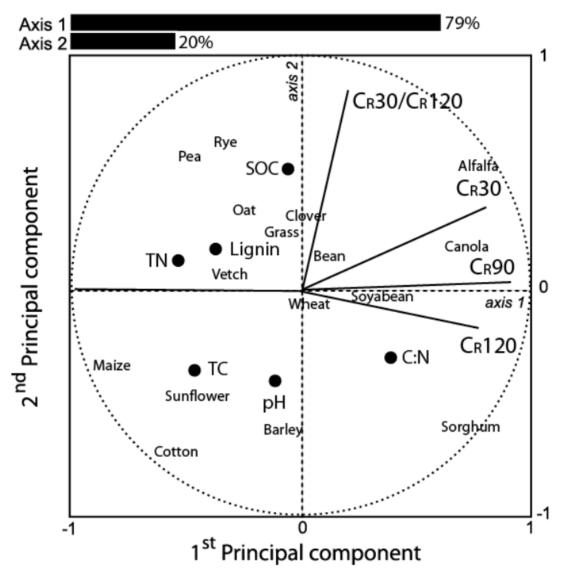


Figure 2.10: Principal component analysis generated using the CO_2 emission variables (C_R 30= cumulative CO_2 emissions for 30 days; C_R 90= 90 days; C_R 120= 120 days; 1-(C_R 30/120) = one minus 30 to 120 ratio as a stability index) and the soil, organic matter variables and crops as display.

2.4. Discussion

2.4.1. Causes of variation in residue decomposition and soil C building amongst crop types

The different crop types exhibited large variations in CO₂ emissions with legumes emitting the highest cumulative CO₂ emissions, which decreased over time, versus cereals that had lower emissions but of higher stability. Such variations were shown to correlate with the quality of crop residues, which are affected by their genetic composition (Machinet, et al., 2009). Ajwa and Tabatabai (1994) attributed the significantly higher CO₂-C released from alfalfa residues to its ability to fix N, as they had higher initial N concentration (12.6%) compared with that of maize (6.9%) and oilseed crops (1.3%). Greater emissions at high initial N concentration are due to enhanced microbial activity leading to high decomposition and CO₂ emissions (Gezahegn et al., 2016).

The present meta-analysis tends to contradict this, as it pointed to a negative correlation between CO₂ emissions and residue N concentration. This might be due to the fact that N availability enhances C uptake by microbes, and thus humus formation versus C emissions to the atmosphere, (Henriksen and Breland, 1999 and Rousk and Bååth, 2007). The fact that legumes, especially Alfalfa, emitted large amounts of CO₂ despite a high N concentration was most likely due to its low lignin concentration. Carbon stabilization into soils in microbial biomass thus seems to be favoured by the supply of residues with a C and N stoichiometric ratio close to that of living microorganisms. Results on maize, sunflower and cotton tend to show that carbon and lignin rich residues experience low decomposition rates which constitute a second route of carbon stabilization into soils.

2.4.2. The impact of crops on the loss of soil carbon through priming

Recent studies have indeed shown that soil organic matter decomposition by decomposers can be stimulated by the addition of fresh organic matter resulting in an increase in soil respiration beyond C addition, a condition known as 'priming' (Fontaine et al. 2003; Kuzyakov, 2010). In the present study, 43% of the respiration data points showed 120 days cumulative emissions beyond C addition (Appendices A. 1), which points to the existence of significant C losses from soil organic matter. For instance, from 25% for maize, sunflower and beans to over 55% for sorghum, alfalfa and canola, with differences between legumes, cereals and oilseed crops being non-significant at p<0.05.

Several authors have suggested that chemically recalcitrant residues, such as those rich in carbon and lignin, decompose more slowly than residues with low lignin and high N concentrations, leading to enhanced C stabilization into soils and increased soil organic matter (Johnson et al. 2007; Berg and McClaugherty, 2008). However, several other authors such as Stewart et al. (2015), pointed out that high lignin residues are used inefficiently by the soil microbial community that decompose SOM (priming effect) to acquire key nutrients resulting in much greater respiration losses and less C stabilization into soils. The present study which points to a tendency for CO₂ emissions to decrease with increase in residue lignin and N concentration shows that high lignin and high N concentration may limit priming and foster C stabilization into soils by microbes. Moreover, the present data showed that lignin concentration minimally impacted the temporal stability of the emissions, but they decreased more sharply overtime at higher initial residue C concentration.

2.4.3. The impact of soil properties on CO_2 emissions

Soil texture and pH had significant impact on cumulative CO₂ emissions from crop residues. The higher cumulative CO₂ emissions under clayey soils could be due to favorable living conditions for decomposers than under coarser soil conditions. Schmatz, et al., (2017) also found higher C emissions from clay soils as compared to sandy-silt soils due to high organic carbon concentration in clayey soils and enhanced water retention capability, thus favoring the activity of microorganisms responsible for residue break-down. In contrast, many previous studies stated that clay soils offered physical protection and mineral adsorption of C constituents. However, this was not confirmed by the present meta-analysis since clay soils experienced higher CO₂ emissions per gram of residue C added. The addition of labile organic materials (crop residues) to clayey soils, with higher organic carbon, could result in more CO₂ emission (priming effect) than from sandy or silty soils. In addition, such an increase in CO₂ emissions with increasing clay concentration might (as suggested before) come from higher soil moisture and bacteria populations favoring more rapid turnover of residue C.

Slightly acidic soils (pH of 5.5-6.4), experienced consistently higher CO₂ emissions (>50%) than the other pH levels irrespective of incubation time as conditions for fungi and bacteria are not optimal (Hågvar, 1994; Stott and Martin, 1989). This is the pH range where most nutrients are available in the soil, which would favour proliferation of microbes. Another possible reason for the higher CO₂ emissions could be the liming effect of residues that foster the priming of soil organic matter (Wang et al. 2017; Yaowu et al. 2016). Murungu et al. (2011), stated that

mildly acidic soil pH (pH=6.1) can be further decreased by addition of residues (i.e., cover crops) with long-term use, while soils with higher pH values than those of residues are known to suffer a decrease in soil pH after residue treatment (Murungu et al., 2011).

2.4.4. Climate impact on CO₂ emissions

The finding of this study pointed to higher residue CO₂ emissions under tropical conditions than under the other climates throughout the incubation days. This is because tropical climates tend to experience high temperature and rainfall conditions that are conducive for the production of high plant biomass and microbial activity for decomposition. On the other hand, soils of subtropical or temperate climates have lower rainfall and cooler temperature that limit microbial activity and the biochemical processes involved in residue decomposition (Ontl and Schulte, 2012). As the data used in the present study come from laboratory experiments with controlled conditions of temperature and humidity, the higher decomposition rates and cumulative CO₂ emissions under tropical climates could be due to favourable conditions for microbial activity.

2.5. Conclusions

The analysis of 394 laboratory trials worldwide revealed that on average, legumes exhibited significantly higher CO₂ emissions than cereals, oilseed, and fibre crops, with alfalfa emitting 2.7 times more CO₂ than maize after 120 days. Additionally, legumes showed lower stability of CO₂ emissions over time (i.e., the highest decrease from 30 to 120 days) followed by grasses, cereals, and oilseed crops. Amongst the two models for enhanced C sequestration into soils, there was a tendency for CO₂ emissions to decrease with increase in residue lignin. This contrasted with previous studies showing that high lignin concentration enhanced microbial decomposition and thus C outputs from soils i.e., alfalfa (low lignin and high N concentration) versus maize residue (high lignin and low N concentration). Thus, providing soils with residues with high lignin such as maize may improve SOM and C uptake by microbes (a key process in humus formation).

A large proportion of the variance in the data remained however unexplained thus calling for further analysis of variables such as soil nutrients, microbial communities, and quality of lignin, among others. More work also needs to be done on selecting crop cultivars for generating superior ones in our fight against land and climate degradation. Finally, the question of whether the carbon remaining into the soil after 120 days of incubation will be stabilized to contribute

to soil carbon stocks calls for further research to understand the fate and underlying mechanisms of C sequestration into soils.

3. CHAPTER THREE:

CO₂ EMISSION, NITROGEN MINERALIZATION FROM ROOT AND SHOOT RESIDUES OF DIFFERENT SORGHUM CULTIVARS IN SOIL

Abstract

The retention of crop residues into the soil is essential for maintaining soil organic carbon (SOC) and nitrogen (N) stocks. While numerous studies focused on the decomposition of residues from single crops, the difference between crop cultivars and/or crop parts has received little attention. This incubation study was carried out to determine the variations in CO₂ emission and N release patterns, due to the decomposition in soils of roots and shoots from different sorghum cultivars. Root and shoot residues (0.25g; n=3) from five sorghum cultivars (AS8, KZ5246, LP4403, OS-POTCH, and MAMOLOKWANE) were ground to 2 mm, and mixed with 100 g of soil in a 500 ml sealed plastic container and incubated at 25°C and 50% soil field capacity. The amount of CO₂-C was estimated after trapping in NaOH at day 0, 7, 14, 28, 42, 56, 84, and 120. The amount of NH₄⁺-N and NO₃⁻-N were analyzed using a Thermo Scientific Gallery Discrete Autoanalyzer. The CO₂-C emissions were consistently higher in shoots than in roots. After 120 days, emitted overall mean C values varied with a range of 21.9 mg CO₂-C kg⁻¹ of the initial added C in roots and 24.3 in shoots for all cultivars (between 44.85 and 7.85 mg CO₂-C kg⁻¹ added C in roots and between 49.9 and 5.85 mg CO₂-C kg⁻¹ added C in shoots for OS-POTCH and KZ5246 residues), respectively. In roots, the initial total N concentration was 1.7% on average and N values varied between 1.1 and 2.3% (KZ5246 and OS-POTCH). While in shoots, the initial total N concentration was 1.9% on average and values varied between 1.4 and 2.4% (KZ5246 and OS-POTCH). Net N immobilization was observed for low-quality residue (i.e., KZ5246 and AS8 root) at the initial stages of incubation, then remineralization happened. Over time N mineralization decreased, with consistently higher N releases in shoots compared to root residues. After 120 days, NH₄⁺-N mineralization varied between 18.9 and 4.59 mg N/kg of the initial added N in shoots, and between 16.65 and 3.95 mg N/kg added N in root residues for OS-POTCH and KZ5246, respectively. While NO₃-N mineralization ranged between 22.66 and 11.46 mg N/kg of the initial added N in shoots of OS-POTCH and KZ5246, then 20.7 and 9.29 mg N/kg added N in root residues of OS-POTCH and KZ5246. These results suggest shoot residues (i.e., OS-POTCH) to have more available N for plants uptake, while root residues (i.e., KZ5246) can be more commendable for adding N and C stocks to the soil.

Keywords: Sorghum cultivar decomposition, N mineralisation, C emission, Soil quality, C stocks.

3.1. Introduction

The emissions of carbon dioxide (CO₂) and nitrous oxide (N₂O) to the atmosphere through agricultural activities are becoming increasingly challenging, with declining soil fertility and contributing to climate change, globally (Muñoz, et al., 2010; Follett et al., 2001). This is evident from the accelerating rates of residue carbon (C) and nitrogen (N) outputs to the atmosphere, through decomposition and mineralization (Kan et al., 2021; Bationo et al., 2017). Apparently, more than a third of N and C losses through gaseous emissions are anthropogenic (Paul et al., 2018; Macharia et al., 2020). This is related to massive expansion of cropland in sub-Saharan Africa, Asia and America, particularly in the last few decades (Hong et al., 2021). The total anthropogenic CO₂ emissions from fossil plus land-use change amounted to 38.9 ± 2.9 GtCO₂ for the years 2011–2020 decade, reaching 38.0 GtCO₂ yr⁻¹ in 2020, and rapidly increased to 39.3 GtCO₂ yr⁻¹ by the end of 2021 (Friedlingstein et al., 2021; Lienert, 2021; Gür, 2022), The IPCC AR6 assessment has estimated that total anthropogenic CO₂ emissions cut of 1.4 GtCO₂ each year on average, would be required in order to reach zero CO₂ emissions by 2050 (Friedlingstein et al., 2021; IPPC, 2022). Ball et al. (2008) found 1.5 to 35 times higher fluxes of N₂O from no-till compared to tilled soils. Plant biomass production in agriculture links the enhancement of N₂O and CO₂ atmospheric air with the rate at which C and N are cycled within crop-soil systems. There is a need to develop strategies to increase C and N storage in soils and reduce emissions of these greenhouse gases from agricultural lands.

The retention of crop residues during the off-season and incorporating them through tillage during planting season is commonly practiced by small-scale farmers under rain-fed cropping in Sub-Saharan Africa, where 43 % of the area is arid and semi-arid (Wang et al., 2018). While the residues left on the surface could be burnt or fed on by livestock, incorporation could increase SOM storage. Crop residue C is transferred to the soil through root materials (i.e., decaying roots or root exudates) and surface litterfall, which includes residues after harvest (Henning et al., 1996). The residues may vary in their biochemical qualities due to differences in crop species and/or parts (Kriaučiūnienė et al., 2012). The variation in crop residue is dependent on whether the plant part is a supportive tissue (i.e., stem), conductive tissue (leaf), or protective tissue (root tissue, Abiven et al., 2005; Ma and Petersen, 2003), with great variation in macromolecules such as cellulose, hemicellulose or lignin and the constituents of C and N (Abiven and Ajwa, 2005). Thus, roots or shoots show specific patterns of decomposition in relation to peculiarities in their biochemical composition (Kriaučiūnienė et al., 2012; Abiven et al., 2005). Hence, the measurable organic pools in the root and shoot

residues are assumed to have different decomposition dynamics, ranging from the faster labile fractions to the slowest recalcitrant molecules.

Crop materials are composed of different proportions, which presumably influences the decomposition (Johnson et al., 2007). The levels of labile components have been found to determine the initial rates of decomposition, while the insoluble components are responsible for long-term C storage (Broder and Wagner, 1988). Machinet (2009); Hadas et al. (2004) identified the chemical cell wall features of crop tissues such as lignin concentration to negatively correlate with the decomposition rate. In addition, the C: N ratio of crop residues is often used as an index to assess whether the residues will release or immobilize inorganic N (Hadas et al., 2004). Crop materials with a low N or high C: N ratio are expected to initially result in N immobilization. Johnson et al. (2007) observed materials such as alfalfa and aboveground material of other crops with a low C: N ratio or low lignin: N to have faster decomposition rates. However, the C: N ratio can be a useful indicator of substrate decomposability in N deficient rather than N-rich soils (Bonanomi et al., 2017; Xu et al., 2017). While the total crop N concentration can be a good predictor for the N mineralization potential, and hemicellulose C concentration explains most of the C mineralization (Jensen et al., 2005).

Numerous studies have presented different observations for different parameters, which are attributed to varied factors influencing residue decomposition. Bonanomi et al. (2017); Xu et al. (2017); Johnson et al. (2007); Gorissen and Cotrufo (2000); and Franck et al. (1997), identified soil moisture content and abiotic factors to significantly contribute to the decomposition process, discrediting the accuracy of residue C: N ratio in predicting decomposition rate where there is enough N available for microbial activity. Xu et al. (2017), showed wheat residues to have retained 43% of the initial C, followed by maize (40%) and soyabean (37%) residues, with 78%, 73%, and 69%, and of the remaining N observed in wheat, soyabean, and maize residues, respectively after 12 months of decomposition. In support, Vachon and Oelbermann (2011) also estimated a great amount of N mineralized in the soyabean crop (12 g N m⁻²), compared to the maize crop (9 g N m⁻²).

Cover crops and leguminous crop residues have N fixation capacity, hence, their lower C: N ratio leads to high mineralization, more especially at the initial stages of decomposition (Fageria et al., 2007). Li et al. (2020); and Hu et al. (2018) studied leguminous cover crop residues (i.e., clover and vetch) and found comparable results, with average net N mineralization of 33% from the leguminous cover crops and 20% from the non-legumes.

Moreover, Hadas et al. (2004); Green and Blackmer (1995) explained the rapid decomposition in soyabean than corn residues by a higher proportion of soluble components in soyabean. For the non-legumes, Li et al. (2020) found the largest proportions of N immobilized (10% and 14% of N input for ryegrass and fodder radish, respectively) at the early stages (14 days) for the shoots and the largest immobilization for root treatments at the later stages (30 days) of decomposition. These observations were ascribed to a higher initial N concentration and a narrower C: N ratio in legume crop residues (Hu et al., 2018).

Li et al. (2020) further identified leguminous residues to have 41% lower C: N ratios than nonleguminous, with 7 to 69% higher N concentrations in shoots compared with the roots of the same species. The decomposition rates of non-legume residues, particularly cereal, tend to be slower than those for legumes due to the variations in biochemical properties. For instance, soyabean had the most rapid rate of decomposition, losing 68% of its total organic matter over the course of 32 days compared with 47 and 42% found in wheat and corn residues, respectively, (Broder and Wagner, 1988). In support, Gezahegn et al. (2016) observed 66 % of the added C decomposition in soyabean residue compared to 46% in maize, with net N mineralization of 7.4 to 98.4 mg/kg in soyabean, while maize residue showed N immobilization (10.75 to 3.69 mg/kg soil) within the 60 days of incubation. Murungu et al. (2011) showed higher CO₂-C emissions in oats (1750 mg C/kg), followed by peas (1250 mg C/kg) then 1000 mg C/kg in vetch residues, with oats having 15 mg N /kg compared to 65 mg N /kg of added N found in peas and vetch residues after 60 days of controlled incubation. Moreover, the decomposition rate was higher in the pearl millet compared to guinea grass residues, with 87 and 56 kg ha⁻¹ N, respectively released after 34 days (Soratto et al., 2019). Diack and Scott (2016) also found greater mean cotton residue mass loss (45%) than those of peanut (40%), and sorghum (34%), and further observed great decomposition variability in sorghum (Sorghum bicolor L. Moench) cultivars, with 32% of mass loss for Triumph-266, 24% for GW-744BR and 20% for NKing-300, at the early decomposition stages (Diack and Scott, 2016). Sorghum is among the five important cereal crops in the world (after wheat, rice, barley, and maize) and ranks third in South Africa. It uses relatively lower agronomic inputs such as fertilizers and irrigation because of drought-resistance and adaptation stability, compared to other crops. Of the different cereal residues, sorghum is among the slowest in the rate of decomposition, suggesting that it has the greatest potential to increase soil carbon storage and minimize gas emissions.

Agricultural systems that are based on sorghum production add residues to the soil through above-ground and root biomass, which may vary in terms of biochemical properties and decomposition rate. Li et al. (2020) reported that the net C mineralization from shoot residues of cover crops after 100 days corresponded to 53-62% of the C applied, and the proportion was higher than that of roots (18-39% of applied C). Lu et al. (2003) also reported that 73 and 56% of the straw and root residues, respectively, decomposed over 240 days of incubation at 15°C. The higher C and N mineralization rates in shoot than root residues, were attributed to differences in biochemical composition such as lignin concentration and insoluble constituents, and the residue interactions to soil (i.e., aggregate formation), which is known to physically protect the organic C from biodegradation (Lu, et al., 2003; Puget and Drinkwater, 2001). Li et al. (2020) and Hu et al. (2018) found that the N release from shoot residues corresponded to 42% of the initially added N compared to 28% N mineralized from roots during 180 days of incubation. Whereas some studies observed lack of significant differences in the chemical composition (i.e., C concentration) of residues from cultivars of the same crop species (Daudu et al., 2009; Lehman et al., 2008). Indicating that significant C concentrations may be determined at a species level rather than genotype (Poorter et al. (2012). Machine et al. (2009) studied different cultivars of maize roots (isogenic F2, F292, and mutant F2bm1, F292bm3), and found significantly lower total amounts of mineralized C in F2bm1 (27.5% of added C) than in F2 (37.2%), while those in F2 were significantly lower than in F292 (45.8%) and F292bm3 (46.0%) at p \leq 0.05. These C mineralization results were explained by lower levels of cell wall polysaccharides and higher initial lignin in F2 and F2bm1. While the sharper decrease in soil mineral N because of net immobilization in F292 and F292bm3 after 36 days of incubation, was explained by lower C: N ratio (or higher N concentration) (Machine et al., 2009). Overall, all these observations were attributed to great variations in biochemical properties of crop residues (Lynch et al., 2016; Machine et al., 2009).

Genetic manipulation of sorghum has resulted in the development of improved cultivars for higher biomass and yield, resistance to weeds and droughts. The residues of these improved cultivars may vary in terms of biochemical properties and rate of decomposition and C storage when added to the soil. Hence, this study builds on developments in sorghum research towards the selection of cultivars with high roots and shoot biomass production, to select the superior cultivars that will increase soil C storage and nitrogen cycling. The objective of this study was to determine CO₂ emission and nitrogen mineralization from the root and shoot residues of different sorghum cultivars in the soil. This was achieved through an incubation study, with

environmental factors (i.e., moisture and temperature) and soil variables being held constant to avoid confounding effects.

3.2. Method and materials

3.2.1. Site description

The soil used in the incubation was collected from an arable field located at Ukulinga Research Farm of the University of KwaZulu-Natal, South Africa. The farm is located at latitude 29.667'S and longitude 30.406'E at an elevation of 811 m above sea level. The soil in the study site was clay loam in texture and classified as a Westleigh soil form (Soil Classification Working Group, 1991) or Plinthic Acrisols (IUSS Working Group WRB, 2014). Soil samples were collected from a depth of 0-15 cm at random points using a soil auger, then mixed thoroughly to form a composite sample. This was then air-dried and ground to pass through a 2 mm sieve. A sub-sample of 0.5 kg was taken for determination of soil physical and chemical properties. The hydrometer method explained by Okalebo et al., (2002) for particle size analysis was used, and pH was determined in water or 1M KCl using a Hanna Ph microprocessor meter (Model 211). Total N and C were determined using an automated LECO-CNS Trumac analyzer, and the AMBIC-2 method, as modified by Hunter (1974) and Van der Merwe et al., (1984) was used for P analysis, extractable (1M KCl) calcium and magnesium in the soil were displaced with neutral 1.0 N NH₄Oac solution (Moral, 2010). While the soil bulk density was determined on the undisturbed field soils (0-10cm depth) following the core method by Blake and Harte (1986). The physicochemical properties of the soil are shown in Table 3.1.

Table 3.1: Soil physicochemical properties for incubation study

| Soil Property | Values ±SD |
|---|-------------------|
| Bulk density (g cm ⁻³) | 1.24±0.006 |
| Sand (%) | 30.0±0.061 |
| Silt (%) | 34.9±0.058 |
| Clay (%) | 35.1±0.015 |
| Texture | clay loam |
| Ph (KCl) | 4.8±0.015 |
| Exchangeable acidity (cmol kg ⁻¹) | 0.047 ± 0.001 |
| Total carbon % | 1.9 ± 0.067 |
| Total nitrogen % | 0.18 ± 0.01 |
| Phosphorous (mg kg ⁻¹) | 11 ± 0.051 |

| Potassium (mg kg ⁻¹) | 114 ± 0.168 |
|----------------------------------|------------------|
| Calcium (mg kg ⁻¹) | 1294 ± 0.176 |
| Magnesium (mg kg ⁻¹) | 389 ± 0.156 |

3.2.2. Sorghum plant material

The residues were selected from ten cultivars of sorghum that proved to have high biomass production from a previous biomass allocation field study (Zengeni et al., 2021). The sorghum accessions used represent a considerable genetic pool as they were collected from different provinces across South Africa and possibly exhibit divergent adaptation (Table 3.2). The sorghum accessions were still in developmental stages, and were previously evaluated for drought tolerance and diversity. The plants were grown under drought-stressed and nonstressed (well-watered) conditions. In the non-stressed regime, the plants were watered to field capacity (FC) whenever average soil moisture content fell to 80% of FC. The volumetric soil moisture content was allowed to drop to 30% of FC in the drought stressed treatments before watering back to FC. After harvest the residues were analyzed for initial acid detergent lignin and cellulose concentrations using the filter bag technique (ANKOM-200 technology method) by AOAC (1984). Total initial C and N concentrations were also determined by the use of an automated LECO Trumac CNS Auto-analyser Version 1.1x. These results informed the selection and categorization of five cultivars based on their biomass production, C: N, and lignin: N ratios (Chapter 3, Table 3.3). Thus, in the end, we had two varieties with high, two with low and one with medium values of biochemical composition.

Table 3.2: Sources of the ten sorghum cultivars used in this study

| No. | Cultivar | Source | Attributes |
|-----|-------------|---------------|---------------------|
| 1 | AS8 | ACCI | Experimental hybrid |
| 2 | AS15 | ACCI | Experimental hybrid |
| 3 | AS16 | ACCI | Experimental hybrid |
| 4 | AS18 | ACCI | Experimental hybrid |
| 5 | OS-POTCH | ARC | Experimental hybrid |
| 6 | Mamolokwane | ARC | Landrace |
| 7 | LP4403 | Limpopo | Landrace |
| 8 | NW5430 | Northwest | Landrace |
| 9 | KZ5246 | KwaZulu-Natal | Landrace |
| 10 | NW5393 | Northwest | Landrace |

ACCI=African Centre for Crop Improvement, ARC=Agricultural Research Council of South Africa.

Incubation setup:

An incubation experiment was set up using a completely randomized design with 11 treatments replicated 3 times per crop. The sorghum root or shoot residues of five genotypes of cultivars AS8, KZ5246, LP4403, Mamolokwane, and OS-POTCH were used in this study. These cultivars were selected from the above-mentioned experiment (based on high biomass). Ground (<2 mm) root or shoot residues (0.25 g) of each genotype were mixed with 100 g of soil in 100 ml plastic containers, slowly wetted to fill up 50% pore space, and placed in a 500 ml airtight plastic container.

3.2.3. CO₂-emission determination

A vial containing 25 ml of 1 M NaOH solution was also placed inside the plastic container to trap CO₂. The containers were closed so that they were airtight and incubated in the dark in a constant temperature room set at 25°C. Fresh NaOH was added to each plastic container at each sampling. The amount of CO₂-C trapped in the NaOH solution was determined by titration against 0.5M HCl with phenolphthalein indicator, after precipitating carbonates with BaCl₂ at 0, 7, 14, 28, 42, 56, 84, and 120 days of incubation. The crop residues had different amounts of C in their tissues, therefore the results of CO₂-C emitted were normalized by expressing them as mg CO₂- C kg⁻¹ C added. The net CO₂-C emitted was obtained by calculating the differences in the values of the biomass treated soil and control, while cumulative CO₂-C was calculated as the sum of all previous measurements.

3.2.4. Mineral nitrogen determination

The incubated treatments were also analyzed for NH₄⁺-N, NO₃⁻-N. Samples (2.0 g) from each treatment were removed from the plastic containers at different incubation times (0, 7, 14, 28, 42, 56, 84, and 120 days) and suspended in 20 ml of 2M KCl, shaken for 1 hour at 400pm followed by 10 minutes of filtration. The concentration of NH₄⁺-N and NO₃⁻-N in the extracts were analyzed using Thermo Scientific Gallery Discrete Autoanalyzer (Scientific Thermo Fisher 2014). Net NH₄⁺-N and NO₃⁻-N were obtained by the difference between the values of the control and the biomass-treated soil. The net mineral N was calculated as the sum of NH₄⁺-N, and NO₃⁻-N concentrations released from that treatment after subtracting the control. Different residues contained different concentrations of N in their tissues therefore, the results of N mineralized were normalized and represented as mg N kg⁻¹ N added.

3.3. Statistical analyses

The data on all parameters studied were subjected to two-way analysis of variance (ANOVA) for each sampling time (plant part * cultivar), and the least significant difference (LSD) test at the 0.05 probability level was used to compare significant differences among treatment means, using GenStat 18th edition (Payne et al., 2017). Spearman rank correlations were determined between cumulative CO₂-C or total mineral N and biochemical properties of sorghum cultivars.

3.4. Results

3.4.1. Initial biomass production, and biochemical properties of shoot and root residues of incubated sorghum cultivars

The different sorghum cultivars showed significant variation in biomass production and biochemical properties, with less differences between shoot and root residues of each cultivar. The LP4403 shoots had the highest initial biomass (2800 g m⁻²), while Mamolokwane roots had the lowest of 1032 g m⁻². The OS-POTCH shoots had significantly higher N (2.37%) being insignificantly different to its roots, while KZ5246 and AS8 roots showed a lower mean initial total N (1.19%), with no significant difference between their shoot parts. Further, no significant differences were observed between all residue treatments in lignin and cellulose concentrations. However, roots of KZ5246 and AS8 showed significantly higher C: N (average of 30.6) and lignin: N ratio (average of 25), followed by their shoot parts with less acute differences. Shoots of OS-POTCH and MAMOLOKWANE had lower C: N and lignin: N ratios than the other treatments, while LP4403 exhibited intermediate values (Table 3.3).

Table 3.3: Biomass yield and biochemical properties of shoot and root residues of different sorghum cultivars.

| Treatment part | Cultivar | Biomass | Total C | Total N | Lignin | Cellulose | C: N | Lignin: N |
|----------------|-------------|----------------------|------------------------|------------------------|--------|-----------|----------------------|----------------------|
| | | (g m ⁻²) | | % | | | | |
| Root | AS8 | 1692 ^{bc} | 38.88 ^{bcde} | 1.237 ^{cde} | 29.91 | 6,33 | 29.75 ^{ab} | 24.44 ^{ab} |
| Root | OS-POTCH | 1464 ^{bc} | 41.31 ^{abcde} | 2.298 ^a | 25.56 | 7,04 | 18.06 ^{de} | 11.15 ^d |
| Root | LP 4403 | 1960 ^{abc} | 41.79^{abcd} | 1.991 ^{abc} | 30.57 | 8,40 | 21.84 ^{cde} | 16.21 ^{bcd} |
| Root | KZ5246 | 1128° | 36.02^{de} | 1.149 ^{def} | 29.31 | 7,92 | 31.43 ^{ab} | 25.64a |
| Root | MAMOLOKWANE | 1032° | 42.09^{abcd} | 2.246^{ab} | 26.54 | 8,53 | 18.84 ^{de} | 11.80 ^d |
| Shoot | AS8 | 2468ab | 36.50^{cde} | $1.430^{\rm cd}$ | 28.77 | 8,67 | 27.22abc | 20.10^{abcd} |
| Shoot | OS-POTCH | 2052abc | 41.35 ^{abcde} | 2.367ª | 26.12 | 4,39 | 17.87 ^{de} | $11.40^{\rm d}$ |
| Shoot | LP 4403 | 2800^{a} | 42.14 ^{ab} | 1.985° | 29.05 | 4,76 | 21.26 ^{cde} | 14.64 ^{cd} |
| Shoot | KZ5246 | 1560 ^{bc} | 30.33^{ef} | 1.357 ^{cd} | 32.40 | 6,96 | 22.47 ^{bcd} | 24.30 ^{ab} |
| Shoot | MAMOLOKWANE | 2212 ^{ab} | 42.12 ^{ab} | 2.050^{abc} | 28.18 | 6,15 | 22.18 ^{bcd} | 14.21 ^{cd} |

^{*}The alphabets represent the significant difference at p<0.05.

3.4.2. The effect of decomposition on CO₂-emission of shoot and root residues of different sorghum cultivars

Generally, CO₂-C emissions increased at the initial stages of incubation and peaked at day 14 with the highest being 157 mg CO₂-C kg⁻¹ C added for OS-POTCH shoots, followed by Mamolokwane shoots (144 mg CO₂-C kg⁻¹ C added). The KZ5246 roots showed the lowest peak of 75 mg CO₂-C kg⁻¹ C added in CO₂ emission (day 14). The CO₂ emissions were higher for shoot than the root residues throughout the incubation period (Fig. 3.1). At the later stages of the incubation (from day 56), all treatments firstly showed drastic decreasing trends of CO₂ emissions, as OS-POTCH shoots emitted higher CO₂ (98.6 mg CO₂-C kg⁻¹ C added) than other treatments, with no significant difference with its roots (93 mg CO₂-C kg⁻¹ C added). A steady decreasing trend was observed thereafter, as all treatments showed less than 50 mg CO₂-C kg⁻¹ C added at day 120, with both root and shoots of KZ5246 showing the lowest CO₂ emission (5.9 mg CO₂-C kg⁻¹ C added), at the end of the incubation.

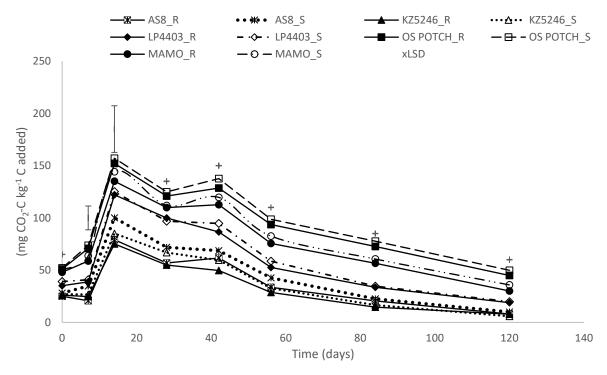


Figure 3.1: CO₂-C emission from roots (R) and shoots (S) of different sorghum cultivars incubated over a 120-day period. Bars represent the LSD (p<0.001)

3.4.3. The effect of decomposition on N mineralisation of shoot and root residues of different sorghum cultivars

Root residues of KZ5246 and AS8 exhibited N immobilization (3 to 1.8 and 2 to 0.5 mg N kg⁻¹) at the initial stages of incubation (from day 0 to day 7), respectively, while other treatments resulted in mineralisation of NH₄⁺-N (Fig. 3.2). Shoot residues had higher NH₄⁺-N than roots for each cultivar over the incubation period. Hence, OS-POTCH shoots had the highest NH₄⁺-N release, with KZ5246 shoots having the lowest N mineralization than the shoots of the other cultivars throughout the whole incubation. Moreover, KZ5246 roots exhibited the lowest NH₄⁺-N release followed by AS8 roots, while OS-POTCH roots had the highest NH₄⁺-N release compared to roots of the other treatments, throughout the incubation. NH₄⁺-N mineralization further varied significantly between the same parts of different cultivars over time. On day 42, NH₄⁺-N peaked at 28.8 mg N kg⁻¹ for OS-POTCH shoots, with KZ5246 shoots showing the lowest NH₄⁺-N (14 mg N kg⁻¹) than other shoot treatments. Moreover, KZ5246 roots maintained lower values of 8.6 mg N kg⁻¹ added N, while OS-POTCH roots showed the highest (25.6 mg N kg⁻¹) N mineralization compared to other root treatments (day 42). Steady decreasing trends of NH₄⁺-N were observed from day 56, following the pattern of high OS-POTCH shoots (22.4 mg N kg⁻¹), followed by Mamolokwane, then LP4303 treatments,

compared to lower KZ5246 roots (6.7 mg N kg⁻¹). All treatments approached the equilibrium stage from day 84 till the end of the incubation. Precisely, OS-POTCH cultivar showed the maximum N mineralization (18.9 and 16.6 mg N kg⁻¹) in both shoots and roots, respectively, while KZ5246 and AS8 roots showed the minimum values (3.9 and 5.7 mg N kg⁻¹), respectively, with less acute differences between their parts, at day 120.

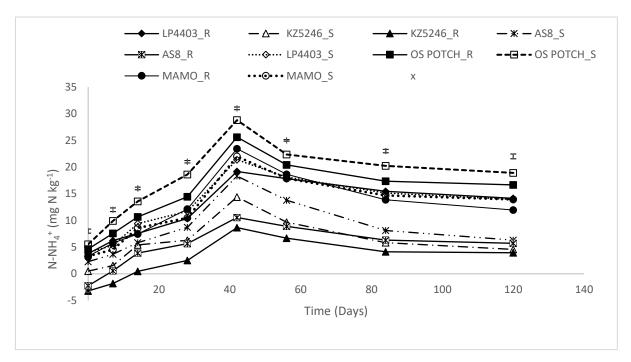


Figure 3.2: NH₄⁺-N concentration from roots (R) and shoots (S) of different sorghum cultivars incubated over a 120-day period. Bars represent the LSD (p<0.001)

Figure 3.3 subsequently revealed that KZ5246 and AS8 roots also exhibited NO₃⁻-N immobilization of 6 to 2 and 5 to 1 mg N kg⁻¹ added N, from day 0 to day 14. The shoot residues also had high NO₃⁻-N than roots for each cultivar, throughout the incubation study (Fig. 3.2). The OS-POTCH shoots exhibited the highest NO₃⁻-N release, while KZ5246 followed by AS8 shoots showed the lowest NO₃⁻-N than the shoots of other cultivars. Moreover, KZ5246 roots exhibited significantly lower NO₃⁻-N mineralization compared to other root treatments, while OS-POTCH showed the highest NO₃⁻-N release throughout the incubation period. Increasing trends of NO₃⁻-N were observed in all high-quality residue treatments from day 7 and peaked at 26.9 mg N kg⁻¹ for OS-POTCH shoots, at day 56, while KZ5246 roots were significantly lower (9.5 mg N kg⁻¹) than other treatments. At the later stages of incubation, all the treatments approached the equilibrium stage from day 84 to day 120. Precisely OS-POTCH followed by

Mamolokwane shoots exhibited higher NO₃-N of 22.7 and 20.5 mg N kg⁻¹ N added respectively, while KZ5246 roots showed lower NO₃-N (9 mg N kg⁻¹) than other treatments (day 120).

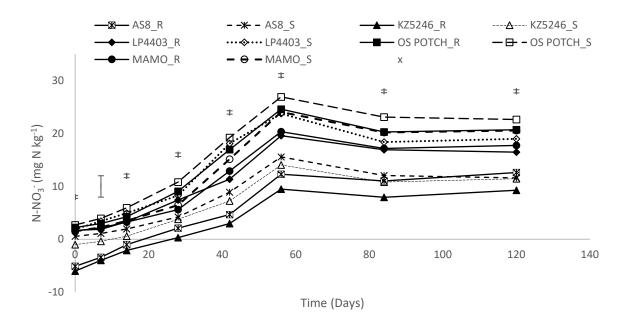


Figure 3.3: NO₃⁻-N concentration from roots (R) and shoots (S) of different sorghum cultivars incubated over a 120-day period. Bars represent the LSD (p<0.001)

3.4.4. Net mineral N and cumulative CO₂ emission in soil amended with root and shoot residues of different sorghum cultivars

Significant differences in cumulative net CO₂-C emissions were also observed among the sorghum residues (p < 0.001, Table 3.4). Thus, OS-POTCH shoots released the highest cumulative net CO₂-C emissions of 771.5 mg kg⁻¹ soil, followed by its roots (734.5 mg kg⁻¹ soil), while KZ5246 and AS8 treatments showed the lowest cumulative net CO₂-C emissions with no significant difference between each other. The LP4403 treatments showed intermediate cumulative net CO₂-C compared to other cultivars, with no significant difference between its parts. In terms of net total mineral N, KZ5246 roots showed lower values (4.59 mg kg⁻¹ soil), followed by AS8 (7.25 mg kg⁻¹ soil), while OS-POTCH roots (25.68 mg kg⁻¹ soil) showed higher net total N release compared to roots of other cultivars. Finally, OS-POTCH shoots had a significantly higher net total N mineralization of 29.7 mg/kg soil than other treatments (Table 3.4).

Table 3.4: Net mineral N and cumulative CO₂ emission of shoot and root residues of different cultivars of sorghum residues, after 120 days of incubation.

| Treatment part | Cultivar | Net CO ₂ -C | Net Cumulative CO ₂ -C | Net NH ₄ ⁺ -N | Net NO ₃ -N | Net mineral N |
|----------------|-------------|------------------------|-----------------------------------|-------------------------------------|------------------------|----------------------|
| Root | AS8 | 38.97 ^d | 305,6 ^d | 4.19 ^{de} | 3.33 ^{cd} | 7,25° |
| Root | KZ5246 | 38.85 ^d | 319,5 ^d | 2.67 ^e | 1.92 ^d | 4,59 ^d |
| Root | LP4403 | 61.99 ^{bcd} | 487,5° | 11.82 ^{bc} | 9.15 ^{abc} | 20,97 ^{abc} |
| Root | OS-POTCH | 92.97ª | 734,5 ^b | 14.67 ^{ab} | 11.01 ^{ab} | 25,68a |
| Root | MAMOLOKWANE | 81.52 ^{ab} | 645,4 ^b | 12.07 ^{abc} | 8.02 ^{abcd} | 20,09 ^{abc} |
| Shoot | AS8 | 47.23 ^{cd} | $378,6^{d}$ | 8.38 ^{cd} | $6.47^{\rm abcd}$ | 14,85 ^{bcd} |
| Shoot | KZ5246 | 35.53 ^d | 282,5 ^d | 3.03^{de} | 4.86^{bcd} | 7,89 ^d |
| Shoot | LP4403 | 63.90^{abcd} | 509,5° | 12.38 ^{abc} | 10.56^{ab} | $22,94^{ab}$ |
| Shoot | OS-POTCH | 93.62a | 771,5 ^a | 17.12 ^a | 12.56 ^a | 29,68 ^a |
| Shoot | MAMOLOKWANE | 75.04 ^{abc} | 649,7 ^b | 11.92 ^{bc} | 10.88 ^{bc} | $22,80^{ab}$ |
| | LSD | 5.87 | 108.3 | 3.18 | 4.04 | 6.56 |

LSD=least significant difference at 5%; figures with same letter within a column are not different at 5% LSD.

The CO₂-C emissions showed negative significant correlation with C: N ratio (r= -0.82) and lignin: N ratios (r= -0.95), at p < 0.05 over 120 days (Table 3.5). However, CO₂ -C emission was not significantly correlated with biomass production. Positive significant correlations were observed between CO₂ -C and initial total N and C concentrations (r= 0.95; r= 0.74, p<0.05), respectively. Similar positive relationships were observed between net N mineralization with initial N and C concentrations (r= 0.95 and r= 0.7) at p < 0.05, over 120 days). Net mineral N negatively correlated with C: N and lignin: N ratios (r= -0.89; r= -0.95, p<0.05), respectively, over the incubation study.

Table 3.5: Correlation matrix of cumulative CO₂-C emissions, total N mineralisation and initial biochemical properties of the root and shoot residues of incubated sorghum cultivars.

| | CO ₂ -C | Total min N | Initial C | Initial N | Lignin | C: N | Lignin: N | Biomass |
|--------------------|--------------------|-------------|-----------|-----------|--------|-------|-----------|---------|
| CO ₂ -C | 1,00 | | | | | | | |
| Total min N | 0,86* | 1,00 | | | | | | |
| Initial C | 0,74* | 0,70 | 1,00 | | | | | |
| Initial N | 0,95* | 0,95* | 0,76 | 1,00 | | | | |
| Lignin | -0,86* | -0,65 | -0,66 | -0,72 | 1,00 | | | |
| C: N | -0,82* | -0,89* | 0,47 | -0,92* | 0,51 | 1,00 | | |
| Lignin: N | -0,95* | -0,95* | -0,79 | -0,99* | 0,77 | 0,88 | 1,00 | |
| Biomass | -0,07 | 0,36 | 0,12 | 0,07 | 0,07 | -0,05 | -0,13 | 1,00 |

^{*}Marked correlations are significant values at p < 0.05.

3.5. Discussion

The large variations in CO₂-C emissions and N release patterns between root and shoot residues after soil incorporation may be due to the effects of their biochemical composition. The highest cumulative CO₂-C emissions in both shoot and roots of POTCH were explained by the highest N tissue and lowest lignin: N ratios in these treatments, while the residues of KZ5246 with the lowest N and highest lignin: N (low-quality) showed the lowest cumulative CO₂-C emissions. This view was supported by the positive correlation of CO₂-C with initial N concentration and the negative correlation with the lignin: N ratio (Table 3.5). Hence, several studies attributed the observed variations to diverse genetic variation within the residues of different cultivars and even crop parts (Lynch et al., 2016; Johnson et al., 2007 Jensen, et al., 2005; Nicolardot, 2001). Carbon assimilation in the residue was associated with their genetic control, which is affected by internal C metabolism and growth dynamics (Weier et al., 1993). Low initial N concentration (<2%) was identified to limit the activity of decomposers, consequently, with widened C: N ratio residues (low-quality) slowing the decomposition rate (Machine et al., 2009; Nicolardot, 2001). However, Johnson et al. (2007); Franck et al. (1997); and Gorissen and Cotrufo (2000) observed a weak correlation between the C: N ratio and decomposition potential of crop residues. Instead, soil proximity was identified to promote microbial interaction with residues and their quick responses to residue addition, leading to early decomposition of soluble substrates (Johnson et al., 2007). In the current study, all the ground residues were incorporated into the same soil (good proximity), such that this effect could have been minimal.

The higher CO₂-C emissions observed at the initial stages of incubation and peaks at day 14, followed by a gradual decrease over time, were related to previous studies stating that labile

organic matter such as water-soluble C and N and other labile substrates including organic acids, amino acids, and simple sugars, are readily available and easily decomposable after residue incorporation to the soil. Moreover, the gradual decrease in CO₂-C observed, showing less than 50 mg CO₂-C kg⁻¹ C added in all treatments (by day 120), was associated with depletion of the labile C substrates. The recalcitrant fraction of SOC such as lignin, cellulose, amongst others tends to decompose slowly and produce less CO₂-C emissions over time. This view was supported by the negative correlation of CO₂-C with and initial lignin: N ratio of the treatments (Table 3.5). In support, Hu et al. (2018) found 58% net release of C from roots and shoots residues during the last 90 to 120 days of incubation. This could be due to variation in biochemical make-up of shoots and root tissues, as shoots can be made up of the identified labile syringyl-units' linkages built-in lignin, which could be easily decomposed than the guaiacyl units in roots, which may form recalcitrant polymers of condensed aryl-aryl lignin linkages (Xu et al. 2017; Talbot et al., 2012; Bahri et al., 2006). Comparative results were produced by Lu, et al. (2003), with 72.3±2.4% of straw residue decomposed, compared with 58.3±1.1% of root residue (p<0.05) at the end of 240 days of incubation. Thus, belowground C has been observed to be better retained in soils than C from aboveground plant parts (Jensen, et al., 2005). Hu et al. (2018) also showed 43% of shoot C and 32% of root C emitted as CO₂ during the first 10 days. Qiua et al. (2015) found >70% of the initial litter-derived dry matter to be decomposed within the first 10 days, with 40% of root C and half of straw C being lost within 60 days of incubation. Greater C mineralization observed could explain an increase in demand for N of the microbial decomposers (Li et al., 2020). While the lower mineralization of root C could be related to their highly recalcitrant C pool influenced by the presence of the suberin fractions (Bending, 1998; Li et al., 2020). Hu et al. (2018) also found that shoot C mineralized faster than root C in the initial phase of decomposition, but after 180 days of incubation, the release of C did not differ between shoots and roots. Based on the current results, cumulative CO₂-C in root and shoot residues of AS8 and KZ5246 would result in higher C storage while OS-POTCH and MAMOLOKWANE will result in higher emissions, with limited differences between the plant parts, when incorporated into the soil. The slow CO₂-C in the former two corresponded with immobilization of N and was further supported by the positive correlation between CO₂-C and Net mineral N.

Immobilization is commonly observed within the initial stages of incubation, just after the application of residues to the soil. This may happen after low N and/or high C: N ratio crop material is incorporated into the soil as it is locked up in microbial biomass (Mary et al., 1996).

The NH₄⁺-N and NO₃⁻-N immobilization found in KZ5246 and AS8 roots at the initial stages of incubation in this study (from day 0 to day 14), can be explained by lower initial N concentrations (1.2 and 1.1%) and higher C: N ratios (30 and 31:1) in both treatments, respectively (Table 3.3). These results agreed with several previous studies (Li, et al., 2013; Jensen, et al., 2005; Curtin et al., 1998). Abiven., (2005) also observed a maximal net immobilization of wheat leaf (31.8 mg N kg⁻¹ dry soil) at day 17. A C: N ratio >25:1 (AS8 and KZ5246 roots) is known to result in net N immobilization, while those with less than 20:1 (OS-POTCH) may result in a high rate of decomposition and net N mineralization (Lynch et al., 2016; Heal et al., 1997). The lack of N immobilization beyond 14 days was attributed to enough C released as CO₂, lowering the C: N ratio to a range that is favourable for mineralization. N mineralization has been identified to occur mostly after significant decomposition of crop residues (Myers et al., 1994; Seneviratne et al., 2000). Furthermore, Machine et al. (2009) observed F292 and F292bm3 sorghum cultivars to have a sharper decrease in soil mineral N than F2 and F2bm1, due to net immobilization happening after 36 days, this was explained by the polysaccharides and lignin concentrations. However, Li et al. (2020), showed nonleguminous crops, specifically shoot residues to have higher immobilization than the leguminous and/or cover crops, due to their higher N concentration.

High-quality residues exhibited increasing trends of N mineralization from the initial stages of incubation and peaked at day 42 to day 56 in the current study. Precisely, OS-POTCH shoots showed maximum NH₄⁺-N and NO₃⁻-N mineralization. Thereafter, all treatments gradually decreased, then steady trends of N mineralization were observed towards the end of the incubation. Similarly, Bending et al. (1998) found the rapid release of mineral N following incorporation of the shoot materials. The rapid N turnover was observed within the first 30 days of the incubation in shoot residues of cover crop, while only during the first 7 days for the roots, with a slower rate. Hu et al. (2018) also found a net N-mineralization of 42% of N added in shoot residues, while only 28% for N in roots. Thus, previous studies observed N mineralization rates to be higher in shoot than root residues (Johnson et al., 2007; Seneviratne et al., 2000). The mineralization of N may not be affected by lignin under non-limiting conditions of N (Seneviratne et al., 2000). Moreover, these observations were associated with the easily degradable nitrogenous substances in high-quality shoot residues (Maheshwari et al., 2014; Johnson et al., 2007). In addition, AS8 roots in the current study showed lower NH₄⁺-N trends compared to their shoot parts, which could be due to the slow rate of decomposition of recalcitrant components.

3.6. Conclusion

The study concluded that carbon sequestration potential and nutrient mineralization varied significantly amongst the residue treatments of different sorghum cultivars. The incorporation of roots and shoot residues from each cultivar had significant effects on CO₂-C emission and N mineralization. The main conclusions drawn from this incubation study were that lower CO₂-C emissions, and lower N mineralization after immobilization of mineral N into microbial biomass were greatly influenced by the low-quality sorghum cultivars KZ5246 and AS8. Secondly, there was high proportional contribution of C and slower release patterns of N in roots with recalcitrant components, compared to their corresponding shoot parts with easily degradable nitrogenous substances.

Based on the results of the current study, higher biomass production cultivars (i.e., AS8 and LP4403) can improve SOC stocks due to high accumulation of organic materials in soils, however, shoot parts tend to decompose faster and emit C. Considering that KZ5246 and AS8 root were the lowest cumulative CO₂-C emitters, these cultivars could be the most ideal for carbon sequestration (C stocks), while OS-POTCH and MAMOLOKWANE are less ideal, but would be beneficial for N cycling. However, the results suggest further investigation on the microbial influence on crop residue decomposition under open environmental conditions, (where soil moisture and temperatures are not controlled), including the contribution of the soil physicochemical parameters to residue decomposition potential.

4. CHAPTER FOUR:

DECOMPOSITION OF ROOT AND SHOOT RESIDUES OF DIFFERENT SORGHUM CULTIVARS IN A WHEAT FIELD IN KWAZULU-NATAL, SOUTH AFRICA

Abstract

Decomposition of sorghum residues may impact soil organic carbon (C) and nitrogen (N) mineralization, with effects on soil quality and climate change mitigation. Despite the existence of numerous studies on crop residue decomposition in soil, the impact of crop cultivars on decomposition potential is not well established. In the present litterbag study, five sorghum cultivars (AS8, KZ5246, LP4403, OS-POTCH, and MAMOLOKWANE) separated into roots and shoots were studied under field conditions to assess the effects of their biochemical properties on decomposition potential. The experiment was set up in a randomized complete block design in a winter-wheat field, with triplicates to allow for destructive sampling at 0, 14, 28, 42, 56, 84, 112, and 168 days. Decomposition potential was assessed based on the percentage weight of residues remaining, over time. The remaining residues were analyzed for total C and N concentrations, using the LECO Trumac auto-analyzer, and for lignin concentration using the ANKOM-200 technology method. The dry matter (DM) remaining of all five sorghum cultivars and parts, either roots or shoots decreased with time. At day 42, >50% of the initial DM had decomposed in high-quality residues. By the end of the incubation (168 days), less than 12.5% residue DM remained in all residue cultivars and parts. MAMOLOKWANE and OS-POTCH shoots in particular had the lowest (average 0.9 %) DM remaining. On the other hand, increasing trends of C concentration were observed in the initial stages of decomposition, with AS8 (both roots and shoots) showing the highest concentrations (57.5 and 45%), respectively, compared to other cultivars. On day 168, the MAMOLOKWANE shoot showed lower C concentration (2%) compared to its root and other cultivars. In terms of total N concentration, all residue treatments rapidly decreased to <1 % N, from day 14 to 28. At the end of the incubation, OS-POTCH shoots showed lower N concentration (0.9%) than its roots and other treatments. While high C: N and lignin: N ratios were observed at the initial stages of decomposition, KZ5246 followed by AS8 root had the highest ratios. Over time, all the treatments, maintained lignin: N and C: N ratios of less than 25. Overall, KZ5246 and AS8 root parts would be useful for retaining C and N balance in soils.

Keywords: Carbon dioxide, Decomposition, Drymatter remaining, Sorghum residues,

4.1.Introduction

The carbon (C) and nutrient dynamics in soil have attracted unprecedented global attention, in recent times, due to the escalating amounts of carbon dioxide (CO2) emissions and nutrients released during the decomposition of organic materials in an agroecosystem. In the past few decades, global CO₂ and N₂O concentrations in the atmosphere increased at rates of 0.5% and 0.3% per year, respectively (Wang et al., 2013; Tongwane et al., 2016). The total soil organic carbon (SOC) in South Africa (SA) (5.6 Pg C) is ca. 0.37% of the global terrestrial SOC (1500 Pg C) (Venter et al., 2021). Agriculture is a key contributor to climate change, globally, being responsible for about 14% of all GHG emissions (National Climate Change Response white paper, 2021). The sorghum residues (12%) retained in the fields after harvest in SA, in 2012, contributed about 2150 tonnes of CO₂ emissions (DAFF, 2013; Tongwane et al., 2016). Global warming, as a result of high CO₂ emissions, causes climate change, marked by great variability in annual precipitation and high annual temperatures (Helme, 2001). On the other hand, nutrients cycles are altered, contributing to poor soil fertility, especially in sub-Saharan Africa (SSA) (Ajwa and Tabatabai, 1994). This mostly affects the communal farmers who cannot afford chemical fertilizers and rely upon rain-fed agriculture for sustainable crop production. The potential of decomposition associated with CO₂ emissions along with N mineralization is often used as a proxy in evaluating the potential of plant residues to balance nutrient dynamics in soil.

In most agricultural practices in SA, crop residues are either retained on the surface after harvest (i.e., as mulch) or incorporated into the soil (during tillage) (Fu, 1987; Abiven et al., 2005; Rezig, 2014). The retention of crop residues after harvesting was reported to contribute to lower CO₂ emissions (13%) compared to the application of mineral fertilizers (87%) in SA soils (Tongwane et al., 2016). The decomposition of crop residues is therefore affected by their management and species differences. Residues of cereal crops left on the field after harvesting were found to emit higher CO₂ (72%) than 28% emitted by legumes, oilseeds, vegetables, and other field crop residues in SA, in 2012 (Tongwane et al., 2016). Thus, maize emitted the highest CO₂ (60.6%) than wheat, sunflower, soyabean, and sorghum (10.8, 2.5, 1.0, and 0.3%), respectively (Tongwane et al., 2016). Such high emissions in cereal crop residues were attributed to their high biomass and large total areas they are planted on (Mathew et al., 2012;

Tongwane et al., 2016). Where the same amounts of residues were considered between the crops, Xu et al. (2017) found soyabean to significantly have a lower proportion of remaining mass (43%, p<0.05) than maize (48%, p<0.05) and wheat (55%, p<0.05) residues in a litterbag decomposition experiment. Legumes tend to have lower lignin, C: N, and lignin: N than cereal crops. Thus, Sainju et al. (2005), reported that ryegrass and grazing vetch had C: N of 29: and 9:1 respectively, making the decomposition of legumes more rapid. In addition to species differences, there could also be variations between cultivars of the same crop species, in terms of biochemical composition and its resultant effects on the decomposition of these residues. Villegas-Pangga, (2000) reported significant variation in decomposition potential of residues from different cultivars of rice straw, with *Soc Nau* showing a high rate of decomposition (38.4%) followed by *Suakoko8* (30.3%) then *Intan* with 14.2%. Overall, these were attributed to differences in the genetic composition of different crops as a result of their C: N; lignin: N ratios and lignin concentration amongst other factors.

Half of the human food consumption in SSA is from cereals (such as maize, wheat, rice, millet, and sorghum) (Tongwane et al., 2016). Sorghum is mostly grown both for food security in low-input smallholder areas and for commercial purposes, as input for beer brewing and in biofuel production (Prakasham et al., 2014). Potential variation in biochemical composition of the sorghum cultivars, and between their shoot and root residues could affect their decomposition and mineralization of carbon and nitrogen. Sorghum lignin concentration and composition may vary due to natural mutation in the genes (Prakasham et al., 2014). For instance, the sweet and forage stems of sorghum cultivars contain large quantities of soluble and insoluble carbohydrates (Prakasham et al., 2014). In addition, brown midrib sorghum forages usually contain less lignin (< 6%) due to modifications in their lignin biosynthesis pathway. While sweet sorghum has 45% cellulose, 27% hemicellulose, high biomass sorghum cultivar ranges from 27 to 52% cellulose, 17 to 23% hemicellulose, and 6.2 to 8.1% lignin concentration (Prakasham et al., 2014). Therefore, identifying crop cultivars that sequester high C and retain and supply nutrients, has been identified as an important strategy to mitigating climate change and improving soil fertility in the SSA agroecosystem.

Decomposition may alter the compositional quality of sorghum crop residues over time, with a potential to impact their C and nutrient release patterns in soil (Gunnarsson and Marstorp, 2002). Kriaučiūnienė (2012), reported a decrease in hemicellulose and cellulose concentrations with time due to utilization as microbial carbon and energy sources, while lignin concentration was resistant to microbial attack. The lower quality of root residues makes them more resistant to decomposition, and may contribute to the build-up of C stock, and affect nutrient balance in

the soil, than shoot residues (Spain and Hodgen, 1994). However, the differences between root and shoot residues could depend on cultivars. Hence, the objective of this study was to assess the decomposition pattern of shoot versus root residues of different sorghum cultivars, under field conditions. The findings of this study will be essential in identifying cultivars that have greater potential for increased storage of carbon in soils, with positive contributions to carbon sequestration and available N especially from those with high biomass production.

4.2. Method and Materials

4.2.1. Site description

A litterbag experiment was conducted at the University of KwaZulu-Natal research farm, Ukulinga, located at the Pietermaritzburg campus (KZN), in South Africa (-29°, 66' S and 30°.40' E and about 768 m of altitude above the sea level). This area has warm to hot summers with a mean monthly maximum temperature of 26°C, and mild winters with a mean monthly minimum temperature of 8.8°C (Swemmer et al., 2007; Zeglin et al., 2007), and annual rainfall of 694 to 738 mm (Morris and Fynn, 2001; Zeglin, et al., 2007; Mwadzingeni et al., 2016). The soils at this site were classified as Dystric Regosols according to the Soil and Terrain Database for southern Africa (McGranahan et al., 2016), with 30.7% clay, pH 4.8 (KCl), 2.5% organic carbon (OC), and 0.18% total N, (Chikuvire et al., 2018; Phophi et al., 2017).

4.2.2. Wheat planting design:

A BWI62 wheat cultivar was grown in the field, divided into three blocks, based on slope positions. Each plot was 5 x 5 m in size, with 1 m separating distance between the plots. The trial was tilled and supplemented with a basal fertiliser (2:3:2); N: P: K, at 25 kg N ha⁻¹ after disking, as recommended for normal wheat growth. Each plot was made up of 10 wheat rows, with about 45 cm of inter-row and 10 cm intra-row spacing.

4.2.3. Litterbag experimental layout and design:

The five different sorghum cultivars (AS8, KZ5246, LP4403, OS-POTCH, and MAMOLOKWANE), selected and characterised in the previous chapter were used in this study. These residues were oven-dried at 65°C temperature for approximately 72 hours and chopped into 2–4 mm lengths, then 10g of each treatment were evenly distributed into a 20 × 40 cm nylon organza bag of <1 mm pores, before stapling and weighing the initial residue mass. The experiment was laid out in a split-plot design with the main plot factor being sorghum plant parts with two levels (either shoots or roots), while the subplot factor was

different sorghum cultivars with five levels (AS8, KZ5246, LP4403, OS-POTCH, and MAMOLOKWANE). The treatments were completely randomized within the three blocks.

Then sorghum residues were buried in the prepared wheat growing plot, with the aim to monitor the decomposition potential of different sorghum cultivars partitioned into roots and shoots, under field conditions. In each block, nine subplot holes of about 10 cm deep, with a width of the size of the dimension of the litterbags were opened by a spade, with 1m spacing separating the subplots. In each subplot hole, separate 5 litterbags of the five cultivars (either root or shoot per subplot-hole) were evenly placed in one slot (with no contact, to avoid contamination). Then they were buried using the surrounding soils, to create contact between the litterbags and the soil. There was enough replication for destructive sampling. Average temperatures and rainfall during the study period are shown in Appendix: D 1., (WorldWeatherOnline, 2021). Supplemental irrigation was applied using sprinkler irrigation, while weed control was done by hand hoeing, when necessary.

Overall, 80 litterbags were then recovered from all the plots (i.e., roots and shoots of 5 different cultivars) at different sampling times of 0, 14, 28, 42, 56, 84, 112, and 168 days. At each sampling period, the ten litterbags collected were gently brushed to ensure that the adhering soil was removed, then transported to the laboratory. The collected litterbag samples were oven dried (70°C for 48 hours) and measured for dry weight remaining, which represented the percentage residue weight remaining after decomposition. These were then analyzed for total C and N using the LECO auto-analyser. The residues lignin and cellulose concentrations were also analyzed by the filter bag technique (ANKOM-200 technology method).

4.3. Statistical analyses

All the data on percentage residue and biochemical properties were subjected to analysis of variance (ANOVA), and treatment means were separated using the least significant difference (LSD) test at p< 0.05. Correlation matrixes were calculated among the variables, to determine the strength of associations between biochemical properties and percentage residue remaining at p<0.05, using Statistica 10.0 software (Table 4.3).

4.4. Results

4.4.1. The effect of decomposition on remaining dry weight of different sorghum residues.

The dry-matter (DM) remaining of all five sorghum cultivars and parts, decreased with time (Fig. 4.1 and Table 4.1). Generally, the rate of weight loss was higher for high-quality cultivars, specifically the shoot parts (Table 4.1). On day 14, 60.6 and 62.9% of initial OS-POTCH and Mamolokwane shoot DM remained, respectively (Fig. 4.1). Root parts of OS-POTCH and Mamolokwane residue, by comparison, had high DM remaining than the shoots, however, they both (roots and shoots) exhibited rapid DM decomposition than the residues of other treatments. At day 42, high-quality residues had less than 50% DM remaining, with Mamolokwane shoots (26.8 %) and roots (34%) having the lowest DM remaining, followed by both shoots and roots (31.8%, 43%) of OS-POTCH, respectively, compared to AS8 and KZ5246 (both roots and shoots) (>50% DM remaining). The percentage weight loss showed a 64.9% increase between Mamolokwane and AS8 shoots (from 47.9 to 16.8 % DM remaining), respectively. While an increase of 55.5% (from 57.79 to 25.7 % DM remaining) between their root parts, respectively, were observed at day 56. Moreover, AS8 had the highest while Mamolokwane had the lowest DM remaining compared to other residue cultivars at day 56. DM remaining exhibited decreasing trends for all the treatments, from day 56 to day 112. Hence, at the end of the incubation (day 168), there was <12.5% residue DM remaining for all the treatments. In comparison, Mamolokwane and OS-POTCH shoots had the lowest DM remaining (0.88 and 0.91 %) than their roots (1.8 and 4.5%), respectively, and were also lower than the other residue treatments.

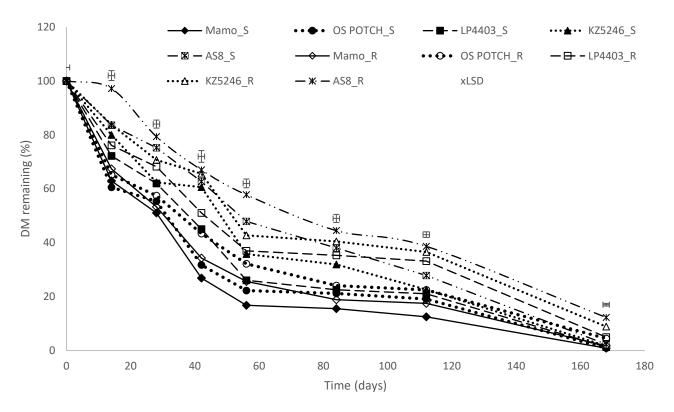


Figure 4.1: Change in dry weight remaining of different sorghum residue treatments (Mamo is Mamolokwane: "R" is root and "S" is shoot).

Table 4.1: Regression equations of percentage of the shoot and root residues of sorghum cultivars remaining over time (x = incubation time in days)

| Cultivar | Part | Equation | \mathbb{R}^2 |
|----------|-------|-------------------------|----------------|
| AS8 | Shoot | $y = 132.63e^{-0.019x}$ | 0.876 |
| AS8 | Root | $y = 111.57e^{-0.012x}$ | 0.961 |
| KZ5246 | Shoot | $y = 127.24e^{-0.021x}$ | 0.905 |
| KZ5246 | Root | $y = 104.59e^{-0.013x}$ | 0.930 |
| LP4403 | Shoot | $y = 114.15e^{-0.022x}$ | 0.899 |
| LP4403 | Root | $y = 101.95^{-0.014x}$ | 0.878 |
| OS POTCH | Shoot | $y = 105.58e^{-0.024x}$ | 0.900 |
| OS POTCH | Root | $y = 88.585e^{-0.015x}$ | 0.914 |
| MAMO | Shoot | $y = 97.527e^{-0.025x}$ | 0.942 |
| MAMO | Root | $y = 91.291e^{-0.019x}$ | 0.847 |

4.4.2. The effect of decomposition on chemical composition of different sorghum residues. The quality of residues of different sorghum cultivars, determined by the initial C, N, lignin concentrations as well as C: N and lignin: N ratio differed significantly over time. For instance, at day 14, an increase in C concentrations was observed, with AS8 and LP4303 roots being the highest (57.5 and 54%), compared to their shoots (45 and 33.9%), respectively, and other residue treatments (Fig. 4.2). At day 56, C concentration peaked at 60% for KZ5246 root, being higher than its shoots (48.5 % C). Thereafter, decreasing C trends were observed in all the treatments, as a rapid decrease in KZ5246 roots (36% C), to even lower than it shoots (43 %C) was observed at day 84. Then all residue treatments showed decreasing trends, while AS8 (both root and shoots) showed a constant pattern towards the end of the incubation. Ultimately, at day 168, AS8 (both root and shoot) had higher C concentration (32.6 and 29.5%C), respectively, while Mamolokwane shoots had lower C concentration (2%) compared to its root and other treatments.

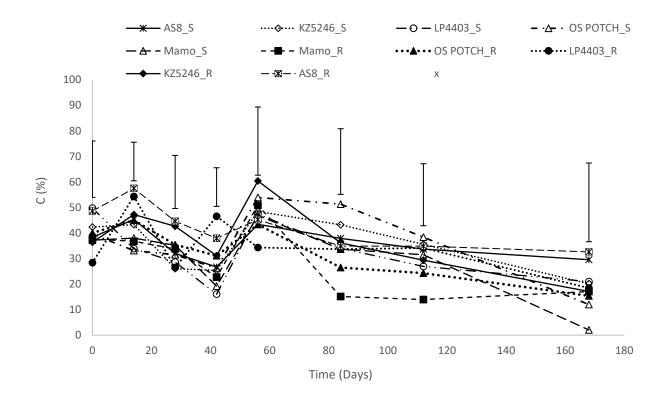


Figure 4.2: Change in C concentration of different sorghum residue treatments with time. (Mamo is Mamolokwane: "R" is root and "S" is shoot).

The lignin concentration of different treatments decreased over time (Fig 4.3). A higher decrease of 24% lignin concentration (27.6 to 20.9 %) was observed in Mamolokwane shoots, than the 11% decrease found in its root parts between day 14 to day 28 (Fig 4.3). This led to lower lignin concentration found in Mamolokwane shoots compared to other treatments, at day 28. However, at the end of the incubation, Mamolokwane shoots had the highest lignin concentration (20%) than its roots, while AS8 (both roots and shoots) had lower lignin concentration (16%) than the other treatments at day 168.

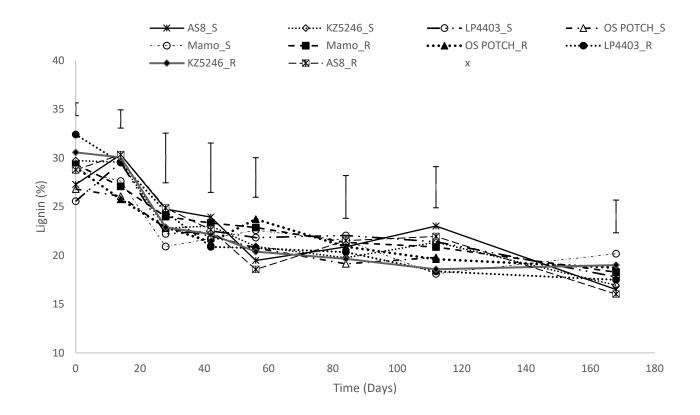


Figure 4.3: Change in lignin concentration of different sorghum residue treatments with time. (Mamo is Mamolokwane: "R" is root and "S" is shoot).

In terms of total N concentration (Fig. 4.4), all the treatments rapidly decreased to less than 1.3 % N, at the early stages (day 14 to 28). As from day 42, increasing trends of N concentration were observed in all cultivars, as LP4303 roots showed a higher increase (1.6 %N), compared to its shoots (0.6 %N) and other treatments. The N concentration remained more or less constant between days 84 to 112 for all treatments, with OS-POTCH shoots having higher N concentration (average of 2.6 %N). At the end of the incubation (day 168), N concentration decreased significantly to less than 1.5 %N for all sorghum treatments.

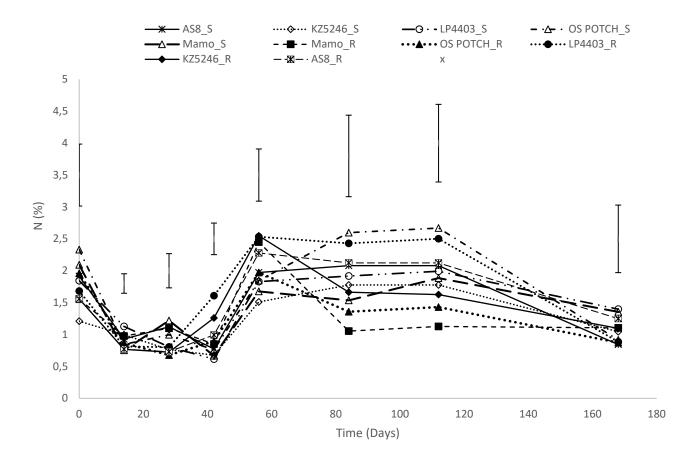


Figure 4.4: Change in N concentration of different sorghum residue treatments with time. (Mamo is Mamolokwane: "R" is root and "S" is shoot).

The above trends resulted in decomposition having a significant impact on sorghum residue C: N ratio over time (Fig 4.5). Initially, the highest C: N ratios were observed on day 14, with KZ5246 roots having the highest ratio (59), while AS8 shoots showed the lowest ratio of 41.9. Thereafter, decreasing trends of C: N ratios were observed, as all treatments, maintained C: N ratio of 25 or less from day 84, then remained more or less constant until the end of the incubation (day 168).

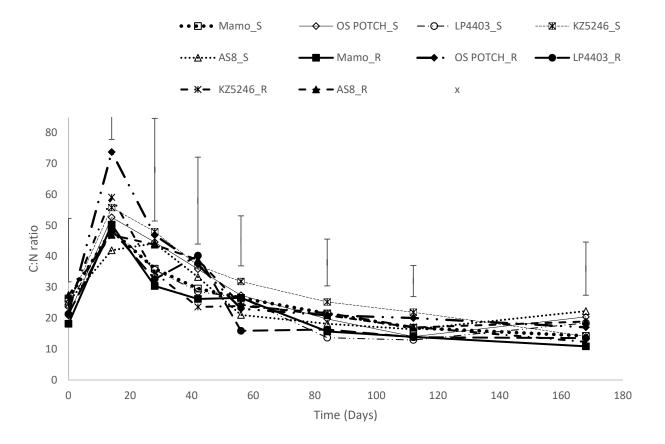


Figure 4.5: Change in C: N ratio of different sorghum residue treatments with time. (Mamo is Mamolokwane: "R" is root and "S" is shoot).

Lastly, the lignin: N ratio also showed significant variations in different sorghum treatments, over time (Fig. 4.6). Higher lignin: N ratio of 38.8 was observed for AS8 roots, while Mamolokwane shoots exhibited the lowest (28), at the early stages of decomposition (day 14). However, a 77.7% decrease of lignin: N ratios was observed for KZ5246 roots, followed by AS8 roots with 66%, between day 42 to day 56. Overall, AS8 root and Mamolokwane shoot exhibited the lowest lignin: N ratio (13 and 15), respectively, compared to other treatments, by the end of the incubation (day 168).

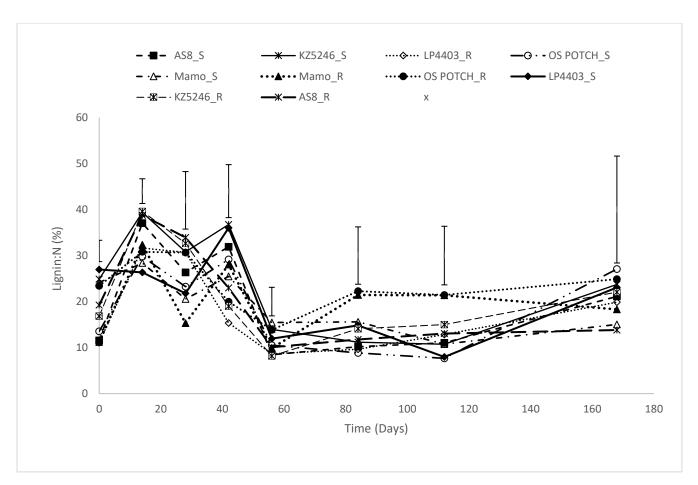


Figure 4.6: Change in lignin: N ratio of different sorghum residue treatments with time. (Mamo is Mamolokwane: "R" is root and "S" is shoot).

Table 4.2 indicates correlations between the residue DM remaining with initial biochemical properties of different sorghum treatments. DM remaining showed no significant correlations with initial total C and N. Negative correlations were observed with temperature and rainfall (r= -0.81 and r= -0.75), respectively, at p < 0.05 over 168 days. However positive correlations were found between DM remaining and initial lignin concentration (r= 0.14), C: N (r= 0.21) and also with lignin: N ratios (r=0.20), at p < 0.05, over time.

Table 4.2: Correlation matrix table of percent drymatter and initial biochemical properties of the roots and shoots residues of different sorghum cultivars.

| | Total C | Total N | Total P | Lignin | C: N | Lignin: N | Biomass | Temp | Rainfall | DM remaining |
|-----------------|---------|---------|---------|--------|-------|-----------|---------|--------|----------|-----------------|
| Total C | 1,00 | | | | | | | | | |
| Total N | 0,79* | 1,00 | | | | | | | | |
| Total P | 0,31* | 0,29 | 1,00 | | | | | | | |
| Lignin | -0,70* | -0,72 | -0,19 | 1,00 | | | | | | |
| C: N | 0,51* | -0,92 | -0,17 | 0,51 | 1,00 | | | | | |
| Lignin: N | -0,84* | -0,99* | -0,30* | 0,77* | 0,88* | 1,00 | | | | |
| Biomass | 0,11* | 0,01 | 0,18 | 0,24* | 0,05 | 0,03 | 1,00 | | | |
| Temp | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 1,00 | | |
| Rainfall | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,49* | 1,00 | |
| DM remaining | -0,14 | -0,22 | -0,15 | 0,14* | 0,21* | 0,20* | 0,02 | -0,81* | -0,75* | 1,00 |

DM remaining: Dry matter remaining of residues of different sorghum cultivars of the shoot and root after 168 days; - Total \dot{C} : Initial residue total carbon, - Total N: Initial residue total nitrogen; Temp: mean temperatures during the incubation period (168 days); Rainfall: mean rainfall during the incubation period (168 days), Biomass: initial residue biomass; -*: Marked correlations are significant at p < 0.05.

4.5. Discussion

4.5.1. Residue weight remaining over time

The difference in dry-matter remaining between the different sorghum residues treatments is likely due to low quality (high lignin: N and C: N ratios) in AS8 and KZ5246 cultivars, mainly their roots compared to their shoots and those of OS-POTCH and Mamolokwane. Villegas-Pangga (2000) and Prakasham et al. (2014) attributed the differences of decomposition potential to genetic variation between different sorghum treatments. Relative to the initial stages (14 days), the high rate of decomposition in OS-POTCH and Mamolokwane residues, specifically their shoots, maybe due to their low C: N and lignin: N ratios, which exposed them to fast-growing soil microorganisms that rapidly degraded them (Geisseler et al., 2011). Studies show that the high N concentration of high-quality residues (i.e., OS-POTCH and Mamolokwane treatments) enhances microbial activity to initiate decomposition (Hadas et al., 2004; Gezahegn, et al., 2016). Hence, the high initial N concentrations (>2%) in OS-POTCH and Mamolokwane shoot played a significant role in promoting early decomposition mostly in highly soluble constituents (Swift et al., 1979; Bending, 1998; Kriaučiūnienė, 2012; Fioretto et al., 2014). Similar results were found by Ruhland et al. (2018), where residues of a wild-type sorghum cultivar showed low mass loss (average 50.8%) than the bmr6 gene (57.4%) and

bmr12 (61.7%). These results were attributed to higher initial N concentrations (1.19; 1.4%), lower initial lignin: N (1.8; 2.1) and C: N ratios (30.2, 25.8) of bmr6 and bmr12 mutants, respectively, compared to the wild-type cultivar (Spain and Hodgen, 1994; Daudu et al., 2009; Ruhland et al., 2018).

In this study, the less than 50% DM remaining found in most sorghum cultivars, precisely high-quality residues (Mamolokwane, and OS-POTCH treatments), at day 56, could be due to narrowed lignin: N ratios (less than 20). Previous studies identified that lignin: N ratios of less than 25, tend to increase residue decomposition over time (Daudu et al., 2009; Zwahlen et al., 2003; Osono and Takeda, 2004; Kamota et al., 2014). Furthermore, lignin is known to increase concentrations of manganese, which is essential for some lignin-degrading enzymes in the later stages of decomposition (Berg et al., 1995; Fioretto, et al., 2014). Henceforth, in this study, the <12.5% residue DM remaining for all the treatments by the end of the incubation, was attributed to the degradation of the recalcitrant constituency. Moreover, the favourable climatic factors, such as the high rainfall (236 mm) and good average temperature (22 °C) in January 2020 (Appendix D. 1), stimulated microbial activity and greatly contributed to the later decomposition.

In addition, variation in DM remaining was observed between the roots and shoots, with KZ5246 and AS8 (roots) showing higher DM remaining compared to shoots of other treatments, over time. These observations could have been influenced by complex mass fractions in roots than the other plant parts (i.e., stem, cobs, leaves, or husks) (Xue et al., 2012). Bending, (1998); Abiven, (2005) explained the roots' highly recalcitrant C pool by the presence of the suberin, which forms complex barriers when associated with lignin leading to reduced rate of decomposition. In support, Yanni et al. (2011) showed roots of maize Bt gene type (Bacillus thuringiensis) to have less decomposition because of their high lignin concentration (11.7%), than its leaves (3.3%). On the other hand, shoots materials were found to have higher N concentration, carbohydrate and water-soluble C and N constituents, which constitute the most labile components of plant residues (Bending, 1998).

4.5.2. Carbon mineralization over time

Generally, net C and N mineralization, C: N, and lignin: N ratios of residues decreased over time (168 days), regardless of their initial residue quality. For instance, constant decreasing trends of C were observed in all the treatments as from day 56, down to a minimum value of 2% C found in Mamolokwane shoots, by the end of the incubation. These observations were

similar to what was observed for DM loss, in this study. Lynch et al. (2016), explained the faster C mineralization found in sorghum-sudangrass (86% average), to be due to greater contact with soil microbial biomass. Contrary, retention of sorghum residues is known to reduce C loss and contribute to SOC stocks (Lynch et al., 2016). Hence, the increased C, at day 14 in our study, with AS8 roots showing higher total C (57.5 %), compared to their shoots (45 %) were attributed to their high initial C concentration (38.8 %), despite the high biomass production of 2198 g.m⁻² (Table 3.3), in shoots. However, Mathew, et al., (2017) reported higher biomass production potential in carbon-rich clayey soils of tropical humid areas to contribute to C transfer to soils. The higher total C concentration (32.6 roots and 29.5 % shoots) found in AS8 treatments, compared to the 2% found in Mamolokwane shoots at the end of the incubation (day 168), were attributed to higher C: N and lignin: N ratios in AS8 treatment, which were found to decompose slower and release less C and N over time. Apparently, over time C can be non-lignified, becoming more accessible to microbial activity, also, the ability of decomposer to decompose soil organic matter for their living (priming effect), may lead to increased C loss beyond C addition (Fontaine et al. 2003; Abiven and Ajwa, 2005; Kuzyakov, 2010).

4.5.3. Nitrogen mineralisation

Initially, all cultivars (roots and shoots) rapidly decreased to < 1.3 percent of N, from day 14 to day 28. This suggests high demand of N for microbial activity in the early stages of decomposition, where soluble and easily degradable N compounds were mineralized (Recous, 1994). The decreased N concentration (<1.5 %) observed with all sorghum treatments at day 168, in our study, implies the net mineralization of N (Lynch et al., 2014). On the contrary, from day 42, there were increasing trends in N concentrations for all cultivars. The net gains in total N concentration can be explained by the immobilization of exogenous N after initial losses of soluble or readily mineralizable N (Fox et al., 1990; Recous, 1994; Kiem and Kögel-Knabner, 2003). Previous studies also found that the decomposition of plant residues and mineralization of N can be reduced by lignin concentrations of greater than 15% (Recous, 1994; Daudu et al., 2009). Moreover, residues with higher initial C: N ratios (more than 20:1) can mineralize N at slower rates than those with lower initial ratios (Heal et al., 1997; Lynch et al., 2014). Abiven et al. (2005), obtained maximal net immobilization of 31.8 mg N/kg soil for wheat leaf (C: N ratio of 59.3), at day 17 and the highest net mineralization of 65.1 mg N/kg soil was obtained for soyabean leaf (C: N ratio of 10.7), at day 100.

4.5.4. Carbon to nitrogen ratio

The high C: N ratios observed at day 14, with KZ5246 root having the highest ratio (59), with a 5% decrease between its shoots were associated with high initial C: N residues, following the utilization of initially available N concentrations, leading to widened C: N ratio (Hadas, et al., 2004; Wilson and Raymer, 1992). In support, Spain and Hodgen, (1994) observed a high initial C: N ratio (171) of sugarcane residues which increased to a maximum value of 241 after 42 to 75 days. Contrary, a decrease in C: N ratios from residues of maize were observed by Lynch et al., (2016), in support, Kamota et al. (2014) observed different maize cultivars, where the C: N ratio of DKC75-15B decreased from 17.6 to 16.7, while CRN3505 was from 16.4 to 14.1, between 8 to 12 weeks of the incubation. The decreasing trends of C: N observed for all the treatments of less than 25.5 from day 84, approaching more or less constant trends towards the end of the incubation in our study, were attributed to the release of insoluble C, following less microbial biomass C effects.

4.5.5. Lignin to nitrogen ratio

Lastly, the decomposition of plant residues and mineralization of N can be reduced by a lignin: N ratio greater than 23 to 25 (Daudu et al., 2009). Fioretto et al. (2014) identified high lignin constituents associated with N to form new and stable complexes, leading to a reduced rate of decomposition. Also, lignin is likely to degrade at the later stages of decomposition only after the mass loss of insoluble substances, hence, in this study lower lignin: N ratio (13) was found in AS8 roots, at day 168 (Abiven et al. 2005).

4.6. Conclusion

Initial roots and shoots residue quality (i.e., initial C, N, lignin concentrations and C: N, lignin: N ratios) of different sorghum cultivars affected C and N mineralization rates and DM loss, via decomposition. Two main conclusions can be drawn from this field litterbag study. The first conclusion was that KZ5246 and AS8 treatments (i.e., low-quality sorghum cultivars) had higher DM remaining, which increases OM and C stocks stabilization in soils, over time. While increasing N concentrations were observed with LP4403 and OS-POTCH treatments in most cases, which may contribute to N cycle and plants N uptake in soils. The second conclusion was that there was high proportional contribution of roots to the SOM, due to lower rates of material degradation. As a result, N availability and C stabilization in soils is expected more in roots than shoot residues.

These results yield a better understanding of C and N allocation within plant parts and potentially to the soils and could be used as criteria for the selection of sorghum cultivars with low decomposition potential for soil quality and climate change mitigation purposes. Amongst the five sorghum cultivars under study, KZ5246 and AS8 (i.e., root parts), would be more useful to build-up of soil organic matter due to low decomposition potential. While the other treatments, precisely, those of OS-POTCH and Mamolokwane (i.e., shoot parts) with high biomass production would be useful for nutrients (like N) availability in soils.

However, the existence of multiple interactions coming from climatic, microbial, and soil factors, were also observed to influence the decomposition potential. In our case, high rainfall and temperatures of 22°C summer season (January 2020 in SA, KZN) presented increased decomposition and high C and N mineralization.

The results suggest further investigation on the main controlling factors of crop residue decomposition under controlled environmental conditions. Further investigation is necessary in quantifying the effect of soil microorganisms on the terrestrial carbon cycle, up to a global scale.

5. CHAPTER FIVE:

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1.General Discussion

From Silver and Miya, (2001) it was learnt that the prediction of crop residue decomposition in soils can be obtained by simultaneously considering crop residue quality (i.e., C: N, and lignin: N ratio), climate (i.e., temperature and moisture), and soil factors (i.e., soil texture and pH) which determine the fate of C and N inputs and outputs in soils (Johnson et al., 2007). Hence, the highest CO₂ emissions were observed in the meta-study from legumes (with alfalfa exhibiting the highest emissions) as compared to cereals (maize showing the lowest emissions). These observations were linked to the incubation and litterbag studies which presented higher dry-matter (DM) loss, and N mineralization for high-quality sorghum cultivars (i.e., OS-POTCH) and shoot part residues. The variation in CO₂ emissions and nutrients release from residues of different crop types and/or cultivars emanate from differences in their structural compositions (El-Naggar, et al., 2019). It was also observed from previous research that the rate of crop residue decomposition is determined by crops' genetic structure, and chemical composition of different plant parts, i.e. either supportive tissue (stem), conductive tissue (leaf), or protective tissue (root) (Ma and Petersen, 2003). Previous studies indicate that legumes, unlike cereals, benefit from bacterial N fixation that favors residue decomposition (Johnson, et al. 2007; Gezahegn, et al. 2016). Moreover, greater legume decomposition might come from the observed higher initial residue N concentration (Gezaheg, et al. 2016). These authors observed legume tissue N concentration of 2.5% as compared to maize (<2% N). Leguminous and more easily biodegradable substrates have been identified to have lower values of lignin in most cases (Trinsoutrot et al., 2000).

The increased CO₂ emissions observed in the meta-analysis under low residue N, but higher C concentration could be attributed to C substrate solubility. The readily available substrates are unlikely to be affected by N-limitation of the microbial growth to decompose, but more likely to influence the production of extracellular enzymes (Jingguo and Bakken, 1997). These require relatively little N to maintain the maximal rate of decomposition (Schimel and Weintraub, 2003). Other studies found labile substrates to easily decompose and release C and N at the early stages of decomposition, due to stimulated microbial activity (Bending, 1998; Jensen et al., 2005). These observations corresponded to the results of the decomposition studies, which were associated with initial higher C loss through microbial respiration with high amounts of available carbon that were readily utilized by decomposers. Another explanation for high CO₂ emission from high C concentration residues is the fostering of microbial activity and the decomposition of supposedly stable organic matter due to the higher

availability of energy released from the decomposition of fresh organic matter (Fontaine et al., Nature 2004). Moreover, the results of this study showed maize residues to have the highest stability index of CO₂ emissions. Thus, the CO₂ emitted from the added residue C in soils was identified to emanate from the amount of dry material decomposed over time (Lynch et al., 2014). Decomposition tends to be retarded by highly insoluble substrates resulting in high C sequestration potential over the long term.

In the incubation study, root residues of KZ5246 and AS8 (1.1 and 1.2 % N) respectively, exhibited N immobilization at the initial stages of incubation (from day 0 to day 7), while other cultivars resulted in the ready release of NH₄⁺-N. Similar patterns of NO₃⁻-N immobilization were observed for KZ5246 (6 to 2 mg N kg⁻¹ added N), and AS8 roots (5 to 1 mg N kg⁻¹ added N), from day 0 to day 14, respectively. In addition, CO₂-C emissions showed a significant increase at the initial stages of incubation (peaked at day 14), with the highest emissions observed in OS-POTCH shoots (2.4 % N), while KZ5246 roots (1.1 % N) showed the lowest. In support, N immobilization was observed immediately after the addition of maize residues (C: N of 60: 1) in soils, in a study by Sakala et al. (2000). Huang et al. (2004) found sugarcane stalk with a higher C: N ratio to presumably stimulate NH₄⁺-N immobilization, while Abiven and Recous, (2007) observed lower N immobilization for brachiaria and soyabean residues that had high N concentrations (i.e., smaller C: N ratios). Several studies found the decomposition rate of the labile fraction to be strongly associated with the C: N ratio than of the stable fraction, hence, crop residues with lower C: N ratios have higher mineralization of nutrients in soils (Sakala et al., 2000; Silver and Miya 2001; Huang et al., 2004; Abiven and Recous, 2007; Johnson et al., 2007; Lynch, 2016; Stegarescu et al., 2020; and da Silva et al., 2021). On the other hand, the decomposition potential of root residues could also not be explained solely by the C: N ratio, due to their complex structural components and the presence of the suberin in their lignified tissues which provides more recalcitrant material than shoots (Soon and Arshad, 2002; Hofmann et al., 2009; Rasse et al., 2005).

Moreover, a rapid CO₂ evolution within the first 10 to 28 days of incubation, which then slowed down due to recalcitrant and less decomposable compounds at the later stages of decomposition (i.e., cellulose, lignin, lipids) was observed by Stegarescu et al., (2020) and Sakala et al., (2000). These could be attributed to high lignin fractions presented in the oilseed radish (33 %) observed by Jensen et al., (2005). The high lignin: N ratios in both AS8 (24) and KZ5246 (25.6) cultivars in this study which were more resistant to decomposition compared to other residues, as recalcitrant substrates tend to degrade at the later stages. Thus, lignin is a relatively

recalcitrant organic constituent of fibrous material in plant tissue, known to reduce decomposition rates by its resistance to enzymatic attack, which is likely to appear only after the consumption of the more labile substrate forms (Silver and Miya, 2001). The lignin: N ratio has been reported as an important factor influencing decomposition (Moore et al., 1999 and Rasse et al., 2005). In the field litterbag experiment, root residues exhibited significantly higher DM remaining, compared to shoots of the same cultivars. In support of this, De Neergaard et al. (2002) found roots of clover to be 2.5 times higher in lignin concentration than in shoots. Puttaso et al. (2011) also observed oilseed radish residue to have the highest DM remaining (1620 kg ha⁻¹) compared to ryegrass, and oat residues (1040, and 880 kg ha⁻¹), respectively. A similar result was observed by Salume et al. (2020), where oilseed rape had a lower decrease in DM compared to rye and wheat crop residues. These observations were linked to highly lignified tissues in root parts, resulting in highly retarded decomposition, hence, their higher potential to contribute to soil C stocks than shoots (Talgre et al., 2017). On the other hand, highly lignified residues can be used inefficiently by the soil microbial community that decompose SOM to acquire key nutrients leading to higher respiration losses than the added C in soils (priming effect). The ability of microbial activity to decompose soil organic matter for their living can be stimulated by the addition of fresh organic matter resulting in an increase in soil respiration beyond C addition, which is referred to as 'priming' (Fontaine et al. 2003; Kuzyakov, 2010). The priming effect may vary with soil and residue variables, as Cheng et al (2014) identified the root priming effect of soyabean to be consistently higher than that of wheat. These were linked to the present meta results study (Appendices A. 1), as 43% of the respiration data points showed 120 days cumulative emissions beyond C addition, which points to the existence of significant C losses from soil organic matter. All crop types experienced priming in the studies reviewed, with C losses over residue C from 25% for maize, sunflower and beans to over 55% for sorghum, alfalfa and canola. In addition, the environmentally stressed crop parts can produce relatively slow decomposing roots, because of their adaptive strategies of tissue defence against the decomposers (Foy, 1984; Kumar and Goh, 2003).

The meta-analysis results further showed higher residue CO₂ emissions under tropical climates (i.e., tropical clayey soils) than temperate regions, with clayey soils exhibiting significantly higher cumulative CO₂ emissions as compared to sandy soils (after 30 days). Generally, soils found in low latitudes are associated with high annual temperature and precipitation, which explains the higher decomposition of SOC in tropical compared to template regions (Xu et al., 2015). Moreover, the aggregates in clay soils are known for greater capabilities to store water,

resulting in increased microbial activity and decomposition of the available C (Xu et al., 2015). Furthermore, all undecomposed root materials within the soil (i.e., non-humic matter) are likely to be found under clayey soils associated with the larger surface area which has the advantage of entrapping organic matter in the micropore soil matrix, making it inaccessible to microbial attack (McClellan et al., 2012; Van Veen and Kuikman, 1990, Bloomfield et al. 1993). In addition, as far as the variation in sorghum cultivars, the environmental conditions (moisture) that the biomass of this study was obtained may play a role in decomposition rates. As, drought tolerance material is expected to be filled with lignin. Zengeni et al. (2021) observed drought tolerance capability in sorghum residues grown under dry conditions. These observations suggest a significant difference in performance (biomass quality) among genotypes level due to the genetic diversity reflecting their background (Bhutta et al., 2006; Poorter et al., 2011; Zengeni et al., 2021).

Furthermore, leaching of Ca by exposure to increased soil acidity has been shown to lead to altered permeability of root membranes (Zhao et al., 1987), decreased solubility of organic compounds (Vance and David, 1991), with direct changes in enzyme activity (Haynes and Swift, 1988; Foy, 1984) and microbial community (Nodar et al., 1992; Xu et al., 2019). However, the adsorption of organic substrates appeared to decrease with incrEAsing soil pH (Zhao et al., 1987), which explains the higher cumulative CO₂ emissions found under slightly acidic soils, followed by neutral and alkaline, while strongly acidic soils were lower in all cases, in the meta-analysis study. Overall, the rate of CO₂ emission and N release patterns in soils were probably changed by the impacts of selective soil and crop residue components that decompose independently from laboratory to field, and to the global meta-analysis studies.

5.2.Conclusion And Recommendations

Thus, decomposition potential and CO₂ emissions vary for different crop types, with significant impact imposed by soil and environmental factors, globally. Secondly, CO₂ emissions and nutrients release (N mineralization) through decomposition, varied for root and shoot residues of different sorghum cultivar, under field and laboratory-controlled conditions. These findings show that the incorporation of cereal crop types, precisely, roots and shoot residues from sorghum cultivars of known quality (i.e., KZ5246 and AS8 with low quality, LP4403 with intermediate, and OS-POTCH and MAMOLKWANE with high quality) has significant effects on decomposition potential. Lower cumulative CO₂-C emissions and N mineralization are

greatly influenced by the low-quality sorghum cultivars KZ5246 and AS8 (i.e., roots), which are attributed to their recalcitrant components, compared to their corresponding shoot parts, followed by OS-POTCH and MAMOLOKWANE cultivars with easily degradable substances.

Drought stress causes variation within and among the crops as they have different responses to the effects of drought. Sorghum suffered the least effects of drought on biomass production while millet incurred the least reduction in grain yield production due to their relative ability to maintain viable assimilate partitioning even under water stress (Killi et al., 2017).

The study recommends farmers to consider utilization of the selected superior crop types (i.e., low quality) with low decomposability potential and high stability index to contribute to soil carbon stocks, to fight against climate and land degradation. However, this would require additional N fertilizer to achieve sustainable yield for food security. Amongst the 17 crops studied in the global meta-analysis, sorghum (Sorghum bicolor L. Moench) ranks fifth of the most produced cereal crops after wheat, rice, barley, and maize, globally, and third in South Africa. As per the global trends pointed in our meta-analysis study, the crop types such as pea over alfalfa in leguminous crops, and maize over sorghum in cereal crops, and cotton over canola in oil crops foster C stabilization into soils by soil microbes through their high lignin and high N concentration, decreasing CO₂ emissions. Based on the current incubation study, OS-POTCH sorghum cultivar (i.e., shoot residues) is suggested to be considered as a good N source for plant uptake purpose, while KZ5246 followed by AS8 treatments root residues are the most commendable sorghum cultivars for N and C stocks to the soil. Lastly, roots were considered as the highly resistant crop part to decomposition, hence, their high DM remained compared to shoots presented on the field decomposition study. Thus, the system of leaving selective residues in soils during the off-season and incorporating them through tillage during planting is highly feasible and commended to smallholder farmers. These recommendations aim at improving soil quality and mitigating climate change and its effects in SSA and in a global scale.

Future research should focus more on the biological aspect, precisely, the microbial biomass and/or microbial communities and microbial activity influence. Further analysis of variables such as soil nutrients, environmental factors, and quality of lignin, and of other organic matter compounds is needed. More work also needs to be done on selecting crop cultivars for generating superior ones in mitigating against soil and climate degradation.

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6. APPENDICES:

Appendix A. 1: Proportion of data points showing 120 days cumulative emissions beyond C addition.

| Crop | % |
|-----------|----|
| Alfalfa | 58 |
| Barley | 44 |
| Bean | 25 |
| Canola | 63 |
| Clover | 27 |
| Cotton | 44 |
| Grass | 48 |
| Maize | 25 |
| Oat | 50 |
| Pea | 33 |
| Rice | 50 |
| Rye | 58 |
| Sorghum | 56 |
| Soyabean | 48 |
| Sunflower | 25 |
| Vetch | 38 |
| Wheat | 36 |

Appendix B. 1: Sample sizes of crop quality (n), soil and climatic factor categories in association with residue carbon emission variables.

| | | C _R 30 | | C _R 90 | | | C _R 120 | | | 1-(C _R 30/120) | | | |
|--------------------------------------|--------------------|-------------------|-------|-------------------|-----|-------|--------------------|-----|--------|---------------------------|-----|------|-------|
| | | n | Mean | STDEV | n | Mean | STDEV | n | Mean | STDEV | n | Mean | STDEV |
| Overall | | 394 | 196,3 | 193.3 | 394 | 518,8 | 461.9 | 394 | 914,2 | 765.1 | 394 | 0,81 | 0.07 |
| | Cereal | 195 | 171,0 | 185,9 | 195 | 473,9 | 445,4 | 195 | 846,6 | 777,1 | 195 | 0,82 | 0.07 |
| Crop type | Grass | 54 | 217,0 | 185,8 | 54 | 529,7 | 426,7 | 54 | 946,8 | 741,0 | 54 | 0,79 | 0.06 |
| Стор туре | Legume | 120 | 228,0 | 203,2 | 120 | 586,7 | 505,7 | 120 | 1003,0 | 758,3 | 120 | 0,78 | 0.08 |
| | Oil | 25 | 196,0 | 211,9 | 25 | 519,2 | 459,2 | 25 | 944,0 | 742,5 | 25 | 0,83 | 0.07 |
| Residue lignin | High | 73 | 173,0 | 165.2 | 73 | 457,0 | 402.3 | 73 | 816,9 | 694.2 | 73 | 0,80 | 0.07 |
| Concentration | Low | 119 | 218,0 | 188.9 | 119 | 602,3 | 524.3 | 119 | 1055,0 | 853.0 | 119 | 0,84 | 0.05 |
| | High | 142 | 144,9 | 159.0 | 142 | 447,0 | 424.9 | 142 | 837,9 | 777.4 | 142 | 0,78 | 0.07 |
| Residue C: N Ratio | Low | 103 | 273,7 | 223.4 | 103 | 686,9 | 537.4 | 103 | 1163,1 | 814.7 | 103 | 0,80 | 0.07 |
| | Medium | 59 | 201,1 | 214.7 | 59 | 484,9 | 449.7 | 59 | 856,8 | 734.8 | 59 | 0,80 | 0.08 |
| TC | High | 284 | 201.8 | 198.7 | 284 | 524,6 | 450.5 | 284 | 927,3 | 759.9 | 284 | 0,80 | 0.08 |
| TC | Low | 110 | 181.9 | 168.9 | 110 | 503,6 | 635.6 | 110 | 880,2 | 803.9 | 110 | 0,82 | 0.07 |
| | Clayey | 121 | 224,4 | 179.0 | 121 | 587,5 | 440.7 | 121 | 1060,6 | 777.5 | 121 | 0,81 | 0.07 |
| Soil texture | Silt | 130 | 191,1 | 215.6 | 130 | 505,8 | 523.9 | 130 | 894,3 | 819.4 | 130 | 0,82 | 0.08 |
| | Sandy | 137 | 178,2 | 184.7 | 137 | 454,7 | 417.8 | 137 | 789,9 | 699.2 | 137 | 0,79 | 0.08 |
| | Alkaline | 133 | 178,1 | 170.0 | 133 | 487,2 | 410.7 | 133 | 869,2 | 712.5 | 133 | 0,82 | 0.07 |
| | Neutral | 57 | 195,4 | 165.4 | 57 | 532,3 | 362.0 | 57 | 919,4 | 613.8 | 57 | 0,79 | 0.07 |
| Soil Ph | Slightly acidic | 86 | 262,1 | 203.3 | 86 | 634,6 | 560.3 | 86 | 1140,2 | 874.0 | 86 | 0,78 | 0.07 |
| | Strongly acidic | 57 | 175,3 | 222.3 | 57 | 414,7 | 443.4 | 57 | 709,9 | 687.7 | 57 | 0,79 | 0.09 |
| | High | 39 | 149,4 | 148.9 | 39 | 356,7 | 529.6 | 39 | 591,4 | 695.8 | 39 | 0,81 | 0.07 |
| Soil organic carbon Concentration | Low | 153 | 157,5 | 188.9 | 153 | 421,3 | 430.9 | 153 | 746,9 | 725.8 | 153 | 0,81 | 0.07 |
| Concentration | Medium | 101 | 230,3 | 184.9 | 101 | 595,2 | 432.0 | 101 | 1066,4 | 746.6 | 101 | 0,81 | 0.07 |
| | Subtropical | 198 | 192,5 | 181.8 | 198 | 522,8 | 427.3 | 198 | 912,2 | 736.5 | 198 | 0,81 | 0.07 |
| Climate | Temperate | 101 | 149,8 | 213.8 | 101 | 430,3 | 536.5 | 101 | 779,7 | 828.1 | 101 | 0,82 | 0.08 |
| | Tropical | 95 | 253,4 | 193.7 | 95 | 604,5 | 444.7 | 95 | 1061,2 | 755.8 | 95 | 0,78 | 0.08 |

Appendix C. 1: Sample sizes of crop type categories associated with residue carbon emission variables.

| Residue | | | C _R 30 | | C _R 90 | | | C _R 120 | | | 1-(C _R 30/120) | | |
|-----------|-----------|-----|-------------------|-------|-------------------|-------|--------|--------------------|-------|--------|---------------------------|------|-------|
| Crop type | Crop | N | Mean | STDEV | N | Mean | STDEV | n | Mean | STDEV | n | Mean | STDEV |
| | Overall | 394 | 196,3 | 193,3 | 394 | 518,8 | 461,95 | 394 | 914,2 | 765,07 | 394 | 0,81 | 0,07 |
| | Barley | 9 | 177,7 | 156,8 | 9 | 547,2 | 436,1 | 9 | 1005 | 778,6 | 9 | 0,84 | 0,05 |
| | Maize | 59 | 85,8 | 124,3 | 59 | 275,1 | 319,8 | 59 | 495 | 541,7 | 59 | 0,84 | 0,08 |
| | Oat | 8 | 212,1 | 190,8 | 8 | 501,9 | 425,5 | 8 | 872,7 | 724,5 | 8 | 0,79 | 0,06 |
| Cereal | Rice | 10 | 251,9 | 255,7 | 10 | 657,1 | 662,3 | 10 | 1211 | 1196,6 | 10 | 0,82 | 0,08 |
| | Rye | 12 | 199,3 | 190,8 | 12 | 501,6 | 453,5 | 12 | 824,2 | 729,5 | 12 | 0,77 | 0,04 |
| | Sorghum | 16 | 261,1 | 218,8 | 16 | 714 | 496,7 | 16 | 1303 | 855,9 | 16 | 0,83 | 0,08 |
| | Wheat | 81 | 196,3 | 194,8 | 81 | 533,6 | 452,3 | 81 | 950,7 | 791,8 | 81 | 0,81 | 0,07 |
| Grass | Grass | 54 | 217 | 184,9 | 54 | 529,7 | 420,3 | 54 | 946,8 | 734,2 | 54 | 0,79 | 0,06 |
| | Alfalfa | 24 | 359 | 262,7 | 24 | 796 | 515,0 | 24 | 1319 | 801,0 | 24 | 0,76 | 0,07 |
| | Bean | 12 | 223,7 | 239,9 | 12 | 538,7 | 471,0 | 12 | 962,1 | 667,0 | 12 | 0,80 | 0,06 |
| I | Clover | 26 | 184,2 | 139,6 | 26 | 580,6 | 661,0 | 26 | 940,3 | 866,5 | 26 | 0,78 | 0,06 |
| Legume | Pea | 27 | 184,2 | 152,7 | 27 | 452 | 343,8 | 27 | 809 | 554,1 | 27 | 0,78 | 0,07 |
| | Soyabean | 23 | 207,8 | 157,0 | 23 | 577,5 | 401,7 | 23 | 1039 | 715,6 | 23 | 0,81 | 0,10 |
| | Vetch | 8 | 190,2 | 182,6 | 8 | 531,1 | 433,4 | 8 | 870,4 | 696,1 | 8 | 0,80 | 0,05 |
| - | Canola | 8 | 293,5 | 327,3 | 8 | 670,5 | 717,3 | 8 | 1211 | 1184,5 | 8 | 0,79 | 0,07 |
| Oil | Cotton | 9 | 135,5 | 98,8 | 9 | 442,5 | 281,0 | 9 | 841,1 | 513,9 | 9 | 0,85 | 0,03 |
| | Sunflower | 8 | 166,5 | 208,7 | 8 | 454,2 | 451,7 | 8 | 792,5 | 667,4 | 8 | 0,84 | 0,10 |

Appendix D. 1: Average temperatures (°C) and rainfall at the study site (Ukulinga-Pietermaritzburg in KZN, South Africa), over the experimental period.

| | July | August | September | October | November | December | January |
|-------------------|------|--------|-----------|----------|----------|----------|-----------|
| | | | Y | ear 2019 | | | Year 2020 |
| Temperatures (°C) | 17 | 18 | 19 | 21 | 20 | 21 | 22 |
| Rainfall (mm) | 2,8 | 14,2 | 41,2 | 37,4 | 236 | 145,3 | 236 |

Appendix E. 1: Summarized database compiled using data collected from ISI journal papers showing references included in database with author, country, crops type, soil texture and climatic zones under which the studies were conducted

| No. | Author | Country | Crop type | Soil texture | Climate |
|----------|-------------------------------|--------------|---|---------------------|-------------|
| 1 | Abiven et al. (2005) | Brazil | Brachiaria; Rice; Sorghum; Soyabean; Wheat | Clayey | Tropical |
| 2 | Abiven and Recous (2007) | Brazil | Brachiaria; Rice; Sorghum; Soyabean; Wheat | Clayey | Tropical |
| 3 | Abro et al. (2011) | China | Maize | Clayey | Subtropical |
| 4 | Ajwa and Tabatabai (1994) | USA | Alfalfa; Maize; Sorghum; Soyabean | Silt | Subtropical |
| 5 | Angers and Recous (1997) | France | Rye; Wheat | Silt | Temperate |
| 6 | Anguria et al. (2017) | Uganda | Cowpea; groundnut; millet; sorghum | (blank) | Subtropical |
| 7 | Arunachalam et al. (2003) | India | Bean and pea | Sandy | Tropical |
| 8 | Aulakh et al. (1991) | USA | Vetch and wheat | Silt | Tropical |
| 9 | Begum et al. (2014) | Australia | Canola; Sorghum; Soyabean; Wheat | Clayey | Subtropical |
| 10 | Bertrand, et al. (2006) | France | Wheat | Silt | Subtropical |
| 11 | Blaise & Bhaskar (2003) | India | Cotton | clayey, | Subtropical |
| 12 | Cayuela et al. (2009) | Italy | Cotton; wheat | Sandy | Subtropical |
| 13 | Clark et al. (2007) | Australia | Alfalfa; Wheat | Clayey | Subtropical |
| | | China | | | - |
| 14 | Cong et al. (2015) | | Bean; Maize; Wheat | Sandy | Temperate |
| 15 | Corbeels et al. (2000) | Morocco | Sunflower; Wheat | Clayey | Temperate |
| 16 | Curtin et al. (2008) | New Zealand | Barley; Wheat | Silt | Subtropical |
| 17 | Datta et al. (2019) | India | Maize; Rice; Wheat | Silt | Subtropical |
| 18 | De Neergaard et al. (2002) | Denmark | Clover; Grass | Silt | Temperate |
| 19 | Duong et al. (2009) | Australia | Wheat | Sandy | Subtropical |
| 20 | Finn et al. (2015) | Australia | Alfalfa; Grass; Wheat | clayey, Silt& sandy | Subtropical |
| 21 | Fruit et al. (1999) | France | Wheat | Silt | Subtropical |
| 22 | Gezahegn et al. (2016) | Malaysia | Maize; Soyabean | Sandy | Subtropical |
| 23 | Ghimire et al. (2017) | USA | Canola; Oat; Pea | Clayey | Subtropical |
| 24 | Havstad et al. (2010) | Norway | Barley; Clover; Grass; Meadow; Wheat | Silt | Subtropical |
| 25 | Henriksen and Breland (2002) | Norway | Barley; Clover; Wheat | Silt, sandy | Temperate |
| 26 | Jha et al. (2012) | India | Wheat | Clayey | Subtropical |
| 27 | Jin et al. (2008) | China | Peanut; Wheat | Silt | Tropical |
| 28 | Johnson et al. (2017) | USA | Alfalfa; Cuphea; Maize; Soyabean; Switchgrass | Silt | Subtropical |
| | · · · | China | | | • |
| 29 | Juan et al. (2009) | | Rice; Wheat | (blank) | Subtropical |
| 30 | Khalil et al. (2005) | Bangladesh | Bean; wheat | clayey, Silt &sandy | Tropical |
| 31 | Li et al. (2013) | China | Maize; Soyabean | Silt | Temperate |
| 32 | Lou et al. (2007) | China | Rice | Clayey | Tropical |
| 33 | Luxhoi et al. (2002) | Denmark | Clover; Grass; Rye | Sandy | Tropical |
| 34 | Machinet et al. (2009) | France | Maize | Silt | Temperate |
| 35 | Machinet et al. (2011) | France | Maize | Clayey | Temperate |
| 36 | Magid et al. (2004) | Denmark | Clover; Radish; Rye; Sugarcane; Vetch | Sandy | Temperate |
| 37 | Marstorp and kirchmann (1991) | Sweden | Clover | Sandy | Temperate |
| 38 | Martens (2000) | USA | Alfalfa; Canola; Maize; Oat; Prairie | Sandy | Subtropical |
| 39 | Moreno-Cornejo et al. (2014) | Spain | Pepper | Silt | Subtropical |
| 40 | Muhammad et al. (2011) | Australia | Cotton; Maize; Sorghum, Sugarcane | Clayey | Subtropical |
| 41 | Müller et al. (2003) | Denmark | Barley; Clover; Grass; Rape | Sandy | Temperate |
| 42 | Mungai and Motavalli (2006) | Kenya | Grass; Maize; Soyabean | clayey, Silt | Temperate |
| 43 | Murungu et al. (2011) | South Africa | Oat; Peas; Vetch | Sandy | Temperate |
| 44 44 | Nourbakhsh (2006) | Iran | Alfalfa; Wheat | Clayey | Subtropical |
| | · · · | | | | - |
| 45 46 | Pascault et al. (2010) | France | Alfalfa; Rape; Wheat | Clayey | Temperate |
| 46 | Quemada and Cabrera, (1995) | USA | Clover; Oat; Rye; Wheat | Sandy | Subtropical |
| 47 | Raiesi; (20065) | Iran | Alfalfa; Wheat | Clayey | Subtropical |
| 48 | Redin et al. (2014) | Brazil | Maize; Sorghum; Wheat, Soyabean; Sunflower; Vetch | Sandy | Tropical |
| 49 | Schmatz et al. (2017) | Brazil | Pea; Vetch; Wheat | clayey, sandy | Tropical |
| 50 | Shahande et al. (2011) | USA | Switchgrass | Silt | Subtropical |
| 51 | Shi et al. (2013) | Australia | Barley; Grass | Silt | Subtropical |
| 52 | Stewart et al. (20157) | USA | Maize; Sorghum; Soyabean; Sunflower; Wheat | Silt | Temperate |
| 53 | Vachon and Oelbermann (2011) | Argentina | Maize; Soyabean | Silt | Subtropical |
| 54 | Vahdat et al; (2010) | Iran | Alfalfa; Barley; Clover; Grass; Wheat | Clayey | Subtropical |
| 55 | Wang et al. (2004) | Australia | Brigalow; Grass; Sugarcane; Wheat | Sandy | Subtropical |
| 56 | Xu et al. (2006) | Australia | Alfalfa; Chickpea; Medic; Wheat | Sandy | Tropical |
| 57 | Zaccheo et al. (2002) | Italy | Alfalfa; Maize | Sandy | Temperate |
| | Zeng et al. (2010) | China | Maize; Peanut; Poplar | Sandy | Temperate |