SHORT-TERM EFFECTS OF CLIMATE SMART MANAGEMENT PRACTICES ON SORGHUM (Sorghum bicolor (L.) Moench) PRODUCTIVITY, SOIL ORGANIC CARBON FRACTIONS AND AGGREGATE STABILITY IN CONTRASTING AGRO-ECOLOGICAL ZONES IN SOUTH AFRICA

Kaya Mrubata

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School Agriculture, Earth and Environmental Sciences

College of Agriculture, Engineering and Science

University of KwaZulu-Natal

Pietermaritzburg Campus

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DECLARATION 1 – PLAGIARISM

I, …Kaya Mrubata……………………………….., declare that

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Prof P. Muchaonyerwa (Supervisor)

Signed:[redacted]……………. Date ...014/07/2025………………

Dr AD Nciizah (Co-supervisor)

ABSTRACT

Addressing climate change calls for the adoption of climate smart practices including the use of resilient, underutilized crops, to increase yields especially on marginal soils, which are widely used by smallholder farmers, particularly in sub-Saharan Africa. A short-term study was conducted to determine the effects of planting date, tillage system, sorghum (Sorghum bicolor (L.) Moench) cultivar and rotations on crop productivity and selected quality parameters of two contrasting soils in KwaZulu-Natal (KZN) and Free State (FS) Provinces (sand clay loam and sandy loam, respectively) of South Africa. The experiment consisted of two sorghum cultivars (Pan8816 and Macia), sown in December (First planting date) and in January (Second planting date) the three years 2020/21 and 2021/22 and under conventional tillage (CT) and no-till (NT), with sorghum-cowpea (Vigna unguiculate (L.) Walp)-sorghum and sorghum-sorghum-sorghum rotations arranged in a randomized complete block design (RCBD) with a 2×2×2×2 factorial experiment.

In KZN, none of the factors, except cultivar in the second season had significant effects on yield and the uptake of nitrogen (N), phosphorus (P), and potassium (K) in either season. Pan8816 had higher yield and uptake of N, P and K than Macia, in the second season. In FS, Pan8816 took up 1.74 times more P than Macia, while early-planted sorghum had significantly higher yield and uptake of N, P and K than when planted late in both seasons. Additionally, sorghum plants grown under CT had significantly higher yield and K uptake than under NT. The main effects of planting date, tillage, season and their interactions did not affect microbial biomass carbon (MBC) in FS, except under CT, where in season one MBC was 3.8 times higher than in season two, when the sorghum was planted late. In KZN, only tillage affected MBC with NT resulting in 7% higher MBC than CT. No treatment effects were observed on β-glucosidase. Phosphomonoesterase activity was higher in season 1 than season two at both sites.

Late planting under CT (26.75%) resulted in higher water stable aggregates (WSA) in the >250 µm size class, while NT affected WSA of the size range 53-250 µm and < 53 µm than under CT soils, in FS. In KZN, the interaction between planting date and tillage had significant influence on both > 250 µm and 53 µm – 250 µm aggregate sizes, while the main treatments did not. However, late planting resulted in higher <53 µm fraction compared to early planting. Soils from early-planted sorghum under CT had significantly ($p < 0.05$) higher WSA in both the 53 µm -250 µm and in the > 250 µm than late planting under CT and NT for both tillage systems. Planting date had a significant effect on the <53 µm aggregate size in both sites. In FS, there were no treatment or interactive effects on SOC and the same was observed for all the carbon fractions in KZN. Late planted soils had 6.3% higher OC than early planted soils in KZN. Fine particulate organic carbon did not respond to planting date, tillage or their interaction at both sites. In FS, late planting resulted in a 24.1 % increase in coarse particulate organic carbon (cPOC) compared to early planting.

Soil microbial properties were influenced by interactions among PD, tillage and seasonal characteristics in FS while in KZN, tillage effects were predominant. These findings suggest that these climate smart agricultural practices improve aggregate stability, while other effects are site specific, with greater responses of sorghum productivity in the drier FS area, while soil OC and microbial properties were improved in wetter KZN. In the drier FS, early planting under CT is critical for sorghum productivity, while in the wetter KZN, selecting the appropriate cultivar (e.g. PAN8816), is more essential on sorghum productivity, at least in the short-term. Early planting under NT gives a more metabolically active microbiome in the FS soils while CT and season 2020/21 does the same for soils in KZN. Season 2020/21 also resulted in higher numbers and abundance of microbe species in KZN.

Keywords: Aggregate stability, conservation agriculture, enzyme activity, microbial activity, mineral associated carbon

DECLARTION 2 – PUBLICATIONS

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DEDICATION

This thesis is dedicated to:

OoRhadebe, ooMmthimkhulu, ooNdlebe ntle zombini

Namampondo, ooThahla Kandayeni

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CHAPTER 1: GENERAL INTRODUCTION

The agriculture sector needs to produce more products from less land and water resources to provide for a growing population in an urbanized world (FAO, 2017) under the difficult conditions of a changing climate (Lal, 2013). The decline in arable land is caused by increased degradation due to erosion, nutrient mining, and pollution, and the associated negative impacts on soil quality (Lal, 2015). Climate change poses direct and indirect threats to food security through its influence on extreme whether events (drought and flooding), soil properties, water resources, and crop production (Fischer et al. 1996; Gornall et al. 2010; Lal, 2013; Masipa 2017). These effects include changes in the hydrological balance, increased soil erosion, loss of soil organic carbon (SOC), reduced fertility and plant available water, and prevalence of pests and diseases (Gornall et al. 2010 & Lal, 2013). The vulnerability of agriculture to changes in climate (Lal 2013) is exacerbated by projections that point towards difficult times for land users, especially those in the developing world like Sub-Saharan Africa (SSA) (Lal, 2019). The increased frequency of extreme weather events that will lead to a reduction in yields are some of the difficulties affecting the developing world (Lal, 2013). For this reason, the development and adoption of practices to increase agricultural productivity and at the same time mitigate climate change is paramount. Conservation agriculture (CA) is a viable option to ensure improved and sustainable production levels without exacerbating land degradation and compromising the environment (Aziz et al., 2013; Busari et al., 2015; Ngachan et al. 2018).

Conservation agriculture entails the integrated management of available natural resources and other inputs to maximize efficiency (Choudhury et al, 2016) through minimal soil disturbance, permanent soil cover, and crop rotations (Hobbs et al, 2008). Palm et al. (2014) asserted that CA can improve soil physical, chemical, and biological properties, and overall ecosystem services such as regulation of climate and water. As such, CA is regarded as a promising technology to enhance productivity, improve and maintain soil quality and protect the environment including marginal lands, which have been converted to agricultural lands especially in smallholder settings. The effective implementation of CA primarily influences SOC, which in turn creates conditions that are necessary for soil organisms to perform nutrient cycling (Busari et al. 2015). Soil biological properties are good indicators of soil quality due to their sensitivity to changes in soil conditions linked to management practices. The most frequently measured biological indicators of soil quality include SOC, microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), microbial biomass phosphorus (MBP), and activity of enzymes involved in the cycling of C, N, P, and soil protein index (Bennding et al. 2000; Veum et al. 2014 & Wander et al. 2019). Soil enzymes tend to react rapidly to changes in management because they are involved in metabolic reactions and as such can be used as early indicators of soil quality decline or improvement (Veum et al. 2014). Tillage practices affect soil enzyme activity, which ultimately affects yield sustainability (Rao at al. 2014). Rao et al. (2014) continue to state that different cropping systems, residue management and tillage influence soil conditions like moisture, pH, organic carbon and soil structure. Knowledge of the various ways in which management practices influence soil quality and therefore its productivity is imperative at a time when the soil resource is threatened by degradation and climate change.

According to Bolo et al. (2021), reduced tillage and residue retention have been shown to significantly increase microbial biomass phosphorus (MBP) in long-term CA studies in Kenya, USA and Asia. This improvement has been attributed to higher levels of SOC, nutrients and reduced disturbance. Logah et al. (2010) concurred that SOC and nutrients are very important sources of energy for microbial populations. According to Moore et al. (2000), MBC and MBN respond positively to crop rotation and plant cover because of the amount and diversity of residues, proportion of decomposable organic matter, soil structure and microclimate amongst other factors.

The availability of nutrients to crops at the appropriate time is another factor that can be improved through the modification of land use practices and cropping systems. According to Logah et al. (2010) management practices should be designed to synchronize nutrient release particularly P, with peak crop demand periods. Phosphorus is vulnerable to fixation by oxides of aluminium (Al) and iron (Fe), precipitation with solution Al and Fe, and immobilization by microbes through transformation reactions that allow for the use of carbon as an energy source (Zhang et al. 2018 and Arenberg & Arai, 2019). The MBP is a useful pool of P to limit its fixation on soil colloids, and Gichangi et al. (2010) reported that increasing the microbial P improves the use efficiency of P fertilisers. They go further and state that the labile fractions of P are made available to plants through desorption from soil colloids. Zhang et al. (2018), further state that MBP can be released into the available pool during biomass turnover, a process that depends on factors such as soil moisture, fertilizer addition and season. The influence of CA in improving soil quality and productivity has been reported (Moore et al. 2000; Logah et al. 2010; Suner et al 2014 and Page et al. 2020), and the findings appear to be dependent on climatic and soil characteristics.

Soil quality, the capacity of a soil to perform multiple functions, is often estimated by the determination of certain physical, chemical, and/or biological properties and their interactions and relating them to a particular function (Northcliff, 2002; Bongiorno 2017). However, soil quality parameters have been shown to depend on the quantity and quality of the organic matter inputs. For instance, increased organic matter resulting from residue retention improves soil physical and biological properties, which is good for crop productivity. Good soil aggregation means good porosity that leads to good aeration and infiltration which supports crop absorption of water and nutrients for growth. Sorghum can grow on low fertility soils with limited input for food and industrial use in breweries, production of animal feed, and as feedstock for biofuel production (Meki et al. 2013; Malobane et al. 2018 & Ikazaki et al. 2020). Areas that are currently suited to maize in the African savannah may in the future become more suitable for sorghum, due to climate change (Lal, 2013). While a lot of research has been done in maize-based CA systems globally including in Southern Africa (Nyagumbo et al. 2020), limited work has been reported in the literature for sorghum-based (*Sorghum bicolor*) CA systems.

Research done by Meki et al. (2013) and Malobane et al. (2018) on sweet sorghum, showed that when cultivated under CA, the crop can enhance SOC while lowering its global warming potential. These benefits are closely linked to the role of SOC in regulating soil functions through its distribution across different fractions (Luo et al. 2017). Stable SOC fractions contribute to improved soil physical properties, whereas labile forms – such as particulate organic matter, water soluble C, and microbial biomass – play a central role in nutrient cycling. The balance among these fractions, and their interaction with aggregate stability and nutrient cycling, is strongly influenced by soil type and prevailing climatic conditions (Sarker et al. 2018). For instance, clay-rich soil protects SOC within aggregates, while sandy soils provide limited aggregation benefits. Similarly, areas with higher rainfall typically promote greater biomass production and organic matter inputs compared to drier regions. Understanding these dynamics across contrasting agro-ecological zones is therefore critical for assessing the potential impacts of climate change on both crop productivity and soil quality. Despite this importance, limited research has examined how SOC fractions and functions in soil biology and nutrient cycling are influenced within sorghum-based CA systems.

Given that soil organic carbon and its functional fractions play a key role in maintaining soil productivity and resilience, understanding these dynamics is critical in the context of ongoing pressures such as climate change, land degradation and population growth. Generating empirical and practical data on the sustainable use of natural resources is crucial for ensuring agricultural productivity, environmental sustainability, and system resilience. In this context, the study seeks to elucidate the influence of selected conservation agriculture technologies on soil physical and biological quality and resilience and crop growth in sorghum-based production systems in different agro-ecological zones in South Africa. The use of short-term study is important as a selling point of CA to smallholder farmers by highlighting the immediate benefits of CA.

1.1. Research questions

(a) What are the effects of selected climate smart agriculture practices on the growth and yield of two sorghum cultivars produced under dryland conditions in sub-humid and semi-arid regions in South Africa?

(b) How do tillage and planting date influence the composition of soil organic carbon, its functional fractions and aggregate stability in the short term within sorghum-based conservation agricultural systems across contrasting soil and climates in South Africa.

(c) How do tillage practices and planting date influence soil microbial activity in sorghum-based systems across two agro-ecological zones in South Africa?

1.2. Aims and objectives

The study aims to determine the effects of planting date, tillage, and crop rotation on selected soil biological properties, sorghum growth and water stable aggregate in two contrasting agro-ecological zones of South Africa. The specific objectives to be determined are:

(a) The effects of planting date, tillage, and crop rotation on grain yield and nutrient uptake of two sorghum cultivars grown on sandy clay loam soils in sub-humid and semi-arid regions in South Africa.

(b) The effects of planting date and tillage on SOC and its fractions and water stable aggregates in a short-term sorghum-based CA system on two contrasting soils and climatic conditions in South Africa.

(c) The effect of tillage and planting date on enzyme activity (β-glucosidase, acid phosphatase), microbial biomass carbon and functional diversity of two contrasting soils under sorghum in South Africa.

1.3. Hypotheses

(a) Planting date, tillage, cultivar and crop rotation will affect the grain yield and nutrient uptake of sorghum in sandy clay loam soil in sub-humid and semi-areas in South Africa.

(b) The SOC and its fractions and water stable aggregates are affected by tillage and planting date in two agro-ecological zones.

(c) Tillage and planting date influences the activity of β-glucosidase, acid phosphatase, microbial biomass carbon and functional diversity of soil microbes in a sorghum-based systems at two sites in South Africa.

CHAPTER 2: SORGHUM BIOTIC AND ABIOTIC STRESS-RESPONSE, IMPROVEMENT OF SOIL QUALITY AND FOOD SECURITY UNDER CLIMATE SMART AGRICULTURAL PRACTICES IN SUB-SAHARAN AFRICA: A REVIEW

2.1.Introduction

The world faces a challenge of simultaneously supporting animal and crop production, health and well-being, and biodiversity restoration under the increasing constraints of unreliable rainfall, increased temperatures, and increased weather fluctuations (Alabi et al 2023). These challenges are particularly critical in the context of the rising global population and the need to increase food production from finite land resources (Thierfelder et al. 2014). The condition is most apparent in the developing world where economies are heavily reliant on agricultural production. Climate change has exacerbated these challenges and a holistic approach that includes cultivar selection, choice of planting time, and integration of nutrient management, is required to address challenges of water conservation, pests and diseases and mitigate greenhouse gas (GHG) emissions. Climate smart agriculture (CSA), which is an approach to sustainably intensify productivity, limit environmental pollution and increase resilience to climate change is one such approach (Rosenstock et al. 2016, Taylor 2018, Chandra et al 2018, Basara et al. 2021 and Mizik 2021). The strategies of CSA include those to combat climate change by reducing agriculture's GHG emissions, increase sustainable production, adapting to climate change, and increasing resilience to weather extremes (Alabi et al. 2023). The aim of CSA practices is to reduce poverty and enhance food security, increase the productivity and resilience of agriculture and ecosystem functions, and promote economic development (Alabi et al 2023). The CSA approach has been explored at varying scopes and scales throughout the African continent. Integrated soil fertility management to increase yields, crop-stress modifications to develop tolerant varieties, the role of gender, socioeconomics and biodiversity and contribution of precision agriculture to farming practices in South Africa, Ghana and Kenya (Makate et al. 2019, Ighodaro et al. 2020, Mazarire et al. 2020, Basara et al. 2021).

One major aspect of CSA, which is a potential solution to these challenges, is conservation agriculture (CA). CA is based on three principles i.e. crop diversification through rotations and/or intercropping to ensure natural fertilization and the control of pest and diseases (Strauss et al. 2021); minimum soil disturbance to improve soil quality (Page et al. 2020) and maintaining a permanent organic soil cover with crop residues (Nyanga et al. 2020). The improvements in soil physical, chemical and biological properties allow soil microorganisms to perform their nutrient turnover reactions under conducive conditions. The need to increase cereal production by 70% to meet the food requirements of a growing global population by 2050 (Liaqat et al. 2024) needs to be coupled with practices like CA that have shown potential to improve soil quality and SOC storage, which mitigates GHG emissions. While maize-based CA systems have been studied and have shown positive results, there is need to understand their contribution to sorghum-based CA systems, considering that sorghum is more tolerant to drought than maize. The vulnerability of agriculture to climate change in SSA has made adaptation efforts a policy imperative (Alabi et al 2023). This paper presents as review of the suitability of a sorghum production system to tackle food security and enhance soil quality in Sub-Saharan Africa in the context of smallholder farmers facing unreliable climate on marginal soils and a lack of resources.

2.2.Sorghum: A crop for a changing climate

2.2.1. Overview

Sorghum (*Sorghum Bicolor* L. Moench) is a crop that has shown potential to achieve this imperative. It has been shown to be able to grow on poor soil under warming climates with minimal inputs. This is because of the traits it possesses such as being C4 plant with high photosynthetic efficiency, self-pollination, valuable nutrients and health benefits (Liaqat et al. 2024) and ability to thrive under biotic and abiotic stresses (Borrel et al 2022). According to Njinju et al (2022) sorghum uses 332 kg of water to produce 1 kg of dry matter compared to 368 kg and 541 kg needed by maize and wheat, respectively, which makes it an ideal crop for climate smart agriculture. This means that sorghum has high water use efficiency than both maize and wheat being able to produce more dry matter from little water. The ability of sorghum to grow under conditions of reduced nutrient availability, moisture and high temperatures makes it the ideal crop to grow on marginal soils under conditions of a changing climate. Originating from tropical Africa sorghum is C4 plant that grows well in the warmer temperatures of the region (Rutasiyire et al. (2021). It is therefore important to find ways to maintain and improve environmental conditions suitable for growing sorghum. For this reason, this literature review aims to find solutions and opportunities to sorghum production from studies primarily in SSA.

According to Wang et al. (2016), domesticated grain sorghum originated from central and northeast Africa in what is now Sudan and Ethiopia. It is now well distributed around the world in Africa, Asia, the Americas and Australia (Doudu et al. 2003 and Wang et al. 2016) where it is mainly grown for food and feed. Africa produces approximately 20 million tonnes of sorghum yearly making it the region's second most important cereal (Taylor, 2003). Global production levels for sorghum by region shows that the majority occurs on the African content followed by the Americas (Figure 2.1). This production is mostly concentrated in areas in the west and east Africa in countries like Ethiopia, Kenya Burkina Faso and Ghana in smallholder agricultural settings.

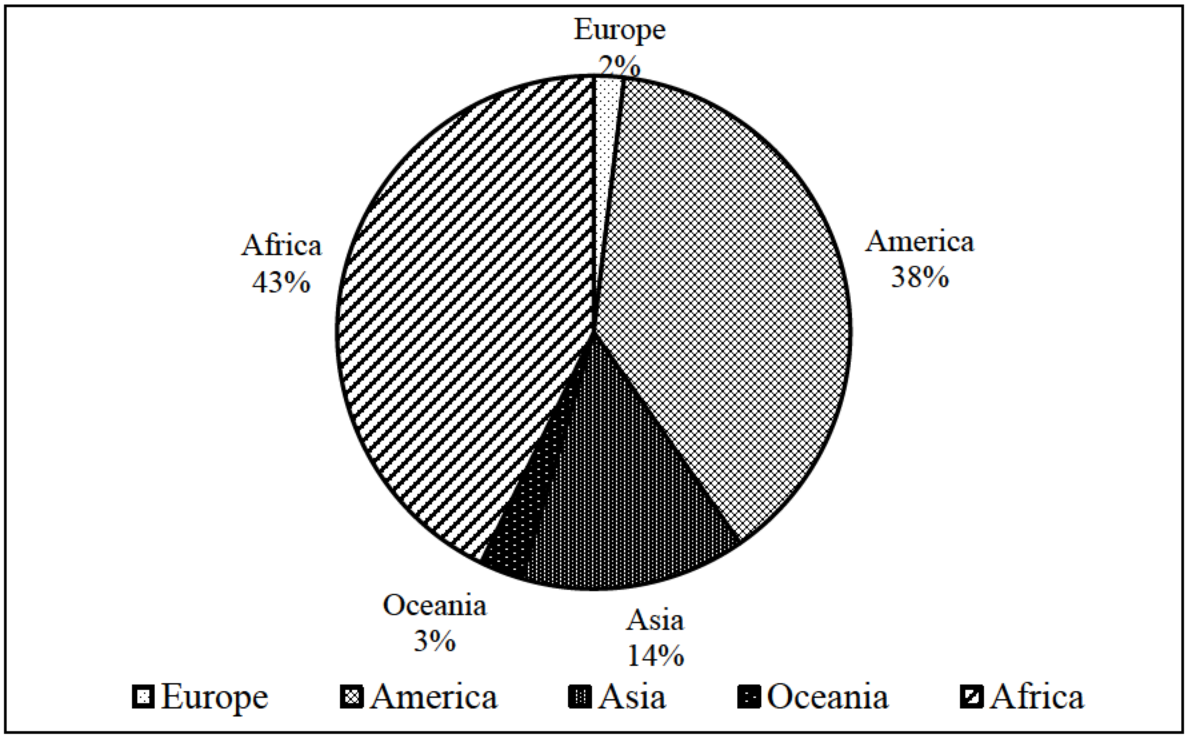

Figure 2. 1: Sorghum production levels by region.
Source: Liaqat et al. 2024

2.3 Sorghum for the improvement of food security.

Climate change threatens the achievement of the sustainable development goals of reducing poverty and increasing food security especially in third world countries where livelihoods and food production is vulnerable like sub-Saharan Africa (SSA) (Alabi et al. 2023). The climate of the region is semi-arid with frequent occurrence of droughts spells that make agriculture a challenging endeavour. Branca et al. (2011) stated in a working paper, that to maintain food security, agricultural systems needed to be modified towards increasing productive capacity and stability of smallholder production. This can be achieved by ensuring that management practices are tailored to the prevailing conditions of smallholder farmers like lack of inputs and poor fertility soils by selecting the appropriate crops for the edaphic and climatic conditions. Smallholder farmers in SSA are facing poverty, limited access to technology and capital, reliance on rain fed production and insufficient research and extension services (Alabi et al. 2023). According to predictions, crop yields will decrease by

30-82% by the end of the 21st century and food production will decrease by 1-5% per ten-year period (Tadese et al. 2021). According to Datta (2022), long-term predictions indicate that yearly incomes could decrease by 15-18% under irrigation and 20-25% under dryland due to climate change. These point towards difficult times for farmers especially smallholder and subsistence farming because of their reliance on dryland production. This means that they cannot absorb the shocks that come with unreliable rainfall and temperature extremes.

The poorer and most vulnerable population dependant on agriculture will feel the impact of climate change such as reduced water availability and increasing the likelihood of floods, droughts and heatwaves (Datta et al. 2022). In Africa in particular, yields are expected to decrease by 20% by 2050 (Recha et al. 2022), specifically the yields of maize, sorghum and millet are expected to be reduced by 5%, 14.5% and 9.6% respectively, at the end of 21st century (Tadesse et al. 2021). The World Bank conducted a study of the population dynamics of SSA, South Asia and Latin America and found that climate change will cause large scale within country and cross-border migration, which would create crowded slums (Datta et al 2022). This will affect people in these areas in various ways such as poverty, health and crime. Moreover, Datta et al. (2022) continue to state that more than 140 million people in the three regions, which account for more than half of the population, are most likely to be internally displaced between now and 2050 and this will make it difficult for governments to provide economic and social development.

Sorghum has many uses like as a source of food, feed, and biofuel (Ndlovu et al. 2021). Interest has risen again in sorghum due to its valuable nutrients, active components, potential health benefits, and its gluten-free characteristic (Liaqaat et al 2024). In a world where healthy lifestyles are all the rage, crops such as, sorghum with all its health benefits will be in draw interest. Sorghum is a hardy crop capable of surviving in low fertility soils and tolerant to climate change (Chadalavada et al. 2021). It is also important to note that even though sorghum is a hardy crop it can still be impacted by stresses that come with climate extremes. These do not only affect the yield of the crop but also the nutritional quality by reducing the essential nutritional components of the grain (Chadalavada et al. 2021). These stresses also affect the economics of sorghum production because when growth and development is hindered the quality and therefore the profit that can be extracted is lowered.

Chadalavada et al. (2021) state that strategies like selecting appropriate varieties and shifting planting dates could be beneficial without compromising the nutritive value of the crop. However, these strategies have to be designed carefully with the available resources and conditions in the area considered lest they prove unbeneficial. Management strategies can be designed in a number of ways in order to achieve particular objectives. According to Gatechew et al. (2023), a different approach of using crop models to find an ideotype of the tolerant variety and focusing breeding efforts on that cultivar is method of selecting a suitable variety. Crop simulation models are used to study the interactions between genotype, management, and environment that help to develop an ideotype for a target environment and locations (Gatechew et al 2023). The environment where a simulation is run and management thereof tend to affect a certain crop differently owing to the genetic material of that variety.

According to Branca et al. (2011), another technology that has gained attention in the bid to increase smallholder food security is the adoption of sustainable land management (SLM). They state that the benefits of these SLM technologies are an increase in food production with the available soil water resources, restoration of soil fertility, enhancing the resilience of farming systems to climate change and improving carbon sequestration. SLM with the benefits it imparts in the soil is a sure way the putting a stop to land degradation and hopefully reversing its impacts in the future. Moreover, adoption of technologies like SLM have the added benefit of improving biodiversity conservation and mitigation of climate change (Branca et al 2011, Liaqat et al 2024).

There is an interconnected relationship between smallholder agriculture and climate change with visible effects on each other (Tabe-Ojong et al. 2023). This is due to various factors, including but not limited to adverse weather conditions, suboptimal farming practices, input choices, crop diversification, and the prevalence of crop diseases and insect pests (Liaqaat et al 2024). Hence, it is imperative to find and adopt the use of alternative crops that have tolerance to climate extremes to ensure for food security. The growing population, projected to exceed nine billion by 2050, needs cereal production to increase by approximately 70% (Liaqaat et al 2024). Sorghum is the ideal alternative crop due to its ability to grow with minimal inputs on poor soils and its tolerance to temperature and water stresses (Chadalavada et al. 2021). The advantage is that sorghum was first domesticated in SSA and therefore knowledge of its cultivation is vast within the region. Screening sorghum crops for stress resistance will allow for the development of resistant varieties to ensure that sorghum is produced sustainably even on marginal soils and adverse climatic conditions (Zheng et al. 2024). Understanding of the response of sorghum to biotic and abiotic stresses of temperature, water, nutrient availability, acidity and salinity could go a long way towards maximizing the sustainability of sorghum productivity.

2.3.1. Impact and response of sorghum to temperature stress.

Sorghum is a warm-weather crop that grows well in soil temperatures above 15°C (Roozeboom and Prasad, 2019 and Rutayisire et al. 2021). Cold/chilling stress is recognized as temperatures between 0-15°C (Rutayisire et al. 2021), and heat stress (HS) is when the temperature exceeds 32°C (Prasad et al. 2021), impede the growth and development of the crop. The predicted temperature increases of 3.7-4.8°C in the present century (Prasad et al. 2021 and Ndlovu et al. 2024) threatens to exacerbate HS especially in semi-arid regions of Sub-Saharan Africa (SSA). The effects of HS occur during pre-flowering and post-flowering stages and manifest as reduction in stem height, panicle size and number of leaves (Ndlovu et al. 2024). This coincides with the vegetative stage when the crop is growing leaves, which are vital in the interception of radiation and photosynthesis, and roots, which are important for absorption of nutrients and crop stand. The grain filling stage is also affected by HS, which ultimately leads to lower yield because the higher temperatures hinder the accumulation of starch and increase in moisture.

In the semi-arid regions of the world sorghum is an important cereal which needs temperatures of 21-35 °C for emergence, 26-34 °C for vegetative growth and development and 25-28 °C for reproductive growth (Prasad et al 2008). Future climate projections point towards short and long episodes of high temperatures that could influence sorghum yields (Prasad et al. 2021). It is therefore important to understand the impacts of season long and short episodes of heat stress on the physiology, reproductive biochemistry, growth, and yield of grain sorghum (Prasad et al 2008, Djanaguiraman et al. 2014). According to Prasad et al. (2008) temperatures above 33°C during the day and 28 °C at night during early stages of panicle development, induce floret and embryo abortion. In Africa, such conditions are common and coupled with poor soils and a lack of resources the impact of climate change will potentially be severe. Moreover, the majority of the population in Africa are smallholder or subsistence farmers' dependant on dryland cropping for their livelihoods.

Sorghum experiences cold temperature stress at temperatures below 15 ºC (Yu et al. 2004, Maulana and Tesso 2013, Casto et al. 2021). According to Rutasiyire et al. (2021), this occurs during the early developmental stages. The developmental stages include leaf expansion, panicle formation and grain filling which are concerned with biomass and grain yield accumulation. Cold temperatures affect sorghum by injuring embryos of germinating seeds that imbibe cold water (Casto et el. 2021). Freezing can result in cold hardening with the crop producing reactive oxygen species (ROS) leading to membrane damage and protein denaturing (Jeyasri et al. 2021). ROS are oxygen derivatives produced during plant reactions such as photosynthesis and respiration (Wang et al. 2024). According to Wang et al. (2024) when maintained at the low levels, ROS can act as signals for plant stress but can be detrimental to plant growth and development in excess amounts. Moreover, Jeyasri et al. (2021) stated that chilling stress such as reduced leaf expansion and wilting affects reproductive development and causes chlorosis and ultimately necrosis. Improvement of cold tolerance of crops like sorghum has the potential to raise yields by expanding the geographical area of cultivation and extending the growing season (Maulana and Tesso, 2013, Casto et al. 2021).

2.3.2 Impact and response of sorghum to water stress

Drought tolerance, which is the ability of the crop to withstand water deficit, is one of the advantages of sorghum and is affected by many genetic factors like morphological ones such a deep root system or physiological ones like quiescence in addition to environmental factors (Amelework et al. 2015; Wagaw et al. 2019). For example, early maturing varieties are more sensitive to water stress during the vegetative stage compared to late maturing varieties because it occurs when the plants have ability to recover when moisture is restored (Gano et al. 2021). Amelework et al. (2015) also found that cultivars from drier areas had higher drought tolerance than their humid environments counterparts. According to Jeyasri et al. (2021), rainfall affects crop production in rainfed agriculture as it controls salinity and acidic properties. When water and oxygen in the atmosphere mix with greenhouse gasses it produces acid rain lowering the soil acidity resulting in unavailability of nutrients for crops.

According to Devnarain et al. (2016), drought, which is mostly experienced during the early vegetative and reproductive stages negatively, affects physiological functions, inflorescence development and leaf growth of sorghum (Jeyasri et al. 2021). Losses due to drought can be around 36% during the vegetative stage and 55% during the reproductive stage (Yahaya 2023). The early growing stages are particularly vulnerable to failure due to stand loss after emergence before seedling establishment (Abreha et al. 2022). Abreha et al. (2022) stated that drought affects starch and energy synthesis by increasing respiration rate. Moreover, after germination drought can reduce transpiration and photosynthesis through leaf senescence (Amelework et al. 2015 and Gano et al. 2021). When occurring after flowering drought can affect biomass, stem sugar accumulation, and ultimately grain yield (Gano et al. 2021).

Excessive water (waterlogging and/or submergence) is another abiotic stress that can affect sorghum growth and development (Zhang et al. 2019); as the anaerobic soil conditions affect the respiration of plants roots (Sharma et al. 2021). Under waterlogged conditions, plants are affected by reduced levels of oxygen and photosynthesis, which ultimately affects the production of adenosine triphosphate (ATP) (Sharma et al. 2021 and Rane et al. 2021). Waterlogging also affects the nutrient uptake mechanisms (Majeed et al. 2023) and enzymatic systems and hormone levels (Vandana et al. 2024). Sub-Saharan Africa is a region characterized by distinct rainy and dry seasons with the rainy season sometimes being accompanied by flooding (Zheng et al 2024). Sorghum plants have several mechanisms to resist water stress drawn from their physiology and genotype. They can reduce leaf area as a means of avoiding moisture loss that comes with drought (Gano, et al. 2021). Choudhary et al. (2020) found that sorghum minimizes water loss by reducing leaf area under conditions of high vapour pressure deficit. The resilience of sorghum to flooding makes it suitable for cultivation in SSA than other cereals (Hadebe et al. 2017).

2.3.3. Impact and tolerance of sorghum to acid stress.

Soil acidity is a consequence of the leaching of base cations (Ca^{2+}, Mg^{2+} and Na^{+}) and the subsequent accumulation of H^{+} , Fe^{3+}, $Mn^{2+}$and Al^{3+} ions in the soil (Kisinyo et al. 2019, Agegnehu et al. 2021). Aluminium toxicity is a major stress to sorghum productivity especially in acid soils which take up to 40% of arable land worldwide (Mola 2021). In sub-Saharan Africa acid soils account for about a third of productive land (Agegnehu et al. 2021). The use of ammonium fertilizers and acid rain worsen the toxicity of aluminium ion in the root-zone leading to limitations in plant productivity (Tari et al. 2013). Aluminium in acid soils hinders the uptake of water and minerals leading to a reduction in plant vigour and yield (Mola 2021). The mechanisms of plant tolerance to aluminium toxicity due to soil acidification generally come in two ways. The first is the exclusion of Al^{3+} from the rhizosphere through the release of organic acids and the second is the chelation of Al^{+3} inside the root and shoot cells (Tari et al. 2013, Mola 2021 and Agegnehu et al. 2021). Sorghum is part of the first group of plants that uses exudation of organic acids to chelate aluminium in the rhizosphere (Tari et al. 2013, Mola 2021). Acid soil management is a major challenge in many countries of sub-Saharan African but the gap can be reduced by learning from countries like Brazil where they were able to convert about 60 million ha of acid soil with crops and pastures using a variety of technologies (Agegnehu et al. 2021).

2.3.4 Impact and response of sorghum to salinity

Salt stress affects plants in a number of ways including ionic and osmotic effect, nutrient and hormonal imbalance, and production of ROS (Jeyasri et al. 2021, Zheng et al 2024). Salt stress lowers the percentage and raises the duration of germination, causes chlorosis and impairs photosynthesis (Tari et al. 2013). Plant growth is influenced by the accumulation of sodium, chloride, calcium, magnesium and sulphate ions that cause ionic, osmotic, and oxidative stresses (Tari et al. 2013, Jeyasri et al. 2021). Sodium and chloride ions are the most harmful to the growth and development of sorghum (Tari et al. 2013, Mola 2021). Sodium has been reported to interfere in metabolic processes like protein translation, transcription and enzyme activity leading to osmotic stress (Jeyasri et al. 2021). According to Liaqat et al (2024), when exposed to sodium ions, salt tolerant sorghum plants increase the expression of the gene that encodes potassium transporters leading to a low Na^+/K^+ ratio which promotes optimal plant growth. Mola et al. (2021) showed that some genotypes of sorghum are tolerant to salt stress at the germination stage while others can tolerate salt stress at the seedling growth stage. Sodic soils also deflocculate and disperse the soil because of the high sodium content, which increases erodibility (Page et al. 2021).

2.3.5 Impact and response of sorghum to biotic stress

The living part of the soil and environment can also have an impact on the growth of sorghum. Bacterial, fungal and viral diseases, insect pests and parasitic weeds are among the biotic stresses that affect sorghum plants either in isolation or combination resulting in reduced productivity and ultimately economic loss (Liu et al. 2016). Yield losses due to biotic stresses have been estimated to be about 35% (Baillo et al 2019). Rizvi et al. (2021) stated that the impact of these soil organisms could be beneficial or negative. Phosphorus solubilizing microorganisms (PSM) play a vital role in the suppression of phytopathogens that cause severe sorghum yield losses (Rizvi et al. 2021). The PSM offer an inexpensive and environmentally friendly biotechnological substitute to chemical P fertilizers and hazardous pesticides during their management of P nutrition and sorghum phytopathogens (Kumar et al

2020, Rizvi et al. 2021). This is another way of attaining sustainable production systems using inexpensive and multi-use growth promoting organisms. The PSMs are particularly interesting because they can work with other plant growth promoting organisms to reduce the negative effects of injudicious application of inorganic fertilizers while simultaneously enhancing the crop tolerance to environmental stress (Rizvi et al. 2021). Moreover, the rhizospheric microbes work in the soil-plant interface to improve nutrient uptake and use efficiency, nodulation and plant development advancement, resistance to abiotic and biotic stress and reduction of environmental contamination, which ultimately improves agricultural sustainability (Kumar et al 2020). According to Li et al. (2008), abiotic stress like drought and high temperature can lead to biotic stresses through their encouragement of outbreaks of phytophagous insects that cause insect attacks and insect-feeding diseases. For instance, aphids can kill sorghum by crops removing juices from plant tissues and injecting a potent salivary toxin when they feed on them (Li et al 2008). Management strategies that improve soil microbial diversity and functioning may contribute significantly to crop yield improvement. In addition to the management strategies, selection of appropriate varieties could also contribute.

Plants can develop a new metabolic homeostasis in response to a particular stress and continue to grow without suffering injury (Mola, 2021). The resistance of cereals to biotic stresses has been correlated to the increase of phenolic compounds and activities of phenolic related enzymes (Dicko et al. 2005). Plants have that evolved genes that encode transcription factors (TF) that regulate their stress-response are good candidates for crop improvement (Baillo et al. 2019). A considerable amount of research has been done on TF's role in crop improvement in important cereals but studies on sorghum are limited (Baillo et al. 2019). Mola et al. (2021) reviewed the genetic diversity of Ethiopian sorghum landraces for stress resistance and found that a gap still exists in the genetic potential of sorghum. Baillo et al. (2019) stated that the small genome size of sorghum makes it ideal for genomic and functional studies of plant growth under stress condition. They continue to assert that response strategies involve changes at the molecular, biochemical and physiological level and controlled by genes encoding transcription activators and repressors that regulate downstream stress-induced genes and pathways. Considering the negative effects of temperature and water stress, acidity, salts and biotic factors, climate smart agricultural approaches could make a valuable contribution to sorghum productivity and soil quality improvement.

2.4 Climate smart agriculture and soil quality

Soil health describes the ability of the soil system to function as an ecosystem whilst soil quality defines the capacity of the soil function as part of an ecosystem (Brady and Weil, 2008, Ponge 2015). Soil health is a measure of the natural capacity of a soil to respond to agricultural management and provide other ecosystem services and soil quality refers to the physico-chemical and biological properties of soil and how they relate to management practices and soil functions (Recha et al 2022). The concept of soil quality defines the capacity of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation (Gallandt et al. 1999). Improvement of soil quality and spatio-temporal management of all production factors requires the maintenance of increased levels of production without further environmental damage (Cassam et al 1999).

Climate smart agriculture comprises of three connected pillars of productivity, adaptation and mitigation using management practices like, reduced or zero tillage, residue retention, water and nutrient management and crop diversification (Datta et al. 2022). This has the potential of increasing soil organic matter as the most important aspect. Optimum levels of OM are indicators of the extent of management practices improving soil architecture and composition. For example, reduced and no-till agriculture minimizes soil disturbance and soil organic matter decomposition rates and encourages higher fungal and earthworm biomass (Bai et al. 2019). The coexistence of the living and non-living parts renders the soil conducive for its ecosystem services such as being habitable to various creatures and modifier of the environment. The balance between carbon inputs and outputs plays a role as a soil quality indicator in agricultural systems for soil productivity and climate change mitigation and adaptation (Bai et al. 2019). It is important to analyse and understand which specific soil quality parameters that are affected by land use and which agricultural practices are linked to declining soil quality parameters (Recha et al. 2022).

In a study conducted to study the effects of CSA practices on soil fertility, moisture content and crop yields in Ethiopia, Tadesse et al. (2021) found high nutrient availability, which they attributed to the mineralization of the returned crop residues. Crop residues serve as a barrier between the soil surface and erosive forces of weather, blankets for the maintenance of optimum temperature and conservation of moisture and a source of slow-release nutrients. Datta et al (2022) reported increased organic carbon in soils under conservation tillage than under conventional tillage, which they explained by noting that that the CA system had 8.1 t/ha rice residues retained. According to Paustine et al. (2016), the conversion of natural forests and grassland results in losses of soil carbon in the range of 0.5-2 Mg per hectare per year and is not advisable in a time of changing climate. However, this becomes difficult considering the need to increase production for a growing population. Hence the need to find ways of intensifying production systems in a sustainable manner (Paustine et al. 2016, Recha et al 2022 and Alabi et al 2023). In sub-Saharan Africa, small-scale farmers realise the need for farming methods that can absorb, to some extent, the shocks of a changing climate and have adopted the climate smart agriculture approach (Okoronkwo et al. 2024). This adoption is seen in farmers from low rainfall areas switching to drought tolerant crops and some have started diversifying their cropping systems and even changing planting dates to suit the variability of rainfall (Abegunde et al. 2019).

According to Singh and Singh (2017), traditional agricultural practices such as intercropping, crop rotation, traditional organic composting, agroforestry and integrated crop-animal farming form the basis of CSA and have the potential to enhance crop productivity and mitigate climate change. Bai et al. (2019), when incorporating biochar addition in their climate smart agricultural practices SOC content increased by 39% compared to cover crops and conservation tillage, which resulted in 6% and 5% SOC content, respectively. The addition of biochar affects SOC dynamics by improving soil aggregation and the physical protection of aggregate-associated organic matter from microbial attack and increasing the pool of stubborn organic substrates leading to low decomposition rate and substantial negative priming (Bai et al. 2019). Earthworm abundance and biomass was found to be 60-70% higher under CSA compared to conventional agriculture (Jat et al. 2022). This is because conventional agriculture exposes the earthworms to predators and decreases their food by accelerating organic matter decomposition (Jat et al. 2022). The same authors reported increased mean weight diameter of aggregates under CSA through the increase in TOC, reduced soil disturbance and increasing earthworm numbers.

Another factor that can influence soil properties of a particular soil is the type and age of the crop growing in the soil through the secretion of root exudates. The quantity and quality of root exudates is determined by type and growth stage of plant growing in that soil (Ma et al. 2022) and this affects the diversity and activity of microbes, biochemical processes and enzyme activities (Jat et al 2021). For example, the density and diversity of soil fungi, which is important for nutrient cycling, transformation and availability, is influenced by the residues retained (Choudhary et al. 2022). Tillage and crop rotation practices play a role in the distribution of microbes in soil though soil disturbance (Orrù et al., 2021). Soil enzymes are the key players in biochemical processes of organic matter decomposition of and nutrient cycling acting as catalysts and strongly influence energy transformation, environmental quality, and agronomic productivity (Jat et al. 2021). Moreover, changes in soil enzymes determine nutrient availability and crop productivity (Jat et al 2021). Higher enzyme activity (dehydrogenase, urease, protease, phosphatase, β-glucosidase) was reported under no till than conventional tillage system in sorghum cultivation under subtropical conditions (Jat et al 2021). According to Recha et al. (2022), soil quality indicators must be applicable to the wide range of environments and incorporate diverse soil properties from physical, chemical and biological.

CA has the potential to alleviate food insecurity and sustain agriculture but a good understanding of yield response to the inherent soil characteristics is needed (Ren et al. 2023). For instance, Ren et al. (2023) found that yield increases under CA were greatest in areas with coarse textured soils in the tropics. Abdallah et al. (2021) stated that CA management can directly affect water holding capacity (WHC) directly through increasing SOM and indirectly by changing the pore size distribution to increase pore connectivity the hydrophilic property of SOM renders it capable of holding water. According to Scopel et al. (2013), soil erosion can be reduced by a factor of 2-20 through no-till depending on the slope and texture in tropics and through stability of top soil aggregates and improved infiltration due increase SOM in the temperate zones.

2.5 Summary

Sub-Saharan Africa is among the regions of the world that will be impacted by climate change more than other regions due to several factors like population size, reliance on rain fed agriculture in a semi-arid environment, lack of land and inputs and socio-economic and political challenges. Governments in the region lack the capacity to provide basic needs like food, health care, education, shelter and employment for the ever-growing populations because of socio-economical and geo-political pressures. The predicament of SSA requires a holistic approach that will address all the challenges giving them equal significance.

Climate smart agriculture is an approach that aims to sustainably intensify agricultural production, increase and maintain biodiversity, eliminate environmental pollution and reduce greenhouse gas emissions using cost effective means. Sustainable and increased production is a requirement for improved food security. This can be achieved through cultivation of nutritious and stress tolerant crops in suitable locations. Sorghum is one such crop, which is native and therefore adapted to SSA conditions. It boasts tolerance to high and cold temperatures, excess and deficient water, poor fertility, acidity, salinity and heavy metal toxicity and local famers possess the requisite knowledge to grow it successfully. The challenge of providing for the population of SSA now and into the future needs the participation of everyone involved in the sourcing of production inputs, all the farmers (commercial, emerging and smallholder/subsistence) and policy makers in government.

CSA is not fix it all solution to climate related challenges but requires the integration of local and scientific knowledge when selecting the appropriate CSA practice on a particular agro-ecology (Were et al. 2016). Sub-Saharan Africa is experiencing of shortage of nutrients and low nutrient use efficiency due to land degradation, which is caused to a lack of crop residue retention and insufficient use of mineral and organic inputs to offset the loss of organic matter, and the nutrients lost with harvests (Gram et al. 2023). Zougmore et al. (2018), citing the intergovernmental panel on climate change (IPPC) state that the food growing seasons in southern and western Africa may be shortened by 20% by 2050 leading to a 40% decline in cereal yields. For this reason, adoption of climate smart agriculture is vital for food security.

The adoption of CSA technologies and practices like conservation tillage to reduce soil disturbance, residue retention to increase nutrient inputs and green manuring to increase nitrogen and phosphorus contents in Africa have been shown to be the reasons behind improved soil nutrient status, enhanced SOC, improve aggregation and subsequently crop yields (Kichamu-Wachira et al. 2021). However, this adoption is not yet spread all over the continent to the desired levels due to several factors. There is lack of attention put into interventions and technologies aimed at ensuring that agricultural systems in SSA can adapt to climate change and variability (Zougmore et al. 2018).

CHAPTER 3: PLANTING DATE AND TILLAGE EFFECTS ON YIELD AND NUTRIENT UPTAKE OF TWO SORGHUM CULTIVARS GROWN IN SUB-HUMID AND SEMI-ARID REGIONS IN SOUTH AFRICA

(A version of this paper was published in frontiers in Agronomy journal).

Abstract

Sorghum is emerging as a viable option to increase food production under changing climate due to its resilience to drought and marginal soils. Appropriate planting date, crop cultivars, type of tillage and rotations, under contrasting climatic conditions, could make significant contribution on the effective management of sorghum under changing climatic conditions. A short-term study was carried out to investigate the effects of planting date, crop cultivar, tillage type and rotations on the growth and primary nutrient uptake of sorghum in contrasting climatic regions. The study was conducted in Free State - (FS) and KwaZulu Natal (KZN) Provinces of South Africa over two seasons (2020/21 and 2021/22). The two cultivars (Pan8816 and Macia) were sown in December (first planting date) and in January (second planting date) under conventional tillage (CT) and no-till (NT) and with two rotations (Sorghum-Cowpea-Sorghum and Sorghum-Sorghum-Sorghum) resulting in a 2×2×2×2 factorial experiment. In KZN, in both the first (2020/21) and second (2021/22) seasons, the effects of all the factors, except cultivar in the second season, were not significant on yield, and uptake of nitrogen (N), phosphorus (P) and potassium (K). In the second season, Pan8816 (4.40 t/ha) produced 3.3 times higher yield than Macia (1.32 t/ha), and took up higher N, K and P. In FS, the early planted sorghum (1.15 t/ha for season one; 3.39 t/ha for season two) had significantly higher yield than late planted sorghum (0.434 and 0.91 t/ha for seasons one and two, respectively). Furthermore, the early-planted crops took up higher N, K and P than when planted late, while Pan8816 took up 1.74 times more P than Macia. Plants grown under CT (2.61 t/ha) produced significantly higher yield than under NT (1.70 t/ha), with plants under CT taking up significantly more K than those grown on NT. These findings suggest that in the drier FS (annual average rainfall 401 mm), early planting under conventional tillage, while in the wetter KZN (annual average rainfall 799 mm), selecting

the appropriate cultivar (PAN8816), are essential for sorghum grain yield, at least in the short-term.

Keywords: climate change conservation agriculture, food security, small-scale famers, Sub-Saharan Africa

3.1. Introduction

The agriculture sector needs to produce more food from less land and water resources to provide for a growing population with an increasing per capita income in an urbanized world (FAO, 2017) under the difficult conditions of a changing climate (Lal, 2013). Climate change poses direct and indirect threats to food security through its influence on soil properties, water resources, and crop production (Fischer et al. 1996; Gornall et al. 2010; Lal, 2013; Masipa 2017). These effects include, but are not limited to, loss of SOC, reduced fertility and plant available water, the prevalence of pests and diseases, changes in the hydrological balance, and increased soil erosion (Gornall et al. 2010; Lal, 2013). According to Lal (2013), agriculture is vulnerable to changes in climate, and projections point towards challenging times for farmers and land users, especially those in the developing world, especially sub-Saharan Africa (SSA) (Lal, 2019). It is therefore important to develop agricultural technologies and strategies that minimize the effects of climate change and can enhance and sustain soil productivity levels under adverse climatic conditions. This can be achieved through the selection of the appropriate crops to be grown under systems modified to suit the prevailing conditions in a particular region.

Conservation agriculture (CA) continues to be promoted as a suitable strategy that can ensure improved and sustainable production levels without compromising the environment or exacerbating land degradation (Aziz et al, 2013; Busari et al, 2015). In essence, CA entails the integrated management of available natural resources and other inputs to maximize efficiency (Choudhury et al, 2016) through minimal soil disturbance, permanent soil cover, and crop rotations (Hobbs et al. 2008). For maximum benefits to be realized, all the three principles of CA must be applied simultaneously. When applied appropriately, CA improves soil porosity, which in turn reduces runoff and erosion hence enhancing biomass production. CA can increase yield by up to 56% compared to conventional tillage due to increased soil organic matter and reduced evapotranspiration (Thierfelder and Wall, 2012; Wakindiki et al. 2018). However, smallholder farmers often struggle to maintain the requisite amount of crop residue for soil cover due to competing uses. Moreover, most soils under smallholder farming are too poor to support optimum biomass production. However, the inclusion of crop rotation in no-till systems may boost biomass production thus ensuring the retention of optimum levels of residues, which may increase yield.

Whilst it is generally agreed that conversion from conventional tillage to no-till has potential to increase crop productivity, its effectiveness is influenced by soil and climate factors. For instance, Toliver et al. (2012) analyzed data from 442 paired tillage experiments across the United States, examining six crops and various environmental factors such as geographic location, annual precipitation, soil texture, and time since conversion from tillage to no-till. The results indicated that no-till generally resulted in similar or higher yields than conventional tillage for sorghum and wheat. However, environmental factors influenced yield variations between the two tillage methods for other crops. Moreover, no-till tended to produce similar or greater yields than conventional tillage in warmer and more humid regions with loamy soils. Conversely, in drier regions with sandy soils, no-till yields were often lower than conventional tillage yields. This suggests that CA may be less beneficial on sandy; however, there is not sufficient evidence on this in South Africa. Moreover, the effects of CA may be mediated by environmental conditions and cropping systems. Therefore, this study aims to show that no-till can be a viable and beneficial agricultural practice, particularly for certain environmental conditions and resilient crops like sorghum.

Due to climate change, areas that are currently suited to maize in the African savannah may become more favorable for sorghum in the future (Lal, 2013). Summer crop production in South Africa is shifting from current production areas due to changes in rainfall and temperature regimes (Weepener et al.2014). Sorghum thrives on low fertility soils with limited inputs for food and industrial use in breweries, production of animal feed, and as feedstock for biofuel production (Meki et al. 2013 & Ikazaki et al. 2020). Moreover, sorghum is the fifth most important cereal crop after wheat (*Triticum aestivum)*, rice (*Oryza sativa)*, maize (*Zea mays)* and barley (*Hordeum vulgare)* (Bakari et al. 2023) with high tolerance to heat and drought. The crop can remain dormant during dry periods (Pinzi & Dorado, 2011). During the period 1998 to 2020, 58-64 million tons of sorghum was grown on 43.2 million hectares of land globally, which equates 6.4% of total cereal growing land and 2.7% of total cereal production (Bakari et al. 2023).

Despite this huge potential, there is a critical knowledge gap. Very few studies have reported the performance of sorghum under CA across diverse environments and soils particularly sandy soils in South Africa. Although South Africa is traditionally a maize growing nation, climate change suggests that the baseline could shift towards drought tolerant crops such as sorghum as a coping mechanism. Adapting sorghum-planting dates to synchronise with potential shifts in rainfall and temperature patterns, which vary across seasons, has been proposed as an adaptation strategy. However, there is little evidence regarding the effectiveness of this approach in South Africa. To address this gap, this study aimed to compare the effects of growing two sorghum cultivars on two different planting dates and two tillage practices on sorghum growth, nutrient uptake and yield in two areas with contrasting soil and climatic conditions over two seasons. This study will provide valuable insights for optimizing sorghum production under CA in South Africa.

3.2. Materials and methods

3.2.1 Site description

The study was conducted in Houtnek village (-29.0264; 26.8014) in Thaba Nchu, Free State and in Qongqo village (-28.0434; 31.6183) near the town of Nongoma, Kwa-Zulu Natal provinces, South Africa (Figure 3.1). Free State was chosen for the climate and potential of adding the underutilized sorghum in the farming activities of the small-scale farmers of the region and KZN was selected because the emerging famers in the region have had some success with conservation agriculture. The town of Thaba Nchu forms part of the Mangaung Metropolitan Municipality together with Bloemfontein and Botshabelo. The town is located at 1531m above sea level and receives approximately 692 mm of rainfall annually with an annual average temperature of 15.2 ºC (Muthelo et al. 2019). The predominant farming activities are livestock (cattle and sheep) production and to a lesser extent cropping (oil seed crops). The choice of this site was for possible introduction sorghum into the cropping activity. The area is characterized by duplex soils of the Valsrivier form, which translate to the chromic luvisol in the word reference base (WRB) for soil resources classification (van Huyssteen, 2020). These soils typically have dark brown to reddish brown topsoil and moderate to strong structured subsoil (Land type survey staff 1973-2004). The Highveld grassland vegetation dominates the area.

Nongoma is one of five towns that constitute the Zululand District Municipality. Annual rainfall in the area is reported to range from 800 to 1000 mm due to variation in topography, and the average temperature ranges between a minimum of 4 ºC to a maximum of 20 ºC (ZDM, 2018). Soils in the area are of the Swartland form (Protocalcic haplic luvisol in WRB) (van Huyssteen, 2020) with dark brown to grey topsoil transitioning to a moderate to strong blocky structured subsoil on weathering rock (Land type survey staff 1973-2004). The vegetation is described as the Natal lowveld bushveld. Majority of agricultural activity in the area is maize and beans. The climatic characteristics of the two sites are presented on Table 3.1.

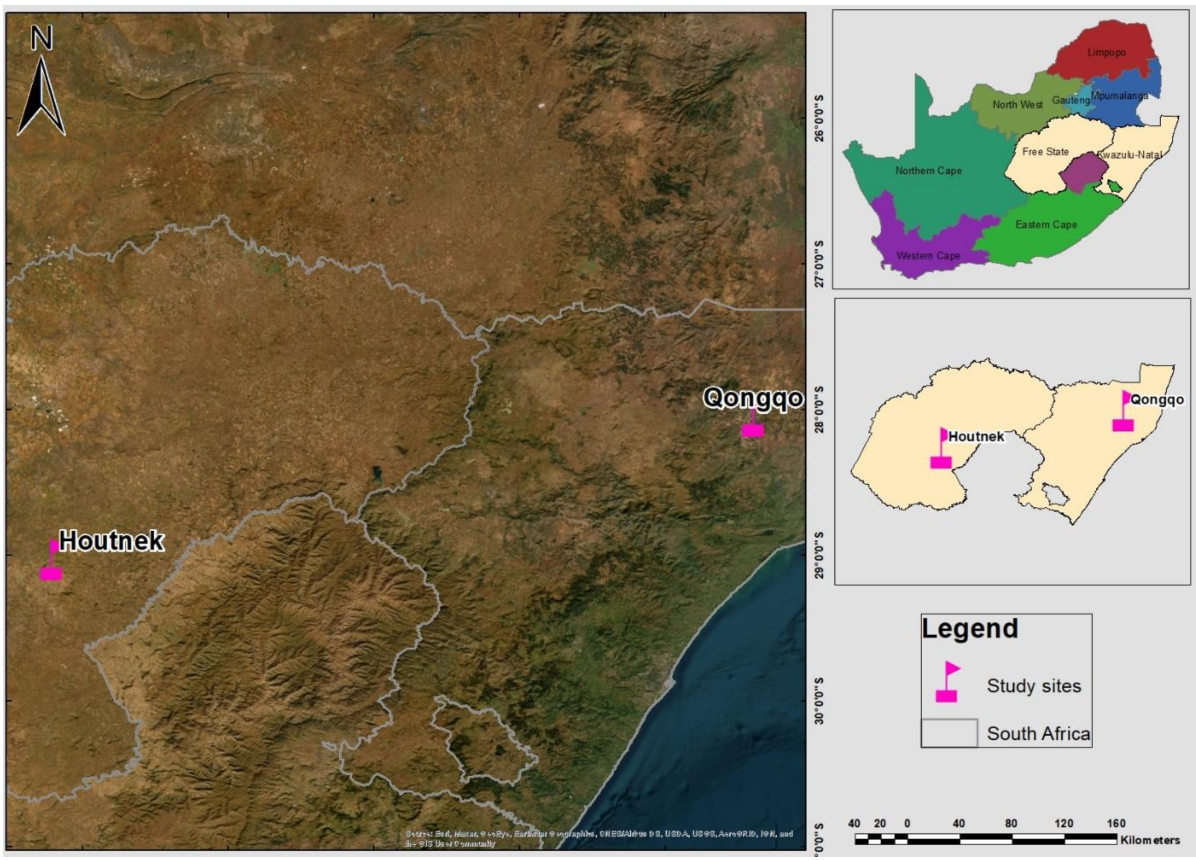

Figure 3. 1: Map showing the study sites within Free State and KwaZulu Natal Provinces of South Africa.

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Table 3. 1: Maximum and minimum monthly temperatures and rainfall in KZN (2013-2022) and FS (2013-2021).

	Kwa-Zulu Natal			**Free State**		
Month	**Max T (°C)**	**Min T (°C)**	**Rain (mm)**	**Max T (°C)**	**Min T (°C)**	**Rain (mm)**
Jan	24.8	18.4	67.9	34.7	15.9	77.1
Feb	27.4	20.3	78.7	32.6	15.1	94.7
Mar	27	19.7	102	30.9	12.7	71.1
Apr	25.3	17.2	173	27.1	8.33	60.6
May	24.7	15.4	41.2	25.3	3.13	16.6
Jun	23.6	13.4	7.77	22.3	-1.03	8.24
Jul	22.8	12.5	46.8	22.1	-1.69	12.4
Aug	22.8	13.7	34.1	24.0	0.65	8.69
Sep	20.7	13.6	54.9	26.2	4.82	8.89
Oct	21.4	14.5	103	31.6	9.39	30.3
Nov	21.9	15.4	119	32.8	11.5	59
Dec	23.4	17.2	131	34.2	14.5	87.4
Annual average	23.8	15.9	799	28.7	7.77	401

Source: Mrubata et al. 2024

3.2.2 Soil sampling and characterization

Soil samples were collected from the 0–20 cm depth at each site for initial characterization prior to land preparation. The samples were taken following the grid sampling procedure to ensure that the whole 1 ha was covered and then mixed into a composite prior to analysis The soil analysis results were used as a guide for fertilizer application recommendations. Soil pH and EC was measured in a 1:1.25 soil: water suspension using a laboratory pH meter and particle size distribution by the hydrometer method (Beretta et al. 2014). Essentially a soil-water-calgon suspension was used to separate particles of different sizes based on their settling velocity as determined by Stoke's law. Total N was determined by dry combustion using a Leco CN-2000 analyzer (Wright & Bailey, 2001) and P by the bray 1 method of Bray and Kurts (1945).

The 1M neutral ammonium acetate method (van Reeuwijk, 2002) was used to determine CEC. A 1M ammonium acetate solution (NH4OAc) at pH 7 was used to extract the cations from the soil samples and the solution was shaken for two hours and centrifuged to separate the liquid and solid phases and analysis of cations was done by inductively coupled plasma-optical emission spectrometry (ICP-OES). The CEC was determined as the sum total of the determined cations. The determination of soil organic carbon followed the Walkley-Black method (Wang et al. 2012), where soil organic matter was oxidized with 1N potassium dichromate in the presence of sulphuric acid and the percentage amount of organic carbon calculated from the amount of dichromate remaining after titration with ferrous sulphate.

Selected characteristics of the study soils are shown on Tables 3.2. Soils from both study areas show that the two sites were acidic, with KZN (pH 5.17) being more acidic than FS (pH 5.71) and the EC fell in the range of non-saline in both FS (3.0 mS/m) and KZN (7.79 mS/m) (USDA-NRCS, 2020). The KZN soil had more cations than the soil from FS. Total N was similar at KZN (0.13%) and & FS (0.11%), while the soil in KZN had more P (6.59 mg/kg) than the 2.88 mg/kg P in the FS soil. The soil in FS was sandy loam and had more sand (74%) and less clay (20%) than the soil from KZN, which was sandy clay loam and had 56 % sand and 38 % clay.

Table 3. 2: Initial soil properties of the two study sites.

Soil property	Free State	KwaZulu-Natal
pH H_2O	5.71	5.17
EC (mS/m)	3.05	7.79
Exchangeable Ca (mg/kg)	455	868
Exchangeable Mg (mg/kg)	156	196
Exchangeable K (mg/kg)	217	232
Exchangeable Na (mg/kg)	6.88	14.0
CEC meq/100g	4.13	4.93
OC (%)	0.745	2.37
Total N (%)	0.108	0.134
Avail P (mg/kg)	2.88	6.59
Clay (%)	20	38
Silt (%)	6.0	6.0
Sand (%)	74	56
Texture	Sandy loam	Sandy clay loam

3.2.3 Agronomic practices

The two cultivars of sorghum used in this study were the bronze-grained, medium to late maturing PAN8816, known to be highly resistant to leaf disease and head smut and the early to a medium maturing open-pollinated variety (OPV), Macia. The common characteristic of the cultivars is that they are both low tannin, semi-dwarf hybrids.

Mono-Ammonium Phosphate (MAP) was used as the pre-plant fertilizer and Lime Ammonium Nitrate (LAN) as the topdressing fertilizer at 3-4 weeks after planting. MAP was applied at a rate of 15.41 kg/block and LAN at a rate of 11.76 kg/block in FS. In KZN the application rates were 13.44 kg of MAP per block and 10.08 kg of LAN per block. The selective herbicide used was Sorgomil® Gold 600sc at 174.72 ml per block in FS and 201.6

ml per block in KZN, owing to the difference in texture between the 2 sites. Cypermethrin 200 EC was applied for the control of stalk borer at 23.5 ml per block and Decis® forte for the control of armyworm at a rate of 4.032 ml per block. The herbicide SLASH PLUS 540 SL was used at the start of each season for the control of perennial weeds at least 7 days prior to plant at a rate of 83.36 ml per block.

3.2.4 Experimental set up

Sorghum was sown in 6 x 4.5 m plots with 1 m spaces between them and 4 m between the tillage systems and 2 m between planting dates resulting in each block being 28 × 24 m or 672 m^2. Each plot consisted of 7 rows with 45 cm inter-row spacing.

The experiment was laid out in completely randomized block design with a strip-strip-split-plot treatment layout (Gomez & Gomez, 1984) and replicated three times. The factors of tillage and planting date were assigned to the vertically and horizontally arranged main strips. The two vertical strips were divided between conventional tillage (CT) and no-till (NT) while the horizontal strips were randomly assigned to early planting (December) and late planting (January). This vertical – horizontal arrangement of the strips resulted in intersection plots, which were split in two for the assignment of the two crop rotations, sorghum –sorghum-sorghum (S-S-S) and sorghum –cowpea-sorghum (S-C-S). The rotations were further split in two to accommodate both cultivars (PAN8816 and Macia) used in the trial. This resulted in 16 treatment combinations that were replicated three times. The field layout is visually depicted in the graphic below (Figure 3.2), showing a single block out of the total of three.

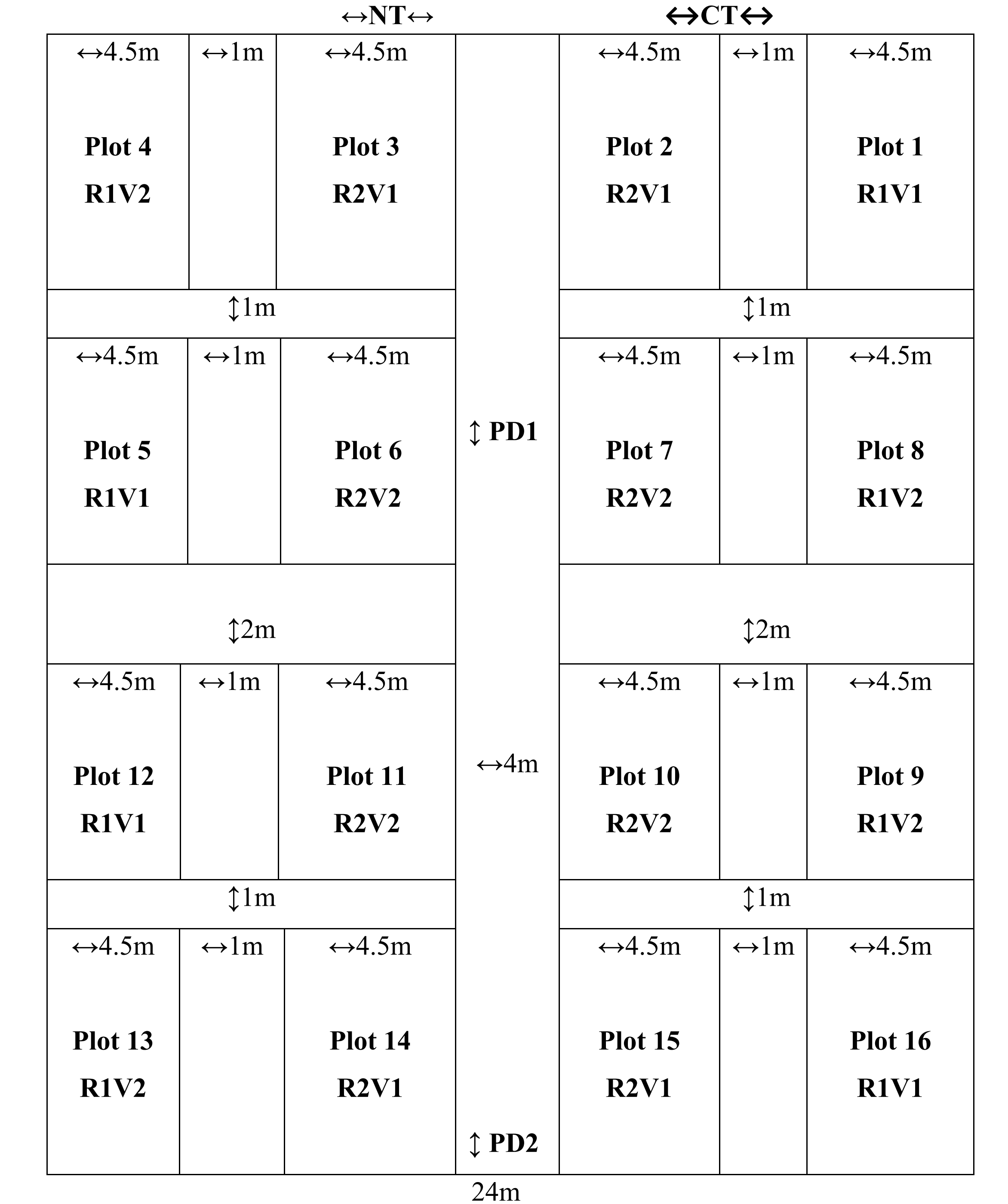

Figure 3. 2: Field map showing 1/3 blocks with dimensions and treatments per plot.

Where: CT=Conventional tillage; NT=No-till; PD=Planting date; V1=PAN8816; V2=MACIA; R1=S-C-S; R2=S-S-S

3.2.5. Measurements

During the months of May and June in 2021 and 2022 plant height and grain yield were determined. Plant height was determined at harvest (end of the season) using a graduated rod from the bottom the highest leaf. Plants that fell within the boundaries of a 4 m x 3 m (three middle rows) net plot were harvested at the end of the growing season to determine grain yield at 12%. The harvested panicles from each plot were threshed and weighed to determine yield. At the end of the season, leaves were taken from three random plants (same plants as for plant height) for leaf tissue analysis for nitrogen, phosphorus and potassium following the method of Zekri at al (2015).

3.5.6. Statistical data analysis

The field trial was made up of 2 planting dates, 2 tillage systems, 2 cultivars and 2 rotations making the experiment a 2×2×2×2 factorial. The study was replicated three times and laid out in three blocks. The data from the field trial was subjected to Analysis of variance (ANOVA) with the four factors as fixed effects and mean separation performed using least significant difference (LSD) at a confidence interval of 95%. The analysis was performed using JMP Pro Version 17 (JMP Statistical Discovery LLC 2022).

3.3. Results

3.3.1. Effects planting date, tillage and sorghum cultivar on plant height in FS

Yield parameters, panicle length and 1000 seed weight did not show significant differences across treatments in FS. The three-way interaction effect of planting date, tillage and sorghum cultivar did not have significant effects ($p>0.05$) on plant height in the first season. Only the tillage × cultivar interaction effects, and the main effects of planting date and cultivar significantly ($p<0.05$) affected plant height in FS. Greater plant heights were observed in early than late-planted crop. Under NT, Pan8816 had significantly taller plants than Macia when planted early (Figure 3.3). The only difference in plant height between the tillage systems was for Macia, when planted early, where CT had taller plants than NT (Figure 3.3).

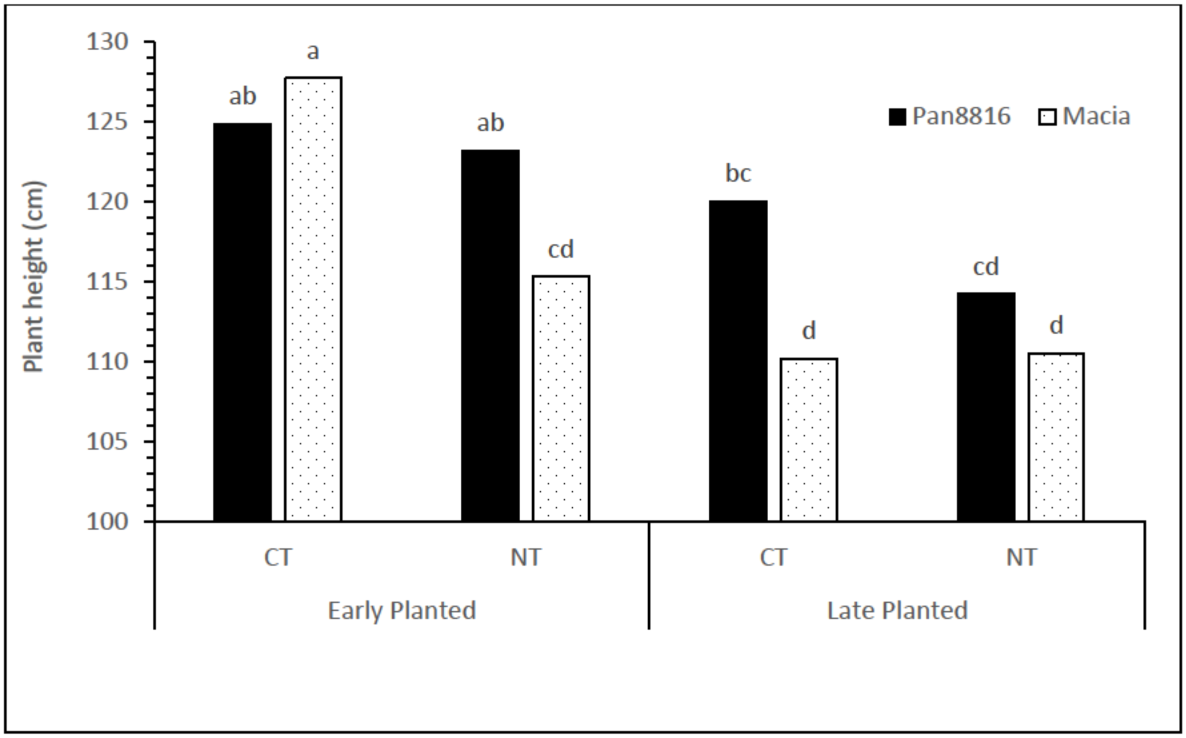

Figure 3. 3: Effect of planting date×tillage×cultivar on plant height in FS in season 2021/22. Different letters on the bars indicate significant differences at $p < 0.05$

Under NT, the sorghum-cowpea-sorghum rotation resulted in significantly shorter plants than the sorghum-sorghum-sorghum rotation when planted early (Figure 3.4). When planted late, there were no significant differences between the rotations irrespective of tillage system (Figure 3.4). Moreover, when planted late, the sorghum-cow pea-sorghum rotation resulted in significantly taller plants than the sorghum-sorghum-sorghum rotation, for PAN8816 but not Macia (Figure 3.5).

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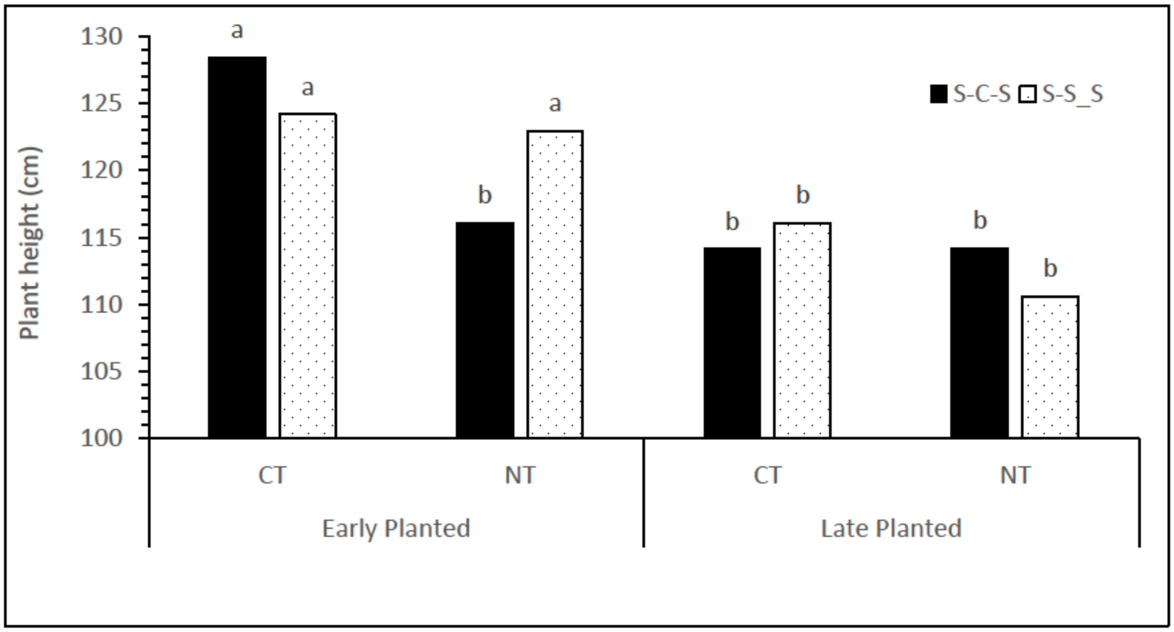

Figure 3. 4: Effect of planting date×tillage×rotation on plant height in in FS in season 2021/22 Different letters on the bars indicate significant differences at $p < 0.05$

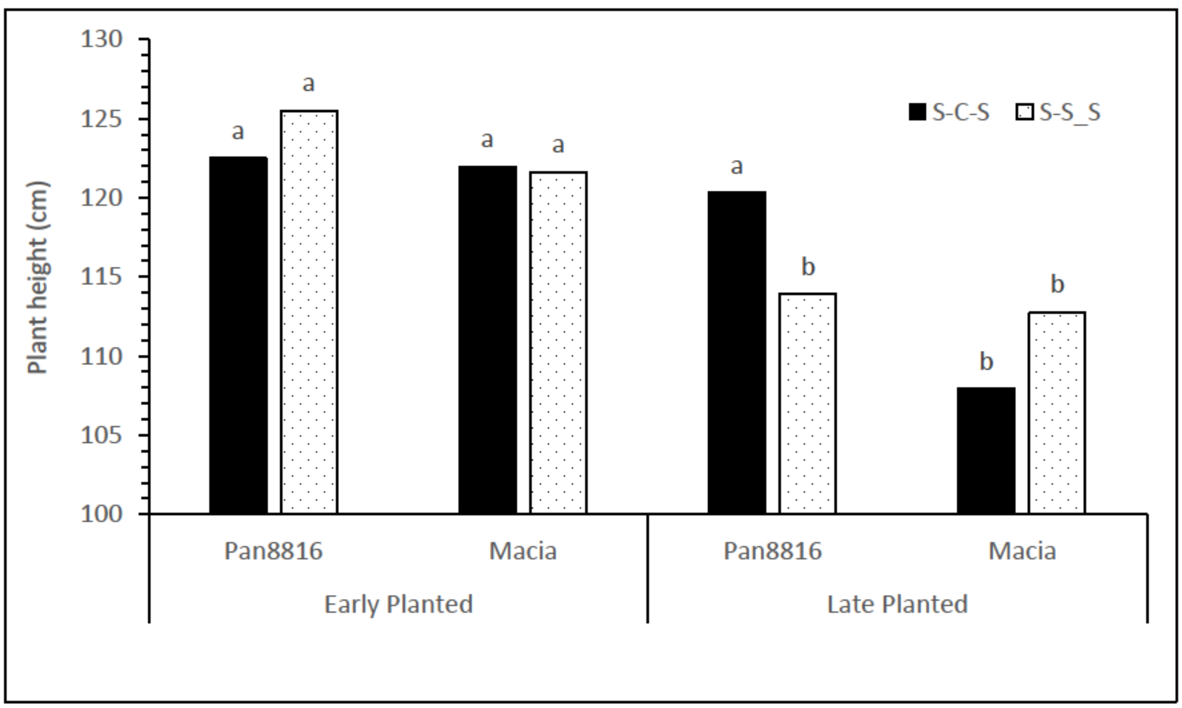

Figure 3. 5: Effect of planting date×cultivar×rotation on plant height in FS in Season 2021/22. Different letters on the bars indicate significant differences at $p < 0.05$.

3.3.2. Effects planting date, tillage and sorghum cultivar on plant height in KZN

Only the planting date × tillage interaction had a significant effect on plant height in KZN in the first season. There were no significant tillage effects for early-planted crops, while for late planting; NT resulted in taller plants than CT (Figure 3.6). Late planting resulted in significantly shorter plants only under CT but not under NT (Figure 3.6). For the second season (2021/22), the 4-way interaction effects of planting date × tillage × cultivar × rotation were significant ($p<0.05$) on plant height. In the second season, PAN8816 resulted in taller plants than Macia, in the sorghum-cowpea-sorghum rotation, under CT but not under NT, for both planting times (Figure 3.7). The PAN8816 also had taller plants than Macia, in the sorghum-sorghum-sorghum rotation under NT only when planted early, with no differences for late planting. Overall, the Pan8816 (140 cm) was generally taller than Macia (113 cm) in the second season (Figure 3.7).

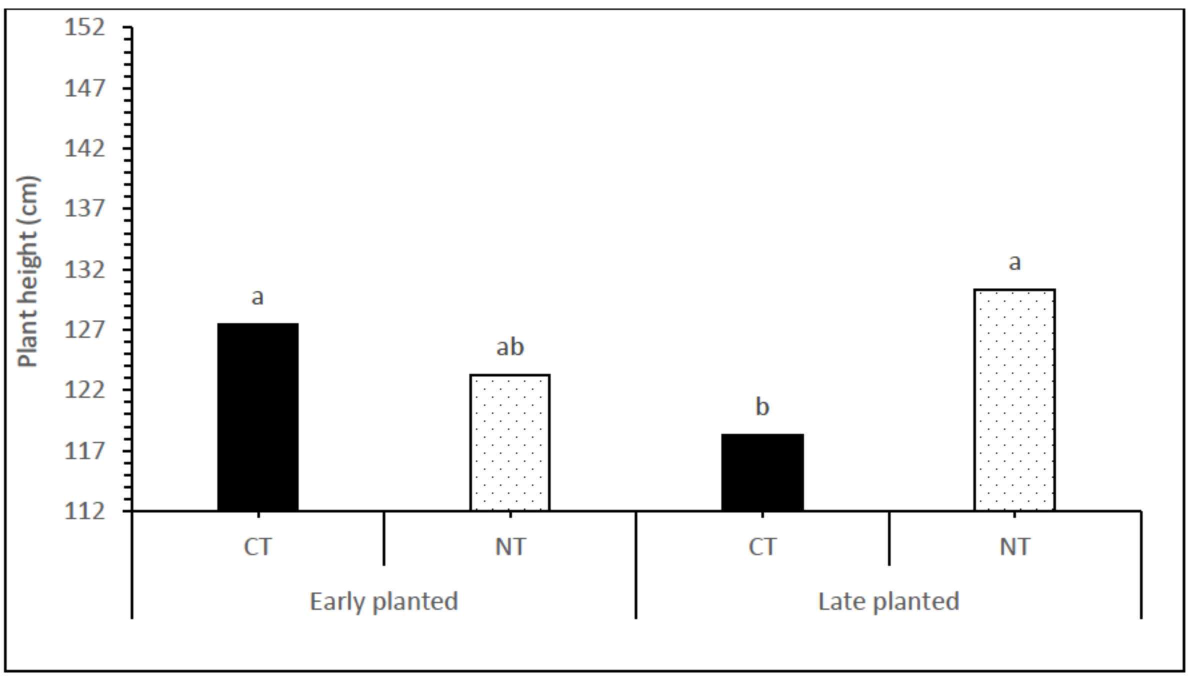

Figure 3. 6: Effect of planting date×tillage on plant height in KZN in 2020/21.
Different letters on the bars indicate significant differences at $p < 0.05$.

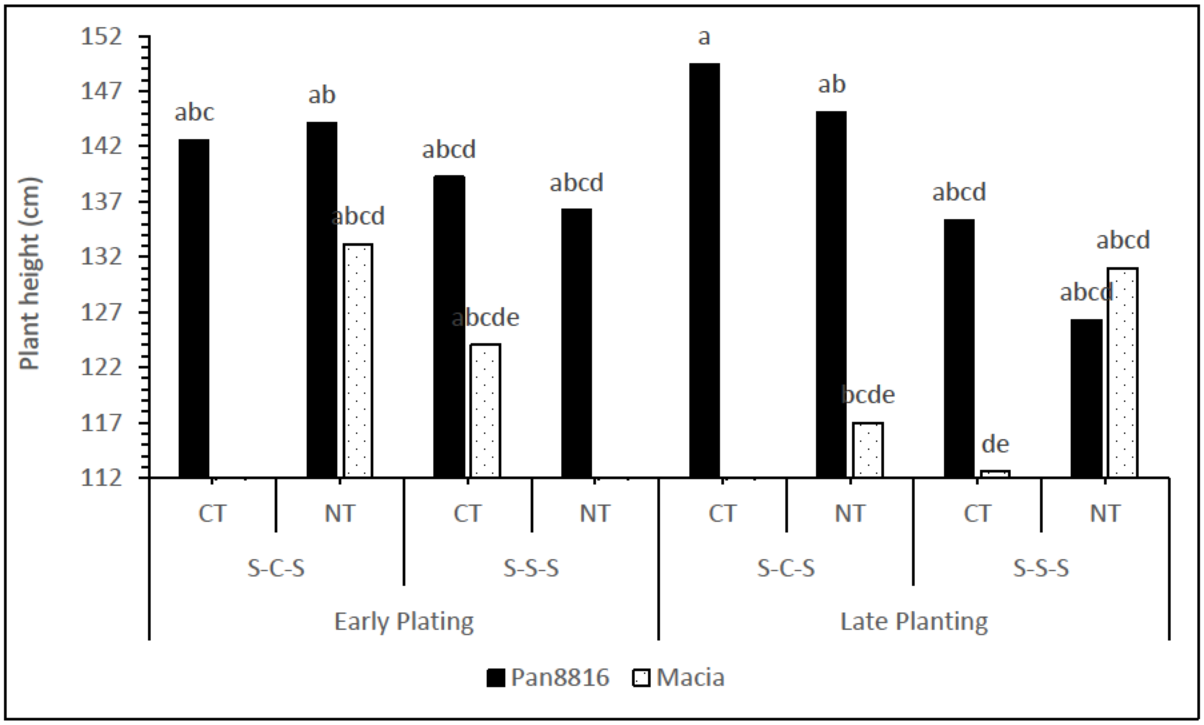

Figure 3. 7: Effect of plating date×tillage×cultivar×rotation on plant height in KZN in season 2021/22.
Different letters on the bars indicate significant differences at $p < 0.05$.

3.3.3. Grain yield and primary nutrient uptake in FS

There were no interactive effects of PD, tillage, cultivar on yield in FS for the first season. The early planted sorghum (1.15 t/ha) had 2.66 times higher yield than late planted (0.434 t/ha) sorghum in the first season. In the second season (2021/22), early planting (3.39 t/ha) resulted in significantly ($p<0.05$) higher yield than late planting (0.91 t/ha), while plants grown under CT (2.61 t/ha) produced significantly higher yield than under NT (1.70 t/ha) in FS (Figure 3.8).

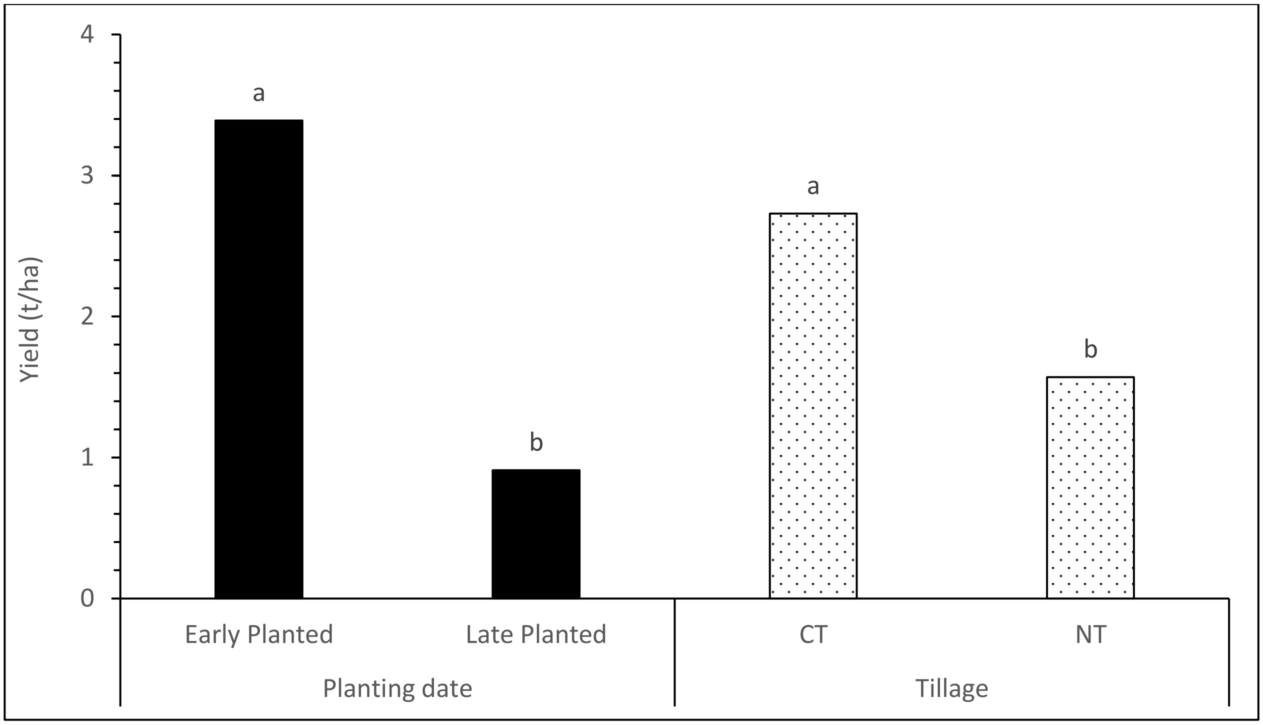

Figure 3. 8: Effect of planting date and tillage on yield in FS in Season 2021/22.
Different letters on the bars indicate significant differences at $p < 0.05$.

Early planting resulted in significantly ($p<0.05$) higher uptake of all three nutrients (Figure 3.9), i.e., higher N (16 kg/ha), K (15 kg/ha) and P (3 kg/ha) uptake than late planted crops.

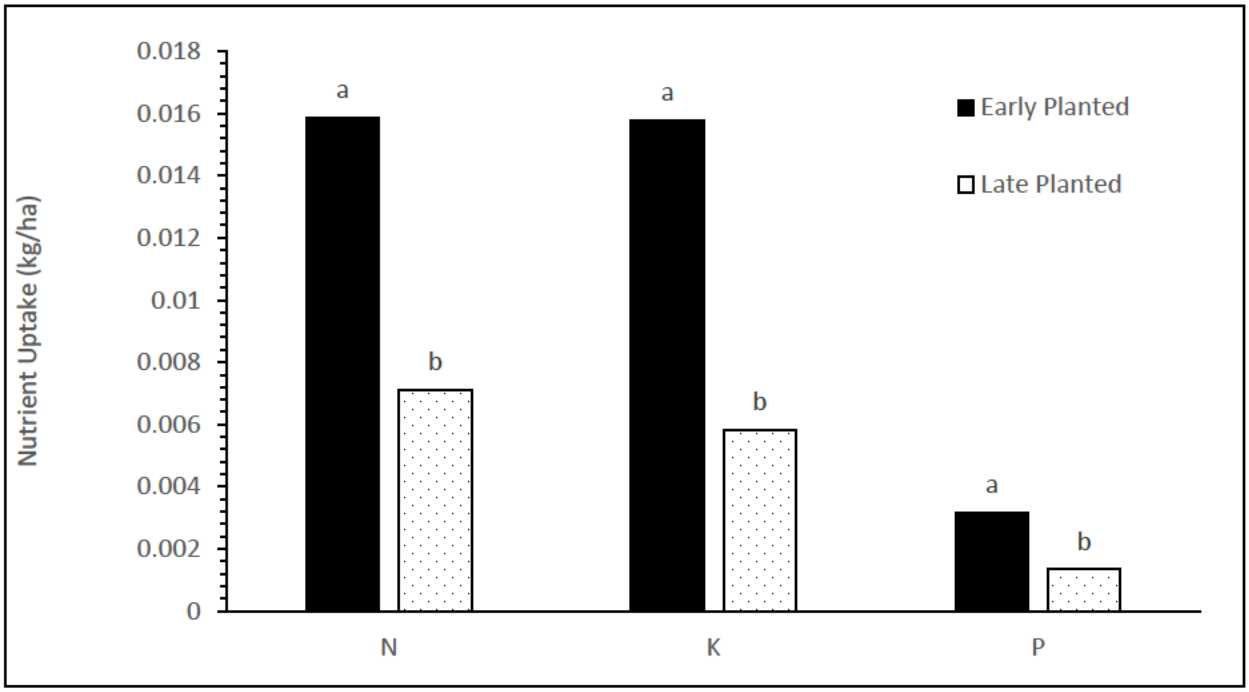

Figure 3. 9: Effect of planting date on NPK uptake in FS in 2020/21.
Different letters on the bars indicate significant differences at $p < 0.05$.

Plants grown under CT took up significantly more K (17 kg/ha) than those grown on NT plots in FS (8 kg/ha), in the second season. Plants grown under CT took up 36 Kg N/haand 2.3 Kg P/ha and plants grown under NT took up 24 Kg N/ha and 1.4 Kg P/ha. Planting date was again the only treatment that had significant effect ($P < 0.05$) on all the three measured nutrients, in FS, in the 2021/22 season. Early planted sorghum took up significantly more uptake of N, P and K than late-planted sorghum (Figure 3.10). Effects of cultivar on uptake of N, P and K were not significant, with Pan 8816 taking up 3.4 kg N/ha, 2.0 kg P/ha and 16.0 kg K/ha, while Macia took up 2.6 kg N/ha, 2.0kg P/ha and 8.0kg K/ha, in FS.

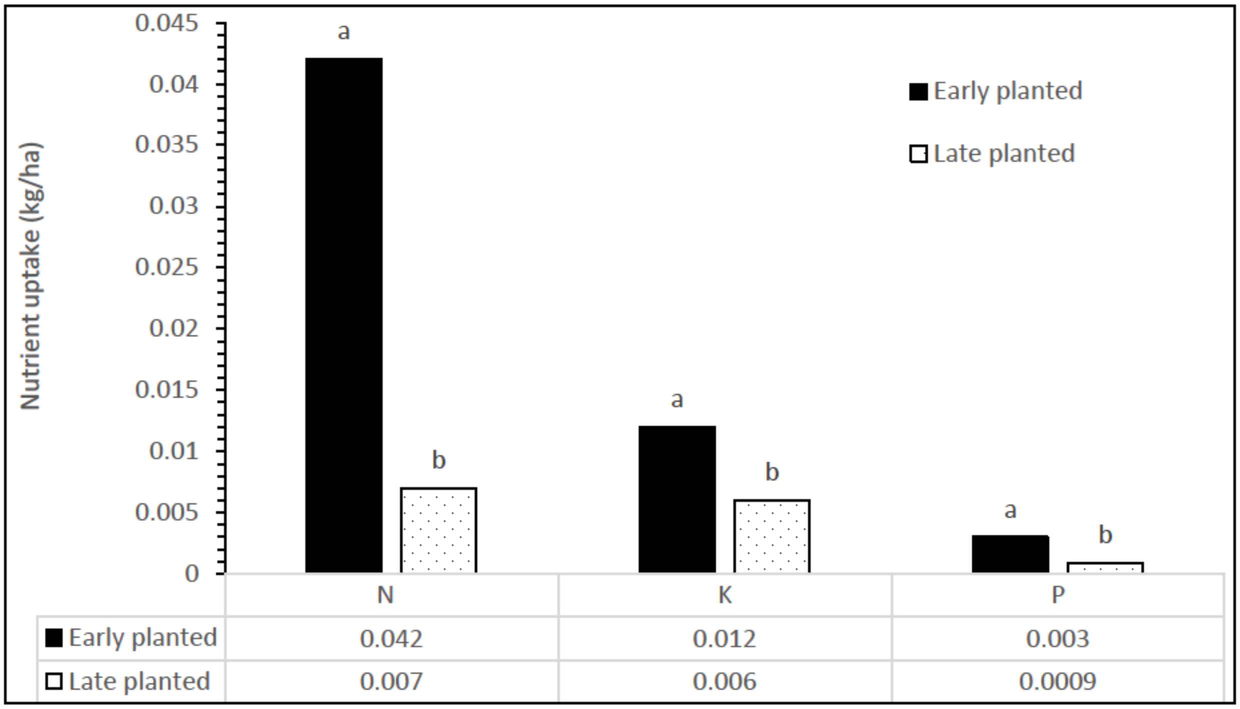

Figure 3. 10: Effect of planting date on NPK uptake in FS in 2021/22.
Different letters on the bars indicate significant differences at $p < 0.05$.

3.3.4. Grain yield and primary nutrient uptake in KZN

The main and interaction effects did not significantly affect ($p>0.05$) sorghum grain yield and uptake of N, P and K in KZN in both seasons, except cultivar in the second season, where Pan8816 (4.40 t/ha) produced 3.3 times higher yield than Macia (1.32 t/ha) (Figure 3.11). Cultivar had a significant effect ($p<0.05$) of on N, P and K uptake in KZN, for both seasons, with Pan8816 having higher of N (84 kg/ha), K (64 kg/ha) and P (8 kg/ha) uptake when compared to Macia in the second season (Figure 3.12).

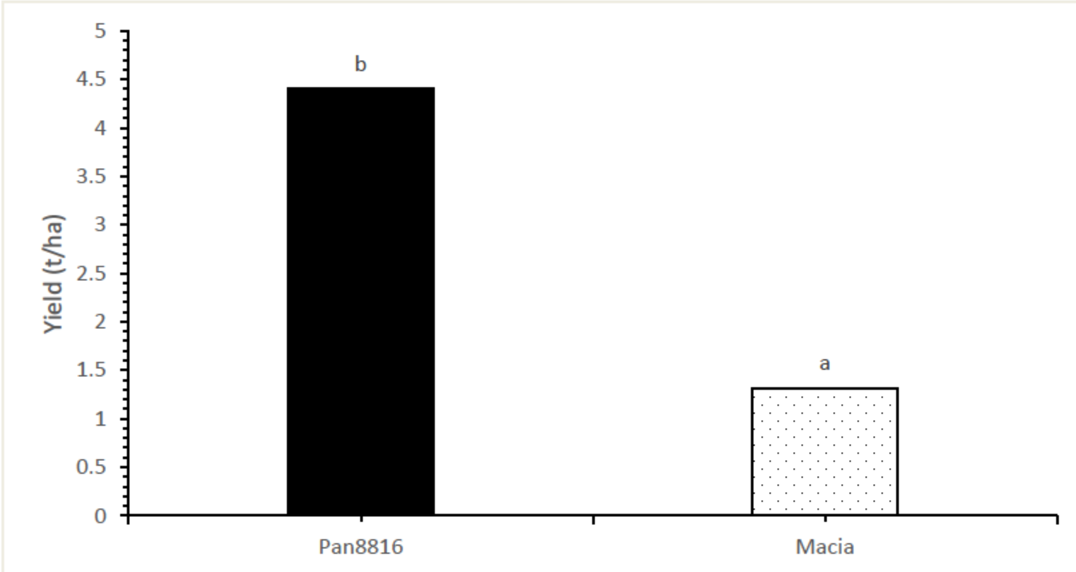

Figure 3.11: Effect of cultivar on grain yield in KZN for the 2021/22 season.
Different letters on the bars indicate significant differences at $p < 0.05$.

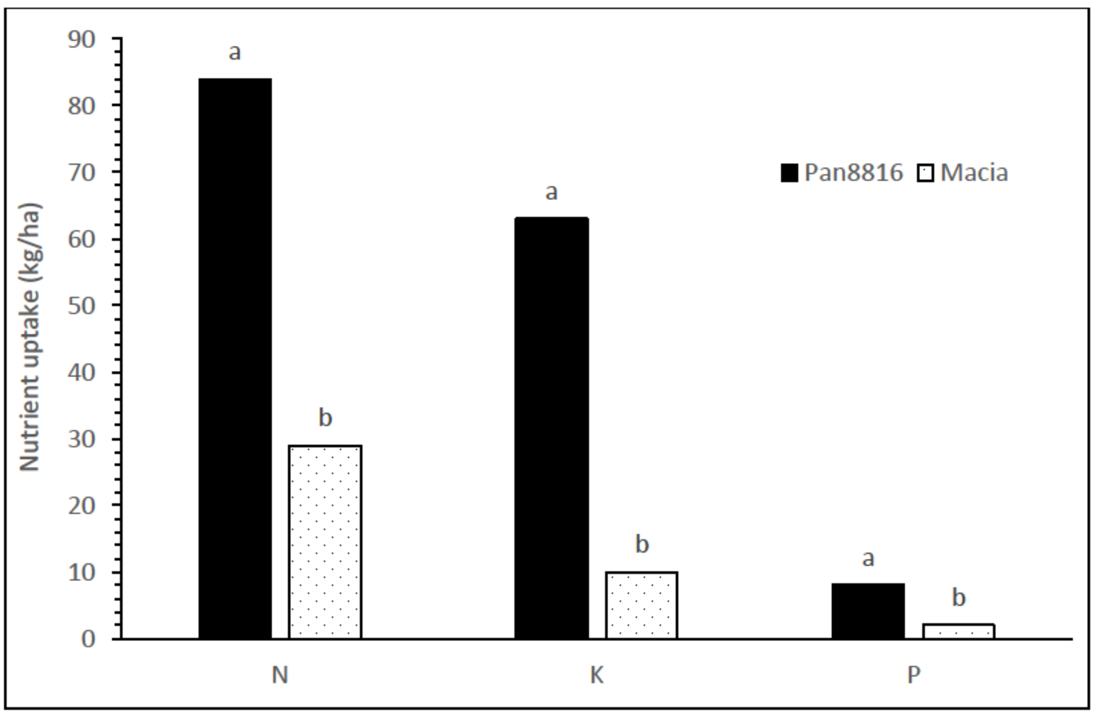

Figure 3. 11: Effect of cultivar on N, P and K uptake in KZN in 2021/22.
Different letters on the bars indicate significant differences at $p < 0.05$.

3.4. Discussion

Planting date, cultivar and planting date × tillage were the most influential factors affecting plant height across all treatments. Early planted crops consistently grew taller than the late-planted crops due to the more conducive environmental conditions for growth such as temperature and moisture. Optimal environmental conditions positively influence plant growth (Irawan et al. 2022). This effect was particularly pronounced in the drier FS province. Early planting in FS allowed plants to benefit from availability of more soil moisture for extended periods. Conversely, the absence of significant height differences between early and late planting in KZN province, which generally receives higher rainfall, and a naturally longer growing season supports this observation. Table 3.1 shows that the annual rainfall of KZN (799 mm) is higher than of FS (401 mm).

Sorghum cultivar also played a role in plant height. Pan8816 consistently produced taller sorghum plants than Macia in both seasons and locations. This suggests inherent genetic differences among the cultivars (Irawan et al. 2022). Pan8816 is a medium to late maturing hybrid whilst Macia is early to medium maturing open-pollinated variety (OPV) (Weepener et al. 2014, Hadebe et al. 2017). Pan8816 being a hybrid has been bred to have the best traits for a number of different parent lines while the OPV Macia only have the traits of the parent because the pollination occurs naturally. According to Gosh et al. (2015), differences in plant height among cultivars are due to genetic differences. While planting date and tillage interaction influenced plant height in KZN during the first season, cultivar and the interaction of all treatment factors had significant effects in the second season. This suggests that selecting the most appropriate cultivar, such as the taller PAN8816 in this study is more critical than time of planting in KZN under a particular set of conditions. Furthermore, regional adaptability also plays a role in the plant height of sorghum crops (Gosh et al. 2015 and Sebetha & Modisapudi, 2019).

The longer season when planting early in FS during both seasons resulted in higher yield than late planting because it allows for greater biomass accumulation, flowering and grain filling. This aligns with findings by Shoyer et al. (1998) and Hadebe et al. (2017) who reported that planting after the first rains in dry to semi-arid summer grain growing regions results in much greater yields. Sorghum thrives in soil temperatures around 15 °C at a depth of 10 cm (Murdy et al. 1994). However, late planting in FS coincides with the occurrence of frost, usually occurring between March and April (Moeletsi et al. 2016). These cooler temperatures impact sorghum emergence and early growth by delaying panicle initiation and flowering, ultimately reducing yield (Emendack et al. 2021). In the current study, the prevailing temperatures in FS fell below the optimal range reported by other researchers (Balota et al. 2010 and Emendack et al. 2021) (Table 1). Moreover, sorghum tends to do well in warmer climates (Sebetha and Modisapudi 2019). The higher yields observed under conventional tillage can be attributed to the relatively short period during which no-till was practised at the site. Findings by Pittelkow et al. (2015) suggested the full benefits of no-till agriculture may take up to four years to be realized. The observed similarity between yield and uptake of N, P and K, suggested that the enhanced uptake of these nutrients under conventional tillage contributed to the greater yield.

Nitrogen is the most limiting nutrient for sorghum production (Teshome et al. 2023). Tillage practices and cultivar selection can significantly affect short-term nitrogen use (Ramadhan and Mushin 2021). In this study, conventionally tilled plots exhibited higher porosity and lower bulk density compared to NT plots. Another notable observation was the similar N and P uptake patterns across both sites. Similarly, Ortas et al. (1996) found that ammonium-based nitrogen fertilisers like monoammonium phosphate (MAP) used in this study promoted the uptake of phosphorus. Therefore, the MAP used as the pre-planting fertilizer in this study, implies greater availability of phosphorus owing to the ammonia in the MAP.

The significantly higher yield of PAN8816 compared to Macia is likely due to its greater uptake of N, P and K. This enhanced uptake could be attributed to inherent genetic differences between the cultivars. In KZN, cultivar was the most prominent factor influencing yield. Pan8816, a hybrid, has been demonstrated to have small canopy size, which allows it to maintain a higher chlorophyll content (Manyathi et al. 2014), which in turn translates to increased levels of photosynthesis ultimately supporting higher yields. The lack of tillage, time of planting and rotation effects in KZN could be due to the higher rainfall received in the region. This translates to greater available moisture throughout a longer growing season compared to FS.

A drawback of this short-term study is the potential for increased competition between sorghum crops and weeds for vital resources such as nutrients and moisture (Giller et al. 2009). Despite applications of pre-planting and post-planting herbicides, field observations showed a relatively high incidence of weeds. Challenges like poor germination, prevalence of pests and nutrient immobilization tend to affect short-term studies (Giller et al 2009). These challenges arise due to the complex interaction between crop requirements, climate and soil characteristics. For instance, the presence of plant residues can cause temporary nutrient immobilization by soil microbes especially when the climatic conditions allow (Dabeux & Sollenberger, 2020).

3.5. Conclusion

This study identified key factors influencing sorghum performance in some parts of FS and KZN. In FS, planting date and cultivar, significantly influenced plant height, yield and nutrient uptake. Early planting and selection on PAN8816 cultivar is the most favourable combination to avoid frost and maximise yield. In KZN, cultivar was the primary factor, influencing yield, with Pan8816 outperforming Macia. However, planting date had minimal impacts in KZN, suggesting a greater flexibility for farmers. Therefore, farmers at the KZN site can plant sorghum across the tested dates without significant yield penalties. This study shows that there is potential of growing sorghum in FS, especially when planted early to avoid the frost season. Additionally, PAN8816 is recommended at both sites, especially under conventional tillage. However, longer-term studies are necessary to determine the influence of no-till practices, as the benefits may not be realised within the two-season timeframe of this study.

CHAPTER 4: SOIL ORGANIC CARBON FRACTIONS AND WATER STABLE AGGREGATES AT TWO CONTRASTING AGRO-ECOLOGICAL ZONES UNDER SHORT-TERM SORGHUM-BASED CONSERVATION AGRICULTURE IN SOUTH AFRICA.

Abstract

Soil organic matter is important for maintaining soil quality and ensuring sustainable crop production. This study investigated the effects of planting date, tillage and sampling time on the concentration of SOC and its fractions and water stable aggregates (WSA) in sorghum-based systems at two contrasting sites in South Africa i.e., Free State (FS) and KwaZulu Natal (KZN) provinces. In FS, neither treatments nor interactions significantly influenced total SOC or its fractions, except coarse particulate organic carbon (cPOC). Late planting resulted in 24.1 % more cPOC than early planted soils. According to Plaza-Bonilla et al. (2014) saturation of carbon in mineral fraction of soil results in additional inputs of carbon through management practices being more in the labile carbon fraction. Similarly, in KZN, carbon fractions showed no clear treatment effects though, late planted soils had 6.3% more OC than early planted soils. Fine particulate organic carbon (fPOC) did not respond to planting date, tillage or their interaction at both sites. Soil protein concentration was not significantly affected by planting date and tillage, but the first season (0.235 mg/g) had significantly higher compared to the second season (0.172 mg/g). Soils under NT had 7.64 % more water stable aggregates (WSA) in the 53 µm – 250 µm and 5.9 % more WSA in <53 µm aggregate size fraction than soils under CT. In FS, late planted plots, under CT, had greater WSA in the > 250 µm aggregate size fraction, while NT treatments had higher WSA in the 53-250 µm and <53 µm size classes. In KZN, early planting combined with CT resulted in the highest WSA in both the >250 µm and 53–250 µm aggregate classes, while NT consistently had higher proportion of the <53 µm fraction than CT. The study highlights the role of management practices in influencing soil aggregation and carbon stabilization, and that the impact is more site-specific, in the short term. Monitoring soil carbon levels in conjunction with biological and physico-chemical properties such as soil protein and WSA

is essential for improving soil health and long-term productivity in conservation agriculture systems.

Keywords: Aggregate stability, mineral-associated organic matter, particulate organic matter, soil quality.

4.1 Introduction

Loss of organic matter is one of the drivers of soil degradation (Lal 2020). Soil organic carbon (SOC) is vital in understanding land degradation, climate change and most importantly food production (Lal 2020, Pravalie et al. 2021). SOC is essential for water and nutrient storage, microbial activity, ultimately land productivity in terms of stabilizing crop yields food, and fibre supply (Jackson et al. 2017). The SOM, of which SOC is a major constituent, is a complex collection of decomposing plant matter and plant-microbial compounds in varying sizes, level of degradation and spatio-temporal availability (Chen et al. 2013; Canisares et al. 2023). The different SOM fractions (Canisares et al. 2023), are classified into labile and non-labile forms that differ in resistance to decomposition (Ramesh et al. 2019) and therefore contribute in different ways to soil productivity. The labile SOM fraction, including particulate SOM (POM), water soluble SOM and microbial biomass, is sensitive to land use changes and is therefore useful in monitoring changes in SOC dynamics within agricultural ecosystems (Pravalie et al. 2021). The POM, which is larger than 53 µm in particle size, is made up of decomposed plant material not directly assimilable by microbes (Lavelle et al. 2019). Conversely, the mineral-associated organic matter (MAOM) is non-labile SOM, which is less than 53 µm, is comprised of compounds that have been transformed by microbes and protected from decomposition by chemically bonding with mineral surfaces (Lavelle et al. 2019). The distribution of SOM fractions in the soil, which is affected by management practices, determines the benefits that can be derived in the particular soil. Adoption of management practices to increase and preserve organic matter continues to be advocated for (Alvaro-Fuentes et al. 2008; Paul et al. 2013; Dhaliwal et al. 2020).

Land use management practices are linked to SOC dynamics, the activity of microbes and soil health in general (Nocita et al. 2011; Jackson et al. 2017; Kopecky et al. 2021). The scientific community is advocating for the adoption of practices like reduced tillage, residue retention, and crop rotation that are collectively known as conservation agriculture, in order to derive benefits including the improvement of soil organic matter reserves (Cordova et al. 2018, Dhaliwal et al. 2019, Kauer et al. 2021). These land use and management strategies that slow decomposition and maintain or accumulate SOM (reduced tillage, crop rotations, residue retention, crop selection and integrated nutrient management), are preferable (Kauer et al 2021). In a world threatened by a changing climate, increases in SOM can ensure sustainable food production (Kane et al. 2021), through its influence on soil structure, water and nutrient holding capacity and hydraulic conductivity (Kopecky et al. 2022). These benefits are influenced by the concentrations of SOM and its different fractions. Cordova et al. (2018) reported that the quality of residues retained has a direct effect on the stabilization and mineralization of mineral-associated organic matter (MAOM). Mixing retained crop residues into the soil matrix increases decomposition of SOM and mineralization of nutrients due to exposure optimal moisture, temperature and microbial decomposers (Dhaliwal et al. 2019). Understanding how different components of SOM accumulate under various cropping systems will be helpful to guide management of SOM in order to influence its potential benefits, which include improved soil structure (Cotrufo et al. 2022).

Soil structure influences movement of water, gasses and nutrients through the profile and provides a habitat for organisms that live in the soil (Wang et al, 2015). Soil aggregation is affected by flocculation and binding of soil particles by various biotic and abiotic agents (Okolo et al. 2020 and Zhang et al 2022) while the stability of these aggregates is influenced by factors like plant diversity, biological activity, SOM and tillage practices (Wang et al, 2015). Soil tilth, the necessary soil condition for seedling germination and emergence and subsequently crop growth, is dependent on optimum aggregate stability (Munkholm et al. 2019, Okolo et al. 2020 and Keller et al. 2023). Aggregate stability is in turn influenced by factors that include reduced tillage, integrated nutrient and water management, crop residue retention, high OM content and increased biological activity (Zhang et al. 2014, Wang et al. 2015 and Okolo et al. 2020).

The different pools of SOM contribute directly or indirectly to soil structure, including the role of soil organisms in soil structure development. For example, the symbiotic relationship between arbuscular mycorrhizal fungi (AMF) and plants on terrestrial ecosystems produces an active carbon fraction glycoprotein that binds soil aggregates (Zhang et al. 2014, Ren et al. 2022 and Zhang et al. 2022). The activity of the AMF is influenced by fertilization with nitrogen and phosphorus through effects on yield, root colonization and aluminium and calcium phosphates (Ren et al. 2022). The aggregation of soil particles mitigates the evolution of greenhouse gasses (GHG) through protection and stabilization of organic carbon (Gonzalez-Chavez et al. 2010 and Ren et al. 2022). Plants that are grown in a particular soil also affect aggregate stability through the action of plant roots physically enmeshing and/or biochemically binding soil particles using their secretions (Ren et al. 2022). Microorganisms also stabilize soil structure through carbohydrates produced by bacteria and secretion of hydrophobic substances by fungi that enhance water repellency of aggregates (Gonzalez-Chavez et al. 2010 and Ren et al. 2022). The contribution of CA on soil microbial activity has been documented in several studies including those in South Africa (Mukumbareza et al., 2015; Mukumbareza et al., 2016).

While many studies have been done in maize-based CA systems, in sub-Saharan Africa, similar studies on sorghum are limited. This study seeks to gather data on the response of soil properties to alternative management practices and growth of sorghum in regions of varying climate and soils in South Africa. Underutilized crops like sorghum have a role to play in food security and thus studies on how they may be influenced by changing climatic and edaphic factors are required to ensure sustainable production. Sorghum is increasingly recognized as a climate resilient crop due to its drought tolerance and low nutrient requirements, compared to maize. Therefore, the objective of the study was to determine effects of different planting dates and tillage systems on soil organic carbon pools, easily extractable glomalin, and aggregate stability of soils under sorghum-based CA at two contrasting sites in South Africa. The study will contribute to a deeper understanding of how planting date, tillage and their interactions influence the dynamics of aggregate binding agents in the short-term and what environmental factors modify soil architecture in dryland cropping systems under contrasting agro-ecological zones.

4.2 Materials and method

4.2.1. Study site

The study was conducted in the two villages: Houtnek in Thaba Nchu and Qongqo in Nongoma. Detailed descriptions of the prevailing agricultural activities, climatic conditions and soil properties are provided in Chapter 3.

4.2.2. Soil sampling and analysis

Soil samples were collected from an on-going sorghum trial at the two study sites, detailed in Chapter 3. Composite samples were obtained from each replicate plot with sampling designed to assess the effects of planting date, tillage and sampling time on soil properties. The factors cultivar and rotation were excluded from this analysis as their influence on soil physical properties is generally expressed over considerable amount of time (Mtyobile et al. 2020) than the timeframe of the current study. The samples were collected from the 0- 20 cm depth from four plots and mixed into one composite sample resulting in 4 samples per block of 16 plots resulting in a total of 12 samples per site. The soil samples were placed in cooler box for transport to the laboratory where they stored in a fridge at 4°C until analysis. Sampling was done in 2021 for initial determination of easily extractable glomalin and in 2022 and 2023 to monitor changes in the levels of soil protein because of the treatments. Soil samples were taken at the end of the trial after harvesting of the sorghum crop to measure the effects of the treatments on the levels of SOC fractions and water stable aggregates.

4.2.3. Soil Protein

The method described by Wright and Upadhyaya (1996) was used to extract soil protein. One gram of soil was placed in a centrifuge tube with 8 ml of 20 mM sodium citrate, vortexed and autoclave at 121°C for 30 minutes. The sample was then centrifuged at 5000 x *g* for 15 minutes and the supernatant was collected in screw-top bottles and stored at 4°C. The absorbance of the extract was measured at 590 nm of Bio-Rad protein dye reagent on 96 well flat-bottom microplates with bovine serum albumin standards of 1-5 µg protein per well (Wright and Upadhyaya, 1996). The soil protein obtained in units of volume was then converted to mass units by the multiplying it with the sample density.

4.2.4. Carbon fractions

The SOC was determined following the Walkely-Black method (Wang et al. 2012). The samples were then fractionated following the method used by Sainepo et al. (2018). Air-dried samples (20 g) were placed in 250 ml plastic bottles and 70 ml of sodium hexametaphosphate ($Na_6(PO_3)_6$) solution was added and the mixture was shaken on a rotary shaker for 15 hours. After shaking, the contents were passed through two sieves (250 and 53 µm) to separate into > 250 µm; 250 - 53 µm µm and < 53 µm fractions and then oven dried at 50 °C for 48 hours. The material collected for each fraction was weighed and analysed for SOC following the Walkley-black method. Carbon fraction per gram of soil was calculated according to the equation:

$$\text{SOC in fraction} = \frac{(\text{mass of fraction} \times \text{Carbon per fraction})}{\text{mass of soil}}$$

4.2.5. Water stable aggregates

The wet sieving apparatus (Eijkelkamp ART. No 081301, Giesbeek) was used to determine the water stable aggregates of soil samples collected at the end of the trial, based on fractionation into the three aggregate size classes (< 250 µm; 53 µm – 250 µm and < 53 µm).

Air-dried aggregates (4 g) were placed in 8 sieves (60-mesh screen), which were agitated with distilled water inside metal can for 3 minutes then collected and oven dried at 105 °C for 24 hours. The next step was to replace the distilled water in the cans with a dispersing solution of 2 g/l of $Na_6(PO_3)_6$ for soils with pH > 7 or NaOH for soils with pH < 7. The same agitation process was followed for the dispersing solution but for 8 minutes and sample was collected and oven-dried under similar conditions to distilled water fractionation. The equation below was used to compute percentage water stable aggregates:

$$\%\mathrm{WSA} = \frac{\mathrm{Mass}_{\mathrm{dispersing\ solution}}}{\mathrm{Mass}_{\mathrm{dispersing\ solution}} + \mathrm{Mass}_{\mathrm{water}}} \times 100$$

Where: WSA= water stable aggregates

4.2.6. Data analysis

The data for each site were subjected to three-way ANOVA using the JMP Pro Version 17 statistical software with planting date, tillage and sampling time as fixed effects (JMP Statistical Discovery LLC 2022). The means were separated by means of Fischer's least significant difference at $p<0.05$ level. A correlation analysis was done to determine the relationship between the aggregate size fractions, SOC and its fractions using Pearson's correlation.

4.3 Results

4.3.1. Effect of planting date and tillage and season on soil protein in FS and KZN

The main effects of planting time and tillage and the interaction effects did not affect soil protein in either FS or KZN ($p > 0.05$) (Table 4.1). Season significantly influenced soil protein levels in FS but not in KZN, with soils sampled in 2021 having 36. % higher concentration (0.234 mg/g), than in 2022 (0.172 mg/g) (Figure 4.1). Samples taken in 2023 had soil protein (0.201 mg/g) which was comparable to both 2022 and 2021.

Table 4. 1: ANOVA of effects of planting date, tillage and sampling time and interactions on soil protein in FS and KZN.

Treatment	df	Soil protein (mg/g)	
		FS	KZN
Planting date	1	0.80	0.92
Tillage	1	0.43	0.39
Season	2	0.03*	0.05
Planting date*Tillage	2	0.40	0.37
Planting date*Season	2	0.32	0.21
Tillage*Season	2	0.94	0.88
Planting date*Tillage*Season	3	0.39	0.67

*indicates significant difference at P < 0.05. df= degrees of freedom.

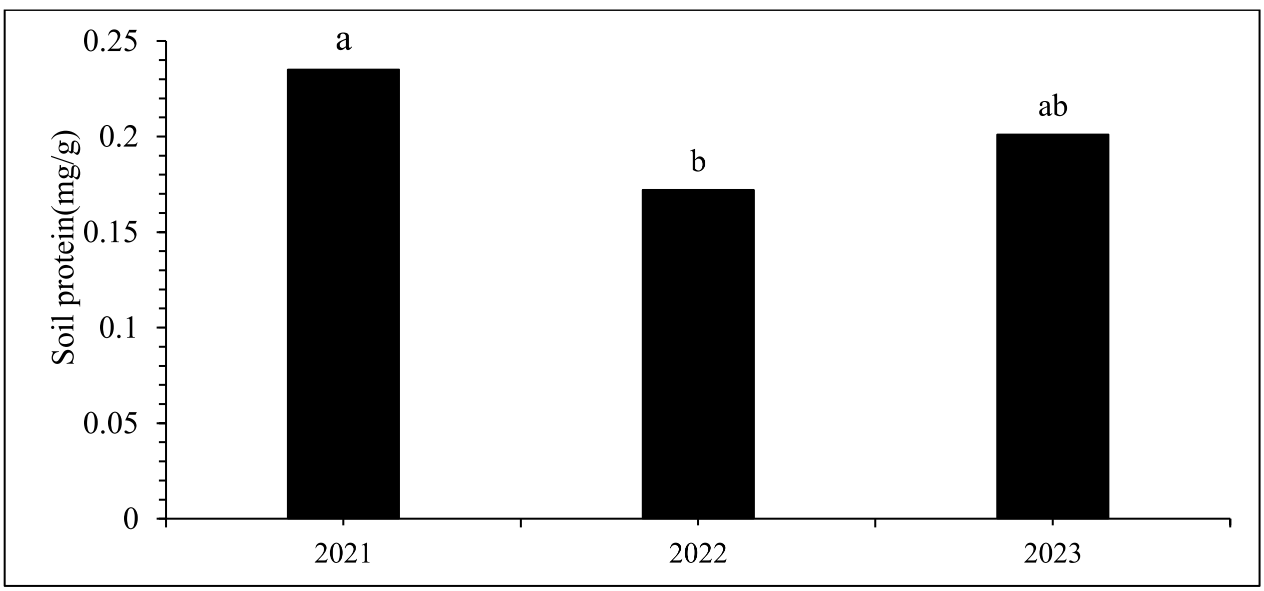

Figure 4. 1: Effect of sampling time on easily extractable glomalin in FS.
Different letters on top of the bars indicate significant difference at p < 0.05.

4.3.2. Effect of planting date and tillage on soil organic carbon and carbon factions in FS and KZN.

Planting date and tillage, including their interaction, had no significant effect on SOC levels in FS (Table 4.2). In KwaZulu-Natal (KZN), only planting date had a significant effect on SOC (Table 4.2). Late planted soil had 6.3 % more SOC than early planted soils in KZN (Table 4.3). None of the treatments significantly influenced the levels of the cPOC, fPOC, or MAOC in KZN (Table 4.3). In contrast, in FS planting date significantly affected cPOC (Table 4.2). In FS, late planting resulted in 24.1% higher coarse particulate organic matter than planting early (Table 4.4) while neither treatments nor their interaction had effects on the cPOC fraction in KZN (Table 4.4).

Table 4. 2: ANOVA table of the effects of planting date, tillage on soil organic and carbon fractions in FS and KZN in 2023.

Treatment	**df**	**% SOC**		**cPOC**		**fPOC**		**MAOC**	
		p-value							
		FS	**KZN**	**FS**	**KZN**	**FS**	**KZN**	**FS**	**KZN**
Planting date	1	0.726	0.042*	0.044*	0.408	0.744	0.278	0.689	0.571
Tillage	1	0.775	0.339	0.133	0.509	0.874	0.480	0.396	0.768
PD × tillage	1	0.243	0.059	0.562	0.689	0.070	0.475	0.395	0.248

*Indicates significant difference at P < 0.05. cPOC - coarse particulate organic matter, fPOC - fine particulate organic matters and MAOC - mineral associated organic matter, PD – Planting date

Table 4. 3: Effects of planting date, tillage and PD × tillage on soil organic carbon in FS and KZN in 2023.

Treatments	Levels	% OC in soil	
		FS	KZN
Planting date	Early planting	0.720^{ns}	2.055^{b}
	Late planting	0.745^{ns}	2.189^{a}

Tillage	CT	0.742 ^{ns}	2.094 ^{ns}
	NT	0.722 ^{ns}	2.150 ^{ns}

Different superscripts between treatment levels in columns indicate significant difference at P < 0.05. PD= Planting date, CT= conventional tillage, NT= No-till

Table 4. 4: Effects of planting date and tillage on coarse particulate organic carbon in FS and KZN in 2023.

Treatments	Levels	% cPOC in soil	
		FS	KZN
Planting date	Early planting	0.222^{b}	0.252 ^{ns}
	Late planting	0.283^{a}	0.374 ^{ns}
Tillage	CT	0.231^{ns}	0.362 ^{ns}
	NT	0.274 ^{ns}	0.264 ^{ns}

Different superscripts between treatments in columns indicate significant difference at P < 0.05. cPOC = coarse particulate organic carbon, CT= conventional tillage, NT= No till.

Planting date, tillage and their interaction (PD × tillage) had no significant effects on the levels of fPOC at either site. However, a consistent trend was observed across treatments with late planting and no-till generally associated with higher fPOC concentrations in KZN compared to early planting and no-till practices (Table 4.5). In both sites, no significant treatment effects were observed on MAOC levels (Table 4.6). A similar trend was observed in FS with early planting and conventional tillage having higher values of MAOC.

Table 4. 5: Effects of planting date and tillage on fine particulate organic carbon in FS and KZN in 2023.

Treatments	Levels	% fPOC in soil	
		FS	KZN
Planting date	Early planting	0.045^{ns}	0.410 ^{ns}
	Late planting	0.039 ^{ns}	0.548 ^{ns}
Tillage	CT	0.041 ^{ns}	0.435 ^{ns}
	NT	0.044 ^{ns}	0.523 ^{ns}

Different superscripts between treatments in columns indicate significant difference at P < 0.05. fPOC= fine particulate organic carbon, CT= conventional tillage, NT= No till.

Table 4. 6: Effects of planting date and tillage on mineral associated organic carbon in FS and KZN in 2023.

Treatments	Levels	% MAOC in soil	
		FS	KZN
Planting date	Early planting	0.452^{ns}	1.393^{ns}
	Late planting	0.422^{ns}	1.266^{ns}
Tillage	CT	0.470^{ns}	1.297^{ns}
	NT	0.404^{ns}	1.363^{ns}

Different superscripts between treatments in columns indicate significant difference at P < 0.05. MAOC= mineral associated organic carbon, CT= conventional tillage, NT= No till.

4.3.3. Effect of planting date and tillage on water stability of selected aggregates

The interaction between planting date and tillage significantly affected the stability of macroaggregates (> 250 µm) at both sites, while no significant main effects of planting date or tillage were observed for this size class individually (Table 4.7). Tillage affected the water stability of aggregates in the 53 – 250 µm size class in FS, whereas in KZN, neither planting date nor its interaction with tillage had any effect on aggregates in the same size class (Table 4.7). The stability of the smallest aggregates (<53 µm) was influenced by both planting date and tillage in FS while in KZN, only planting date had a significant effect (Table 4.7).

Table 4. 7: ANOVA on effects of planting date, tillage and their interaction on the WSA of different aggregate sizes in FS and KZN.

Treatment	**df**	**> 250 µm**		**53 µm – 250 µm**		**< 53 µm**	
		FS	**KZN**	**FS**	**KZN**	**FS**	**KZN**
Planting date	1	0.073	0.082	0.114	0.057	0.042*	0.045*
Tillage	1	0.339	0.086	0.003*	0.082	0.048*	0.193
PD × Tillage	1	0.040*	0.043*	0.239	0.049*	0.059	0.119

*indicates significant difference at P < 0.05.

Late planted soils under CT had higher WSA in the > 250 µm aggregate size class (26.75%), significantly exceeding all the other treatment combinations (Figure 4.2) in FS. Soils under NT had greater WSA in both the 53 µm – 250 µm and <53 µm size classes compared to soils under CT in FS (Figure 4.3). Additionally, late planting resulted in higher water stable aggregates in the <53 µm size class than early planted soils in FS (Figure 4.4).

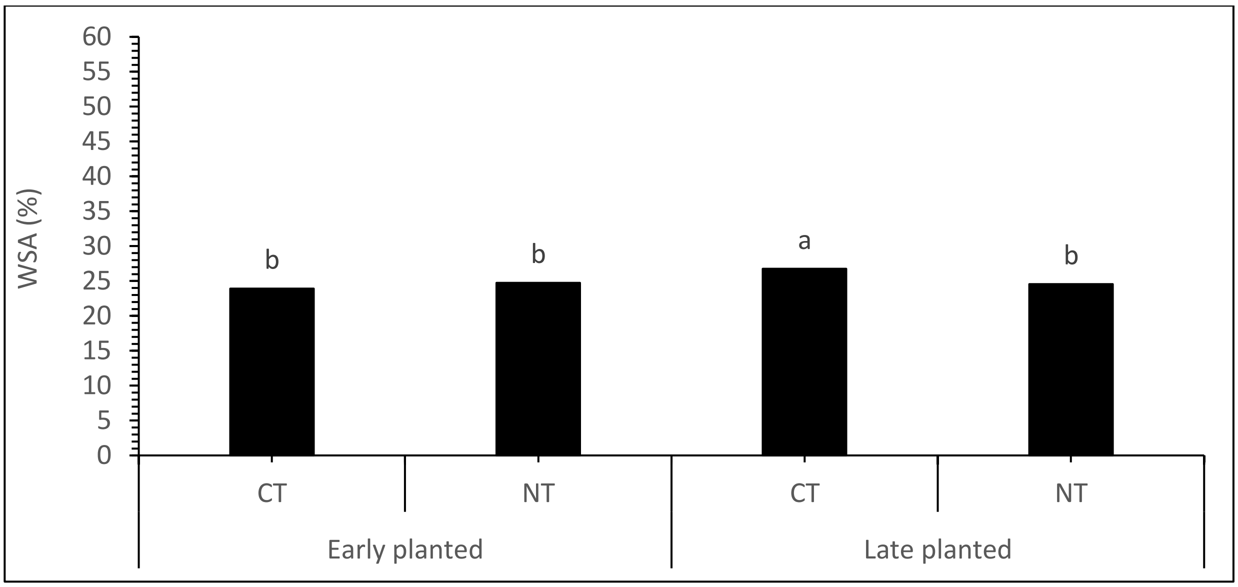

Figure 4. 2: Effect of planting date and tillage on WSA of the >250 µm aggregate size class in FS. Different letters on the bars indicate significant difference at $p < 0.05$.

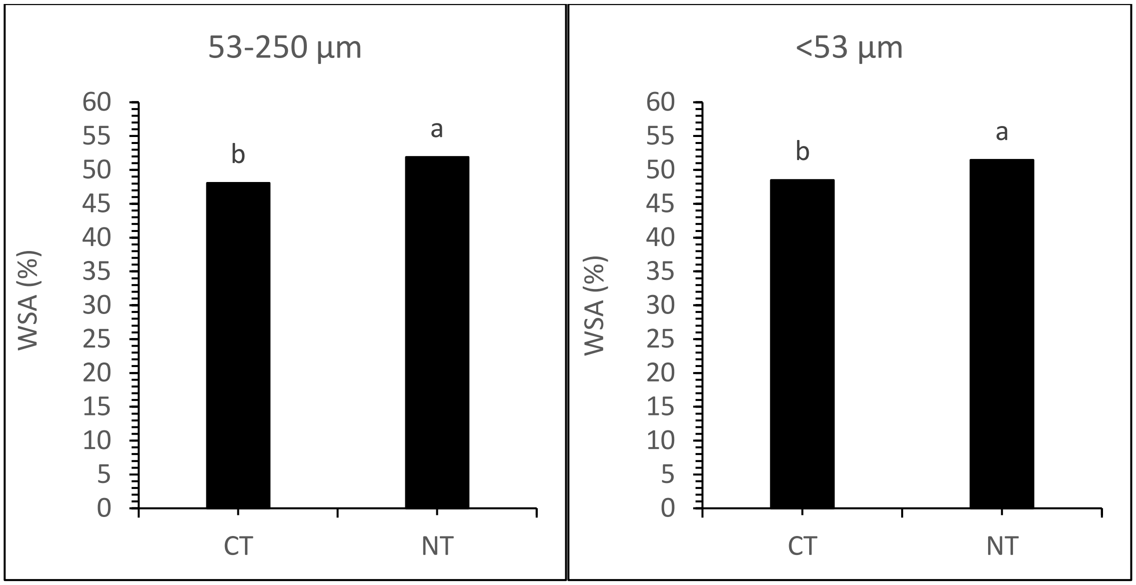

Figure 4. 3: Effect of tillage on the WSA of the 53-250 µm and <53 µm aggregate size classes in FS. Different letters on the bars indicate significant difference at $p < 0.05$.

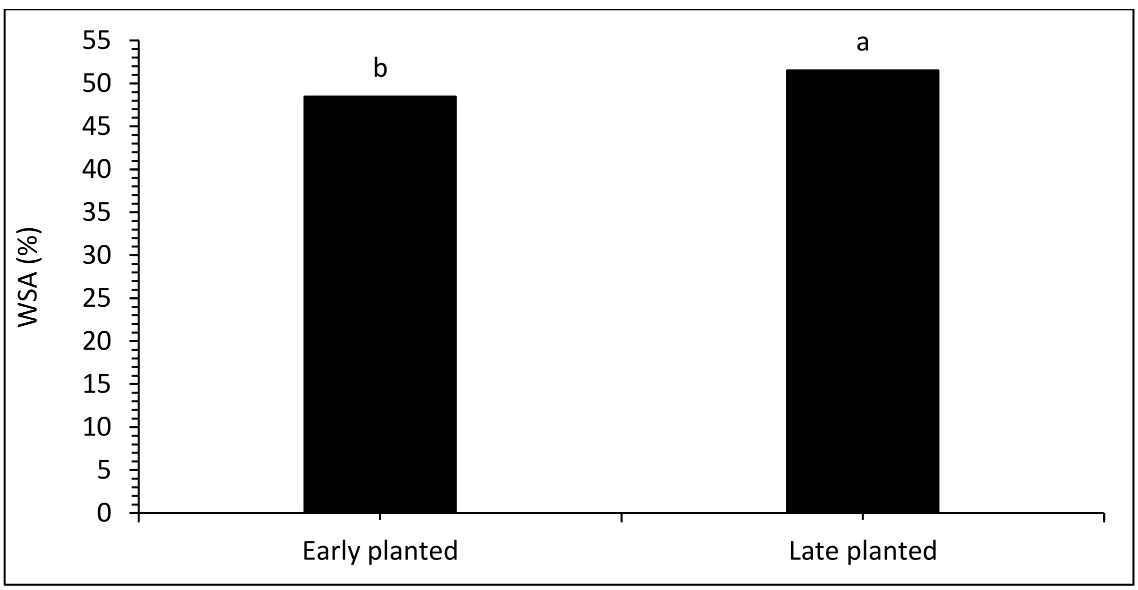

Figure 4. 4: Effect of planting date on WSA of the <53 µm aggregate size class in FS. Different letters on bars indicate significant difference at $p < 0.05$.

Late planted soil had a significantly higher WSA <53 µm than early planted soil (Figure 4.5). Early planted soil under CT had significantly ($P < 0.05$) higher WSA in > 250 µm than the rest of the treatment combinations (Figure 4.6) in KZN. Similarly, in the 53-250 µm class, early-planted soils under CT (27.88 %)   had significantly ($P < 0.05$) higher WSA compared to the other treatment combinations (Figure 4.7).

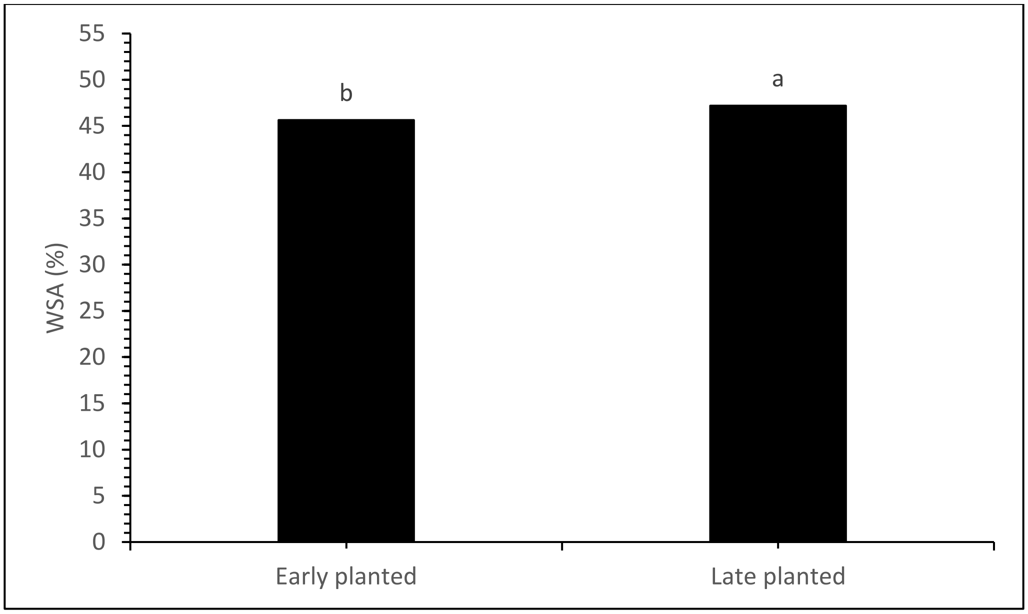

Figure 4. 5: Effect of planting date on WSA of the <53 µm aggregate size class in KZN.
Different letter on bars indicate significant difference at $p < 0.05$.

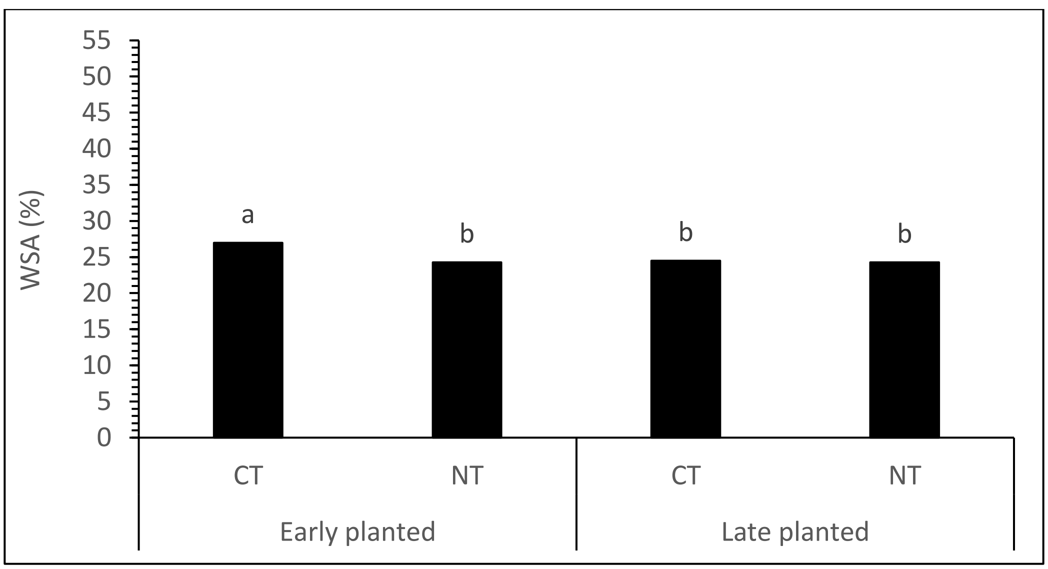

Figure 4. 6: Effect of planting date and tillage on WSA of the >250 µm aggregate size class in KZN.
Different letters on bars indicate significant difference at $p < 0.05$.

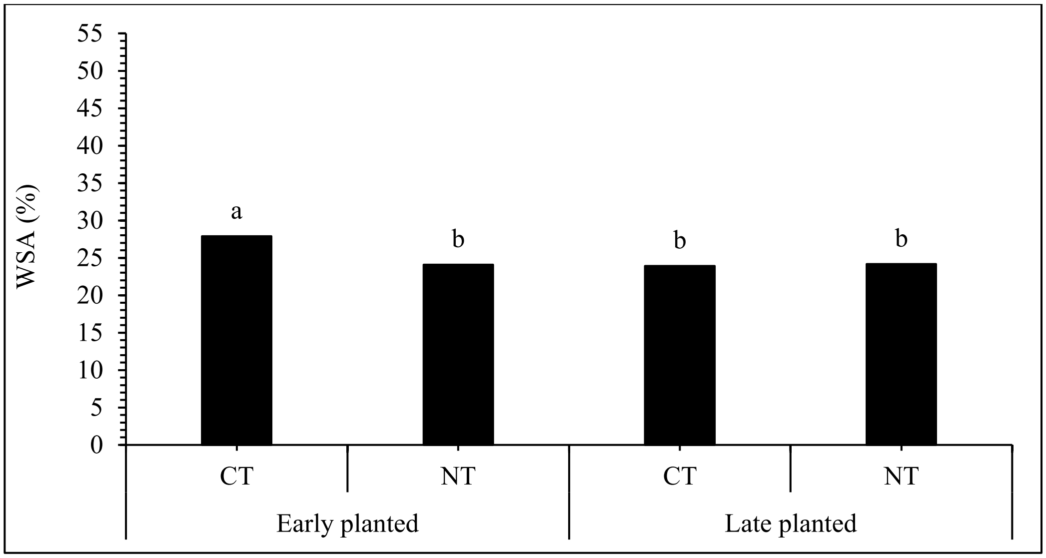

Figure 4. 7: Effect of planting date and tillage on WSA of the 53-250 µm aggregate size class in KZN.
Different letters on bars indicate significant difference at $p < 0.05$.

4.3.4 Correlations between OC, soil protein, carbon fractions and water stable aggregation.

Correlations show a moderate correlation between OC and WSA >250 µm, weak correlation between OC and WSA <53 µm, negligible correlation between soil protein and WSA >250 µm, moderate correlation cPOC and WSA 53-250 µm and a negative relationship between OC and cPOC and OC and WSA 53-250 µm in FS (Table 4.8). Soil organic C was correlated significantly positively with WSA >250 and WSA <53 µm, and negatively with WSA 53-250 µm, while WSA >250 was significantly correlated negatively with 53-250 µm at FS. In KZN (Table 4.9), there was a moderate correlation between the OC and WSA <53 µm and negligible between cPOC and fPOC. Additionally, there was a perfect positive correlation between WSA >250 µm and WSA 53-250 µm and negative relationship between OC and the aggregate sizes >250 µm, 53-250 µm and fPOC and MAOC and WSA >250 µm and WSA <53 µm and WSA 53-250 µm and WSA <53 µm. Soil organic C was correlated significantly positively with WSA <53 µm, and negatively with WSA >250 and WSA 53-250 µm. The

WSA >250 and 53-250 µm were significantly correlated positively, and were both significantly negatively correlated with WSA <53 µm at KZN.

Table 4. 8: Correlations between OC, soil protein, carbon fractions and water stable aggregation in FS.

	OC	**SP**	**cPOC**	**fPOC**	**MAOC**	**WSA >250 µm**	**WSA 53-250 µm**	**WSA <53µm**
OC	1							
SP	0.19	1						
cPOC	-0.49	-0.39	1					
fPOC	-0.06	-0.38	0.28	1				
MAOC	-0.08	0.19	0.29	-0.09	1			
WSA >250 µm	**0.60**	**0.52**	-0.34	-0.19	0.27	1		
WSA 53-250 µm	**-0.70**	-0.32	0.42	0.20	-0.12	**-0.81**	1	
WSA <53µm	0.50	0.01	-0.41	0.23	0.20	0.16	-0.07	1

Table 4. 9: Correlations between OC, soil protein, carbon fractions and water stable aggregation in KZN.

	OC	**SP**	**cPOC**	**fPOC**	**MAOC**	**WSA >250 µm**	**WSA 53-250 µm**	**WSA <53µm**
OC	1							
SP	-0.24	1						
cPOC	0.38	-0.34	1					
fPOC	0.34	0.16	0.61	1				
MAOC	0.13	-0.3	-0.30	-0.77	1			
WSA >250 µm	**-0.49**	0.38	-0.26	-0.20	0.25	1		
WSA 53-250 µm	**-0.54**	0.42	-0.31	-0.23	0.24	**0.99**	1	
WSA <53µm	**0.67**	-0.4	0.40	0.34	-0.19	**-0.93**	**-0.95**	1

4.3 Discussion

In this study organic carbon declined from the initiation of the study to the last season (Tables 3.2 and 4.3), which contrasts with the findings of Kumar et al. (2018), who reported improvements in OC under zero tillage together with residues retention in a 2-year study on wheat. Kumar et al. (2018) also observed that conservation practices improved the labile pool of OC when compared to conventional tillage without residue retention. In contrast, Jat et al. (2019) reported low organic carbon levels in the less than 2 mm aggregate size class under no-till. The reduction in OC over three years of the current study could be due to the conversion from previously uncultivated grazing land in Free State and exposure of OC to microbes in KZN that comes with tillage. In Free State, the conversion of the previously uncultivated land likely lead to OC losses through the volatilization of organic compounds driven by changes in moisture, temperature, vapour pressure and pH (Curtin et al. 2012). The exposure of organic carbon that was previously protected within soil aggregates to microbial attack through tillage processes leads to OC decline in KZN. This is consistent with Mando et al. (2005) who noted that tillage accelerates OC breakdown through its effect on soil moisture and aeration, which in the long term leads to the undesirable effect of OC decline.

Soil protein is an aggregate binding agent that contributes to aggregate stability in soils (Malobane et al. 2021). According to Dai et al. (2015), soils under NT have greater soil protein content compared to soils under conventional and alternating tillage treatments. The lack of treatment effects suggests that the contribution of this parameter on soil aggregate stability could be limited at these sites in the short-term. The higher soil protein in season one compared to season two in FS was likely due to shifting from previously undisturbed to conventionally tilled land, at this site, while there were no seasonal effects in KZN where the soil was previously tilled. Tilling of the soil, fertilization and sowing of various seeds can alter soil processes and properties of the previously undisturbed soils (Fokom et al. 2012). In this study, the Houtnek site in FS was initially uncultivated and used for cattle grazing, with sorghum introduced in the current study. The higher soil protein in season one compared to season two in FS is consistent with the findings of Lombardo et al. (2019) and Mubekaphi (2019) who state that soil disturbance leads to increased hydrolysis and reduced production of GRSP due to the disruption of the arbuscular mycorrhizal fungal (AMF) hyphae. The lack of response of soil protein to any of the treatments and their interaction in KZN, could be attributed to site's long-term agricultural activity characterized by continuous cereal cultivation with cover crops between seasons. The use of cover crops maintains living roots that can be colonized by the fungi and thus maintaining consistent production of glomalin irrespective of the short-term experimental interventions (Prasad et al. 2018). The seasonal fluctuations in glomalin levels at FS may be attributed to impact of seasonal tillage practices on the integrity and activity of AMF mycelial networks (Lormbado et al. 2019). Fungal synthesis and secretion of soil protein is hindered by intensive tillage operations through physical fragmentation of fungal hyphae resulting reduced active AMF biomass. Moreover, CT encourages aerobic microbes with high metabolic rates whilst NT favours fungal growth and temporary nutrient immobilization. The lack of effects of planting and tillage on the soil protein at both FS and KZN could be explained by the limited sensitivity of glomalin to changes in management. This is because the soil protein molecule is generally stable (Mubekaphi 2019). More sensitive OC fractions are often used to determine the effects of management changes on OC.

Soils under late planting had more mineral associated OC than early planting in FS and there were no treatment or interaction effects in KZN. This was attributed to the fast pathway of biosynthesis of organic matter from simple organic compounds by soil microbes. According to Mikutta et al. (2019), carbon substrate use efficiency of microbes is higher for labile compounds like carbohydrates and proteins than it is for stable compounds like lignin and so more microbial biomass is produced during decomposition of labile compounds. Ye et al. (2019) also states that this rapid transfer to MAOC is modified by fluctuations in oxygen availability and drying-wetting cycles. According to Cotrufo et al. (2022), abiotic processes such as direct sorption mediate the formation of MAOC. In FS, the fraction that was affected by planting date was cPOC, with it being higher for the late-planted soils than early-planted soils likely due to limited decomposition of the residues (Cotrufo and Lavallee et al. 2021). The late-planted soils had a relatively shorter growing season and the cooler temperatures in the site made decomposition of residues slower. Moreover, Yeasmin et al. (2023) monitored the effect of changing from grazing land to cropland in three years and their results revealed that there was more cPOC than MAOC due to the recently deposited and undecomposed residues.

Late planted soils had a significantly higher amount of organic carbon compared to the early-planted soils in KZN. The storage of OC in the soil is done in a two-step process whereby soil particles physically combine plant residues into macro aggregates and then the POC and binding agents are polymerised into micro aggregates which a relatively protected from microbial attack (Malobane et al. 2018 and Shen et al 2021). Yeboah et al. (2016) found higher organic carbon levels in NT plots for the 0-30 cm depth and in the current study, we found higher POC in the 0-20 cm layer. These results show that, in the short-term, no-tillage can be used to improve carbon sequestration in sorghum-based CA systems.

In FS, the higher WSA of the 53 – 250 µm aggregate sizes under NT is consistent with the findings of Malobane et al. (2021) and Pinto et al. (2022) who attributed it to resistance to dispersion resulting from higher OC in the NT plots. According to Pinto et al. (2022) minimum disturbance also improves microbial microhabitat, increase fungal hyphae density, which has a direct effect of reforming clay particles with polysaccharides, and indirectly provide carbon for the microorganisms that produce binding agents. The results of this study also show a positive but weak correlation between fPOC and MAOC and the corresponding aggregates sizes with the MAOC vs 53-250 µm aggregate size.

The higher concentration of water stable aggregates in the >250 and 53-250 um classes under the CT treatment with early planting in KZN than with late planting and the treatment combinations with NT, could not be explained by OC, which was higher with late planting than early. Coarse particulate organic carbon was the only fraction affected in KZN. This suggests that OC remained in the cPOC fraction and did not assimilate into the lower fractions, with gradually increasing stability.

The higher concentration of aggregates in the >250 µm in the CT treatment with late planting in FS, than the other treatment combinations, especially those with NT, followed the same trend as cPOC. The similarity in the trend suggested that the cPOC is protected in the water stable aggregates in the >250um class, stabilizing it. Higher water stable aggregation for early planting (53 µm -250 µm and > 250 µm) under CT conditions in KZN suggested that early planting resulted in greater aggregation while late planting reduces aggregation under CT and time of planting not affect aggregation under NT. The WSA in the < 53 µm was higher under late planting compared to early planting and there were no effects of tillage. These findings are in contradiction with other studies in the literature (Blanco-Canqui and Ruis 2018; Sithole et al. 2019 and Malobane et al. 2021) who found that tillage, more specifically NT, favoured aggregation. The higher stability in the < 53 µm compared to the other sie fractions could be explained by action of soil organic matter and Fe and Al Oxides in forming organo-mineral complexes that enhance the tensile strength and stability of aggregates.

4.5 Conclusion

The study highlights the importance of monitoring soil carbon levels as they pertain to biological and physico-chemical properties and subsequently soil productivity. Aggregate stability responded to opposing treatments in the two sites while the organic carbon content saw a decline over the course of the study. This study recommends monitoring changes in carbon dynamics across different land use changes at varying time scales and modifying factors.

CHAPTER 5: SEASONAL AND MANAGEMENT EFFECTS ON SOIL MICROBIAL ACTIVITY AND FUNCTIONAL DIVERSITY IN SORGHUM-BASED PRODUCTION SYSTEMS IN SOUTH AFRICA

(*a version of this paper was published in Archives of Agronomy and Soil Science Journal*)

Abstract

Microbial properties affect soil productivity and quality depending on environmental and management factors. This study aimed to determine the influence of management practices, climate and soil factors on microbial biomass carbon, functional diversity and enzyme activity in two soils under sorghum production in South Africa. Two planting dates (Early and Late planting), two tillage systems (CT and NT) and two seasons (2020/21 and 2021/2022) resulting in a randomized complete block design with a 2 × 2 × 2 strip-split-plot design. Phosphomonoesterase activity was significantly higher in Season 1 (88.9 and 95.1 µg p-nitrophenol/g soil) compared to Season 2 (80.7 and 82 µg p-nitrophenol/g soil) in FS and KZN, respectively. In FS, late-planted sorghum had 3.8 times higher MBC in season one than late-planted sorghum in season two, under CT. In KZN, NT had 7% higher MBC than that in CT. The average well colour development (AWCD) was higher for CT and season one plots compared to NT and season two in KZN, respectively. Early planting under NT resulted in higher AWCD than late planting under NT. Late planted sorghum under both tillage systems and early-planted sorghum under NT had higher utilization of carbohydrates and polymers than early-planted sorghum under CT in FS. In KZN, during season two, the microbes preferred to utilize carboxylic (1.67 OD) and amino acids (1.82 OD) and amines (1.67) than in season one. The findings indicate that season affected acid phosphatase in both sites. In KZN, tillage affected MBC and planting date × season did the same in FS.

Keywords: Enzyme activity, microbial biomass, microbial diversity, nutrient cycling, tillage

5.1. Introduction

Challenges associated with agricultural management, climate change and environmental factors necessitate the adoption of resilient crops like sorghum. Sorghum (*Sorghum* bicolor [L.] Moench) is increasingly becoming an important crop in addressing climate change owing to its tolerance to drought, and multiple uses including biofuel feedstock, animal feed and phytoremediation of polluted soils. Epelde et al. (2009) showed that sorghum could ameliorate soil pollution while maintaining high biomass yield. This crop has significant resistance to biotic and abiotic stress (Khalifa and Eltahir, 2023). Dunjana et al. (2022) reported that heat and moisture stress, brought about by climate change result in reduced yields. However, there is variation of yield and tolerance to stresses in different genotypes under different climates (Pfeiffer et al. 2019). To ensure successful adoption of sorghum by smallholder famers empirical studies showing its productivity under diverse soil and climate conditions are needed.

Globally, soil health is deteriorating at alarming rates, with nearly one-third of soil experiencing some form of degradation (Wipf et al. 2021). The productivity, sustainability and resilience of agricultural production depend on maintaining high quality soils. Climate smart agricultural practices such as reduced and/or no-till improve soil organic carbon levels, stabilize soil temperature and improve nutrient availability (Jat 2019, Kraut-Cohen et al. 2020 and Wipf et al. 2021). Diversified cropping systems, efficient irrigation practices, and integrated soil fertility management are also esential, for improving soil organic matter and microbial activity (Pratibha et al. 2023, Habig and Swanepoel, 2015, Jat et al. 2021).

Soil microbial activity is essential in maintaining high quality soils (Nazir et al. 2017 and Yu et al. 2023). In a study on the effect of fertilizer and manure application on enzyme activity in a sorghum- based cropping system, Venugopal et al. (2015) reported increased acid phosphatase activity following application of NPK fertilizer together with farmyard manure compared to just NPK fertilizer. This difference was attributed to the enhanced organic matter, which increased organic carbon and nitrogen levels.

Plants and soil microbes share a close relationship that is important both for protection from disease and provision of nutrients (Yuan et al. 2015). This relationship also extends to soil microbial influence on food production and environmental processes (Adetunji et al. 2017). The associations between soil microbes and the rhizosphere are driven by root exudates, which enhance nutrient cycling, plant growth stimulation and resistance to diseases. These interactions subsequently improve soil quality, resulting in healthier and higher yielding crops (Habig and Swanepoel, 2015). However, the composition and activity of the microbial community are highly sensitive to changes in the soil environment. The characteristics of the rhizosphere are shaped by soil physico-chemical and biological properties, plant genetics and management practices, like tillage and residue management (Yuan et al. 2015).

Soil biological properties are the most sensitive indicators for assessing the short-term impact of management practices such as tillage and residue management (Janusauskaite et al. 2013; Chandra et al. 2023). The composition of the microbial community is important for environmental services because its activity influences the breakdown rate of soil organic matter and nutrient turnover (Mir et al. 2023). Microorganisms play a key role in agriculture by improving soil quality, which ultimately enhances productivity (Morugan-Coronado et al. 2022). However, management practices such as tillage can influence microbial biomass and soil organic carbon turnover, which in turn affect enzyme activity (Hoorman & Islam, 2010 and Delgado et al. 2012). Enzyme activities are reported to be higher under NT compared to CT due to improved conditions of moisture, temperature and organic matter (Balota et al., 2011).

Soil enzymes particularly hydrolases, are key indicators of soil fertility as they play a crucial role in the transformation of nutrient elements (Roldan et al. 2005). Examples include protease, phosphatase, urease and glucosidase. Plant species and age influence enzyme activity (Rejzek et al 2012 and Venugopal et al. 2015). For example, acid phosphatase activity usually increases in the rhizosphere of crops such as wheat, onion; tomato and sorghum (Sharma et al. 2023) were it enhances the availability of phosphorus (Rejzek et al.

2012 and Dlugosz et al. 2022). Optimum levels of P support early plant growth and maturity (Acosta-Martinez and Tabatabai, 2011) and its transformation from soil organic matter into available forms depends on phosphatase activity (Margalef et al. 2017). Plants, directly affect soil enzyme activity through root secretions of enzymes (Bais et al 2004), and indirectly by stimulating microbial growth via root exudates containing sugars, amino acids, organic acids, hormones and vitamins (Bais et al 2004; Dlugosz et al 2022). Jat et al. (2021) observed higher activities of dehydrogenase, urease, protease, phosphatase and β-glucosidase activities under no-till compared to conventionally tilled soil under sorghum cultivation.

In South Africa and sub-Saharan Africa numerous studies have focused on sorghum as a crop for human consumption, animal feed and fuel production (Weepener et al, 2014; Hadebe et al. 2017; Sebetha et al. 2019; Ikazaki et al. 2020 and Teshome et al. 2023). These studies explore sorghum production in various ways in order to maximise benefits for both the environment and humanity. The success of these efforts largely depends on selecting appropriate cultivars and adopting suitable management practices that enhance soil physico-chemical and biological properties under diverse climatic and soil conditions. However, there is limited research in sorghum-based conservation agriculture systems on the response of soil biological properties to changes in management practices such as tillage and planting date. Monitoring soil microbial activity is essential to detect short-term changes in soil quality. Furthermore, such data are lacking for South African smallholder farming communities. The objective of this study was to determine the effects of tillage and planting date on microbial biomass carbon and functional diversity (using community level physiological profile) and enzyme activity (β-glucosidase, acid phosphomonoesterase), of two sorghum cultivars under contrasting climatic and soil conditions in South Africa.

5.2. Materials and Methods

5.2.1. Site Description

The study sites were located in the villages of Houtnek (-29.0264; 26.8014) in Thaba Nchu, Free State and Qongqo (-28.0434; 31.6183) in Nongoma, Kwa-Zulu Natal provinces, South

Africa (Chapter 3). The prevailing properties in the two study sites are detailed in Chapter 3 including the climatic and initial soil characteristics (Table 3.1 and 3.2).

5.2.2. Initial Soil Characterization

Soil samples were collected from the 0–20 cm depth at each site for initial characterization using standard methods. Soil pH and EC were determined in a 1:1.25 soil: water suspension. SOC and exchangeable cations were determined with the Walkley-black and 1 molar ammonium acetate method at pH 7, respectively. Total N was measured with the Leco CN-200 Analyzer, available P using the Bray 1 method, and soil texture by the hydrometer method, respectively. A detailed description of the analytical methods is available in Chapter 3.

5.2.3 Field trial

The study was conducted in a sorghum field trial comprising two planting dates, two tillage systems, two cultivars and two rotations as is described in chapter 3. The design is a strip-strip-plot with two tillage systems in the vertical strips, two planting dates in the horizontal strips. Optimum planting date for summer season in South Africa is during the months November and December with planting that falls on before and after this date deemed early and late, respectively.

5.2.3.1 Soil sampling

Soil samples (0-20 cm depth) were collected at the end of each season as composite samples. Three samples per plot (4.5 x 6 m) were taken from four plots and combined into one composite sample, yielding four samples per block for a total of 12 samples per site. This sampling strategy resulted in samples from the strip plots of tillage and planting date and cultivar and rotation could not be tested independently of each other. The samples were transported to the laboratory in a cooler box with ice on the day of sampling and analysis was carried out within two days (Xu and Ge, 2015).

5.2.3.2 Microbial biomass carbon (MBC)

The chloroform fumigation-extraction method described by Witt et al. (2000) was used to determine MBC. Thirty-five g of water-saturated soil was weighed in to 250 ml glass bottles, mixed with 2 ml chloroform, and placed inside 10 L desiccators with 50 ml glass beakers containing 25 ml ethanol-free chloroform. The samples were then incubated for 24 hours in the dark at 25°C after which the chloroform was allowed to evaporate for 30 min. After fumigation, the soils were extracted with 140 ml of 0.5 M K_2SO_4 at 1:4 w/v for 1 hour using an end-to-end shaker at 35 rpm and filtered through Whatman no. 42 filter paper. The unfumigated soil was extracted following the same procedure. Carbon was determined from the soil extracts following the Walkley-black procedure.

5.2.3.3 Community level physiological profile (functional diversity)

The method of Furtak and Galazka (2019) was followed to determine the functional diversity of the microbial communities of the soil samples. A 1 g sample of soil was suspended in 99 ml of sterile solution, shaken for 20 minutes at 15 °C, and then incubated at 4 °C for 30 minutes. A 150 µl volume of the suspension was then inoculated on 96 well biolog microplates and the plates were incubated at 26 °C for one week. The rate of colour change in each well was recorded and the Optical Density (OD) of each well was read on Biolog MicroLog^{TM}3E or MicroStation^{TM} systems every 24 hours for 7 days until the values became constant and the last reading was taken for statistical analysis. Data from each plate reading was normalized by subtracting the Blank (Control) from each plate well.

5.2.3.4 Acid phosphatase activity

The method described by Acosta-Martinez and Tabatabai (2011) was used to determine the phosphatase activity. A 1 g sample of soil was placed in a flask before adding 0.2 ml toluene, 4 ml modified universal buffer (MUB at pH 6.5) and 1 ml of p-nitrophenol phosphate solution. The mixture was then incubated at 37 °C for 1 hour, and 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH were added. The suspension was then filtered, and the intensity of the yellow colour was measured with a spectrophotometer at 400 to 410 nm.

5.2.3.5 β-glucosidase activity

A similar method to that of acid phosphatase was used in the determination of β-glucosidase with a few differences. Firstly, the buffer (MUB) was pH 6 and after incubation 0.1 M Tris-hydroxymethyl amino methane (THAM) buffer at pH 12 was added instead of 4 ml of 0.5 M NaOH prior to filtration (Deng and Popova, 2011).

5.2.4 Statistical analysis

The data on microbial biomass carbon and enzyme activity for each site was subjected to analysis of variance (ANOVA) based on a 2 x 2 x 2 factorial treatment structure to determine effects of planting date (fixed effect), tillage (fixed effect) and season (random effect) on the MBC, functional diversity and enzyme activity from two contrasting climates. The statistical analysis was performed using JMP Pro 17 (JMP Statistical Discovery LLC 2022). The post hoc tests were performed using Fischer's least significant differences (LSD). Principal Component Analysis (PCA) was performed on the data for substrate utilization at 72 h after correction for control absorbance using JMP Pro 17 (JMP Statistical Discovery LLC 2022). Substrate utilization data were also used to calculate Average Well Color Development (AWCD) and Shannon-Weaver Index (H) (Gomez et al., 2006). The AWCD and H for substrate utilization data were subjected to analysis of variance (ANOVA) and the means were separated using the least Significant Differences (LSD) at $p < 0.05$.

5.3. Results

5.3.1 Effect of planting date, tillage and season on microbial activity.

A significant three-way interaction between planting date, tillage, and season was observed for MBC (Table 5.1). In FS, CT combined with late planting during the first season resulted in significantly ($p < 0.05$) higher MBC (155 µg C/g of soil) compared to NT under early planting in season 2, CT and NT under late planting in season 2 (Figure 5.1). Under NT, there were no significant differences in MBC between early and late planting within and between seasons. There were no main or interactive effects on β-glucosidase. However, season had a significant effect on phosphomonoesterase activity (Table 5.1). Season one had significantly higher acid phosphatase activity (9.63 % higher) compared to season two (Figure 5.2).

Table 5. 1: Effect of planting date, tillage and season on microbial activity in soil from FS.

Treatment	**Df**	**p-Value**		
		Microbial biomass C	**β-glucosidase**	**phosphomonoesterase**
PD	1	0.6995	0.7383	0.5677
Tillage	1	0.2103	0.3601	0.4324
Season	1	0.0628	0.0507	**0.0491***
PD x tillage	1	0.535	0.6736	0.5774
PD x season	1	**0.0227***	0.6844	0.6434
Tillage x season	1	0.9751	0.2802	0.1692
PD x tillage x season	1	**0.0443***	0.7495	0.248

*On bold values indicate a significant treatment effect at $p < 0.05$

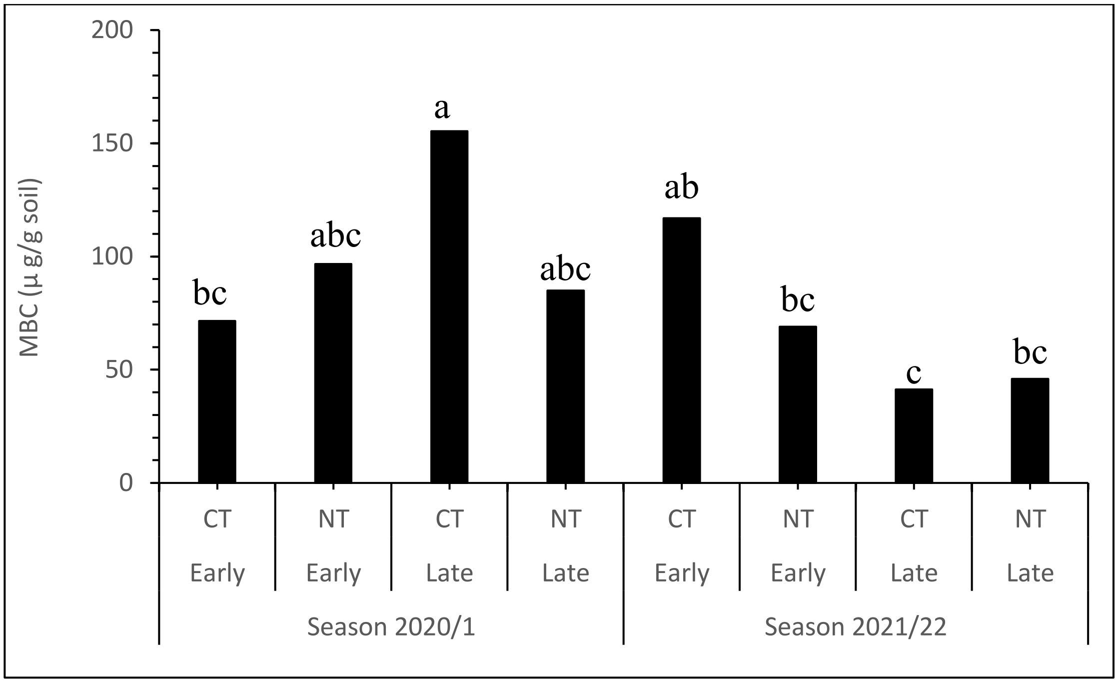

Figure 5. 1: Effect of planting date x tillage x time on MBC in FS soils.
Lowercase letters on the bars indicate significant difference at $p < 0.05$.
MBC=microbial biomass carbon, CT= conventional tillage, NT= no-till

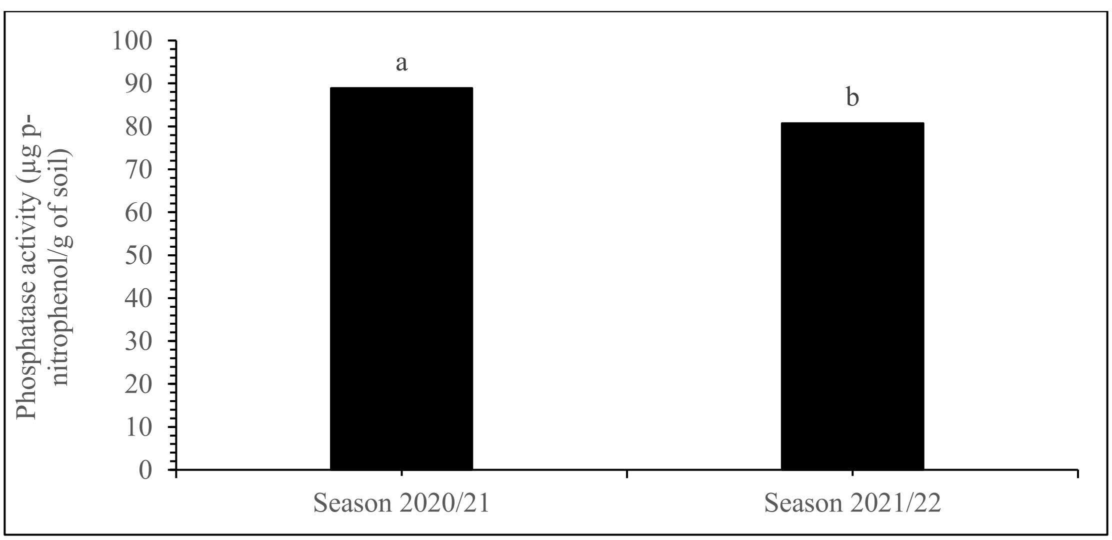

Figure 5. 2: Effect of season on acid phosphatase in FS soil.
Lowercase letters on the bars indicate significant difference at $P < 0.05$.

In KZN, only tillage and season had significant effects on MBC and acid phosphatase activity respectively, with no treatment effects on β-glucosidase (Table 5.2). Soils under NT had significantly higher ($P < 0.05$) MBC (63.71 μg C/g) than those under CT (59.54 μg C/g) (Figure 5.3). Additionally, Season 1 resulted in significantly higher phosphomonoesterase activity than season 2 (Figure 5.4), with no effects of tillage and planting date.

Table 5. 2: Effect of planting date, tillage and season on enzyme activity and microbial biomass carbon of soil from KZN.

Treatment	**Df**	**p-Value**		
		Microbial biomass C	**β-glucosidase**	**phosphomonoesterase**
PD	1	0.9333	0.3929	0.1786
Tillage	1	**0.0260***	0.2305	0.2514
Season	1	0.7139	0.5859	**0.0022***
PD x tillage	1	0.5549	0.8371	0.2689
PD x season	1	0.5663	0.5233	0.2979
Tillage x season	1	0.8775	0.1079	0.3603
PD x tillage x season	1	0.6185	0.5987	0.3688

*on bold values indicate a significant treatment effect at $p < 0.05$

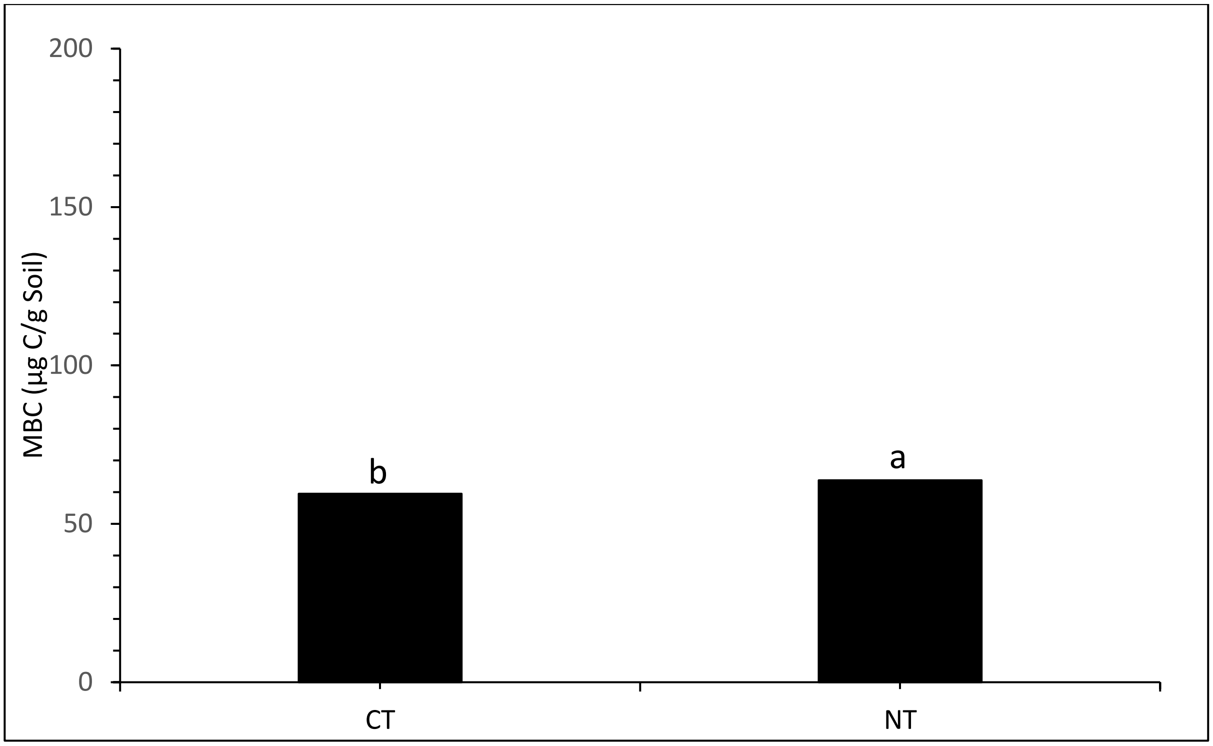

Figure 5. 3: Effect of tillage on MBC in KZN soil.
Lowercase letters on the bars indicate significant difference at $P < 0.05$.
MBC=microbial biomass carbon, CT= conventional tillage, NT= no-till

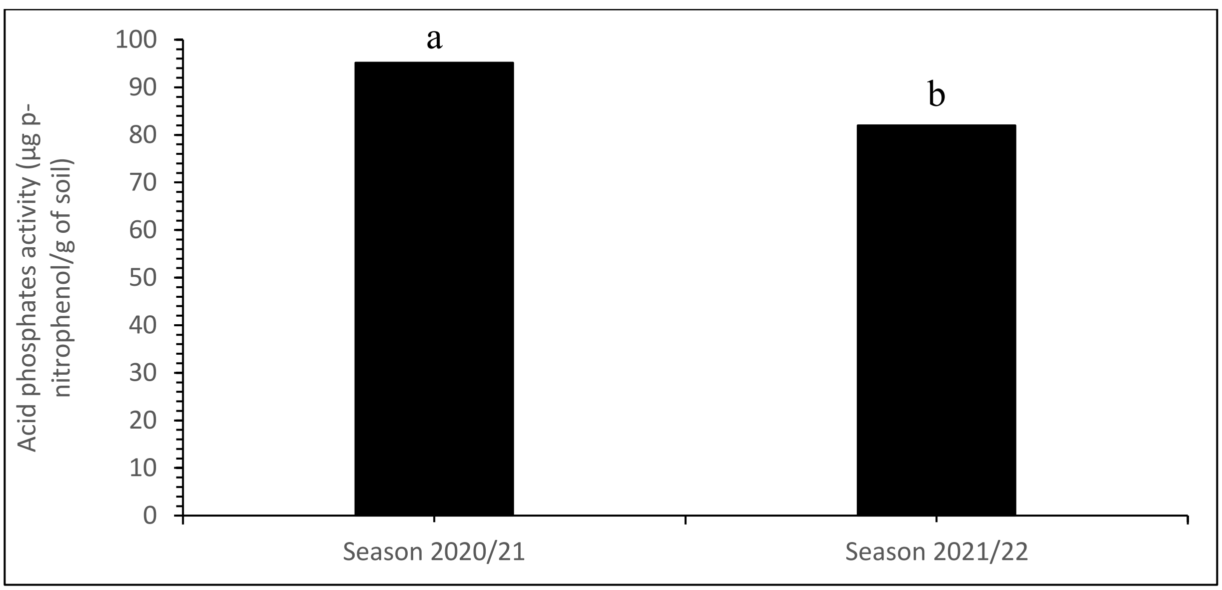

Figure 5. 4: Effect of season on acid phosphatase in KZN soil.
Lowercase letters on the bars indicate significant difference at $P < 0.05$.

5.3.2 Effect of planting date, tillage and season on microbial functional diversity.

No significant treatment effects were observed for AWCD across all treatments, except for the two-way interaction between planting date and tillage in FS and the main effects of tillage and season in KZN (Table 5.3). None of the treatment combinations affected the Shannon weaver index in FS; it was only affected by season in KZN (Table 5.3). In FS, planting early under NT resulted in significantly ($p < 0.05$) higher AWCD compared to late planting under NT while other interactions resulted in comparable results (Figure 5.5). In KZN, CT and season 2020/21 AWCD were significantly higher ($p < 0.05$) than NT and season 2021/22, respectively (Figure 5.6). The Shannon Weaver-Index was significantly higher ($p < 0.05$) in season 2020/21 compared to season 2021/22 in KZN with no other treatment combinations exerting any influence (Figure 5.7).

Table 5. 3: Effect of planting date, tillage and season on the AWCD and Shannon weaver index in FS and KZN.

		FS		**KZN**	
Treatment	**Df**	**p- Value**			
		AWCD	**Shannon-Weaver index (H)**	**AWCD**	**Shannon-Weaver index (H)**
PD	**1**	0.209	0.301	0.414	0.2166
Tillage	**1**	0.591	0.366	**0.049***	0.1508
Season	**1**	0.236	0.213	**0.007***	**0.013***
PD x tillage	**1**	**0.044***	0.063	0.349	0.729
PD x season	**1**	0.823	0.397	0.221	0.061
Tillage x Season	**1**	0.697	0.382	0.080	0.164
PD x tillage x season	**1**	0.344	0.118	0.166	0.730

*on bold values indicate a significant treatment effect at $p < 0.05$

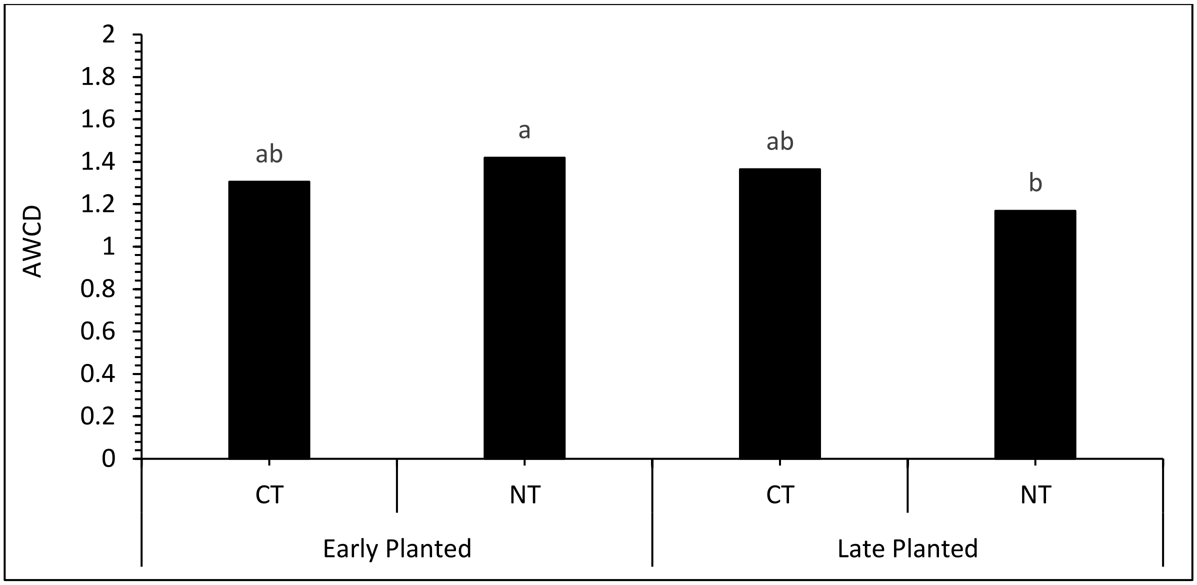

Figure 5. 5: Effect of planting date x tillage on AWCD in FS.
Lowercase letters on bars indicate significant difference at $p < 0.05$.
AWCD= average well colour development, CT= Conventional tillage, NT= No-till

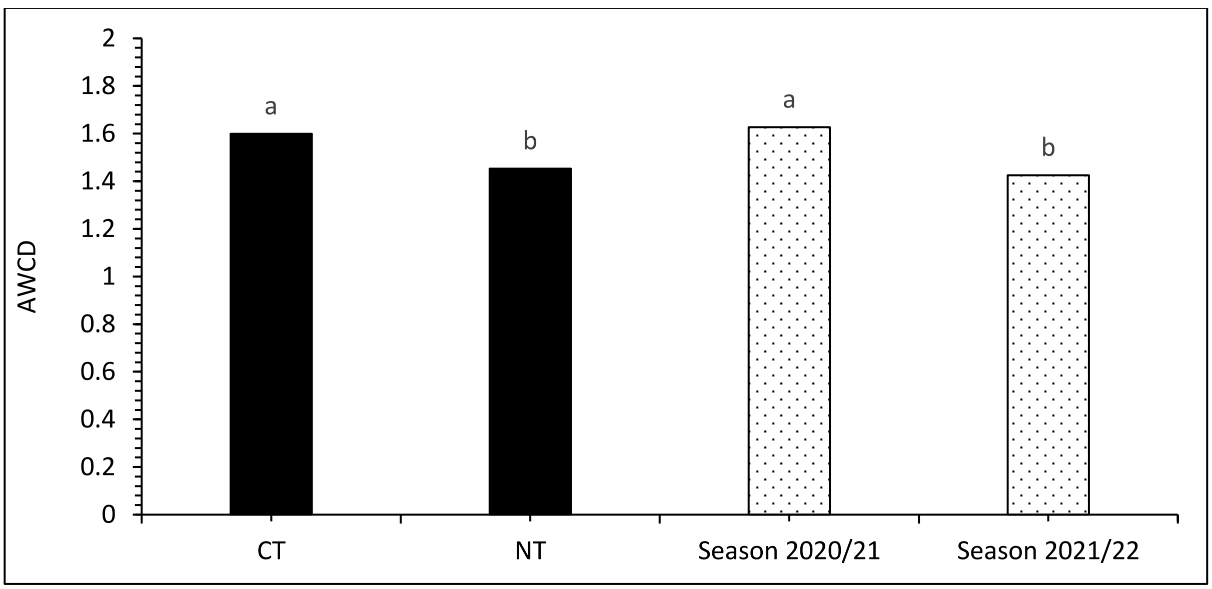

Figure 5. 6: Effect of Planting date and season on AWCD in KZN.
Lowercase letters on bars indicate significant difference at $p < 0.05$.
AWCD= average well colour development, CT= Conventional tillage, NT= No-till

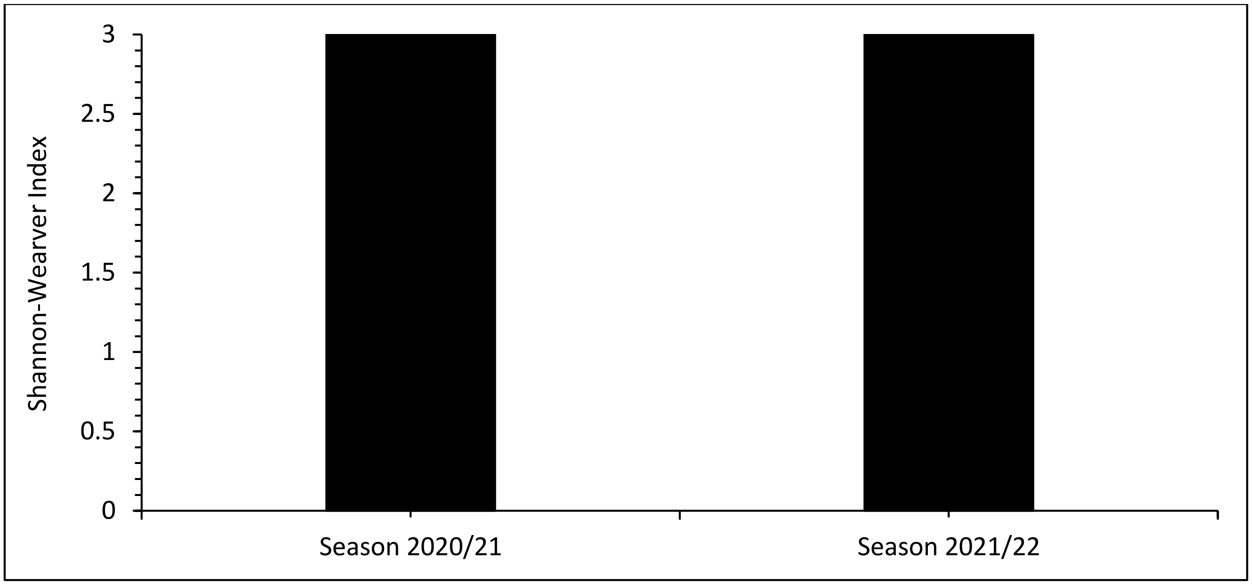

Figure 5. 7: Effect of season on the Shannon weaver index in KZN.
Lowercase letters on bars indicate significant difference at $p < 0.05$.

5.3.3 Effect of planting date, tillage and season on carbon source utilization

Planting date significantly affected ($p < 0.05$) the utilization of carbohydrates and polymers. Additionally, the interactions planting date × tillage and tillage × season significantly influenced the use of carbohydrates, carboxylic acids, amino acids, polymers and amines by microbes in FS soil (Table 5.4). In soils in which planting was late, the microbes utilized 15.67% more carbohydrates than soils in which planting was done early (Figure 5.8). Soils in which planting was early under CT, the utilization of carbohydrates (0.87 OD) and polymers (0.95 OD) was significantly ($p < 0.05$) lower than in all the other PD x tillage treatment combinations (Figure 5.9). In the second season, soil under NT had higher utilization of amino acids (1.83 OD) than the rest of the tillage x season interactions (Figure 5.10). In the second season, soils under NT had higher utilization of carbohydrates (1.23 OD) than CT in the second season (0.99 OD) and NT in season 1 (1.02 OD), though the carbohydrate utilization was similar to CT in season 1 (1.09) (Figure 5.10). Additionally, CT in the first season (1.29 OD) had significantly lower utilization of polymers the all the other interactions (Figure 5.10).

Table 5. 4: Effect of planting date, tillage and season on the carbon source utilization of microbes of soil from FS.

		Carbohydrates	Carboxylic acids	Amino Acids	Polymers	Amines
Treatment	**Df**		**p-Value**			
PD	1	**0.0235***	0.3516	0.1467	**0.0036***	0.0574
Tillage	1	0.4173	0.0614	0.1557	0.4658	0.1413
Season	1	0.2526	0.3228	0.0667	0.0545	0.0718
PD x tillage	1	**0.0069***	0.4077	0.1508	**0.0254***	0.1312
PD x season	1	0.8505	0.4874	0.3019	0.6287	0.7353
Tillage x season	1	**0.0353***	**0.0061***	**0.0145***	**0.0277***	**0.043***
PD x tillage x season	1	0.4647	0.6894	0.2386	0.5347	0.4413

*on bold values indicates a significant treatment effect at $p < 0.05$

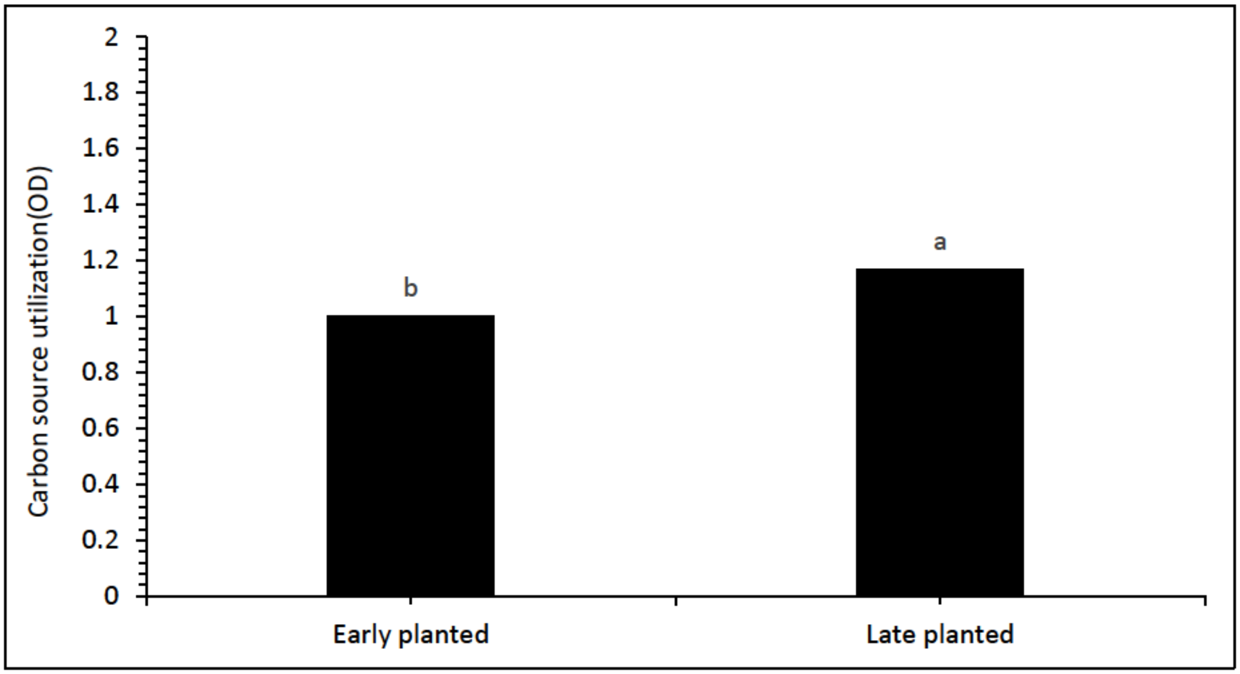

Figure 5. 8: Effect of planting date on use of carbohydrates in FS soil.
Lowercase letters on the bars indicate significant difference at $P < 0.05$
OD= optical density

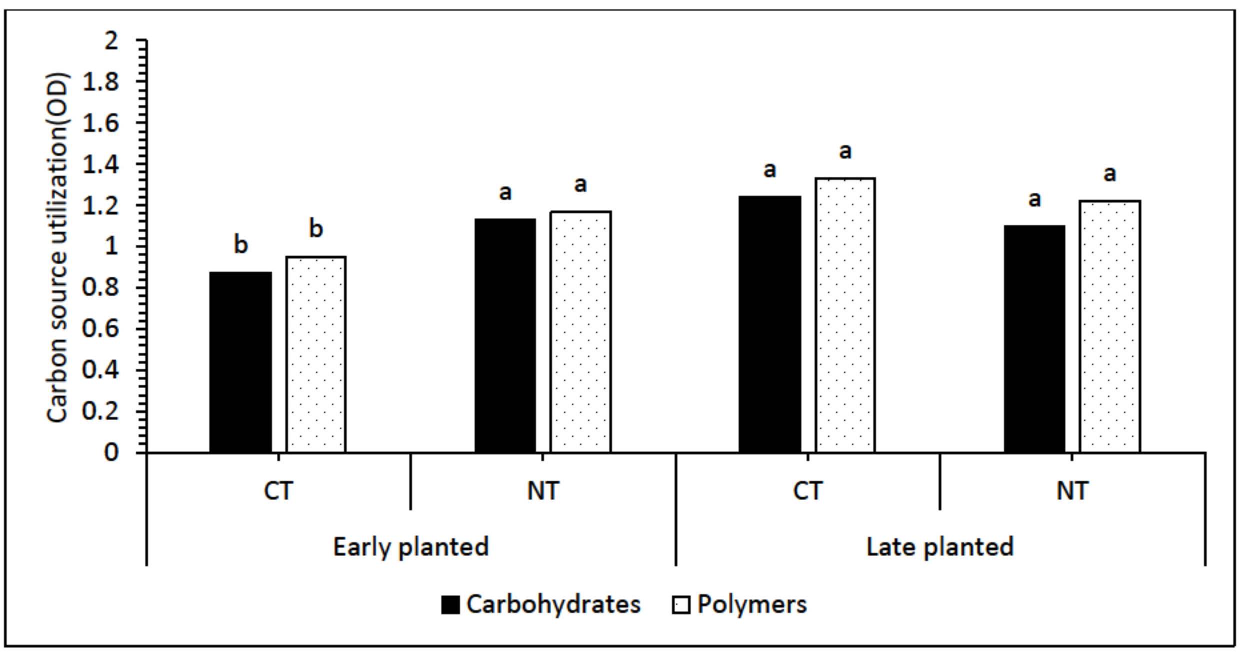

Figure 5. 9: Effects of planting date and tillage on utilization of carbohydrates and polymers in FS soil.
Lowercase letters on the bars indicate significant difference at $P < 0.05$
CT= conventional tillage, NT= No-till, OD= optical density

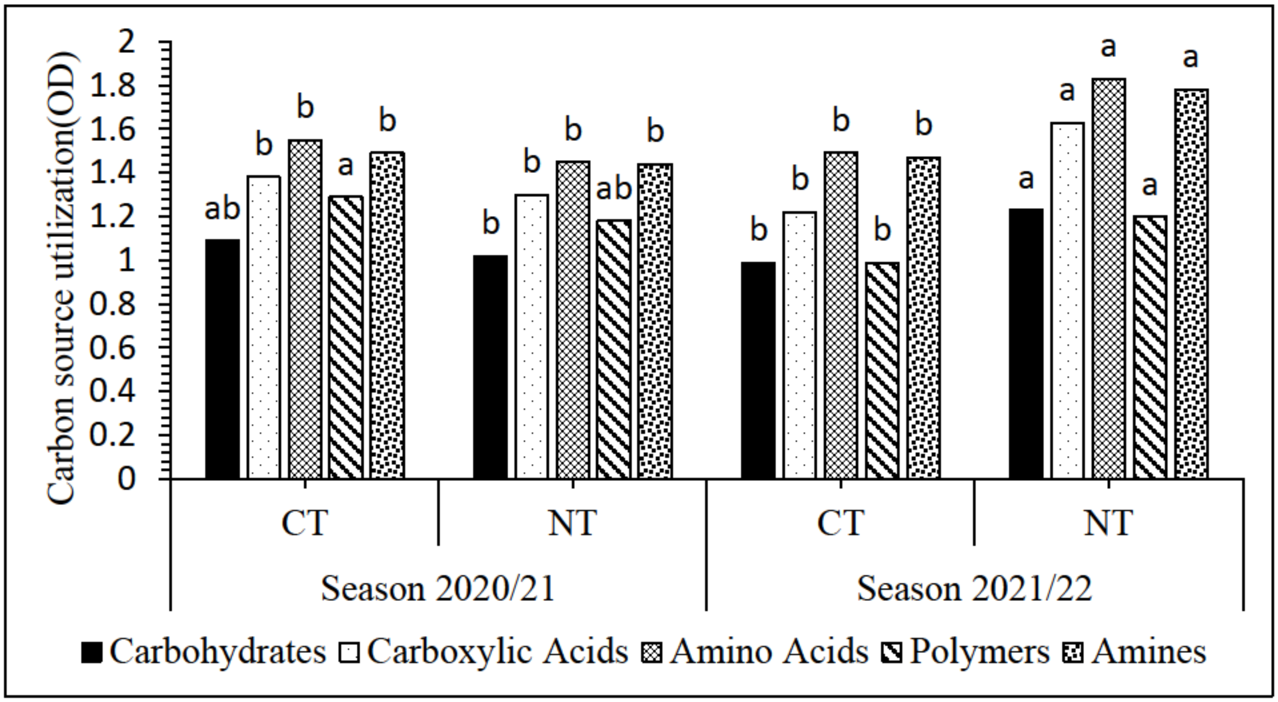

Figure 5. 10: Effect of tillage and season on the utilization of carbohydrates, amino acids and polymers by FS.
Lowercase letters on the bars indicate significant difference at $P < 0.05$
CT= conventional tillage, NT= No-till, OD= optical density

Significant ($p < 0.05$) seasonal effects were observed on the utilization of carboxylic acids, amino acids, amines while the interaction between tillage, and season significantly ($p < 0.05$) affected the utilization of amino acids (Table 5.5). During season two, the microbes utilized significantly ($P < 0.05$) more carboxylic acids (1.67 OD), amino acids (1.82 OD) and amines (1.67 OD) as a carbon source compared to season one (Figure 5.11). In contrast, under NT in the first season soil microbes used significantly (1.35 OD) less amino acids than the rest of tillage x season interactions (Figure 5.12). Neither planting date nor tillage practices significantly affected the carbon utilization patterns of all carbon groups.

Table 5. 5: Effect of planting date, tillage and season on the carbo source utilization of microbes of soil from KZN.

Treatment	Df	Carbohydrates	Carboxylic acids	Amino Acids	Polymers	Amines
			p-Value			
PD	1	0.7965	0.4574	0.2662	0.1304	0.569
Tillage	1	0.2716	0.8119	0.1957	0.8928	0.1498
Season	1	0.4676	**0.0002***	**0.0082***	0.1401	**0.0013***
PD x tillage	1	0.5662	0.1811	0.5212	0.351	0.7661
PD x season	1	0.5767	0.7535	0.4302	0.924	0.0998
Tillage x season	1	0.8113	0.574	**0.0297***	0.1458	0.1074
PD x tillage x season	1	0.0508	0.142	0.4829	0.677	0.7188

*on bold values indicates a significant treatment effect at $p < 0.05$

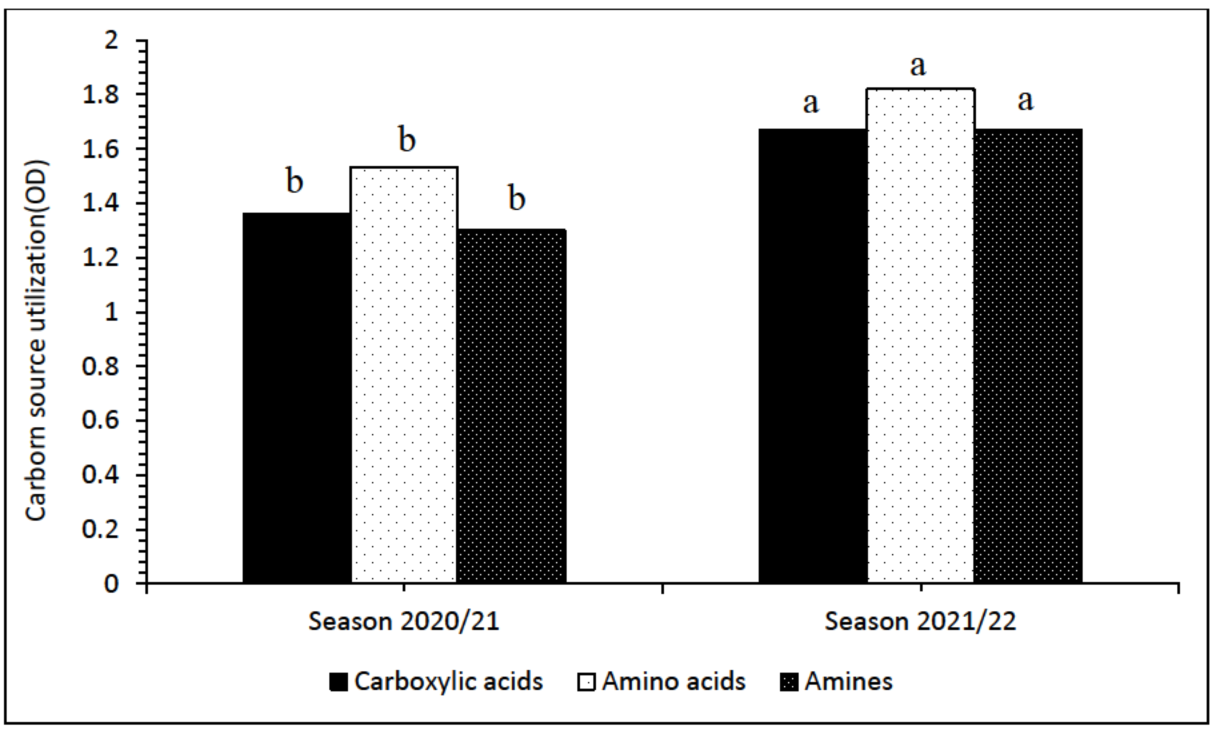

Figure 5. 11: Effect of season on the carbon source utilization of microbes of soil from KZN.
Lowercase letters on the bars indicate significant difference at $P < 0.05$
OD= optical density

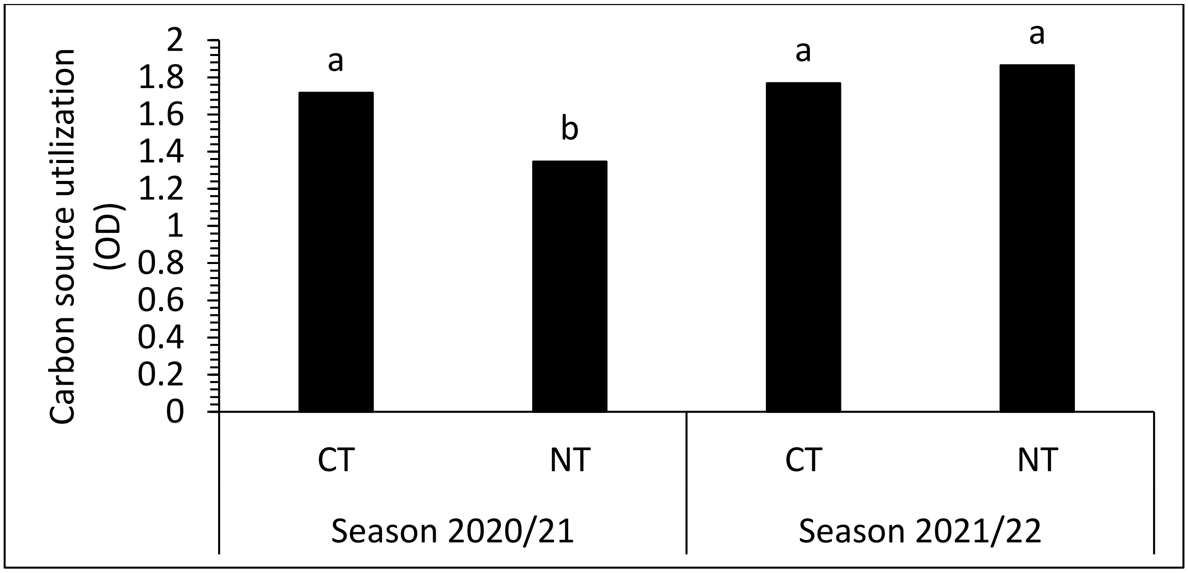

Figure 5. 12: Effect of tillage x season on utilization of amino acids in KZN soil.
Lowercase letters on the bars indicate significant difference at P < 0.05
OD= optical density

5.3.4 Principal component analysis

Figure 5.13 indicate that in FS, CH, CA, P, AWCD, AA, H and E are all negatively correlated to soil protein and βg, WSA250 µm, OC and AP show a negative correlation with >250 µm and WSA53 µm. The parameters CH, CA, P, AWCD, AA, H show similar variation that is explained by PC1 and βg, WSA250 µm, OC have similar variation that is explained that is highest on PC2. In KZN (Figure 5.14), 53-250 µm, >250 µm, P, CA, AA, AP have similar variance explained by PC1 and negatively correlated with the cluster of CH, E, H, S and <53 µm explained by PC2. The variation of WSA53 µm and WSA250 µm and SP is similar and lines on PC2 and is negatively correlated with the PC1 cluster of βg, OC, WSA<53 µm, AWCD, MBC and A.

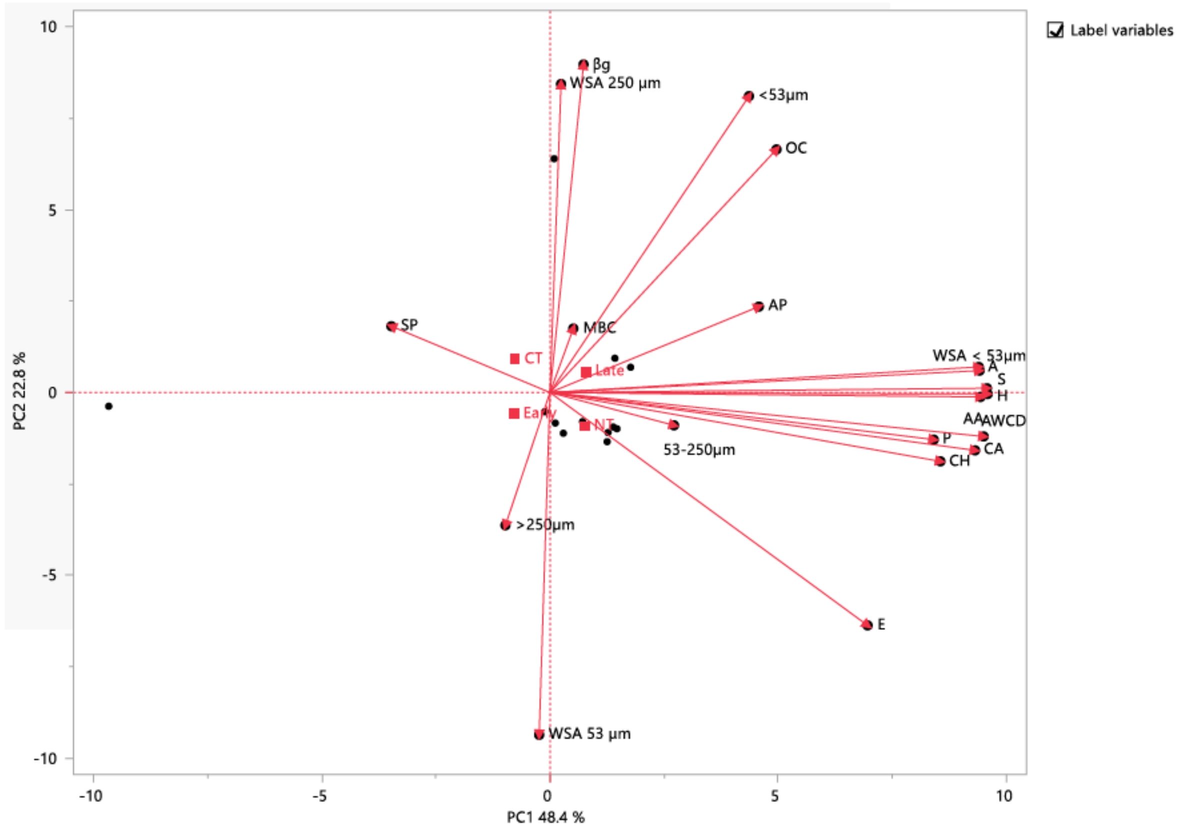

Figure 5. 13: PCA biplot showing correlation between OC, MBC, SP, βg, AP, AA, CH, CA, AA, A, P, WSA250 µm, WSA53 µm, WSA<53 µm, <53 µm, 53-250 µm and >250 µm in FS.

PCA= Principal component analysis, OC= organic carbon, MBC= microbial biomass carbon, SP=soil protein, WSA= water stable aggregates, βg= βetaglucosidase, AP= Acid phosphatase, H= Shannon-Weaver Index, E= Evenness, S= Richness, AWCD= average well color development.

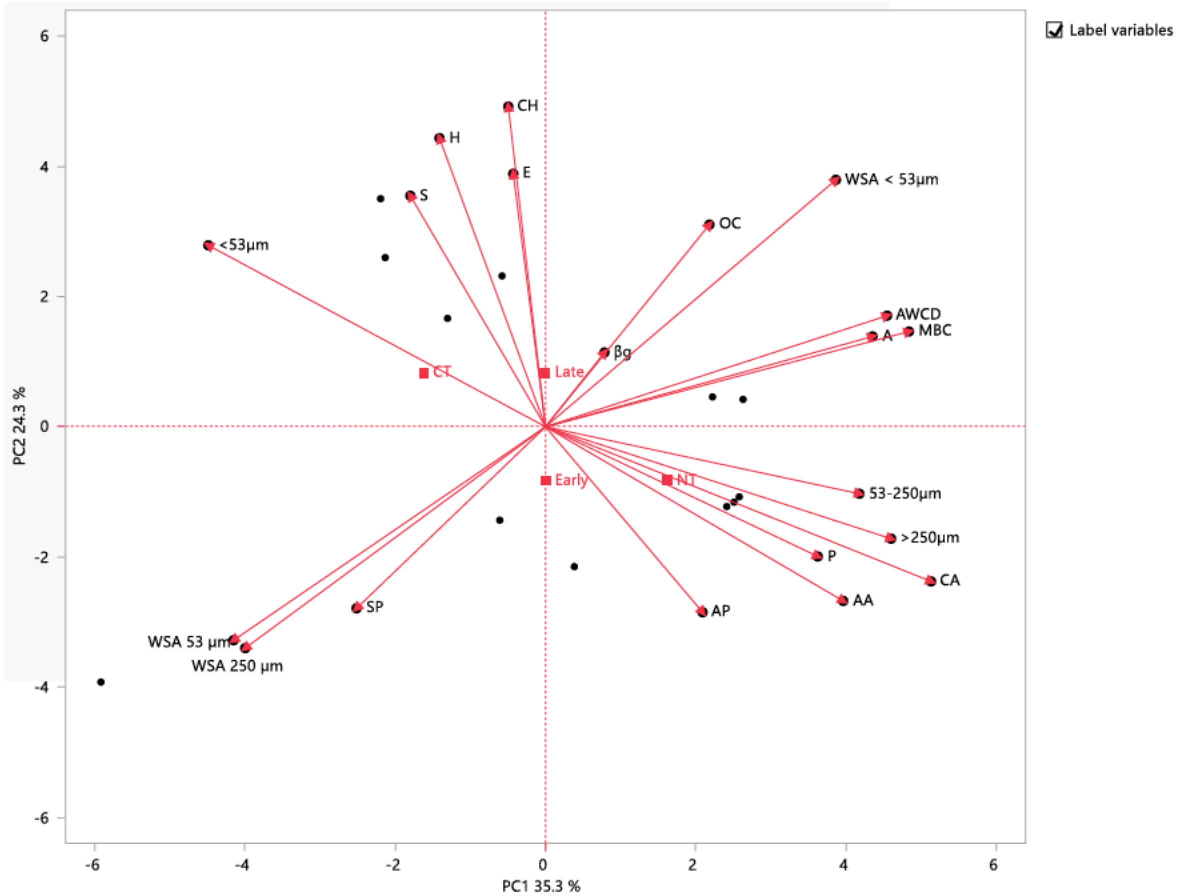

Figure 5. 14: PCA biplot showing correlation between OC, MBC, SP, βg, AP, AA, CH, CA, AA, A, P, WSA250 µm, WSA53 µm, WSA<53 µm, <53 µm, 53-250 µm and >250 µm in FS.
PCA= Principal component analysis, OC= organic carbon, MBC= microbial biomass carbon, SP=soil protein, WSA= water stable aggregates, βg= βetaglucosidase, AP= Acid phosphatase, H= Shannon-Weaver Index, E= Evenness, S= Richness, AWCD= average well color development.

5.4. Discussion

The significant interaction effects of planting date × tillage × season on MBC in FS but not in KZN highlights the influence of site-specific characteristics on MBC. The absence of significant differences between NT treatments across seasons and planting times suggests that NT stabilises MBC. The higher MBC in the first season compared to the second season under late planting may be attributed to the drier conditions at the time of sampling in the second season. According to Moeletsi et al (2022), the area received, 101.7 mm of rain in 2022 compared to 134.2 mm in 2021 during the planting season. The higher MBC suggests greater nutrient cycling from the soil organic matter under these conditions. These effects were more evident under CT than NT. The higher MBC in season one and lower in season two, under late-planted sorghum with CT than early planting suggests that under CT, MBC is more sensitive to season than tillage. These differences under CT could be due to the variation in rainfall received between the two seasons, while NT known for enhancing moisture retention, was less affected. This suggests that under CT, the timing of planting has a significant effect on activity of microorganisms and their effects on organic matter decomposition. The higher MBC under NT compared to CT in KZN soils could be due to the greater moisture storage in this relatively wetter region than FS. This is consistent with findings of Mikanova et al. (2009), who reported higher MBC in soils under NT and minimum tillage system compared to CT. Soils under NT have greater potential to store organic matter compared CT due to less disturbances, whereas CT exposes OM to decomposition, especially in the top soil layers through aggregate breakdown. Engell et al. (2022) found similar results in the 0–10 cm depth followed by the sharp decline with depth. They attributed this to the organic matter input rather than the nitrogen and SOC already in the soil. The lack of impacts of planting date and season in KZN suggest that time does not affect the level of MBC which aligns with the findings of Spedding et al (2004).

In the current study, the treatments and their interactions did not significantly affect β-glucosidase indicating that other factors influenced the activity of this enzyme. β-glucosidase activity is known to be affected by pH, moisture and soil depth (Adetunji et al. 2017) as well as the addition of organic residue that provide substrate (Balota et al. 2011). β-glucosidase activity is generally higher under slightly acidic conditions like those in this study (FS-5.71 and KZN- 5.17) (Acosta-Martinez and Tabatabai, 2000 and Wade et al. 2021). This is however contrary to our results suggesting the activity is modified by other properties such as C:N ratio, type of roots of the crop and soil texture. Tillage practices affects the activity of β-glucosidase through their influence on microbial biomass, substrate availability and soil disturbance (Adetunji at al. 2017).

The higher activity of acid phosphomonoesterase activity in the first season than in the second for both FS and KZN could be due to higher demand for phosphorus, in the wetter season (Acosta-Martinez and Tabatabai (2011). According to Bucher 2007; Balota et al. 2011 phosphatase availability and activity are linked to phosphorus levels optimum for both plant and microorganism growth. The results of this study align with those of Ajeigbe et al. (2018) who reported similar trends. The lack of tillage effects is consistent with the findings of Acosta-Martinez and Tabatabai, (2011) and Balota et al. (2011), who reported that the activity of phosphatases is closely linked to the pH of the soil rather than level of disturbance or organic matter content. In a study involving maize and beans, switching to no-till management resulted in lower acid phosphatase due to increase in pH (Rucabruna et al 2024). Alvey et al. (2001) also reported higher phosphomonoesterase activity in continuous sorghum compared to rotational sorghum. However, the current study could not verify due to limitations of scope. Moreover, there is currently no method available to differentiate phosphatase activity based on its source (Dlugosz et al. 2022).

Pesticide application can negate the benefits of conservation tillage (Arora et al. 2019, Sim et al. 2022 and Mackay et al. 2023). Soil properties also modify the effect of pesticides on soil microbes and overall soil biological properties. The finding is the current study agrees with the findings of Sim et al (2022), who showed the pyrethroid Cypermethrin® increases soil microbial biomass. Pesticides in the current study did not affect the activity of β-glucosidase, similar results were reported by Floch et al. (2011) in an incubation study were β-glucosidase activity varied with time but always returned to original levels after 12 months of incubation.

The significant main effects of tillage and season on diversity indices (AWCD and H) in KZN, suggest that CT and season 1 microbes had higher functional diversity than their counterparts did. This is consistent with the findings of Janusauskaite et al. (2013) and Xiao et al. (2019) who reported higher carbon utilization in CT than NT plots. They attributed this to increased availability of hydrocarbons in CT compared to NT. In this study, the relationship between diversity indices and carbon source utilization was evident as the utilization of carboxylic acids, amino acids and amines was influenced by season while amino acids utilization was also influenced by the interaction of tillage × season. Xiao et al. (2019) explained that the alterations in the microbial environment brought about the effects of tillage in the soil biogeochemical properties, could alter the patterns of substrate utilization by microbes.

In Free State, the AWCD was influenced by the interaction of planting date and tillage while the Shannon-weaver index was not affected by any of the treatments and their interactions. This implies the number of microbes and abundance of different species did not respond to the different soil conditions caused by the treatments. We observed a difference in microbial diversity, according to AWCD, between early and late planting under NT, but no differences between CT and either planting time. These results are consistent with Lupwayi et al. (2001), who also observed increased microbial diversity after one year of implementing zero tillage. The lack of treatment effects on the Shannon weaver index in the FS soils suggests that species richness and evenness remained unaffected. As numerous investigators have highlighted, the Shannon-Weaver index changes depending on whether, richness and/or evenness is more prominent in the community (Hill, 2003, Wolinska et al. 2017, and Roswell et al. 2021). When species richness is high, the Shannon index tends to reflect this richness, but when one species dominates in terms of individuals, the index reflects more on evenness. This suggests that the microbial community in FS may have balanced levels of richness and evenness, making the Shannon index stable across treatments.

Planting date, tillage x planting date and tillage x season, significantly influenced the microbial preference for carbon sources in FS. Planting date affected the use of carbohydrates, while tillage x planting date influenced the utilisation of carbohydrates and polymers, and tillage x season influenced all the five substrates tested. According to Liu et al. (2023), the explanation for this is that NT and season two most likely had higher dissolved organic carbon, which is a key factor affecting microbial preference of carbon sources. In FS soils, microbes predominantly utilised carbohydrates indicating that they preferred simple carbon sources to complex ones. Shutter and Dick (2001) observed similar results when they amended a soil with glucose highlighting the physiological differences in the profile of microbial groups under NT. This suggests that NT fosters a microbial profile favouring the breakdown of simple carbon sources, while tillage may alter these dynamics. In KZN, amino acids, carboxylic acids and amines were the preferred substrates, influenced by season and the interaction between season and tillage. According to Delgado et al. (2012), no-till enhances MBC leading to increased enzyme activity, which promotes organic matter retention. In contrast, CT reduces hydrocarbons, contributing to a decline in organic matter. The fact that carbon source utilization was higher under NT and during the second season in both study sites suggests that conservation agriculture has the potential to protect substrates from microbial attack while improving soil temperature and structure. This supports the idea that studying carbon source utilization is a good indicator of soil quality (Jezierska-Tys et al, 2020).

5.5. Conclusion

The study revealed that tillage, season and to lesser extent planting date, significantly affect biological properties, particularly in the topsoil, which is the most affected by tillage systems. In semi-arid regions, these effects interacted with planting time, tillage, and season, while in sub-humid areas, tillage alone had the predominant influence. The higher metabolic activity exhibited by early planted NT soils in FS and first season CT soils in KZN indicate greater nutrient cycling to benefit sorghum. The study also highlighted the importance of microbial carbon use preferences and levels, which are important for determining appropriate crop rotations with sorghum, suitable fertilization regimes and key abiotic factors to consider when selecting location for cultivation. This is the first study conducted on rural (smallholder/subsistence farming) settings to report on enzyme activity and microbial functional diversity in South Africa.

Based on these findings, we recommended further studies on the effects of other soil parameters that modify the effects of planting date and tillage on biological properties. These parameters could include time since introduction of conservation tillage, soil depth and other site-specific conditions. Additionally, we recommend adopting management interventions tailored to local edaphic and climatic factors to optimize agricultural practices and improve soil health and productivity.

CHAPTER 6: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS.

6.1. Discussion

The prospect of climate change necessitates the adoption of climate smart agriculture approaches such as reduced or no-till agriculture to build up soil organic matter, selecting appropriate planting dates, adaptable cultivars, crop rotations, and management practices that increase and maintain soil quality. This mostly affects the resource poor farmers of sub-Saharan Africa. Hence, the aim of this thesis was to determine if different CSA practices affect the productivity of sorghum, soil organic carbon, microbial activity and functional diversity and aggregate stability in two smallholder farming areas of South Africa with different soils and climates.

The increase in plant height because of planting date, cultivar and planting date × tillage effects in both sites (chapter 3), could have been caused by the crop being exposed to favourable environmental conditions for longer (Irawan et al. 2022). Early planting allows for a longer growing season than late planting and different cultivars differ in plant height owing to differences in the genes (Gosh et al. 2015). The significant influence of planting date, tillage and cultivar in Free State and only cultivar in KZN in terms of yield (chapter 3) shows that the regional adaptability of sorghum crops plays a role in the growth of the crop (Gosh et al. 2015; Sebetha and Modisapudi 2019). Time of planting becomes more important in the cooler temperatures of the Free State so as to avoid the frost season and the warmer climate of KwaZulu Natal favours PAN8816 because it produces more yield regardless of time of planting. However, it needs to be acknowledged that these findings were based on a short-term study. Wight et al. (2012), Pittelkow et al. (2015) and Schlegel et al. (2017) asserted that in order to realize the impact of conservation agriculture one must allow time from the date of inception. Schlegel et al. (2018) conducted a long-term study (1991-2015) and found that NT produced 120 % more sorghum yield than CT, on a silt loam soil under semi-arid conditions. Similar conditions prevailed in FS in this study and the yield was influenced by planting date rather than NT due to the short-term of the study. According to Saini et al. (2018), sowing early affords the crop more favourable climate conditions and enough time for grain filling before the winter cold hits, which was most important in Free State in the current study. The lack of frost in KZN when planting late means either date is suitable without yield penalties.

In KZN, the cultivar factor was the most influential with PAN8816 performing better than Macia (chapter 3). Although it was not significant, PAN8816 produced more yield in FS as well. Several researchers attribute this to genetic differences between cultivars (Gosh et al. 2015, Hadebe et al. 2017 and Ajaj et al. 2021). These differences manifest in various ways such as a greater rate of photosynthesis and assimilation of its products, which are related to certain traits of each cultivar. For example, Manyathi et al. (2014) stated that PAN8816 had a higher chlorophyll content because it has a small canopy and Ajaj et al. (2021) found the superior leaf area of the Rabeh cultivar provided a greater amount of processed nutrients for grains, when compared to Babel and Warka varieties. The increased yield of PAN8816 at both sites is also explicable by the observation that at both sites the nutrient uptake followed similar trends as the yield. A number of factors including available moisture, genotype, soil organic carbon, nutrient availability and soil physico-chemical properties affects sorghum nutrient uptake (Ameen et al. 2017).

The pH, organic carbon, CEC, total N and available P are some of the most important soil properties for sorghum growth and soil health (Agenehu et al. 2016). Soil pH in both sites in the current study was acidic but still in range to support nutrient availability (Hamza 2008, Horneck et al. 2011, Teshome et al 2023). Considering the levels of clay content, CEC and OC in the soil the available P at both sites, was in the right range (2-7 mg/kg) to support the yields we obtained as attested to by Teshome et al 2023. Teshome et al. (2023) stated that 11.8 mg/kg of P is high enough to support sorghum growth for a soil with 47.5% clay, 1.96% OC and 17.0 meq/100g CEC.

Sorghum growth is influenced by edaphic factors like soil organic carbon fractions and aggregate stability, which in turn are affected by the binding agents. In the current study early planting resulted in higher OC in coarse particulate fraction in FS. Results of sorghum yield follow a similar trend, which suggests that planting early under CT is the way to go in FS. Datta et al. (2018) in India found similar correlations between labile organic carbon fractions and sorghum and wheat yield. Aggregation in the size classes 53-250 µm and <53 µm was higher under NT in FS when compared to >250 µm (chapter 4), which agrees with Malobane et al. (2021) and Pinto et al. (2022). This is because there was less carbon in the cPOC fraction compared to fPOC and MAOC. Early planting × CT gave the highest amount of water stable aggregates in the >250 µm and 53-250 µm and late planting affected the <53 µm aggregate size with no direct effects of tillage in either aggregate size. This contradicts Malobane et al. (2021) who found that tillage was the main factor affecting aggregate stability in low carbon soils. However, our results align with Blanco-Canqui and Ruis (2018), who argue that for tillage to be effective, especially in the short-term, it often requires companion practices. Such companion practices include the use of cover crops, animal manure mulches and addition of other organic amendments all of which can help to reduce compatibility and improve aggregate stability.

In this study soil protein, organic carbon fractions and water stable aggregation were determined to elucidate how the management practices modify the soil environment to support sorghum growth. While Lombardo et al. (2019) reported that soil protein can leak from cell walls due to tillage, it did not respond to the treatments (including tillage) or their interaction tillage (chapter 4), which suggests that other factors might be influential. In the treatments of current study, tillage and crop rotation did not influence sorghum yield and the production of soil protein. Hence, there is no relationship between the soil protein and water stable aggregation. The FS site was previously uncultivated grazing land prior to the study, and converting the land to cropping required tilling, which could explain the glomalin dynamics. The major difference between the study sites is in their history with the KZN site being active and FS being new to cropping activities prior to this study. KZN soils have been continually disturbed and glomalin dynamics have been through that whereas glomalin dynamics in FS has been relatively unchanged.

The short duration of the present study means that the influence of no-till cannot be determined conclusively. Malobane et al. (2018) and Shen et al. (2021) explained that carbon sequestration is a two-step process of soil particles physically combining with plant residues into macro-aggregates and then being polymerised into relatively protected micro-aggregates. This is why POC and not MAOC is the ideal indicator of management changes in the short-term (Meloro et al. 2009; Yeboah et al. 2016). Moreover, Yeasmin et al. (2023) monitored the effect land use change from virgin to cropland in three years and their results revealed that there was more POM than MAON due to the recently deposited and undecomposed residues.

Sithole et al. (2019), found that tillage did not affect total soil organic carbon (chapter 4) across treatments but affected the particulate organic matter fraction which they said was because of the sensitivity of the fraction to tillage induced changes. In the presents study, the total SOC decreased over the course of the study at varying levels across the two study sites. The differences between the two sites could be linked to the variations in soil properties like texture particularly clay content, prevailing climate and other site-specific conditions. Kwa-Zulu Natal has more clay which tends to be reactive than sandy soil in Free State. The two sites have differences in climate which can have implications on such properties as soil thermodynamics with certain soils being good conductors if heat which affects soil reactions. According to Blanco-Canqui and Ruis (2018) NT can increase heat conductivity of the soil.

Conventional tillage had higher levels MAOC in FS. The transfer of carbon to mineral protected surfaces is at its most efficient with high C:N ratio crops like sorghum (Cordova et al. 2018, Canisares et al. 2023). Moreover, this rapid transfer of OC to MAOC is controlled by direct sorption which is influenced by drying-wetting cycles and oxygen availability (Ye et al. 2019, Cotrufo et al 2022). This relates when the findings of higher aggregate stability in <53 µm size class which indicates that higher organic carbon is sequestered in this fraction. The results of SOC and its fractions and aggregate fractions could have translated to soil biological activity. The microbial biomass carbon was positively correlated to fPoc in FS, indicating this SOC fraction as a ready substrate for soil microorganisms, with implications on nutrient cycling, where this POC fraction is increased.

The three-way interaction effect of tillage, time of planting and season on soil microbial biomass carbon in FS soil and not KZN (chapter 5), could suggest that this effect is site-specific. The higher MBC under CT in season one in FS indicates that the quality of the season was more influential than tillage. Season one received more rain than season two (Moeletsi et al. 2022), which is conducive for crop growth and microbial activity. The more clayey soil of KZN is could be the reason why it responded to tillage. In KZN, the higher MBC under NT than CT with no other treatment effects was similar to the studies of Mikanova et al. (2009) and Engell et al. (2022). They attributed this to the lack of disturbance, moisture retention and organic matter input. Under CT, the soil is perturbed and the residues exposed to erosion and runoff, which carries away the moisture and soil. The lack of impact of planting date aligns with the findings of Spedding et al. (2004), in their study on a sandy loam to loamy sand in arid conditions; said time did not influence MBC more than tillage. Soil enzymes facilitate the cycling of elements such as carbon, nitrogen and phosphorus by soil microbial biomass.

The lack of treatment effects on β-glucosidase and acid phosphomonoesterase activity at either site contradicted Malobane et al (2020), who found that tillage particularly no-till increased the levels of β-glucosidase and acid phosphatase (chapter 5). However, these findings are consistent with de Almeida et al. (2015) who states that there is less β-glucosidase when the organic material added if of high C:N ratio like sorghum. Acid phosphomonoesterase responded to season at both sites with season one having more activity than season two, which could be explained by moisture availability than changes in its substrate or soil P availability (Kramer and Green, 2000 & Sardans et al. 2008). The moisture was not determined for this study but season one received more rain than season two in FS and the soil in KZN has good moisture retention.

6.2 Conclusion

The study aimed to determine the productivity of growing sorghum under climate smart approaches such as no-till agriculture, manipulation of planting dates and crop rotation in the smallholder and subsistence farming communities in Thaba Nchu and Nongoma in the Free State and Kwa-Zulu Natal provinces of South Africa, respectively. The study also looked at the short-term changes in the soil biological properties and aggregate binding agents and aggregate stability. The following conclusions were drawn:

I. In the drier Free State planting early during i.e. during November-December months guarantees the highest possible yield as it avoids the frost season during the early growing stages of sorghum. Conversely, planting date did not affect the growth of sorghum in KZN which points towards a flexibility on planting time for the resource poor farmers in the region.
II. The study also showed that conventional tillage resulted in higher sorghum yields at least in the short term.
III. The greater performance of Pan8816 over Macia in both study areas shows that cultivar selection is an important part of climate smart technologies.
IV. Over the duration of the study, soil organic carbon declined by 1.74% in Free State and 10.46% in Kwa-Zulu Natal.
V. In KZN, the <53 µm aggregates were more stable than the particulate organic carbon fractions aggregate sizes band their stability was closely linked to the mineral associated organic carbon fraction.
VI. No-till had positive influence on aggregate stability in FS specifically in the 53-250 µm and <53 µm sizes. Additionally, late planting affected the stability of macroaggregates (>250 µm).
VII. Tillage significantly affected MBC in KZN with NT resulting in higher values than CT.
VIII. In FS MBC was influence by the interaction of PD × tillage × season. Furthermore, acid phosphomonoesterase activity was affected by season across both sites, while β-glucosidase activity did not respond to any of the treatments.

IX. Carbon source utilization preferences of the soils from both sites were also highlighted which is a parameter that can useful when selecting rotational crops for sorghum, fertilizer recommendations and local abiotic factors.

Overall, for maximum yields in both sites famers should plant Pan8816 after the first rains (early). Carbon dynamics did not reveal any meaningful response to the response to the treatments and aggregate stability responded to late planting and NT in Free State and CT and early planting in KZN. Soils planted early under no-till in FS and under CT in KZN showed the highest metabolic activity, which indicates that, the sorghum would benefit from the nutrient cycling on offer.

6.3. Recommendations

It is recommended that rural farmers adopt management practices specifically tailored to their local edaphic and climatic factors. Planting early to avoid exposing the crop to frost season during early development is recommended for the drier Free State province, while in KZN, farmers can use either of the planting dates tested by this study. The Pan8816 cultivar is the ideal for these climate smart production systems in both study areas due to its superior yield performance. To fully understand the long-term impacts of these climate-smart approaches, expanding the scope and duration of the study is recommended. Future research should include additional parameters over a longer period to observe the influence of the treatments on soil health and crop productivity. Another aspect to explore in future studies is the dynamic between soil microbes and soil carbon to elucidate the microbes facilitated transformations of soil carbon.

6.4. Relevance of the study to national research and development programmes, and policy.

Ensuring food security for all citizens is a major step towards the eradication of poverty and it is highly dependent on increasing the productivity of agricultural soils. This study, although short-term, provides relevant information about the potential benefits that smallholder farmers can draw from implementing the CSA (no-till, crop rotation, cultivar considerations and planting date manipulation) practices in to their farming activities. These include ability to absorb the impact of crop failures and economic losses by selecting the appropriate seed for their region and sowing it at a climatically suitable time. Reduction of input costs like fertilizers, labour and fuel through the adoption of no-till agriculture and incorporation of crop diversification. Introducing an abiotic and biotic stress resilient and underutilized crop such as sorghum to their production systems has the potential to improve the food base and economic gains. CSA policymakers seeking actionable knowledge acquisition for a wider adoption of sustainable production practices can target similar studies.

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APPENDICES.

Chapter 3 ANOVA Tables.

Table 1: ANOVA table of effect of planting date, tillage, cultivar and interactions on plant height in KZN for season 2020/21

Treatment	df	p-Value
Planting date	1	0.6778
Tillage	1	0.1691
Cultivar	1	0.8314
Planting date × tillage	1	0.0053*
Planting date × cultivar	1	0.6778
Tillage × cultivar	1	0.2990
Planting date × tillage × cultivar	1	0.4017

*Indicates significant difference at $p < 0.05$

Table 2: ANOVA of Effects planting date, tillage and sorghum cultivar on plant height in KZN for season 2021/22

Treatment	df	p-Value
Planting date	1	0.4315
Tillage	1	0.9012
Cultivar	1	<.0001*
Rotation	1	0.2676
Planting date × tillage	1	0.7315
Planting date × cultivar	1	0.2951
Planting date × rotation	1	0.7379
Tillage × cultivar	1	0.4387
Tillage × rotation	1	0.0786
Cultivar × rotation	1	0.4099
Planting date × tillage × cultivar	1	0.3862
Planting date × tillage × rotation	1	0.0356*
Planting date × cultivar × rotation	1	0.1993
Tillage × cultivar × rotation	1	0.1748
Planting date × tillage × cultivar × rotation	1	0.0348*

*Indicates significant difference at $p < 0.05$

Table 3: ANOVA table of effect of planting date, tillage, cultivar and interactions on yield in FS for season 2020/21

Treatment	df	p-Value
Planting date	1	<.0001*
Tillage	1	0.2735
Cultivar	1	0.0837
Planting date × tillage	1	0.0613
Planting date × cultivar	1	0.4081
Tillage × cultivar	1	0.5720
Planting date × tillage × cultivar	1	0.6192

*Indicates significant difference at $p < 0.05$

Table 4: ANOVA table of effect of planting date, tillage, cultivar, rotation and interactions on yield in FS for season 2020/21.

Treatment	**df**	**p-Value**
Planting date	1	<.0001*
Tillage	1	0.0439*
Cultivar	1	0.1079
Rotation	1	0.5124
Planting date × tillage	1	0.1620
Planting date × cultivar	1	0.3932
Planting date × rotation	1	0.4246
Tillage × cultivar	1	0.1537
Tillage × rotation	1	0.1110
Cultivar × rotation	1	0.5700
Planting date × tillage × cultivar	1	0.2195
Planting date × tillage × rotation	1	0.3601
Planting date × cultivar × rotation	1	0.9366
Tillage × cultivar × rotation	1	0.2166
Planting date × tillage × cultivar × rotation	1	0.3917

*Indicates significant difference at $p < 0.05$

Table 5: ANOVA table of the effect of planting date, tillage, cultivar, rotation and interactions on yield in KZN for season 2021/22.

Treatment	df	p-Value
Planting date	1	0.5554
Tillage	1	0.7381
Cultivar	1	<.0001*
Rotation	1	0.5701
Planting date × tillage	1	0.8745
Planting date × cultivar	1	0.3437
Planting date × rotation	1	0.2587
Tillage × cultivar	1	0.9364
Tillage × rotation	1	0.3582
Cultivar × rotation	1	0.3704
Planting date × tillage × cultivar	1	0.7170
Planting date × tillage × rotation	1	0.7668
Planting date × cultivar × rotation	1	0.8761
Tillage × cultivar × rotation	1	0.3206
Planting date × tillage × cultivar × rotation	1	0.5688

*Indicates significant difference at $p < 0.05$

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Planting date and tillage effects on yield and nutrient uptake of two sorghum cultivars grown in sub-humid and semi-arid regions in South Africa

Kaya Mrubata[1,2*], Adornis Dakarai Nciizah[1,2] and Pardon Muchaonyerwa[2]

Introduction: Sorghum is emerging as a viable crop option to increase food production under changing climate due to its resilience to drought and marginal soils. Appropriate planting date, crop cultivars, type of tillage and rotations, under contrasting climatic conditions, could make significant contribution on the effective management of sorghum under changing climatic conditions. A short-term study was carried out to investigate the effects of planting date, crop cultivar, tillage type and rotations on the growth and primary nutrient uptake of sorghum in contrasting climatic regions.

Methods: The study was conducted in Free State - (FS) and KwaZulu Natal (KZN) Provinces of South Africa over two seasons (2020/21 and 2021/22). The two cultivars (Pan8816 and Macia) were sown in December (PD1) and in January (PD2) under conventional tillage (CT) and no-till (NT) and with two rotations (Sorghum-Cowpea-Sorghum and Sorghum-Sorghum-Sorghum) resulting in 2x2x2x2 factorial experiment.

Results and discussion: In KZN, in both the first (2020/21) and second (2021/22) seasons, the effects of all the factors, except cultivar in the second season, were not significant on yield, and uptake of nitrogen (N), phosphorus (P) and potassium (K). In the second season, Pan8816 (4.40 t/ha) produced 3.3 times higher yield than Macia (1.32 t/ha), and took up higher N, K and P. In FS, the early planted sorghum (1.15 t/ha for season one; 3.39 t/ha for season two) had significantly higher yield than late planted sorghum (0.434 and 0.91 t/ha for seasons one and two, respectively). Furthermore, the early-planted crops took up higher N, K and P than when planted late, while Pan8816 took up 1.74 times more P than Macia. Plants grown under CT (2.61 t/ha) produced significantly higher yield than under NT (1.70 t/ha), with plants under CT taking up significantly more K than those grown on NT. These findings suggest that in the drier FS, early planting under conventional tillage, while in the wetter KZN, selecting the appropriate cultivar (PAN8816), are essential for sorghum grain yield, at least in the short-term.

conservation agriculture, climate change, food security, small-scale famers, sub-Saharan Africa

1 Introduction

The agriculture sector needs to produce more food from less land and water resources to provide for a growing population with an increasing per capita income in an urbanized world (FAO, 2017) under the difficult conditions of a changing climate (Lal, 2013). Climate change poses direct and indirect threats to food security through its influence on soil properties, water resources, and crop production (Fischer et al., 1996; Gornall et al., 2010; Lal, 2013; Masipa, 2017). These effects include, but are not limited to, loss of SOC, reduced fertility and plant available water, the prevalence of pests and diseases, changes in the hydrological balance, and increased soil erosion (Gornall et al., 2010; Lal, 2013). According to Lal (2013), agriculture is vulnerable to changes in climate, and projections point toward challenging times for farmers and land users, especially those in the developing world, especially sub-Saharan Africa (SSA) (Lal, 2019). It is therefore important to develop agricultural technologies and strategies that minimize the effects of climate change and can enhance and sustain soil productivity levels under adverse climatic conditions. This can be achieved through the selection of the appropriate crops to be grown under systems modified to suit the prevailing conditions in a particular region.

Conservation agriculture (CA) continues to be promoted as a suitable strategy that can ensure improved and sustainable production levels without compromising the environment and exacerbating land degradation (Aziz et al., 2013; Busari et al., 2015). In essence, CA entails the integrated management of available natural resources and other inputs to maximize efficiency (Choudhary et al., 2016) through minimal soil disturbance, permanent soil cover, and crop rotations (Hobbs et al., 2008). For maximum benefits to be realized, all the three principles of CA must be applied simultaneously. When applied appropriately, CA improves soil porosity, which in turn reduces runoff and erosion hence enhancing biomass production. CA can increase yield by up to 56% compared to conventional tillage due to increased soil organic matter and reduced evapotranspiration (Thierfelder and Wall, 2012; Wakindiki et al., 2018). However, smallholder farmers often struggle to maintain the requisite amount of crop residue for soil cover due to competing uses. Moreover, most soils under smallholder farming are too poor to support optimum biomass production. However, the inclusion of crop rotation in no-till systems may boost biomass production thus ensuring the retention of optimum levels of residues, which may increase yield.

While it is generally agreed that conversion from conventional tillage to no-till has potential to increase crop productivity, its effectiveness is influenced by soil and climate factors. For instance, Toliver et al. (2012) analyzed data from 442 paired tillage experiments across the United States, examining six crops and various environmental factors such as geographic location, annual precipitation, soil texture, and time since conversion from tillage to no-till. The results indicated that no-till generally resulted in similar or higher yields than conventional tillage for sorghum and wheat. However, environmental factors influenced yield variations between the two tillage methods for other crops. Moreover, no-till tended to produce similar or greater yields than conventional tillage in warmer and more humid regions with loamy soils. Conversely, in drier regions with sandy soils, no-till yields were often lower than conventional tillage yields. This suggests that CA may be less beneficial on sandy; however, there is not sufficient evidence on this in South Africa. Moreover, the effects of CA may be mediated by environmental conditions and cropping systems. Therefore, this study aims to show that no-till can be a viable and beneficial agricultural practice, particularly for certain environmental conditions and crops particularly sorghum, which has attracted significant interest due to its resilience to harsh conditions.

Due to climate change, areas that are currently suited to maize in the African savannah may in the future transition to being suitable for sorghum (Lal, 2013). Summer crop production in South Africa is shifting from current production areas due to changes in rainfall and temperature regimes (Weepener et al., 2014). Sorghum thrives on low fertility soils with limited inputs for food and industrial use in breweries, production of animal feed, and as feedstock for biofuel production (Meki et al., 2013; Ikazaki et al., 2020). Moreover, sorghum is the fifth most important cereal crop after wheat (*Triticum aestivum*), rice (*Oryza sativa*), maize (*Zea mays*) and barley (*Hordeum vulgare*) (Bakari et al., 2023) with high tolerance to heat and drought. The crop can remain dormant during dry periods (Pinzi and Dorado, 2011). During the period 1998 to 2020, 58–64 Million tons of sorghum was grown on 43.2 Million hectares of land globally, which equates 6.4% of total cereal growing land and 2.7% of total cereal production (Bakari et al., 2023).

Despite this huge potential, there is a critical knowledge gap. Very few studies have reported the performance of sorghum under CA across diverse environments and soils particularly sandy soils in South Africa. Although South Africa is traditionally a maize growing nation, climate change suggests that the baseline could shift toward drought tolerant crops such as sorghum as a coping mechanism (Soga and Gaston, 2018). Adapting sorghum-planting dates to synchronize with potential shifts in rainfall and temperature patterns, which vary across seasons, has been proposed as an adaptation strategy. However, there is little evidence regarding the effectiveness of this approach in South Africa. To address this gap, this this study aimed to compare the effects of growing two sorghum cultivars on two different planting dates and two tillage practices on sorghum growth, nutrient uptake and yield in two areas with contrasting soil and climatic conditions over two seasons. This study will provide valuable insights for optimizing sorghum production under CA in South Africa.

2 Materials and methods

2.1 Site description

The study was conducted in Houtnek village (-29.0264; 26.8014) in Thaba Nchu, Free State and in Qongqo village (-28.0434; 31.6183) near the town of Nongoma, Kwa-Zulu Natal

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provinces, South Africa. The town of Thaba Nchu forms part of the Mangaung Metropolitan Municipality together with Bloemfontein and Botshabelo. The town is located at 1531m above sea level and receives approximately 692 mm of rainfall annually with an annual average temperature of 15.2 °C (Muthelo et al., 2019). The predominant farming activities are livestock (cattle and sheep) production and to a lesser extent cropping (oil seed crops). The area is characterized by duplex soils of the Valsrivier form, which translate to the chromic luvisol in the word reference base (WRB) for soil resources classification (van Huyssteen, 2020). These soils typically have dark brown to reddish brown topsoil and moderate to strong structured subsoil (Land Type Survey Staff, 1973–2004). The area is dominated by the Highveld grassland vegetation.

Nongoma is one of five towns that constitute the Zululand District Municipality. Annual rainfall in the area is reported to range from 800 to 1000 mm due to variation in topography, and the average temperature ranges between a minimum of 4 °C to a maximum of 20 °C (ZDM, 2018). Soils in the area are of the Swartland form (Protocalcic haplic luvisol in WRB) (van Huyssteen, 2020) with dark brown to grey topsoil transitioning to a moderate to strong blocky structured subsoil on weathering rock (Land Type Survey Staff, 1973–2004). The vegetation is described as the Natal lowveld bushveld. The major agricultural practices in the area livestock and cropping activities mainly beans and maize. The climatic characteristics of the two sites are presented on Table 1.

TABLE 1 Maximum and minimum average Temperature and Rainfall in KZN (2013–2022) and FS (2013–2021).

Month	Kwa-Zulu Natal			Free State		
	Max T (°C)	Min T (°C)	Rain (mm)	Max T (°C)	Min T (°C)	Rain (mm)
Jan	24.8	18.4	67.9	34.7	15.9	77.1
Feb	27.4	20.3	78.7	32.6	15.1	94.7
Mar	27	19.7	102	30.9	12.7	71.1
Apr	25.3	17.2	173	27.1	8.33	60.6
May	24.7	15.4	41.2	25.3	3.13	16.6
Jun	23.6	13.4	7.77	22.3	-1.03	8.24
Jul	22.8	12.5	46.8	22.1	-1.69	12.4
Aug	22.8	13.7	34.1	24.0	0.65	8.69
Sep	20.7	13.6	54.9	26.2	4.82	8.89
Oct	21.4	14.5	103	31.6	9.39	30.3
Nov	21.9	15.4	119	32.8	11.5	59
Dec	23.4	17.2	131	34.2	14.5	87.4
Annual average	23.8	15.9	799	28.7	7.77	401

2.2 Soil sampling and characterization

Soil samples were collected from 0–20 cm depth at each site for initial characterization prior to land preparation. The soil analysis results were used as a guide for fertilizer application recommendations. Soil pH and EC was measured in a 1:1.25 soil: water suspension using a laboratory pH meter and particle size distribution by the hydrometer method (Beretta et al., 2014). Essentially a soil-water-calgon suspension was used to separate particles of different sizes based on their settling velocity as determined by Stoke's law. Total N was determined by dry combustion using a Leco CN-2000 analyzer (Wright and Bailey, 2001).

The 1M neutral ammonium acetate method (Van Reeuwijk, 2002) was used to determine CEC. A 1M ammonium acetate solution (NH4OAc) at pH 7 was used to extract the cations from the soil samples and the solution was shaken for two hours and centrifuged to separate the liquid and solid phases and analysis of cations was done by inductively coupled plasma-optical emission spectrometry (ICP-OES). The CEC was determined as the sum total of the determined cations. The determination of soil organic carbon followed the Walkley-Black method (Wang et al., 2012), where soil organic matter was oxidized with 1N potassium dichromate in the presence of sulphuric acid and the percentage amount of organic carbon calculated from the amount of dichromate remaining after titration with ferrous sulphate.

Selected characteristics of the study soils are shown on Table 2. Soils from both study areas show that the two sites were acidic, with KZN (pH 5.17) being more acidic than FS (pH 5.71) and the EC fell in the range of non-saline in both FS (3.0 mS/m) and KZN (7.79 mS/m) (USDA-NRCS, 2020). The KZN soil had more cations than the soil from FS. Total N was similar at KZN (0.13%) and & FS (0.11%), while the soil in KZN had more P (6.59 mg/kg) than the 2.88 mg/kg P in the FS soil. The soil in FS was sandy loam and had more sand (74%) and less clay (20%) than the soil from KZN, which was sandy clay loam and had 56% sand and 38% clay.

2.3 Agronomic practices

Sorghum was sown in 6 x 4.5 m plots with 1 m spaces between them and 4 m between the tillage systems and 2 m between planting dates resulting in each block being 28 X 24 m or 672 m^2. Each plot consisted of 7 rows with 45 cm inter-row spacing. Mono-Ammonium Phosphate (MAP) was used as the pre-plant fertilizer and Lime Ammonium Nitrate (LAN) as the topdressing fertilizer at 3–4 weeks after planting. MAP was applied at a rate of 15.41 kg/block and LAN at a rate of 11.76 kg/block in FS. In KZN the application rates were 13.44 kg of MAP per block and 10.08 kg of LAN per block. The selective herbicide used was Sorgomil® Gold 600sc at 174.72 ml per block in FS and 201.6 ml per block in KZN, owing to the difference in texture between the 2 sites. Cypermethrin 200 EC was applied for the control of stalk borer at 23.5 ml per block and Decis® forte for the control of armyworm at a rate of 4.032 ml per block. The herbicide SLASH PLUS 540 SL was used at the start of each season for the control of perennial weeds at least 7 days prior to plant at a rate of 83.36 ml per block.

TABLE 2 Initial soil properties of the two study sites.

Soil property	Free State	KwaZulu-Natal
pH H_2O	5.71	5.17
EC (mS/m)	3.05	7.79
Exchangeable Ca (mg/kg)	455	868
Exchangeable Mg (mg/kg)	156	196
Exchangeable K (mg/kg)	217	232
Exchangeable Na (mg/kg)	6.88	14.0
CEC meq/100g	4.13	4.93
OC (%)	0.745	2.37
Total N (%)	0.108	0.154
P (mg/kg)	2.88	6.59
Clay (%)	20	38
Silt (%)	6.0	6.0
Sand (%)	74	56
Texture	Sandy loam	Sandy clay loam

2.4 Experimental set up

The two cultivars of sorghum used in this study were the bronze-grained, medium to late maturing PAN8816, known to be highly resistant to leaf disease and head smut and the early to a medium maturing open-pollinated variety (OPV), Macia. The common characteristic of the cultivars is that they are both low tannin, semi-dwarf hybrids.

The experiment was laid out in completely randomized block design with a strip-strip-split-plot treatment layout (Gomez and Gomez, 1984) and replicated three times. The factors of tillage and planting date were assigned to the vertically and horizontally arranged main strips. The two vertical strips were divided between conventional tillage (CT) and no-till (NT) while the horizontal strips were randomly assigned to early planting (December) and late planting (January). This vertical – horizontal arrangement of the strips resulted in intersection plots, which were split in two for the assignment of the two crop rotations, sorghum –sorghum-sorghum (S-S-S) and sorghum –cowpea-sorghum (S-C-S). The rotations were further split in two to accommodate both cultivars (PAN8816 and Macia) used in the trial. This resulted in 16 treatment combinations that were replicated three times. The field layout is visually depicted in the graphic below (Figure 1), showing a single block out of the total of three.

2.5 Measurements

The following parameters were measured at the end of each season, during the months of May and June in 2021 and 2022, respectively. Plant height was determined at harvest (end of the season) using a graduated rod from the bottom the highest leaf. Plants that fell within the boundaries of a 4 m x 3 m (three middle rows) net plot were harvested at the end of the growing season to determine grain yield. The harvested panicles from each plot were threshed and weighed to determine yield. At the end of the season, leaves were taken from three random plants (same plants as for plant height) for leaf tissue analysis for nitrogen, phosphorus and potassium following the method of Zekri et al (2015).

2.6 Statistical data analysis

The field trial was made up of 2 planting dates, 2 tillage systems, 2 cultivars and 2 rotations making the experiment a 2×2×2×2 factorial. The study was replicated three times and laid out in three blocks. The data from the field trial was subjected to Analysis of variance (ANOVA) and mean separation performed using least significant difference (LSD) at a confidence interval of 95%. The analysis was performed using JMP Pro Version 17 (JMP Statistical Discovery LLC, 2022).

3 Results

3.1 Effects planting date, tillage and sorghum cultivar on plant height in FS

Yield parameters, panicle length and 1000 seed weight did not show significant treatment effects in FS. The three-way interaction effect of planting date, tillage and sorghum cultivar did not have significant effects ($p>0.05$) on plant height in the first season. Only the tillage × cultivar interaction effects, and the main effects of planting date and cultivar significantly ($p<0.05$) affected plant height in FS. Greater plant heights were observed in early (131 cm) than late (110 cm) planted crop, PAN8816 (125 cm) than Macia (116 cm), in the first season, and the trend was the same in the second season. Under NT, Pan8816 had the significantly taller plants than the Macia both when planted early or later (Figure 1), while under CT, the difference was only observed when planted late (Figure 2). The only difference in plant height between the tillage systems was for Macia, when planted early, where CT had taller plants than NT (Figure 2). Under NT, the sorghum-cow pea-sorghum rotation resulted in significantly shorter plants than the sorghum-sorghum-sorghum rotation, under NT but not CT, when planted early but not later (Figure 3). When planted early, there were no significant differences between the rotations irrespective of tillage system (Figure 4). However, when planted late, the sorghum-cow pea-sorghum rotation resulted in significantly taller plants than the sorghum-sorghum-sorghum rotation, for PAN8816 but not Macia (Figure 4).

3.2 Effects planting date, tillage and sorghum cultivar on plant height in KZN

Only the planting date × tillage interaction had a significant effect on plant height in KZN in the first season. There were no significant tillage effects for early planted crops, while for late

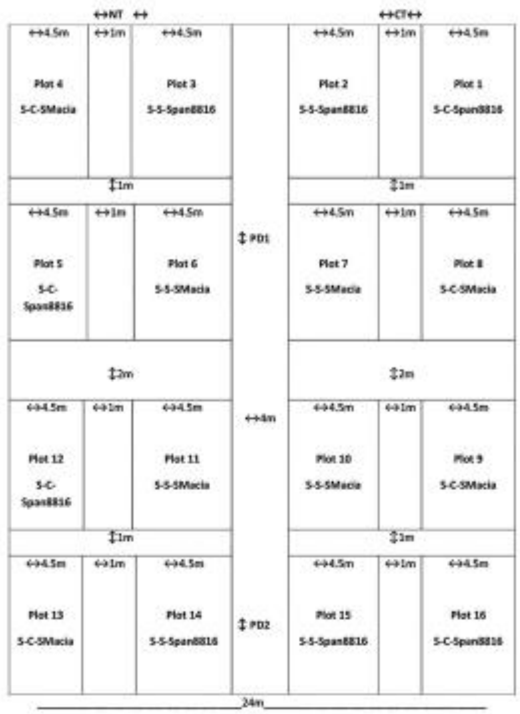

FIGURE 1
Schematic representation of a single block of out of three in the field

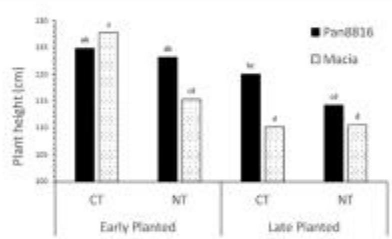

FIGURE 2
Effect of planting date×tillage×cultivar on plant height in FS in season 2021/22. Different letters on the bars indicate significant differences at p < 0.05.

planting, NT resulted in taller plants than CT (Figure 5). Late planting resulted in significantly shorter plants only under CT but not under NT (Figure 5). For the second season (2021/22), the 4-way interaction effects of planting date × tillage × cultivar × rotation were significant ($p<0.05$) on plant height. In the second season, PAN8816 resulted in taller plants than Macia, in the sorghum-cowpea-sorghum rotation, under CT but not under NT, for both planting times (Figure 6). The PAN8816 also had taller plants than Macia, in the sorghum-sorghum-sorghum rotation under NT only when planted early, with no differences for late planting. Overall, the Pan8816 (140 cm) grew significantly taller than Macia (113 cm) in the second season (Figure 6). All the major and interactive treatment effects were not significant for 1000 seed weight and panicle length in both seasons.

3.3 Grain yield and primary nutrient uptake in FS

There were no interactive effects of PD, tillage, cultivar on yield in FS for the first season. The early planted sorghum (1.15 t/ha) had 2.66 times higher yield than late planted (0.434 t/ha) sorghum in the

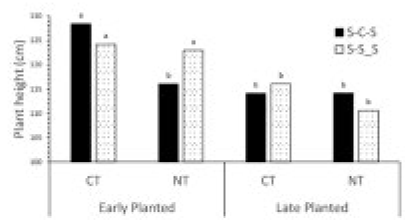

FIGURE 3
Effect of planting date×cultivar×rotation on plant height in in FS in season 2021/22. Different letters on the bars indicate significant differences at $p < 0.05$.

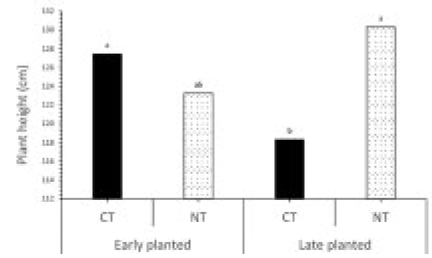

FIGURE 5
Effect of planting date×tillage on plant height in KZN in 2020/21. Different letters on the bars indicate significant differences at $p < 0.05$.

first season. In the second season (2021/22), early planting (3.39 t/ha) resulted in significantly (p<0.05) higher yield than late planting (0.91 t/ha), while plants grown under CT (2.61 t/ha) produced significantly higher yield than under NT (1.70 t/ha) in FS (Figure 7).

Early planting resulted in significantly (p<0.05) higher uptake of all three nutrients (Figure 8), i.e., higher N (16 kg/ha), K (15 kg/ha) and P (3 kg/ha) uptake than late planted crops. There was also a significant effect of cultivar on P uptake where Pan8816 (2.7 kg/ha) took up 1.74 times more than Macia (1.6 kg/ha). All the treatment factors had no significant (p>0.05) effects on the uptake of all the nutrients in KZN for the same season.

Plants grown under CT (17 kg/ha) took up significantly more K than those grown on NT (8 kg/ha) plots in FS, in the second season. Planting date is again the only treatment that has significant effect (P < 0.05) on all the three measured nutrients, in FS, in the 2021/22 season. Early planted sorghum took up significantly more NPK than late planted sorghum (Figure 9).

3.4 Grain yield and primary nutrient uptake in KZN

The interactions and main factor did not significantly affect (p>0.05) sorghum grain yield and uptake of N, P and K in KZN in both seasons, except cultivar in the second season, where Pan8816 (4.40 t/ha) produced 3.3 times higher yield than Macia (1.32 t/ha). Cultivar had a significant effect (p<0.05) of on N, P and K uptake in KZN, for both seasons, with Pan8816 having higher of N (84 kg/ha), K (64 kg/ha) and P (8 kg/ha) uptake when compared to Macia in the second season (Figure 10).

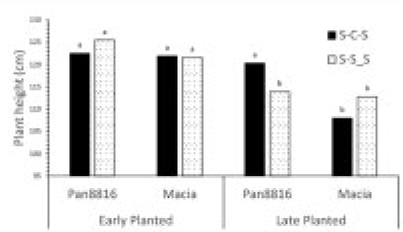

FIGURE 4
Effect of planting date×cultivar×rotation on plant height in FS in Season 2021/22. Different letters on the bars indicate significant differences at $p < 0.05$.

4 Discussion

The planting date, cultivar and planting date × tillage factors were the most influential factors affecting plant height across all treatments. Early planted crops consistently grew taller than their late planted counterparts due to longer growing season with more conducive environmental conditions for growth such as temperature and moisture. Optimal environmental conditions positively influence plant growth (Irawan et al., 2022). This effect was particularly pronounced in the drier FS province. Early planting in FS allowed plants to benefit from availability of more soil moisture for extended periods. Conversely, the absence of significant height differences between early and late planting in KZN province, which generally receives higher rainfall, and a naturally longer growing season supports this observation. Table 1 shows that the annual rainfall of KZN (799 mm) is higher than of FS (401 mm).

Sorghum cultivar also played a role in plant height. Pan8816 consistently produced taller sorghum plants for than Macia in both seasons and locations. This suggests inherent genetic differences among the cultivars (Irawan et al., 2022). Pan8816 is a medium to late maturing hybrid while Macia is early to medium maturing open-pollinated variety (OPV) (Weepener et al., 2014; Hadebe et al., 2017). Pan8816 being a hybrid has been bred to have the best traits for a number of different parent lines while the OPV Macia only have the traits of the parent because the pollination occurs naturally. According to Gosh et al. (2015), differences in plant height among cultivars are due to genetic differences. While planting date and tillage interaction influenced plant height in KZN during the first season, cultivar and the interaction of all treatment factors had significant effects in the second season. This suggests

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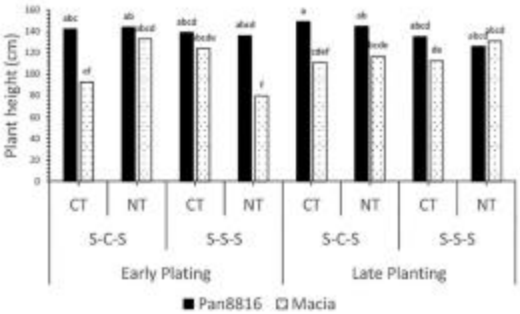

FIGURE 6
Effect of plating date×tillage×cultivar×rotation on plant height in KZN in season 2021/22. Different letters on the bars indicate significant differences at $p < 0.05$.

that selecting the most appropriate cultivar, such as the taller PAN8816 in this study is more critical than time of planting in KZN under a particular set of conditions. Furthermore, regional adaptability also plays a role in the plant height of sorghum crops (Gosh et al., 2015; Sebetha and Modisapudi, 2019).

The higher yield for early planted than late planted crops in Free State in both seasons, is likely due to the longer growing season, which allows for greater biomass accumulation, flowering and grain filling. This aligns with findings by Shoyer et al. (1998) and Hadebe et al. (2017) who reported that planting after the first rains in dry to semi-arid summer grain growing regions results in much greater yields. Sorghum thrives in soil temperatures around 15°C at a depth of 10 cm (Murdy et al., 1994). However, late planting in FS coincides with the occurrence of frost, usually occurring between March and April (Moeletsi et al., 2016). These cooler temperatures impact sorghum emergence and early growth by delaying panicle initiation and flowering, ultimately reducing yield (Emendack et al., 2021). In the current study, the prevailing temperatures in FS fell below the optimal range reported by other researchers (Balota et al., 2010; Emendack et al., 2021) (Table 1). Moreover, sorghum tends to do well in warmer climates (Sebetha and Modisapudi, 2019). The higher yields observed under conventional tillage can be attributed to the relatively short period during which no-till was practised at the site. Findings by Pittelkow et al. (2015) suggested the full benefits of no-till agriculture may take up to four years to be realized. The observed similarity between yield and uptake of N, P and K, suggested that the enhanced uptake of these nutrients under conventional tillage contributed to the greater yield.

Nitrogen is the most limiting nutrient for sorghum production (Teshome et al., 2023). Tillage practices and cultivar selection can significantly affect short-term nitrogen use (Ramadhan and Mushin, 2021). In this study, conventionally tilled plots exhibited higher porosity and lower bulk density compared to NT plots. Another notable observation was the similar N and P uptake patterns across both sites. Similarly, Ortas et al. (1996) found that ammonium-based nitrogen fertilisers like monoammonium phosphate (MAP) used in this study promoted the uptake of phosphorus. Therefore, the MAP used as the pre-planting

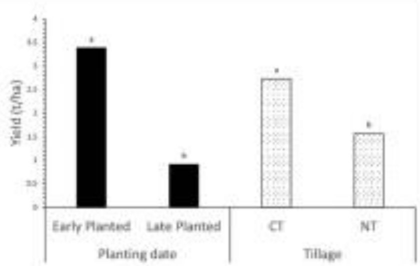

FIGURE 7
Effect of planting date and tillage on yield in FS in Season 2021/22. Different letters on the bars indicate significant differences at $p < 0.05$.

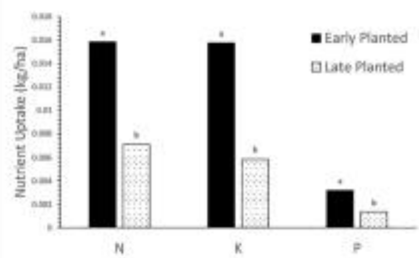

FIGURE 8
Effect of planting date on NPK uptake in FS in 2020/21. Different letters on the bars indicate significant differences at $p < 0.05$.

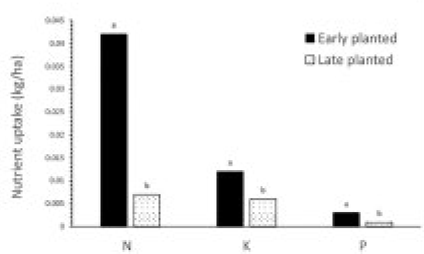

FIGURE 9
Effect of planting date on NPK uptake in FS in 2021/22. Different letters on the bars indicate significant differences at $p < 0.05$.

fertilizer in this study, implies greater availability of phosphorus owing to the ammonia in the MAP.

The significantly higher yield of PAN8816 compared to Macia is likely due to its greater uptake of N, P and K. This enhanced uptake could be attributed to inherent genetic differences between the cultivars. In KZN, cultivar was the most prominent factor influencing yield. Pan8816, a hybrid, has been demonstrated to have small canopy size, which allows it to maintain a higher chlorophyll content (Manyathi, 2014), which in turn translates to increased levels of photosynthesis ultimately supporting higher yields. The lack of tillage, time of planting and rotation effects in KZN could be due to the higher rainfall received in the region. This translates to greater available moisture throughout a longer growing season compared to FS.

A drawback of this short-term study is the potential for increased competition between sorghum crops and weeds for vital resources such as nutrients and moisture (Giller et al., 2009). Despite applications of pre-planting and post-planting herbicides, significant weed presence was observed at both sites. Short-term studies can also be susceptible to challenges like poor germination, higher prevalence of crop pests and nutrient immobilization (Giller et al., 2009). These challenges arise due to the complex interaction

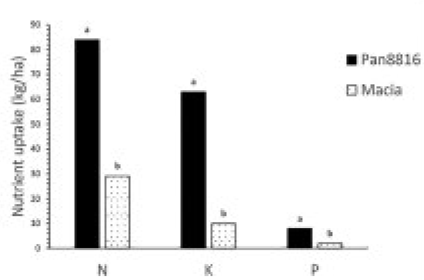

FIGURE 10
Effect of cultivar on N, P and K uptake in KZN in 2021/22. Different letters on the bars indicate significant differences at $p < 0.05$.

between crop requirements, climate and soil characteristics. For instance, the presence of plant residues can cause temporary nutrient immobilization by soil microbes especially when the climatic conditions allow (Dubeux and Sollenberger, 2020).

5 Conclusion

This study identified key factors influencing sorghum performance in some parts of FS and KZN. In FS, planting date and cultivar, significantly influenced plant height, yield and nutrient uptake. Early planting and selection on PAN8816 cultivar is the most favorable combination to avoid frost and maximize yield. In KZN, cultivar was the primary factor, influencing yield, with Pan8816 outperforming Macia. However, planting date had minimal impacts in KZN, suggesting a greater flexibility for farmers. Therefore, farmers at the KZN site can plant sorghum across the tested dates without significant yield penalties. Early planting is recommended for FS to avoid the frost season. Additionally, PAN8816 is recommended at both sites, especially under conventional tillage. However, longer-term studies are necessary to determine the influence of no-till practices, as the benefits may not be realized within the two-season timeframe of this study.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

KM: Data curation, Formal Analysis, Investigation, Methodology, Software, Writing – original draft. AN: Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Validation, Writing – review & editing. PM: Conceptualization, Investigation, Project administration, Resources, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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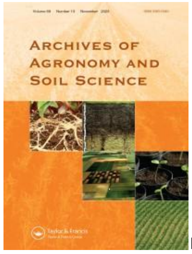

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Seasonal and management effects on soil microbial activity and functional diversity in sorghum-based production systems in South Africa

Kaya Mrubata[a,b], Adornis Dakarai Nciizah[a,c] and Pardon Muchaonyerwa[b]

[a]Agricultural Research Council - Natural Resources & Engineering, Pretoria, South Africa; [b]School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Pietermaritzburg, South Africa; [c]Department of Agriculture and Animal Health, University of South Africa, Roodepoort, South Africa

ABSTRACT

Microbial properties affect soil productivity and quality depending on environmental and management factors. This study aimed to determine the influence of management practices, climate and soil factors on microbial biomass carbon, functional diversity and enzyme activity in two soils under sorghum production in South Africa. Two planting dates, two tillage systems and two seasons resulting in a randomized complete block design with a 2 × 2 × 2 strip-split-plot design. Season 1 had significantly higher phosphomonoesterase activity than season 2. In FS, late-planted sorghum in season one had 3.8 times higher MBC than late-planted sorghum in season two, under CT. In KZN, NT had 7% higher MBC than that in CT. The average well colour development (AWCD) was higher for CT and season one than NT and season two in KZN, respectively. Early planting × NT had higher AWCD than late planting × NT. Late planted sorghum under both tillage systems and early-planted sorghum under NT had higher utilization of carbohydrates and polymers than early-planted sorghum under CT in FS. In KZN, during season two, microbes utilized carboxylic, amino acids and amines than in season one. The findings indicate that PD, tillage and season affect soil microbial properties under sorghum.

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KEYWORDS
Enzyme activity; microbial biomass; microbial functional diversity; nutrient cycling; soil health indicators

Introduction

Challenges associated with agricultural management, climate change and environmental factors necessitate the adoption of resilient crops like sorghum. Sorghum (*Sorghum bicolor* [L.] Moench) is increasingly becoming an important crop in addressing climate change owing to its tolerance to drought, and multiple uses including biofuel feedstock, animal feed and phytoremediation of polluted soils. Epelde et al. (2009) showed that sorghum can ameliorate soil pollution while maintaining high biomass yield. This crop has shown significant resistance to biotic and abiotic stress (Khalifa and Eltahir 2023). Dunjana et al. (2022) reported that heat and moisture stress, brought about by climate change result in reduced yields. However, there is variation of yield and tolerance to stresses in different genotypes under different climates (Pfeiffer et al. 2019). In order to ensure successful adoption of sorghum by smallholder farmers empirical studies showing its productivity under various climates and soils are needed.

Soil health is declining at alarming rates, with nearly one-third of global soils experiencing some form of degradation (Wipf et al. 2021). The productivity, sustainability and resilience of agricultural production rests upon maintaining high-quality soils. According to Jat et al. (2021), climate smart agriculture holds the key to increased productivity levels. Practices such as reduced and/or no-till improve soil organic carbon levels, lower soil temperature fluctuations, increase nutrient levels (Jat et al. 2019; Kruat-Cohen et al. 2020 and Wipf etal. 2021).

Practices that maintain diversified cropping systems (Habig and Swanepoel 2015), integrate efficient irrigation practices and soil fertility (Jat et al. 2021), and improve soil organic matter and microbial activity (Pratibha et al. 2023) are necessary for agricultural sustainability, especially in view of climate change and declining soil fertility. Enhancing overall soil microbial activity is essential in maintaining high-quality soils

CONTACT Kaya Mrubata mrubatak@arc.agric.za; 221120087@stu.ukzn.ac.za Agricultural Research Council - Natural Resources & Engineering, Pretoria, South Africa

(Nazir 2017; Yu et al. 2024). Increased acid phosphatase activity was found to be higher after application of NPK fertilizer and farmyard manure than only NPK fertilizer application in a sorghum system (Venugopal et al. 2015). The authors attributed the difference to the added organic matter, which increased organic carbon and nitrogen levels.

Plants and soil microbes share a close relationship that is important both for protection from disease and provision of nutrients (Yuan et al. 2015). This relationship also extends to soil microbial influence on food production and environmental processes (Adetunji et al. 2017). The associations between soil microbes and rhizosphere, which are driven by root exudates, enhance nutrient cycling, plant growth stimulation and resistance to diseases. These interactions subsequently improve soil quality, resulting in healthier and higher yielding crops (Habig and Swanepoel 2015). However, the composition and activity of the microbial community is highly sensitive to changes in the soil environment. The characteristics of the rhizosphere are shaped by soil physico-chemical and biological properties, plant genetics and management practices, like tillage and residue management (Yuan et al. 2015).

Soil biological properties are the most sensitive indicators for assessing the short-term impact of management practices such as tillage and residue management (Janusauskaite et al. 2013; Chandra et al. 2023). The composition of the microbial community is important for environmental services because its activity influences the rate of breakdown of soil organic matter and nutrient turnover (Mir et al. 2023). Microorganisms play a key role in agriculture by improving soil quality, which ultimately enhances productivity. However, management practices such as tillage can influence microbial biomass and soil organic carbon turnover, which in turn affect enzyme activity (Delgado et al. 2012). No-till has been reported to increase enzyme activities compared with conventional tillage (Balota et al. 2011).

Soil enzymes, particularly hydrolases, are key indicators of soil fertility as they play a crucial role in the transformation of nutrient elements (Roldan et al. 2005). Examples include protease, phosphatase, urease and glucosidase. Type of plant species and its age influence enzyme activity (Rejšek et al. 2012; Venugopal et al. 2015). For example, acid phosphatase activity usually increases in the rhizosphere of crops such as wheat, onion, tomato and sorghum (Sharma et al. 2023) where it enhances the availability of phosphorus (Rejšek et al. 2012). Optimum levels of P support early plant growth and maturity (Acosta-Martines et al. 2011) and its transformation from soil organic matter into available forms depends on Margalef et al. 2017). Plants directly affect soil enzyme activity through root secretions of enzymes (Bais et al. 2004), and indirectly by stimulating microbial growth via root exudates containing sugars, amino acids, organic acids, hormones and vitamins (Bais et al. 2004; Dlugosz et al 2022). Jat et al. (2021) observed higher activities of dehydrogenase, urease, protease, phosphatase and ß-glucosidase activities under no-till compared to conventionally tilled soil under sorghum cultivation.

In South Africa and sub-Saharan Africa (SSA) numerous studies have focused on sorghum as a crop for human consumption, animal feed and fuel production (Weepener et al. 2014; Hadebe et al. 2017,; Ikazaki et al. 2020; Teshome et al. 2023). The studies explore sorghum production in various ways in order to maximise benefits for both the environment and humanity. The success of these efforts largely depends on selecting appropriate cultivars and adopting suitable management practices that enhance soil physico-chemical and biological properties under diverse climatic and soil conditions. However, there are limited studies on the response of soil in sorghum-based conservation agriculture systems. Monitoring soil microbial activity is essential to detect short-term changes in soil quality. Furthermore, such data is most urgently in need for smallholder farmers in SSA because they lack the inputs to elevate their production. Practices like no-till offer cost-effective albeit labour-intensive alternative and shifting plating dates according to variation in climate can minimize losses due to crop failure. Crop rotations and intercropping can minimize fertilizer cost and also improve the diets of communities vulnerable poverty.

The objective of this study was to determine the effects of tillage and planting date on microbial biomass carbon and functional diversity (using community level physiological profile) and enzyme activity (ß-glucosidase, acid phosphomonoesterase), of two sorghum cultivars under contrasting climatic and soil conditions in South Africa.

Materials and methods

Site Description

The study sites were located in the villages of Houtnek (−29.0264; 26.8014) in Thaba Nchu, Free State and Qongqo (−28.0434; 31.6183) in Nongoma, Kwa-Zulu Natal provinces, South Africa (Figure 1). The prevailing properties in the two study sites are detailed in Mrubata et al. (2024) including the climatic (Table 1) and initial soil characteristics (Table 2).

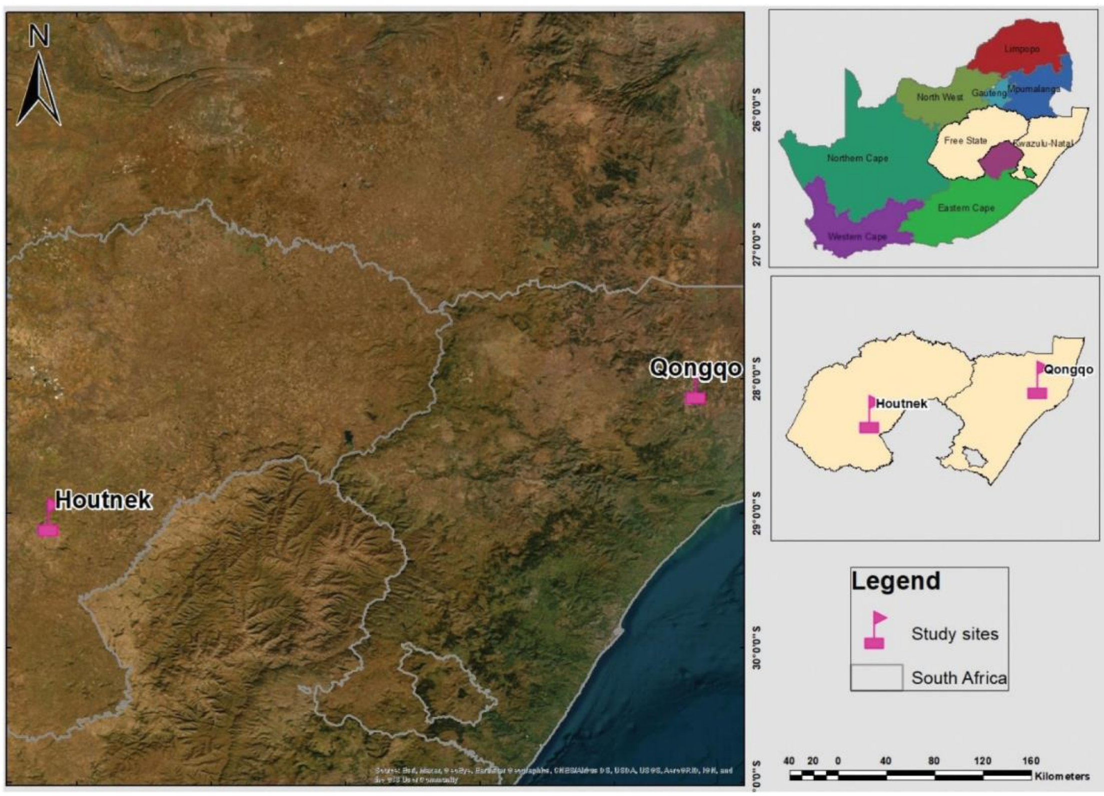

Figure 1. Map showing the study sites within Free State and KwaZulu Natal provinces of South Africa.

Table 1. Maximum and minimum average temperature and rainfall in KZN (2013–2022) and FS (2013–2021).

	Kwa-Zulu Natal			Free State		
Month	Max T (°C)	Min T (°C)	Rain (mm)	Max T (°C)	Min T (°C)	Rain (mm)
Jan	24.76	18.37	67.92	34.66	15.88	77.05
Feb	27.35	20.27	78.72	32.59	15.09	94.69
Mar	27.00	19.69	101.68	30.91	12.67	71.07
Apr	25.25	17.23	172.59	27.14	8.33	60.57
May	24.69	15.41	41.15	25.28	3.13	16.62
Jun	23.57	13.38	7.77	22.29	−1.03	8.24
Jul	22.81	12.49	46.84	22.06	−1.69	12.39
Aug	22.82	13.70	34.14	24.03	0.65	8.69
Sep	20.71	13.62	54.89	26.22	4.82	8.89
Oct	21.40	14.52	103.15	31.63	9.39	30.31
Nov	21.89	15.43	118.67	32.81	11.53	58.98
Dec	23.44	17.16	131.34	34.24	14.49	87.35
Annual average	23.81	15.94	799.04	28.65	7.77	401.13

Source: Mrubata et al. (2024).

Table 2. Initial soil properties of the two study sites.

Soil property	Free State	KwaZulu-Natal
pH H_2O	5.71	5.17
EC (mS m^{-1})	3.05	7.79
Exchangeable Ca (mg kg^{-1})	455	868
Exchangeable Mg (mg kg^{-1})	156	196
Exchangeable K (mg kg^{-1})	217	232
Exchangeable Na (mg kg^{-1})	6.88	14.0
CEC meq 100 g^{-1}	4.13	4.93
OC (%)	0.745	2.37
Total N (%)	0.108	0.134
P (mg kg^{-1})	2.88	6.59
Clay (%)	20	38
Silt (%)	6.0	6.0
Sand (%)	74	56
Texture	Sandy loam	Sandy clay loam

Source: Mrubata et al. (2024).

Initial soil characterization

Soil samples were collected from the 0–20 cm depth at each site for initial characterization using standard methods. Soil pH and EC were determined in a 1:1.25 soil: water suspension, SOC with the Walkley-black and cations using the 1 molar ammonium acetate, method respectively. Total N was measured with the Leco CN-200 Analyzer, available P using the Bray 1 method, and soil texture by the hydrometer method, respectively. A detailed description of the analytical methods is available in Mrubata et al. (2024).

Field trial

The study was conducted in a sorghum field trial comprising two planting dates, two tillage systems, two cultivars and two rotations as is described by Mrubata et al. (2024) (Figure 2). The design is a strip-strip-plot with two tillage systems in the vertical strips, two planting dates in the horizontal strips. Planting in December was designated as early and planting in January was designated as late.

Soil sampling and microbial analysis

Soil sampling

Soil samples (0–20 cm depth) were collected at the end of each season as composite samples. Three samples per plot (4.5 × 6 m) were taken from four plots and combined into one composite sample, yielding four samples per block (colour coded in Figure 2) for 12 samples per site. This sampling strategy resulted in samples from the strip plots of tillage and planting date and cultivar and rotation a significant effect owing to the short-term of the application of the treatments. The samples were transported to the laboratory in a cooler box with ice on the day of sampling and stored at 4°C, analysis was done within two days (Xu et al. 2015).

Microbial biomass carbon (MBC)

The chloroform fumigation-extraction method described by FAO (2024) was used to determine MBC. 35 g of water-saturated soil was weighed in to 250 ml glass bottles, mixed with 2 ml chloroform, and placed inside 10 l desiccators with 50 ml glass beakers containing 25 ml ethanol-free chloroform. The samples were then incubated for 24 hours in the dark at 25°C after which the chloroform was allowed to evaporate for 30 min. After fumigation the soils were extracted with 140 ml of 0.5 m K_2SO_4 at 1:4 w/v for 1 hour using an end-to-end shaker at 35 rpm and filtered through Whatman no. 42 filter paper. The unfumigated soil was extracted following the same procedure. Carbon was determined form the soil extracts following the Walkley-black procedure.

Community level physiological profile (functional diversity)

The method of Galazka and Furtak (2019) was followed to determine the functional diversity of the microbial communities of the soil samples. A 1 g sample of soil was suspended in 99 ml of sterile solution, shaken for

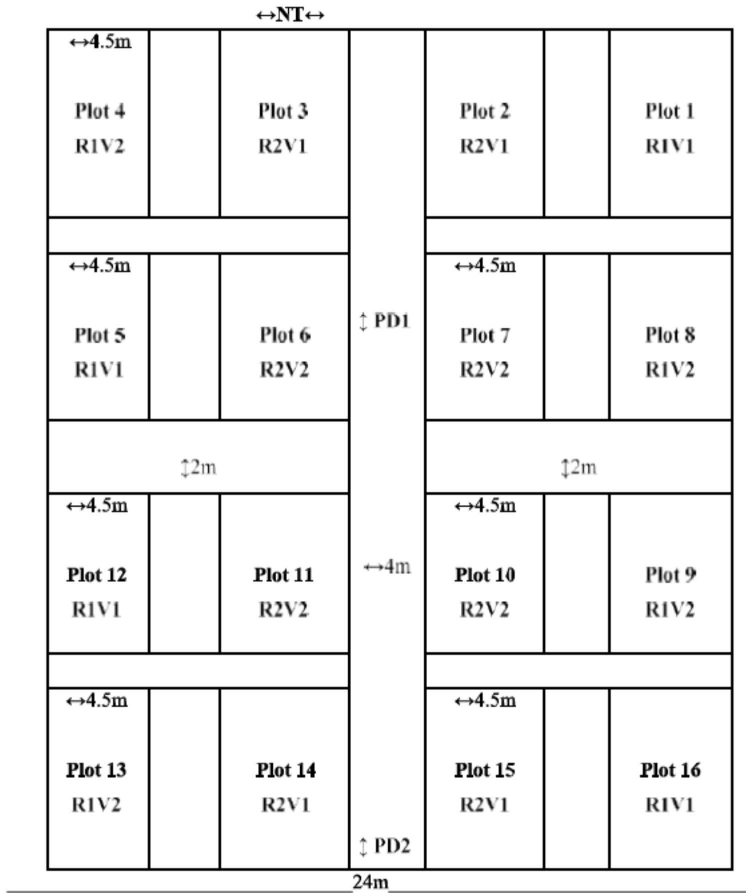

Figure 2. Field map showing 1/3 blocks with dimensions and treatments per plot. Where: CT = Conventional tillage; NT = No-till; PD = Planting date; V1 = PAN8816; V2 = MACIA; R1 = S-C-S; R2 = S-S-S. Source: Mrubata et al. 2024.

20 minutes at 15°C, and then incubated at 4°C for 30 minutes. A 150 µl volume of the suspension was then inoculated on 96 well biolog microplates and the plates were incubated at 26°C for one week. The rate of colour change in each well was recorded and the Optical Density (OD) of each well was read on Biolog MicroLog™3E or MicroStation™ systems every 24 hours for 7 days until the values became constant and the last reading was taken for statistical analysis. Data from each plate reading were normalized by subtracting the Blank (Control) from each plate well.

Enzyme activity

Acid phosphatase. The method described by Acosta-Martines et al. (2011)was used to determine the phosphatase activity. A 1 g sample of soil was placed in a flask before adding 0.2 ml toluene, 4 ml modified universal buffer (MUB at pH 6.5) and 1 ml of p-nitrophenol phosphate solution. The mixture was then incubated at 37°C for 1 hour, and 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH were added. The suspension was then filtered, and the intensity of the yellow colour was measured with a spectrophotometer at 400-t0 410 nm.

β-glucosidase. A similar method to that of acid phosphatase was used in the determination of β-glucosidase with a few differences. Firstly, the buffer (MUB) was pH 6 and after incubation 0.1 M Tris-hydroxymethyl amino methane (THAM) buffer at pH 12 was added instead of 4 ml of 0.5 M NaOH prior to filtration (Deng et al. 2011).

Statistical analysis

The data on microbial biomass carbon and enzyme activity for each site was subjected to analysis of variance (ANOVA) with a randomized complete block design (RCBD) based on a 2 × 2 × 2 factorial treatment structure to determine effects of planting date, tillage and season on the MBC, functional diversity and enzyme activity from two contrasting climates. The statistical analysis was performed using JMP Pro 17 (JMP Statistical Discovery LLC 2022). The post hoc tests were performed using Fischer's least significant differences (LSD) because it offers power and protection when making a few comparisons and the risk of a false positive is relatively low. Principal Component Analysis (PCA) analyzed the data for substrate utilization at 72 h after correction for control absorbance using the JMP. Substrate utilization data were also used to calculate Average Well Color Development (AWCD) and Shannon Weaver Index (H) (Gomez et al., 2006). The AWCD and H for substrate utilization data were subjected to analysis of variance (ANOVA) and the means were separated using the least Significant Differences (LSD) at $p < 0.05$.

Results

Effect of planting date, tillage and season on enzyme activity and microbial biomass carbon

The three-way interaction between planting date, tillage, and season had a significant effect on MBC (Table 3). In FS, CT × late planting × season 2020/21 resulted in significantly ($p < 0.05$) higher MBC (155 µg C/g of soil) than to NT × late planting × season 2021/22, NT × early/late planting × season 2021/22 (Figure 3). There were no main or

Table 3. Effect of planting date, tillage and season on enzyme activity and microbial biomass carbon of soil from FS.

		p-Value		
Treatment	Df	Microbial biomass C	β-glucosidase	phosphomonoesterase
PD	1	0.6995	0.7383	0.5677
Tillage	1	0.2103	0.3601	0.4324
Season	1	0.0628	0.0507	**0.0491***
PD × tillage	1	0.535	0.6736	0.5774
PD × season	1	**0.0227***	0.6844	0.6434
Tillage × season	1	0.9751	0.2802	0.1692
PD × tillage × season	1	**0.0443***	0.7495	0.248

*on bold values indicates a significant treatment effect at $p < 0.05$.

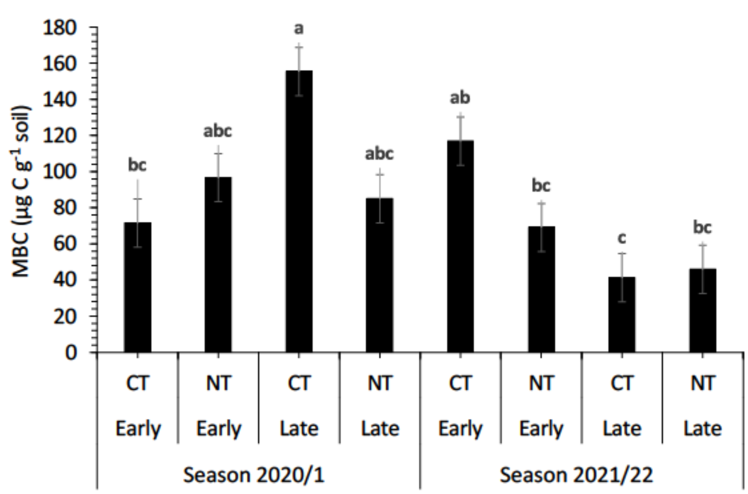

Figure 3. Effect of planting date × tillage × time on MBC in FS soils. Lowercase letters on the bars indicate significant difference at $p < 0.05$. MBC = microbial biomass carbon, CT = conventional tillage, NT = no-till.

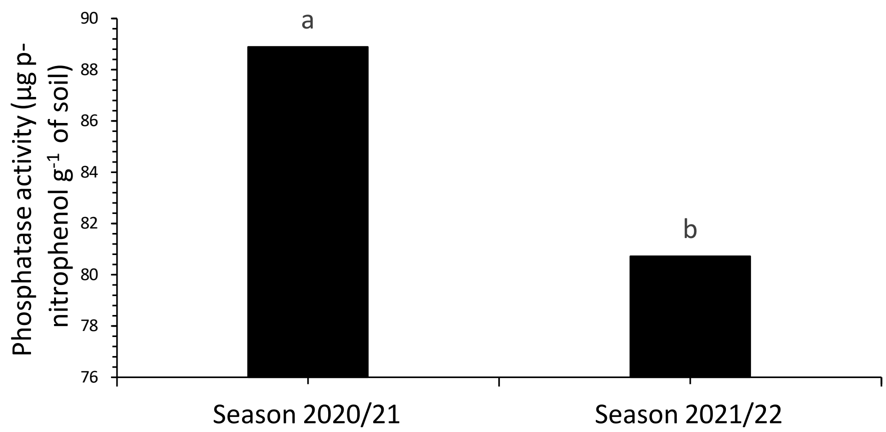

Figure 4. Effect of season on acid phosphatase in FS soil. Lowercase letters on the bars indicate significant difference at $p < 0.05$.

interactive effects on β-glucosidase and only season had a significant effect on phosphomonoesterase activity (Table 3). Season one had significantly higher acid phosphatase (9.63% higher) than to season (Figure 4).

In KZN, only tillage and season had significant effects on MBC and acid phosphatase activity respectively, with no treatment effects on β-glucosidase (Table 4). NT soils had significantly higher ($p < 0.05$) MBC of 63.71 µg C g^{-1} than CT soils (59.54 µg C g^{-1}) (Figure 5). Season 1 resulted in significantly higher phosphomonoesterase activity than season 2 (Figure 6).

Table 4. Effect of planting date, tillage and season on enzyme activity and microbial biomass carbon of soil from KZN.

		p-Value		
Treatment	Df	Microbial biomass C	β-glucosidase	phosphomonoesterase
PD	1	0.9333	0.3929	0.1786
Tillage	1	0.0260*	0.2305	0.2514
Season	1	0.7139	0.5859	0.0022*
PD × tillage	1	0.5549	0.8371	0.2689
PD × season	1	0.5663	0.5233	0.2979
Tillage × season	1	0.8775	0.1079	0.3603
PD × tillage × season	1	0.6185	0.5987	0.3688

*on bold values indicates a significant treatment effect at $p < 0.05$.

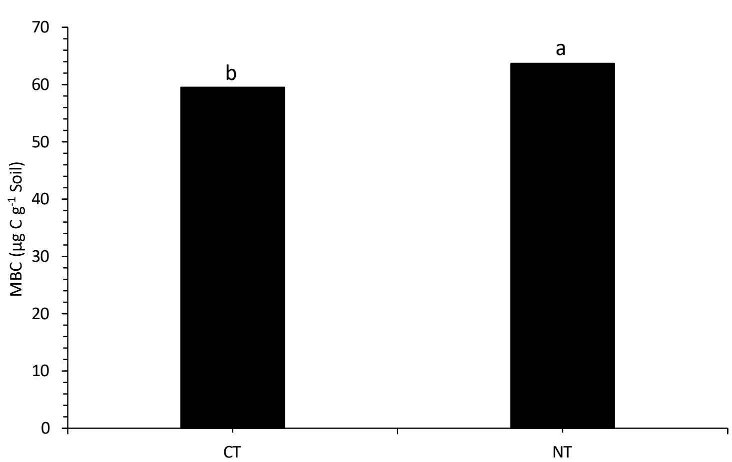

Figure 5. Effect of tillage on MBC in KZN soils. Lowercase letters on the bars indicate significant difference at $p < 0.05$. MBC = microbial biomass carbon, CT = conventional tillage, NT = no-till.

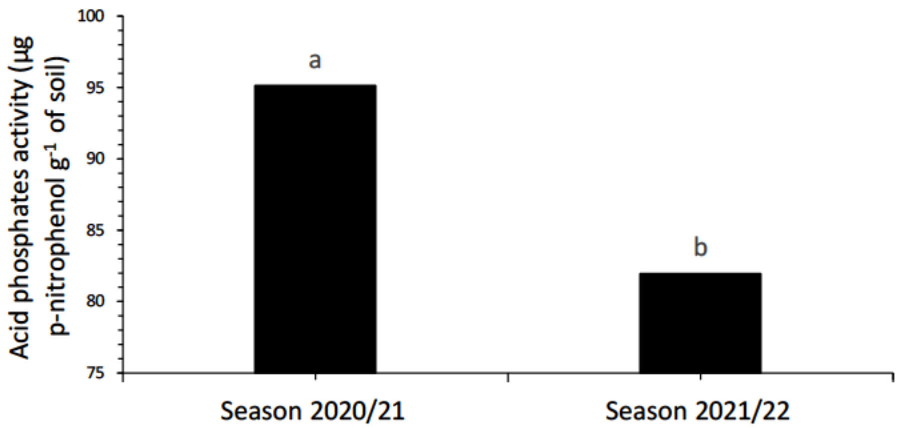

Figure 6. Effect of season on acid phosphatase in KZN soil. Lowercase letters on the bars indicate significant difference at $p < 0.05$.

Effect of planting date, tillage and season on microbial functional diversity

Planting date × tillage in FS and main effects of tillage and season showed a significant effect on AWCD and season on Shannon-Weaver index in KZN (Table 5). In FS, planting early under NT resulted in significantly ($p < 0.05$) higher AWCD compared to late planting under NT while other interactions resulted in comparable results (Figure 7). In KZN, CT and season 2020/21 resulted in significantly ($p < 0.05$) higher AWCD compared

Table 5. Effect of planting date, tillage and season on the AWCD and Shannon weaver index in both Free State and KwaZulu Natal sites.

		FS		KZN	
		p- Value			
Treatment	Df	AWCD	Shannon-Weaver index (H)	AWCD	Shannon-Weaver index (H)
PD	1	0.209	0.301	0.414	0.2166
Tillage	1	0.591	0.366	0.049*	0.1508
Season	1	0.236	0.213	0.007*	0.013*
PD × tillage	1	0.044*	0.063	0.349	0.729
PD × season	1	0.823	0.397	0.221	0.061
Tillage × Season	1	0.697	0.382	0.080	0.164
PD × tillage × season	1	0.344	0.118	0.166	0.730

*on bold values indicates a significant treatment effect at $p < 0.05$.

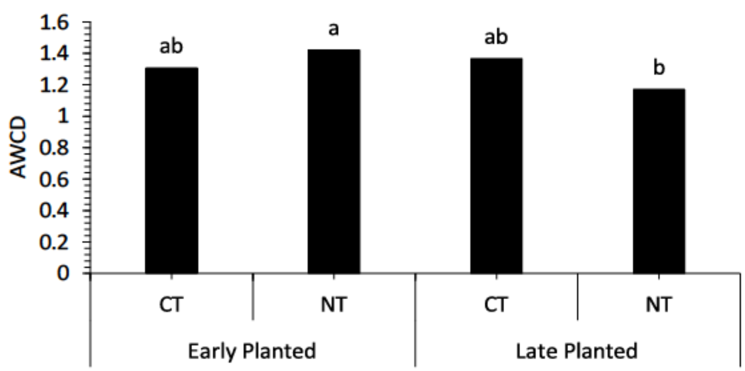

Figure 7. Effect of planting date × tillage on AWCD in FS. Lowercase letters on bars indicate significant difference at $p < 0.05$. AWCD = average well colour development, CT = Conventional tillage, NT = No-till.

NT and season 2021/22 (Figure 8). The Shannon-Weaver Index was significantly higher ($p < 0.05$) in season 2020/21 compared to season 2021/22 in KZN (Figure 9).

The PCA results show that all measured parameters are explained by component 1 at both sites (Figure 10 and 11). In FS AWCD, is positively correlated to carboxylic acids (CA), amino acid (AA) and amines (A) while the Shannon-Weaver index (H) is correlated with carbohydrates (CH), polymers (P) and species evenness (E) and richness (S). In KZN Shannon-Weaver index (H) is correlated with CA, CH and S while AWCD is correlated to AA, A, and E.

Planting date, PD × tillage significantly the utilization of carbohydrates and polymers. Tillage × season influence the utilization of all the carbon sources in FS (Table 6). In late-planted soils, the microbes utilized 15.67% more carbohydrates than in early-planted soils (Figure 12). Early-planted soils utilized less carbohydrate and polymers, than the other PD × tillage treatment combinations (Figure 13). NT × season 2021/22 had higher utilization of carbon sources than all the treatment combinations except for carbohydrates under CT × season 2020/21 (Figure 14).

There were significant ($p < 0.05$) seasonal effects on the utilization of carboxylic acids, amino acids and amines while the interaction between tillage and season significantly ($p < 0.05$) affected the utilization of amino acids in KZN (Table 7). Microbes utilized more carboxylic acids, amino acids and amines as a carbon source compared in season 2021/21 than season 2020/21 (Figure 15). In contrast, soil microbes under NT in the first season used significantly less amino acids than the rest of tillage × season interactions (Figure 16).

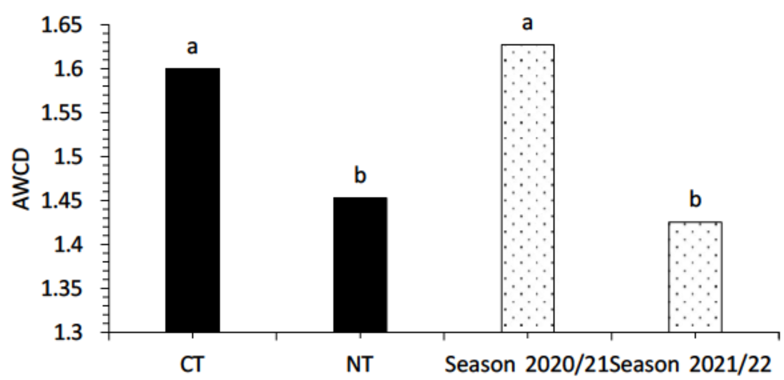

Figure 8. Effect of planting date and season on AWCD in KZN. Lowercase letters on bars indicate significant difference at $p < 0.05$. AWCD = average well colour development, CT = Conventional tillage, NT = No-till.

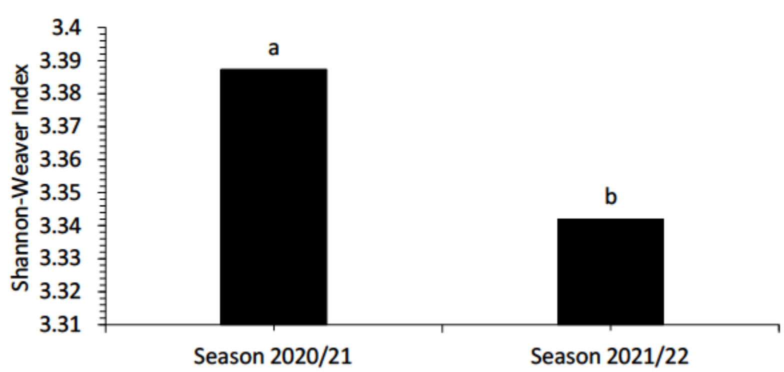

Figure 9. Effect of season on the Shannon weaver index in KZN. Lowercase letters on bars indicate significant difference at $p < 0.05$.

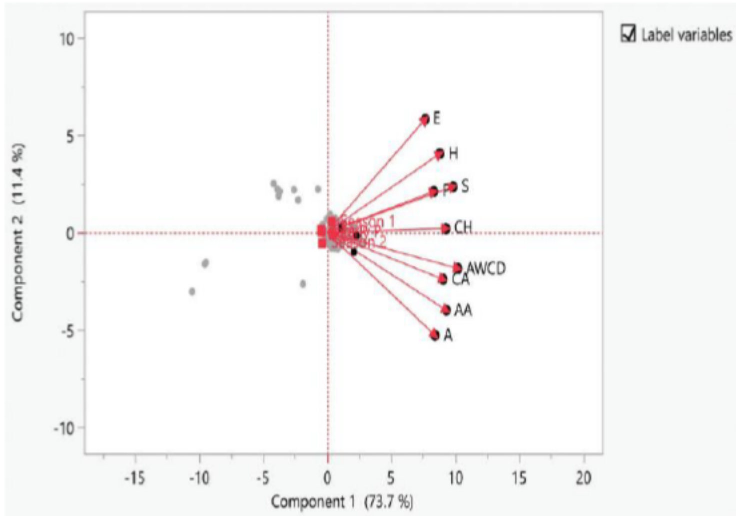

Figure 10. Effect of planting date on use of carbohydrates in FS soil. Lowercase letters on the bars indicate significant difference at $p < 0.05$.

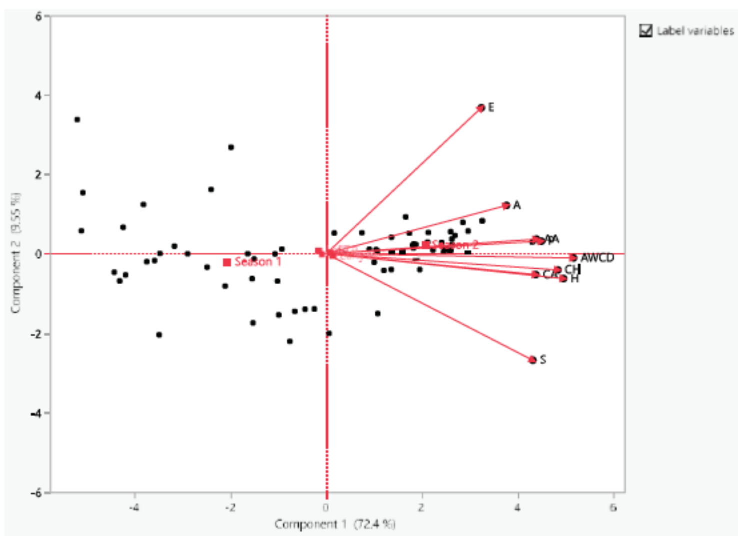

Figure 11. Effects of planting date and tillage on utilization of carbohydrates and polymers in FS soil. Lowercase letters on the bars indicate significant difference at $p < 0.05$. CT = conventional tillage, NT = No-till.

Table 6. Effect of planting date, tillage and season on the carbon source utilization of microbes of soil from FS.

Treatment	Df	Carbohydrates	Carboxylic acids	Amino Acids	Polymers	Amines
		p-Value				
PD	1	0.0235*	0.3516	0.1467	0.0036*	0.0574
Tillage	1	0.4173	0.0614	0.1557	0.4658	0.1413
Season	1	0.2526	0.3228	0.0667	0.0545	0.0718
PD × tillage	1	0.0069*	0.4077	0.1508	0.0254*	0.1312
PD × season	1	0.8505	0.4874	0.3019	0.6287	0.7353
Tillage × season	1	0.0353*	0.0061*	0.0145*	0.0277*	**0.043***
PD × tillage × season	1	0.4647	0.6894	0.2386	0.5347	0.4413

*on bold values indicates a significant treatment effect at $p < 0.05$.

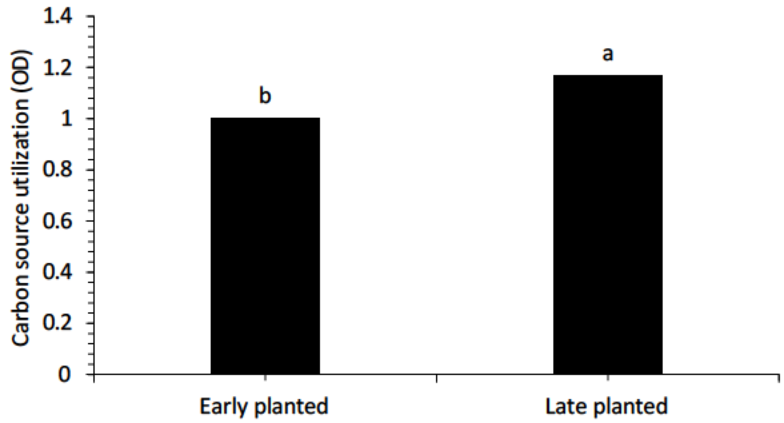

Figure 12. Effect of tillage and season on the utilization of carbohydrates, amino acids and polymers by FS. Lowercase letters on the bars indicate significant difference at $p < 0.05$. CT = conventional tillage, NT = No-till.

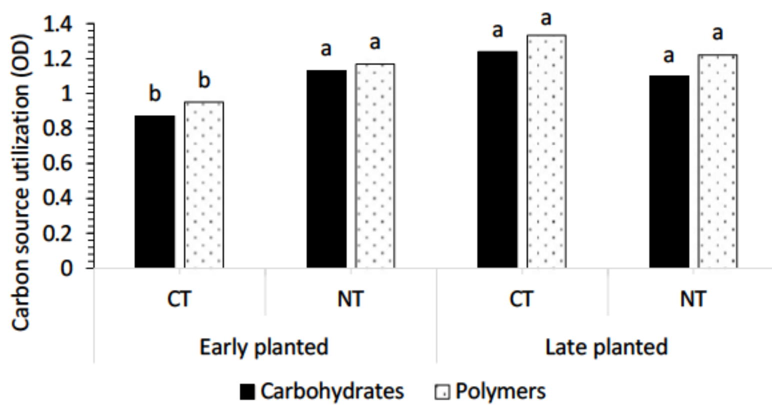

Figure 13. Effect of season on the carbon source utilization of microbes of soil from KZN. Lowercase letters on the bars indicate significant difference at $p < 0.05$.

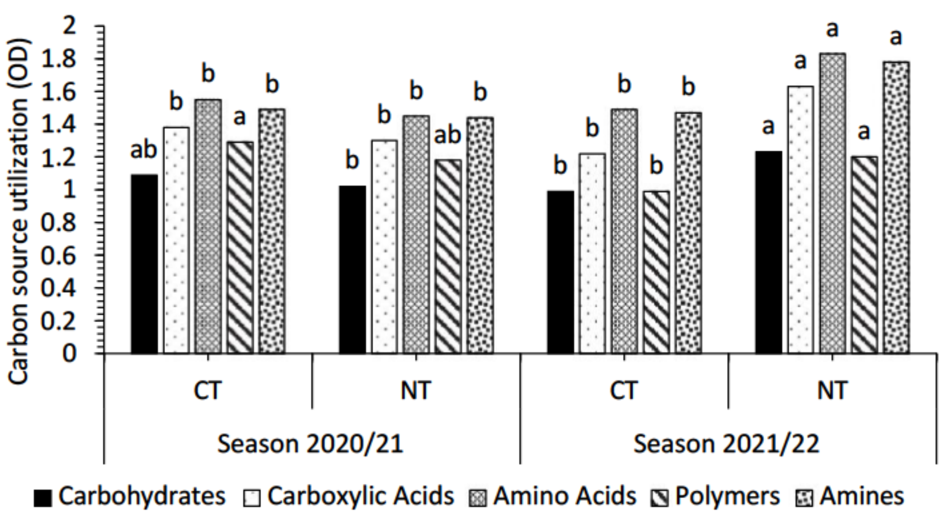

Figure 14. Effect of tillage × season on utilization of amino acids in KZN soil. Lowercase letters on the bars indicate significant difference at $p < 0.05$. CT= conventional tillage, NT= No-till.

Table 7. Effect of planting date, tillage and season on the carbon source utilization of microbes of soil from KZN.

Treatment	Df	Carbohydrates	Carboxylic acids	Amino Acids	Polymers	Amines
		p-Value				
PD	1	0.7965	0.4574	0.2662	0.1304	0.569
Tillage	1	0.2716	0.8119	0.1957	0.8928	0.1498
Season	1	0.4676	0.0002*	0.0082*	0.1401	0.0013*
PD × tillage	1	0.5662	0.1811	0.5212	0.351	0.7661
PD × season	1	0.5767	0.7535	0.4302	0.924	0.0998
Tillage × season	1	0.8113	0.574	0.0297*	0.1458	0.1074
PD × tillage × season	1	0.0508	0.142	0.4829	0.677	0.7188

*on bold values indicates a significant treatment effect at $p < 0.05$.

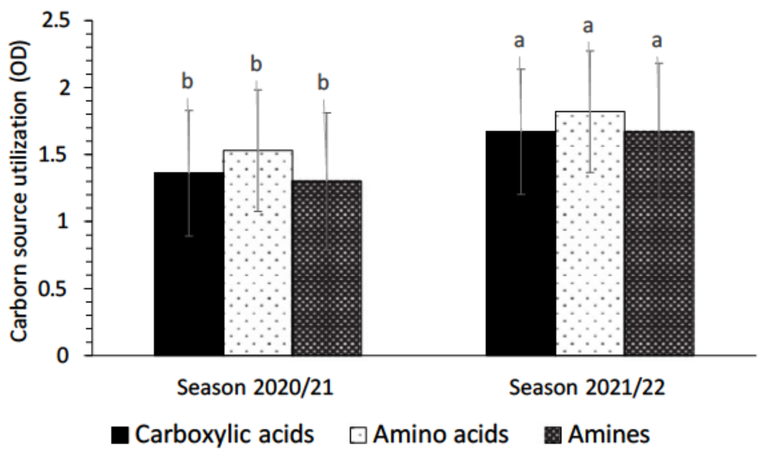

Figure 15. Effect of season on the carbon source utilization of microbes of soil from KZN. Lowercase letters on the bars indicate significant difference at $p < 0.05$.

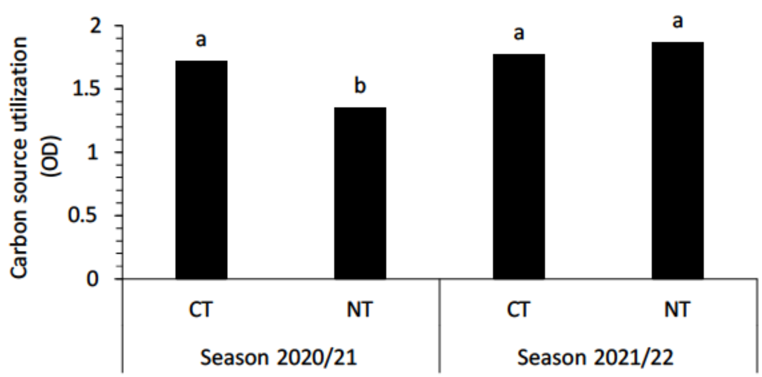

Figure 16. Effect of tillage × season on utilization of amino acids in KZN soil. Lowercase letters on the bars indicate significant difference at $p < 0.05$. CT= conventional tillage, NT= No-till.

Discussion

The significant interaction effects of planting date × tillage × season on MBC in FS but not in KZN highlight the influence of site-specific characteristics on MBC. The absence of significant differences between NT treatments across seasons and planting times suggests that NT stabilises MBC. The higher MBC in the first season compared to the second season under late planting may be attributed to the drier conditions at the time of sampling in the second season. According to Moeletsi et al. (2022), the area received, 101.7 mm of rain in 2022 compared to 134.2 mm in 2021. The higher MBC suggests greater nutrient cycling from the soil organic matter under these conditions. These effects were more evident under CT than NT. The higher MBC in season one and lower in season two, under late-planted sorghum with CT than early planting suggests that under CT, MBC is more sensitive to quality of the season than NT. These differences under CT could be due to the variation in rainfall received between the two seasons, while NT known for enhancing moisture retention, was less affected. This suggests that under CT, the timing of planting has a significant effect on activity of microorganisms and their effects on organic matter decomposition. The higher MBC under NT compared to CT in KZN soils could be due to the greater moisture storage in this relatively wetter region than FS. This is consistent with findings of Mikanova et al. (2009), who reported higher MBC in soils under NT and minimum tillage system compared to CT. Soils under NT have greater potential to store organic matter compared CT due to less disturbances, whereas CT exposes OM to decomposition, especially in the top soil layers through aggregate breakdown. Engell et al. (2022) found similar results in the 0–10 cm depth followed by a sharp decline with depth. They attributed this to the organic matter input rather than the nitrogen and SOC already in the soil. The lack of impacts of planting date and season in KZN aligns with the findings of Spedding et al. (2004), who found that time did not influence the levels of MBC.

In the current study, the treatments and their interactions did not significantly affect ß-glucosidase indicating that other factors influenced the activity of this enzyme. ß-glucosidase activity is known to be affected by pH, moisture and soil depth (Adetunji et al. 2017) as well as the addition of organic residue that provide substrate (Balota et al. 2011). ß-glucosidase activity was low in Free State due to the higher temperatures, that reduce substrate availability by limiting the input of residues in this drier region. ß-glucosidase activity is generally higher under slightly acidic conditions (Acosta-Martinez and Tabatabai 2000; Wade et al.). This is contrary to our results pointing to modifying factors like C:N ratio, type of roots of the crop and soil texture. Tillage practices affects the activity of ß-glucosidase through their influence on microbial biomass, substrate availability and soil disturbance (Adetunji et al. 2017).

The higher activity of acid phosphomonoesterase activity in the first season than in the second for both FS and KZN could be due to higher demand for phosphorus, in the wetter season. Acosta-Martines et al. (2011) assert that Phosphatases availability and activity are linked to phosphorus levels optimum for both plant and microorganism growth (Bucher 2007; Balota et al. 2011). The results of this study align with those of Ajeigbe et al. (2018) who reported similar trends. The lack of tillage effects is consistent with the findings of Acosta-Martines et al. (2011) and Balota et al. (2011), who reported that the activity of phosphatases is closely linked to the pH of the soil rather than level of disturbance or organic matter content. In a study involving maize and beans, switching to no-till management resulted in lower acid phosphatase due to increase in pH (Rocabruna et al. 2024). Alvey et al. (2001) also reported higher phosphomonoesterase activity in continuous sorghum compared to rotational sorghum. However, the current study could not verify due to limitations of scope. Moreover, there is currently no method available to differentiate phosphatase activity based on its source (Dlugosz et al. 2022).

The significant main effects of tillage and season on diversity indices (AWCD and H) in KZN suggest that CT and season 1 microbes had higher functional diversity than their counterparts did. This is consistent with the findings of Janusauskaite et al. (2013) and Xiao et al. (2019) who reported higher carbon utilization in CT than NT plots. They attributed this to increased availability of hydrocarbons in CT compared to NT. In this study, the relationship between diversity indices and carbon source utilization was evident as the utilisation of carboxylic acids, amino acids and amines was influenced by season while amino acids utilisation was also influenced by the interaction of tillage × season. Xiao et al. (2019) explained that the alterations in the microbial environment brought about the effects of tillage in the soil biogeochemical properties could alter the patterns of substrate utilization by microbes.

In Free State, the AWCD was influenced by the interaction of planting date and tillage while the Shannon-weaver index was not affected by any of the treatment and their interactions. We observed a difference in microbial diversity, according to AWCD, between early and late planting under NT, but no differences between CT and either planting time. These results are consistent with Lupwayi et al. (2001), who also observed increased microbial diversity after one year of implementing zero tillage. The lack of treatment effects on the Shannon weaver index in the FS soils suggests that species richness and evenness remained unaffected. As numerous investigators have highlighted, the Shannon index changes depending on whether, richness and/or evenness are more prominent in the community (Hill et al. 2003; Wolinska et al. 2017; Roswell et al. 2021). When species richness is high, the Shannon index tends to reflect this richness, but when one species dominates in terms of individuals, the index reflects more on evenness. This suggests that the microbial community in FS may have balanced levels of richness and evenness, making the Shannon index stable across treatments.

The main effect of planting date, along with the interactive effects tillage × planting date and tillage × season, influenced the microbial preference for carbon sources in FS. Planting date affected the use of carbohydrates, while tillage × planting date influenced the utilisation of carbohydrates and polymers, and tillage × season influenced all the five substrates tested. According to Liu et al. (2023), the explanation for this is that NT and season two most likely had higher dissolved organic carbon, which is a key factor affecting microbial preference of carbon sources. In FS soils, microbes predominantly utilised carbohydrates indicating that they preferred simple carbon sources to complex ones. Shutter and Dick (2001) observed similar results when they amended the soil with glucose highlighting the physiological differences in the profile of microbial groups under NT. This suggests that NT fosters a microbial profile favouring the breakdown of simple carbon sources, while tillage may alter these dynamics. In KZN, amino acids, carboxylic acids and amines were the preferred substrates, influenced by season and the interaction between season and tillage. According to Delgado et al. (2012), no-till enhances MBC leading to increased enzyme activity, which promotes organic matter retention. In contrast, CT reduces hydrocarbons, contributing to a decline in organic matter. The fact that carbon source utilization was higher under NT and during the second season in both study sites suggests that conservation agriculture has the potential to protect substrates from volatilization while improving soil temperature and structure. This supports the idea that studying carbon source utilization is a good indicator of soil quality (Jezierska-Tys et al. 2020).

Conclusion

The study revealed that tillage, season and to lesser extent planting date, significantly affect biological properties, particularly in the topsoil, which is the most affected by tillage systems. It also shows the importance of microbial carbon use preferences and levels, which are important for determining appropriate crop rotations with sorghum, suitable fertilization regimes and key abiotic factors to consider when selecting location for cultivation. Although this is a short-term study, the positive benefits of soil microbiology to conservation agriculture in terms of sustained productivity and reduced cost to farmers of the developing world is apparent. If smallholder farmers are to benefit from CSA practices they need to adopt the relevant practices for their resources.

Based on these findings, we recommended further studies on the effects of other soil parameters that modify the effects of planting date and tillage on biological properties. These parameters could include time since introduction of conservation tillage, soil depth and other site-specific conditions. Additionally, we recommend adopting management interventions tailored to local edaphic and climatic factors to optimize agricultural practices and improve soil health and productivity.

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ORCID

Adornis Dakarai Nciizah http://orcid.org/0000-0002-2481-5218

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