COVER CROP AND NITROGEN FERTILISATION EFFECTS ON SOIL ORGANIC CARBON AND PHOSPHORUS FRACTIONS IN A SILAGE MAIZE-BASED CONSERVATION AGRICULTURE SYSTEM

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PREFACE

The data described in this thesis were collected in Cedara Research Station at Pietermaritzburg, Republic of South Africa from 2022 to 2023. Experimental work was carried out while registered at the School of Agriculture, Earth and Environmental Science, University of KwaZulu-Natal, Pietermaritzburg, under the supervision of Professor P Muchaonyerwa and Dr F Murungu.

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

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

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Professor Pardon Muchaonyerwa

Supervisor

January 2025

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DECLARATION - PLAGIARISM

I, Annitta Mqina, declare that

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2. This dissertation has not been submitted for any degree or examination at any other university.
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ABSTRACT

Purpose Cover crops (CCs) have been demonstrated to enhance soil organic carbon (SOC) and available phosphorus (P); however, there is a notable lack of long-term studies examining their effects on SOC and P fractions, particularly in South Africa. This research aims to understand the impact of nitrogen (N) fertilisation of silage maize in rotation with unfertilised CCs, including their combinations, on SOC and P fractions.

Methods and materials First, a meta-analysis was done to assess how N-fertilised and unfertilised CCs affected SOC and available P under diverse conditions. A field trial experiment was also done to assess the impact of N fertilisation of silage maize in rotation with unfertilised (CCs), including their combinations, on (a) SOC fractions (b) soil P fractions, and (c) other chemical properties. The trial was established in 2003, and the CCs for the current study were established in 2011. The trial was a completely randomised block design, with nine CCs treatments in rotation with maize silage in three replicates, each split for N fertiliser (0 and 120 kg N ha^{-1}) in 2012. Soil samples were collected from 0-5, 5-10, and 10-20 cm depths using beater auger.

Results and discussion The meta-data analysis showed that CCs planted as mono- and bi-culture improved SOC and available P, especially under legumes, except when mixed with broadleaves crops. It was also evident that CCs thrive and are more effective under tropical climates, particularly on loam soils. While SOC was higher under neutral pH, CCs increased available P in acidic soils. Nitrogen fertiliser application on CCs significantly increased SOC and available P than when it was not applied.

From the field study, cover crops significantly affected total organic carbon (TOC) and coarse particulate organic carbon (coarse-POC) at 10 cm, fine-POC at 20 cm, and mineral-associated organic carbon (MAOC) only at 5 cm, with no effects on water-soluble organic carbon (WSOC). The TOC was higher under black oat and stooling rye + vetch. At the same time, POC fractions were higher under forage pea + black oats, stooling rye + vetch, black oats + vetch, black oat and stooling rye, and the MAOC was higher under forage pea, which also increased fine-POC at 10-20 cm when compared with the control. On the other hand, vetch greatly increased the total N. Nitrogen fertilisation had no effects on SOC fractions except for coarse-POC, which was decreased. Total cations were significantly increased by black oat at 10 cm and forage pea + black oat at 10-20 cm. Extractable calcium was not significantly affected by CCs, however, it was higher under black oat + vetch and vetch at 10 cm. Forage pea also increased Ca at 5-10 cm, and it was higher under forage pea + black oat at 10-20 cm. Cover crops decreased soil pH, especially vetch. As a result, P bound to Al and Fe was very high in all CC treatments than AP. Total P was higher under black oat + vetch, stooling rye + vetch and black oat with N fertiliser at 0-5 cm, CCs with and without N fertiliser except for black oat + vetch and forage pea without N fertiliser at 5-10 cm, and it was higher in all CCs in 10-20 cm. While extractable P was not significantly affected, CCs significantly increased soluble P. It was higher under forage pea + black oat, stooling rye + vetch, stooling rye and black oat at 5-10 cm, and forage pea + black oat at 10-20 cm.

Conclusion The findings of the study imply that certain cover crops, like forage pea, black oat, and vetch, enhance SOC, N, and P levels while varying in effectiveness by soil depth. Vetch is especially beneficial in acidic soils, improving fertility, pH, and P retention while promoting carbon sequestration and sustainable nutrient management.

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DEDICATION

I dedicate this dissertation to my late grandmother, MaNdaba Mqina. Growing up I always knew that I wanted to be like her, character-wise and spiritually. She was a wise woman, kind, humble, goal driven and, most importantly, she loved God. I also dedicate this dissertation to my late sister, Ntandokazi Nyangule. We were raised like twins and attended the same grades since Creshe, she was always by my side at every level of my life. I will always appreciate the chance to have these two women in my life.

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CHAPTER ONE

GENERAL INTRODUCTION

1.1. Background

Many farmers in Southern Africa plant maize as a staple grain and for animal feed. White maize, which is mainly used as a staple food, accounts for about 60% of maize produced in South Africa, while yellow maize, which is mainly used as feed for animals, accounts for about 40% (Department of Agriculture, Forestry and Fisheries, 2017). These different types of maize require water and essential nutrients, amongst other resources, to achieve optimum yields. Nitrogen (N) and phosphorus (P) are often the most limiting nutrients for crop yields, as plants require them in significant amounts, but most soils do not supply enough. Many studies have shown that the application of N fertilisers improves crop yield (Iqbal et al., 2014; Oyebiyi et al., 2019; Tsvey et al., 2021). The effectiveness of fertilisation is affected by climatic and soil conditions, together with the source of the N (organic and inorganic fertilisers) (Syamsiyah et al., 2021; Betancur-Corredor et al., 2023). Understanding the dynamics and management of N and P in maize production under conservation agriculture practices is essential.

Conservation agriculture (CA) is mainly defined by minimum soil disturbance, residue retention, crop rotation, cover crops (CCs), and good management of nutrients (Cárceles Rodríguez et al., 2022). Cover crops, including black oats and vetch, are plants that are mostly planted to shield the soil surface from erosion, reduce nutrient leaching and improve soil fertility rather than being harvested (Scavo et al., 2022). Cover crop and main crop residue retention may enhance soil organic carbon (SOC), while legumes can improve N status, which in return improves the soil's physical, chemical and biological properties (Koudahe et al., 2022; Momesso et al., 2022). While some studies in the literature included fertilisation of CCs, practically farmers may only invest in fertiliser for the main crop including maize (*Zea mays* L.). Most of the observed positive effects were with the retention of residues of irrigated maize, whereas some farmers produce the main crop without irrigation and remove the maize for silage. This results in lower residue addition than where both the CC and the maize are irrigated, and fertilised and all the residues retained.

In silage maize-based CA systems, the maize biomass (above ground) is removed to make silage, leaving the roots and residues of the CCs being the only contributors to soil nutrient cycling and organic matter (OM) addition. Fontana et al. (2023) reported that CCs, especially field pea, increased the nutrient content such as N of the soil and maize biomass. This was consistent with Marcillo and Miguez (2015), who reported that legume winter CCs increased maize yields by 30% and 33% under low N fertiliser rates and no-tillage. However, even though clover was reported to improve maize biomass, grain yield and N content, maize exploited N more efficiently under control treatment without a CC (Kramberger et al., 2009). According to Chen and Weil (2011), forage radish enhanced root penetration of maize in compacted soils, while rye improved soil surface water availability, and rapeseed provided both benefits. The implications of these factors on SOC, N and P dynamics are not well understood. Due to limitations in resources, farmers may grow silage maize without N fertilisation, where a legume CC is used. Such a practice could affect the residue addition through maize roots and the CC with significant effects on SOC and P and their pools. There is a need to understand the influence of different unfertilised CCs and fertilised main crops on soil quality in a silage maize system.

1.2. Justification of the study

In sub-Saharan Africa, the production of agriculture is limited by deficiencies of plant essential nutrients and organic carbon (OC) in large portions of the continent (European Commission, 2013). Africa is one of the continents with the highest P depletion (Alewell et al., 2020), with average global losses of about 4-9 kg of P per hectare per year (Hilton, 2020). Conservation agriculture has been introduced in South African agroecosystems to improve SOC among other benefits (Sithole et al., 2016). The addition of crop remains enhances SOC, depending on the amount and quality of the biomass (Blanco-Canqui & Lal, 2009). Both main crop and CC need fertilisers making the practice potentially expensive. As such, the benefits of applying fertilisers only to the main crop, with the CC depending on residual effects need to be explored with a variety of cover crops as sole crops and bicultures. Biculture CCs have been proven to have more pronounced effects than sole CCs, like higher total biomass (Muzangwa et al., 2012) which then increases SOC, however, sole CCs eliminate the risk of competing for resources. The practice could be particularly important in a silage maize-based system, where the biomass of the maize is removed to produce silage. The findings of this study will be beneficial in the selection of the best CCs and their combinations and whether the exclusion of fertilisation of the CCs is practical in silage maize CA systems.

1.3. Research questions

- How does N fertilisation of silage maize and rotation with different species of unfertilised cover crops, as sole and bicultures, affect soil organic carbon fractions?
- How does N fertilisation of silage maize and rotation with different species of unfertilised cover crops, as sole and bicultures, affect soil phosphorus fractions and other soil chemical properties?

1.4. Aim and Objectives

This research aims to understand the effects of nitrogen fertilisation of silage maize in rotation with unfertilised cover crops on soil organic C and P fractions.

- Determine the effects of nitrogen fertilisation of silage maize and rotation with unfertilised cover crops, including their combinations, on soil organic carbon fractions.
- Determine the effects of nitrogen fertilisation of silage maize and rotation with unfertilised cover crops, including their combinations, on soil phosphorus fractions and other chemical properties.

1.5. Hypothesis

- Application of N fertiliser on silage maize and rotation with unfertilised cover crops will increase the content of SOC and its pools.
- Application of N fertiliser on silage maize and rotation of unfertilised cover crops will increase soil P availability and change soil P pools and other soil chemical properties.

CHAPTER TWO

COVER CROPS AND NITROGEN FERTILISATION EFFECTS ON SOIL ORGANIC CARBON AND PHOSPHORUS FRACTIONS: REVIEW

2.1. Introduction

In natural ecosystems, there is usually some vegetation that covers the soil, regulates soil moisture, serves as a storage for plant readily available nutrients, fixes carbon (C), and supports soil fauna (Kaspar et al., 2011). However, due to the demand to grow high yield crops for the growing population and other economic pressures like industrialization, farmers may replace the vegetation with continuous, intense farming, especially in tropical Africa (Abdullahi et al., 2011). Due to the significant demand to produce high yields, many farmers globally have practised conventional farming, used intense tillage, and mostly left the soil bare and exposed, especially in winter. The spaces between rows in some annual (such as maize) and perennial cropping systems for nut or fruit crops are left fallow and tilled for a long period (Kaspar et al., 2011). Conventional tillage also includes the use of inorganic fertilisers such as nitrogen (N) and phosphorus (P) fertilisers, which have drastically increased. These practices have been having an immense negative impact on food crop production and the environment across the globe, including the increase of global warming, soil degradation, pollution of groundwater, and loss of soil nutrients. The inclusion of cover crops (CCs) into fallow spaces or fallow periods is being promoted and adopted worldwide to reduce these negative effects of conventional agriculture.

The pools and bioavailability of soil organic carbon (SOC) and P can be impacted by CCs, because of their ability to enhance organic matter (OM) inputs, microbial diversity, and enzyme activity, which changes the soil's physical, chemical, and biological characteristics and processes (Chen et al., 2023). Farmers use CCs for different reasons, including dead or living mulch. While any plant can be used as a CC, *Fabaceae* (legume) and *Poaceae* (grasses) are generally more frequently used (Abad et al., 2021). Legume crops that are commonly used include alfalfa, vetch, pea, sunn hemp, cowpea, faba bean, soybean, and clover, while commonly used grass crops include barley, rye, buckwheat, oats, ryegrass, wheat, and triticale

(Adetunji et al., 2020). These types of species can be planted as biculture (mixture of legume and grass) or monoculture. The selection of CCs by a farmer is influenced by their qualities, including being simple to plant, having high biomass that covers the soil and supplies sufficient residues quickly, resisting diseases and not serving as a host for diseases that affect cash crops, being easy to terminate, and be economically viable (Adetunji et al., 2020). This chapter aims to examine the literature regarding the impacts of CCs and N fertilisation on SOC and P fractions.

2.2. Cover crops and SOC fractions

Soil OC makes up a big portion of soil organic matter (SOM) with different chemical and physical properties. Cover crops can greatly influence SOC by affecting the decomposition rate and OC input. The viability of terrestrial ecosystems and the cycling of C are both greatly influenced by the dynamics of soil carbon (Kaushal et al., 2021). Organic carbon is classified into labile and stable OC fractions according to their turnover rate. The impacts of CCs on SOC fractions, on the other hand, differ.

Labile OC comprises particulate organic carbon (POC), water-soluble organic carbon (WSOC), microbial biomass OC, and other fractions that decompose rapidly (Muir et al. 2013). Labile OC serves as the energy source for microbes; hence, high labile OC amounts increase microbial community, and this significantly increases nutrient cycling in the soil (Muir et al. 2013). High-quality CC residues and high root biomass might significantly increase labile OC. According to Zhang et al. (2023), the incorporation of summer CCs increased labile OC rapidly, creating hotspots for arbuscular mycorrhizal fungi growth; on the other hand, microbial activity enhanced the availability of labile OC. White et al. (2020) observed that cover cropping led to a 26% increase in permanganate oxidizable labile C while a higher increase was observed at legume plus rye and mustard systems because of the low carbon to nitrogen (C/N) ratio. Labile OC fractions are easily altered by soil management or land use, and CCs with C/N ratio that is low have a higher degradability. Hence, legume CCs tend to have a high decomposition rate. Another study reported that grass crops in the spring increased soil C/N ratio and labile OC more effectively in the short term than legumes (Rosolem et al., 2016). Zhou et al. (2012) reported a significant effect of wheat on soluble OC than oats and control, respectively, due to its higher biomass input, high moisture, and less acidity. Chahal et al. (2020) suggested that labile C and N fractions and rhizodeposits are expected to increase during the flowering stage than the planting stage due to an increase in plant density, size, and root development. Labile

OC also improves soil structure by binding micro-aggregates into macroaggregates, improving water retention and infiltration.

The influence of CCs on stable OC fractions may occur more slowly than other fractions. The type of CC and root distribution and length play a role in stable OC alteration (Lorenz et al., 2005; McClelland et al., 2021). Soil OC stability and the soil's ability to store SOC for a long period are determined by the pool of stable SOC in the soil; hence, this fraction contributes more to SOC sequestration and storage (Zhao et al., 2022). Stable OC fractions take years to decades to decompose due to them being chemically resistant from decomposition (e.g. humus) or being physically protected within aggregates or binding with clay-minerals (mineral-associated organic carbon, MAOC) (Muir et al. 2013). Unlike labile OC fractions, more stable OC fractions are not easily altered by land use or soil management practices. This observation was also supported by Beltran et al. (2018), who found that adding CCs did not significantly impact carbon in the mineral-associated fraction. However, it was observed that CC system with Mucuna (Stizolobium cinereum Piper & Tracy) had a higher C sequestration (0.6 Mg ha-1 yr-1) and MAOC than other cover cropping systems because of the higher biomass input (Bayer et al., 2009). According to Rosolem et al. (2016), the addition of a legume (sunn hemp) preserved a C/N ratio that was more stable which would be more successful in raising soil carbon over time. The maintenance of stable SOC has a significant impact on soil ecosystems, soil fertility, plant productivity, and atmospheric carbon dioxide (CO_2) levels (Zhao et al., 2022). Duval et al. (2016) reported that significant variations between the treatments were only observed in 2009, where the control treatment had the highest MAOC, and explained that CCs effectiveness was probably affected by soil moisture due to reduced rainfall.

2.3 Cover crops and soil phosphorus fractions

Cover crops have been demonstrated in several research studies to affect soil P fractions by stimulating microbial activity and OC. Legume crops can fix their N through a symbiotic relationship and reduce the need for N fertilisers, which can indirectly affect P dynamics in the soil. These crops can also improve SOM and microbial activity, which may influence P availability. Although a large fraction of P is in an inorganic form, a large portion of that P is not available for plant uptake. Hence, it is important to understand how CCs affect P by investigating its fractions. Inorganic P is divided into solution, labile, and non-labile P.

Soluble P is a fraction that is in soil solution readily available to be absorbed by plants. However, this is the smallest fraction, and it can easily be changed by soil management implements and is prone to being eroded and leached (Wortmann et al., 2006). Soluble P can be increased through the decomposition of the residues by microbes releasing the nutrients. It can also increase through P solubilisation from iron (Fe), aluminium (Al), calcium (Ca), and clay minerals. According to Demir (2020), CCs significantly increased available P in both 2013 and 2014 in the topsoil only, where available P was significantly higher in *Festuca rubra* subsp. *Rubra* in 2013 and higher in *Trifolium repens* in 2014. Janegitz et al. (2017) showed that crambe resulted in higher available P, while grain sorghum and peanuts decreased soil maximum P adsorption capacity. High P uptake by CCs and high decomposition rate are important for CCs to significantly affect P availability. Other studies reported that CCs did not significantly affect P availability (Takeda et al., 2009; Acunã & Villamil, 2014). Takeda et al. (2009) observed that CCs did not significantly influence available P, and the control treatment had a greater P availability, followed by rapeseed and rye CCs. Even though Acunã and Villamil (2014) observed no significant influence of CCs on P availability, radish and buckwheat had a greater available P while radish and hairy vetch lowered it, followed by the control treatment. A significant impact of CC (oats) on available P was also observed on topsoil from a study done by Beltrán et al. (2018); however, the addition of oats decreased P availability, especially fertilised oats.

Even though labile P is higher than soluble P in the soil, it still makes up a very minor portion of total P (Wortman et al., 2006). This fraction is easily mineralizable and consists of organic P, secondary phosphate minerals, and adsorbed P (Prasad et al., 2019). Cover crops and microorganisms can mobilise rhizosphere phosphate by lowering soil pH to dissolve calcium-phosphate minerals, increasing organic acid content to desorbed phosphate fixed with aluminium and iron oxides, and mineralizing organic phosphate by promoting phosphate enzyme activity (Maltais-Landry et al., 2014). Dube et al. (2014) found that grazing vetch and oats CCs significantly increased labile P than weedy fallow, and hydrochloric acid extractable inorganic P (HCl-P_i) was higher than sodium bicarbonate extractable inorganic P ($NaHCO_3$-P_i) in both CCs. Grazing vetch is a legume crop; therefore, it decomposes faster than oats, which are grass crops, and higher total biomass input also increases labile-P. According to Rodrigues et al. (2021), brachiaria was the best CC as it was able to recycle more P and increase labile P by 5-20% than other CCs due to its ability to stimulate microbial enzyme activity around the roots and exudate organic acids. Other scientists had different observations. In the study done by Takeda et al. (2009), the authors reported that CCs had no significant impact. However, an increase in labile P pools from May 2005 to October 2007 was observed, and sodium hydroxide extractable inorganic P (NaOH-P_i) was higher than other pools (Takeda et al., 2009). Even the interaction of CCs and tillage systems did not have a significant effect on labile P; however, there was a significant effect when the observation was done over two seasons where there was a significantly higher labile P in the rainy season than in the dry (no rain) season (Carvalho et al., 2014).

Non-labile P is the largest fraction of all the P fractions, and it is unavailable for plant uptake. Although most of this fraction will remain in the non-labile state, a minor portion eventually undergoes chemical reactions to transform into labile P and soluble P (Wortman et al., 2006). Cover crops may not significantly affect P cycling in less weathered soils with low P-fixing capacity, resulting in low P mobilisation (Maltais-Landry et al., 2014). When Maltais-Landry et al. (2014) compared cereals and legumes, cereals produced greater biomass with the same amount of P, but cereals have a high C/P ratio that could increase microbial immobilisation, decreasing residue decomposition and P cycling rates. In the study by Rodrigues et al. (2021), CCs did not have a significant effect on non-labile P in Oxisol-1, which had long cultivation period, low cation exchange capacity (CEC), Al ions, and high SOC and clay content, in 0-10 cm and 10-20 cm depths, and non-labile P in fallow treatment was significantly lower than millet, brachiaria and maize treatments in both depths in Oxisol-2. Soltangheisi et al. (2020) observed that non-labile P on CCs without P fertiliser (755 mg/kg) was significantly lower than on CCs with single superphosphate (812 mg/kg) and CCs with rock phosphate (859 mg/kg). The application of P fertilisers increases soluble P concentration, which might not all be taken up by plants, and that remaining amount gets fixed and becomes unavailable for plant absorption. This further proves that CCs can be used to recycle P and decrease the necessity of high P fertiliser application for the main crop.

2.4. Nitrogen fertilisation and SOC fractions

The addition of N fertiliser is vital for the growth and development of the crop and impacts the dynamics of SOC through various mechanisms, such as changes in the quality of crop residues, microbial diversity and activity, and the structure of the soil. According to Mahal et al. (2019), N fertilisation can both negatively and positively affect SOC. Nitrogen fertilisation might significantly increase the main crop growth, leading to high water absorption and leaving a small amount of water for CCs. This may decrease the development of CCs when they are only dependent on residual moisture. Nitrogen fertilisation increases soil residual N which may then be taken by CCs, increasing productivity and retention of residues in the soil. However, N fertilisation also increases the microorganism community, which will then increase SOC mineralisation. Begum et al. (2021) stated that the highest rate of N fertiliser application enhanced the stocks of labile OC. However, an increase in SOC mineralization may decrease OC sequestered in the soil. According to Jesmin et al. (2021), residues from high N fertilisation treatment were rapidly decomposed by microbes, resulting in greater CO_2 production. The application of N fertiliser could influence the soil aggregate stability, thus influencing OC content. Excessive N may degrade soil structure and increase the risk of erosion, which could potentially decrease the OC content. Mulvaney et al. (2009) reported that N fertilisation decreases OC stocks due to high SOC mineralisation and high OC demand by microbes. Excessive N application without CCs may accelerate SOC decomposition or alter soil microbial communities negatively, reducing SOC stability.

2.5. Nitrogen fertilisation and P fractions

Jian et al. (2020) mentioned that the application of N fertiliser affects the microbial processes responsible for regulating P dynamics, which may enhance or reduce available P, depending on the specific management practices. However, the ability of N fertilisation to alter P dynamics also depends on soil characteristics and N fertiliser rate and type. Nitrogen fertilisation can increase or decrease soluble P. The application of N fertiliser stimulates microbial activity, increasing the mineralization rate of CC residues. The P is released to the soil solution as the residues decompose, increasing soluble P. High N content increases plant growth in turn increasing P uptake. Most of the time, N fertilisation is applied to the main crop, which may result in most P content absorption, leaving a small amount of P to be absorbed by CCs. Zhang et al. (2004) reported that ammonium (NH_4^+-N) fertiliser increased the crop biomass and N and P uptake than nitrate (NO_3-N) fertiliser. Therefore, the type of N fertilisation also influences P dynamics. Their study was consistent with Beltran et al. (2018); they observed a decrease in P availability with N fertiliser application. Nitrogen fertilisation also affects soil pH, which may further influence the solubility of P. Zhang et al. (2004) also reported that NH_4^+-N fertiliser decreased soil pH, which may result in high solubility of P in calcareous soils.

Labile P refers to the pool of P readily available for plant uptake. Like soluble P, N fertilisation can increase labile P by stimulating microbial activity and decomposition of organic matter

(OM), which releases more labile forms of P. This makes P more accessible to plants in the short term. However, rapid mineralisation of OM may result in high soluble P that may leach out to water bodies. According to Mahmood et al. (2021), N fertiliser application significantly decreased labile P by 19%. The decrease of soil pH with N fertiliser application increases aluminium ion (Al^{3+}) and iron ion (Fe^{3+}) in acidic soils. These oxides then bond with the P, decreasing available P for plant uptake.

Non-labile P is bound to soil minerals and OM, and its availability to plants is limited over the short term. Nitrogen fertilisation may also increase or decrease the concentration of non-labile P in the soil. Nitrogen fertilisation may also influence non-labile P by altering soil pH or microbial communities, which can affect the availability of non-labile P. Increased microbial activity stimulated by N can accelerate the decomposition of SOM, which may lead to the release of some non-labile P into labile forms. Low soil pH may stimulate the weathering of phosphate rocks, releasing P to labile and soluble P forms. According to Mahmoon et al. (2021), non-labile P concentrations increased with the addition of the N fertilisation rate, and non-labile P fractions might be involved in long-term P cycling.

2.6. Conclusion

Overall, CCs and N fertilisation significantly affect SOC and P fractions through their respective contributions to SOM dynamics and nutrient availability. The ability of CCs and N fertilisation to alter these SOC and P dynamics also depends on many factors, such as the type of the CC, whether it is mono or bicultural cropping, the type of nitrogen fertiliser, and soil properties. Hence, it is important for effective management strategies to focus on improving the stability of SOC while also sustaining and enhancing crop yield. This emphasises the need for customised management practices that are suitable to specific local conditions. However, the influence of the interaction effects of CCs and N fertilisation on SOC and P fractions is complex and requires meta-data analysis from different regions, and further research, especially on long-term practices.

CHAPTER THREE

COVER CROP AND N FERTILISATION EFFECTS ON SOIL ORGANIC CARBON AND AVAILABLE PHOSPHORUS: META-ANALYSIS

3.1. Introduction

Main greenhouse gases (GHGs) include nitrous oxide (N_2O) and carbon dioxide (CO_2), whereby CO_2 is the primary greenhouse, accounting for about 76% of the effect, and N_2O accounts for 6% (Climate Trade, 2023). Climate change has been one of the major threats to global food security. Soil has been reported to store greater carbon (C) than the atmosphere and vegetation combined; about 2500 Gt of C is stored worldwide, and 1550 Gt of that C is organic (Agriculture & Food, 2021). Carbon from the atmosphere can be reduced by being fixed in the vegetation and microorganisms and stored in soil C pools in soil aggregates. However, it has been reported that woodlands and grasslands are being converted to croplands due to the high rise in the population worldwide and food production (Jian et al., 2020). Several studies reported that the conversion of grassland to cropland resulted in a 36% decrease in SOC, and the conversion of forestland to cropland led to a 25-30% decrease in SOC (Jian et al., 2020). The increase in monoculture practices has also been a concern because of the low soil surface coverage and lack of crop diversity, which leads to a decline in soil fertility, water infiltration, and high runoff and erosion.

On the other hand, phosphorus (P) in the soil exists in both organic and inorganic forms, each with different levels of availability. Phosphorus deficiency results in a decrease in plant growth and development rate, and that results in a low plant yield. An increase in P adsorption sites and a decrease in P desorption can be affected by fixation with aluminium (Al) and iron (Fe) oxides, which also result in lower P availability that can be taken by plants. Tropical and subtropical climate regions with highly weathered soils have been reported to have high P deficiency (Pavinato et al., 2010; Teles et al., 2017). This has resulted in farmers from these regions being dependent on phosphate fertilisers and the need for liming to produce food crops. Due to increasing threats to food security, there is a necessity for a new management system that will improve nutrient cycling and soil properties and reverse C losses from the soil. Cover cropping, crop rotation, conservation tillage (Hallama et al., 2019) and reduced inorganic fertilisation application have been some of the CA practices adopted by some farmers.

Planting cover crops (CCs) is suitable for preventing runoff and soil erosion. Their canopy covers the soil, and their roots bind with the particles of soil, improving soil aggregation and structure while incorporating the residues increases SOC content. Alteration of SOC dynamics is also impacted by the type of the CCs, with grass CCs having higher C and lower N and a higher C/N ratio. Legume CCs have higher N because they can fix their atmospheric N, resulting in a lower C/N ratio and faster decomposition than grass CCs. While other researchers recommend the use of a mixture of legume and grass CCs (Nyfeler et al., 2011), some studies have observed that legume CCs had higher SOC increase than grass CCs (Blanco-Canqui et al., 2012), while other studies reported the opposite (Sainju et al., 2002), these results may be caused by the variation in biomass and C/N ratios between CC species (Thorup-Kristensen et al., 2003; Poeplau & Don, 2015).

Cover crops take up residual nutrients, preventing leaching out or fixation of P, and cover the soil surface which prevents runoff, erosion and weed growth, and improves soil productivity (Farmaha et al., 2020; Hopkins, 2017). When CC residues decompose, they release nutrients such as magnesium (Mg), potassium (K), and calcium (Ca), which are also known as base cations. The basic cations interact with hydrogen ions present on the soil exchange sites. This interaction decreases the availability of free hydrogen ions, thereby increasing the soil pH (Xu et al., 2006) and P availability. The organic matter (OM) from CCs increases the cation exchangeable site and buffering ability of the soil, allowing it to hold more cations at the exchange sites and to resist changes in pH (McCauley et al., 2009). However, OM mineralization contributes to soil acidification through the formation of organic and inorganic acids which release hydrogen ions to the soil. As a result, labile OM contributes more to acidification, whereas stable OM (humic substances, OM bound to inorganic components, biochar) contributes more to buffering capacity and cation exchange capacity (CEC) (McCauley et al., 2009). According to Hallama et al. (2019), plants that can be utilised as CCs are expanding, however, there are some restrictions due to the agronomic requirements of the CCs. Cover crop biomass production, the organic P-mineralising and P-mobilising compounds they exude, how their roots explore the soil, and their interaction with the rhizosphere microbial community vary substantially according to each species (Hallama et al., 2019). The ability of CCs to increase plant-available P and SOC is influenced by various factors, including the species of CCs used, whether they are planted as sole crops or in bicultures, and soil characteristics and climatic conditions. This underscores the need for further analysis of data from different locations, different soil types, varying climate conditions, and a range of CC species. Additionally, in most cases, CCs rely on resources such as nutrients and water left after the main crop is harvested. This may result in low biomass production from CCs. Therefore, the effectiveness of CCs can be impacted by the nutrient content in the soil, which may explain why some farmers choose to add N fertiliser to their CCs while others do not. The study focuses on examining how various unfertilised and N-fertilised CCs, either planted sole or in combination, impact SOC and available P. This investigation is based on data gathered from multiple research articles, considering different soil and climatic conditions.

3.2. Methods and materials

3.2.1. Literature search and study selection

An extensive search of several publications was performed to collect data on the impact of mono- and biculture CCs on SOC and available P globally. Numerous databases were used for searching articles, including Google Scholar, SpringerLink, Science Direct, Scopus and Library Genesis. Several topics were used to search the published journal articles such as "effects of cover crops on SOC", "effect of cover crops on available P", "effects of cover crops on soil fertility", "effects of cover crops on soil quality", and "effects of cover crops on soil nutrients". The combinations of keywords were also used, including "cover crops" AND "soil organic carbon", "cover crops" AND "soil available P", and "cover crop" AND "soil nutrients". The search was run from 2022 to 2024. All the articles selected in this meta-analysis had to meet the following criteria:

i) The investigation must take place in the field in any location. As a result, studies carried out in greenhouses, pots, and controlled laboratory environments were excluded.

ii) It is necessary for the research to include at least one cover crop treatment and a control group that does not receive any modifications. This meta-analysis included different types of CCs.

A summary of the selected articles is represented in Table 3.1.

3.2.2. Data collection and grouping

The effects of different CCs were assessed on the 2 response variables: SOC and available P. The averages of each response variable of interest were extracted from the findings of the chosen articles and organised into an Excel database. Soil OC was expressed as grams per kilograms (g/kg), and SOC expressed as a percentage was converted by multiplying by 10. If solely SOM was available, it was converted into SOC utilising the van Bemmelen factor (1.724) (Pribly, 2010). Available P was expressed in milligrams per kilograms (mg/kg), and bulk density (BD) and soil depth were used to convert available P expressed in kilograms per hectare (kg/ha). Typical BD values were estimated using soil texture classes where it was not provided (Zeri et al., 2018).

Example:

Available P = 20.51 kg/ha, BD = 1.22 g/cm^3, Soil depth = 50 cm

BD = 1.22 × 1000 = 1220 kg/m^3, Soil depth = 50 ÷ 100 = 0.5 m

Ha to kg = 10000 m^2 × 1220 kg/m3 × 0.5 m = 6100000 kg

Available P = 20.51 × 1000000 = 20510000 mg

Available P (mg/kg) = 20510000 mg ÷ 6100000 kg = 3.36 mg/kg

Averages of available P, SOC and initial soil characteristics that were taken from the studies with multiple data collection and studies done in different fields of the same area. The means from a study done in various locations were not averaged.

Data gathered from the reviewed articles comprised the study sites (country name, specific location, and climate), details of the experiments (duration of experiments, design, number of replicates, and the use of N fertiliser on CCs), initial soil characteristics (including soil texture, pH, SOC, total nitrogen (TN), and BD), and information on CCs (species type and scientific name). Where initial soil information was not provided, the information recorded during the study was recorded, except for SOC. Total nitrogen was expressed as g/kg and BD as grams per cubic centimetres (g/cm^3). Independent variables used were CC, soil texture, soil pH, BD and climatic region. The soil texture, pH levels and climates were categorised according to Mkhonza et al. (2019) and summarised in Table 3.2, including categories of CCs. Total N was not included in the statistics because of the limited data. Results that were reported in graphs were extracted using Web Plot Digitizer.

Table 3. 1: Summary of the selected articles

Row ID	Author	Country	Experimental years	MAP (mm)	MAT (°C)
1	Adler et al. (2020)	USA	2016-2018		
2	Nascente et al. (2013)	Brazil	2007-2009	1500	22.7
3	Ball et al. (2020)	Australia	2014-2018	454	22.8
4	Bawa et al. (2021)	USA	2017-2020	655	7.60
5	Sastre et al. (2018)	Spain	2010-2013	390	13.6
6	Mukumbareza et al. (2016)	South Africa	2009-2011	430	18.1
7	Cober et al. (2019)	Canada	2016-2017	780	6.5
8	Chavarria et al. (2016)	Argentina	2011-2014	971	16.5
9	Dinesh et al. (2004)	India	1991-2000	3100	30.1
10	Dong et al. (2024)	China	2018-2022	1250	20.3
11	Moore et al. (2014)	USA	2001-2011	856	9.30
12	Fiorini et al. (2022)	Italy	2016-2019	830	13.1
13	Blanco-Canqui et al. (2019)	USA	2005-2017	693	
14	Acuna et al. (2014)	USA	2010-2011	84.8	11.6
15	Sulok et al. (2014)	Malaysia	2013-2014	3775	
16	Duval et al. (2016)	Argentina	2006-2012	1019	17.5
17	Villamil et al. (2006)	USA	2002-2003		
18	Demir et al. (2019)	Turkey	2014-2016	420	13.4
19	Rigon et al. (2020)	Brazil	2003-2015	1450	
20	Kaur et al. (2024)	USA	2018-2022		
21	Pérez-Álvarez et al. (2015)	Spain	2009-2011	204	12.4
22	Decker et al. (2022)	USA	2017-2021		
23	Welch et al. (2016)	USA	2011-2013	1015	11.3
24	Rosolem et al. (2016)	Brazil	2006-2008	1400	20
25	de Sousa et al. (2017)	Brazil	2015-2016	1100	26.6
26	Souza et al. (2021)	Brazil	2009-2025	1400	17.6
27	Oliveira et al. (2016)	Brazil	2012-2013	112	8.90
28	Thapa et al. (2022)	USA	2019-2026	437	14.1
29	Che et al. (2022)	China	2011-2018	586	11.6
30	Sainju et al. (2015)	Georgia	2010-2013		
31	Demir et al. (2019)	Turkey	2013-2014	686	14.5
32	Demir et al. (2020)	Turkey	2013-2014	686	14.5

33	Zhou et al. (2012)	Australia	2009		21.9
34	Ghimire et al. (2019)	USA	2016-2017	470	15
35	Pareja-Sánchez et al. (2024)	Spain	2018	404	17.5
36	Frasier et al. (2016)	Argentina	2010-2013	700	13
37	Novara et al. (2020)	Italy	2009-2017	516	18
38	Peregrina et al. (2010)	Spain		477	12.9
39	Steele et al. (2012)	USA	1994-2007	1033	14.4
40	Zhang et al. (2022)	USA	2012-2019	975	9.5
41	Wang et al. (2021)	China	2006-2019	578	11.4
42	da Silva Souza et al. (2018)	Brazil	2014	1500	21
43	Brewer et al. (2023)	USA	2014	1500	21
44	Sawchik et al., (2012)	Uruguay	2018-2021	89.7	23.3
45	Torres et al. (2021)	Spain	2003-2014		

MAP: Mean Annual Precipitation, MAT: Mean Annual Temperature, USA: United States of America

Table 3. 2: Categories defining the used environmental, soil conditions and CCs for analysis

Independent variables	Remarks	Class range	Name
MAP (mm $year^{-1}$); MAT, (°C $year^{-1}$)	Hot and dry	MAT > 25 MAP <100	Arid
	Hot and wet.	MAT > 20 MAP > 1500	Tropical
	Warm and arid-humid	MAT ≥10-20 MAP > 100-1110	Subtropical
	Cool and arid-moist	MAT < 10 MAP ≥ 120 - 1000	Temperate
Soil texture (%)	Clay content distribution (%)	<20 20 – 32 >32	Sand Loam Clay
Soil pH	Highly acidic	<4.5	Strongly acidic
	Moderately acidic	4.5 – 6.5	Acidic
	Soils with moderate carbonates	>6.5 – 7.5	Neutral
	Soils with high carbonates	>7.5	Alkaline
Cover crops		Grass Legume Broadleaves	

MAP: Mean Annual Precipitation, MAT: Mean Annual Temperature

3.2.3. Statistical analysis

The R software was used to analyse the collected data statistically. Density plots were utilized to assess publication bias, which quantifies the variability and quality of the effect size (Brien & Hatfield, 2019; Motsi, 2022). In contrast, other researchers have employed funnel plots or histograms for this purpose (Zhao et al., 2022). The study utilised a random-effects model for the meta-analysis, specifically employing the residual maximum likelihood (REML) method through the 'rma' function in R's "metafor" package. In environmental data analysis of meta-analyses, random effects models are typically preferred due to their ability to account for variance heterogeneity. The influence of CCs on the concentration of SOC and available P was evaluated using the natural logarithm response ratio (lnR) as the measure of effect size. This assessment was conducted using the following equation:

$$lnR = \frac{lnCt}{lnCc} \quad (1)$$

where Ct and Cc are the mean responses to CC and no CC, respectively.

The variance (v) of each lnR of the mean soil responses was calculated using the following equation:

$$v = \frac{SD^2 t}{n^2 t} + \frac{SD^2 c}{n^2 c} \quad (2)$$

where SDt and nt are the standard deviation and number of replications (sample sizes) under CC, respectively, and SDc and nc are the standard deviation and sample size under no CC, respectively. The weight was calculated from the inverse of the variation using the following equation:

$$wi = \frac{1}{v} \quad (3)$$

where v is the variance (v) of each lnR of the mean soil response. Finally, the measured average effect size (lnRR) was calculated to generate the overall changes in SOC and AP using the following equation:

$$lnRR = \frac{\Sigma(lnR\ x\ wi)}{\Sigma wi} \quad (4)$$

where lnR is the effect size and wi is the corresponding weight of each comparison of SOC and AP. The 95% confidence interval (CI) for lnRR was calculated as follows:

$$95\%CI = lnRR \pm 1.96S_{lnRR} \quad (5)$$

where, S_{lnRR} is the standard error of lnRR and was calculated as follows;

$$S_{lnRR} = \sqrt{\frac{1}{\Sigma wi}} \quad (6)$$

To assess significant differences between treatments with and without CCs, the 95% confidence interval (CI) was employed. A significant distinction was indicated when the 95% CI did not encompass zero. Conversely, if the 95% CI included zero, it suggested that no substantial difference existed between the presence and absence of CCs.

3.3. Results

3.3.1. Publication bias and the general effect of the parameters of the study

The distribution of the data (effect size) is shown in Figure 3. 1. The variables assessed (SOC and available P) were close to zero, indicating limited biases from the articles used in this study. The overall effect of the evaluated parameters of the study is shown in Table 3. 3. In general, CCs increased SOC by 0.67 [n=40, CI=0.57-0.77] and available P by 7.40 [n=22, CI=4.48-10.32]. Cover crops under different climates increased SOC by 0.67 [n=40, CI=0.57-0.77] and available P by 7.41 [n=22, CI=4.48-10.38]. The different soil textures increased SOC by 0.61 [n=23, CI=0.49-0.73] and available P by 4.82 [n=11, CI=1.63-8]. The different soil pH increased SOC by 0.68 [n=30, CI=0.59-0.78] and available P by 9.61 [n=17, CI=6.63-12.58]. Cover crops with or without N fertiliser application increased SOC by 0.67 [n=31, CI=0.58-0.78] and available P by 6.79 [n=17, CI=3.27-10.30].

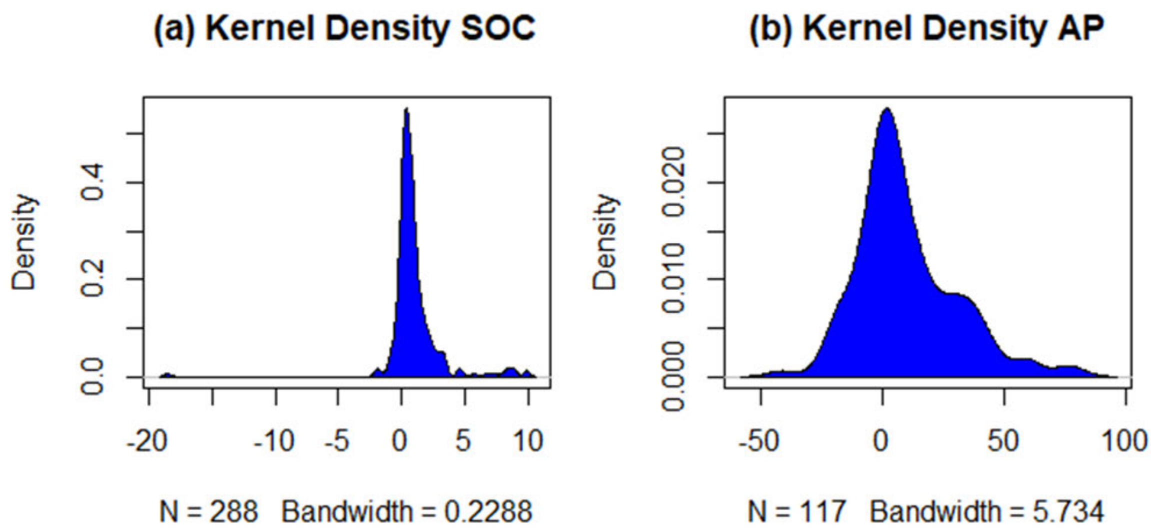


Figure 3. 1: Kernel density plots of effect sizes for (a) soil organic carbon and, (b) available phosphorus.

Table 3. 3: Overall effects of the study of effect sizes of all studied variables in the metanalysis.

	SOC				AP			
	ES	CI	p-value	n	ES	CI	p-value	n
Cover crop	0.67	0.57-0.77	0.001	40	7.40	4.48-10.32	0.001	22
Climate	0.67	0.57-0.77	0.001	40	7.41	4.48-10.38	0.001	22
Texture	0.61	0.49-0.73	0.001	23	4.82	1.63-8.00	0.001	11
N fertiliser	0.67	0.58-0.78	0.001	31	6.79	3.27-10.30	0.001	17
pH	0.68	0.59-0.78	0.001	30	9.61	6.63-12.58	0.001	17

ES= Effect Size; CI= Confidence Interval; SOC = soil organic carbon; AP= available P

3.3.2. Effect of mono- and bi-culture cover crops on SOC and available P

A significant improvement ($p<0.05$) in SOC was observed under mono- and biculture CCs, except under legumes plus broadleaves (Figure 3.2a). A significant improvement in available P was only seen under legumes and broadleaves (Figure 3.2b). Legumes had the highest SOC [1.27, 0.88-1.66] and available P [20.32, 13.81-26.82].

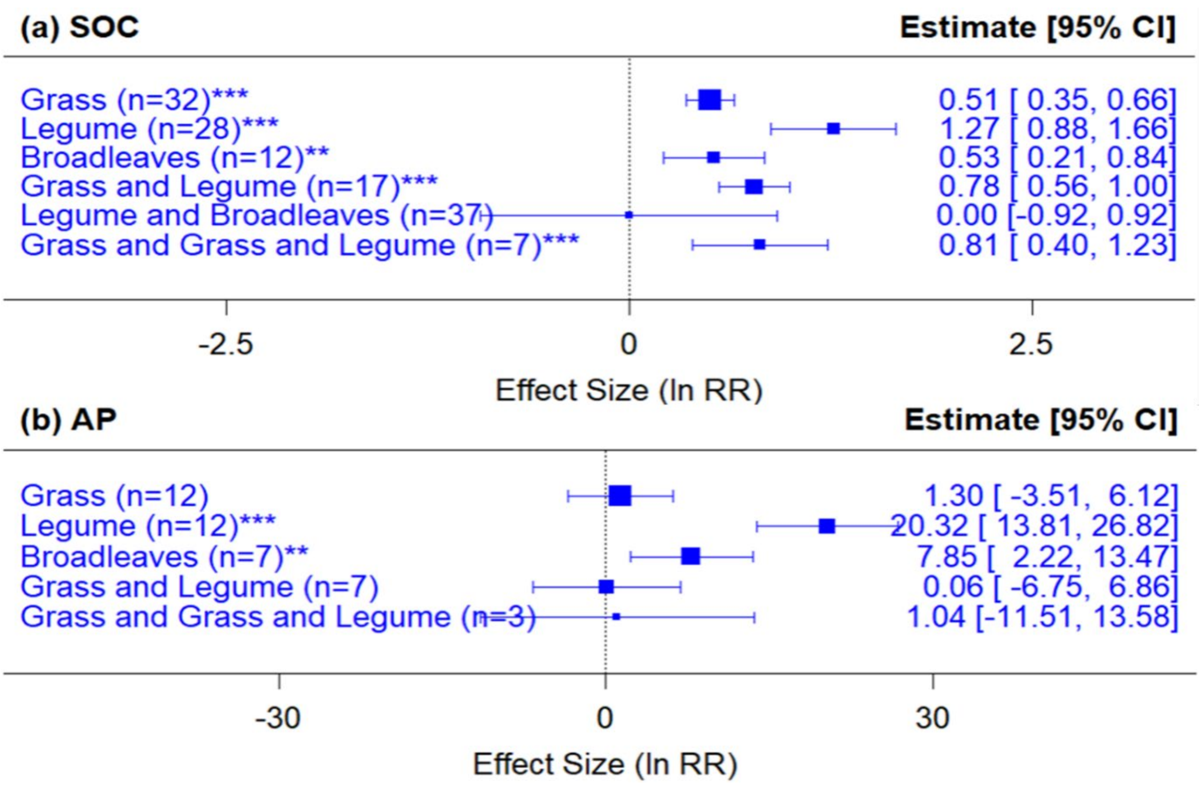


Figure 3. 2: Cover crop type effects on (a) soil organic carbon and, (b) available phosphorus. The horizontal dotted vertical line represents a zero line. The solid blue square indicates the mean ES, while 95% confidence intervals is represented by the error bars.

3.3.3. Effect of different climatic conditions on SOC and available P

Soil OC was significantly improved ($p<0.05$) by CCs under different climates, except under temperate regions (Figure 3.3a). Tropical regions had the highest SOC increase [2.18, 0.39-3.397] (Figure 3.3a). Available P was significantly increased under different climates, except under arid regions (Figure 3.3b). Tropical regions also had the highest increase in available P [50.94, 18.85-83.03] (Figure 3.3b).

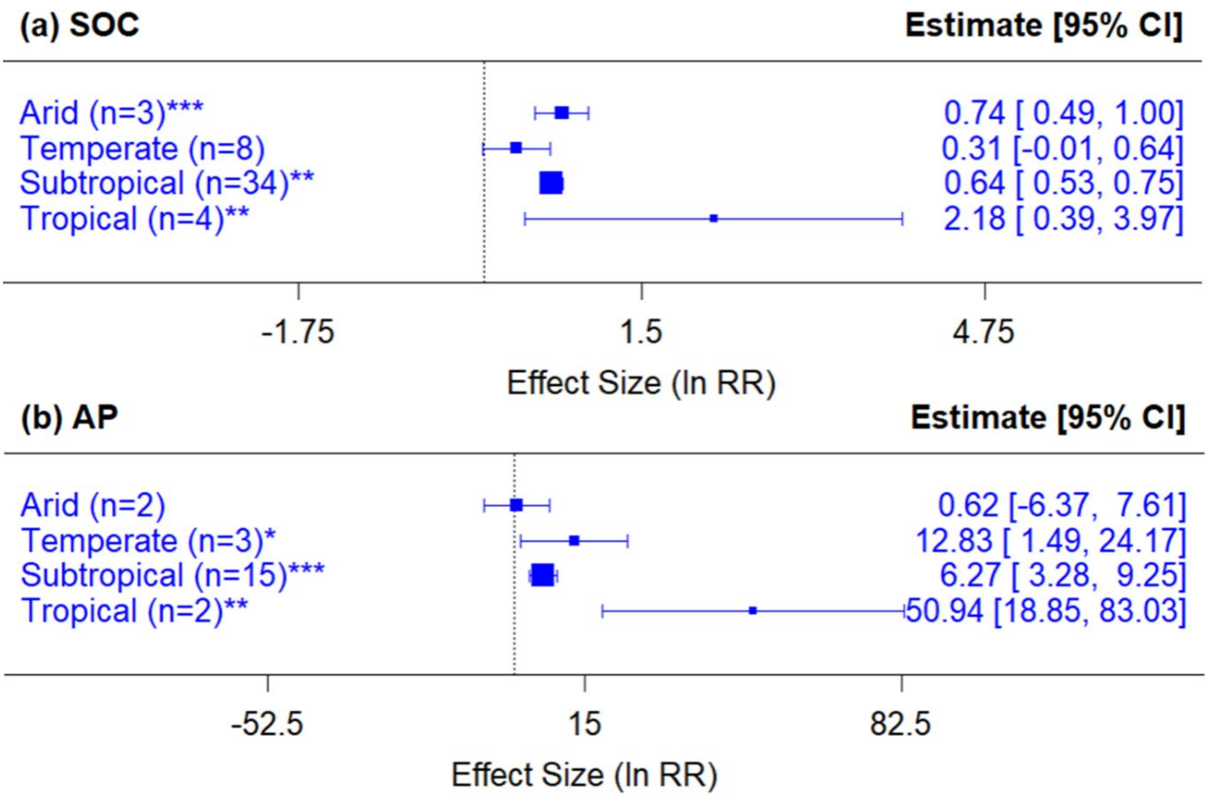


Figure 3. 3: Effects of climate on (a) soil organic carbon and, (b) available phosphorus. The horizontal dotted vertical line represents a zero line. The solid blue square indicates the mean ES, while 95% confidence intervals is represented by the error bars.

3.3.4. Effect of soil textures on SOC and available P

Different soil textures significantly affected ($p<0.05$) SOC (Figure 3.4a) and available P (Figure 3.4b). Loamy soils had the highest SOC [0.70, 0.52-0.88] and available P [9.90, 3.65-16.15], while sandy soils had the lowest increase in SOC [0.52, 0.28-0.76] and decrease in available P [-9.44; -12.77, -6.10] (Figure 3.4).

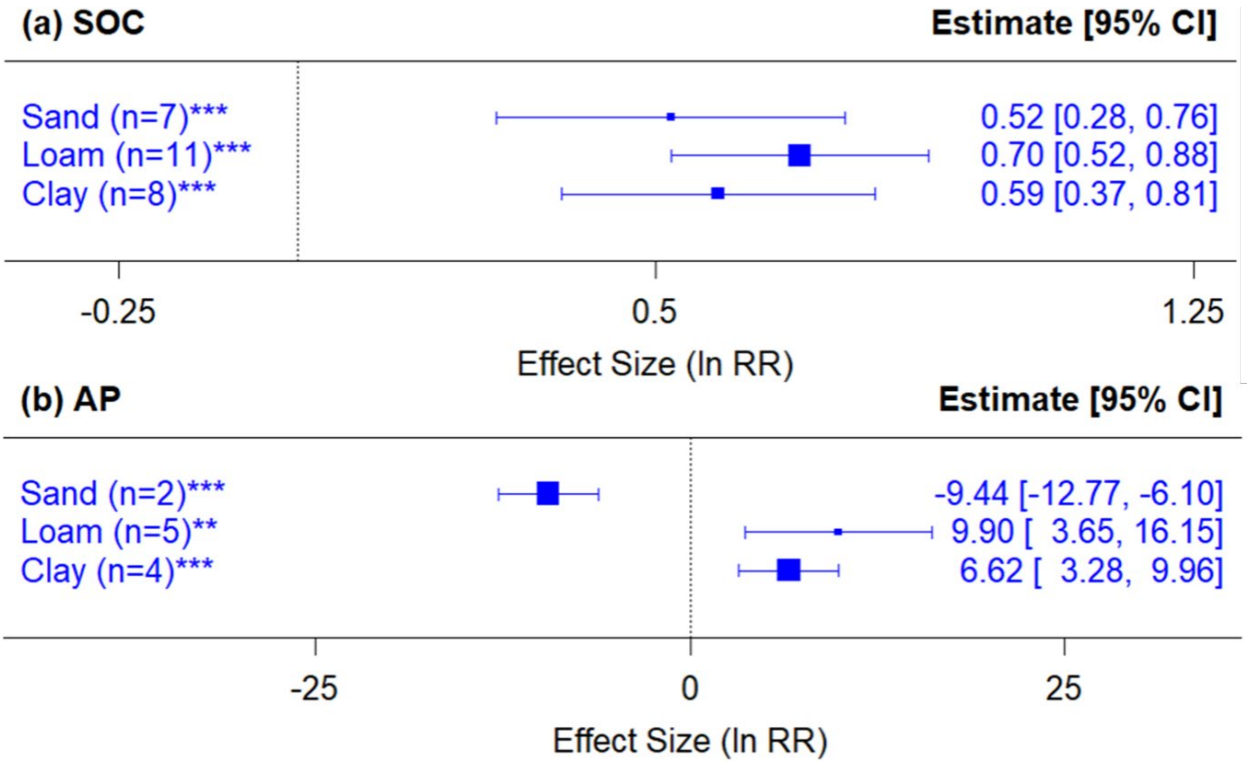


Figure 3. 4: Effects of soil texture on (a) soil organic carbon and, (b) available phosphorus. The horizontal dotted vertical line represents a zero line. The solid blue square indicates the mean ES, while 95% confidence intervals are represented by the error bars.

3.3.5. Effects of different soil pH levels on SOC and available P

Soil pH significantly affected ($p<0.05$) SOC (Figure 3.5a) and available P (Figure 3.5b), with an increase in SOC being significantly higher in neutral soils [1.68, 1.02-2.33] than in acidic soils [0.61, 0.50-0.72]. The increase in available P was higher under acidic soils [10.74, 5.04-16.44] and lower under alkaline soils [8.09, 3.41-12.76] (Figure 3.5b).

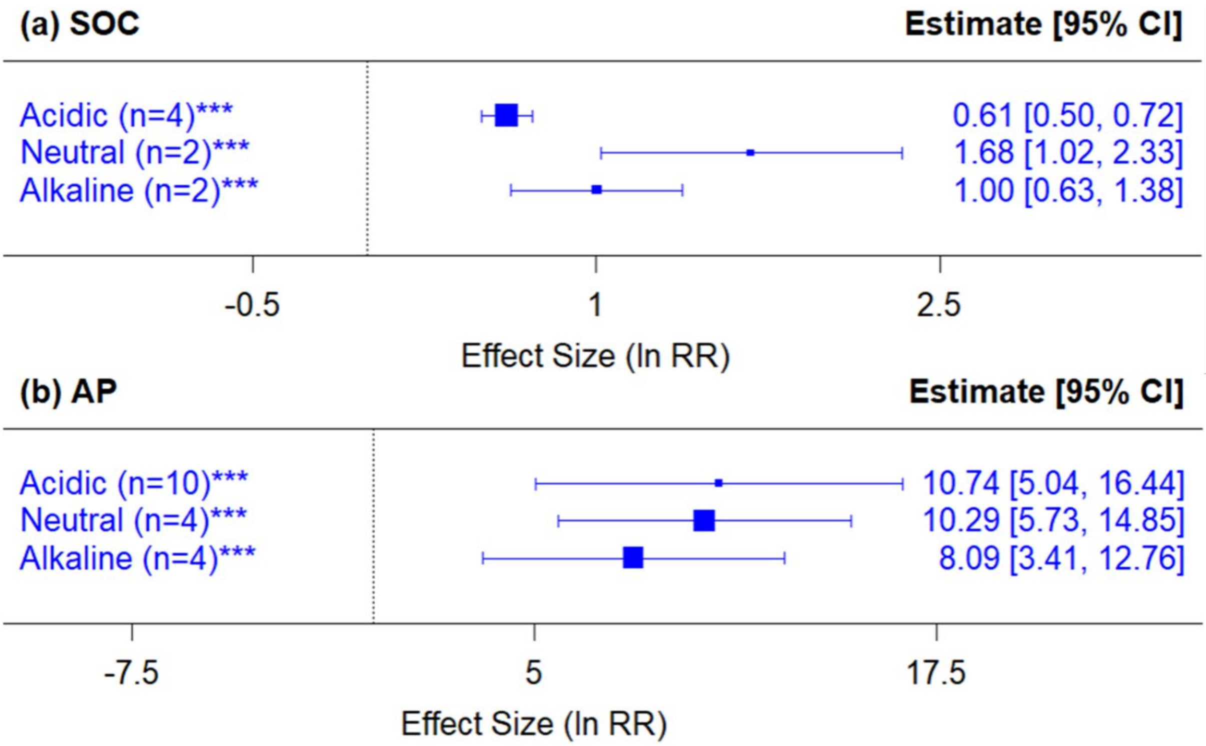


Figure 3. 5: Effects of initial soil pH on cover crop on (a) soil organic carbon and (b) available phosphorus. The horizontal dotted vertical line represents a zero line. The solid blue square indicates the mean ES, while 95% confidence intervals are represented by the error bars.

3.3.6. Effect of N fertiliser application on SOC and available P

Soil OC significantly increased ($p<0.05$) with and without N fertiliser application, where it was higher under N fertiliser application [0.65, 0.51-0.76] (Figure 3.6a), whereas available P was only significantly increased by the application of N fertiliser [16.77, 8.92-24.62] (Figure 3.6b).

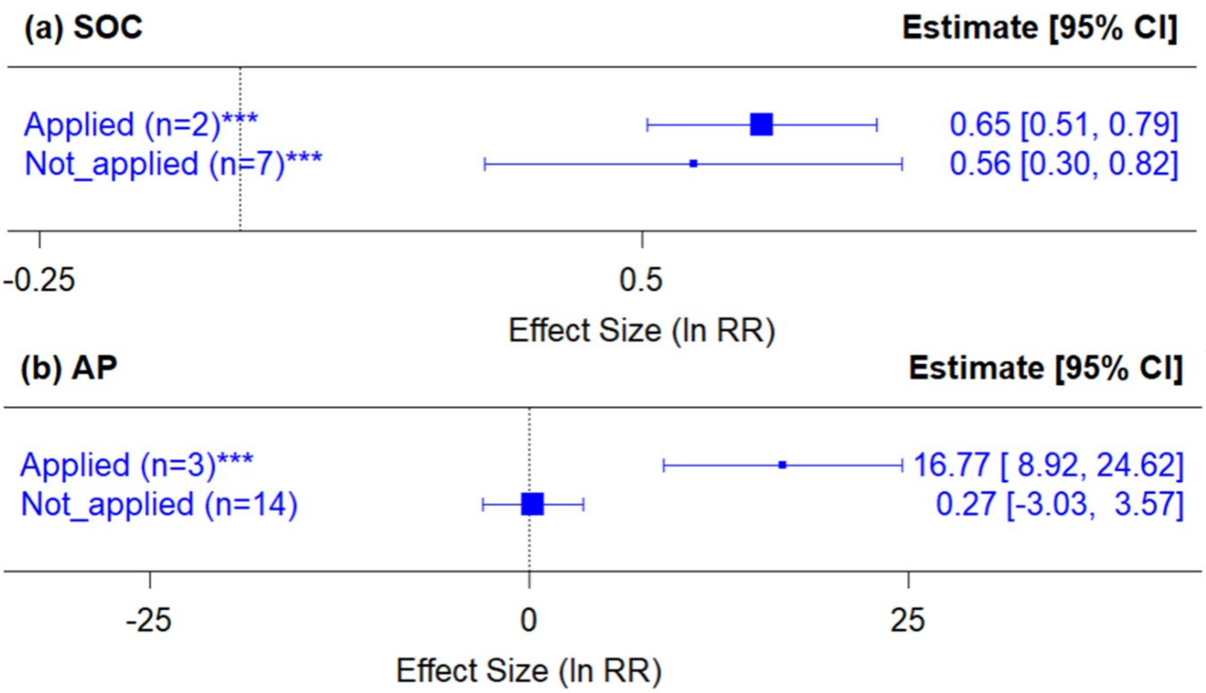


Figure 3. 6: Effects of nitrogen application on (a) soil organic carbon and (b) available phosphorus. The horizontal dotted vertical line represents a zero line. The solid blue square indicates the mean ES, while 95% confidence intervals is represented by the error bars.

3.4. Discussion

The increase in SOC under CCs was because their biomass served as a carbon source when decomposed it became part of the SOC pools. The high SOC observed under legumes might be because they can fix their own N, which may result in a higher total biomass production than other crops especially where the resources are limited. Our results were consistent with Joshi et al. (2023) meta-analysis, who reported that legumes had the highest SOC increase (8.7%) compared to mixtures and non-legume crops. Additionally, Joshi et al. (2023) indicated that the significant positive effect on SOC is due to increased carbon inputs from root exudates produced by living CCs while they are growing, along with the stabilisation of carbon from the biomass of both shoots and roots as decaying CCs break down after they are terminated. Chavarría et al. (2016) also reported that CCs significantly increased SOC, especially in leguminous cropping systems. A combination of legumes and broadleaves crops might not significantly increase SOC, but both legumes and other broadleaves crops, such as brassica, have similar decomposition rates (Koudahe et al., 2022). If both crops have a low C/N ratio, the biomass they contribute might not remain in the soil long enough to significantly increase SOC. The competition between the crops for nutrients may decrease their biomass addition, decreasing OM material. Management practices and environmental conditions also have a significant impact on whether this mixture influences OC long-term storage in the soil (Joshi et al., 2023). The choice between bicultures and sole CCs depends on species combinations and site conditions. Different species have varying root depths and nutrient needs, leading to efficient resource use. For example, a deep-rooted legume like hairy vetch can fix N, while a shallow-rooted cereals like rye scavenges nutrients. Mixing species enhances soil coverage and competition with weeds and supports diverse soil microorganisms. Faster-growing species can protect the soil while slower ones can establish an effective root system. However, bicultures may not always surpass sole CCs, as competition for resources among species can reduce effectiveness.

Overall, the meta-data analysis showed that CCs have the potential to increase available P. The increase in available P in legumes and broadleaves crops may result from organic acid exudation that lowers root-zone pH and chelates metals binding P (Pantigoso et al., 2023), enhanced mycorrhizal associations accessing otherwise unavailable P (Etesami et al., 2021), N fixation by legumes boosting plant growth and root exudates (Qiao et al., 2024), and quick decomposition of residues releasing nutrients. According to Hallama et al. (2022), rhizodeposition of CC roots improved microbial activity, which can speed up the decomposition rate and release of available P. Oberson et al. (1999) reported that legumes such as *Pueraria phaseoloides* enhance microbial biomass and phosphatase activity, resulting in higher available P than grass systems. Cover crops serve as a temporal storage for P, and the P only becomes available when the crop residues decompose, however, the decomposition rate is affected by the C/N ratio and the quality of the CC residues. For example, P stored in crop residues with high C/N might not be immediately available to the next crop due to a low decomposition rate. Grass CCs may not have a strong ability to enhance P availability in a short period compared to legumes or other broadleaved crops because of their wide C/N ratio. According to Honvault et al. (2024), a high C/P ratio in CC residues can also cause microbial P immobilisation, which can have a negative impact on the availability and uptake of P. The effects of bicultures versus sole CCs on available P vary based on plant combinations, their interactions, and local conditions. Bicultures can improve P availability through complementary traits, with one species mobilising P while the other absorbs it. Legumes in biculture can fix N, benefiting companion crops and P cycling. However, competition for the same P pool may reduce overall P availability.

In tropical regions, the combination of moisture and high temperatures provides ideal conditions for a wide variety of CCs to thrive, leading to increased SOC levels. As a result, higher levels of SOC have been observed in tropical climates. Additionally, tropical areas enhance microbial activity, accelerating the decomposition of CC residues. As a result, the effectiveness of CCs is often challenged by their rapid decomposition rates and high nutrient absorption. Therefore, there is still a need for sustainable management practices to maximise CCs benefits in tropical areas. However, the rapid growth of CCs in this climate can make up for these losses by continuously adding SOC. Ogle et al. (2005) found that CCs can enhance SOC storage by 23% in tropical climates under no-tillage. In contrast, Jian et al. (2020) observed that SOC under CCs was higher in temperate climates (18.7%) than in tropical climates (7.2%). On the other hand, temperate climates may limit CCs' growth due to shorter growing seasons (McClelland et al., 2021). Leifeld et al. (2013) observed that fluctuations in temperature and precipitation throughout the year can decrease the effectiveness of CCs to sequestrate carbon compared to warmer climates. However, certain studies have suggested that by implementing improved management practices, such as choosing CC varieties that are suitable and optimising growth periods, the potential for SOC improvement in temperate climates can be significant (Jone et al., 2018).

Janegitz et al. (2017) found that tropical and subtropical regions have very low available P content. However, warm climates in the tropics and subtropics provide ideal conditions for high biomass production, which slowly releases P when decomposed, improving available P over time. Although temperate climates have shorter growing seasons, CCs still manage to produce significant biomass, especially in spring and summer. The restricted development of CCs due to lack of water, decreased microbial activity, low root exudation, and inefficient nutrient cycling in arid climates might have resulted in the low available P observed in these climatic conditions. Arid conditions are often prone to soil crusting, reducing water infiltration and increasing runoff, thereby limiting the moisture available for CCs (Mualem & Assouline, 1996). However, SOC and soil health can be increased by the addition of CCs through improved microbial communities and SOC pools (Acharya et al., 2024).

The high SOC and available P in loamy soils might be because of the good water retention, aeration, aggregates and high microbial activity that are ideal for greater biomass production and OM addition (Dirt Connections, 2023). Loamy soil conditions also provide ideal conditions for microbial activity, increasing CCs decomposition and P mineralisation. The CCs' root exudates are more effective in loamy soils due to the close contact between the roots and the soil. This process makes P more soluble and easily accessible to plants, than in clayey soils where it can get fixed. Jian et al. (2020) observed that the increased SOC under CCs was more retained in fine-textured soil (39.5%) than in coarse-textured soil (11.4%) and medium-textured soil (10.3%). Ye et al. (2024) observed that silt loam soil had the highest available P and CEC. On the other hand, sandy soils have very low water and nutrient-holding capacity (Dirt Connections, 2023) and low microbial activity, which reduces the effectiveness of CCs in increasing SOC and available P. Unlike loamy and clayey soils, sandy soils do not provide the same aggregates that protect SOC from further breakdown (Chivenge et al., 2011); therefore, SOC levels may not significantly increase despite the presence of CCs. The sandy soils also reduce the ability of CCs to improve P cycling due to rapid decomposition due to high aeration (lowering SOC) and leaching of P due to macropores. Macropores function more for drainage such that any free P and that associated with fine particles could be leached or eluviated to deeper soil layers.

Soil pH affects the decomposition of crop residues by enhancing microbial growth, activity, and SOC solubility (Peng et al., 2023). Most plants do well when the pH level is between 6.0 and 7.5 because most of the nutrients are more soluble and readily available for plant uptake (Tătaru-Fărmuş et al., 2018). The availability of nutrients increases biomass production, which increases SOC. The ideal growing conditions in soils with a neutral pH also enable crops to develop extensive root systems that reach nutrients in deeper soil layers. Most microorganisms that break down plant residues and recycle nutrients thrive in these neutral conditions. Malik et al. (2018) reported that soils with a near-neutral pH and lower-intensity management practices can enhance carbon storage potential by promoting greater efficiency in microbial growth. However, acidic soils limit microbial growth, which reduces decomposition rates.

The higher increase in available P in acidic soils may be because CCs can increase available P in acidic soils through biological and chemical processes. Although acidic soils have limited available P due to high fixation and nutrient imbalances, CCs add OM to the soil, which releases P upon decomposition, increasing available soil P. Dube et al. (2014) suggested that residues from CCs can emit organic acids that compete for P sorption sites with orthophosphate, thereby enhancing the quantity of available P in the soil solution. In acidic soils, microbial activity may be restricted (Lei et al., 2024), but the SOC from CCs can stimulate the growth of microbial communities (Castellano-Hinojosa & Strauss, 2020), which are essential for P mineralisation. As a result, CCs residues function as a P source that releases slowly, resulting in a gradual increase in available P levels (Hansen et al., 2022).

The increased SOC and available P after the application of N fertiliser on CCs could be because N fertiliser increases plant growth and root development, leading to higher OM inputs for SOC accumulation and stimulating OM mineralisation, increasing available P. Hu et al. (2023) observed that N fertiliser application increased soil enzyme activities. Therefore, N management is important to control residue decomposition, N mineralisation, and acidification that may limit P availability or result in nutrient losses through leaching. Beltran et al. (2018) observed that N fertiliser application on CCs increased SOC than when it was not applied. They also observed that N fertiliser increased N, which is used by microbes when mineralising CC residues, thereby resulting in high P availability. However, this could also increase the potential for P leaching, particularly in sandy soils (United States Department of Agriculture Natural Resources Conservation Service, 2014).

3.5. Conclusion

Cover crops have shown great potential to increase SOC and available P, especially legumes when grown as a monoculture. This highlights the importance of carefully selecting suitable CCs, especially those that are compatible with biculture systems. Even under different climates, soil textures, and pH levels, CCs have been found to improve SOC and available P through OM addition, stimulation of microorganisms, release of root exudates, and improvement of soil structure. However, further research is necessary to measure the long-term effectiveness of CCs in arid climates and sandy soils, especially when no N fertiliser is applied to the CCs. The goal is to avoid applying fertilisers to CCs and instead use them as temporary storage for remaining nutrients while enhancing the soil's physical, biological, and chemical properties.

CHAPTER FOUR

COVER CROP AND NITROGEN FERTILISATION EFFECT ON ORGANIC CARBON POOLS IN SILAGE MAIZE-BASED CONSERVATION AGRICULTURE SYSTEM

4.1. Introduction

Soil organic carbon (SOC) including its various pools has been utilised as quality indicators for crop production sustainability, ecosystem resilience, and a possible reservoir for atmospheric carbon dioxide (CO_2) (Yang et al., 2012; Poeplau et al., 2015). The labile SOC fraction (active fraction), made up of carbon at different stages of decomposition has a high turnover rate and is easily influenced by management practices (Lucas & Weil, 2012). This fraction comprises water-soluble organic carbon (WSOC) and particulate organic carbon (POC) and serves as a measure of soil quality and provides a readily available source of nutrients for plants. The POC refers to the organic carbon (OC) in fresh and decomposing plant animal matter ranging from 53-200 µm, in particle size, while WSOC refers to the water-soluble and most active (labile) fraction and represents a minor part of total organic carbon (TOC) (Lu et al., 2011). The stable fraction, which is made up of OC that is physically and chemically protected from further decomposition by microorganisms, is not easily affected by land use or management implements but serves as the reservoir of plant nutrients. This fraction (stable) includes the mineral-associated organic carbon (MAOC), which is the most resistant fraction because of its association with fine mineral soil particles and chemical bonding through condensation/complexation and physical occlusion (Cheng et al., 2024). The fraction is highly decomposed and stored in less than 53 µm soil aggregates. The content and distribution of the different SOC fractions in agricultural soils depend on litter input and agricultural management (Beheshti et al., 2012).

The long history of conventional agricultural practices, such as monoculture of high-yielding crops, low biomass input, and intensive tillage and inorganic fertilisers (Beltrán et al., 2018), has resulted in a large area of South African soils having low fertility (Mukumbareza et al., 2016). Conservation agriculture (CA), with minimum soil disturbance, retention of crop residues and cover crops (CCs) is being promoted to improve SOC storage, reduce soil erosion, and improve soil quality. Although cover cropping is an old management practice, it has gained attention on a global scale in recent years (Romdhane et al., 2019), to reverse the effects of severe decline in biodiversity, environmental quality, and agricultural productivity (Delgado & Gantzer, 2015). The addition of CCs has improved the cycling of nutrients and improved various properties of the soil, including an increase in OC, soil structure, water holding capacity (WHC) and infiltration, and cation exchange capacity (CEC) (Wulanningtyas et al., 2021; Koudahe et al., 2022). Cover crops have also helped in reducing runoff and erosion increasing carbon sequestration, conserving soil moisture, controlling weeds, and managing pests (Weerasekara et al., 2017). Furthermore, CCs help increase microbial abundance and diversity in the soil (Vukicevich et al., 2016), including improvement of the growth of arbuscular mycorrhizal fungi, which work in symbiosis with plant roots to absorb more water and nutrients (Weerasekara et al., 2017). The success of CCs in enhancing soil properties, especially physical properties, is not always consistent (Blanco-Canqui & Jasa, 2019), and their significant effect on soil properties takes a long time to be realised.

Some studies have reported that more than 10 years is required for CCs to significantly enhance soil properties, depending on soil types, climatic conditions, farming system, and CC type (Blanco-Canqui & Jasa 2019). Cover crops like radish, faba beans, and peas have been reported to improve microorganism abundance and diversity, while rye and vetch improve soil moisture (Adetunji et al., 2020). The amount and quality of CC residues significantly influence alterations in soil properties, particularly in the various fractions of SOC.

Most winter CCs, including legumes and grasses, are commonly rotated with summer cereal crops like maize, with residues of the main crop and CCs being retained. However, some farmers produce maize for silage and only the residues of the CC are retained. Moreover, fertilisation of the CC is often considered as an extra cost such that farmers consider only fertilising the main crop. The impacts of the winter CCs and fertilisation of the silage-maize on improving soil quality, especially SOC fractions, are not clearly understood. Positive effects of CCs could benefit the main crop, especially where the effects of fertilisation of the silage-maize, are not significant. This study determined the effects of nitrogen fertilisation of silage maize in rotation with unfertilised CCs on SOC fractions in a conservational agriculture system.

4.2. Methods and materials

4.2.1. Study site

The research was carried out at the Cedara Research Station of the KwaZulu-Natal Department of Agriculture Development in Pietermaritzburg (-29.5342, 30.2668; 1051 m above sea level). The slope was about 2%, with a north west-facing direction. The study area experiences a subtropical climate with 830 to 1140 mm of annual rainfall (985 mm mean annual precipitation, MAP), moderate to hot summers, cold winters with frost, and mean minimum and maximum temperatures of 16.2°C and 21.5°C, respectively (18.85 mm mean annual temperature, MAT) (Arathoon & Magubane, 2023). The soil is relatively deep, highly weathered and leached, resulting in good drainage and poor chemical fertility. It is classified as Hutton (Hu1200) soil type (Soil Classification Working Group, 2018), which is equivalent to Ferrasols (IUSS Working Group, 2022).

4.2.2. Treatments and Experimental Design

The conservation agriculture trial, on which this study was based, started in 2003 with rotations of silage maize and individual winter CCs. Some changes were made in 2010 to include grass-legume CC combinations. Subsequently, in 2012, CCs plots were split for top-dressed N; either 0 or 120 kg N ha^{-1} (as urea) was applied once plants were knee-high (approximately 6 weeks after planting). The trial implements a no-till system for planting both the CCs and maize. An Aitchison planter is used to plant the CCs in April; the growing period is throughout winter, and they are killed in September using Glyphosate; the dry residues are left on the soil surface. The CCs do not receive any fertiliser or irrigation and are not grazed. Maize (for silage) is planted in November or December, once rainfall has raised topsoil water content sufficiently. Weeds are controlled using Glyphosate throughout the maize-growing season. The maize is removed around March every year, followed by CCs again. The size of each plot is 20 m * 12 m, and the split plot size is 20 m * 6 m. The trial has nine treatments, with three replicates, split for N fertiliser (120 and 0 kg N ha^{-1}). Treatments are (with seeding rate): black oats (50 kg ha^{-1}, *Avena strigose*); stooling rye (60 kg ha^{-1}, *Secale cereale*); forage pea (120 kg ha^{-1}, *Pisum sativum*) which was planted in 2019 in the plots that were previously planted with white clover (*Trifolium repens*); vetch (22 kg ha^{-1}, *Vicia sativa*); black oats (50 kg ha^{-1}) and vetch (22 kg ha^{-1}); stooling rye (60 kg ha^{-1}) and vetch (22 kg ha^{-1}); forage pea (120 kg ha^{-1}) and black oats (50 kg ha^{-1}). There were two control plots per replicate: control with no weeds (T8) and control with weeds (T9), weeds are controlled in June with Glyphosate. The maize was removed using a harvester for silage, leaving the roots, while those of CCs were retained. *Ampligo and Steward* were used to control Stalk borer and Fall Army Worm in the silage-maize annually. Maize and cover crops biomass data was extracted from Manson and Thurtell reports (2013a, 2014b, 2015c, 2016d, 2017e, 2019f). Manson and Thurtell measured these biomasses by harvesting the crops in the middle row per plot and weighing them, and the yield was expressed as t/ha. Statistical analysis was done from the data from 2012 to 2019 where the number of years was taken as replicates. The data from 2012 to 2018 was reported in Table 4. 2 for biomass observed after the trial was split for different N fertiliser application rates. The data from 2019 was extracted from Manson and Thurtell (2019) report as it is and reported in Table 4. 3 for biomass observed after white clover was replaced by forage pea. The statistics analysis for CCs and maize biomass was done on the earlier reports. The p-value indicates the probability that the differences are significant (and not the LSD). The lower the p-value the greater the probability of the difference. Where differences between means are greater than the LSD, the differences are significant.

4.2.3. Soil sampling

Soil samples were taken from the inter-row spaces at 0-5, 5-10 and 10-20 cm depths using a beater auger after the maize was harvested. About 30 subsamples from each depth per treatment were collected after silage maize was harvested and thoroughly mixed. The samples were then air-dried for 6 days and ground to pass a 2 mm sieve before being analysed.

4.2.4 Particle Size Analysis

The hydrometer method was used to analyse particle size (Okalebo et al., 2002). About 50 g of air-dried soil was transferred, with 50 ml of Calgon and 500 ml of distilled water, into a stirring cup, and stirred for 5 minutes. The mixture was then transferred into a 1000 ml sedimentation cylinder and was brought to volume by distilled water. A blank (Rb) was also prepared following all the steps without soil. The content's temperature was determined using a thermometer, which was used to determine the settling time. The suspension was then mixed with a plunger, and the density was measured using a hydrometer while timing. The first density (R1) was read at a determined time, and R1-Rb was equivalent to silt plus clay. The suspension temperature was measured and mixed as before and allowed to stand for the determined time. The hydrometer was placed in a cylinder 30 seconds before the determined time for a second reading (R2). The content was then passed through a 53 µm sieve and retained sand was oven-dried at 105°C and then sieved with 500 and 250 µm and a receiver at the bottom. Masses of coarse, medium, and fine sand fractions were then measured. The particle size distribution of the soil is summarised in Table 4. 1. The soil was clayey in texture. Cover crop treatments and N fertilisation did not affect soil texture, therefore, the data was showed.

Table 4. 1: Soil texture at different depths

Depth (cm)	Clay (%)	Silt (%)	Sand (%)	Soil Textural Class
0-5	42.1	19.1	38.8	Clay
5-10	42.9	17.3	39.8	Clay
10-20	43.5	17.3	39.2	Clay

The absence of letters indicates that the particle size distribution did not significantly differ at $p<0.05$.

For soil moisture correction, the moisture content of the air-dried soils was assessed by drying 10 g of each sample overnight using an oven at 105°C. The calculated moisture content of each sample was then used for moisture correction of all the analyses based on the air-dried soil samples.

4.2.5 Total organic carbon (TOC) and total nitrogen (TN)

Total OC was measured with the Walkley-Black method (Walkley & Black, 1934). About 0.25 g of soil was put into a 500 ml Erlenmeyer flask. Then 10 ml of potassium dichromate solution was added, and the mixture was stirred and left to stand for 20 minutes in a fume hood with the fan running. A blank was prepared following the same steps, starting with the addition of potassium dichromate. After 20 minutes, 170 ml of deionised water was added to the mixture, followed by 10 ml phosphoric acid (H_3PO_4) and 0.2 g sodium fluoride (NaF) and mixed well. The samples were then titrated with ferrous ammonium sulphate (FAS), with five drops of ferroin indicator added before titration. The blank was titrated first to standardise the FAS solution and determine the titration endpoint. Initially, the blank was golden brown, turning to a dark brownish-black colour upon the addition of FAS. The volume of FAS used was recorded. Nitrogen was assessed following the Automated Dumas dry combustion method (Manson & Roberts, 2000) and the LECO CNS 2000 instrument was used. A ceramic crucible with soil and vanadium pentoxide was put into a furnace and burnt in with oxygen at 1350°C. Nitrogen was then measured in a thermal conductivity cell.

4.2.6. Water-soluble organic carbon

For WSOC extraction, a 1:2 ratio of air-dried soil and distilled water was added into a centrifuge tube and shaken for 1 hr (Tao & Lin, 2000; Scaglia & Adani, 2009). The samples were centrifuged for 10 minutes at 4000 rpm and filtered into glass bottles. About 10 ml of the filtrate was put into a conical flask and OC content was determined using the Walkley-Black method (Walkley & Black, 1934).

4.2.7. Particulate organic carbon fractionation

The POC was measured using the Cambardella and Elliot (1992) method, and it was fractionated into coarse- ($_c$POC) and fine-POC ($_f$POC). A 100 ml centrifuge tubes were filled with 20 g of soil and 70 ml of sodium hexametaphosphate. The samples were shaken for 15 hours, and the mixture passed through a 250 µm sieve and then a 53 µm sieve, which was stacked below the 250 µm sieve and transferred to labelled 100 ml beakers. The soil particles that were retained in 250 µm sieve were analysed for $_c$POC and the ones in 53 µm were analysed for $_f$POC. The samples were dried using an oven for 48 hrs at 105 °C and grounded to pass a 50 µm sieve. Thereafter, the Walkley-Black method (Walkley & Black, 1934) was used to measure OC from 250 and 53 µm soil aggregates.

4.2.8. Mineral-associated organic carbon

MAOC was assessed by subtracting POC from TOC for each treatment per soil depth.

MAOC (%) = TOC (%) – POC (%)

POC (%) = fine-POC (%) + coarse-POC (%)

MAOC = stable pool

POC = labile pool

TOC = overall pool

4.2.9. Other soil chemical properties

4.2.9.1. Extractable calcium (Ca), magnesium (Mg), and acidity

Extractable Ca and Mg were measured following the procedures outlined by Manson and Roberts (2000). A sample cup was filled with 2.5 mL of soil and 25 mL of 1M potassium chloride (KCl) and mixed for 10 minutes at 400 revolutions per minute (r.p.m) using multiple stirrer. Whatman No.1 filter paper was then used to filter the content and 20 mL of 0.0356M strontium chloride ($SrCl_2$) was used to dilute 5 mL of the pipetted filtrate to determine Ca and Mg using atomic absorption. After, 10 ml was pipetted, and 10 ml of distilled water was added with 4 drops of phenolphthalein. The mixture was then titrated using 0.005 M sodium hydroxide (NaOH) to measure exchangeable acidity.

4.2.9.2. Extractable potassium (K)

A sample cup containing 2.5 mL of air-dried soil and 25 mL of Ambic-2 solution was stirred using multiple stirrer for 10 minutes at 400 r.p.m. (Manson & Roberts, 2000). The Whatman No.1 filter paper was used to filter the content and 20 mL of distilled water was used to dilute the 5 mL pipetted filtrate to measure extractable K using atomic absorption.

4.2.9.3. Acid saturation and total cations

Acid saturation was calculated as extractable acidity/CEC x100 (Manso & Roberts, 2000). The CEC was determined by adding base cations (Ca^{2+}, Mg^{2+} and K^{+}) and acidity (Manson & Roberts, 2000). The base cations were added to determine total cations (Manson & Roberts, 2000).

4.2.10. Statistical analysis of data

Two-way Analysis of Variance (ANOVA) was used to statistically analyse the data on the effects of CC treatments and N fertilisation on the CC biomass and silage maize yield and the measured parameters per depth. The Tukey test and LSD at a 5% level were used to separate between the treatment means using the Genstat 20th Edition.

4.3. Results

4.3.1. Average silage maize and cover crop biomass across the seasons

Cover crop biomass was significantly ($p<0.05$) different between the treatments and N fertiliser significantly affected ($p<0.05$) the biomass (Table 4. 2). The controls had significantly lower biomass than the CC biomass, except white clover. Black oats + clover, stooling rye + vetch and black oats biomass was significantly greater than that of vetch, stooling rye, white clover, and the controls (Table 4. 2). The CC treatments and N fertilisation interaction significantly (p<0.05) affected silage-maize biomass production (Table 4. 2). Silage-maize planted on CC treatment rows with the addition of N fertiliser had significantly higher biomass than without N fertiliser, except on vetch -N, black oats + vetch -N and stooling rye -N (Table 4. 2).

Table 4. 2: Cover crop and silage-maize biomass production from 2012 to 2018 as affected by cover crop treatments and nitrogen fertilisation

Factor	Biomass production (t ha^{-1})		
	Cover crop	Silage-maize	
		+N	-N
Control (T8)	0.000a	14.0de	8.48abc
Control (T9)	0.347a	12.6de	6.78a
Vetch	1.682b	14.4de	12.71de
White clover	0.441a	13.9de	7.19ab
Stooling rye	1.390b	13.4de	7.14ab
Black oats	2.604c	14.1de	7.29abc
Black oats + vetch	4.039d	14.8e	11.22cde
Black oats + clover	2.955c	14.9e	7.39abc
Stooling rye + vetch	2.775c	14.5de	10.74bcd
LSD	0.496	2.179	
+ N	2.015b		
- N	1.592a		
LSD	0.2337		

Different lowercase letters represent significant variation between cover crop treatments and nitrogen fertilisation within each parameter at p<0.05. There was no significant variation in the interaction of treatments. +N, 120 kg N ha^{-1} and -N, 0 kg N ha^{-1}; Control (T8), no weeds and Control (T9), weeds; LSD, Least Significant Difference.

Nitrogen fertilisation decreased CC biomass, and the control (T9) and forage biomass were significantly decreased by N fertiliser (Table 4.3). Cover crop treatments with black oats with and without N fertiliser had the highest biomass (Table 4.3). Cover crop treatments and N fertilisation interaction significantly affected silage-maize biomass at a significant level of 10% (P=0.85) (Table 4.3). While N fertilisation significantly increased silage maize biomass, except at stooling rye + vetch and vetch the biomass was still increased (Table 4.3). Silage maize planted at black oats + vetch +N row had the highest silage-maize biomass (Table 4.3). Cover treatments with vetch and without N fertiliser increased silage-maize biomass (Table 4. 3).

Table 4. 3: Cover crop and silage maize biomass production from 2019 as affected by different nitrogen fertilisation rates

Factor	Biomass production (t ha^{-1})			
	Cover crop		Silage-maize	
Cover crop	+N	-N	+N	-N
Control (T8)	0	0	# 10.70	# 6.57
Control (T9)	# 0.010	# 0.062	# 10.21	# 6.56
Vetch	0.166	0.304	9.80	9.17
Forage pea	# 0.436	# 1.059	# 10.59	# 6.88
Stooling rye	0.057	0.115	# 9.75	# 5.75
Black oats	2.061	2.333	# 9.41	# 6.08
Black oats + vetch	3.035	3.207	# 11.14	# 8.49
Forage pea + black oats	2.976	3.498	# 10.26	# 7.27
Stooling rye + vetch	0.450	0.770	10.26	8.88
LSD (0.05)	0.5738		1.771	

"#" indicates the significant effect of N fertiliser on biomass production per cover crop (p = 0.004). +N, 120 kg N ha^{-1} and -N, 0 kg N ha^{-1}; Control (T8), no weeds and Control (T9), weeds; LSD, Least Significant Difference.

4.3.2. Total organic carbon and total nitrogen

Cover crop treatments had a significant ($p<0.05$) impact on TOC except at 10-20 cm depth (Table 4.4). At 0-5 cm, TOC under stooling rye + vetch and black oats was significantly higher than under control (T8), while under the control (T9) TOC did not significantly differ from under the CC treatments (Table 4. 4). At 5-10 cm, TOC under stooling rye + vetch, forage pea + black oats, black oats, and control (T9) were significantly higher than under control (T8), while under control (T9) TOC was not significantly different from the CCs (Table 4. 4). The addition of N fertiliser did not significantly affect ($P<0.05$) TOC at all depths (Table 4. 4).

Cover crop treatments had a significant ($p<0.05$) effect on total N only at 0-5 cm depth (Table 4. 5). The total N was higher under vetch (Table 4. 5). The addition of N fertiliser significantly ($p<0.05$) increased total N only in the 0-5 cm depth (Table 4. 5).

Table 4. 4: TOC as influenced by cover crops and nitrogen fertilisation at different depths under silage-maize

Factors	TOC (%)		
	0-5 cm	5-10 cm	10-20 cm
Cover crop			
Control (T8)	4.27a	4.23a	5.25
Control (T9)	6.61ab	6.02b	4.86
Vetch	5.34ab	5.07ab	4.57
Forage pea	6.51ab	5.11ab	4.65
Stooling rye	5.33ab	5.44ab	6.36
Black oats	6.83b	6.05b	5.95
Black oats + vetch	6.57ab	5.09ab	4.59
Forage pea + black oats	5.56ab	5.98b	6.40
Stooling rye + vetch	7.40b	5.98b	4.92
LSD (Cover crop)	1.488	1.014	1.129
Nitrogen fertiliser			
+ N	5.84	5.32	5.40
- N	6.25	5.57	5.16
LSD	0.701	0.478	0.532

Different lowercase letters represent significant variation between cover crop treatments and nitrogen fertilisation per parameter at $p<0.05$. The absence of letters indicates that the treatments did not significantly vary at $p<0.05$, and there was no significant variation in the interaction of treatments. +N, 120 kg N ha^{-1} and -N, 0 kg N ha^{-1}; Control (T8), no weeds and Control (T9), weeds; LSD, Least Significant Difference.

Table 4. 5: Nitrogen as influenced by cover crops and nitrogen fertilisation at depths under silage-maize

Factors	N (%)		
	0-5 cm	5-10 cm	10-20 cm
Cover crop			
Control (T8)	0.267b	0.210	0.155
Control (T9)	0.212a	0.188	0.188
Vetch	0.322c	0.225	0.177
Forage pea	0.265b	0.182	0.160
Stooling rye	0.282bc	0.192	0.175
Black oats	0.280bc	0.207	0.188
Black oats + vetch	0.290bc	0.210	0.182
Forage pea + black oats	0.283bc	0.205	0.188
Stooling rye + vetch	0.303bc	0.220	0.190
LSD (Cover crop)	0.027	0.028	0.031
Nitrogen fertiliser			
+ N	0.29b	0.21	0.18
- N	0.27a	0.20	0.17
LSD	0.013	0.013	0.014

Different lowercase letters represent significant variation between cover crop treatments and nitrogen fertilisation per parameter at $p<0.05$. The absence of letters indicates that the treatments did not significantly vary at $p<0.05$ and there was no significant variation in the interaction of treatments. +N, 120 kg N ha^{-1} and -N, 0 kg N ha^{-1}; Control (T8), no weeds and Control (T9), weeds; LSD, Least Significant Difference.

4.3.3. Water-soluble organic carbon and Particulate organic carbon fractions

The CC treatments and N fertilisation did not significantly ($p<0.05$) affect WSOC at all soil depths (Table 4. 6). Cover crop treatments significantly ($p<0.05$) affected $_c$POC, except at 10-20 cm depth (Figure 4. 1). At 0-5 cm, $_c$POC was significantly higher under forage pea + black oats, black oats, stooling rye, black oats + vetch and stooling rye + vetch than under control (T9) and forage pea, but not significantly different from control (T8) (Figure 4. 1). At 5-10 cm, $_c$POC under stooling rye was significantly higher than under vetch, forage pea, control (T9), and black oats + vetch (Figure 4. 1). While $_c$POC under control (T9) did not significantly differ from under all other CC treatments, $_c$POC under control (T8) was significantly higher than under black oats + vetch (Figure 4. 1). Nitrogen fertilisation only significantly affected $_c$POC at 0-5 cm (Figure 4. 2), where zero N fertilisation resulted in higher $_c$POC than where N fertiliser is applied. Cover crops affected $_f$POC ($p<0.05$) at all depths (Figure 4. 3). At 0-5 cm, $_f$POC under stooling rye and stooling rye + vetch was significantly higher than under control (T8) and forage pea, and $_f$POC under control (T8) was significantly lower than control (T9) and cover crops except forage pea (Figure 4. 3). At the same time, control (T9) was not significantly different from cover crops. At 5-10 cm, $_f$POC under stooling rye and forage pea + black oats were significantly higher than under control (T8), while $_f$POC under control (T8) was not different from under other CCs and control (T9) did not differ from all the cover crop treatments (Figure 4. 3). At 10-20 cm, $_f$POC under forage pea + black oats were significantly higher than under black oats + vetch, vetch, control (T8) and forage pea, while under control (T9) it did not significantly differ from all the CCs (Figure 4. 3). Nitrogen fertiliser did not affect $_f$POC at 0-5 cm (+N, 1.37; -N, 1.54), 5-10 (+N, 1.15; -N, 1.08) and 10-20 cm (+N, 0.76; -N, 0.71) (data not shown).

Table 4. 6: WSOC as influenced by cover crops at depths under silage maize

Factors	WSOC (%)		
	0-5 cm	5-10 cm	10-20 cm
Cover crop			
Control (T8)	0.487	0.356	0.340
Control (T9)	0.567	0.289	0.231
Vetch	0.465	0.421	0.280
Forage pea	0.493	0.391	0.235
Stooling rye	0.510	0.344	0.248
Black oats	0.513	0.414	0.250
Black oats + vetch	0.219	0.384	0.414
Forage pea + black oats	0.536	0.337	0.299
Stooling rye + vetch	0.437	0.429	0.336
LSD (Cover crop)	0.4022	0.2702	0.2046
Nitrogen fertiliser			
+ N	0.467	0.350	0.281
- N	0.472	0.397	0.304
LSD	0.1896	0.1274	0.0965

The absence of letters indicates that the treatments did not significantly vary at $p<0.05$, and there was no significant variation in the interaction of treatments. +N, 120 kg N ha^{-1} and -N, 0 kg N ha^{-1}; Control (T8), no weeds and Control (T9), weeds; LSD, Least Significant Difference.

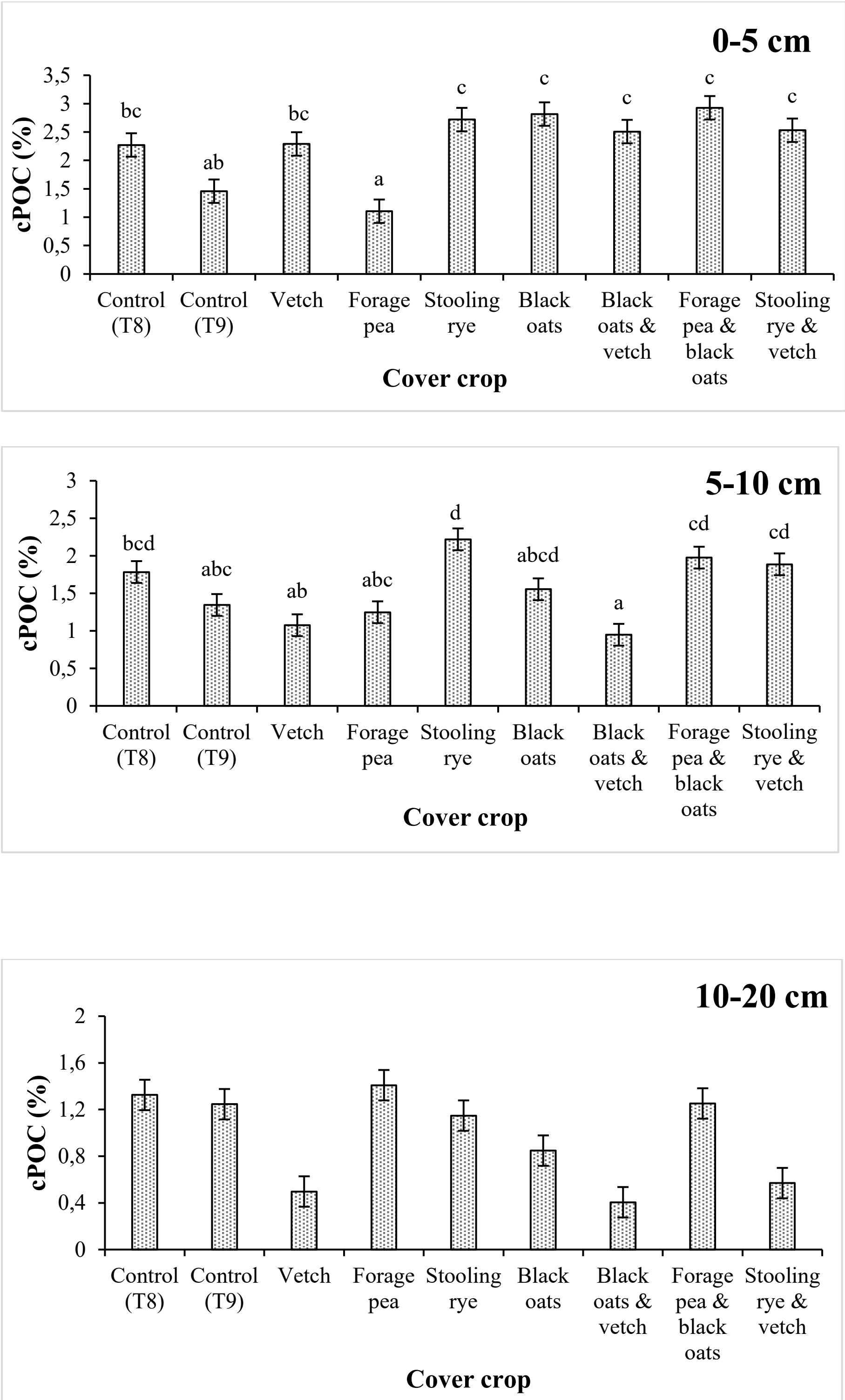


Figure 4. 1: Coarse POC as affected by cover crops at different depths under silage maize. Different lowercase letters represent significant variation at $p<0.05$ and the error bars represent standard error difference. The absence of letters indicates that the treatments did not significantly vary at $p<0.05$ and there was no significant variation in the interaction of treatments. Control (T8), no weeds and Control (T9), weeds.

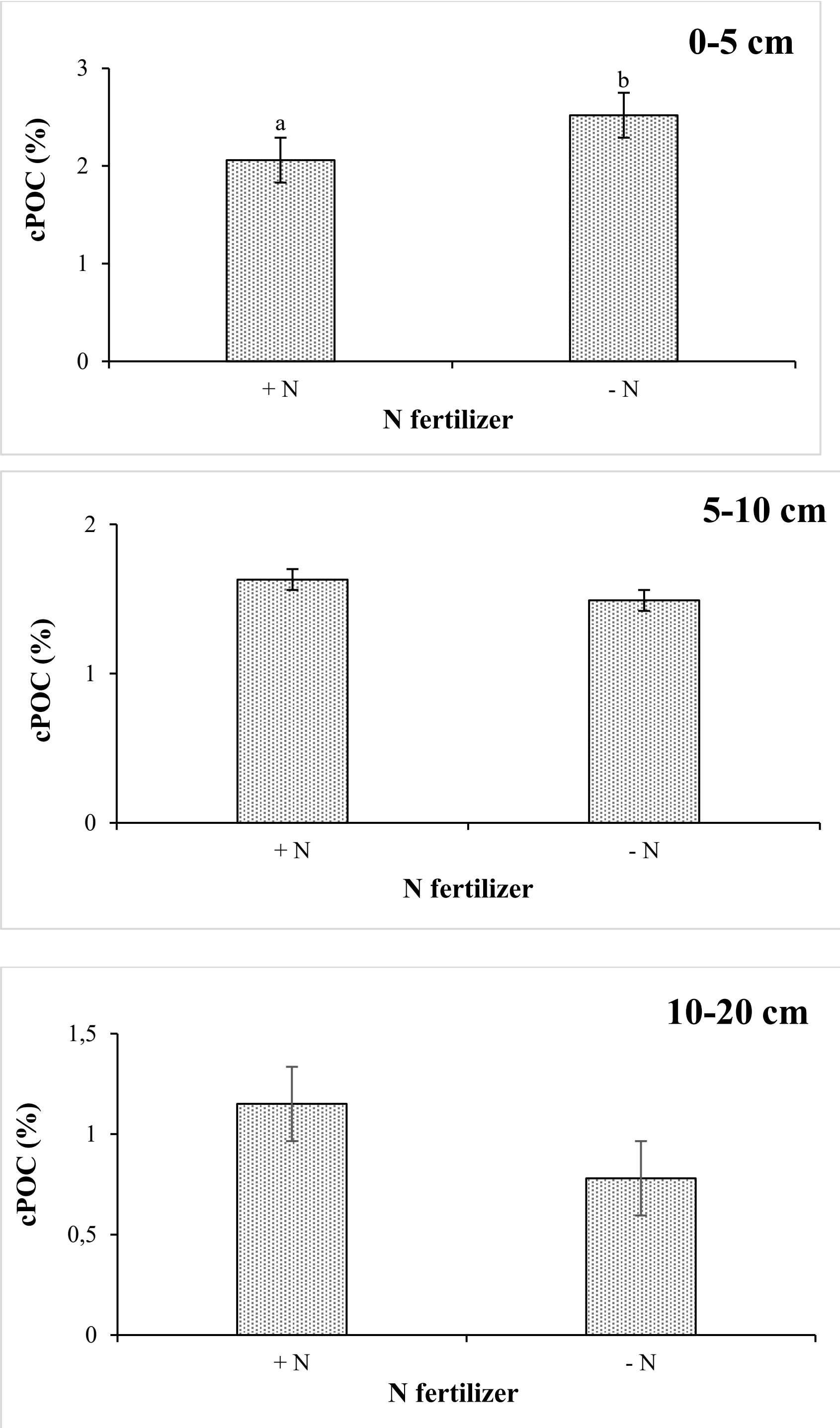


Figure 4. 2: Coarse POC as affected by nitrogen fertilisation at different depths under silage maize. Different lowercase letters represent significant variation at $p<0.05$ and the error bars represent standard error difference. The absence of letters indicates that the treatments did not significantly vary at $p<0.05$ and there was no significant variation in the interaction of treatments. +N, 120 kg N ha^{-1} and -N, 0 kg N ha^{-1}.

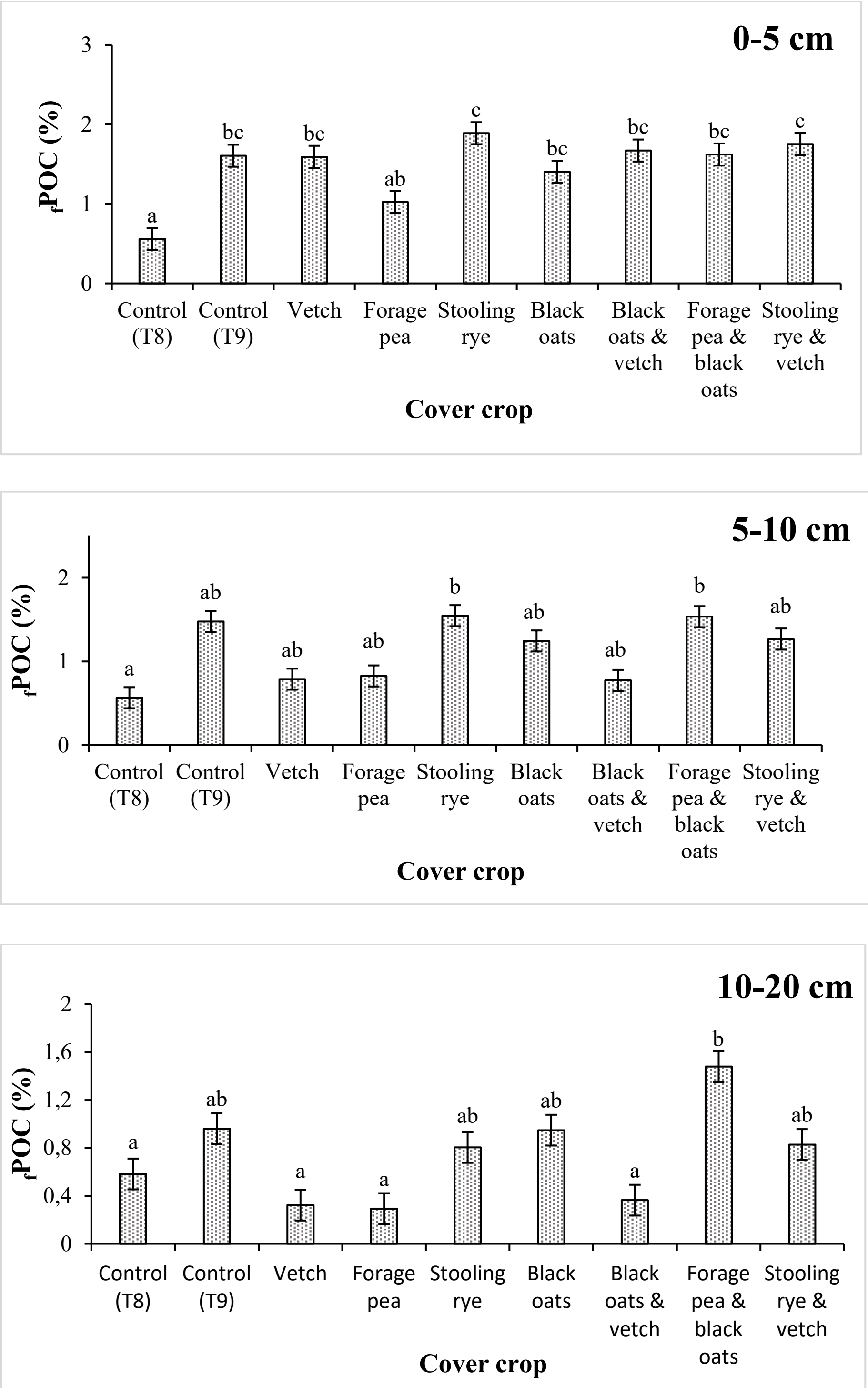
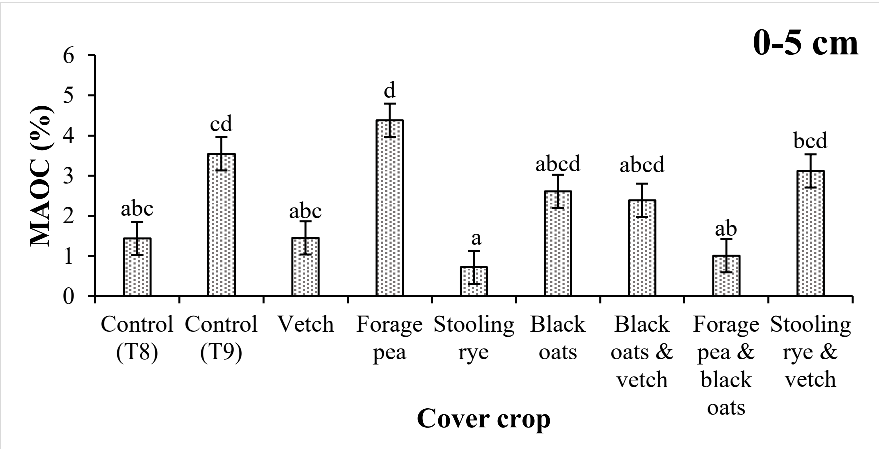
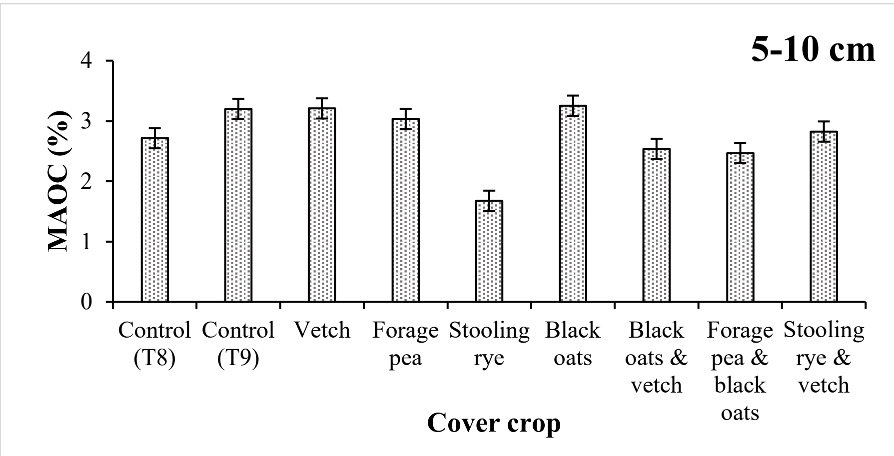


Figure 4. 3: Fine POC as affected by cover crop and nitrogen fertilisation at different depths in silage maize. Different lowercase letters represent significant variation at $p<0.05$ and the error bars represent standard error difference. The absence of letters indicates that the treatments did not significantly vary at $p<0.05$ and there was no significant variation in the interaction of treatments. Control (T8), no weeds and Control (T9), weeds.

4.3.5. Mineral-associated organic carbon

Cover crop treatments only significantly influenced MAOC (p<0.05) at 0-5 cm depth (Figure 4. 4). At that depth (0-5 cm), MAOC under forage pea was significantly higher than under vetch, forage pea + black oats, stooling rye and control (T8), while MAOC under control (T9) was significantly higher than under forage pea + vetch and stooling rye. Nitrogen fertiliser did not significantly influence (P<0.05) MAOC at all depths (Figure 4. 4). Nitrogen fertiliser did not affect (p<0.05) MAOC at 0-5 (+N, 2.41; -N, 2.18), 5-10 (+N, 2.54; -N, 3.00) and 10-20 cm (+N, 3.50; -N, 3.67) (data not shown).





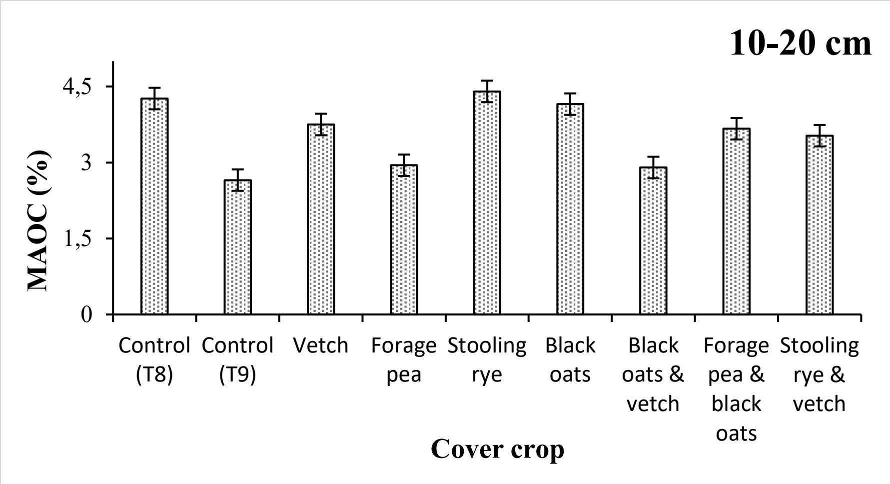


Figure 4. 4: Mineral-associated organic carbon as affected by cover crop and nitrogen fertilisation at different depths under silage maize. Different lowercase letters represent significant variation at $p<0.05$ and the error bars represent standard error difference. The absence of letters indicates that the treatments did not significantly vary at $p<0.05$ and there was no significant variation in the interaction of treatments. Control (T8), no weeds and Control (T9), weeds.

4.4. Discussion

4.4.1. Cover crop biomass and silage-maize biomass as affected by CC treatments and N fertiliser

The legume-grass combination in this study had a higher biomass from 2012 to 2018, followed by black oat. A combination of legume-grass crops was more effective than planting one crop as it allows a greater combination use of environmental resources (Tahir et al., 2023). High N increases plant growth, hence, there was a significant increase when N fertiliser was applied (Table 4. 3). The interaction of CC biomass and high N fertiliser significantly increased silage-maize biomass, especially in rows where the combination of CCs was planted because of the high biomass they produced (Table 4. 4). Cover crops are not irrigated; therefore, they depend on the remaining water after the silage-maize. According to Manson (2020), N fertiliser application increased maize biomass, which might have increased the nutrients and water absorption, reducing the nutrients and water content; this may have affected the CCs biomass production in 2019. Nitrogen fertiliser significantly increased silage-maize biomass, except where silage-maize was rotated with vetch. According to Manson (2020) these observations might be due to the vetch ability to fix N. When the vetch is decomposed, the N is released to the soil, reducing the N fertiliser significant effect.

4.4.2. Total OC and nitrogen as affected by CC treatments and N fertiliser

The higher TOC under stooling rye + vetch and black oats than the control (T8) could be explained by biomass addition from the CCs and their higher C/N ratios. This was supported by the results of average biomass addition over the years. Both black oats and stooling rye are grass crops, and their ability to increase SOC may be due to their high biomass production, high C concentration, and higher C/N ratio (Sainju et al., 2002). The higher biomass increases added C, while the higher C/N slows down the rate at which the residues decompose in the soil. However, TOC under control (T8) was also lower than the other control (T9), black oats + vetch, forage pea, forage pea + black oats, vetch, and stooling rye. Cover crops had higher biomass (Table 4. 2 and 4. 3) than the controls, which resulted in higher TOC than control (T8). The higher TOC under control (T9) than control (T8) may be due to biomass addition due to the moss that grew in the control (T9) plot. At 5-10 cm, control (T8) also had the lowest TOC than CCs and control (T9) because control (T9) also had a higher biomass than control (T8). These results show that the root biomass at 10-20 cm was not effective enough to affect TOC. According to Mazzoncini et al. (2011), incorporating CCs in crop rotation often significantly enhances the soil organic matter (SOM) amount. This was consistent with observations by López-Vicente et al. (2020), who recommended using CCs as the best solution to increase SOM. Integrating CCs into agricultural production systems has been reported to potentially offer a more sustainable alternative (Frasier et al., 2016), with SOC being significantly increased, particularly in the top 10 cm soil depth (Restovich et al., 2019). This was contrary to the results of Acuña and Villamil (2014), who reported that CCs did not significantly improve TOC because the study was done after one growing season.

In this study, vetch was a legume crop that showed a potential to increase nitrogen in the soil. This research was consistent with the findings of Chavarría et al. (2016), where N was significantly higher under CC mixture including vetch. Vetch as a legume fixes about 85-89 kg N/ha; in that way, it does not take too much N from the soil, and when the residues are returned to the soil, it increases N content. This might also be the reason why stooling rye + vetch had the highest TOC, because high N improves plant growth in that way increasing carbon content in the plant that is then stored in SOC pools. The addition of N fertiliser increased N concentration at 0-5 cm depth, and this might be due to how the fertiliser was applied. High N content also improves microbial activity in that way, increasing nutrients and OC mineralisation. Hence, vetch alone had lower OC and high N.

4.4.3. WSOC as affected by CC treatments and N fertiliser

Singh and Kumar (2021) found that CCs significantly improved WSOC by 25% compared to no CCs, but only before planting the maize. This contrasted with our findings, as CCs did not affect WSOC. According to Singh and Kumar (2021), their observations might be due to early spring freeze-thaw and high soil moisture, which increased microbial activity leading to high WSOC; however, that results in WSOC being susceptible to translocation to deeper soil layers. Cover crops showed a potential to increase TOC but also showed potential to increase total N content, which might have increased OC mineralisation, resulting in WSOC that is prone to leaching. Grebliunas et al. (2016) stated that because WSOC is a highly mobile fraction of DOC, it frequently leaches into deep layers of the soil or aquatic ecosystems within days of plant death, which likely explains why our results showed no significant differences between the treatments on WSOC. Our results supported those of Grebliunas et al. (2016), however, they suggested that the short duration of CCs may have influenced the absence of significant variation on WSOC. In contrast, Peregrina et al. (2010) reported a significant improvement in WSOC within a short term of CC management due to the different CCs (resident vegetation dominated by annual grass and forbs, including *Bromus mollis* L., *Hordeum murinum* L., *Diplotaxis erucoides* (L.) DC., *Sonchus asper* (L.) Hill, *Sonchus oleraceus* L., *Veronica latifolia* L., *Conyza canadensis* (L.) Cronquist., and *Papaver hybridum* L.; and sown 'Aurora Gold' blue fescue, *Festuca longifolia* Thuill.) used.

4.4.4. POC fractions as affected by CC treatments and N fertiliser

The alteration of POC fractions by CCs showed they are indeed susceptible to changes in carbon inputs (Tirol-Padre & Ladha, 2004). These results show that the root biomass at 10-20 cm was also not effective enough to affect all the SOC fractions. This study shows that retaining crop residues increase POC in the soil, however, forage pea as a legume crop has a low C/N ratio which results in high mineralisation of OC, in that way decreasing $_c$POC. Maize biomass was higher on the control (T8) than the control (T9) (Table 4.2 and Table 4.3), therefore, the maize root biomass distribution might explain the increase in $_c$POC under control (T8) than in control (T9). The addition of N fertiliser decreased $_c$POC at 0-5 cm. Nitrogen fertiliser increased maize biomass, which might have increased water and nutrient uptake by maize, leaving less fewer nutrients and moisture for CCs, affecting their biomass production. This decreased the residues returned in the soil, decreasing POC. Nitrogen fertiliser might have also improved microbial activities, increasing SOC mineralisation. However, the results show that N fertiliser was not effective enough to affect other SOC fractions. Stooling rye and stooling rye + vetch showed the potential to increase $_{f}$POC than the controls due to high TOC in these CCs, and the high TN helped in decomposing stooling rye by stimulating microbes, resulting in high sequestration of $_{f}$POC. At 5-10 cm depth, the results suggest that the effectiveness of CCs declined as the soil depth deepened, which is consistent with TOC and TN results. This might be due to the decrease in CC residues at deeper layers as they were left on the soil surface and lower the activity of microorganisms. Peregrina et al. (2010) observed that CCs affected POC except at 15-25 cm depth. The distribution of crop roots also plays a role in SOC content and its dynamics which might explain the results at 10-20 cm. Belmonte et al. (2018) and Restovich et al. (2019) also observed that CCs affected POC. Belmonte et al. (2018) found that CCs enhanced both coarse and fine POC; however, the interaction of CCs and tillage decreased POC fractions therefore, tillage was not suitable for POC availability. Restovich et al. (2019) also reported that CCs affected POC in all soil depths where it was higher under oats and vetch, while the addition of N fertiliser on maize did not affect POC. According to Duval et al. (2016), how long the CCs have been incorporated in a trial is also important, as they observed that three years of carbon input by CCs was not enough to have an impact on the most dynamic and labile OC fractions.

4.4.5. MAOC as affected by CC treatments and N fertiliser

Mineral-associated organic carbon is a stable OC fraction. As a result, it was only influenced by CCs at 0-5 cm depth. Cover crops effect on MAOC varied from what was observed from TOC and POC as it was higher under forage pea and lower under forage pea + vetch than the controls. However, increased TOC and TN might have resulted in significant differences observed in MAOC between CC treatments at 0-5 cm depth. According to Ding et al. (2006), residues from various plant types do not contribute to the production of humus to the same degree, and there are multiple mechanisms influencing the OM sequestration in the soil. Restovich et al. (2019) also found that CCs did not influence MAOC at all depths and N fertiliser affected MAOC only at 0-5 cm. Beltrán et al. (2018) found that CCs affected TOC and POC fractions but not MAOC and that N fertiliser addition increased TOC and $_{f}$POC while it decreased $_{c}$POC.

4.4.6. Basic soil properties

How the studied basic soil properties were affected by the incorporation of CCs and nitrogen fertilisation was a somewhat complex (Appendix Table 1, 2, 3, 4, 5 & 6). Only Ca was not affected by CCs at 0-5 cm (Appendix Table 1), CCs did not affect all the parameters at 5-10 cm (Appendix Table 1, 4, & 6), and only K was not affected by CCs at 10-20 cm depth (Appendix Table 5). The heavy rainfall (985 mm mean annual precipitation) in Cedara at KwaZulu-Natal might also have played a role in this variation. Even though Ca was only affected at 10-20 cm soil depth, it was greater than both Mg and K in all depths due to its high adsorption strength which made it less susceptible to leaching (Ahmed et al., 2025). High SOC content increases cation adsorption sites which might be the reason why black oats had the highest total cations at 10 cm depth. Cover crop treatments with black oats showed the potential to increase base cations at 0-5 cm depth, but forage pea and forage pea + black oats showed potential to increase base cations at 5-10 cm depth. Vetch showed the potential to increase acid saturation and decrease base and total cations. During TN mineralisation, hydrogen ions that are released might increase soluble aluminium ions and decrease base cations, and vetch had the highest TN (McCauley et al., 2009; Hamidi et al., 2021). The significant difference in acid saturation, Ca, Mg, and total cations at 10-20 cm depth might be caused by leaching due to 985 mm mean annual rainfall received in Cedara.

4.5. Conclusion

Under the circumstances of this study, the CC biomass and maize yield increased, especially where N fertiliser was applied. However, the low biomass of CCs in 2019 shows that the low availability of water and N fertiliser application after the main crop may have a huge negative impact to the production of CC biomass. Overall, the winter CCs showed great potential for increasing TOC, TN, $_c$POC, $_f$POC, and MAOC in a no-till silage-maize monoculture. Black oats, stooling rye + vetch at 0-5 cm depth and black oat at 5-10 cm showed greater TOC. Vetch, black oats, stooling rye, forage + black oat, black oat + vetch and stooling rye + vetch had higher TN. At 0-5 cm, forage pea + black oat, stooling rye +vetch and black oats had higher POC fractions, while black oat and stooling rye also showed potential to increase $_c$POC and $_f$POC, respectively. Forage pea + black oat and stooling rye also had high POC fractions at 5-10 cm depth layer, while stooling rye showed the potential to increase $_c$POC only. Forage pea showed a potential to increase $_c$POC at 10-20 cm and MAOC at 0-5 cm. However, the production of dry matter was likely insufficient to change the organic content at lower depths. Furthermore, CCs had little effect on WSOC. In addition, N fertiliser was found to be ineffective in affecting all OC fractions at all depths. Winter CCs can improve soil quality and crop productivity under limited irrigation and fertilisation. The improvement in soil quality was mainly in the upper 10 cm. However, more research is needed to further understand the influence of management practices and climate variability on the benefits and efficiency of winter CCs.

CHAPTER FIVE

COVER CROP AND NITROGEN FERTILISATION EFFECTS ON PHOSPHORUS FRACTIONS IN SILAGE MAIZE-BASED CONSERVATION AGRICULTURE SYSTEM

5.1 Introduction

Phosphorus (P) is among the most limiting essential plant elements for crop production, together with nitrogen (N) and potassium (K) (Harman, 2017). Phosphorus plays a crucial role in the growth and reproduction of the plant by storing and transferring energy produced through photosynthesis (Malhotra et al., 2018). It is required in significant quantities and absorbed by plants in its inorganic form as phosphoric acid (H_3PO_4), dihydrogen phosphate ($H_2PO_4^-$) and hydrogen phosphate (HPO_4^{2-}) (Glaser & Lehr, 2019). Soil P fractions include organic P (P in the organic matter (OM), and microbial biomass) and inorganic P like soluble P, P fixed in aluminium (Al) and iron (Fe) oxides (Nziguheba et al., 1998), calcium (Ca), clay minerals and primary P minerals such as P fixed in apatite rocks. Hence, a significant proportion of P is unavailable for plant uptake. Considering that soil P is non-renewable, understanding P dynamics in agricultural systems is important for effective management.

According to Hilton (2020) and Alewell et al. (2020), P is increasingly depleted in soils worldwide, even in regions that use high inorganic fertilisers, with South America, Eastern Europe, and Africa having the highest depletion rates (Alewell et al., 2020). The factors influencing soil P availability include soil properties such as texture and pH, climate (precipitation), mineralogy, OM, and fertiliser application (Muindi, 2019). Areas with high rainfall, such as those in KwaZulu-Natal, tend to have acidic soils due to the leaching of basic ions (such as K^+, Na^+, Mg^{2+} and Ca^{2+}), erosion and runoff, especially on sand soils (Raath et al., 2018). A high solubility of Al and Fe in acidic soils (below pH 5.5) and Ca at high pH (greater than pH 7.5) results in precipitation with P, causing its immobilisation. Higher clay content results in greater P fixation on adsorption sites (Machado et al., 2012; Poswa, 2016). Fertiliser P has been used to increase P availability and maximise agricultural production. However, high P fertiliser inputs have been reported to be both inefficient and unsustainable in the long term for agricultural production and environmental quality (Van der Salm et al., 2017). Another issue is that chemical P fertilisers are very expensive in developing countries, such as those in Africa, where farmers cannot afford the high costs (Alewell et al., 2020). As such, strategies that recycle the applied P can improve fertiliser use efficiency and reduce farmers' costs and financial loss. Conservation agriculture (CA) is one such strategy that has been promoted to improve agricultural sustainability by increasing and maintaining high yields and profits because of conserving soil resources, protecting the environment and responding to climate change (Donovan, 2020; Dube et al., 2014).

Conservation agriculture includes practices such as zero tillage, cover crops (CCs), permanent soil cover with crop residues and different crop varieties through rotation or intercropping (Donovan, 2020). Dube et al. (2014) reported that winter cover CCs could improve P availability in the soil as they observed a positive interaction between CC biomass and total P and some labile P content. Cover crops absorb nutrients from the soil, especially perennial CC roots, which can reach deeper depths, absorbing P. In this way, P is not lost through leaching and joining groundwater and other water bodies. When CC residues are incorporated into the soil, the P is released from them through decomposition and becomes available for the main crop next season (Damon et al., 2014). The residues of CCs are decomposed by microbes using the N they consume. The application of N fertiliser increases microorganism activity, increasing the decomposition rate and releasing nutrients to the soil to be used by other plants. Cover crops had higher biomass than the controls (Chapter 4). The preliminary findings from Chapter 4 showed that CCs have the potential to increase total organic carbon (TOC) and its fractions, except water-soluble organic carbon (WSOC), and total nitrogen (TN). The results showed that black oat and stooling rye + vetch had the potential to increase TOC, while forage pea + black oat, stooling rye + vetch, black oat + vetch, black oat and stooling rye increased particulate organic carbon (POC) fractions; and forage pea had high mineral-associated organic carbon (MAOC). The N fertiliser application to the silage maize significantly decreased coarse POC and increased N content. In most CA, the remains of the main crop and CCs are both retained. However, there are limited studies where only CC residues are retained while only the main crop is fertilised and residues are removed, as is the case with silage maize. The higher SOC and its fractions could limit the sorption of P through competitive sorption sites. Moreover, the lower labile SOC fractions could be a result of rapid decomposition of legume residues, mineralising nutrients, including P. Therefore, the influence of the CCs, including the bicultures, on available P and other P fractions needs to be understood in CA systems especially those with silage maize, where the maize biomass is removed.

5.2. Methods and materials

5.2.1. Study site experimental design

This research was conducted from the same running trial described in Chapter 4 and the soil samples that were taken in the previous chapter were used for this study. For soil moisture correction the soils (10 g) were dried in an oven overnight at 105 °C, and the moisture content of the air-dried soils was calculated.

5.2.2 Soil pH (KCl)

Soil pH was measured following the method from Manson and Roberts (2000). About 25 mL of 1M potassium chloride (KCl) and 10 mL of soil were mixed in a sample cup, and the mixture was stirred with multiple stirrer for 5 minutes at 400 revolutions per minute (r.p.m). A gel-filled combination glass electrode was used to measure the pH while stirring after it had been left to stand for 30 minutes.

5.2.3 Total P

Total P was analysed using the Kovar and Pierzynski (2009) method. About 2 g of soil (<0.5 mm) and 20 ml nitric acid (HNO_3) were mixed in a 250 ml volumetric flask for each sample before heating at 203°C using a preheated sand bath in the acid hood for 40 minutes to oxidize soil organic matter (SOM). After adding 30 ml of 70% perchloric acid ($HClO_4$) and heating until the insoluble material became white sand, distilled water was put into the mixture to get it to volume, and the sediments were left to settle. About 2 ml of the content was transferred to a 50 ml flask and mixed with five drops of *p*-nitrophenol indicator. Drops of 5M sodium hydroxide (NaOH) were added to adjust the solution's pH till the colour changed from colourless to yellow. After, 4 ml of colour reagent B was put into the mixture to develop the heteropoly blue colour, and distilled water was added to get the solution to the volume and mixed. From the 10 ppm P working solution 0, 1, 2, 4 and 6 ml standards were pipetted into 50 ml volumetric flasks respectively. Then, the indicator was added, pH was regulated, and the content was brought to the volume using the above steps. The samples were left to stand for 10 minutes for colour development, and the absorbance was measured at 880 nm using a UV spectrometer. The standards were analysed first to get the standard curve to calculate the P concentration (ppm).

5.2.4. Extractable P

Extractable P was measured following the method from Manson and Roberts (2000). A sample cup containing 2.5 mL soil and 25 mL Ambic-2 was stirred using multiple stirrer for 10 minutes at 400 r.p.m. The contents were filtered using Whatman No.1 filter paper, and 2 ml was pipetted to measure P by a modification of the molybdenum blue procedure (Hunter, 1974).

5.2.5. Phosphorus fractionation

Total P was analysed using the Zhang & Kovar (2000) method. To extract the soluble and loosely bound P, 50 ml of 1M ammonium chloride (NH_4Cl) and 1 g soil were added to centrifuge tubes (100 ml) and shaken for 30 minutes. After centrifuging the tubes, the supernatant was poured into a volumetric flask (50 ml) and filled with deionised water to the volume. To extract aluminium phosphates (Al-P), 50 ml of 0.5M ammonium fluoride (NH_4F) (pH 8.2) was added to the residues and shaken for an hour. Following the centrifugation of the tubes, the aliquot was poured into a volumetric flask (100 ml). After two rounds of washing with 25 ml sodium chloride (NaCl), the soil residues were centrifuged. Aluminium phosphates P extracts were added to the washings, and distilled water was used to fill the volumetric flask to the volume. Iron phosphates (Fe-P) were then extracted from the residues by adding 50 ml of 0.1M sodium hydroxide (NaOH) and shaken for 17 hours. Following the centrifugation of the tubes, the aliquot was poured into a volumetric flask (100 ml). Following two rounds of washing with 25 ml NaCl, the samples were centrifuged. After mixing the washings with the Fe-P extract, deionised water was added to fill the flask to the volume.

The residues were then heated at 85°C in a water bath with 40 ml of 0.3M disodium citrate ($Na_2C_6H_5O_7$) and 5 ml of 1M sodium bicarbonate ($NaHCO_3$). After, reductant-soluble P was extracted by adding 1 g sodium dithionite ($Na_2S_2O_4$) and stirring. After another 15 minutes of heating, the samples were centrifuged. After transferring the aliquot into a volumetric flask (100 ml), the residue underwent two centrifugations and was rinsed with 25 ml of NaCl. Reductant-soluble P extracts were mixed with the washings, and distilled water was added to fill the flask to the volume. After that, the flasks were exposed to air to oxidize $Na_2S_2O_4$.

To extract calcium phosphates (Ca-P), 50 ml of 0.25M sulfuric acid (H_2SO_4) was poured into the remains and shaken for an hour. After centrifuging for 10 minutes, the aliquot was poured into a volumetric flask (100 ml). After two rounds of washing with 25 ml of NaCl, the soil was centrifuged. After mixing the washings with Ca-P extracts, deionised water was added to fill the flask to the volume.

To analyse, 2 ml of the extract was pipetted into a 100 ml beaker for analysis, along with 8 ml of distilled water and 15 ml of 0.8M boric acid (H_3BO_3) for Al-P extracts. The beakers holding the extracts of Ca-P and Fe-P were filled with 5 drops of a p-nitrophenol indicator. Drops of 2M hydrochloric acid (HCl) for Fe-P extracts and 2M NaOH for Ca-P extracts were added till the colour of the indicator changed to adjust their pH. Each beaker received 10 ml of colour reagent and was left to stand for 50 minutes for colour development. Six standards were prepared for each extracted P fraction, whereby 0, 1, 2, 4, 6 and 8 ml of the 100 mg P/L were pipetted to the six volumetric flasks respectively. The volumetric flasks were then brought to volume using the extractant solution. The UV spectrometer was used to analyse the samples for absorbance at 640 nm. The standards were analysed first to get the standard curve to calculate the P concentration (parts per million, ppm) from the extracts.

5.2.6. Statistical analysis of data

Genstat 20th Edition software was used to do a two-way Analysis of Variance (ANOVA) on data about the impacts of CC treatments and N fertilisation for each variable per soil depth. A Tuckey test was then used to distinguish the treatment means at a significance level of 0.05.

5.3. Results

5.3.1. Soil pH

Cover crop treatments significantly affected ($p<0.05$) soil pH at all depths (Table 5. 1). Soil pH was significantly lower under vetch in all depths, while soil pH under the control (T8) was significantly higher in the upper 10 cm depth (Table 5. 1). The soil pH was significantly higher under forage pea + black oats than under the cropping systems with vetch, and vetch also had a significantly lower soil pH than the controls and forage pea at 10-20 cm. Nitrogen fertilisation also significantly affected the pH in the top 5 cm by significantly decreasing the pH (Table 5. 1).

Table 5. 1: Soil pH as influenced by cover crop treatments and nitrogen fertilisation at different soil depths in silage maize

Factors	pH		
	0-5 cm	5-10 cm	10-20 cm
Cover crop			
Control (T8)	5.04b	4.84c	4.81bc
Control (T9)	4.75ab	4.63abc	4.78bc
Vetch	4.65a	4.34a	4.44a
Forage pea	4.88ab	4.67bc	4.75bc
Stooling rye	4.84ab	4.60abc	4.56ab
Black oats	4.98ab	4.70bc	4.62abc
Black oats + vetch	4.72ab	4.57abc	4.54ab
Forage pea + black oats	4.85ab	4.78c	4.89c
Stooling rye + vetch	4.69ab	4.47ab	4.55ab
LSD (Cover crop)	0.236	0.187	0.179
Nitrogen fertiliser			
+ N	4.52a	4.56	4.69
- N	5.13b	4.68	4.64
LSD	0.111	0.088	0.085

Different lowercase letters represent significant variation within each soil depth at $p<0.05$, and no significant variation was observed in the interaction of the treatments. +N, 120 kg N ha^{-1} and -N, 0 kg N ha^{-1}; Control (T8), no weeds and Control (T9), weeds; LSD, Least Significant Difference.

5.3.2. Total P

The interaction of CC treatments and N fertilisation significantly impacted ($p<0.05$) total P at 0-5 and 5-10 cm depth (Table 5. 2). At 0-5 cm, total P under black oats + vetch +N was significantly higher than under other CC treatments, followed by stooling rye + vetch +N (Table 5. 2). The total P under control (T8) was significantly lower than other CC treatments, irrespective of whether there was N fertiliser on the silage maize or not (Table 2). However, the total P under Control (T9) with or without N was not different from under black oats, vetch -N, forage pea +N and forage pea + black oats -N (Table 5. 2). At 5-10 cm, total P under black oats + vetch +N was significantly higher than other CC treatments (Table 5. 2). While total P under control (T9) +N and control (T8) with and without N was not significantly different from under forage pea -N and black oats + vetch -N, while total P under control (T9) -N was higher than under forage pea + black oats -N, stooling rye -N, and under stooling rye + vetch -N (Table 5. 2). The N fertiliser significantly increased total P at 0-5 (+N, 1051b; -N, 1006a) and 5-10 cm (+N, 997b; -N, 977a). Cover crop treatments affected total P at 10-20 cm soil depth at $p<0.05$, where black oats, black oats + vetch and stooling rye had a significantly higher total P than under both controls (Table 5. 3). The total P under black oats was not different from vetch and stooling rye + vetch, while total P under control (T8) was significantly lower than the total P under CCs and control (T9). However, N fertilisation did not influence total P at the 10-20 cm soil depth (Table 5. 3).

Table 5. 2: Total P (mg/kg) as affected by cover crops and nitrogen fertilisation at different depths in silage maize

Cover crops	0-5 cm		5-10 cm	
	- N	+ N	- N	+ N
Control (T8)	847a	919b	939a	931a
Control (T9)	1067hi	1069hi	961b	930a
Vetch	1080hi	1005de	1056f	999d
Forage pea	985d	1060ghi	936a	1025e
Stooling rye	949c	1036fg	971bc	975c
Black oats	1068hi	1086ij	1057f	1046f
Black oats & vetch	1008de	1143k	936a	1072g
Forage pea & black oats	1058gh	1026ef	962b	1000d
Stooling rye & vetch	991d	1113j	972bc	998d
LSD (Cover crop x N)	15.0		6.27	

Different lowercase letters represent significant variation within each soil depth at $p<0.05$, and no significant variation was observed in the interaction of the treatments. +N, 120 kg N ha^{-1} and -N, 0 kg N ha^{-1}; Control (T8), no weeds and Control (T9), weeds; LSD, Least Significant Difference.

Table 5. 3: Total P as influenced by cover crops and nitrogen fertilisation at 10-20 cm depth in silage maize

Factors	Total P (mg/kg)
Cover crop	
Control (T8)	861a
Control (T9)	906b
Vetch	938bc
Forage pea	945c
Stooling rye	995d
Black oats	1017d
Black oats & vetch	997d
Forage pea & black oats	945c
Stooling rye & vetch	932bc
LSD (Cover crop)	22.9
Nitrogen fertiliser	
+ N	946
- N	951
LSD	10.8

Different lowercase letters represent significant variation within each soil depth at $p<0.05$, and no significant variation was observed in the interaction of the treatments. +N, 120 kg N ha^{-1} and -N, 0 kg N ha^{-1}; Control (T8), no weeds and Control (T9), weeds; LSD, Least Significant Difference.

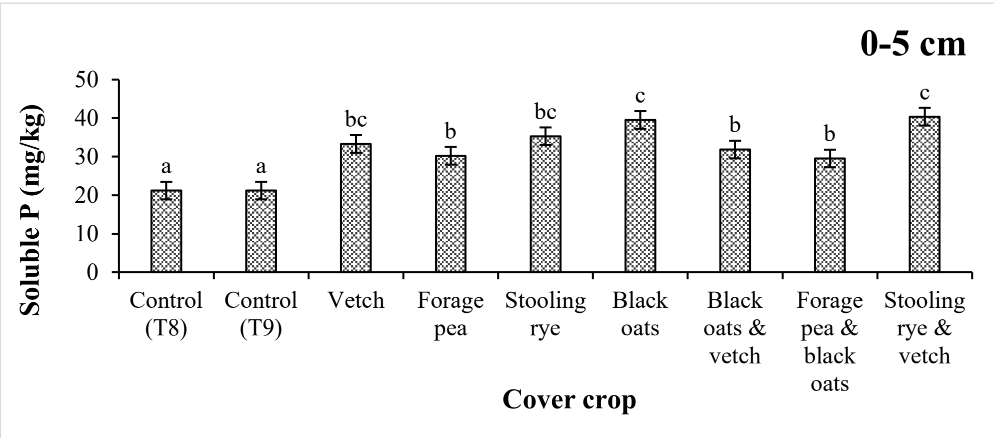
5.3.3. Extractable P

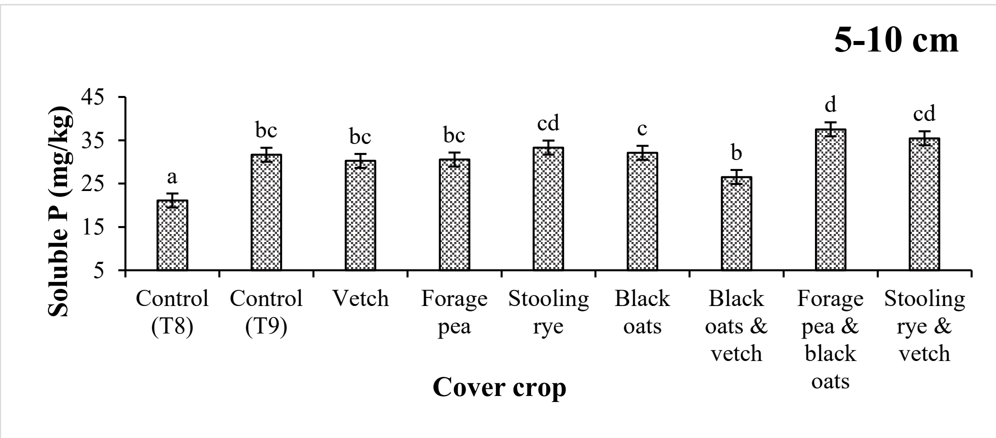
No significant influence ($p>0.05$) of CC treatments was found on extractable P at all soil depths and ranged from 14.5 to 17.7 mg kg^{-1} in the 0-5 cm, from 12.5 to 30.8 mg kg^{-1} at 5-10 cm, and from 9.77 to 16.1 mg kg^{-1} at 10-20 cm soil depth (data not shown). Data not shown, N fertilisation did not affect ($p<0.05$) extractable P at 0-5 cm (+N, 16.3; -N, 16.4), 5-10 (+N, 17.2; -N, 21.3) and 10-20 cm (+N, 11.6; -N, 12.6).

5.3.4. Phosphorus fractionation

5.3.4.1. Soluble P

Cover crop treatments significantly affected soluble P at all soil depths ($p<0.05$) (Figure 5. 1). At 0-5 cm, soluble P under CCs was significantly higher than under both controls, and black oats and stooling rye + vetch had the highest soluble P (Figure 5. 1). At 5-10 cm, soluble P under forage pea + black oats were significantly higher than under black oats + vetch, vetch, forage pea, black oats and controls (Figure 5. 1). The control (T8) had significantly lower soluble P than the CCs and control (T9), and soluble P under control (T9) was not different from under CCs except under forage pea + black oats (Figure 5. 1). At 10-20 cm, soluble P under forage pea + black oats and control (T9) was significantly higher than under control (T8) and other CCs (Figure 5. 1). Soluble P under control (T8) was not significantly different from the CCs except forage pea + black oats, while vetch had the lowest soluble P (Figure 5. 1). Nitrogen fertilisation did not affect ($p<0.05$) soluble P at 0-5 (+N, 30.6; -N, 32.2), 5-10 (+N, 30.7; -N, 31.2) and 10-20 cm (+N, 27.6; -N, 29.9).





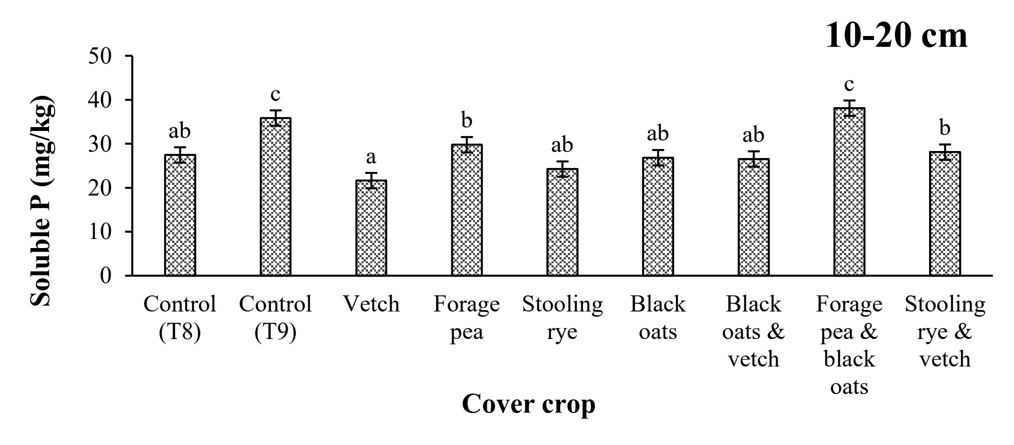


Figure 5. 1: Cover crops and nitrogen fertilisation effects on soluble P at different soil depths. Lowercase letters represent significant variation between cover crop and N treatment per soil depth at p<0.05 and the error bars indicate standard error difference. There was no significant variation in the interaction of treatments at p<0.05.

5.3.4.2. Aluminium bound P

A significant variation (p<0.05) was found between CC treatments and N fertilisation interaction at 0-5 cm soil depth (Figure 5. 2). Where it was observed that treatments with black oats, irrespective of whether there was N fertiliser on the silage maize or not, had the highest Al-P than other CC treatments (Figure 5. 2). While the controls irrespective of whether there was N fertiliser on the silage maize or not, had lower Al-P except control (T8) -N (Figure 5. 2). Cover crops significantly (p<0.05) affected Al-P at 5-10 and 10-20 cm depth intervals (Figure 5. 2). At 5-10 cm, Al-P was significantly higher under control (T8) than control (T9), black oats + vetch and black oats, while control (T9) did not significantly differ from the CCs (Figure 5. 2). At 10-20 cm, Al-P was significantly higher under stooling rye but not different from Al-P under the controls, while black oats and black oats + vetch had significantly lower Al-P than the controls (Figure 5. 2). Nitrogen fertilisation significant impact at 0-5 cm was only observed under black oats and control (T8), where it significantly decreased Al-P under these treatments (Figure 5. 2). Nitrogen fertilisation did not affect (p<0.05) Al-P at 5-10 (+N, 149; -N, 148) and 10-20 cm (+N, 184; -N, 186).

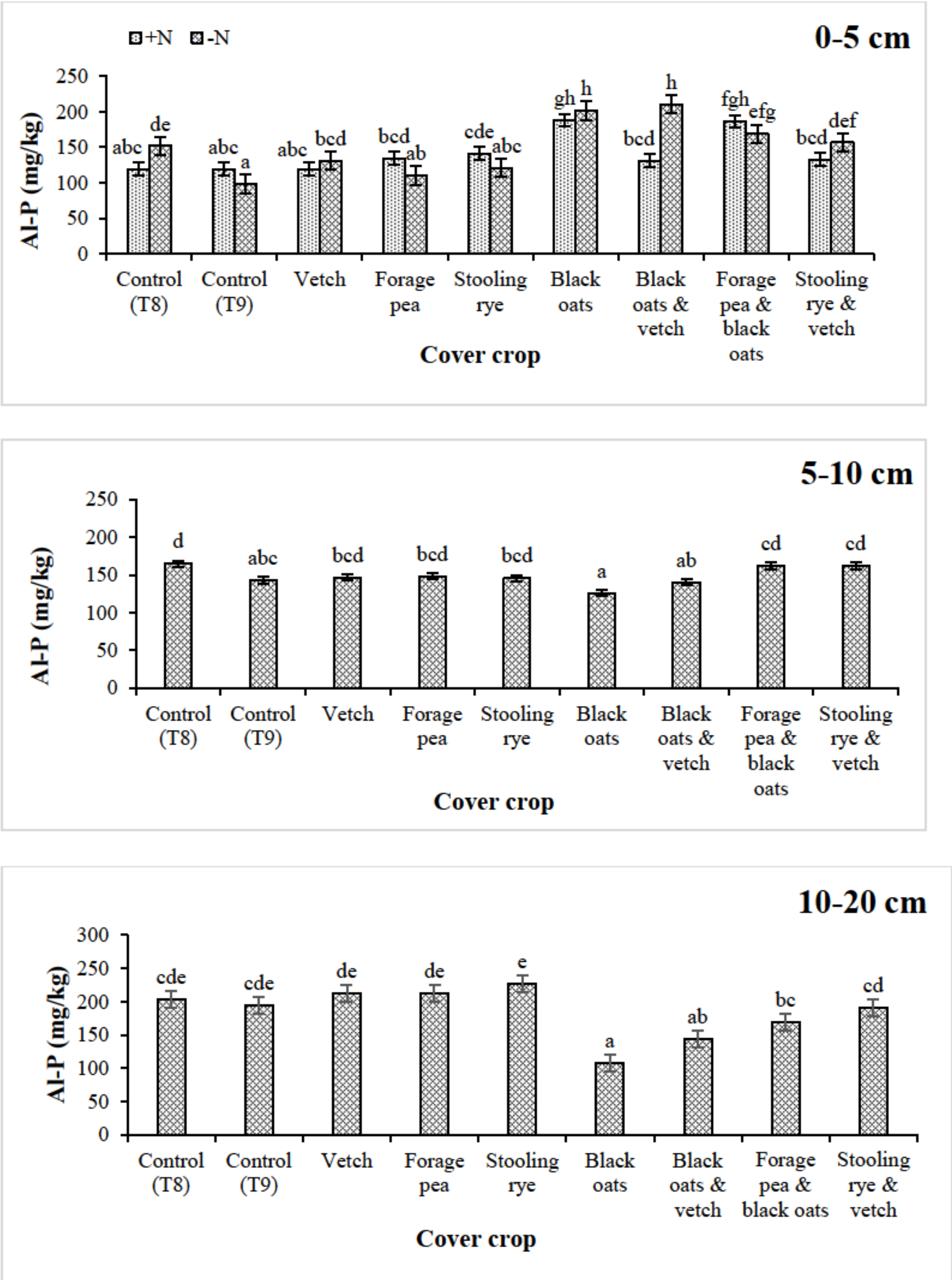
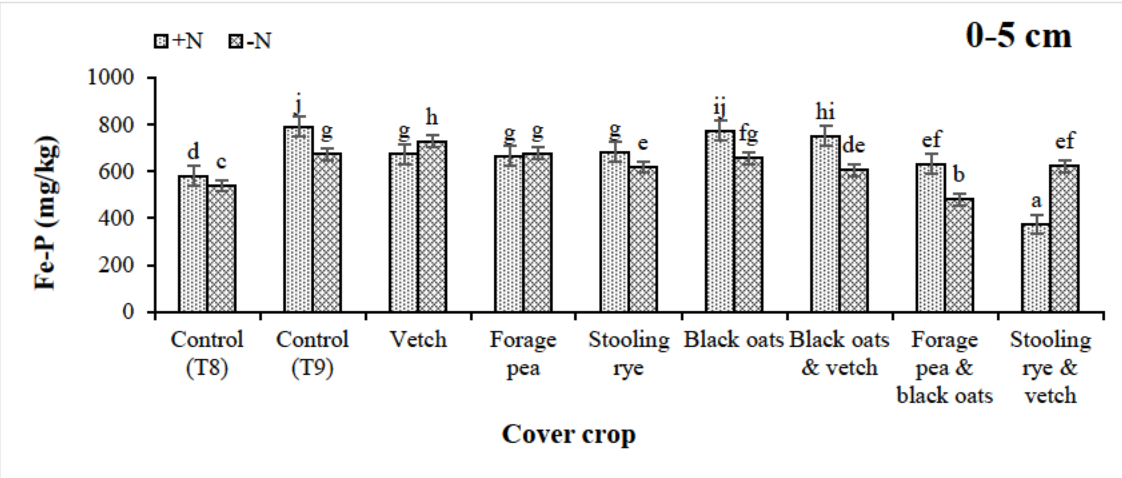
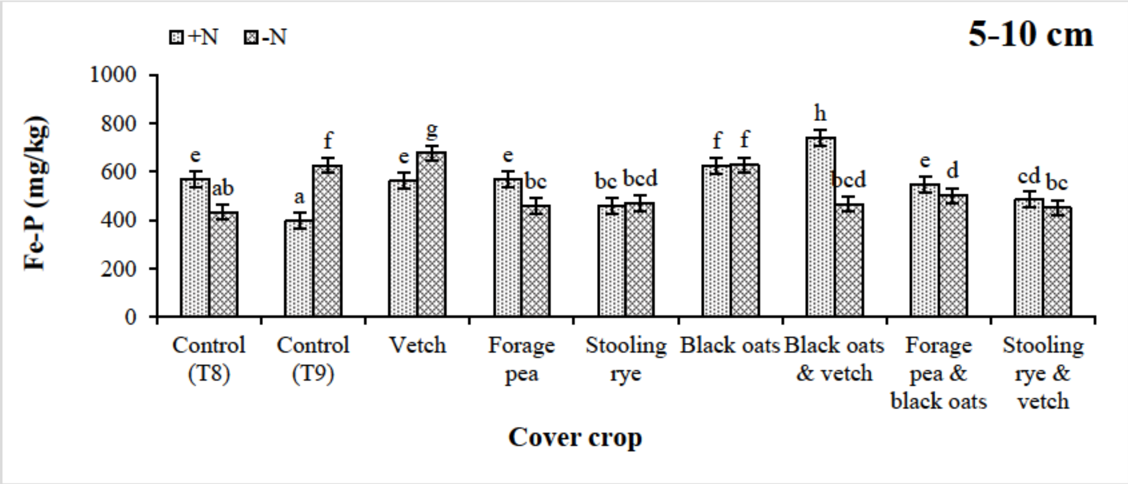


Figure 5. 2: Cover crops and nitrogen fertilisation effects on Al-P at different soil depths. Lowercase letters indicate significant variation between cover crop and N treatment per soil depth at $p<0.05$ and the error bars indicate standard error difference. Cover crop and N fertilisation were only shown where significant variation was observed. +N, 120 kg N ha^{-1} and -N, 0 kg N ha^{-1}.

5.3.4.3. Iron bound P

The interaction of CC treatments and N fertilisation significantly influenced ($p<0.05$) Fe-P at all soil depths (Figure 5. 3). At 0-5 cm, Fe-P was significantly higher under control (T9) +N than other CC treatments (Figure 5. 3). While control (T9) -N had significantly higher Fe-P than forage pea + black oats -/+N, stooling rye + vetch -/+N, stooling rye -N, black oats + vetch -N and control (T8) -/+N. Control (T8) irrespective of whether there is N fertiliser in silage-maize or not, had a higher Fe-P than forage pea + black oats -N and stooling rye + vetch +N (Figure 5. 3). At 5-10 cm, black oats + vetch +N had the highest Fe-P than under other CC treatments, while control (T9) +N and control (T8) -N had significantly lower Fe-P than other CC treatments (Figure 5. 3). On the other hand, control (T9) -N was only significantly lower than vetch -N and black oats + vetch -N, and control (T8) +N was significantly lower than black oats -/+N, control (T9) -N, vetch -N and black oats + vetch +N (Figure 5. 3). At 10-20 cm, Fe-P was higher under stooling rye + vetch + N and forage pea + black oats – N while Fe-P was significantly lower under control (T8) -N (Figure 5. 3). Fe-P under control (T9) -N was significantly higher than under black oats + vetch -/+N, control (T8) -/+N, control (T9) +N and forage pea -N, while Fe-P under control (T8) +N and control (T9) +N was significantly higher than under black oats + vetch -N, forage pea -N and control (T8) -N (Figure 5. 3).





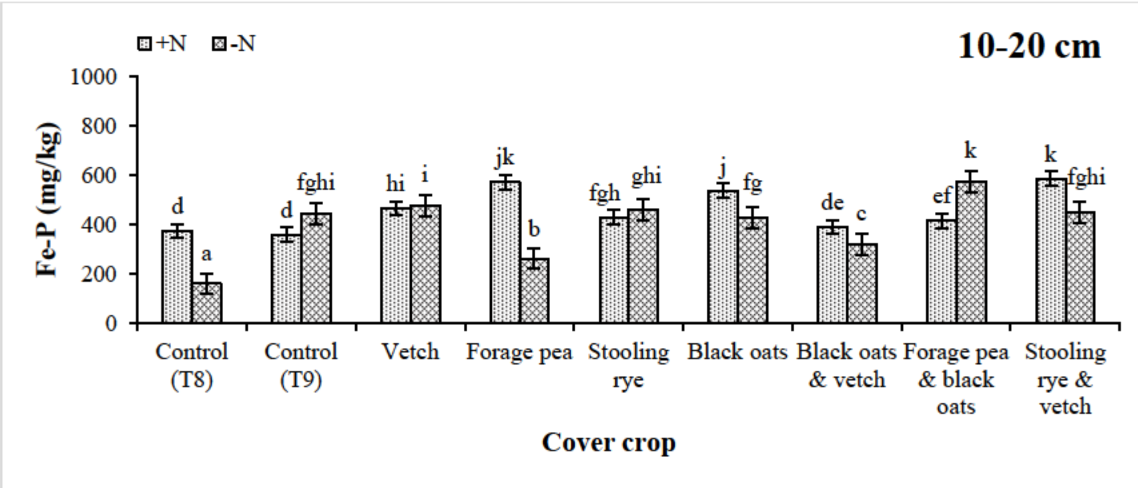


Figure 5. 3: Cover crops and nitrogen fertilisation effects on Fe-P at different soil depths. Different lowercase letters represent significant variation between cover crop and N treatment per soil depth at $p<0.05$, and the error bars indicate standard error difference. +N 120 kg N ha^{-1} and -N, 0 kg N ha^{-1}.

5.3.4.4. Reductant-soluble and calcium-bound phosphorus

Cover crop treatments significantly impacted ($p<0.05$) reductant-soluble P at all soil depths (Table 5. 4). At 0-5 cm, reductant-soluble P was significantly higher under forage pea + black oats than vetch, while under the controls, reductant-soluble P was not significantly different from under CCs (Table 5. 4). At 5-10 cm, reductant-soluble P under stooling rye +vetch was significantly greater than other CC treatments (Table 4). While reductant-soluble P under control (T8) was significantly higher than black oats, control (T9), forage pea + black oats, forage pea and vetch (Table 5. 4). On the other hand, reductant-soluble P under control (T9) was significantly lower than under control (T8) and stooling rye + vetch. At 10-20 cm, reductant-soluble P under stooling rye was significantly higher than control (T8), while reductant-soluble P under control (T8) was not significantly different from under CC treatments (Table 5. 4). Nitrogen fertilisation did not affect ($p<0.05$) reductant-soluble P (Table 5. 4).

Cover crop treatments significantly affected ($p<0.05$) Ca-P at all soil depths (Table 5. 4). At 0-5 cm, Ca-P under black oats + vetch, black oats, forage pea, vetch and stooling rye was significantly higher than under forage pea + black oats, while it was not significantly different under the controls (Table 5. 4). At 5-10 cm, Ca-P under black oats and vetch were significantly higher than forage pea + black oats while not significantly different from the controls (Table 5. 4). At 10-20 cm, Ca-P under forage pea + black oats and stooling rye + vetch was significantly higher than under control (T8), while under control (T9) it was not different from the CCs (Table 5. 4). Nitrogen fertilisation did not affect ($p<0.05$) Ca-P (Table 5. 4).

Table 5. 4: Reductant-soluble P as influenced by cover crops and nitrogen fertilisation at different soil depths in silage-maize

Factors	Reductant-soluble P (mg/kg)			Ca-P (mg/kg)		
	0-5 cm	5-10 cm	10-20 cm	0-5 cm	5-10 cm	10-20 cm
			Cover crop			
Control (T8)	34.2ab	62.5b	91.7a	20.1ab	10.3ab	4.97a
Control (T9)	35.8ab	39.0a	97.3ab	15.9ab	10.7ab	7.88abc
Vetch	30.1a	34.2a	99.8ab	22.4b	20.9b	15.0bc
Forage pea	40.7ab	37.4a	95.7ab	24.0b	17.4ab	11.4abc
Stooling rye	39.8ab	49.6ab	103.0b	22.2b	16.7ab	5.40ab
Black oats	37.4ab	43.1a	97.3ab	26.7b	21.8b	10.7abc
Black oats & vetch	35.0ab	47.9ab	94.9ab	28.4b	12.3ab	6.62ab
Forage pea & black oats	41.45b	38.2a	98.2ab	5.37a	8.58a	16.6c
Stooling rye & vetch	33.4ab	81.2c	102.2ab	13.4ab	12.2ab	16.6c
LSD (Cover crop)	6.88	10.22	6.56	9.550	7.233	5.911
			Nitrogen fertiliser			
+ N	38.0	48.6	97.9	21.13	13.14	9.31
- N	34.9	47.7	97.7	18.51	15.91	11.85
LSD	3.24	4.82	3.09	4.502	3.410	2.787

Lowercase letters indicate significant variation between cover crop and N treatment per soil depth at $p<0.05$, and there was no significant variation in the interaction between cover crops and N fertiliser. +N, 120 kg N ha^{-1} and -N, 0 kg N ha^{-1}; Control (T8), no weeds and Control (T9), weeds; LSD, Least Significant Difference.

5.4. Discussion

5.4.1. Soil pH as affected by CC treatments and N fertiliser

The reduction in soil pH with the inclusion of CCs may be due to the acidic root exudates, and organic acids production from the mineralisation of the OM (Nardi et al., 2000). This also indicates a significant leaching of base ions at 20 cm depth due to the heavy rain (985 mm) received in Cedara at KwaZulu-Natal. Hydrogen ions tend to behave like trivalent ions; hence, they have higher adsorption strength than base cations (Summers, 2016), and the ability of CCs to reduce the soil pH depends on the number of base cations absorbed. Control (T8) had the lowest biomass which might be why it had higher soil pH due to less organic acid production. On the other hand, vetch had the highest total N (Chapter 4), and when N is converted to inorganic-N, soil pH decreases. These results were consistent with the findings of Demir (2020), where CC inclusion affected pH at 0-20 cm depth from the study done in 2014. In contrast, Acuna and Villamil (2014) observed that CCs did not affect pH, and Crespo et al. (2024) stated that the insignificant impact of grass CCs on soil pH when they evaluated the effect of CCs (oat and triticale) and P fertilisation (0 P and 0-20-0 P) from sites 1 and 2 in their study could be because of the lack of N fertilisation. When ammonium-N fertiliser is applied to the soil, the ammonium is converted to nitrate in that way releasing hydrogen ions that can make the agricultural soils more acidic (Reeves & Liebig, 2016). Because these processes are happening in the topsoil, it tends to have the lowest pH. Hence, in our results, the addition of nitrogen decreased pH, even though it was insignificant. Zhou et al. (2019) found that as soil depth increases, soil pH tends to rise and subsequently decrease. According to Zhou et al. (2019) the low soil pH in the topsoil is mostly affected by precipitation which leads to leaching of basic cations, and the increase of soil pH in the middle section of soil profiles may be due to available iron and manganese nodules that react with hydrogen ions. Soil pH is an important factor that influences soil fertility because of its ability to control nutrient availability including P availability, as well as to affect microbial activity and plant root growth (Demir, 2020).

5.4.2. Total P as affected by CC treatments and N fertiliser

The insignificant effect of N fertiliser on total P might be due to the urea being applied as topdressing. The soil used was acidic causing fixation of most of the P, which would not be significantly affected by the urea application. Research conducted by scientists has demonstrated that a positive correlation exists between SOC and P (Cao, 2013; Hawkins et al., 2022; Zhang, 2023). This is due to the enhanced P availability in the soil through the chelation of polyvalent cations by organic acids and other decay products. This may explain the higher total P content under black oats + vetch +N, stooling rye + vetch +N, black oats +N and vetch -N than under the controls at 0-5 cm depth. The moss at control (T9) also showed a potential to increase total P than control (T8) because it had higher biomass. Yuan et al. (2024) suggested that the distribution of P can be influenced by various complex factors. The inclusion of crop residues in the soil can have a positive or negative impact on P levels, as it can promote either adsorption or desorption of P through different physical, biological and mechanical mechanisms (Mabagala & Mng'ong'o, 2022; Yang et al., 2019). Additionally, SOC can compete with P for adsorption sites, potentially occupying sites that would otherwise adsorb P. However, the capacity for P adsorption can also be affected by the type of OC present as well as the soil type. Hutton soil is rich in Al and Fe oxides; these oxides adsorb phosphate ions, reducing P availability. At 5-10 cm, most of the CCs also showed a potential to increase total P than the controls. The controls without N fertiliser had higher total P than the controls with N fertiliser. Nitrogen fertiliser increases the biomass of the plant but also increases mineralisation of the residues releasing P to the soil solution where it can be leached away, especially in places with high rainfall like KwaZulu-Natal, with Cedara receiving mean annual rainfall of 985 mm. The significant effect of CC treatments and N fertiliser at 10 cm might also be due to microorganism activity tending to be higher at the topsoil because of the addition of the residues. In contrast, when Christopher et al. (2021) found that CCs (annual ryegrass, cereal rye, cereal + rapeseed, oats + radish, and alfalfa) did not significantly affect total P on Alfisols and Mollisols, and Che et al. (2022) also reported that CCs (February orchid, rye, hairy vetch, February orchid + hairy vetch, rye + hairy vetch) inclusion on Fluro-aquic soil did not significantly affect total P. The difference could be due to differences in CCs used and the less weathered soil they used. At 10-20 cm, CCs had greater total P than the controls, while the control (T9) had a greater total P than the control (T8) due to higher CCs biomass. The control (T9) also showed a potential to increase total P unlike control (T8), which again might be due to the moss weed that grew from control (T9).

5.4.3. Phosphorus fractions as affected by CC treatments and N fertiliser

Phosphorus fractions are also vital for maintaining soil production (Carvalho et al., 2014**)** and Al, Fe, and Ca are one of the main P adsorbents. Cover crops and N fertilisation affected the P fractions, including soluble P, Al-P, Fe-P, Ca-P, and reductant-soluble P. However, how these fractions were affected by CC treatments and N fertiliser varied. A decrease in soil pH leads to a reduction in hydroxyl-ion activities, leading to a higher formation of iron and aluminium phosphate (Hanyabui et al., 2020). Consequently, this results in lower concentrations of soluble P, Ca-P, and reductant-soluble P. Thus, there is a greater capacity for P sorption and a decreased chance of CC absorption because of the increased abundance of the Al and Fe oxides compared to P (Christopher et al., 2021). Cover crop systems with black oats showed a potential to increase Al-P at 0-5 cm, while control (T9) -N had the highest Al-P than control (T9) +N and control (T8) with and without N fertiliser. Cover crop residues affect Al-P content through the production of organic acids mentioned above. Cover crops with black oats had a high biomass, which may have increased the organic acids content produced. However, the Al-P content under CC treatments with black oats decreased with the increase in soil depth and this might be due to the root distribution. The high Al-P under control (T8) at 5-10 cm might be due to aluminium having higher adsorption strength and the leaching of base cations. At 10-20 cm, Al-P was higher under stooling rye, vetch, and forage pea than under controls. This might be due to the high biomass, which resulted in the solubility of Al through organic acid production. The CCs potential to increase Al-P at 0-5 cm was consistent with Fe-P results and this is because of the high biomass of the CCs produced. Most CCs, including control (T9) and N fertilisation interaction, showed a potential to increase Fe-P except at 5-10 cm depth where the effect varied. The results observed at 5-10 cm depth may be due to the chemical (soil pH), biological (microbial activity), and physical (soil texture) mechanisms that influence P availability. Nonetheless, the CCs demonstrated the potential to enhance the solubility of P, particularly at a depth of 0-5 cm. This aligns with the findings of TOC and soil organic carbon (SOC) fractions, especially forage pea + black oat, stooling rye + vetch, stooling rye and black oats which increased POC fractions. This is because, during the decomposition of residues, negatively charged functional groups like carboxyls and phenols are released. These groups then interact with Al^{3+} and Fe^{3+} to reverse the complexation of P with Al^{3+} and Fe^{3+} (Hanyabui et al., 2020). Most CCs also showed a potential to increase Ca-P at 10 cm depth than the controls, probably because when crop residues are mineralised calcium is released and bonds with soluble-P. While in the upper 10 cm, forage pea + black oats showed a potential to decrease Ca-P. This might be due to organic acids produced, lowering soil pH, and solubilising fixed P with Ca. At 0-5 cm, stooling rye + vetch showed a potential to reduce reductant-soluble P probably due to the ability of OM to increase the desorption of P. While vetch showed a potential to decrease reductant-soluble P at 10 cm depth, vetch as a legume crop tends to have a high decomposition rate and the desorbed P gets is highly susceptible to leaching, which probably explains the low reductant-soluble P content.

Cover crop treatments did not affect extractable P which aligns with water-soluble organic carbon findings (Chapter 4). Debicka et al. (2015) mentioned that SOM has the potential to influence the availability of P by competing for the adsorption sites. When labile OC decompose organic acids are released and compete for adsorption sites and reduce P sorption. The desorbed P can be either leached or fixed with Al^{3+} and Fe^{3+}, probably why there was no significant effect observed on extractable P. According to Beltran et al. (2018), CCs decreased the quantity of P available in the soil, most likely because the absorbed P was still held in the residual tissues. Blanco-Canqui and Jasa (2019) also evaluated the long-term effects of grass (cereal rye and sorghum) and legume (Austrian winter peas and soybean) CCs on available P and found no significant differences, however, their study was done on Aksarben silty clay loam, which received 693 mean annual rainfall. In contrast, Demir (2020) reported a significant increase in P availability with grass (*Festuca rubra rubra* L. and *Festuca arundinacea*) and legume (*Vicia villosa* and *Trifolium meneghinianum* and *Trifolium repens* L.) CCs inclusion at 0-20 cm depth in a cherry orchard that received 685 rainfalls; however, CCs did not affect available P in the deeper layer. Hallama et al. (2019) conducted a meta-analysis on the role of CCs on P cycling and noted that legumes were effective in mobilising P, most likely because legumes have higher P requirements due to the mechanisms involved in N fixation. However, in this study, grass CCs showed a potential to increase available P due to high biomass produced, even though it was significant. Chen et al. (2023) found that available P was greater under ryegrass and that ryegrass decreased inorganic P fractions, including $NaHCO_3$-P, NaOH-P and HCl-P. The high rainfall received in Cedara probably resulted in leaching of P, limiting CCs effectiveness in increasing available P. Carvalho et al. (2014) also observed that moisture content also affects inorganic P availability; $NaHCO_3$-P and NaOH-P were higher under CCs during the rainy season, however, the area received a 252 mm mean maximum rainfall which is lower than the rainfall received in our study.

5.5. Conclusion

This research examined how CC treatments and N fertilisation impacted P fractions under silage maize-based CA systems in acidic soil. The result suggested that both CCs and N fertilisation had the potential to enhance total P and P fractions. The study demonstrated that CC systems with black oats and N fertiliser had a great potential to increase total P and P fractions. Total P was higher under black oats + N, while black oats and forage pea + black oats showed a potential to increase soluble P. On the other hand, black oats with and without N fertiliser and black oats + vetch increased Al-P. The weeds that grew from the control (T9) also showed the potential to increase P availability. While vetch decreased soil pH. Further research is still needed to study the complex effects of CCs on the adsorption and desorption of P.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

6.1. General Discussion

Climate change and soil degradation have been proven to decrease food production globally, which is a problem because of a growing demand for food due to the growing population. Cover cropping management is one of the conservational agriculture practices that was proposed to mitigate this problem. Even though many articles are reporting positive results about CCs, there are still limitations which is why not all farmers have adapted this management practice. Cover crops have been proven to enhance SOC, plant available nutrients, and improve microbial community and diversity, and other physiochemical properties. However, many factors must be considered for CCs to be effective, including their traits, their compatibility with the main crop and when planted as biculture, climate and soil conditions, and economic viability. Most of the time CC depend on nutrients and moisture remaining after the main crop is harvested and water they receive when it rains. With climate change on the rise, rainfall is becoming scarce in other places, therefore, CCs resistant to drought should be implemented. These factors have resulted in inconsistent effectiveness of CCs. In this thesis, the effect of CCs, planted as mono- and biculture, on SOC and available P was evaluated by conducting a meta-analysis. Furthermore, CCs and N fertiliser application on silage-maize effect on SOC and P fractions were evaluated from a field trial that has been running for over a decade.

Grass and legume crops are normally used as CCs. However, other broad-leaf crops like brassica can be used and were evaluated for the meta-analysis. Meta-analysis results showed that CCs, especially legumes enhanced SOC except when they were mixed with broad-leaved crops. It was also evident that unfertilised CCs can enhance SOC under different climatic conditions, soil texture and pH levels. However, SOC was higher under tropical climate, in loam soil with neutral pH. Cover crops thrive in these conditions because of the high moisture, available nutrients, and microbial activities. Our experiment only focused on the effect of legume and grass crops also planted as mono- and bi-culture and rotated with N-fertilised silage-maize. The study demonstrated that CCs effect on SOC fractions is complex. Cover crops significantly affected TOC and $_c$POC at 10 cm, $_f$POC at 20 cm and MAOC only at 5 cm, except WSOC. Total OC was higher under black oat and stooling rye + vetch, POC fractions were higher under forage pea + black oats, stooling rye + vetch, black oats + vetch, black oat and stooling rye, and MAOC was higher under forage pea. On the other hand, vetch showed a great potential to increase TN. Nitrogen fertilisation did not have much effect on SOC fractions, except on $_{c}$POC, which was decreased by N fertiliser addition. Cover crops also altered base cations (Mg, K, and Ca), which is a good observation, especially in places with low pH due to high rainfall like KwaZulu-Natal. Black oat increased Mg and total cations, black oat + vetch and vetch increased Ca, while forage pea + black oat showed a great potential to increase basic cations and total cations at deep soil depth. While N fertilisation increased acid saturation, which may reduce Ca, Mg, and K levels, indicating a potential leaching effect.

A decline in soil available P and high fertiliser costs due to the demand have been reported by several studies. Meta-analysis showed that only legume and broadleaved crops planted as monoculture had a great potential to increase available P, however, CCs without N fertiliser did not significantly increase available P. It was higher in tropical climates, loamy soils, and acidic soils. This was a positive observation because soil pH plays a huge role in P absorption and desorption. Our experiment was performed in acidic soil; even though CCs altered pH, it was still low, especially under vetch. As a result, P bound to Al and Fe was very high. This probably resulted in the effect of CCs on extractable P not being significant. However, CCs and N fertiliser interaction showed potential to increase TP at 10 cm, and CCs showed potential to increase TP at 10-20 cm and soluble P at 20 cm. Total P was higher under black oat + vetch, stooling rye + vetch and black oat with N fertiliser at 0-5 cm, CCs with and without N fertiliser except for black oat + vetch and forage pea without N fertiliser at 5-10 cm, and it was higher in all CCs in the 10-20 cm soil depth. All cover CCs increased soluble P at 0-5 cm, and it was higher under forage pea + black oat, stooling rye + vetch, stooling rye and black oat at 5-10 cm, and forage pea + black oat showed potential to increase soluble P at 10-20 cm. Forage pea + black oat, stooling rye + vetch, stooling rye and black oat effectiveness on soluble P correlated with the one observed on POC fractions.

6.2. Conclusion

The results of this research emphasise the significance of using CCs as a sustainable farming method to address the issues related to soil degradation and nutrient loss considering climate change (high rainfall intensity and extreme hot temperatures) negatively affects crop production, together with increasing SOC storage. Although CCs generally enhanced SOC and P levels, there were variations in the performance of different species and crop combinations.

From the meta-analysis, it was observed that legumes led to higher SOC and available P levels. On the other hand, in our experiment grass crops, when planted as mono- or bi-culture, generally indicated the potential to increase SOC fractions, soluble and total P. In conclusion, the study indicates that while cover cropping is promising to enhance soil health and fertility, its implementation should be suitable for that area, considering environmental conditions and cropping systems.

6.3. Recommendations

Black oat and stooling rye in combination with vetch need to be considered as CC mixtures to enhance TOC, while forage pea with black oat, stooling rye with vetch, black oats with vetch, or a combination of black oat and stooling rye are options to improve the labile OC fractions, particularly POC, with vetch being important for enhancing TN. The findings of this study suggested that, instead in increasing N fertiliser rates, farmers may need to use CC combinations to improve SOC fractions, while black oat with vetch, stooling rye with vetch, or black oat along with N fertiliser, may need to be used to elevate total P in the topsoil (0–5 cm), while incorporation of the CCs may be required to enhance TP in the 10–20 cm depth. Combinations of forage pea with black oat, stooling rye with vetch, stooling rye alone, or black oat, may need to be considered to enhance soluble P across various soil depths. While CCs can influence soil pH, it may be essential to apply lime or other amendments to address persistent acidity, particularly when using vetch, which can further lower pH levels. For synergistic benefits, forage pea with black oat and stooling rye with vetch, may be applied to improve both POC fractions and soluble P.

Additional research is necessary to optimise CC combinations and management practices in various conditions. Customising CC management to the specific soil texture and pH of the area is important. Given the increasing variability of rainfall patterns due to climate change, it is important to prioritise drought-tolerant CCs. Continuation of long-term field trials is essential to determine the diverse impacts of CCs on soil properties, such as carbon sequestration and nutrient availability, as well as their compatibility with the main crops, to refine CC management practices for different agroecosystems. While N fertilisation on main crops may benefit CCs, it requires careful management. Providing education to farmers regarding the long-term advantages of CCs and conducting cost-benefit analyses for various cover cropping systems could help attract a wider range of farmers.

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APPENDICES

Appendix Table 1 Acid saturation and Ca as affected by cover crops and nitrogen fertilisation at different soil depths in silage-maize

Cover crops	0-5 cm		5-10 cm	
	Acid saturation (%)	Ca (mg/kg)	Acid saturation (%)	Ca (mg/kg)
Black oats & vetch	2.167	3495	3.167	1096
Vetch	4.167	1346	10.667	877
Control (T8)	1.333	1503	1.500	1149
Forage pea	1.167	1476	2.333	2594
Forage pea & black oats	1.333	1372	1.333	1238
Stooling rye	1.667	1321	3.000	1028
Black oats	0.833	1511	11.833	1164
Control (T9)	3.667	1184	2.333	1045
Stooling rye & vetch	2.333	1437	5.000	1049
LSD	2.894	2027.867	9.99	1431.787
+ N	3.52	1251.52	4.5	1058.06
- N	0.63	2002.83	4.6	1439.84
LSD	1.364	955.945	4.71	674.951

The absence of letters indicates that the treatments did not significantly vary at $p<0.05$ and there was no significant variation in the interaction of treatments. +N, 120 kg N ha^{-1} and -N, 0 kg N ha^{-1}.

Appendix Table 2 Acid saturation and Ca as influenced by cover crops and nitrogen fertilisation in silage maize

Depth (cm)	Cover crops	Acid saturation (%)		Ca (mg/kg)	
		+ N	- N	+ N	- N
10-20 cm	Black oats & vetch	5.000abc	4.333abc	904ab	961ab
	Vetch	8.000a	7.000ab	855b	855b
	Control (T8)	1.333c	2.333bc	1136ab	1040ab
	Forage pea	1.667bc	2.667abc	1149ab	979ab
	Forage pea & black oats	1.000c	1.000c	1134ab	1257a
	Stooling rye	4.000abc	4.333abc	940ab	930ab
	Black oats	1.667bc	3.000abc	1052ab	1018ab
	Control (T9)	1.333c	3.000abc	1047ab	1108ab
	Stooling rye & vetch	3.667abc	4.000abc	967ab	954ab
LSD	Cover crop × N fertiliser	3.017		196.964	

Different lowercase letters represent significant variation between cover crop treatments and nitrogen fertilisation per parameter at $p<0.05$. +N, 120 kg N ha^{-1} and -N, 0 kg N ha^{-1}.

Appendix Table 3 Total cations, Mg and K affected by cover crops and nitrogen fertilisation at 0-5 cm depth in silage maize

Cover crops	Total cations (cmolc)		Mg (mg/kg)		K (mg/kg)	
	+ N	- N	+ N	- N	+ N	- N
Black oats & vetch	8.760cd	11.000abcd	228.2b	288.1ab	86.5de	152.0abcde
Vetch	7.793d	10.933abcd	202.0b	299.8ab	77.7e	143.4bcde
Control (T8)	9.120bcd	12.463a	253.8ab	296.3ab	117.6cde	300.3a
Forage pea	9.620abcd	11.430abc	251.6ab	308.8ab	95.4cde	191.8abcde
Forage pea & black oats	9.047bcd	11.367abc	256.5ab	303.6ab	96.3cde	242.2abc
Stooling rye	8.443cd	10.547abcd	253.8ab	297.3ab	102.2cde	235.0abcd
Black oats	9.753abcd	12.217ab	291.6ab	349.9a	96.4cde	289.0ab
Control (T9)	8.247cd	9.540abcd	225.8b	260.6ab	93.7cde	170.7abcde
Stooling rye & vetch	8.787cd	10.883abcd	243.4ab	311.6ab	103.8cde	162.4abcde
LSD (cover crop × N fertiliser)	1.776		64.151		81.675	

Different lowercase letters represent significant variation between cover crop treatments and nitrogen fertilisation per parameter at $p<0.05$. +N, 120 kg N ha^{-1} and -N, 0 kg N ha^{-1}.

Appendix Table 4 Total cations and K as influenced by cover crops and nitrogen fertilisation in silage maize

Depth (cm)	Cover crops	Total cations ($cmol_c$/L)		K (mg/kg)	
		+ N	- N	+ N	- N
5-10 cm	Black oats & vetch	7.903ab	8.460ab	47.89ab	82.23ab
	Vetch	6.277b	7.160ab	36.42b	55.03ab
	Control (T8)	8.203ab	8.857ab	56.64ab	129.40ab
	Forage pea	8.300ab	8.137ab	55.67ab	350.00a
	Forage pea & black oats	8.347ab	9.527ab	60.15ab	170.51ab
	Stooling rye	7.363ab	7.923ab	55.79ab	97.56ab
	Black oats	8.433ab	13.573a	40.85ab	139.62ab
	Control (T9)	7.187ab	7.630ab	42.78ab	120.61ab
	Stooling rye & vetch	7.577ab	7.717ab	47.09ab	74.19ab
LSD	Cover crop × N treatment	3.659		168.090	

Different lowercase letters represent significant variation between cover crop treatments and nitrogen fertilisation per parameter at $p<0.05$. +N, 120 kg N ha^{-1} and -N, 0 kg N ha^{-1}.

Appendix Table 5 Total cations and K as influenced by cover crops and nitrogen fertilisation in silage maize

Depth (cm)	Cover crop	Total cations ($cmol_c$/L)	K (mg/kg)
10-20	Black oats & vetch	6.863ab	72.52
	Vetch	6.405a	44.42
	Control (T8)	7.903ab	49.30
	Forage pea	7.800ab	55.14
	Forage pea & black oats	8.392b	66.28
	Stooling rye	6.818ab	52.43
	Black oats	7.413ab	59.89
	Control (T9)	8.043b	72.66
	Stooling rye & vetch	7.017ab	42.62
	LSD	1.001	36.353
N fertiliser	+ N	7.39	39.70
	- N	7.42	74.80
	LSD	0.472	17.137

Different lowercase letters represent significant variation between cover crop treatments and nitrogen fertilisation per parameter at $p<0.05$. The absence of letters indicates that the treatments did not significantly vary at $p<0.05$ and there was no significant variation in the interaction of treatments. +N, 120 kg N ha^{-1} and -N, 0 kg N ha^{-1}.

Appendix Table 6 Mg as affected by cover crops and nitrogen fertilisation at different soil depths in silage maize

Cover crop	Mg (mg/kg)	
	5-10 cm	10-20 cm
Black oats & vetch	193.9	157.3ab
Vetch	151.3	139.1a
Control (T8)	206.1	183.7ab
Forage pea	444.1	175.9ab
Forage pea & black oats	230.1	198.7b
Stooling rye	195.9	165.8ab
Black oats	223.0	182.8ab
Control (T9)	195.7	207.4b
Stooling rye & vetch	191.8	168.0ab
LSD	238.681	33.745
+ N	190.68	175.75
- N	260.84	175.09
LSD	112.515	15.908

Different lowercase letters represent significant variation between cover crop treatments and nitrogen fertilisation per parameter at $p<0.05$. The absence of letters indicates that the treatments did not significantly vary at $p<0.05$ and there was no significant variation in the interaction of treatments. +N, 120 kg N ha-1 and -N, 0 kg N ha-1.