

Reducing inorganic nitrogen inputs for small-scale sugarcane growers in KwaZulu-Natal using legume species (*Vigna unguiculata* and *Cajanus cajan*)

By

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Dissertation submitted in fulfilment of the academic requirements for the degree of Master of Science in the Discipline of Biological Sciences, School of Life Sciences, College of Agriculture, Engineering, and Science at the University of KwaZulu-Natal, Westville.



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Preface

The research contained in this thesis was completed by the candidate based in the Discipline of Biological Sciences, School of Life Sciences of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Westville Campus, South Africa. The study was conducted from January 2022 to January 2025, under the supervision of Dr Syd Ramdhani and Prof Anathi Magadlela.

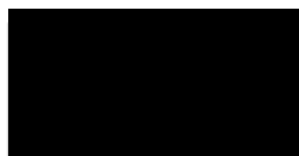
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Date 06 February 2025

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As the supervisor I have approved this thesis/dissertation for submission.



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Prof. Anathi Magadlela (Supervisor)

Declarations: Plagiarism

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Table of Contents

Preface.....	2
Declarations: Plagiarism	3
Acknowledgements.....	4
Abstract	7
General overview of chapters.....	9
List of Tables	10
List of Figures	10

List of Abbreviations.....	11
Chapter 1: General Introduction.....	12
1.1 Background	12
1.2 Rationale of the Study	14
1.3 Aim and Objectives.....	14
1.4 Research questions.....	15
1.5 References	16
Chapter 2: Literature Review	21
2.1 Sugarcane production.....	21
2.2 Effect of chemical fertilizers.....	22
2.3 Soil degradation	23
2.3 Incorporating legumes in sugarcane plantations	24
2.3.1 Biological N fixation.....	24
2.3.2 Sustainable N management.....	25
2.3.3 Association with plant growth promoting bacteria	25
2.4 Study species.....	26
2.5 References	27
Chapter 3	37
A leguminous boost: <i>Vigna unguiculata</i> cover cropping enhances extracellular enzyme activities	37
and bacterial communities in sugarcane plantation soils	37
Abstract	38
3.1 Introduction	39
3.2 Material and Methods	41
3.2.1 Soil collection sites.....	41
3.2.2 Experimental design, growth conditions, and soil nutrient analysis	42
3.2.3 Soil bacterial extraction	43
3.2.4 Bacterial amplification and identification.....	44
3.2.5 Soil enzyme activities assay.....	44
3.2.5.1 Phosphatase and β -glucosidase activities	44
3.2.5.2 Nitrate reductase activity.....	45
3.2.6 Total plant nutrition.....	46
3.2.6.1 Calculations of percentage nitrogen derived from the atmosphere (% NDFA).....	46

3.2.6.3 Carbon construction costs (Cw)	47
3.2.6.4 Relative growth rate	48
3.2.6.5 Root: Shoot ratio.....	48
3.2.7 Statistical analysis	48
3.3 Results.....	48
3.3.1 <i>Vigna unguiculata</i> growth kinetics	48
3.3.2 Bacterial identification	49
3.3.3 Soil enzyme activities	50
3.3.4 Soil Nutrient Analysis	50
3.4 Discussion.....	51
3.5 Conclusion.....	53
3.6 References	54
Tables and figures	60
Chapter 4	70
<i>Cajanus cajan</i> cover cropping improves the abundance of plant growth-promoting bacteria and enhances associated enzyme activities in sugarcane plantation soils	70
Abstract	70
4.1 Introduction	71
4.2 Material and Methods	74
4.2.1 Soil collection sites.....	74
4.2.2 Experimental design, growth conditions, and soil nutrient analysis	74
4.2.3 Total plant nutrition.....	75
4.2.3.1 Calculations of percentage nitrogen derived from the atmosphere (% NDFA).....	75
4.2.3.2 Specific Nitrogen /Phosphorus assimilation and utilization rate.....	76
4.2.3.3 Carbon construction costs (Cw).....	76
4.2.3.4 Relative growth rate	77
4.2.3.5 Root: Shoot ratio.....	77
4.2.4 Soil bacterial extraction and identification	77
4.2.5 Soil enzyme activities analysis.....	78
4.2.5.1 Phosphatase and β -glucosidase activity	78
4.2.5.2 Nitrate reductase activity.....	79
4.2.6 Statistical analysis.....	80
4.3 Results.....	80
4.3.1 <i>Cajanus cajan</i> growth kinetics	80

4.3.2 Bacterial identification	80
4.3.3 Soil Enzymes Activities	81
4.3.4 Soil nutrient analysis	81
4.4 Discussion.....	82
4.5 Conclusion.....	85
4.6 References	86
Tables and figures	91
Chapter 5	99
5.1 General conclusion	99
5.2 Future recommendations	100
5.3 References	100

Abstract

Declining soil fertility and health pose a significant threat to food security and agricultural sustainability. In South Africa, sugarcane production plays a crucial role in the economy, contributing significantly to gross domestic product (GDP) through exports and providing income for many smallholder growers. However, between 2007 and 2017, sugarcane production in the country declined by 52%. Despite being among the largest sugarcane-producing provinces, smallholder growers in KwaZulu-Natal have experienced substantial reductions in yield, threatening both their livelihoods and the broader economic stability of the sector. One of the primary causes of declining sugarcane production is the widespread practice of continuous monoculture, which depletes soil nutrients, reduces microbial diversity, and increases soil acidity. Monoculture selects for crop-specific microbes, leading to a decline in beneficial bacterial populations and their associated enzyme activities, which are essential for nutrient cycling. This degradation in soil health underscores the urgent

need for sustainable agricultural practices, such as the integration of leguminous cover crops, to restore soil fertility and improve sugarcane productivity.

The use of legumes as cover crops has been widely recognised for enhancing soil organic carbon, improving nutrient cycling, and increasing microbial activity. Among leguminous species, *Cajanus cajan* and *Vigna unguiculata* are particularly valuable due to their ability to fix atmospheric nitrogen, improve soil nutrient dynamics, and enhance microbial diversity. This study aimed to evaluate the impact of *C. cajan* and *V. unguiculata* as sustainable cover crops on soil nutrient dynamics in sugarcane plantations managed by smallholder growers in KwaZulu-Natal. The study was conducted under greenhouse conditions, with temperature ranging from 13–16 °C at night to 28–35 °C during day, with humidity levels between 70% and 80%. Soil samples were collected from sugarcane plantations in Gingindlovu, Mvutshini, Mpembeni, Hibberdene, and Mzinto to serve as the growth substrates. Pre-planting and postharvest soil sample were analysed for nutrient concentrations, nutrient cycling bacteria, along with associated extracellular enzyme activities. The results demonstrated that both legume species contributed to improvements in soil health. Post-harvest, soil pH slightly increased across all plantation sites, counteracting the acidity induced by continuous sugarcane monoculture. The abundance of plant growth-promoting bacteria, including *Arthrobacter*, *Agrobacterium*, *Bacillus*, *Lysinibacillus*, *Caulobacter*, and *Pseudomonas*, increased significantly. Plants grown in Hibberdene and Mzinto showed a high reliance on nitrogen derived from the atmosphere. Furthermore, *C. cajan* and *V. unguiculata* accumulated high shoot biomass despite low soil nutrition in the sampled soils.

The growth of *C. cajan* and *V. unguiculata* in the sampled soils indicates that microbial activity and soil enzyme functions facilitated nutrient mineralisation and solubilisation, making

essential nutrients more available for plant uptake and leading to increased total plant biomass. Overall, this study highlights the potential of leguminous cover crops in mitigating soil degradation caused by sugarcane monoculture. The incorporation of *C. cajan* and *V. unguiculata* into sugarcane production systems can enhance soil microbial diversity, improve nutrient cycling, increase soil pH, and ultimately contribute to the long-term sustainability of smallholder sugarcane farming in KwaZulu-Natal.

General overview of chapters

Chapter 1 Outlines the contribution of small-scale growers in sugarcane production in KwaZulu-Natal, highlights factors that contribute to declining yield and production and the use of the legumes to enhance production while reducing the use of chemical inputs, the significance of the study, aims, and objectives of the study.

Chapter 2 Literature review gives detailed overview of sugarcane and contribution of smallscale growers to food security, challenges affecting small-scale growers and the importance of incorporating legumes as cover crop during fallow periods in sugarcane plantations.

Chapter 3 Focuses on the biological and chemical benefits of using *Vigna unguiculata* as a sustainable cover crop on soil quality and nitrogen availability in sugarcane plantations managed by small-scale growers in KwaZulu-Natal by identifying nutrient cycling bacteria along with their associated soil enzyme and their contributions to the soil nutrition of different plantations soils.

Chapter 4 Examines the biological and chemical benefits of using *Cajanus cajan* as a sustainable cover crop on soil quality and nitrogen availability in sugarcane plantations managed by small-scale growers in KwaZulu-Natal by identifying nutrient cycling bacteria and their associated soil enzyme and their contributions to the soil nutrition of different plantations soils.

Chapter 5 Discusses the highlights and future recommendations of the current study.

List of Tables

Table 3.1: Biomass, plant mineral nutrition, and growth kinetics of 54-old-day *Vigna unguiculata* plants grown in sugarcane plantation soils collected from Gingindlovu, Mvutshini, Mpembeni, Hibberdene, and Mzinto, KwaZulu-Natal, South Africa, page 67-68.

Table 3.2: Nutrient cycling bacteria (accession number and percentage identity) isolated from sugarcane plantation soils in Gingindlovu, Mvutshini, Mpembeni, Hibberdene and Mzinto, preplanting and post-harvest of *Vigna unguiculata* species, page 69-71.

Table 3.3: Soil mineral nutrition (N, P, K, Ca, Mg) and chemistry (pH, acid saturation, exchange acidity, total cations) of 54-day-old *Vigna unguiculata* plants grown in soils from Gingindlovu, Mvutshini, Mpembeni, Hibberdene, and Mzinto sugarcane plantations in KwaZulu-Natal, South Africa, page 73.

Table 4.1: Biomass, plant mineral nutrition, and growth kinetics of 54-day-old *Cajanus cajan* species grown in sugarcane plantation soils from Gingindlovu, Mvutshini, Mpembeni, Hibberdene, and Mzinto, KwaZulu-Natal, South Africa, page 98-99.

Table 4.2: Primary nutrients (N, P, K), secondary nutrients (Ca, Mg) and soil chemistry (pH, acid saturation, exchange acidity, and total cations) of 54-day-old *Cajanus cajan* species grown in sugarcane plantation soils from Gingindlovu, Mvutshini, Mpembeni, Hibberdene, and Mzinto, KwaZulu-Natal, South Africa, page 102.

List of Figures

Figure 3.1: Soil enzyme activities in sugarcane plantation soils from 1. Gingindlovu, 2. Mvutshini, 3. Mpembeni, 4. Hibberdene, and 5. Mzinto, KwaZulu-Natal, South Africa,

measured pre- and post-planting and harvest of *Vigna unguiculata*. **A.** The acid phosphatase, **B.** alkaline phosphatase, **C.** glucosidase, and **D.** nitrate reductase activity, page 74.

Figure 3.2.1 Map showing the location of sampling sites in the South Coast and North Coast of KwaZulu-Natal province, South Africa, page 44.

Figure 4.1: Heat Map showing genera isolated from pre-planting and post-harvest soils collected from sugarcane plantations across Gingindlovu, Mvutshini, Mpembeni, Hibberdene, and Mzinto, KwaZulu-Natal, South Africa, page 100.

Figure 4.2: Soil enzymes activities of 54-day-old *Cajanus cajan* species grown in soils collected from 1. Gingindlovu, 2. Mvutshini, 3. Mpembeni, 4. Hibberdene, and 5. Mzinto sugarcane plantations in KwaZulu-Natal, South Africa. Enzymes activities include: **A.** acid phosphatase, **B.** alkaline phosphatase, **C.** glucosidase, **D.** nitrate reductase activity, page 103.

List of Abbreviations

ANOVA	Analysis of Variance
BLAST	Basic Local Align Search Tool
BNF	Biological Nitrogen Fixation
CAF	Central Analytical Facilities
Ca	Calcium
DNA	Deoxyribonucleic Acid
GDP	Gross Domestic Product
K	Potassium
Mg	Magnesium

N	Nitrogen
NCBI	National Centre for Biotechnology Information
NDFA	Nitrogen Derived from the Atmosphere
NDFS	Nitrogen Derived from the Soil
P	Phosphorus
pH	Potential of hydrogen
PCR	Polymerase Chain Reaction
SNAR	Specific Nitrogen Assimilation Rate
SNUR	Specific Nitrogen Utilisation Rate
SPAR	Specific Phosphorus Assimilation Rate
SPUR	Specific Phosphorus Utilisation Rate
TCP	Tricalcium Phosphate

Chapter 1: General Introduction

1.1 Background

Developing countries rely on agriculture to support livelihoods (FAO, 2009). The agricultural sector is primarily dominated by small-scale growers (Elleboudt, 2012). However, large-scale growers contribute 95% of the market share, while small-scale growers remain insignificant contributors, as they sell most of their crops through informal trade (Liebenberg, 2013; Thamaga-Chitja and Morojele, 2014; Stats SA, 2020). Cockburn et al. (2014) reported that production among small-scale growers is lower than that of large-scale growers in South Africa, due to poor soil quality (Williams, 1999). As previously stated by Williams (1999) poor soil quality has been largely impacting, which has led high reliance on fertilizers to maximise

sugarcane production (Eweg et al., 2009). Though synthetic fertilisers are applied to improve yields, other factors such as continuous monoculture and weed infestations reduce sugarcane production (Cockburn et al., 2014).

While monocropping is a common agricultural practice, it is associated with economic losses due to soil degradation, as it depletes soil nutrients, alters soil pH, and reduces organic matter content, ultimately decreases crop yield (Belete and Yadete, 2023). The effects of continuous monoculture were observed through declined maize yields after 60 years of monoculture (Belete and Yadete, 2023), underscoring the need to implement alternative practices that can contribute to high production while improving soil quality and sustainability (Liebman, 2000). Ramouthar et al. (2014) suggested that using legume species as cover crops is a sustainable alternative to breaking the sugarcane monoculture cycle. Legume cover cropping benefits growers by interrupting pest and disease cycles, suppressing weeds, increasing water infiltration rates, and fixing atmospheric N (Garside and Bell, 2007; Ramouthar et al., 2014). Njoka et al. (2003) reported increases in tuber yields after the use of velvet bean as a cover crop. The ability of legumes to form symbiotic associations with beneficial microbes contributes to soil fertility, reducing dependence on synthetic fertilizers (Ramouthar et al., 2014). Additionally, Varco et al. (1989) reported that the incorporation of vetch residues was associated with greater releases of soil inorganic nitrogen. Lastly, the use of cover crops has been shown to reduce nitrate-N losses by 11% in some cropping systems (Strock et al., 2004). Oswald and Ransom (2001) observed decreased *Striga* population in maize after planting *C. cajan* in Kenya. In Ghana maize yield increased by 75 % after rotating maize with *C. cajan* (Adjei-Nsiah, 2012). Furthermore, Ae (1990) reported increased soil P availability under *C. cajan* meaning that species effectively solubilized P.

1.2 Rationale of the Study

The drastic decline of small-scale growers over the past decades has a negative impact in sugar production and food security (Dubb, 2016). In 2012/13 season 22453 sugarcane growers were registered however only 47% of them submitted cane (SASA, 2013). The decline of sugarcane production can be attributed to poor farming practices (Dubb, 2016). Low yield of sugarcane in Mona and Sonkombo is linked to late application of weed control (Zulu et al., 2019). In addition, the high costs of fertilizers have been pointed out by growers to be one of the key constraints in sugarcane production (Zulu et al., 2019). *Cajanus cajan* and *Vigna unguiculata* play a key role in enhancing soil quality and health (Odeny, 2007; Kebede and Bekeko, 2020). These species can fix atmospheric nitrogen in association with plant growth-promoting rhizobacteria, which are involved in nutrient cycling, plant growth promotion, and plant protection against pathogens (Lopez et al., 1987; Peoples et al., 1995; Odeny, 2007; Kebede and Bekeko, 2020). Furthermore, these legumes help control pests and diseases, suppress weeds, reduce nitrogen leaching from the soil, and increase organic matter levels (Garside and Bell, 2007). The contributions of these species as cover crops have been studied in various agroecosystems, including coffee (Venzon et al., 2006), maize (Sogbedji et al., 2006), and sorghum (Beshir et al., 2019). However, their role in sugarcane farming systems remains largely unknown, particularly in South Africa.

1.3 Aim and Objectives

This study aims to evaluate the impact of *Vigna unguiculata* and *Cajanus cajan* as sustainable cover crops on soil nutrient dynamics in sugarcane plantations managed by small-scale growers in KwaZulu-Natal. To the assess the growth physiology of these legume species on the sugarcane plantation soils and determine whether these legumes are well suited to the soil conditions of the sampled plantations.

The objectives of the study are as follows:

- Assess changes in soil parameters before and after planting *Vigna unguiculata* and *Cajanus cajan* in different sugarcane plantation soils in KwaZulu-Natal.
- Isolate and identify nutrient-cycling bacterial strains before and after planting *Vigna unguiculata* and *Cajanus cajan*.
- Analyse nutrient-cycling enzyme activities in plantation soils of small-scale sugarcane growers in KwaZulu-Natal before and after planting *Vigna unguiculata* and *Cajanus cajan*.
- To determine the growth of the species by assessing their growth kinetics and reliance on NDFA and NDFS for N nutrition.

1.4 Research questions

- How do soil parameters change before and after planting *Vigna unguiculata* and *Cajanus cajan* in sugarcane plantation soils managed by small-scale growers in KwaZulu-Natal?
- What are the dominant nutrient-cycling bacterial strains present in the soil before and after planting *Vigna unguiculata* and *Cajanus cajan*?
- How do nutrient-cycling enzyme activities in the soil differ before and after the introduction of *Vigna unguiculata* and *Cajanus cajan*?
- Can *Vigna unguiculata* and *Cajanus cajan* maintain growth in nutrient deficient sugarcane plantation soils?

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Chapter 2: Literature Review

2.1 Sugarcane production

Sugarcane (*Saccharum officinarum* L.) is a perennial grass mainly grown in tropical and subtropical regions (Silva et al., 2019). It is an essential crop worldwide due to its economic and nutritional significance (Zulu et al., 2019). It has been reported that sugarcane contributes about 80% of global sugar production (Islam et al., 2018; Yang et al., 2019). In addition, sugarcane is used to produce ethanol and various by-products, including biofuel, cane fibers, molasses, and rum (Silva et al., 2019). According to Heinrichs et al. (2017), approximately 27 million hectares are planted with sugarcane worldwide, with Brazil contributing 10 million hectares. Brazil, India, and China are the world's largest sugarcane producers, while African countries contribute only 5% of the total global sugar production (FAO, 2019; Thibane et al., 2023). The main African countries producing sugarcane include South Africa, Kenya, Sudan, Swaziland, Mauritius, and Zambia (Thibane et al., 2023). In South Africa, the sugar industry is associated with significant socio-economic development and contributes notably to the GDP (Zulu et al., 2019; Thabane et al., 2023). Additionally, sugarcane in the country is produced by both commercial and small-scale farmers, mainly in the KwaZulu-Natal and Mpumalanga provinces (SASA, 2019).

In 2011, the sugar industry noted that there were 29 130 registered sugarcane growers in KwaZulu-Natal, with 93% of them being small-scale growers (SASA, 2011; Cockburn et al., 2014). Small-scale growers heavily rely on sugarcane production for their livelihood (Thibane et al., 2023). However, sugarcane production has been declining over the years due to decreasing yields among small-scale growers (Zulu et al., 2019). Several factors constrain sugarcane yield and production among small-scale growers (Eweg, 2005). A survey conducted

in 2009 in South Africa and Mauritius demonstrated that poor replanting rates and weed infestations contribute to low yields among small-scale growers (Eweg et al., 2009). Additionally, climate change and lack of knowledge also contribute to low yields (Eweg et al., 2009). Mthimkhulu and Miles (2017) reported that growers need to analyze their soil regularly for optimal crop production since sugarcane assimilates more nutrients for growth. Therefore, planting sugarcane repeatedly places heavy demands on soil reserves, leading to soil fertility deterioration (Mthimkhulu and Miles, 2017). Furthermore, soil organic matter is also reduced due to monocropping (Belete and Yadete, 2023). The deterioration of soil fertility necessitates the use of chemical fertilizers (Krishnaraj and Dahale, 2014).

2.2 Effect of chemical fertilizers

Chemical fertilizers improve soil status by enriching it with nutrients that it lacks (Sharma and Chetan, 2017). Nitrogen (N) is a key constraint for continuous sugarcane production (Yang et al., 2019). The proper amount of nitrogen fertilizer can significantly increase sugarcane yield (Gopalasundaram et al., 2012). Therefore, this implies that small-scale growers do not apply enough fertilizers for optimal sugarcane production (Eweg, 2005). Thibane et al. (2023) noted that some small-scale growers apply chemical fertilizers late due to income shortages, while others cannot afford them at all, which results in low yield and production. Research indicates that small-scale growers have been applying 420 kg ha⁻¹ of fertilizers instead of the 610 kg ha⁻¹ required for optimal sugarcane production (Thibane et al., 2023). Owino (2019) suggested that growers must invest in fertilizers to improve sugarcane output. Although chemical fertilizers may be beneficial, excessive nitrogen application can lead to delayed maturity, poor leaf quality, and low sugar content in sugarcane (Muchow et al., 1996; Sharma and Chetan, 2017). In China, approximately 500-700 kg/mm² of nitrogen fertilizer was applied per crop season to enhance production, but nitrogen recovery in sugarcane ranged from 20-40%, which

was very low, with most nitrogen lost in the soil (Chapman et al., 1994; Yang et al., 2014; Li et al., 2015; Yang et al., 2019). These nitrogen losses occur through leaching, volatilization, dinitrogen (N_2) emissions, and runoff (Rasiah et al., 2003; Thorburn et al., 2005), which are detrimental to the environment (Ju et al., 2009). Furthermore, excessive application of chemical fertilizers leads to soil degradation, nutrient imbalance, non-point pollution, eutrophication, and increased costs (Ojeniyi, 2002; Ju et al., 2009; Chen et al., 2014).

2.3 Soil degradation

Soil degradation refers to the loss of soil quality for specific functions (Blum, 2008). The reduction in soil pH contributes to soil nutrient deficiency by reducing many essential nutrients like ammonium (NH_4^+), calcium (Ca^{2+}), and potassium (K^+) (Aprile and Lorandi, 2012; Zungu et al., 2020). In addition, in acidic soils phosphorus (P) form insoluble complexes with cation such as aluminium, iron and manganese rendering it unavailable for plant uptake (Vance, 2001). Zungu et al. (2020) reported that low pH reduces the microbial richness and diversity. Microbes play a vital role in enhancing soil quality and plant growth through secretion of plantpromoting compounds (Kennedy et al., 2004; Zungu et al., 2020). For instance, microbes belonging to *Bacillaceae* have been reported to improve nutrient availability through solubilizing P and K, producing phytohormones, enhancing N fixation, and preventing pathogen developments (Vendan et al., 2010; Pérez-Fernández, and Alexander, 2017.; Zungu et al., 2020). Changes in microbial communities will negatively affects nutrient cycling and availability. Therefore, soil rehabilitation strategies must be implemented to enhance soil quality and sustainability in sugarcane plantations in KwaZulu-Natal.

2.3 Incorporating legumes in sugarcane plantations

Legumes are known to perform multiple functions and contribute to food security (Atnaf et al., 2015). They are highly rich in protein, carbohydrates, and vitamins (Dube and Fanadzo, 2013). Incorporating legumes as cover crops will enhance soil quality and health and also will contribute to sustainable agriculture (Khan et al., 2021). Planting legume cover crops during the sugarcane fallow period will break the cycle of pests, diseases, suppress weeds, and increase the microbial communities (Ramouthar et al., 2014). They protect the environment by reducing loss of nutrients via leaching (Reeves, 1994). Khan et al. (2021) reported that cover crops increase the soil organic matters. In addition, their symbiotic relationship with the microbes facilitates nutrient cycling and N fixation, reducing the reliance on chemical fertilizers (Prajapati et al., 2023). This occurs through the association with microbes that enables legumes to convert the atmospheric nitrogen (N_2) into simple usable forms through biological fixation process (Dabney et al., 2010).

2.3.1 Biological N fixation

Nitrogen is an essential nutrient for plant growth and development; it plays a key role in many physiological and metabolic processes (Fathi, 2022). It is a constituent of proteins, amino acids, and nucleic acids (Fathi, 2022). Its deficiency delays the phenological development in plants and reproductive stages, resulting in low crop yield or loss of crop (Fathi and Zeidali, 2021). Although, N is abundant in the atmosphere, but it is unavailable to plants. Barbieri et al. (2000) reported that the atmospheric nitrogen (N_2) makes up to 78%, however, nitrogen cannot be assimilated by plants unless it is converted to plant usable forms such as nitrate (NO_3^-) and ammonium (NH_4^+) (Guo et al., 2019). Legumes can convert the atmospheric N_2 into plant usable forms through their symbiotic relationship with the rhizobial bacteria found in the nodules of the legume (Soumare et al., 2020). The bacteria that form a symbiotic relationship

with the legumes belong to *Azospirillum*, *Rhizobium*, and *Bradyrhizobium* genus (Zahran, 2001). The reduction of atmospheric nitrogen (N_2) to ammonium or nitrate is catalysed by the nitrogenase enzymes and this process utilizes 16 ATP molecules (Bloom et al., 1992; Kumar, 2014). The following equation is for the conversion of atmospheric nitrogen (N_2) to ammonia according to Bloom et al., 1992: $N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi$.

2.3.2 Sustainable N management

Incorporating legume to sugarcane plantations will enhance soil fertility and health, reducing the dependence to chemical inputs (Adjei-Nsiah, 2012). For instances, Maize grown after pigeon pea, were perceived by growers to look greener, grow fast, and yield more with 75% increase (Adjei-Nsiah, 2012). Red clover grown in rotation with corn and oats contributed 73 to 115 kg N ha⁻¹ to the subsequent corn (Stute and Posner, 1995). According to Kelner and Vessey (1995) alfalfa contributed 121 kg N ha⁻¹ in the soil in Manitoba. In addition, wheat yield increased by 66% to 114% after alfalfa in the Northern Plains (Hoyt, 1990). Furthermore, this forage legume is also effective in scavenging nutrients from deeper soil profile therefore can assimilate the lost nutrients in the environment (Adjei-Nsiah, 2012). Studies have indicated that decomposing legume cover crops have positive effect in promoting soil fertility by releasing fixed N and reducing the need for fertilizers (Dabney et al., 2010; Jani et al., 2015).

2.3.3 Association with plant growth promoting bacteria

Numerous microorganisms have a significant effect on soil fertility and health and enhance plant growth (Zungu et al., 2020). Some of legumes are chosen due to their well-known abilities to establish association with nutrient cycling and plant promoting rhizobacteria (PGPR) such as *Azotobacter*, *Arthrobacter*, *Alcaligenes*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Klebsiella*, and *Pseudomonas* (Glick, 1995; Saharan and Nehra, 2011). They enhance plant

growth without contaminating environment by inducing resistances against plant pathogens, fixing and solubilizing nutrients, producing phytohormones, siderophores, and protection enzymes such as glucanase, chitinase, and ACC-deaminase (Choudhary et al., 2011; García-Fraile et al., 2015; Vejan et al., 2016). Castignetti and Smarrelli (1986) reported PGPR prevent proliferation of pathogens by secreting siderophores, these molecules will bind to iron which is available in soil, thus preventing any pathogens from proliferating because of lack of iron (O'Sullivan and O'Gara, 1992). The PGPR can produce extracellular enzymes that are responsible for mineralization and cycling nitrogen (N), carbon (C), and phosphate (P) (Zungu et al., 2020). β -D-glucosaminidase and asparaginase enzymes have an effect in N mineralization and increase N assimilation in plants through hydrolysing chito-oligosaccharides and converting asparagine into aspartic acid into and ammonia (Hill et al., 1967; Mega et al., 1972; Zungu et al., 2020). Phosphatase activities are involved in P cycling and mineralization. Phosphorus is solubilized by hydrolysing the phosphoric acid monoester to produce the phosphate anion (Turner et al., 2002). While the β -D-glucosidase, β -D cellobiohydrolase, and dehydrogenase are responsible for carbon cycling (Henriksson et al., 1998).

2.4 Study species

Vigna unguiculata L. (Walp) is an essential legume that is mainly cultivated in the tropical and subtropical regions (Kebede and Bekeko, 2020). In developing countries including South Africa it is used as a food source because of its high nutritional value (Popelka et al., 2004). *Vigna unguiculata* is rich in protein, carbohydrates, and vitamins (Alemu et al., 2016). Besides its nutritional value *V. unguiculata* is a versatile crop that can be cultivated as a cover crop due to its contribution in soil health (Alemu et al., 2016). *Vigna unguiculata* has ability convert the atmospheric N₂ into plant usable forms (Simunji et al., 2019). It can fix approximately 70 to

350 kg N ha⁻¹ and contribute 40 to 80 kg N ha⁻¹ to the soil (Singh, 1997). For instance, Bado et al. (2006) reported that *V. unguiculata* fixed about 50 to 115 kg N ha⁻¹ which increased the yield of succeeding sorghum by 290%. In addition, *V. unguiculata* can improve soil quality by suppressing weed, reducing soil erosion, and preventing pathogens development. According to Kebede (2020) incorporating *V. unguiculata* as ground cover suppressed weed and protected soil from erosion. *Vigna unguiculata* is a suitable cover crop to enhance soil fertility and sustainability due to its positive contributions in agriculture.

Cajanus cajan is a perennial legume primary used as food, animal feed, and incorporated into soil system to enhance soil fertility (Sharma et al., 2011). Integrating *C. cajan* as a cover crop into sugarcane plantations will enhance nutrient availability, increase soil microbes, reduce weed and pathogen development (Sharma et al., 2011). *Cajanus cajan* can fix up to 235 kg N ha⁻¹ and contribute more N per unit area from decomposing plant biomass (Peoples et al., 1995). In Tanzania, they observed that the yield of unfertilized intercropped maize with *C. cajan* generally equalled to the yield of fertilized sole maize, this suggest that *C. cajan* contributed to yield of maize (Myaka et al., 2006). In addition, in West Africa maize yield increased by 32.1% when *C. cajan* was used as a cover crop (Sogbedji et al., 2006). Incorporation of *C. cajan* in coffee plantations enhanced soil properties and reduced weed (Venzon et al., 2006). *Cajanus cajan* is recommended cover crop in sugarcane plantations to enhance sugarcane yield and production while conserving the environment.

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Chapter 3

A leguminous boost: *Vigna unguiculata* cover cropping enhances extracellular enzyme activities and bacterial communities in sugarcane plantation soils

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Abstract

The growing global population has heightened the pressure on agriculture to ensure food security, leading to increased reliance on chemical fertilizers to boost crop productivity. The use of chemical fertilisers and continuous monoculture have been reported reduce soil health and affect belowground communities. To address these challenges, sustainable alternatives, such as legume cover crops during fallow periods, are crucial. This study evaluated the impact of *Vigna unguiculata* as a cover crop on soil nutrient dynamics in sugarcane plantations managed by smallholder growers in KwaZulu-Natal. Additionally, it explores the growth physiology of this legume species when grown in the sampled soils. Soils were collected from five sugarcane plantations (Gingindlovu, Mvutshini, Mpembeni, Hibberdene, and Mzinto) and analyzed pre-planting and post-harvest for soil nutrients, bacterial composition, and enzyme activities. The growth kinetics of *V. unguiculata* grown in the sampled soils were determined. Post-harvest soils were rich in plant growth promoting bacteria belonging to the *Pseudomonas*, *Caulobacter*, *Lysinibacillus* and *Arthrobacter* genera. The phosphatase and β -glucosidase activities increased in Gingindlovu, Mvutshini, and Mpembeni, while nitrate reductase activity increased across all soil collection sites. Though the nitrogen and phosphorus concentrations of the soils across all collection sites decreased in post-harvest soils, there were slight increases in soil pH. *Vigna unguiculata* produced higher shoot biomass than root biomass in all soil collection sites and relied on nitrogen derived from the atmosphere in Mpembeni, Hibberdene and Mzinto soils. The findings of this study highlight the potential of *V. unguiculata* to improve soil health by enhancing nutrient cycling, bacterial diversity, and enzyme activity in sugarcane plantation soils. The integration of *V. unguiculata* in sugarcane production systems could serve as a practical, ecologically sound strategy for mitigating the adverse effects of continuous monoculture and promoting long-term soil sustainability.

Key words: *Vigna unguiculata*, plant-growth promoting rhizobacteria, acidic soils, enzyme activities, small-scale growers, monoculture, sustainability

3.1 Introduction

The rising global population is increasing the demand for food, placing pressure on the agricultural sector to increase food production (Malekela et al., 2024). However, declining soil fertility and poor soil health, exacerbated by unsustainable farming practices, are contributing to reduced crop yields (Messerli, 2006; Styger et al., 2007; Harvey et al., 2014; Herrera et al., 2021). These challenges are particularly evident in sub-Saharan Africa, where declining soil fertility has led to reduced maize production (Tovihoudji et al., 2023). Furthermore, sugarcane growers in KwaZulu-Natal, South Africa, struggle with low yields due to poor soil quality and inadequate management practices (Cockburn et al., 2014).

To improve productivity, the agricultural sector has increasingly relied on synthetic fertilizers. However, the high cost of fertilizers, combined with limited access for small-scale farmers, has made consistent use difficult (Obeng et al., 1990; Mhando and Itani, 2007; Mthembu et al., 2019). Moreover, research shows that up to 60% of nitrogen fertilizers applied to crops are lost through leaching and other processes, contributing to environmental degradation (Vallis et al., 1996; Weier, 1998; Thorburn et al., 2005). Continuous monoculture by smallholder sugarcane growers further depletes soil health (Ramouthar et al., 2014), emphasizing the need for sustainable farming solutions.

One promising approach is integrating legume cover crops into sugarcane plantations to enhance soil fertility and reduce environmental impacts (Eweg et al., 2009; Delgado and Gantzer, 2015; Weerasekara et al., 2017). Legume cover crops, such as *Vigna unguiculata* improve soil chemical properties, and reduce erosion (Reeves, 1994; Dabney et al., 2010).

These crops also play a critical role in suppressing weeds, decreasing pesticide use, and enhancing soil health by improving nutrient cycling through their interaction with soil microbes (Herridge et al., 2005; Lal, 2015). Additionally, legume cover crops increase the availability of nitrogen for subsequent crops by releasing nitrogen through biomass decomposition (Dabney et al., 2010).

Vigna unguiculata has been reported to fix up to 240 kg ha⁻¹ of nitrogen and contribute substantial nitrogen to following crops (Bado et al., 2006; Kebede and Bekeko, 2020). Furthermore, these legumes such as *V. unguiculata* improve soil organic matter content, pH, nutrient levels and the abundance of beneficial microbes (Khan et al., 2021). Microbial communities play a vital role in soil health, influencing enzyme activities necessary for nutrient cycling. Phosphatase enzymes, involved in phosphorus cycling, are produced by plants and soil microbes, enhancing nutrient bioavailability (Duff et al., 1994; Ilakiya et al., 2023). Microbial communities associated with legumes have been reported to increase phosphatase activity in nutrient-deficient soils, further improving nutrient cycling and supporting soil health (Zhu et al., 2012; Alori et al., 2017; Magadlela et al., 2023).

Despite the known benefits of *V. unguiculata* as a cover crop, its potential in sugarcane plantations has not been thoroughly explored. This study seeks to evaluate its impact on soil nutrient dynamics in small-scale sugarcane plantations in KwaZulu-Natal. Specifically, the study aims to: Assess the growth and nitrogen source reliance of *V. unguiculata* in sugarcane plantations and determine the contribution of *V. unguiculata* to soil chemical and biological properties. *Vigna unguiculata* will be planted in soils collected from various sugarcane plantations in KwaZulu-Natal. Soil bacterial communities and associated enzymes and soil

chemical properties will be assayed pre- and post-harvest. We hypothesised that *V. unguiculata* will be able to maintain growth in nutrient poor sugarcane plantation soils while contributing to soil nutrient dynamics by improving the abundance of beneficial bacteria and their associated enzyme activities.

3.2 Material and Methods

3.2.1 Soil collection sites

Soil samples were collected from five different small-scale sugarcane plantations in KwaZuluNatal province, South Africa. These included two plantation sites from the South Coast:

Hibberdene (30°35'37.1214" S, 30°31'30.2916" E), and Mzinto (30°16'41.0262" S, 30°39'47.9658" E). The other three sites located on the North Coast of KwaZulu-Natal were from Gingindlovu (28°56'29.3" S 31°34'58.9" E), Mvutshini (28° 50' 52.9" S 32°00'09.0" E), and Mpembeni (28°51'25.7" S 31°58'19.4" E). In May 2022, twenty random soil samples were collected per site at the depth of 10-20 cm. Pre-planting soils to be used for enzyme assays and bacterial identification were stored in sterile ziplock bags, placed on ice during transportation to the laboratory and stored at 4 °C prior analysis.

Sampling Sites

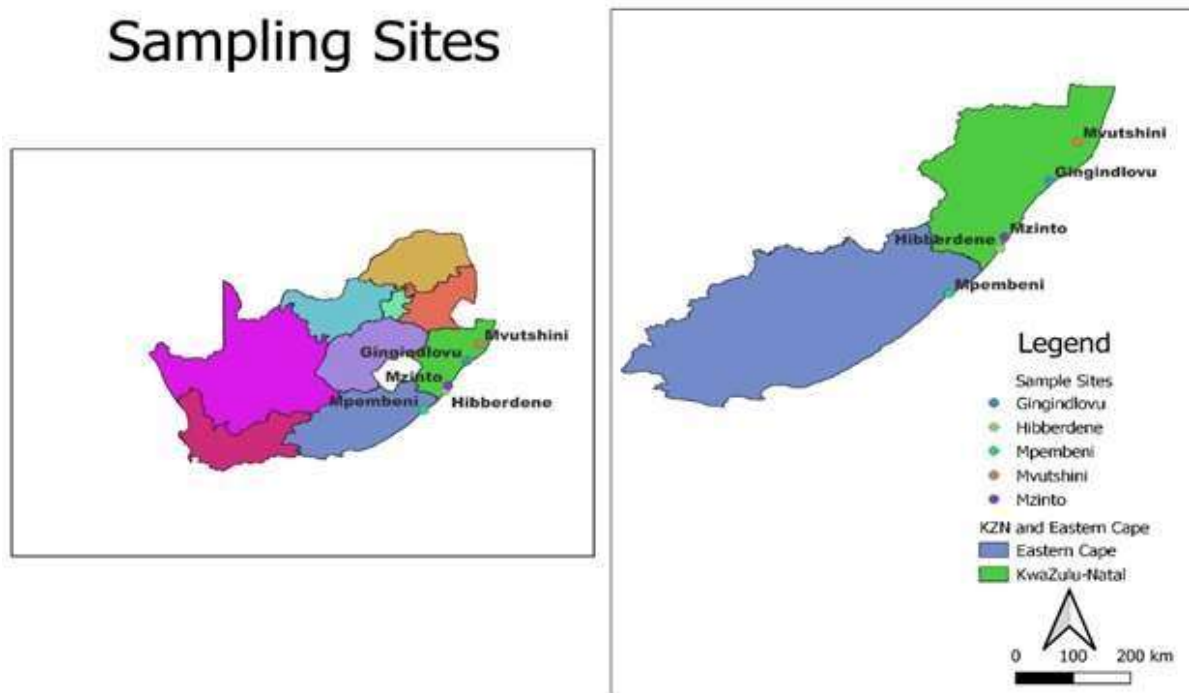


Figure 3.2.1 Map showing the location of sampling sites in the South Coast and North Coast of KwaZulu-Natal province, South Africa.

3.2.2 Experimental design, growth conditions, and soil nutrient analysis

Vigna unguiculata L. (Walp) seeds used in this study were purchased at AGT foods, Marji Mizuri Farm, Ingomankulu, KwaZulu-Natal. To stimulate seeds germination, seeds were soaked in warm water overnight. Seeds were then germinated in petri dishes lined with moist filter paper under sterile conditions, stored in the dark at room temperature for 2 days. After germination, *V. unguiculata* seedlings were transferred into 10 cm diameter pots filled with soil from different sugarcane plantations and planted at depth of 1–2 cm. The experiment was laid in a random block experimental design with each plantation site replicated 10x giving a total of 50 experimental pots. The experiment was conducted under the greenhouse conditions at the University of KwaZulu-Natal, Westville campus, in the School of Life Sciences building, South Africa (13–16 °C and 28–35 °C night and day temperatures, respectively; with humidity

ranging from 70% to 80%). The plants were irrigated everyday using an automated irrigation system. The initial and final harvesting took place after 14 and 54 days after seedling emergence, respectively. Plants were rinsed using distilled water and separated into roots and shoots, then oven dried at 80 °C for a week. Dry weights were recorded, and dried plant material was ground into fine powder using a pestle and mortar. The ground plant material was sent for

C and N isotope and P analysis at the Archaeometry Department University of Cape Town, South Africa and Central Analytical Facilities, Stellenbosch University, South Africa respectively. Post-harvest soils for enzyme assays and bacterial identification were stored in sterile ziplock bags and kept at 4 °C. For nutrient analysis, three kg of soil samples from each plantation pre-planting and post-harvest were air dried and sent to the South African Sugarcane Research Institute (SASRI) Fertiliser Advisory Service (FAS). These samples were analysed for primary nutrients (N, P, K), secondary nutrients (Ca and Mg), pH, acid saturation, exchange acidity, and total cations.

3.2.3 Soil bacterial extraction

For each sugarcane plantation soil, three-fold serial soil dilutions were conducted where 10 g of soil sample was added to a volumetric flask containing 100 ml of autoclaved distilled water under sterile conditions. Fifty to hundred microliters of each dilution were used to inoculate different nutrient agar plates. Tricalcium phosphate media plates were used to isolate and grow phosphate solubilizing bacterial strains while Simmon citrate and Jensen media were used for N-cycling and N-fixing bacterial strains, respectively. The plates were incubated at 30 °C for 3–7 days. Thereafter, colonies were sub-cultured into clean plates to form pure colonies.

3.2.4 Bacterial amplification and identification

Pure colonies extracted from different sugarcane plantation soils were amplified using the polymerase chain reaction (PCR). Primers used in the PCR were 63F (5'-CAGGCCTAACACATGCAAGTC -3') and 1387R (5'-GGGCGGTGTGTACAAGGC -3'). A series of chemicals were added to autoclaved 2 ml Eppendorf tubes making total of 25 μ L PCR reaction. The PCR reaction mixture contained 12.5 μ L of the EmeraldAmpGT master mix (Takara Bio Inc Supplied by Separations, South Africa), 8.5 μ L of sterile milliQ water, 0.5 μ L of forward and reverse primers, and 3 μ L of diluted bacterial pure colony. Amplification was carried out using a T100 Thermal Cycler (Bio-Rad, USA) under the following conditions: initial denaturation at 94°C (5 min), followed by 30 cycles of denaturation at 94°C (30 s), annealing at 55°C (30 s), and extension at 72°C (2 min), with additional extension at 72°C (10 min). The PCR results were visualized on 1% m/v agarose gel electrophoresis using TBE buffer and observed in dark room under UV light. Positive amplified amplicons were sent to Inqaba Biotech Pty. Ltd., Pretoria, South Africa, for sequencing. The resulting sequences were edited and subjected to BLASTN searches for identification using the National Center for Biotechnology Information (NCBI) database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) (accessed 15 November 2024).

3.2.5 Soil enzyme activities assay

3.2.5.1 Phosphatase and β -glucosidase activities

The acid phosphatase activity, alkaline phosphatase activity, and β -glucosidase activity were conducted using the fluorescence-based method adapted from Jackson et al. (2013). Five grams of soil from each sugarcane plantation were added to flask covered with foil containing 100 ml of autoclaved distilled water, homogenized at medium speed in a shaker for 2 hours, and stored

in the fridge overnight. 4-MUB-phosphate (acid and alkaline phosphate activity), 4-MUB- β glucopyranoside (glucosidase activity) standards, 100 mM bicarbonate buffer, and 4-MUBphosphatase substrate were prepared and stored in the fridge. Different solutions were transferred into 96-well microplates and incubated for 1 hour at 30°C. Each sample run consisted of three replicates, with 200 μ L of soil sample plus 50 μ L substrate. Substrate controls had three replicates with 200 μ L buffer plus 50 μ L substrate. Sample controls consisted of two replicates with 200 μ L of soil sample plus 50 μ L buffer. Quenched standards included three replicates with 200 μ L of soil sample plus 50 μ L standard, and standard controls had three replicates with 200 μ L buffer plus 50 μ L standard. To stop the reactions, 5 μ L of 0.5 M NaOH was added to each well. Then, an Apex Scientific microplate reader was used to measure the fluorescent absorbance at 450 nm. Notably, the pH of the standard and buffer were adjusted to 5 before determining acid phosphatase activity.

3.2.5.2 Nitrate reductase activity

The nitrate reductase activity was conducted using the method adapted from Bruckner et al., 1995. Five grams of soil from different sugarcane plantations were added into a conical flask covered with foil containing 5 ml of autoclaved distilled water, 4 ml of 0.9 mM 2,4 DNP, 1 ml of 25 mM KNO₃. The solution was vigorously mixed before being incubated for 24 hours at 30 °C. After incubation, in each sample 10 ml of 4 M KCl was added and thoroughly mixed then passed through filter paper. The enzymatic reaction was initiated by adding 2 ml of filtrates into 1.2 ml of 0.19 M of ammonium chloride buffer (pH 8.5) and 0.8 ml of colour reagent containing 1% sulfanilamide, 1 N HCl, and 0.02% N-(1-naphthyl) ethylenediamine dihydrochloride (NEDD). Then solution was incubated for 30 min at 30 °C in the dark. The absorbance was measured at 520 nm using an Agilent Cary 60 UV-Vis spectrophotometer

(Agilent, Santa Clara, CA, USA). The amount of nitrite (NO_2) released into the medium was expressed as $0.1 \mu\text{mol h}^{-1} \text{g}^{-1}$.

3.2.6 Total plant nutrition

3.2.6.1 Calculations of percentage nitrogen derived from the atmosphere (% NDFA)

Nitrogen isotope analyses were measured at the Archeometry Department, University of Cape Town, South Africa. According to Herridge et al. (1995) ^{15}N natural abundance technique is based on the differences in the natural abundance of the stable isotope of N (^{14}N and ^{15}N) which is between atmospheric nitrogen and other sources of nitrogen such as soil. The heavier ^{15}N isotope occurs more in the soil regions while the lighter ^{14}N isotope occurs more in the atmosphere region (Mariotti, 1983). The isotope ratio of N was calculated using:

$$\delta = 1000\text{‰} (R_{\text{sample}}/R_{\text{standard}})$$

Where R is the molar ratio ^{15}N to ^{14}N of the samples and standards. For each milled sample of approximately 2 mg weighed into 8 x 5 mm tin capsules (Elemental Micro-analysis, 30 Devon, UK) on a Sartorius microbalance (Goettingen, Germany) combusted in a Fisons NA 1500 (Series 2) CHN analyser (Fisons Instruments SpA, Milan, Italy). Nitrogen isotope ratios were determined on a Finnigan Matt 252 mass spectrometer (Finnigan MAT GmbH, Bremen, Germany), connected to a CHN analyser by a Finnigan MAT Conflo control unit. Three standards were used to correct the samples for machine drift, namely, two in-house standards (Merck Gel and Nasturtium) and the IAEA (International Atomic Energy Agency) standard $(\text{NH}_4)_2\text{SO}_4$. According to Shearer and Kohl (1986), the percentage derived from the atmosphere was calculated as:

$$\% \text{NDFA} = 100((\delta^{15}\text{N}_{\text{reference plant}} - \delta^{15}\text{N}_{\text{legume}}) / (\delta^{15}\text{N}_{\text{reference plant}} - \beta)).$$

The β -value is $\delta^{15}\text{N}$ natural abundance of the N derived from BNF of *V. unguiculata*, grown in a nitrogen-free culture, that was determined as -2.60% .

3.2.6.2 Specific Nitrogen /Phosphorus assimilation and utilization rate

The net specific nitrogen/phosphorus assimilation and specific nitrogen/phosphorus utilization rates were calculate using the equations derived from (Nielsen et al., 2001):

$$\text{SNAR} = (N_2 - N_1 / t_2 - t_1) * [(\log_e R_2 - \log_e R_1) / (R_2 - R_1)]$$

$$\text{SPAR} = (P_2 - P_1 / t_2 - t_1) * [(\log_e R_2 - \log_e R_1) / (R_2 - R_1)]$$

$$\text{SNUR} = (W_2 - W_1 / t_2 - t_1) * [(\log_e N_2 - \log_e N_1) / (N_2 - N_1)]$$

$$\text{SPUR} = (W_2 - W_1 / t_2 - t_1) * [(\log_e P_2 - \log_e P_1) / (P_2 - P_1)]$$

Where N and P represent the total nitrogen and phosphorus content in the plant, t is the duration it took for a plant to grow, R is the root dry weight as described by Nielsen et al. (2001).

3.2.6.3 Carbon construction costs (Cw)

Carbon construction cost (Cw) was calculated using the equation modified by Peng et al. (1993):

$$Cw = (C + kN/14*180/24) (1/0.89) (6000/180)$$

Where Cw is the total carbon construction cost of tissues, C is the total concentration of C, k is the reduction state of N substrate (for $\text{NH}_3 = -3$), and N is the total organic N content of tissue as described by Williams (1987). The numerical value 14 represent the atomic number of N, 180 is a conversion factor from moles to grams of glucose, 24 are the electrons in a glucose molecule; 0.89 is an estimate of growth efficiency and the fraction 6000/180 is a constant conversion factor from g^{-1} dry weight to mmol C g^{-1} DW for glucose.

3.2.6.4 Relative growth rate

The relative growth rate was calculated using an equation derived from Agren and Franklin (2003):

$$RGR = [(\ln W_2 - \ln W_1) / t_2 - t_1]$$

Where W represents dry weights and t is the time it took for a plant to grow.

3.2.6.5 Root: Shoot ratio

The root-to-shoot ratio was calculated using an equation derived from Agren and Franklin, 2003:

$$\text{Root: shoot} = D_R/D_S$$

Where D_R represents the root dry weight and D_S is the shoot dry weight.

3.2.7 Statistical analysis

R-studio software (version 3.6.2) was used to compare means of soil nutrients, enzyme activities, plant mineral nutrition, growth kinetics and plant biomass, measured pre-planting and post-harvest of *V. unguiculata* using the analysis of variance (One-way ANOVA). Tukey's multiple comparisons post hoc test was conducted when significant results were observed ($p < 0.05$). The Shapiro-Wilk and Levene's tests were used to test assumptions for normal distribution and homogeneity of variance, respectively. Where assumptions were not met, a non-parametric test was used (Kruskall-Wallis test).

3.3 Results

3.3.1 *Vigna unguiculata* growth kinetics

The growth kinetics of *Vigna unguiculata* grown in soils from the sampled sugarcane plantation sites are presented in Table 3.1. *Vigna unguiculata* grown in soils collected from

Mzinto had significantly higher total plant biomass, shoot biomass, and root biomass compared to those from other sites. However, there were no significant differences ($p \geq 0.05$) in the root-to-shoot ratio of *V. unguiculata* grown in soils from Mpembeni, Hibberdene, and Mzinto. The phosphorus concentration in *V. unguiculata* grown in Hibberdene and Mzinto soils was higher than in plants grown in Gingindlovu, Mvutshini, and Mpembeni. Although there was no significant difference ($p \geq 0.05$) in plant nitrogen (N) concentration between plants grown in Hibberdene and Mzinto, these concentrations were higher than in plants grown in Gingindlovu, Mvutshini, and Mpembeni. *Vigna unguiculata* grown in Mpembeni, Hibberdene, and Mzinto soils relied more on atmospheric N fixation than plants grown in Gingindlovu and Mvutshini soils. There were no significant differences ($p \geq 0.05$) in the relative growth rate (RGR) of plants across all collection sites. However, the SPAR of plants grown in soils from Mpembeni, Hibberdene, and Mzinto was significantly ($p \leq 0.05$) higher than in those from Gingindlovu and Mvutshini. The SNAR was significantly ($p \leq 0.05$) higher in plants grown in Mvutshini and Hibberdene than in other collection sites.

3.3.2 Bacterial identification

The molecular identification of bacteria isolated from pre-planting and post-harvest soils across all sugarcane plantation sites is presented in Table 3.2. In Gingindlovu, pre-planting soils contained a single bacterial isolate belonging to the *Pseudomonas* genus. However, postharvest soils had six bacterial isolates from the *Pseudomonas*, *Caulobacter*, and *Lysinibacillus* genera. In Mvutshini, pre-planting soils contained five bacterial isolates belonging to the *Paraburkholderia*, *Pseudomonas*, *Burkholderia*, and *Chromobacterium* genera, while postharvest soils contained three bacterial isolates from the *Pseudomonas* and *Bacillus* genera. In Mpembeni, pre-planting soils contained four bacterial isolates from the *Burkholderia* and *Pseudomonas* genera. Post-harvest soils in Mpembeni had three bacterial isolates, all

belonging to the *Pseudomonas* genus. In Hibberdene, pre-planting soils contained two bacterial isolates from the *Pseudomonas* genus, whereas post-harvest soils contained four bacterial isolates belonging to the *Agrobacterium*, *Pseudomonas*, and *Lysinibacillus* genera. In Mzinto, preplanting soils contained five bacterial isolates from the *Pseudomonas* and *Arthrobacter* genera. Post-harvest soils had seven bacterial isolates, also belonging to the *Arthrobacter* and *Pseudomonas* genera.

3.3.3 Soil enzyme activities

The enzyme activities in pre-planting and post-harvest soils from Gingindlovu, Mvutshini, Mpembeni, Hibberdene, and Mzinto are presented in Figure 3.1. Post-harvest, acid phosphatase activity increased across all sites except for Mzinto, with significant differences observed in Mvutshini, Mpembeni, and Hibberdene. Alkaline phosphatase activity significantly increased in post-harvest soils from Gingindlovu, Mvutshini, and Mpembeni. Similarly, glucosidase activity significantly increased across all sites except for Hibberdene and Mzinto. Additionally, nitrate reductase activity showed a significant increase across all sites post-harvest.

3.3.4 Soil Nutrient Analysis

The nutrient concentrations and relative acidity of pre- and post-harvest soils is represented in Table 3.3. Post-harvest, soil N and P concentrations showed an insignificant decrease across all sites, except for P concentration in Mzinto, which showed a significant decrease. Additionally, K concentration decreased in Hibberdene, Gingindlovu, Mzinto, and Mvutshini soils, while Mpembeni soils remained unchanged, with no significant differences detected across all sites. Although the differences were insignificant, a slight increase in secondary nutrient concentrations (Ca and Mg) was observed post-harvest across all sites, except for Mvutshini and Mpembeni soils, which displayed a decrease in Ca concentration. All soil

samples from different sites remained acidic, with pH ranging from 4.67 to 5.94. However, post-harvest, pH levels increased, becoming less acidic (Gingindlovu: 4.80 to 4.85, Mvutshini: 4.67 to 4.77, Mpembeni: 4.94 to 5.43, Hibberdene: 5.13 to 5.94, and Mzinto: 4.88 to 5.82). Acid saturation (%) and exchange acidity decreased across all sites, with insignificant differences observed, except for Gingindlovu and Mpembeni soils. Furthermore, total cation concentrations showed a slight increase, but no significant differences were observed across all sites, except for Mpembeni soils.

3.4 Discussion

Vigna unguiculata was able to maintain growth in nutrient deficient sugarcane plantation soils, while enhancing belowground bacterial communities and associated enzyme activities. *Vigna unguiculata* may have secreted root exudates that supported the proliferation of plant growth promoting bacterial genera such *Arthrobacter*, *Agrobacterium*, *Bacillus*, *Lysinibacillus*, *Caulobacter*, and *Pseudomonas* genera. The isolated bacterial strains have been reported to enhance plant growth through mineral solubilisation (phosphate and zinc), nitrogen fixation, indole acetic acid (IAA) production, and siderophore secretion (Glick, 1995; Bell et al., 2005; Matiwane et al., 2019; Verma et al., 2020; Ozdogan et al., 2022). The presence of bacterial strains with N fixing properties may have contributed to the increased %NDFA in *V. unguiculata* plants growing in Mpembeni, Hibberdene and Mzinto. Soils collected from Hibberdene and Mzinto may have a higher abundance of bacterial genera with N fixing traits and conditions that support reliance on Biological Nitrogen fixation (BNF) as Ngonini et al. (2025) reported a higher reliance on NDFA in *Vicia sativa* plants growing in the abovementioned soils.

In Mpembeni soils, the acid phosphatase activity of post-harvest soils was higher than preplanting soils, indicating that *V. unguiculata* used P cycling enzymes as a strategy to meet the demands of BNF. According to Magadlela et al. (2016), high P availability supports BNF, an energy-intensive process requiring P for ATP generation. *Vigna unguiculata* plants growing in Hibberdene and Mzinto had a higher P assimilation rate compared to other soil collection sites, indicating that the plants in these sites assimilated more P to support BNF. The low SPUR and SNUR from *V. unguiculata* plants growing in Gingindlovu and Mvutshini may indicate used nutrients from the soils sparingly to maintain growth, supporting the low reliance on NDFA.

Variations in the nutrient concentrations of soils collected from the sugarcane plantation sites elicited different responses in the growth of *V. unguiculata* plants, showing the influence of soil conditions on cover crops and the resilience of *V. unguiculata*. In Mzinto, the SNAR was lower, supporting the higher reliance on NDFA coinciding with Matiwane et al. (2019).

In Hibberdene, *V. unguiculata* has a high NDFA, NDFS and SNAR, indicating that the plants in this site switch N sources, as supported by Sithole et al. (2019). Though variations were recorded in plant response, a slight increase in pH was observed across all soil plantations after planting *V. unguiculata*, consistent with findings by Balota and Chaves (2011) following the incorporation of cover crops. Acidic soils, which constrain root health and nutrient availability (Van Antwerpen et al., 2023), lead to the binding of nutrients such as P to cations like aluminium, iron, and manganese rendering them unavailable for plant uptake (Lin et al., 2012). Thus, increasing pH could have been a means to increase the bioavailability of P in post-harvest soils and may have been more significant with a longer growth period. Acid and alkaline phosphatase activities are vital for P mineralization and cycling (Magadlela et al., 2023).

Gianfreda and Rao (2014) explained that P is produced by hydrolysing the phosphoric acid monoester to phosphate anions.

Increased acid and alkaline phosphatase activities were observed in acidic and nutrient deficient soils from Gingindlovu, Mvutshini, and Mpembeni plantations. These findings align with Magadlela et al. (2023), who reported increased phosphatase activity under P deficiency and acidic conditions. Moreover, nitrate reductase activity increased across all sites (Figure 3.1), aiding in the degradation and hydrolysis of chitin to inorganic nitrogen (Gianfreda and Rao, 2014). Microbial and enzyme activities contributed to *V. unguiculata*'s growth in nutrient deficiency and acidic soils by enhancing nutrient bioavailability and uptake.

3.5 Conclusion

Vigna unguiculata had a positive effect on sugarcane plantation soils. Its association with plant growth promoting rhizobacteria enhanced nutrient mineralization and solubilisation under nutrient-deficient and acidic soil conditions. *Vigna unguiculata* contributed to increased soil pH, soil enzymes activities, and improved %NDFA, all which are good indicators of improved soil health. It is important to allow *V. unguiculata* plants to decompose and release store nutrients back into the soil, as they exhibited high total plant concentrations. Incorporating *V. unguiculata* as cover crop is an effective strategy for conserving and recycling soil nutrients for following cash crop, while also contributing to food security and a sustainable future.

Authors contributions

Conceptualization, M.A; methodology, M.A; M.M, performed the growth trials, data analysis and draft manuscript; review and editing, M.A; supervision and funding acquisition, M.A.

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Conflict of interest

We declare no conflict of interest with regards to this study.

Data availability

The data can be made available upon request from the corresponding author Prof. Anathi Magadlela at [REDACTED]

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Tables and figures

Table 3.1: Biomass, plant mineral nutrition, and growth kinetics of 54-old-day *Vigna unguiculata* plants grown in sugarcane plantation soils collected from Gingindlovu, Mvutshini, Mpembeni, Hibberdene, and Mzinto, KwaZulu-Natal, South Africa. Data are presented as mean $\pm$ SE, n = 3. Different superscript indicates significant difference ($P \leq 0.05$).

Parameters	Study sites				
	Gingindlovu	Mvutshini	Mpembeni	Hibberdene	Mzinto
Biomass (g)					
Total plant biomass	0.788±0.158 ^b	0.769±0.296 ^a	0.772±0.131 ^b	0.699±0.060 ^c	2.164±0.622 ^d
Shoot biomass	0.283±0.051 ^a	0.226±0.105 ^a	0.201±0.043 ^b	0.174±0.128 ^b	0.483±0.151 ^a
Root biomass	0.141±0.029 ^b	0.075±0.020 ^a	0.153±0.041 ^b	0.154±0.020 ^b	0.305±0.050 ^c
Root: Shoot ratio	0.493±0.038 ^b	0.418±0.135 ^b	0.804±0.205 ^a	0.890±0.044 ^a	0.712±0.151 ^a
Plant mineral nutrition					
Total plant P (µmol P g ⁻¹)	15.02±0.299 ^d	16.95±0.113 ^{cd}	18.86±0.164 ^c	43.43±0.944 ^a	36.23±0.507 ^b
Total plant N (mmol N g ⁻¹)	1.43±0.033 ^b	1.33±0.027 ^b	1.53±0.026 ^c	2.40±0.040 ^a	2.43±0.191 ^a
NDFa (%)	0.63±1.825 ^b	3.05±0.686 ^b	49.15±0.174 ^c	54.14±0.731 ^a	63.14±6.459 ^a
NDFa (mmol N g ⁻¹)	0.009±0.000 ^b	0.008±0.000 ^b	0.010±0.000 ^b	0.015±0.000 ^a	0.015±0.000 ^a
NDFS (mmol N g ⁻¹)	0.009±0.000 ^b	1.325±0.033 ^b	1.524±0.033 ^b	2.385±0.057 ^a	2.418±0.088 ^a
Growth kinetics					
RGR (g/day)	0.198±0.047 ^a	0.066±0.043 ^a	0.292±0.044 ^a	0.259±0.022 ^a	0.129±0.037 ^a
Carbon cost (mmol C/g DW)	0.696±0.004 ^a	0.695±0.004 ^a	0.714±0.002 ^a	0.643±0.013 ^b	0.421±0.010 ^c
SPAR (mg P g ⁻¹ root DW day ⁻¹)	38.28±0.590 ^d	36.07±0.245 ^d	55.35±0.485 ^c	123.1±2.682 ^a	81.43±1.419 ^b
SPUR (mg P g ⁻¹ plant DW day ⁻¹)	0.079±0.000 ^a	0.059±0.000 ^c	0.111±0.001 ^b	0.053±0.001 ^e	0.039±0.000 ^d

SNAR	30.91±12.67 ^c	115.0±3.094 ^a	41.67±0.921 ^c	71.43±1.743 ^b	23.56±0.884 ^c
(mg N g ⁻¹ root DW day ⁻¹)					
SNUR	0.986±0.149 ^a	0.786±0.165 ^a	1.984±0.025 ^a	2.928±1.506 ^a	3.396±0.087 ^a
(mg N g ⁻¹ plant DW day ⁻¹)					

Table 3.2: Nutrient cycling bacteria (accession number and percentage identity) isolated from sugarcane plantation soils in Gingindlovu, Mvutshini, Mpembeni, Hibberdene and Mzinto, pre-planting and post-harvest of *Vigna unguiculata* species

Study sites	N-fixing		N-cycling		P-solubilizing	
	Pre	Post	Pre	Post	Pre	Post
Gingindlovu		<i>Pseudomonas angilliseptica</i> (Acc: MH185879.1, %ID: 99.85)		<i>Pseudomonas</i> sp. (Acc: MT35450 7.1, %ID: 100.00)	<i>Pseudomonas frederiksberginsis</i> (Acc: MT378522.1, %ID: 99.80)	<i>Pseudomonas</i> sp. (Acc: MT354507 .1, %ID: 100.00)
		<i>Caulobacter</i> sp. (Acc: MN:030294.1, %ID: 98.77)				<i>Lysinibacillus</i> sp. (Acc: MN33531 0.1, % ID: 98.12)
		<i>Pseudomonas helmanticensis</i> (Acc: MT322955.1, %ID: 100.00)				

Mvutshini	<i>Paraburkholderia</i> <i>phymatum</i> (Acc: HE864336.1, % ID: 100.00)	<i>Pseudomonas</i> sp. (Acc: MK575026.1, %ID: 99.82)	<i>Pseudomonas</i> <i>nitroreducens</i> (Acc: KY03884.1, %ID: 99.60)	<i>Pseudomonas</i> <i>koreensis</i> (Acc: ON428965.1, % ID: 100.00)		
	<i>Pseudomonas</i> <i>koreensis</i> (Acc: ON428965.1, % ID: 100.00)	<i>Bacillus</i> sp. (Acc: OP890999.1, % ID: 94.2)	<i>Chromobacterium</i> <i>piscinae</i> (Acc: LR 634122.1, % 99.90)			
	<i>Burkholderia</i> sp. (Acc: MK612762.1, %ID: 100.00)					
Mpembeni	<i>Burkholderia</i> sp. (Acc: KT390908.1, %ID: 98.30)	<i>Pseudomonas</i> sp. (Acc: HQ403189.1, % ID: 97.8)	<i>Burkholderia</i> <i>cepacian</i> (Acc: MN 691121.1, %ID: 99.	<i>Pseudomonas</i> <i>koreensis</i> (Acc: MT629852.1,	<i>Burkholderia</i> sp. (Acc: KT390908.1, %ID: 98.30)	<i>Pseudomonas</i> sp. (Acc: AF368755.1, %ID: 96.40)

70)

%ID: 100.00)

Pseudomonas
protegens (Acc:
MT505104.1,
%ID: 100.00)

Hibberdene	<i>Pseudomonas</i> sp.	<i>Bacterium</i> clone	<i>Pseudomonas</i>	<i>Pseudomonas poae</i>	<i>Lysinibacillus</i>
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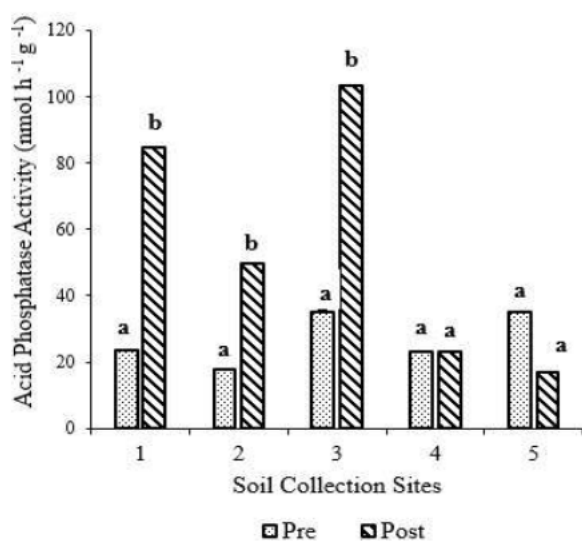
	(Acc: MT35450 7.1, % ID: 99.30)	BOX_G7 (Acc: KU196585.1, % ID: 99.69) <i>Agrobacterium tumefaciens (Rhi zobium radoibacter)</i> (Acc: MN826527.1, %ID: 98.31)		<i>fluorescens</i> (Acc: CP027561.1, % ID: 100.00)	(Acc: OR122193.1, %ID: 99.90)	<i>xylaniticus</i> (Acc: MT605496.1, %ID: 100.00)
Mzinto	<i>Pseudomonas</i> sp. (Acc: MT35450 7.1 %ID: 99.90)	<i>Bacterium</i> clone (Acc: KU196585.1, %ID: 99.96)	<i>Arthrobacter</i> sp. (Acc: MN081011.1, %ID: 100.00)	<i>Pseudomonas</i> sp. strain ATCC (Acc: CP041753.1, % ID: 98.62)	<i>Arthrobacter</i> sp. (Acc: MN081011.1, %ID: 100.00)	<i>Arthrobacter</i> sp. (Acc: MH66931 1.1, % ID:98.95)
	<i>Arthrobacter</i> sp. (Acc: MN08101 1.1, %ID: 99.90)	<i>Bacterium</i> clone (Acc: KU196585.1, %ID: 99.85)		<i>Pseudomonas</i> sp. (Acc: MT354507.1, %ID: 100.00)	<i>Pseudomonas</i> sp. (Acc: MT354507.1, %ID: 99.90)	<i>Pseudomonas</i> sp. (Acc: HQ40318 0.1, % ID:97.42)
				<i>Pseudomonas</i> sp. (Acc: MN400232.1, % ID: 99.59)		

Table 3.3: Soil mineral nutrition (N, P, K, Ca, Mg) and chemistry (pH, acid saturation, exchange acidity, total cations) of 54-day-old *Vigna unguiculata* plants grown in soils from Gingindlovu, Mvutshini, Mpembeni, Hibberdene, and Mzinto sugarcane plantations in KwaZulu-Natal, South Africa. Numerical values are presented as mean $\pm$ SE (n = 3). Different letters in each row denote significant differences ($P \leq 0.05$) among soil sites.

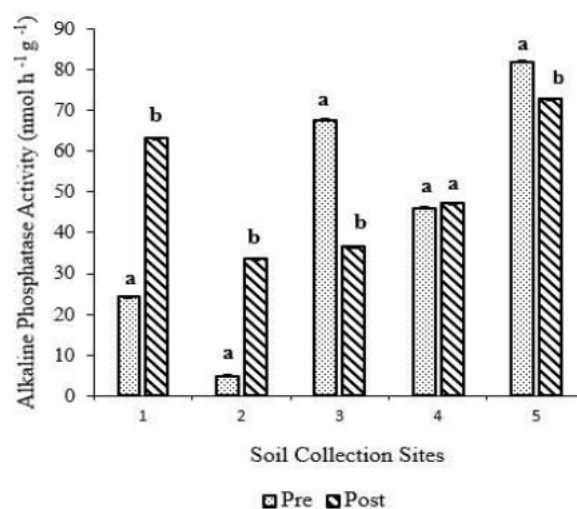
Parameters	Study sites					
	Gingindlovu	Mvutshini	Mpembeni	Hibberdene	Mzinto	
Soil nutrient (mg/L)						
N concentration	Pre	0.055±0.005 ^b	0.085±0.005 ^a	0.055±0.005 ^b	0.060±0.000 ^a	0.070±0.000 ^a
	Post	0.020±0.000 ^b	0.045±0.005 ^b	0.015±0.005 ^c	0.045±0.005 ^b	0.035±0.005 ^c
P concentration	Pre	3.80±0.100 ^d	4.30±0.200 ^a	3.45±0.150 ^d	9.40±0.100 ^b	10.7±0.400 ^a
	Post	2.65±0.250 ^c	2.10±0.300 ^c	1.85±0.050 ^c	7.50±0.500 ^a	6.10±0.300 ^b
K concentration	Pre	55.00±3.000 ^b	33.00±0.000 ^c	18.50±0.500 ^a	100.50±4.50 ^a	61.00±3.000 ^b
	Post	54.50±0.500 ^c	29.00±1.000 ^e	18.50±1.500 ^a	76.50±1.500 ^b	45.00±3.000 ^d
Ca concentration	Pre	220.0±4.000 ^a	1503±1256.5 ^a	452.0±297.0 ^a	387.0±156.0 ^a	603.5±83.50 ^a
	Post	269.0±10.00 ^a	282.5±1.500 ^d	260.0±4.00 ^d	842.0±55.00 ^b	724.0±41.00 ^a
Mg concentration	Pre	132.5±3.500 ^b	79.00±4.000 ^c	39.00±0.000 ^d	141.5±2.500 ^b	166.5±12.50 ^a
	Post	138.5±11.50 ^b	105.5±0.500 ^d	97.00±10.00 ^c	259.5±14.50 ^a	179.0±3.000 ^a
Soil chemistry pH	Pre	4.80±0.050 ^a	4.67±0.030 ^a	4.94±0.295 ^a	5.13±0.045 ^a	4.88±0.345 ^a
	Post	4.85±0.065 ^c	4.77±0.075 ^c	5.43±0.045 ^b	5.94±0.055 ^c	5.82±0.095 ^b
Acid saturation (%)	Pre	11.15±0.65 ^c	20.85±0.05 ^a	14.30±0.90 ^a	1.400±0.00 ^d	1.150±0.15 ^d
	Post	5.850±0.65 ^b	13.55±0.15 ^b	2.650±0.55 ^d	0.750±0.05 ^e	0.950±0.05 ^e
Exchange acidity	Pre	0.32±0.020 ^b	0.54±0.020 ^a	0.19±0.005 ^c	0.06±0.000 ^e	0.06±0.000 ^e

(cmol/L)		Post 0.17 ± 0.010^d	0.39 ± 0.005^b	0.06 ± 0.010^d	0.05 ± 0.000^e	0.05 ± 0.000^e
Total cations	Pre	2.88 ± 0.015^c	2.59 ± 0.090^c	1.30 ± 0.045^d	4.21 ± 0.050^b	5.26 ± 0.285^a
(cmol/L)	Post	2.92 ± 0.140^c	2.84 ± 0.005^c	2.26 ± 0.085^{cd}	6.71 ± 0.400^a	5.36 ± 0.250^b

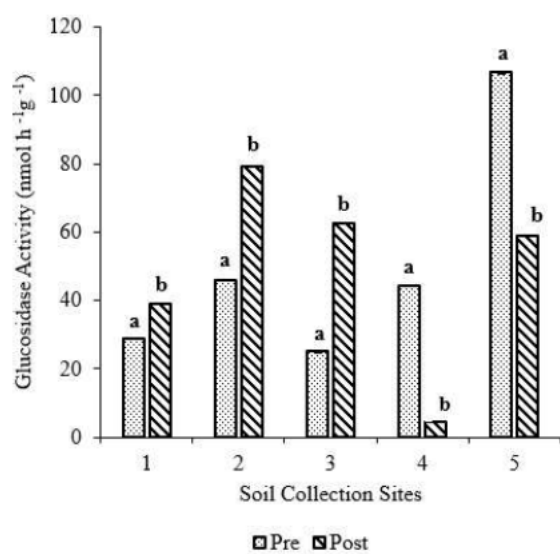
A.



B.



C.



D.

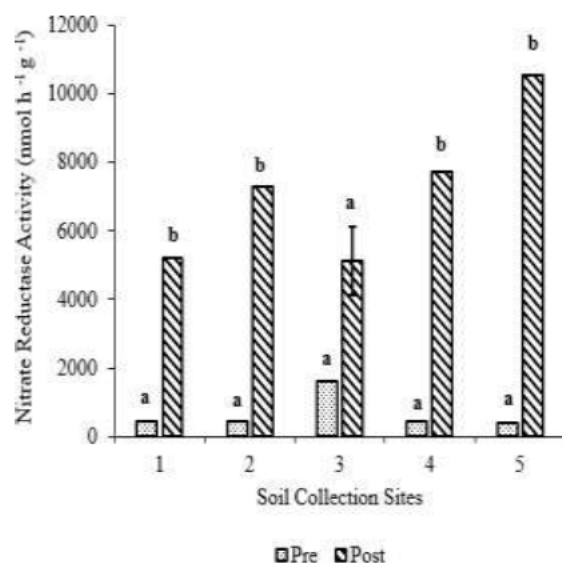


Figure 3.1: Soil enzyme activities in sugarcane plantation soils from 1. Gingindlovu, 2. Mvutshini, 3. Mpembeni, 4. Hibberdene, and 5. Mzinto, KwaZulu-Natal, South Africa, measured pre- and post-planting and harvest of *Vigna unguiculata*. **A.** The acid phosphatase, **B.** alkaline phosphatase, **C.** glucosidase, and **D.** nitrate reductase activity. Different superscripts denote significant difference ($P \leq 0.05$, $n = 3$, mean $\pm$ SE).

Chapter 4

Cajanus cajan cover cropping improves the abundance of plant growth-promoting bacteria and enhances associated enzyme activities in sugarcane plantation soils

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Abstract

Smallholder growers in KwaZulu-Natal practice continuous monoculture in sugarcane production, leading to declining yields due to its negative impact on soil nutrient dynamics. This study evaluates the potential of *Cajanus cajan* as a sustainable cover crop to enhance soil bacterial composition and extracellular enzyme activities in sugarcane plantations managed by smallholder growers in KwaZulu-Natal. Additionally, it examines the growth physiology of this legume species in the sampled

soils. Soils were collected from sugarcane plantations in Gingindlovu, Mvutshini, Mpembeni, Hibberdene, and Mzinto and used as growth substrates for *C. cajan*. The study assessed nutrient-cycling bacterial composition, extracellular enzyme activity, and soil chemical characteristics before planting and after harvesting *C. cajan*. The results showed that *C. cajan* exhibited varied growth across all collection sites, with a high reliance on nitrogen derived from the atmosphere (NDFA) in soils from Hibberdene and Mzinto. Post-harvest soils had an increased abundance of bacterial genera, including *Pseudomonas*, *Pleomorphomas*, *Methylocystis*, *Burkholderia*, *Paraburkholderia*, *Steroidobacter*, *Mucilaginibacter*, and *Sphingomonas*. Nitrate reductase activity increased across all sites, while acid phosphatase activity was higher in post-harvest soils from Gingindlovu, Mvutshini, Mpembeni, and Hibberdene. Alkaline phosphatase activity increased in post-harvest soils from Gingindlovu and Mvutshini, whereas glucosidase activity was higher in post-harvest soils from Gingindlovu, Mvutshini, and Mpembeni. Additionally, *C. cajan* increased soil pH across all collection sites. These findings highlight the potential of *C. cajan* to improve soil health by enhancing nutrient cycling, microbial diversity, and enzyme activity in sugarcane plantation soils. The Widespread adoption of *C. cajan* in sugarcane production systems could serve as a practical, ecologically sound strategy for mitigating the adverse effects of continuous monoculture and promoting long-term soil sustainability.

Keywords: *Cajanus cajan*; Sugarcane monoculture; Soil bacterial composition; Extracellular enzyme activity; Cover cropping

4.1 Introduction

In 2013, South Africa was ranked as the 18th largest sugarcane producer. However, between 2007 and 2017, a 52% decrease in production was recorded (Stats SA, 2017). Declines in sugarcane production pose a significant challenge to the South African economy, as the sector contributes notably to GDP through exports and serves as an important source of income, particularly for smallholder growers

(Zulu et al., 2019). Despite being among the leading sugarcane-producing provinces, smallholder growers in KwaZulu-Natal have reported declines in production, threatening their livelihoods and the South African economy (Thibane et al., 2023). According to Zhang and Govindaraju (2018), sugarcane is primarily grown in continuous monoculture, which may be a major contributing factor to the declines in production. Continuous monoculture significantly threatens agriculture by depleting soil nutrients, ultimately reducing plant growth and development (Belete and Yadete, 2023). The continuous monoculture of sugarcane has been reported to induce soil acidity and reduce the abundance of ecologically important bacterial genera along with their associated enzyme activities (Tayyab et al., 2021).

Plants select microbes through the secretion of organic compounds in root exudates, which promotes rhizosphere colonisation (Marschner et al., 2002; Chen et al., 2016). The continuous monoculture of a single crop selects for crop-specific microbes, reducing microbial diversity, increasing the likelihood of diseases, and decreasing the abundance of beneficial bacteria (Wu et al., 2017). Soil biogeochemical processes and nutrient dynamics are affected by monoculture, as microbes produce extracellular enzymes that play a key role in nutrient cycling (Burns and Dick, 2002; Xiao et al., 2022). This highlights the importance of considering cover cropping in sugarcane plantations. The use of legumes as cover crops has been reported to improve soil health by enhancing soil organic carbon, fertility, nutrient cycling, and microbial activity (Govaerts et al., 2007; Kamanga et al., 2014). These soil nutrient enhancements are largely due to crop residues such as leaf litter and leftover roots after harvest, which increase decomposition rates and, consequently, enhance nutrient cycling, making nutrients available for uptake by succeeding crops (Govaerts et al., 2007; Venkatesh et al., 2017; Zhang et al., 2023).

Cajanus cajan is a perennial legume primarily used as food, fodder, and integrated into cropping systems for soil fertility restoration (Aihou et al., 2006; Odeny, 2007). The integration of *C. cajan* as a cover crop in sugarcane plantations will introduce new root exudates, which will increase microbial diversity and composition, thereby improving extracellular enzyme activities (Lami et al., 2020; Xiao et al., 2023). Additionally, *C. cajan* can serve as an additional source of income, as it is valuable for grain and fodder production (Odeny, 2007; Sharma et al., 2011). According to Sharma et al. (2011), *C. cajan* can fix up to 235 kg N ha⁻¹ and produce more nitrogen per unit area from decomposing plant biomass compared to other legumes, making it a beneficial cover crop for succeeding sugarcane.

Being a leguminous plant, *C. cajan*'s association with plant growth-promoting bacteria has the potential to enhance nutrient mineralisation and solubilisation (Matiwane et al., 2019). Despite its potential, the contribution of *C. cajan* in South African sugarcane plantations remains unknown. This study aims to evaluate the impact of *C. cajan* as a sustainable cover crop on soil bacterial composition and extracellular enzyme activities in sugarcane plantations managed by smallholder growers in KwaZulu-Natal. It also seeks to understand the growth physiology of this legume species in the sampled soils. The objectives of the study include the extraction and identification of nutrient-cycling bacteria, their associated enzymes, and analysis of soil chemical characteristics in pre-planting and post-harvest of *C. cajan*. We hypothesise that *C. cajan* will increase the abundance of beneficial bacterial genera and extracellular enzymes in the sampled soils, improving soil quality for succeeding sugarcane crops. Additionally, *C. cajan* growth and its contribution to soil nutrient cycling will be affected by the soil characteristics of the sampled soils.

4.2 Material and Methods

4.2.1 Soil collection sites

Soil samples were collected from five different smallholder sugarcane plantations in KwaZulu-Natal province, South Africa. These included two plantation sites from the South Coast: Hibberdene (30°35'37.1214" S, 30°31'30.2916" E) and Mzinto (30°16'41.0262" S, 30°39'47.9658" E). The other three sites, located on the North Coast of KwaZulu-Natal, were Gingindlovu (28°56'29.3" S, 31°34'58.9" E), Mvutshini (28°50'52.9" S, 32°00'09.0" E), and Mpembeni (28°51'25.7" S, 31°58'19.4" E).

Twenty random soil samples were collected per site at a depth of 10-20 cm. Pre-planting soils, to be used for enzyme assays and bacterial identification, were stored in sterile ziplock bags, placed on ice during transportation to the laboratory, and stored at 4°C prior to analysis.

4.2.2 Experimental design, growth conditions, and soil nutrient analysis

Cajanus cajan seeds used in this study were purchased from AGT Foods, Marji Mizuri Farm, Ingomankulu, KwaZulu-Natal. To promote seed germination, the seeds were soaked in warm water overnight, placed in Petri dishes lined with moist filter paper under sterile conditions, and kept in the dark at room temperature for 2 days. Once germinated, *C. cajan* seedlings were transferred into 10 cm diameter pots filled with soil from the soil collection sites, and planted to a depth of 1–2 cm. The experiment followed a random block design, with each plantation site replicated 10 times, resulting in a total of 50 experimental pots. The study was conducted in a greenhouse at the University of KwaZulu-Natal, Westville campus, South Africa, with temperatures ranging from 13–16 °C at night to 28–35 °C during the day, and humidity between 70% and 80%. Plants were irrigated daily using an automated irrigation system. The initial and final harvest took place 14 and 54 days after seedling emergence, respectively.

After harvesting, plants were rinsed with distilled water and separated into roots and shoots. The plant material was oven-dried at 80 °C until a constant weight was maintained. Dry weights were recorded, and the dried plant material was ground into fine powder using a pestle and mortar. The ground material was sent to the Archaeometry Department at the University of Cape Town for C and N isotope analysis, and to the Central Analytical Facilities at Stellenbosch University for P analysis. Post-harvest soil samples for enzyme assays and bacterial identification were stored in sterile ziplock bags at 4 °C. For nutrient analysis, 3 kg of soil from each plantation, both pre-planting and post-harvest, were airdried and sent to the South African Sugarcane Research Institute (SASRI) Fertiliser Advisory Service (FAS). These samples were analysed for primary nutrients (N, P, K), secondary nutrients (Ca and Mg), pH, acid saturation, exchange acidity, and total cations.

4.2.3 Total plant nutrition

4.2.3.1 Calculations of percentage nitrogen derived from the atmosphere (% NDFA)

Nitrogen isotope analyses were measured at the Archeometry Department, University of Cape Town, South Africa. According to Herridge et al. (1995) ¹⁵N natural abundance technique is based on the differences in the natural abundance of the stable isotope of N (¹⁴N and ¹⁵N) which is between atmospheric nitrogen and other sources of nitrogen such as soil. The heavier ¹⁵N isotope occurs more in the soil regions while the lighter ¹⁴N isotope occurs more in the atmosphere region (Mariotti, 1983). The isotope ratio of N was calculated using:

$$\delta = 1000\text{‰} (R_{\text{sample}}/R_{\text{standard}})$$

Where R is the molar ratio ¹⁵N to ¹⁴N of the samples and standards. For each milled sample of approximately 2 mg weighed into 8 x 5 mm tin capsules (Elemental Micro-analysis, 30 Devon, UK) on a Sartorius microbalance (Goettingen, Germany) combusted in a Fisons NA 1500 (Series 2) CHN

analyser (Fisons Instruments SpA, Milan, Italy). Nitrogen isotope ratios were determined on a Finnigan Matt 252 mass spectrometer (Finnigan MAT GmbH, Bremen, Germany), connected to a CHN analyser by a Finnigan MAT Conflo control unit. Three standards were used to correct the samples for machine drift, namely, two in-house standards (Merck Gel and Nasturtium) and the IAEA (International Atomic Energy Agency) standard $(\text{NH}_4)_2\text{SO}_4$. According to Shearer and Kohl (1986), the percentage derived from the atmosphere was calculated as:

$$\% \text{NDFA} = 100((\delta^{15}\text{N}_{\text{reference plant}} - \delta^{15}\text{N}_{\text{legume}}) / (\delta^{15}\text{N}_{\text{reference plant}} - \beta)).$$

The β -value is $\delta^{15}\text{N}$ natural abundance of the N derived from BNF of *C. cajan*, grown in a nitrogenfree culture, that was determined as -2.60% .

4.2.3.2 Specific Nitrogen /Phosphorus assimilation and utilization rate

The net specific nitrogen/phosphorus assimilation and specific nitrogen/phosphorus utilization rates were calculate using the equations derived from (Nielsen et al., 2001):

$$\text{SNAR} = (\text{N}_2 - \text{N}_1 / t_2 - t_1) * [(\log_e R_2 - \log_e R_1) / (R_2 - R_1)]$$

$$\text{SPAR} = (\text{P}_2 - \text{P}_1 / t_2 - t_1) * [(\log_e R_2 - \log_e R_1) / (R_2 - R_1)]$$

$$\text{SNUR} = (\text{W}_2 - \text{W}_1 / t_2 - t_1) * [(\log_e \text{N}_2 - \log_e \text{N}_1) / (\text{N}_2 - \text{N}_1)]$$

$$\text{SPUR} = (\text{W}_2 - \text{W}_1 / t_2 - t_1) * [(\log_e \text{P}_2 - \log_e \text{P}_1) / (\text{P}_2 - \text{P}_1)]$$

Where N and P represent the total nitrogen and phosphorus content in the plant, t is the duration it took for a plant to grow, R is the root dry weight as described by Nielsen et al. (2001).

4.2.3.3 Carbon construction costs (Cw)

Carbon construction cost (Cw) was calculated using the equation modified by Peng et al. (1993):

$$C_w = (C + kN/14 \cdot 180/24) (1/0.89) (6000/180)$$

Where C_w is the total carbon construction cost of tissues, C is the total concentration of C, k is the reduction state of N substrate (for $\text{NH}_3 = -3$), and N is the total organic N content of tissue as described by Williams (1987). The numerical value 14 represent the atomic number of N, 180 is a conversion factor from moles to grams of glucose, 24 are the electrons in a glucose molecule; 0.89 is an estimate of growth efficiency and the fraction 6000/180 is a constant conversion factor from g^{-1} dry weight to mmol C g^{-1} DW for glucose.

4.2.3.4 Relative growth rate

The relative growth rate was calculated using an equation derived from Agren and Franklin (2003):

$$\text{RGR} = [(\ln W_2 - \ln W_1) / t_2 - t_1]$$

Where W represents dry weights and t is the time it took for a plant to grow.

4.2.3.5 Root: Shoot ratio

The root-to-shoot ratio was calculated using an equation derived from Agren and Franklin, 2003:

$$\text{Root: shoot} = D_R/D_S$$

Where D_R represents the root dry weight and D_S is the shoot dry weight.

4.2.4 Soil bacterial extraction and identification

For each sugarcane plantation soil, serial soil dilutions were performed in triplicate, where 10 g of soil sample was added to a volumetric flask containing 100 ml of autoclaved distilled water under sterile conditions. Fifty to one hundred microlitres of each dilution were used to inoculate different nutrient agar plates. Tricalcium phosphate media plates were used to isolate and grow phosphate-solubilising bacterial strains, while Simmon citrate and Jensen media were used for N-cycling and N-fixing

bacterial strains, respectively (Ndabankulu et al., 2022). The plates were incubated at 30°C for 3–7 days. Afterward, colonies were repeatedly subcultured into clean plates to form pure colonies.

Pure colonies extracted from different sugarcane plantation soils were amplified using polymerase chain reaction (PCR). The primers used in the PCR were 63F (5'- CAGGCCTAACACATGCAAGTC -3') and 1387R (5'- GGGCGGTGTGTACAAGGC -3'). The PCR reaction mixture contained 12.5 µL of the EmeraldAmpGT master mix (Takara Bio Inc, supplied by Separations, South Africa), 8.5 µL of sterile milliQ water, 0.5 µL of forward and reverse primers, and 3 µL of diluted bacterial pure colony. Amplification was carried out using a T100 Thermal Cycler (Bio-Rad, USA) under the following conditions: initial denaturation at 94°C (5 min), followed by 30 cycles of denaturation at 94°C (30 s), annealing at 55°C (30 s), and extension at 72°C (2 min), with an additional extension at 72°C (10 min). The PCR results were visualised on 1% m/v agarose gel electrophoresis using TBE buffer and observed in a dark room under UV light. Positive amplified amplicons were sent to Inqaba Biotech Pty. Ltd., Pretoria, South Africa, for sequencing. The resulting sequences were edited and subjected to BLASTN searches for identification using the National Center for Biotechnology Information (NCBI) database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) (accessed 15 November 2024).

4.2.5 Soil enzyme activities analysis

4.2.5.1 Phosphatase and β -glucosidase activity

The acid phosphatase activity, alkaline phosphatase activity, and β -glucosidase activity were measured using the fluorescence-based method by Jackson et al. (2013). Five grams of soil from each sugarcane plantation were added to flasks covered with foil, containing 100 ml of autoclaved distilled water. The mixture was homogenised at medium speed in a shaker for 2 hours and stored in the fridge overnight. The 4-MUB-phosphate (for acid and alkaline phosphatase activity), 4-MUB- β -glucopyranoside (for

glucosidase activity) standards, 100 mM bicarbonate buffer, and 4-MUB-phosphatase substrate were prepared and stored in the fridge. Different solutions were transferred into 96-well microplates and incubated for 1 hour at 30°C. Each sample run consisted of three replicates, with 200 µL of soil sample and 50 µL of substrate. Substrate controls consisted of three replicates with 200 µL of buffer and 50 µL of substrate. Sample controls consisted of two replicates with 200 µL of soil sample and 50 µL of buffer. Quenched standards included three replicates with 200 µL of soil sample and 50 µL of standard, while standard controls consisted of three replicates with 200 µL of buffer and 50 µL of standard. To stop the reactions, 5 µL of 0.5 M NaOH was added to each well. The fluorescent absorbance at 450 nm was then measured using an Apex Scientific microplate reader. It is important to note that the pH of the standard and buffer was adjusted to 5 before determining acid phosphatase activity.

4.2.5.2 Nitrate reductase activity

The nitrate reductase activity was conducted using the method adapted from Bruckner et al., 1995. Five grams of soil from different sugarcane plantations were added into a conical flask covered with foil containing 5 ml of autoclaved distilled water, 4 ml of 0.9 mM 2,4 DNP, 1 ml of 25 mM KNO₃. The solution was vigorously mixed before being incubated for 24 hours at 30 °C. After incubation, in each sample 10 ml of 4 M KCl was added and thoroughly mixed then passed through filter paper. The enzymatic reaction was initiated by adding 2 ml of filtrates into 1.2 ml of 0.19 M of ammonium chloride buffer (pH 8.5) and 0.8 ml of colour reagent containing 1% sulfanilamide, 1 N HCl, and 0.02%

N-(1-naphthyl) ethylenediamine dihydrochloride (NEDD). Then solution was incubated for 30 min at 30 °C in the dark. The absorbance was measured at 520 nm using an Agilent Cary 60 UV-Vis spectrophotometer (Agilent, Santa Clara, CA, USA). The amount of nitrite (NO₂) released into the medium was expressed as 0.1 µmolh⁻¹g⁻¹.

4.2.6 Statistical analysis

R-studio software (version 3.6.2) was used to compare means of soil nutrients, enzyme activities, plant mineral nutrition, growth kinetics and plant biomass, measured pre-planting and post-harvest of *C. cajan* using the analysis of variance (One-way ANOVA). Tukey's multiple comparisons post hoc test was conducted when significant results were observed ($p < 0.05$). The Shapiro-Wilk and Levene's tests were used to test assumptions for normal distribution and homogeneity of variance, respectively. Where assumptions were not met, a non-parametric test was used (Kruskal-Wallis test).

4.3 Results

4.3.1 *Cajanus cajan* growth kinetics

The growth kinetics of *C. cajan* are represented in Table 4.1. Total plant biomass showed differences among the sites, with the highest value recorded in Mzinto, while shoot biomass was greater in Mvutshini. Total phosphorus concentrations were highest in Mzinto and lowest in Mpembeni. Total nitrogen concentration was significantly higher in Mvutshini and Mzinto, while Hibberdene had the lowest nitrogen concentrations. Plants grown in Mzinto and Mvuthi had the highest and lowest %NDFA, respectively. The nitrogen derived from the soil followed a similar trend to total nitrogen, with higher values in Mzinto and Mvutshini. The relative growth rate was highest in Mzinto and lowest in Hibberdene. The SPAR was significantly higher in Mzinto compared to the other sites, whereas the SNAR was highest in Hibberdene.

4.3.2 Bacterial identification

The *Pseudomonas* genus was the most dominant across all study sites, isolated from pre-planting and post-harvest soils (Figure 4.1). Post-harvest soils in Mpembeni, Hibberdene, and Mzinto had more *Pseudomonas* isolates. In Gingindlovu, pre-planting soils contained *Pseudomonas*, while post-harvest soils had *Pleomorphomonas*, *Methylocystis*, and *Burkholderia* genera (Figure 4.1). In Mvutshini,

preplanting soils had *Pseudomonas*, *Burkholderia*, *Paraburkholderia*, and *Chromobacterium*, while postharvest soils had *Pseudomonas*, *Paraburkholderia*, and *Steroidobacter* genera (Figure 4.1). Mpembeni's pre-planting soils had *Pseudomonas* and *Burkholderia* genera, while post-harvest soils had *Pseudomonas*, *Paraburkholderia*, *Mucilaginibacter*, and *Sphingomonas* genera (Figure 4.1). In Hibberdene, the *Pseudomonas* genus was isolated in pre-planting soils, and *Pseudomonas* and *Caulobacter* were isolated from post-harvest soils (Figure 4.1). In Mzinto, pre-planting soils had *Pseudomonas* and *Arthrobacter* genera, while post-harvest soils had the *Pseudomonas* genus (Figure 4.1).

4.3.3 Soil Enzymes Activities

The acid phosphatase activity of post-harvest soils in Gingindlovu, Mvutshini, Mpembeni, and Hibberdene was significantly higher than in pre-planting soils. However, in Mzinto, pre-planting soils exhibited significantly higher acid phosphatase activity than post-harvest soils (Figure 4.2A). The alkaline phosphatase activity of post-harvest soils was significantly higher than pre-planting soils in Gingindlovu and Mvutshini. In contrast, in Hibberdene and Mzinto, alkaline phosphatase activity was significantly higher in pre-planting soils than in post-harvest soils (Figure 4.2B). Post-harvest soils in Gingindlovu, Mvutshini, and Mpembeni showed significantly higher glucosidase activity than preplanting soils. Conversely, in Hibberdene and Mzinto, pre-planting soils had significantly higher glucosidase activity than post-harvest soils (Figure 4.2C). Across all soil collection sites, post-harvest soils had significantly higher nitrate reductase activity than pre-planting soils (Figure 4.2D).

4.3.4 Soil nutrient analysis

The soil nutrient concentrations and relative acidity of pre-planting and post-harvest soils are represented in Table 4.2. The primary and secondary nutrient concentrations of pre-planting and postharvest soils varied across the study sites in KwaZulu-Natal. Nitrogen concentrations decreased

postharvest across all sites. Phosphorus concentrations were highest in Mzinto and Hibberdene before plant growth and declined across all sites. Potassium concentrations were highest in Hibberdene pre-planting and decreased across all sites, with the most notable reductions observed in Mvutshini and Hibberdene. Soil pH showed an increasing trend post-harvest across all sites, with the most notable changes in

Hibberdene and Mzinto. Acid saturation decreased at all sites, with the lowest values recorded in Mzinto and Hibberdene post-growth. Exchange acidity declined across all sites, with Gingindlovu and Mvutshini showing the highest pre-growth values. Total cation concentrations were highest in Mzinto before pre-planting and increased in Hibberdene post-harvest, while other sites showed smaller changes.

4.4 Discussion

Cajanus cajan shows varied growth in soils collected from sugarcane plantations in northern and southern KwaZulu-Natal while promoting the proliferation of beneficial bacteria and increasing extracellular enzyme activities. Soils collected from sugarcane plantations in Hibberdene and Mzinto had high nitrogen (N) and phosphorus (P) concentrations, which may have led to a higher P assimilation in *C. cajan* growing in these soils. Plants grown in Mzinto soils had high plant biomass, root biomass, and relative growth rate (RGR), which may have been attributed to the higher concentrations of primary nutrients such as P and N. Compared to Hibberdene and Mzinto, *C. cajan* plants growing in soils collected from Gingindlovu, Mvutshini, and Mpembeni had lower reliance on nitrogen derived from the atmosphere (NDFA). Similarly, high NDFA percentages have been reported in *Vicia sativa* grown in soils collected from sugarcane plantation soils in Mzinto and Hibberdene (Ngonini et al., 2025). The higher reliance on NDFA in plants grown in Mzinto and Hibberdene may be attributed to the higher P concentrations in pre- and post-harvest soils.

According to Magadlela et al. (2016), high P availability supports biological nitrogen fixation (BNF), an energy-intensive process requiring P for ATP generation. The lower concentrations of P in soils from Gingindlovu, Mvutshini, and Mpembeni may not have been sufficient to support a high reliance on NDFA for N nutrition, as similar findings were reported in *V. sativa* grown in the same soils (Ngonini et al., 2025). Thus, the high plant P concentrations in *C. cajan* plants grown in Mzinto and Hibberdene are attributed to higher soil P and a higher specific P assimilation rate (SPAR). Matiwane et al. (2019) reported that specific N assimilation rate (SNAR) and NDFA calculations revealed that *Vigna radiata* relied on both NDFA and nitrogen derived from the soil (NDFS). In the current study, a high specific N assimilation rate (SNAR) was reported for *C. cajan* plants in Hibberdene, which may indicate that plants grown in Hibberdene relied on both NDFS and NDFA. Lower SNAR values were reported in Mzinto, which may indicate that plants grown in Mzinto relied primarily on NDFA for N nutrition.

Although *C. cajan* plants showed varied growth across the sampled sites, they improved the abundance of plant growth-promoting (PGP) bacteria and soil enzyme activities in all collection sites. In all soil collection sites, bacterial genera that were previously not isolated from pre-planting soils were isolated in post-harvest soils. According to Walker (2003) and Sasse and Martinoia (2018), different plant species produce distinct root exudates that attract diverse microbial communities, enabling plants to thrive in different environments (Lyu et al., 2021). Thus, the introduction of *C. cajan* in sugarcane plantation soils may have facilitated the selection of bacterial genera that were not previously selected for by sugarcane. Root exudates provide carbon sources for microorganisms (Baudoin et al., 2003), which may have led to metabolically inactive or dormant bacteria becoming active (van Vliet, 2015), thereby increasing bacterial diversity in post-harvest soils.

Legumes are known to mobilise P (Hinsinger et al., 2011), and the abundance of P-solubilising genera such as *Pseudomonas*, *Burkholderia*, and *Paraburkholderia* in *C. cajan* post-harvest soils may have supported the P demands of *C. cajan* (Amri et al., 2023), enhancing its reliance on NDFA, particularly in Mzinto and Hibberdene soils. The abundance of P-cycling bacteria in *C. cajan* post-harvest soils also increased phosphatase activity (Adetunji et al., 2017), as reflected by higher acid phosphatase activities in post-harvest soils in Gingindlovu, Mpembeni, Mvutshini, and Hibberdene. Similar results were reported by Mbonambi et al. (2024), who observed high phosphatase activities in *Canavalia rosea* grown in coastal hypersaline soils. This could suggest that *C. cajan* roots secreted acid phosphatase as a response to P-deficient and acidic soils (Li et al., 1997). According to Li et al. (2021), soil N concentration may have a dominant effect on alkaline phosphatase activity due to the role of N in synthesising acid and alkaline phosphatases (Mooshammer et al., 2014).

Furthermore, genes encoding alkaline phosphatase activity regulate P starvation, making alkaline phosphatase activity dependent on soil N and P (Li et al., 2021). Thus, the higher alkaline phosphatase activity in pre-planting soils compared to post-planting soils in Mpembeni, Hibberdene, and Mzinto may be attributed to higher soil N and P concentrations. Although alkaline phosphatase activity is generally more active at pH 9–11 (Browman and Tabatabai, 1978), Bergkemper et al. (2016) reported that acidic soils increase the abundance of genes encoding alkaline phosphatase. Thus, the lower alkaline phosphatase activity in post-harvest soils in Mvutshini, Hibberdene, and Mzinto may be attributed to a higher soil pH. According to Martinez and Tabatabai (1997), β -glucosidase plays a role in the degradation of glucosides present in plant debris. The higher β -glucosidase activity in postharvest soils from Gingindlovu, Mvutshini, and Mpembeni suggests that plant roots in these soils senesced and decomposed at a faster rate compared to those in Hibberdene and Mzinto soils.

Being a perennial plant, *C. cajan* contributes to N cycling through root decomposition, which increases nitrate reductase activity. The findings of this study suggest that a longer cultivation period is required for *C. cajan* to significantly enhance soil enzyme activities and bacterial diversity, thereby contributing to soil nutrient dynamics. Furthermore, field studies should be conducted to validate these findings under natural field conditions.

4.5 Conclusion

This study demonstrates that *C. cajan* thrives in nutrient-deficient and acidic soils by switching nitrogen sources from nitrogen derived from the soil (NDFS) to nitrogen derived from the atmosphere (NDFA). As a perennial plant, it develops deep root systems that release diverse root exudates, supporting the proliferation of beneficial bacterial genera and enhancing extracellular enzyme activities, thereby contributing to the restoration of sugarcane plantation soils. Its ability to increase microbial diversity, acid phosphatase and nitrate reductase activities, and soil pH underscores its potential to improve soil health in sugarcane plantation soils. To maximise these benefits, it is recommended that *C. cajan* biomass be left to decompose in the soil, enriching it with essential nutrients for subsequent crops. Integrating *C. cajan* as a cover crop between sugarcane cycles can help mitigate the long-term effects of monoculture by improving soil fertility, stimulating microbial activity, and promoting sustainable agricultural practices. The widespread adoption of *C. cajan* in sugarcane production systems could offer a practical, ecologically sound strategy for enhancing soil resilience and long-term agricultural sustainability.

Authors contributions

Conceptualization, M.A; methodology, M.A; M.M, performed the growth trials, data analysis and draft manuscript; review and editing, M.A; supervision and funding acquisition, M.A.

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Conflict of interest

We declare no conflict of interest with regards to this study.

Data availability

The data can be made available upon request form the corresponding author Prof. Anathi Magadlela at

████████████████████.

4.6 References

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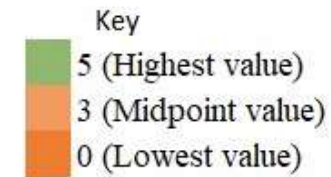
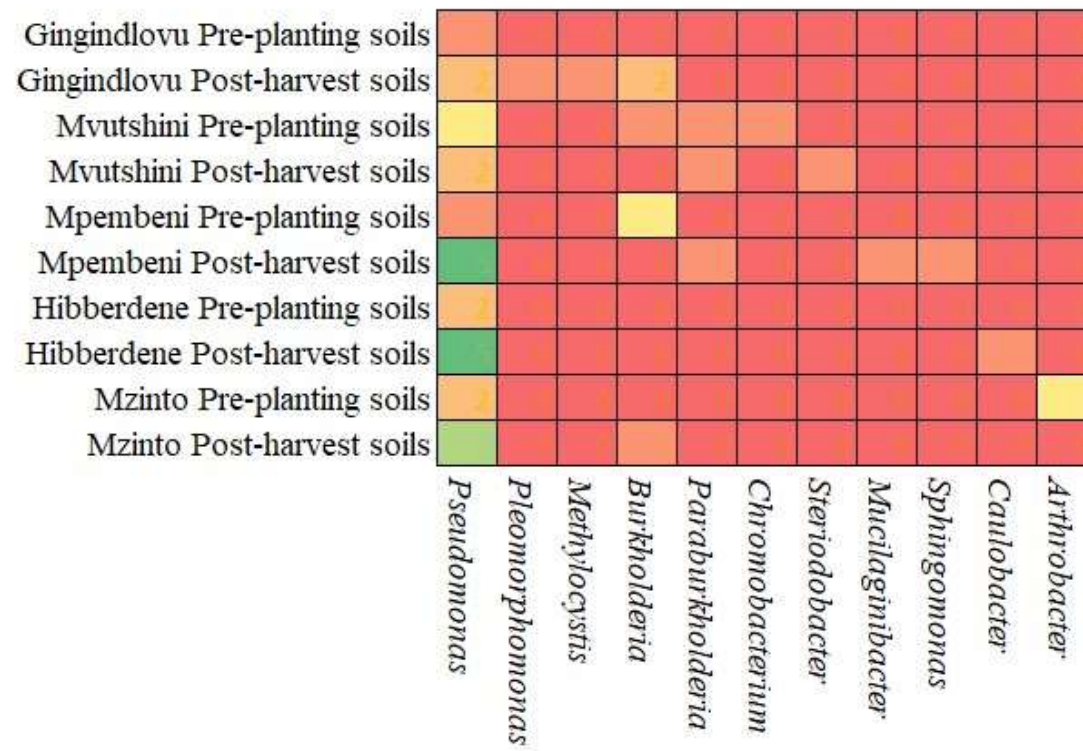
Tables and figures

Table 4.1: Biomass, plant mineral nutrition, and growth kinetics of 54-day-old *Cajanus cajan* species grown in sugarcane plantation soils from Gingindlovu, Mvutshini, Mpembeni, Hibberdene, and Mzinto, KwaZulu-Natal, South Africa. Values are expressed as mean $\pm$ SE, n = 3. Different superscript denote significant differences among soil sites ($P \leq 0.05$).

Parameters	Study sites				
	Gingindlovu	Mvutshini	Mpembeni	Hibberdene	Mzinto
Biomass (g)					
Total plant biomass	0.532 $\pm$ 0.066 ^a	0.572 $\pm$ 0.028 ^a	0.669 $\pm$ 0.189 ^{ab}	0.515 $\pm$ 0.102 ^{ab}	0.734 $\pm$ 0.158 ^{ab}
Shoot biomass	0.141 $\pm$ 0.016 ^a	0.256 $\pm$ 0.029 ^a	0.223 $\pm$ 0.064 ^{ab}	0.128 $\pm$ 0.023 ^a	0.196 $\pm$ 0.046 ^{ab}
Root biomass	0.069 $\pm$ 0.017 ^{ab}	0.046 $\pm$ 0.005 ^{ab}	0.113 $\pm$ 0.034 ^{ab}	0.089 $\pm$ 0.015 ^{ab}	0.121 $\pm$ 0.017 ^{ab}
Root: Shoot ratio	0.475 $\pm$ 0.073 ^{ab}	0.181 $\pm$ 0.033 ^{ab}	0.555 $\pm$ 0.129 ^{ab}	0.698 $\pm$ 0.018 ^{ab}	0.650 $\pm$ 0.059 ^{ab}
Plant mineral nutrition					
Total plant P ($\mu\text{mol P g}^{-1}$)	21.34 $\pm$ 0.378 ^c	20.64 $\pm$ 0.390 ^c	17.86 $\pm$ 0.159 ^d	39.63 $\pm$ 0.453 ^b	50.42 $\pm$ 0.050 ^a
Total plant N (mmol N g ⁻¹)	1.63 $\pm$ 0.088 ^b	2.73 $\pm$ 0.371 ^a	1.67 $\pm$ 0.033 ^b	1.37 $\pm$ 0.033 ^b	2.60 $\pm$ 0.058 ^a
NDFA (%)	25.88 $\pm$ 0.269 ^{ab}	3.05 $\pm$ 0.059 ^a	12.53 $\pm$ 0.317 ^b	47.70 $\pm$ 1.186 ^a	65.17 $\pm$ 0.084 ^a
NDFA (mmol N g ⁻¹)	0.010 $\pm$ 0.001 ^b	0.017 $\pm$ 0.002 ^a	0.010 $\pm$ 0.000 ^b	0.009 $\pm$ 0.000 ^b	0.016 $\pm$ 0.000 ^a
NDFS (mmol N g ⁻¹)	1.623 $\pm$ 0.088 ^b	2.716 $\pm$ 0.369 ^a	1.656 $\pm$ 0.033 ^b	1.358 $\pm$ 0.033 ^b	2.584 $\pm$ 0.057 ^a
Growth kinetics					
Growth rate (mg/day)	0.010 $\pm$ 0.001 ^b	0.011 $\pm$ 0.001 ^b	0.012 $\pm$ 0.003 ^b	0.010 $\pm$ 0.002 ^b	0.014 $\pm$ 0.003 ^b
RGR (g/day)	0.086 $\pm$ 0.007 ^a	0.200 $\pm$ 0.042 ^a	0.129 $\pm$ 0.023 ^a	0.069 $\pm$ 0.011 ^a	1.057 $\pm$ 0.770 ^a

Carbon cost (mmol C/g DW)	0.782±0.048 ^a	0.696±0.172 ^a	0.858±0.000 ^a	0.832±0.014 ^a	0.769±0.004 ^a
SPAR (mg P g ⁻¹ root DW day ⁻¹)	41.23±0.738 ^a	52.16±0.991 ^a	40.62±0.364 ^d	70.74±0.817 ^b	151.23±0.149 ^c
SPUR (mg P g ⁻¹ plant DW day ⁻¹)	0.062±0.001 ^b	0.079±0.001 ^c	0.083±0.000 ^a	0.035±0.000 ^d	0.0475±0.000 ^e
SNAR (mg N g ⁻¹ root DW day ⁻¹)	357.9±19.75 ^c	714.2±98.53 ^b	68.5±15.40 ^c	611.1±15.44 ^a	92.61±2.10 ^c
SNUR (mg N g ⁻¹ plant DW day ⁻¹)	1.174±0.059 ^a	1.019±0.062 ^a	1.237±0.015 ^a	0.995±0.394 ^a	1.315±0.004 ^a

Site



Bacterial genera

Figure 4.1: Heat Map showing genera isolated from pre-planting and post-harvest soils collected from sugarcane plantations across Gingindlovu, Mvutshini, Mpembeni, Hibberdene, and Mzinto, KwaZulu-Natal, South Africa.

Table 4.2: Primary nutrients (N, P, K), secondary nutrients (Ca, Mg) and soil chemistry (pH, acid saturation, exchange acidity, and total cations) of 54-day-old *Cajanus cajan* species grown in sugarcane plantation soils from Gingindlovu, Mvutshini, Mpembeni, Hibberdene, and Mzinto, KwaZulu-Natal, South Africa. Values are expressed as mean $\pm$ SE, (n = 3). Different superscript denote significant differences among soil sites ($P \leq 0.05$).

Parameters		Study sites				
		Gingidlovu	Mvutshini	Mpembeni	Hibberdene	Mzinto
Soil nutrient (mg/L)						
N concentration	Pre	0.055±0.005 ^a	0.085±0.005 ^a	0.055±0.005 ^a	0.060±0.000 ^a	0.070±0.000 ^a
	Post	0.020±0.000 ^a	0.035±0.005 ^b	0.020±0.000 ^a	0.050±0.000 ^a	0.040±0.000 ^b
P concentration	Pre	3.80±0.100 ^c	4.30±0.200 ^a	3.45±0.150 ^a	9.40±0.100 ^b	10.7±0.400 ^a
	Post	0.02±0.000 ^d	2.40±0.000 ^b	2.35±0.250 ^b	5.90±0.800 ^c	6.65±1.050 ^c
K concentration	Pre	55.00±3.000 ^b	33.00±0.000 ^c	18.50±0.500 ^d	100.5±4.500 ^a	61.00±3.000 ^b
	Post	53.50±2.500 ^c	27.50±1.500 ^e	19.50±1.500 ^f	86.50±0.500 ^a	52.50±1.500 ^d
Ca concentration	Pre	220.0±4.000 ^a	1502.5±1256 ^a	452.0±297.0 ^a	387.0±156.0 ^a	603.5±83.50 ^a
	Post	258.0±5.000 ^d	272.0±10.000 ^b	163.5±9.500 ^e	949.5±65.50 ^b	755.5±24.50 ^c
Mg concentration	Pre	132.5±3.500 ^b	79.00±4.000 ^c	39.00±0.000 ^d	141.5±2.500 ^b	166.5±12.50 ^a
	Post	126.5±3.500 ^b	109.0±5.000 ^d	62.00±4.000 ^e	273.0±23.000 ^a	178.5±9.500 ^a
Soil chemistry						
pH	Pre	4.80±0.050 ^a	4.67±0.030 ^a	4.94±0.295 ^a	5.13±0.045 ^a	4.88±0.345 ^a
	Post	4.97±0.010 ^b	4.76±0.010 ^b	4.95±0.005 ^a	5.67±0.135 ^c	5.74±0.090 ^b
Acid saturation (%)	Pre	11.15±0.65 ^c	20.85±0.05 ^a	14.30±0.90 ^a	1.40±0.00 ^d	1.15±0.15 ^d
	Post	4.55±0.05 ^d	12.05±1.05 ^b	7.75±2.15 ^b	0.65±0.05 ^e	0.90±0.00 ^e
Exchange acidity (cmol/L)	Pre	0.32±0.020 ^b	0.54±0.020 ^a	0.19±0.005 ^c	0.06±0.000 ^a	0.06±0.010 ^d
	Post	0.13±0.005 ^d	0.33±0.000 ^b	0.12±0.030 ^d	0.05±0.000 ^a	0.05±0.000 ^d
Total cations	Pre	2.88±0.015 ^c	2.59±0.090 ^c	1.30±0.045 ^d	4.21±0.050 ^b	5.26±0.285 ^a

(cmol/L) Post 2.74 ± 0.065^c 2.75 ± 0.075^c 1.56 ± 0.050^c 7.40 ± 0.525^a 5.51 ± 0.210^b

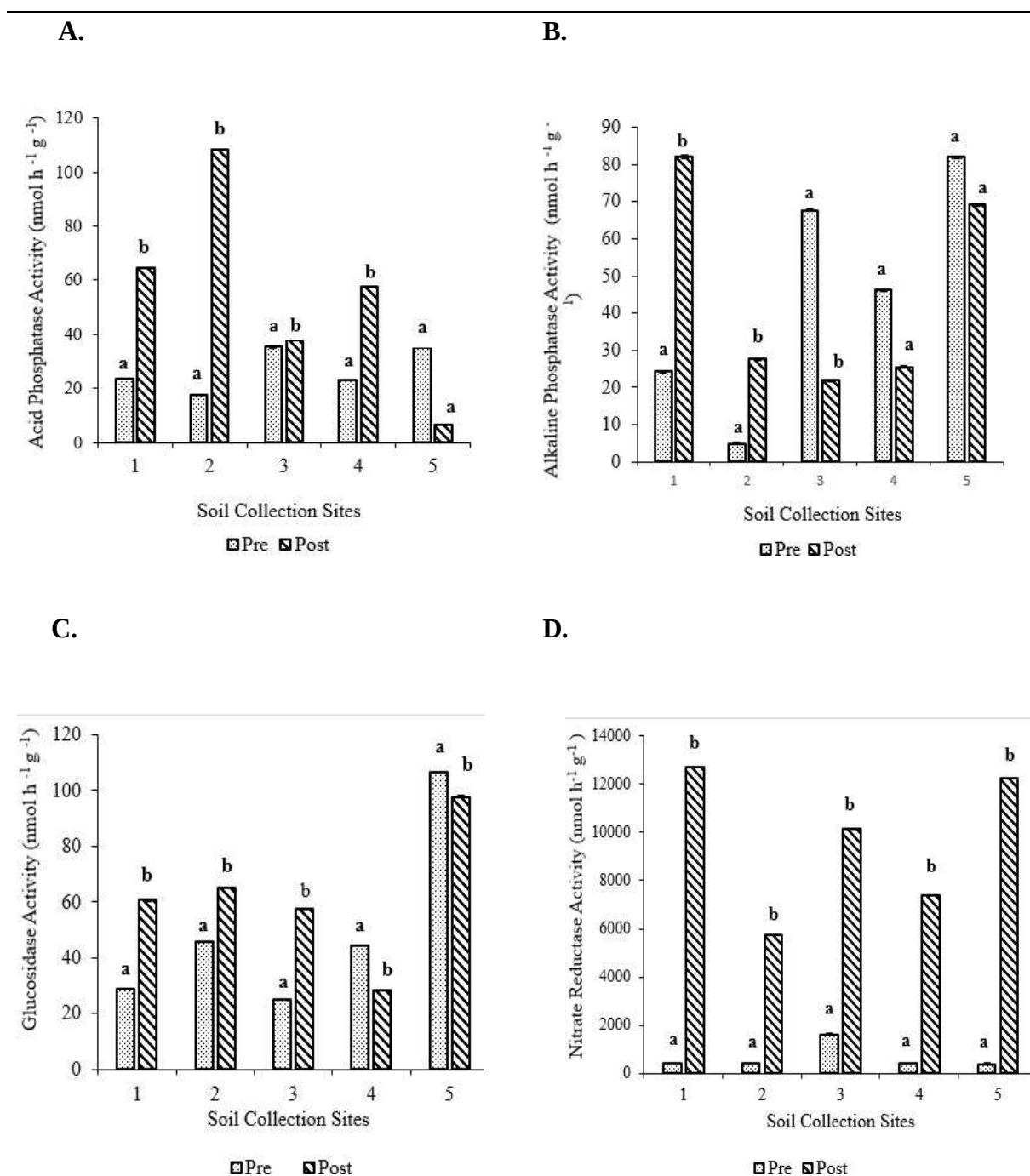


Figure 4.2: Soil enzymes activities of 54-day-old *Cajanus cajan* species grown in soils collected from 1. Gingindlovu, 2. Mvutshini, 3. Mpembeni, 4. Hibberdene, and 5. Mzinto sugarcane plantations in KwaZulu-Natal, South Africa. Enzymes activities include: **A.** acid

phosphatase, **B.** alkaline phosphatase, **C.** glucosidase, **D.** nitrate reductase activity. Different letters denote the significant differences among the soil sites (mean $\pm$ SE, n = 3, $P \leq 0.05$).

Chapter 5

5.1 General conclusion

Cover crops contribute to agricultural production and enhance soil quality (Sharma et al., 2018). They improve physical, chemical, and biological properties of the soil (Sharma et al., 2018). Additionally, cover crops suppress weeds, reduces N loss from soil profile, break pest and disease cycle, and increase organic matter (Reddy, 2016; Kaye and Quemada, 2017). Integrating legume as cover crops reduces need for chemical inputs due to contribution to soil nitrogen through nitrogen fixation in association with soil microbes (Tosti et al., 2014). Furthermore, they increase the abundance and diversity of soil microbes (Sharma et al., 2009). Under nutrient-deficient and acidic soils conditions, microbes which are involved in nutrient cycling can proliferate, allowing them to thrive despite nutrient limitations (Weerasekara et al., 2017).

In this study *Cajanus cajan* and *Vigna unguiculata* contributed to increased soil pH across all sugarcane plantation soils. Both species increased the abundance and diversity of microbial communities, with *Pseudomonas*, *Burkholderia* and *Bacillus* being the most dominant genera. The presence of *Pseudomonas* strain contributed to P solubilisation making it available for plant assimilation. High specific assimilation rates in Mzinto soils contributed to increased nitrogen derived from atmosphere (%NDFA). In addition, both legumes effectively fixed the atmospheric nitrogen. Both species increased the acid phosphatase activity in Gingindlovu, Mvutshini, Mpembeni, and Hibberdene while nitrate reductase activity increased across all plantation sites. The legume species assimilated N and P to sustain growth and development. However, assimilated nutrients can be released back to soil through the decomposition residues when incorporated into the soil. The results of the study demonstrate that *C. cajan* and *V.*

unguiculata enhanced soil health in small-scale sugarcane plantations by increasing the number of microbial communities, soil enzyme activities, and reducing soil acidity. The chemical and biological contributions and adaptations of these species in sugarcane plantations can help alleviate the long-term effects of sugarcane monoculture and enhance sustainability.

5.2 Future recommendations

The current study demonstrated the chemical and biological contributions of *C. cajan* and *V. unguiculata* as sustainable cover crop in sugarcane plantation soils under greenhouse-controlled conditions over a short duration. Field trials conducted over extended period could provide further insight into how cover crops influence plantation soils during fallow period. Rhodes et al. (2009) observed that crops made significant changes in the soil within 6 months, thus long-term experiments would help ensure that observed trends are not transient. Future studies should focus on identifying the decomposition rates of these two legumes residues and their contribution to soil fertility. Understanding the decomposition rates will aid in planning crop rotation and managing fertilizer inputs, as the rate at which legume decompose affects the pace of nitrogen release in the soil.

5.3 References

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