Mucuna pruriens L. (DC) and *Arachis hypogaea* (L.) enhance soil biological and chemical properties of nutrient-deficient small-scale sugarcane soils

By

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Preface

The research contained in this dissertation was completed by the candidate based in the discipline of Biological Sciences, School of Life Science of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Westville Campus, South Africa. This study was carried out under the supervision of Prof. A. Magadlela.

The contents of this dissertation have not been submitted in any form to another university, and except where the work of others is acknowledged in the text, the results reported represent original work by the candidate.

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As the supervisor, I have approved this dissertation for submission

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I, Sikhanyiso Khwela (217079525), declare that:

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Abstract

Most of the rural population in South Africa rely on agriculture for food security and livelihood. In KwaZulu-Natal, sugarcane production plays a significant role in the livelihoods of small-scale farmers in rural areas. However, there has been a decline in sugarcane productivity and yield due to soil-health-related problems caused by long-term monoculture and excessive chemical fertilizer use. Legume cover cropping is an attractive and sustainable alternative to reduce overreliance on synthetic nitrogen (N) fertilizers since legumes increase soil N levels through symbiotic N fixation. *Mucuna pruriens* L. (DC) and *Arachis hypogaea* L. have been reported to fix approximately 34-242 kg N ha^{-1} and 150-200 kg N ha^{-1}, respectively. Furthermore, these legume species have been reported to provide ecosystem services such as nutrient cycling, weed suppression, soil erosion control, increased organic matter, and microbial diversity. Therefore, this study investigated the effects of cultivating *M. pruriens* and *A. hypogaea* on the soil chemical characteristics, bacterial community composition, and enzyme activities of five different acidic and nutrient-deficient sugarcane plantation soils in KwaZulu-Natal, South Africa. Additionally, the study evaluated the N-source preference, biomass accumulation, and plant nutrition of *M. pruriens* and *A. hypogaea* growing in these acidic and nutrient-deficient sugarcane soils. Post-harvesting, the soil pH increased across all study sites for both legume species. Both *M. pruriens* and *A. hypogaea* sanctioned for N-cycling, N-fixing, and P solubilizing bacteria with the dominant strains belonging to the *Burkholderia* and *Pseudomonas* genera. The increase in P solubilizing bacteria, alkaline and acid phosphatase activity assisted with the demands of biological N fixation, allowing *M. pruriens* and *A. hypogaea* to fix 26-58% and 40-70% of N from the atmosphere, respectively. Overall, the findings of this study showed that *M. pruriens* and *A. hypogaea* contribute to soil health improvement by reducing soil acidity and increasing the diversity of nutrient-cycling bacteria and their associated enzyme activities in small-scale sugarcane soils.

General overview of chapters in this dissertation

This dissertation consists of five chapters arranged as follows

Chapter 1: The importance and role of small-scale farmers in addressing food security in the KwaZulu-Natal province, the use of legumes to increase productivity and minimize fertilizer inputs, the rationale, aims, and objectives of the study.

Chapter 2: Literature review expands on the role of small-scale farmers in addressing food security and highlights the detrimental effects of synthetic N fertilizer in sugarcane farming, and the contributions of legume crop rotation to soil fertility between sugarcane cycles. Furthermore, an overview of the impact of legumes on soil microorganisms and their associated enzyme activities was discussed.

Chapter 3: Focused on investigating the contributions of *Mucuna pruriens* as a cover crop in different small-scale sugarcane soils in KwaZulu-Natal by identifying nutrient-cycling bacteria and their associated soil extracellular enzyme activity, and their contributions to soil nutrition. The growth adaptations of *Mucuna pruriens* across the different small-scale sugarcane soil were also explored with a particular focus on plant-microbe symbiosis, N source preference, nutrient (N and P) assimilation, and utilization rates.

Chapter 4: Highlighted the contributions of *Arachis hypogaea* as a cover crop in different small-scale sugarcane soils in KwaZulu-Natal by identifying nutrient-cycling bacteria and their associated soil extracellular enzyme activity, and their contributions to soil nutrition. The growth adaptations of *Arachis hypogaea* across the different small-scale sugarcane soil were investigated with a particular focus on plant-microbe symbiosis, N source preference, nutrient (N and P) assimilation, and utilization rates.

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List of abbreviations

Ammonia= NH_3

Biological Nitrogen Fixation= BNF

Calcium= Ca

Carbon= C

Dinitrogen= N_2

Dry weight= DW

Iron= Fe

Magnesium= Mg

Nitrogen= N

Percent nitrogen derived from the atmosphere= %NDFA

Phosphorus= P

Polymerase chain reaction= PCR

Plant growth promoting rhizobacteria= PGPR

Potassium= K

Small-scale growers= SSGs

Specific nitrogen assimilation rate= SNAR

Specific phosphorus assimilation rate= SPAR

Specific nitrogen utilization rate= SNUR

Specific nitrogen utilization rate=SPUR

Chapter 1: General introduction

1.1 Background

Agriculture plays a crucial role in driving economic growth and ensuring food security in developing countries (Pawlak and Kołodziejczak, 2020). In Africa, small-scale growers (SSGs), who account for an estimated 73% of the rural population, are the main driving force of agricultural production (Rapsomanikis, 2015). These farmers typically own less than 2 hectares of land but are responsible for approximately 80% of food production (Lowder et al., 2016; Bachewe et al., 2017). However, the productivity of SSGs has been hindered by constraints such as soil acidification and nutrient deficiency resulting in yield losses (Shoge et al., 2022). Moreover, SSGs are characterised by minimal farm inputs due to high fertilizer costs and over-reliance on government grants.

Most of the rural population in South Africa relies on agriculture for food security and income (Hlatshwayo et al., 2023). As such, the South African agricultural sector is mostly dominated by SSGs who cultivate a variety of food and cash crops for subsistence (Mkuhlani et al., 2020). Sugarcane is a key cash crop primarily cultivated by SSGs in the Mpumalanga and KwaZulu-Natal (KZN) provinces and is essential to their livelihoods (Thibane et al., 2023; Zulu et al., 2024). However, sugarcane monoculture is costly for resource-constrained farmers, as it requires high amounts of chemical fertilizers (van Antwerpen et al., 2023). Furthermore, the nitrogen (N) recovery of sugarcane is normally <40%, while the rest of the applied fertilizer is lost to the environment (Vieira-Megda et al., 2015). Consequently, sugarcane monoculture coupled with high fertilizer input has been shown to deplete soil health, resulting in negative plant-soil feedback that hinders the productivity and yield of sugarcane (Pankhurst et al., 2003; Huang et al., 2013; Li et al., 2014; Bai et al., 2019; Li et al., 2022). Therefore, it is imperative that SSGs adopt better soil management practices that can improve soil health and enhance cane yield (Deeb et al., 2021).

Numerous studies have highlighted the benefits of using legume in sustainable cropping systems (Tonito et al., 2006; Park et al., 2010; Blanco-Canqui et al., 2011; Otto et al., 2020). Park et al. (2010) revealed that fixed N by legumes could supply up to the fourth ratoon of sugarcane in Australia, thus allowing for reductions in fertilizer use for each ratoon while maintaining sugarcane yields. According to Otto et al. (2020), legume cover cropping increased sugarcane yield by up to 22%, enhanced soil health, and decreased weed populations. Similar findings have been reported in maize-legume cropping systems where legume crop rotations increased maize yield and enhanced soil fertility (Adeleke and Haruna, 2012; Thierfelder et al., 2013; Mupangwa et al., 2017). The presence of legume crops has been reported to enhance microbial diversity (Jiao et al., 2019; Zhang et al., 2019), and Pankhurst et al. (2003) observed that a legume break crop reduced the population of detrimental microbes. A study by Mbuthia et al. (2015) reported significant increases in microbial communities and associated enzyme activities under hairy vetch cover cropping and Mula-Michel et al. (2023) reported increased microbial biomass under soybean cover cropping. The abovementioned increases in microbial activity and soil enzyme activity contribute significantly to the cycling of essential nutrients such as N, P, and potassium (K) (Zhao et al., 2014). For instance, under acidic conditions, P forms insoluble complexes with cations making it unavailable for plant assimilation (Giesler et al., 2002). Soil microbes and enzyme activities contribute to the availability of P by solubilizing and mineralizing P, facilitating an active P cycle and plant growth (Zhu et al., 2012; Alori et al., 2017).

1.2 Rationale of the study

Most agricultural systems are deficient in micronutrients, particularly N, which is the most limiting nutrient resulting in poor crop production. Legumes can fix atmospheric dinitrogen into ammonia, in this way, they contribute to sustainable agriculture by enhancing soil fertility. *Mucuna pruriens* and *Arachis hypogaea* have been reported to fix approximately 34-242 kg N ha^{-1} and 150-200 kg N ha^{-1}, respectively, highlighting their potential to increase N inputs and reduce the use of chemical fertilizers. Furthermore, these legumes have been shown to improve soil structure, increase organic matter, suppress weeds, and foster the persistence of beneficial soil microorganisms, making them an attractive option for sugarcane cover cropping (Zannopoulos et al., 2024). Despite this, there is limited information on the contributions of *M. pruriens* and *A. hypogaea* cover crops in sugarcane farming systems in South Africa, especially in the KwaZulu-Natal province. A better understanding of the biological and chemical contributions of legume cover crops in small-scale sugarcane farmlands will have relevance to N flow management and food security in the KwaZulu-Natal province and South Africa as a whole. In addition, this research aligns with goal number 2 of the United Nation’s sustainable development goals, which aims to improve food security, and promote sustainable agriculture.

1.3 Aims and Objectives

Thus, this study aimed to investigate the biological and chemical contributions of *M. pruriens* and *A. hypogaea* cover crops across five nutrient-poor sugarcane plantations in KwaZulu-Natal.

The objectives of the study are:

1. To study the survival strategy, N preference (soil N or atmospheric N_2), and N contribution of *M. pruriens* and *A. hypogaea* growing in soils from five small-scale sugarcane plantations.

2. To elucidate shifts in microbial community composition by identifying nutrient cycling bacteria pre- and post-*M. pruriens* and *A. hypogaea* planting and harvesting

3. To explore the soil enzyme activities of soils pre- and post-*M. pruriens* and *A. hypogaea* planting and harvesting

4. To evaluate the soil health status by assessing soil nutrient parameters pre- and post-*M. pruriens* and *A. hypogaea* planting and harvesting

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Chapter 2: Literature review

2.1 Challenges in overcoming food insecurity

The global human population is forecasted to reach 9 billion by 2050 (Adam, 2021). Concurrently, the likelihood of extreme climate events is expected to rise due to climate change (Emmerson et al., 2016). This combination has the potential to create a global food crisis, as there is limited arable land to feed the ever-growing population. Projections suggest that by 2050, approximately 130 million people could be food insecure due to climate change, with the majority of these people in vulnerable communities (World Economic Forum, 2023). Currently, food insecurity continues to be a major problem in Sub Saharan Africa (Hlatshwayo et al., 2022). Despite being considered a food secure nation, South Africa still faces food insecurity at a household level (Pereira et al., 2014), with over 16% of the population lacking access to food in 2019 (StatsSA, 2019). Furthermore, the South African population surpassed 63 million in 2024, likely widening the gap between crop demand and supply (StatsSA, 2024). Consequently, the agricultural sector faces a major productivity problem since only 10-12% of the total land surface is suitable for crop production (Strauss et al., 2021).

To cope with the effects of limited arable land on crop production, farmers tend to favour the monoculture of high value cash crops over crop rotations as these provide higher yields and profits with comparably lower management costs (Andres et al., 2016; Loh et al., 2022). One might argue that this is beneficial to small-scale farmers who typically own <2 ha of land and play a significant role in the production of cash and food crops (Lowder et al., 2016; Mkuhlani et al., 2020). Agro-economically important crops such as wheat, maize, and sugarcane are cultivated under monoculture practices in South Africa, with sugarcane being an important cash crop in the KwaZulu-Natal province (Marais et al., 2012; Mthembu et al., 2019; Thibane et al., 2023). According to Chen et al. (2023) long-term monoculture depletes soil health and increases the persistence of host-specific plant diseases. This negative plant-soil feedback can continue for years leaving behind a legacy of low diversity of functional microbial communities, low nitrogen and soil organic carbon (SOC) consequently leading to nutrient-deficient soils and thus decreased productivity of sugarcane (Huang et al., 2013; Li et al., 2014; Bai et al., 2019; Li et al., 2022)

Sugarcane monoculture has been shown to decrease plant vigour and stunt plant growth leading to yearly declines in sugarcane production (Chen et al., 2012; Tayyab et al., 2021). These declines can be attributed to the secretion of allelochemicals and root exudates that alter the bacterial community composition, negatively affecting nutrient cycling and sugarcane growth (Sheng et al., 2012; Gao et al., 2019; Xu et al., 2021). As a consequence of deteriorating soil health, farmers use synthetic fertilizers to sustain the high nutrient demands of sugarcane, especially in nutrients such as N, P and K (van Antwerpen et al., 2023). However, the fertilizer recovery of sugarcane typically does not exceed 40% for N (Vieira-Megda et al., 2015) and 10-45% for P (Adesemoye and Kloepper, 2009). This is costly for resource constrained small-scale farmers and results in the pollution and degradation of terrestrial and water ecosystems (Vance, 2001; Waddington et al., 2004; Abd-Alla et al., 2014).

2.2 The duality of synthetic nitrogen fertilizers

Synthetic N fertilizer is vital to ensuring the productivity and yield of crops under intensive agriculture (Zhang et al., 2013; He et al., 2021). Current recommendations for synthetic N fertilizer use in sugarcane are governed by the expected crop yield (Thorburn et al., 2011; Otto et al., 2013), since proper management of N has been reported to enhance height, stem diameter as well as the number of tillers, all of which are closely linked to sugarcane yield (Trivelin et al., 2002; Prado and Pancelli, 2008; van Heerden et al., 2010). Excessive application of synthetic N fertilizers contributes to significant N losses and environmental problems such as eutrophication and groundwater contamination (Tilman et al., 2001; Galloway et al., 2008; Raza et al., 2021). Acidic soils are likely to become more acidic under excessive N additions (Alekseeva et al., 2011; Kissel et al., 2020). The reduced soil pH obstructs the availability of phosphate, which form insoluble complexes with cations like Al^{3+} and Fe^{2+} rendering them unavailable for plant assimilation (Vance et al., 2003). In addition, soil acidification directly affects soil microbial community composition (Geisseler and Scow, 2014).

Long-term N fertilization has been shown to decrease microbial biomass, thus further influencing the composition and functionality of microbial communities within the soil (Treseder, 2008), and some of these structural and functional changes can persist even after N fertilization has been discontinued (Bowman et al., 2018). Soil biological activities like aeration and enzyme activities can be influenced by N fertilization (Tyagi et al., 2022). Since soil microbial communities have been shown to be particularly sensitive to agricultural practices, and are therefore, a good indicator of soil health (Bedano et al., 2021; Wang et al., 2021), their perturbation has a significant impact on nutrient cycling and bioavailability. Therefore, there is a need to develop better N fertilizer management regimes that can increase N use efficiency in sugarcane and augment the soil N pool without compromising both yield and soil fertility (Tenelli et al., 2019). Additionally, van Antwerpen et al. (2024) reported low adoption levels of better management practices in the KZN province, further highlighting the need for soil rehabilitation strategies that improve soil fertility in sugarcane plantations.

2.3 The contribution of legumes in soil fertility management

Following cereals, legumes are the most valuable food source and contribute to food security globally (Mashungwa, 2019). Nutritionally, legumes are a rich source of protein, carbohydrates, and vitamins and are thus considered a significant dietary component for addressing food security (Jimenez-Lopez et al., 2023). Legumes play a key role in soil fertility management due to their ability to form symbiotic interactions with soil-borne bacteria, so-called rhizobia, enabling them to fix atmospheric dinitrogen (N_2) into plant available forms (Makaure et al., 2023). In addition, they contribute to sustainable agriculture through the control of weeds, pests and diseases, improvement of soil biological, chemical and physical properties, and enhancing the productivity of the succeeding crop.

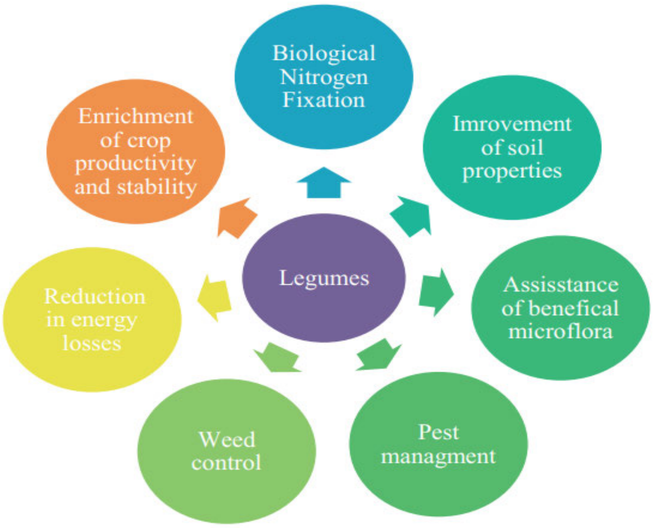

Figure 2.1: Contributions of legumes to soil health and sustainable agriculture (adapted from Rani et al., 2019))

2.4 Biological nitrogen fixation (BNF)

Nitrogen is one the most limiting nutrients for plant growth and development (Chen et al., 2020) as it forms a crucial part of the plant's physiological processes such as protein synthesis and photosynthesis (Alori et al., 2017). Paradoxically, while N is abundant in the atmosphere as N_2, it is unavailable for plant assimilation as plants can only assimilate nitrites, nitrates and ammonia (NH_3) from the soil (Horel et al., 2019). Legumes can fix this atmospheric N_2 into

NH_3 by establishing symbiotic relationships with *Rhizobium* bacteria in specialized root structures called nodules (Roy et al., 2020). In this way, legumes ameliorate soil fertility by depositing fixed N into the soil (Vanlauwe et al., 2019). The only bacteria capable of fixing atmospheric nitrogen are those that produce the nitrogenase enzyme, which is necessary to catalyze the conversion of atmospheric N_2 into NH_3 (Montañez et al., 2009). Since BNF is an energy intensive process, the energy and electrons required to reduce atmospheric N_2 to NH_3 typically come from plant photosynthates, glycolysis, and Krebs cycle chain. The net equation below shows the aforementioned conversion of atmospheric N_2 into NH_3

$$N_2 + 8H+ + 8e- + 16MgATP \rightarrow 2NH_3 + H_2 + 16MgADP + 16Pi$$

2.5 BNF contribution to soil N in cropping systems

Globally, total N fixation is estimated to be about 175 Tg, of which the legume-rhizobia symbiosis accounts for 80 Tg while industrially fixed N from producing fertilizers accounts for the remaining 88 Tg (Kebede, 2021). Legumes, on average, fix 20-200 kg N ha^{-1} yr^{-1} (Shah et al., 2021), thus the introduction of legumes in cropping systems provides economically feasible and sustainable ways of decreasing fertilizer inputs and improving the soil nutrient status (Park et al., 2010; Aschi et al., 2017; Hufnagel et al., 2020). Moreover, the cultivation of legumes as cover crops, intercrops or rotational crops has been reported to have the dual benefit of reducing N leaching and increasing the N recovery of companion or subsequent crops (Reckling et al., 2016). Numerous studies have reported the contribution of legumes to the sustainable management of N in agroecosystems. For example, hairy vetch provided an equivalent of 50- 250 kg N ha^{-1} of fertilizer N to the subsequent corn crop (Seo et al., 2000; Crandall et al., 2005). A study by Blanco-Canqui et al. (2011) demonstrated that in a wheat-legume rotation, soybean and sunn hemp increased soil N levels by 258 kg N ha^{-1} and 279 N kg ha^{-1}, respectively compared to no cover crop treatment. Park et al. (2010) reported that fixed N by legumes can supply up to the fourth ratoon of sugarcane in Australia, which allowed for reductions in fertilizer use for each ratoon while maintaining sugarcane yields. This is of great importance, especially in areas where synthetic fertilizers are scarcely available or expensive.

Table 2.1: Studies that have reported the increase in N content and subsequent fertilizer value of legume cover crops (adapted from (Dabney et al., 2010))

Legume cover crop	Total N increase (kg ha-1)	N fertilizer equivalent (kg ha-1	Main crop	Reference
Hairy vetch	209	100	Corn	(Ebelhar et al., 1984)
Hairy vetch	153	97	Grain Sorghum	(Hargrove, 1986)
Crimson clover	108	99	Corn	(McVay et al., 1989)
Hairy vetch	128	123	Grain Sorghum	(McVay et al., 1989)
Hairy vetch	103	75	Corn	(Blevins et al., 1990)
Bigflower vetch	67	65	Corn	(Blevins et al., 1990)
Hairy vetch	151	164	Maize	(Dou and Fox, 1994)
Red clover	134	186	Maize	(Dou and Fox, 1994)
Sesbania	131	67	Maize	(Sharma and Behera, 2009)
Clover and burr medic	112	40	Vineyard	(Ovalle et al., 2010)

2.6 The impact of legume cultivation on functional microbial communities

Soil microorganisms play an important role in global nutrient cycling and are involved in the processes of organic matter decomposition, and formation of stable soil structure (Kanté et al., 2021). The ecological network of microbial communities is dynamic and radically responds to soil management practices. Long-term monoculture simplifies and reduces the stability of the ecological network of soil microbial communities, which can be linked to declines in soil fertility (Zhang et al., 2024). The inclusion of legumes in crop rotations has often been associated with higher functional microbial diversity compared to monoculture (Faucon et al., 2017; Zhou et al., 2017). Indeed, legumes can establish associations with a multitude of nutrient-cycling and plant growth-promoting rhizobacteria (PGPR) such as *Agrobacterium*, *Acinetobacter*, *Bacillus*, *Enterobacter*, *Mycobacterium*, *Paenibacillus*, and *Pseudomonas* (Martínez-Hidalgo and Hirsch, 2017).

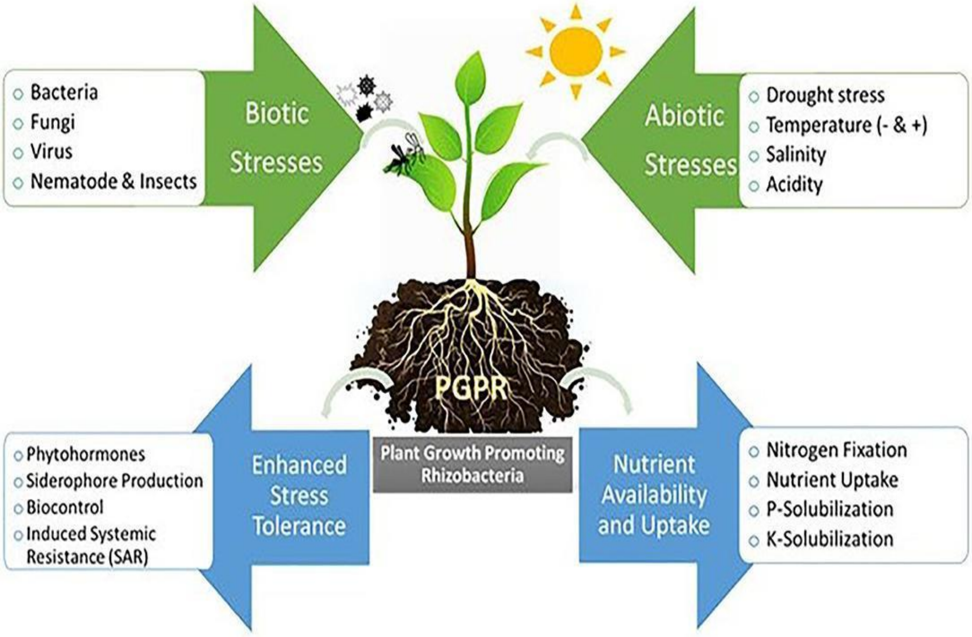

Figure 2.2: Benefits mediated by plant growth-promoting rhizobacteria on host plants (adapted from (Shah et al., 2021)).

Plant growth-promoting rhizobacteria can enhance plant growth through either direct or indirect mechanisms. Direct mechanisms include N fixation (Tan et al., 2015), nutrient solubilization (P, potassium, iron, and zinc) (Etesami and Adl, 2020), secretion of one or more phytohormones such as auxin, cytokinins, gibberellins, and ethylene (Maheshwari et al., 2015), and production of siderophores (Sayyed et al., 2012). Indirect plant growth promotion occurs through biocontrol activity against various biotic and abiotic stresses via induced systemic resistance, upregulating the expression of osmo-protectants, and production of antimicrobial and antioxidant compounds (Aloo et al., 2023). Additionally, some PGPR can suppress ethylene via the synthesis of an enzyme called 1-aminocyclopropane-1-carboxylate deaminase (Karnwal et al., 2024). A study by Lupwayi et al. (1998), reported increased soil and rhizosphere bacterial diversity and functionality in wheat-legume rotations compared with wheat monoculture. Co-inoculation studies with multiple bacterial species in legumes have been shown to enhance plant growth, yield, nitrogenase activity, nutrient availability, and increased resistance to plant pathogens (Schwartz et al., 2013; Kaschuk et al., 2022; Ijaz et al., 2023). Qin et al. (2017) observed increased soil microbial populations and enzyme activity under a potato-legume rotation system, highlighting the influence exerted by legumes on soil microorganisms and their associated enzyme activities.

2.7 The effect of legume cultivation on soil enzyme activities

Soil microorganisms exude extracellular enzymes to regulate the mineralization of organic matter in the soil, ultimately facilitating soil nutrient availability (Allison and Vitousek, 2005; Sinsabaugh et al., 2008). Soil extracellular enzyme activities are highly sensitive to management practices and are therefore considered important indicators of soil fertility and nutrient cycling (Zhou et al., 2021). Common nutrient cycling enzymes include C-cycling (α-1,4-glucosidase, β-1,4-glucosidase, β-1,4-xylosidase), N-cycling (β-1,4-N-acetylglucosaminidase, L-Leucine-7-amido-4-methyl coumarin, urease, nitrate reductase) and P-cycling (phosphatases) (Liu et al., 2024). An important enzyme in C cycling β-1,4-glucosidase, catalyses the hydrolysis and biodegradation of glucosides present in plant debris into glucose molecules (Martinez and Tabatabai, 1997). Phosphatases play a crucial role in liberating phosphatases from organic compounds (Nannipieri et al., 2011), and nitrate reductase is involved in the initial steps of N assimilation through the reduction of nitrate to nitrite (Srivastava, 1980). According to Veloso et al. (2019), leguminous crops favour organo-mineral associations and microaggregate formation which makes them a good option for increasing soil enzyme activity. Maltais-Landry. (2015) reported increased phosphatase activity due to fava bean, pea, and purple vetch cover cropping. This coincides with other studies that have reported increased soil enzyme activity following legume crop rotation (Mbuthia et al., 2015; Qian et al., 2015; Qi et al., 2020; Yang et al., 2021)

2.8 Overview of study species

2.8.1 Velvet bean

Mucuna pruriens L. (DC) commonly known as velvet bean is a vigorous annual climbing legume that originated in southern China and eastern India, where it was cultivated as a vegetable crop (Duke, 2012). It is now widely distributed in tropical and subtropical regions, being cultivated as forage and cover crop in Asia, America, Africa, and the Pacific Islands (Lampariello et al., 2012). *Mucuna pruriens* has been reported to show remarkable tolerance to abiotic stresses such as low soil fertility, drought, and soil acidity (Sathyanarayana et al., 2016). It is essential in sustainable agriculture as the crop can fix approximately 34-242 kg N ha^{-1} through BNF (Sanginga, 2003; Muoni et al., 2019). Crop residues of *M. pruriens* have a low C:N ratio and low lignin, resulting in rapid decomposition in the soil and a more readily release of N for plant assimilation. According to Mhlanga et al. (2015) residues of *M. pruriens* supplied about 170 kg N ha^{-1}, which yielded maize returns comparable to maize that received 70 kg N ha^{-1} in ammonium nitrate fertilizer. This highlights that using *M. pruriens* in crop rotation systems can facilitate soil N build up, which may be of significance to SSGs in South Africa where fertilizer use is not widespread and intensive compared to other sugarcane producing countries.

Mucuna pruriens is considered one of the most allelopathic plants (Ortiz Ceballos et al., 2012; Travlos et al., 2018), which has been linked to the exudation of levodopa or L-DOPA (L-3,4-dihydroxyphenylalanine) from leaves and the rooting system (Fujii, 2003; Adler and Chase, 2007). Also, due to its fast growth and high biomass accumulation, *M. pruriens* outcompetes weeds for growth elements and shades them from light, resulting in decreased weed presence (Teasdale et al., 1998). Cover crops that accumulate significant biomass have been shown to reduce soil erosion and nutrient losses associated with high rainfall intensity (Kaye and Quemada, 2017). Furthermore, *M. pruriens* provides multiple benefits to small-scale farmers such as increased yields in the subsequent crop (Kaizzi et al., 2004; Masikati et al., 2014; Mhlanga et al., 2015), conserves soil moisture (Altieri and Nicholls, 2012), and increases C sequestration (Barthès et al., 2004; Muoni et al., 2019). In general, *M. pruriens* in fallow systems improves soil biological, chemical, and physical properties, making it an excellent cover crop (Boateng, 2005).

2.8.2 Groundnut

Groundnut (*Arachis hypogaea* L.) is an annual legume that originated in South America but is now an agro-economically important grain and oilseed crop in tropical and subtropical regions worldwide (Seijo et al., 2007). Like most legumes, groundnut can fix atmospheric N via bacterial symbiosis in root (Graham and Vance, 2003; Furlan et al., 2017), and groundnut crops have been reported to fix 150-200 kg N ha^{-1} (Toomsan et al., 1995). Therefore, its cultivation contributes to sustainable agriculture by improving the soil N status. Several studies have showed N contributions of groundnut to cropping systems ranging from 19 to 205 kg N ha^{-1} in varying climatic and cropping conditions (Dakora et al., 1987; Pimratch et al., 2004; Nyemba and Dakora, 2010; Puangbut et al., 2011; Konlan et al., 2013; Mokgehle et al., 2014). This highlights that the N contributions of groundnut through BNF have the potential to reduce synthetic N fertilizer use in cropping systems (Van Kessel and Hartley, 2000; Vance, 2001).

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Chapter 3

Mucuna pruriens (L.) DC. cover cropping reduces soil acidity and promotes beneficial plant growth promoting rhizobacteria in small-scale sugarcane soils

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Abstract

There is growing concern about major agricultural regions relying on monoculture and extensive chemical fertilizer use, as these practices often lead to soil fertility loss, structural imbalances, and reduced nitrogen use efficiency. Incorporating legume cover crops during the bare fallow period presents a sustainable way to maximize sugarcane yields while mitigating the detrimental effect of sugarcane monoculture. This study aimed to determine the effects of planting *Mucuna pruriens* on the biological and chemical properties of five different sugarcane plantation soils in KwaZulu Natal. Soils collected pre- and post-*M. pruriens* were analysed for chemical properties, microbial composition, and extracellular enzyme activities. *Mucuna pruriens* increased the pH across all study sites. There were variations in the enzyme activities of post-harvest soils across all study sites. Empembeni post-harvested soils had the highest alkaline phosphatase and nitrate reductase activity, while Gingindlovu exhibited the highest acid phosphatase and β–glucosidase activity. *Mucuna pruriens* shifted the microbial profile of post-harvest soils and selected for Pseudomonadaceae and Burkholderiaceae in all study sites. This study revealed that *M. pruriens* contributes to soil improvement by reducing soil acidity and promoting nutrient-cycling bacteria.

Keywords: *Mucuna pruriens*, plant-microbe symbiosis, nitrogen fixation, soil acidity, monoculture, small-scale growers

3.1 Introduction

Approximately two-thirds of agricultural land has been degraded in the past 50 years, and 40% is considered seriously degraded, significantly impacting food security (Bindraban et al., 2012). According to USAID (2024), food production needs to increase by 60% to feed the global population by 2050. Furthermore, 820 million people have been reported to be food insecure (FAO et al., 2019), with 21% of the people living in Africa (FAO, 2018), underscoring the urgent need for interventions to enhance food security, particularly in southern Africa. Food insecurity remains a significant developmental challenge in southern Africa (Hlatshwayo et al., 2022). Despite the well-developed agricultural system in South Africa, approximately 16% of the population experiences food deprivation (StatsSA, 2019), a situation further exacerbated by declining soil fertility and low crop yields faced by small-scale growers (SSGs) in the country (Mthembu et al., 2019). According to StatsSA. (2021), food security primarily affects people in rural communities and non-metro areas, thus 70% of SSGs rely on subsistence farming as their source of food and income (Hlatshwayo et al., 2023).

Sugarcane is the main cash crop in the KwaZulu-Natal (KZN) province, and SSGs rely on its cultivation for their livelihoods (Thibane et al., 2023; Zulu et al., 2019). Zulu et al. (2019) reported declining sugarcane yields and productivity due to unsustainable farming practices, impacting the livelihoods of SSGs. Sugarcane monoculture has been shown to deplete plant-specific soil nutrients and increase the persistence of soil-borne pathogens (Chen et al., 2018). As a result, farmers need to apply chemical fertilizers to rejuvenate soil nutrients and maintain appreciable sugarcane yields (van Antwerpen et al., 2023). Chemical fertilizers contribute to soil and environmental deterioration and are costly for resource-limited SSGs (Mthembu et al., 2019). Moreover, the applied chemical fertilizers do not necessarily ensure enhanced soil fertility and productivity, since a substantial amount of the fertilizer is lost through surface runoff and leaching (Adesemoye & Kloepper, 2009; Rosenstock et al., 2014). Continued reliance on chemical fertilizers is not a solution for sustainable agriculture, underscoring the need for management practices that can increase sugarcane productivity and improve soil health to ensure food security and alleviate poverty. Cover cropping has emerged as an agronomic practice that can sustainably improve the productivity of cropping systems, with an increasing number of studies highlighting its benefits (Blanco-Canqui et al., 2015; Adentuji et al., 2020).

The cultivation of cover crops (CCs) during the fallow period has been shown to provide various benefits in agroecosystems, such as soil erosion control, improvement of soil and water quality, reduction of nutrient losses, and control of pests and weeds (Wittwer et al., 2017). In particular, the inclusion of leguminous CCs can significantly enhance nitrogen (N) levels in the soil, reducing N fertilizer requirements for the subsequent crop (Etesami, 2022). Park et al. (2010) reported that fixed N can supply up to the fourth ratoon of sugarcane, while legume residues decompose quicker (C/N <20), contributing to increased N availability in the soil (Radicetti et al., 2017; Thilakarathna et al., 2016; Jani et al., 2016). Additionally, leguminous CCs increase organic matter in the soil, providing energy for microbial growth and activity, which plays a role in nutrient cycling, and release of inorganic nutrients necessary for plant growth (Baležentienė, 2012; Kujur & Kumar Patel, 2014). The cultivation of leguminous CCs has been reported to increase microbial biomass C, arbuscular mycorrhizal fungi population, bacteria population, and soil enzyme activities compared to control under varying environmental and management practices (Blanco-Canqui et al., 2015; Mbuthia et al., 2015; Mukumbareza et al., 2015).

Mucuna pruriens (L.) DC. is a dinitrogen (N_2) fixing legume with remarkable tolerance to low soil fertility, drought, and soil acidity (Burle et al., 1992; Duke, 2012; Hairiah, 1992). Its use as a cover crop has been reported to reduce fertilizer use while maintaining crop yields due to its high biomass accumulation and subsequent residue decomposition and N mineralization (Kaizzi et al., 2004; Masikati et al., 2014; Mhlanga et al., 2015). In addition, *M. pruriens* demonstrates high weed suppression ability, reduces soil erosion, and prevents nutrient losses associated with bare fallows (Kaye & Quemada, 2017). Despite its potential benefits, *M. pruriens* remains an underutilized legume, thus its impact on soil nutrition, microbial communities, and enzyme activity remains unknown in South African sugarcane plantations. Understanding the growth physiology, N and P nutrition of *M. pruriens* growing in sugarcane plantations will aid in understanding its application as a cover crop. This study aims to establish if *M. pruriens* can be used as a cover crop in nutrient-deficient small-scale sugarcane plantations. The objectives of this study include 1) Determining soil chemical characteristics of soils collected in five sugarcane plantations in KwaZulu Natal, pre-planting and post-harvesting of *M. pruriens*, 2) Identifying N-fixing, N-cycling, and phosphorus (P) solubilizing bacteria present in the soils pre-planting and post-harvesting of *M. pruriens*, and 3) Assaying the N, P, and carbon (C) cycling enzymes in the collected soils pre-planting and post-harvesting *M. pruriens*.

3.2 Materials and Methods

3.2.1 Soil Collection

Soil samples were collected from five small-scale sugarcane plantations located at different geographical locations in KZN, South Africa. Northern KZN included three sites Mvutshini (28° 50’ 52.9” S 32° 00’ 09.0” E), Gingindlovu (28° 56’ 29.3” S 31° 34’ 58.9” E), and Empembeni (28° 51’ 25.7” S 31° 58’ 19.4” E). The remaining two sites were from southern KZN, which included Umzinto (30°16’ 41.03” S, 30° 39’ 47.97” E) and Hibberdene (30° 35’ 37.12” S, 30° 31’ 30.29” E). From each site, experimental soil was collected from 10 random points at a depth of 0-30 cm, maintaining a distance of 2 m apart. Subsequently, the ten soil samples were homogenised to create a composite sample for a more accurate representation of the soil microbial community composition. Pre-planting soil samples for microbial identification and enzymatic assays were placed in sterile zip-lock bags, stored on ice in the field, and then kept at 4°C until further analysis.

3.2.2 Plant growth trials

Seeds of *M. pruriens* were sourced from AGT Foods Africa, Marji Mizuri farm, KZN. The seeds were soaked in warm water overnight to break seed dormancy and initiate seed germination. Thereafter, the seeds were transferred into petri dishes containing moist, sterile filter paper and kept in the dark for four days at room temperature. After germination, the seeds were transferred into 10 cm diameter pots filled with soils from the different plantations. Each plantation had 10 labelled pots in a completely randomized design, resulting in 50 experimental pots. The growth trials were conducted in greenhouse conditions at the University of KwaZulu Natal, Westville campus, School of Life Science Building, South Africa. Average day and night temperatures ranged from 30-35 ^{o}C and 11-15 ^{o}C, respectively. Two sets of harvests were conducted at 15- and 54-days post-seedling emergence. Five plants per plantation (initial and final harvests) were rinsed with distilled H_2O and then separated into leaves, stems, and roots, and dried at 65 ^{o}C until constant weight was maintained. The dried plant material was weighed, ground into powder and sent for C, N, and P analysis. $\delta^{15}N$ isotope analysis was conducted using a LECO-N analyzer (LECO Corporation, St. Joseph, MI, USA) at the Archaeometry Department, University of Cape Town, South Africa, while C and P concentrations were measured using inductively coupled mass spectrometry (ICP-MS) at the Central Analytical Facilities, Stellenbosch University, South Africa.

3.2.3 Soil nutrition analysis

Post-harvest soils from each plantation were homogenised to create a composite sample and a portion of the soil was stored in the fridge at 4 °C for bacterial extraction and identification and enzyme activities. The remaining soils were air-dried, milled, weighed at 1 kg per sample (3 soil samples per plantation) and sent to the Fertilizer Advisory Service (FAS) at the South African Sugarcane Research Institute (SASRI), South Africa for full nutrient, pH, total cation, and exchangeable acidity analysis

3.2.4 Soil Bacterial Extraction and Identification

A three-fold serial dilution was conducted for 10 g of soil samples from each plantation. Briefly, each 10 g soil sample was diluted in 100 ml of autoclaved distilled water and mixed thoroughly. The resulting dilutions were then transferred to selective media plates by inoculating each plate with 100 µl of the dilution. Tricalcium phosphate plates were used for P solubilizing bacteria, while Simmon's citrate and Jensen plates were used for N-cycling and N-fixing bacteria, respectively. The inoculated plates were incubated at 30 °C for 4 to 7 days. After incubation, pure colonies were obtained by repeated streaking into new, clean plates.

Pure bacterial colonies were amplified using a portion of the 16S rRNA gene primers: 63F (5′-CAG GCCTAACACATGCAAGTC-3′) and 1387R (5′-GGGCGGTGTGTACAA GGC-3′). The PCR reaction mixture (25 µl) consisted of; 12.5 µl EmeraldAmpGT Master mix (Separations, South Africa), 8.5 µl milliQ dH_2O, 0.5 µl forward primer, 0.5 µl reverse primer, and 3 µl diluted pure colony. The PCR amplification was carried out as follows: initial denaturation at 94 °C for 5 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and final elongation at 72 °C for 10 min using a T1000 Thermal Cycler (Biorad Laboratories Inc., USA). Thereafter, the resulting PCR products were resolved on a 1% (w/v) agarose gel (Seakem, Lonza, USA) stained with SafeView™ Classic (Applied Biological Materials Inc., Canada) and viewed in a ChemiDoc (Bio-Rad Laboratories Inc., USA). Positive amplicons were sent to Inqaba Biotech Inc, Pretoria, South Africa. The resultant chromatograms were cleaned and subjected to BLASTN using the National Center for Biotechnology Information database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (accessed 28/04/2024)

3.2.5 Extracellular Enzyme Activity

The enzyme activities of β-glucosidase, acid phosphatase, and alkaline phosphatase were assayed using a fluorescence-based method adapted from Jackson et al. (2013). Briefly, 10 g of soil samples (3 soil samples per plantation) were homogenized at low speed in 100 ml autoclaved distilled H_2O for 2 h and stored at 4 °C overnight. Thereafter, the resulting supernatants were transferred into 96-well black microplates prior to adding their respective 4-methylumbeliferyl (MUB)-linked substrates. The sample run consisted of 200 µl soil aliquot and 50 µl substrate, incubated alongside reference standards (200 µl buffer + 50 µl standard), quench standard (200 µl soil aliquot + 50 µl standard), sample control (200 µl soil aliquot + 50 µl buffer), negative controls (200 µl buffer + 50 µl substrate) and blanks (250 µl buffer). Following a 1 h incubation period at 30 °C, the reaction was stopped using 0,5 M NaOH. The fluorescence was measured at 450 nm using a Glomax Multi Plus microplate reader (BioTek, USA) and the activity was expressed as nmol h^{-1} g^{-1}. The buffer and standard were adjusted to pH 5 before determining acid phosphatase activity.

Nitrate reductase activity was determined according to a modified protocol described by (Bruckner et al., 1995). Briefly, 5 g of soil (3 soil samples per plantation) was added to a light-sealed volumetric flask containing a solution of 1 ml of 25 mM KNO_3, 4 ml of 0,9 mM 2,4-dinitrophenol and 5 ml of milliQ dH_2O. Thereafter, the mixture was thoroughly mixed before being incubated in the dark at 30 °C for 24 h. Following incubation, 10 ml of 4 M KCl was added to each sample, mixed thoroughly and the filtered using Whatman number 1 filter paper. The enzymatic reaction was initiated by adding 2 ml of the filtrate to 0.19 M of ammonium chloride (pH 8.5) and 0.8 ml of the colour reagent (1% sulphanilamide, 1 N HCl and 0.2% N-(1-naphthyl) ethylenediamine dihydrochloride (NEDD). The solution was then incubated at 30 °C for 30 min. The absorbance was measured at 520 nm using an Agilent Cary 60UV-Vis spectrophotometer (Agilent, Santa Clara, CA, USA). The nitrite released into the medium was extrapolated from a prepared KNO_3 standard curve and the enzyme activity was expressed as 0.1 µmol h^{-1} g^{-1}.

3.2.6 Growth Rate Calculations

3.2.6.1 Specific N/P absorption rate (SNAR/SPAR)

Total plant N/P was used to calculate the specific N/P absorption rate according to (Nielsen et al., 2001).

$SNAR = (N_2 – N_1 / t_2 – t_1)*[(\log_e R_2 – \log_e R_1) / (R_2 – R_1)]$

$SPAR = (P_2 – P_1/t_2 – t_1)*[(\log_e R_2 – \log_e R_1)/ (R_2 – R_1)]$

Where N, P, t and R represent total N content, total P content, duration of plant growth and root dry weight, respectively.

3.2.6.2 Specific N/P utilization rate (SNUR/SPUR)

Total plant N/P was used to calculate the specific N/P utilization rate according to (Nielsen et al., 2001).

$SNUR = (W_2 – W_1 / t_2 –t_1)*[(\log_e N_2 – \log_e N_1) / (N_2 – N_1)]$

$SPUR = (W_2 – W_1 / t_2 –t_1)*[(\log_e M_2 – \log_e M_1) / (M_2 – M_1)]$

Where W, N, M and t represent the plant DW, total N content, total P content and the duration of plant growth, respectively.

3.2.6.3 Relative growth rate (RGR)

The relative growth rate was calculated using the (ÅGREN & Franklin, 2003) method.

$RGR = [(\ln W_2 – W_1)/t_2 – t_1]$

Where W represents the plant DW accumulated from the initial (W_1) to final (W_2) and t is the time for plant growth.

3.2.6.4 Root: shoot ratio

The root: shoot ratio was calculated using the (ÅGREN & Franklin, 2003) method.

Root: shoot= D_R/D_S

Where D_R is the root dry weight while D_S is the shoot dry weight.

3.2.6.5 Carbon construction cost (Cw)

Carbon construction cost was calculated using a formula modified by (Peng et al., 1993) and further verified by (Mortimer et al., 2005).

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

Where Cw is the total carbon construction cost in the tissues (mmol C g^{-1} DW), C is the total carbon concentration (mmol C g^{-1}), k is the reduced state of the N substrate (i.e. $NH_3 = -3$) and N is the total organic nitrogen content of the tissue (Williams et al., 1987).

The number 14 is the atomic mass of nitrogen, 180 is a conversion factor from mol to g of glucose. The number 24 represents the number of electrons in a glucose molecule; while 0.89 is an estimation of growth efficiency (Williams, Percival et al. 1987), and (6000/180) is a conversion factor for glucose that converts g dw^{-1} to mmol C g^{-1} dw.

3.2.6.6 Percentage N derived from the atmosphere (%NDFA)

The N isotope analyses was conducted at the University of Cape Town Archaeometry Department. The isotopic ratio of N was calculated as $\delta = 1000$ (Rsample/ Rstandard), where R is the molar ratio of ^{15}N and ^{14}N of the samples and standards. Between 2.100 and 2.200 mg of each milled sample were weighed into 8 mm x 5 mm tin capsules (Elemental Micro-analysis, Devon, UK) on a Sartorius microbalance (Goettingen, Germany). The samples were then combusted in a Fisons NA 1500 (Series 2) CHN analyzer (Fisons Instruments SpA, Milan, Italy). The N isotope values for the N gas released were determined on a Finnigan Matt 252 mass spectrometer (Finnigan MAT GmbH, Bremen, Germany), which was connected to a CHN analyzer 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 $(NH_4)_2SO_4$.

Percent of N derived from the atmosphere was calculated according to Shearer and Kohl (1986);

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

Where NFDA is the N derived from the atmosphere and β is the $\delta^{15}N$ natural abundance of N derived exclusively from the biological N fixation of *Pisum sativum* grown in N-free culture. The β value of *M. pruriens* for this study was -2.58%.

3.2.7 Statistical Analysis

Statistical analyses were conducted using RStudio version 4.3.2 (R Core Team, 2024). To address the first objective, data on growth kinetics, plant biomass, and plant mineral nutrition for each sugarcane site was analysed using a one-way analysis of variance (ANOVA). Where significant differences were detected ($p < 0.05$), mean values were further separated using Tukey's multiple comparisons post hoc test. Assumptions of normality and homogeneity of variances were assessed with the Shapiro-Wilk and Levene's tests, respectively. To address the third and fourth objectives, paired samples t-tests were performed to compare the pre-planting and post-harvesting means of soil nutrients and enzyme activities for each sugarcane site.

3.3 Results

3.3.1 Soil Nutrient Analysis

Post harvesting, soil N, P and potassium (K) concentrations were significantly lower across all study sites (Table 3.1). Calcium (Ca) and magnesium (Mg) concentrations showed significant increases across all sites, however, there was a non-significant decrease in Umzinto soils (Table 3.1). The soil pH increased across all sites ranging from 5.57-6.74, with Mvutshini, Umzinto, Empembeni, and Hibberdene increasing significantly, while Gingindlovu showed a non-significant increase (Table 3.1). Additionally, exchangeable acidity and acid saturation were significantly lower in all sites post harvesting, with the exception of Umzinto soils (Table 3.1). The total cations increased significantly in Empembeni and Hibberdene soils, and decreased significantly in Gingindlovu soils (Table 3.1).

3.3.2 Bacterial Identification

The molecular identification of N-fixing, P-solubilizing and N-cycling bacteria in pre-planting and post-harvesting soils revealed a total of 16 and 15 bacterial strains, respectively (Table 3.2). In Mvutshini, pre-planting soils had a total of six bacterial strains which included two N-fixing bacteria (*Paraburkholderia phymatum* and *Paraburkholderia sabiae*), two N-fixing and P solubilizing (*Pseudomonas koreensis* and *Burkholderia sp.*), and two N-cycling bacteria (*Chromobacterium piscinae* and *Pseudomonas nitroreducens*). Post-harvesting, Mvutshini soils were comprised of three strains; two N-cycling (*Burkholderia diffusa* and *Burkholderia ambifaria*), and one N-fixing and N-cycling bacteria (*Caballeronia sp.*). Pre-planting, *Pseudomonas frederiksbergensis* (N-fixing and N-cycling) and *Priestia megaterium* (N-fixing) were the only strains isolated from Gingindlovu soils. Similarly, two strains were harvested post harvesting which included *Pseudomonas sp.* (N-fixing and P solubilizing) and *Paenibacillus sp.* (N-fixing). In Umzinto, pre-planting soils had a total of two N-fixing and P solubilizing bacterial strains (*Achromobacter sp.* and *Pseudomonas sp.*), compared to post-harvesting soils where three strains were isolated; *Pseudomonas umsongensis* (N-cycling), *Pseudomonas sp.* (N-fixing and P solubilizing), and *Rhizobiaceae bacterium* (N-fixing). Pre-planting soils in Empembeni had a total of four bacterial; three P solubilizing strains (*Burkholderia cepacia, Pseudomonas protegens,* and *Burkholderia cenocepacia*) and an N-fixing and P solubilizing strain (*Burkholderia* sp.). Post-harvesting, the number of bacterial strains in Empembeni increased to six; two N-fixing *Pseudomonas* sp., *Burkholderia diffusa* (N-cycling), *Pseudomonas koreensis* (N-fixing and N-cycling) and *Paraburkholderia* *calledonica* (P solubilisation). Pre-planting, Hibberdene soils revealed two N-fixing and P solubilizing bacterial strains (*Pseudomonas sp.* and *Pseudomonas poae*) compared to post-harvesting soils which also had two N-fixing and P solubilizing strains (*Pseudomonas sp.* and *Pseudomonas frederiksbergensis*

3.3.3 Soil extracellular enzyme activity

Post harvesting, alkaline phosphatase activity was significantly higher in Mvutshini, Gingindlovu, and Empembeni, while there was a significant decrease in Umzinto soils (Figure 3.1A). Gingindlovu had the highest acid phosphatase and glucosidase activity compared to all the study sites (Figures 3.2B-C). Nitrate reductase activity was significantly higher across all the study sites (Figure 3.2D).

3.3.4 Plant Nutrition and Growth Kinetics

Plants grown in Empembeni soils had a slightly higher total plant biomass followed by plants grown Umzinto compared to Gingindlovu, Mvutshini and Hibberdene soils. Umzinto had the highest shoot biomass, while Empembeni showed a distinctively high root biomass (Table 3.3). *Mucuna pruriens* grown in Umzinto soils had the highest specific N absorption (SNAR) (Figure 3.2A) and utilization rate (SNUR) (Figure 3.2C). In addition, plants grown in Umzinto soils accumulated the highest total plant N (Figure 3.3B). On the other hand, Hibberdene distinctively had the highest specific P absorption rate (Figure 3.2B) while Umzinto had a slightly higher specific P utilization rate compared to the other plants (Figure 3.2D). Overall, Hibberdene accumulated the highest total plant P (Figure 3.3D). Plants across all soil sites derived most of their N from the soil except *M. pruriens* plants grown in Empembeni (Figure 3.3C). Consequently, Empembeni-grown plants had the highest %NDFA (58.95%) while Umzinto plants had the lowest (26.08%) (Figure 3.3A).

3.4 Discussion

The sugarcane plantation soils sampled in this study were acidic and nutrient-deficient, promoting the proliferation of N-fixing, P solubilizing, and N-cycling bacteria such as *Burkholderia, Pseudomonas,* and *Paenibacillus*. Plant growth-promoting bacteria (PGPB) can enter a dormant state due to various environmental conditions such as soil pH and nutrient deficiency (Marais et al., 2012). Root exudates provide energy for PGPB, which inactivates their dormancy (Baudoin et al., 2005; van Vliet., 2015) and is likely responsible for the increased bacterial diversity observed across all the sites. These results suggest that *M. pruriens* sanctioned for bacteria that aid in nutrient acquisition by increasing its root exudate signals for N and P cycling bacteria. These findings concur with Mula-Michel et al. (2023), who observed higher bacterial diversity in soybean cover crop than in sugarcane monoculture. Considering this, it would be expected that a crop rotation system including sugarcane and *M. pruriens* would exhibit shifts towards beneficial plant growth-promoting and nutrient cycling bacteria, and subsequently an increase in their associated enzyme activities to increase nutrient transformation and mineralization in the soil.

According to the resource allocation model for extracellular enzymes, soil microbes exude extracellular enzymes to mineralize and cycle deficient nutrients, thereby contributing to nutrient bioavailability for plant uptake (Sinsabaugh & Moorhead, 1994). Phosphatases play an important role in P cycling by mineralizing organic P compounds in the soil (Billah et al., 2019). Furthermore, their activity has been reported to be strongly influenced by P status and soil pH (Burns et al., 2013; Fujita et al., 2018). Our results indicate increased alkaline and acid phosphatase activity in acidic and P-deficient sugarcane soils post-*M. pruriens* harvesting, suggesting that P might have been initially bound by cations, and required mineralization by phosphatases. Previous studies have reported higher alkaline and acid phosphatase activity in P-deficient and acidic soils (Liu et al., 2017; Magadlela et al., 2023), supporting the notion that phosphatase activity is regulated by P-deficiency in soils. In addition, legumes require more P to establish symbiotic associations with bacteria for N fixation (Magadlela et al., 2016; Stevens et al., 2019), which could also contribute to the observed increased alkaline and acid phosphatase activity.

Indeed, previous studies have linked increased phosphatase activity to N addition (Margalef et al., 2017; Marklein & Houlton, 2012; Widdig et al., 2019), since phosphatases contain large amounts of N that are lost by the organism during the exudation (Widdig et al., 2019). *Mucuna* *pruriens* increased nitrate reductase activity across all the study sites post-harvesting, likely due to rhizodeposition enriching the soil with N via the decomposition of dead roots (Henneron et al., 2020; Kanté et al., 2021). Legumes have a low C:N ratio and low lignin content resulting in faster decomposition and mineralization of N (Li et al., 2016), leading to increases in N-cycling and subsequently higher nitrate reductase activity. Additionally, root senescence may account for the increased β-glucosidase activity in Gingindlovu and Hibberdene. According to (Martinez & Tabatabai, 1997), β-glucosidase catalyses the hydrolysis and degradation of glucosides present in plant debris, leading to increased mobilization of mineral elements (Himelblau & Amasino, 2001) and ultimately, increased β-glucosidase activity.

Post-harvesting, soil pH increased across all sites. Similar findings were reported by (Balota & Chaves, 2011), who observed increases in soil pH from 4 to 6 after 10 years of legume (*Arachis hypogaea*, *Crotalaria spectabilis*, *Crotalaria breviflora*, *Leucaena leucocephala*, *Mucuna deeringiana*, *Mucuna pruriens*, and *Vigna unguiculata*) cover cropping. Contrary to this, some studies have reported decreased soil pH following legume cover cropping, which was attributed to higher exchangeable ions and the exudation of organic acids and protons in cover crop treatments (McVay et al., 1989; Wang et al., 2021). Nevertheless, the soil pH across all sites remained acidic, and acidic soils have been reported to reduce the bioavailability of nutrients. Acidic soils increase the concentration of cations such as Al^{3+} and Fe^{3+}, which bind P, forming insoluble complexes that render phosphorus unavailable for assimilation by plants (Ferguson & Gresshoff, 2015; Kopittke et al., 2015). Since P regulates bioenergetic processes, its deficiency can limit plant growth and functioning, especially in legumes where P is required for biological N fixation (Magadlela et al., 2020). Umzinto and Hibberdene showed decreased %NDFA, and changed their N preference to mostly soil N. The ability of legumes to switch N sources under nutrient-deficient conditions is an important survival strategy that ensures more energy is made available for plant photosynthesis and biomass accumulation (Sithole et al., 2021). Similar N use patterns were recorded by Magadlela et al. (2020) and Aranjuelo et al. (2014), who observed that *M. pruriens* exhibits decreased N fixation rates under nutrient poor soil conditions. Our results suggest that P was utilised by *M. pruriens* for growth and function, which is further corroborated by the increased P assimilation rates and high total P content of Umzinto and Hibberdene grown plants.

Mucuna pruriens growing in Empembeni soils had the highest %NDFA despite the soils initially having the lowest P concentration. These results suggest that *M. pruriens* adapted to P deficiency by increasing its root biomass, which may have allowed the plant to scavenge for deficient nutrients and efficiently fix N (Neumann & Martinoia, 2002). Studies have shown that under P-deficient conditions, one of the strategies employed by legumes includes modification of the root system architecture, leading to increased root surface area and ultimately, enhanced nutrient acquisition (Thuynsma et al., 2014). Additionally, *M. pruriens* might have remobilized P from older tissues for optimal nodule P concentration for enhance N fixation (Sulieman & Tran, 2015). This may also explain the moderate levels of total plant P observed in the Empembeni-grown plants.

The decreased soil N and P concentrations post-harvesting across all sites can be explained by *M. pruriens* using these nutrients to support its growth and functioning (Weerasekara et al., 2017). Similar findings have been reported by (Zhou et al., 2011) and (Gao et al., 2017), who observed decreased soil nutrient content following cover cropping. However, the assimilated nutrient can be recycled back into soil through the decomposition and mineralisation of *M. pruriens* into the soil as legume residues. According to Mhlanga et al. (2015), residues of *M. pruriens* provided approximately 170 kg N ha^{-1}, producing maize returns comparable to maize that received 70 kg N ha^{-1} in ammonium nitrate fertilizer. Furthermore, N from legume residue decomposition is not easily leached from the soil, constituting a more sustainable form of N that can support the growth of sugarcane (Dinnes et al., 2002; Salmeron et al., 2011)

3.5 Conclusion

In this study, it was evident that *M. pruriens* has the capacity to survive in nutrient-deficient and acidic sugarcane plantation soils through N fixation, and symbiosis with nutrient cycling bacteria and their associated enzyme activities. *Mucuna pruriens* increased the extracellular enzyme activities and bacterial strains to varying levels across the study sites, which are a good indicator of soil health. However, it is recommended that *M. pruriens* be cultivated for longer than 2 months to better assess the long-term impact on soil health. Consequently, the depleted soil nutrients can be replenished by utilizing *M. pruriens* as green manure. Overall, the positive biological and chemical contributions of *M. pruriens* highlight its potential to as a cover crop that can sustainably enhance soil health.

Author contributions

A.M conceived the research idea; S.K performed the growth trials, data analysis and draft manuscript; A.M conducted the review, editing and supervision.

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Conflict of interest

We declare no known conflict of interest.

Data availability

The data can be made available upon request.

3.6 References

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Tables and figures

Table 3.1: Soil nutrient concentrations (N: Nitrogen, P: Phosphorus, K: Potassium, Ca: Calcium, and Mg: Magnesium) and relative acidity (pH, Exchangeable acidity, Total cations, and Acid saturation) in soils collected from Mvutshini, Gingindlovu, Umzinto, Hibberdene, and Empembeni pre- and post-*M.pruriens* planting. Different values represent the mean±SE, with significant differences ($p < 0.05$) within each treatment denoted by different letters.

	Soil Sites					
		Mvutshini	Gingindlovu	Umzinto	Hibberdene	Empembeni
N (ppm)	Pre	0.09±0.00^{a}	0.06±0.00^{a}	0.07±0.00^{a}	0.06±0.00^{a}	0.06±0.00^{a}
	Post	0.08±0.00^{b}	0.04±0.00^{b}	0.05±0.00^{b}	0.05±0.00^{b}	0.04±0.00^{b}
P (mg/L)	Pre	4.29±0.14^{a}	3.81±0.05^{a}	10.69±0.23^{a}	9.41±0.05^{a}	3.41±0.09^{a}
	Post	2.85±0.14^{b}	2.37±0.14^{b}	7.89±0.18^{b}	7.73±0.00^{b}	2.13±0.09^{b}
K (mg/L)	Pre	33.05±0.19^{a}	55.17±1.79^{a}	61.01±1.78^{a}	100.56±2.47^{a}	18.43±0.36^{a}
	Post	24.92±0.34^{b}	50.39±0.37^{b}	25.31±0.90^{b}	70.42±0.71^{b}	17.91±1.05^{a}
Ca (mg/L)	Pre	238.78±4.45^{a}	219.92±2.21^{a}	717.85±18.07^{a}	531.38±6.80^{a}	143.87±6.18^{a}
	Post	333.52±19.67^{b}	269.26±9.77^{b}	690.98±21.75^{a}	752.46±25.89^{b}	205.28±8.97^{b}
Mg (mg/L)	Pre	78.79±2.37^{a}	132.49±2.12^{a}	166.47±7.42^{a}	141.83±1.39^{a}	39.19±0.12^{a}
	Post	94.53±0.61^{b}	107.66±5.74^{b}	150.11±0.43^{a}	200.18±4.54^{b}	51.85±0.46^{b}
pH (CaCl)	Pre	4.67±0.02^{a}	4.80±0.03^{a}	4.88±0.19^{a}	5.13±0.03^{a}	4.94±0.17^{a}
	Post	5.57±0.01^{b}	6.01±0.33^{a}	6.74±0.19^{b}	6.60±0.19^{b}	6.29±0.21^{b}
Exchangeable acidity (cmol/L)	Pre	0.54±0.01^{a}	0.32±0.01^{a}	0.06±0.01^{a}	0.06±0.01^{a}	0.19±0.01^{a}
	Post	0.34±0.05^{a}	0.11±0.02^{b}	0.05±0.00^{a}	0.05±0.00^{b}	0.05±0.00^{b}
Total cation (cmol/L)	Pre	2.59±0.03^{a}	2.88±0.01^{a}	5.26±0.16^{a}	4.21±0.03^{a}	1.30±0.03^{a}

	Post	2.99±0.09[a]	2.64±0.01[b]	4.89±0.11[a]	5.79±0.18[b]	1.63±0.04[b]
Acid saturation (%)	Pre	20.85±0.03[a]	11.13±0.37[a]	11.14±0.07[a]	1.43±0.01[a]	14.32±0.51[a]
	Post	11.49±1.29[b]	3.99±0.31[b]	1.02±0.02[a]	0.87±0.03[b]	3.09±0.08[b]

Table 3.2: Pre-planting and post-*M. pruriens* harvesting nutrient cycling bacteria isolated from soils collected in Mvutshini. Gingindlovu, Umzinto, Hibberdene, Empembeni.

Soil site		**Family**	**Scientific name**	**Accession number**	**Similarity (%)**	**Function(s)**
Mvutshini	Pre-planting	*Burkholderiaceae*	*Paraburkholderia phymatum*	HE864336.1	100	N-fixing
		Burkholderiaceae	*Paraburkolderia sabiae*	MK139731.1	99.80	N-fixing
		Pseudomonadaceae	*Pseudomonas koreensis*	ON428965.1	100	N-fixing P solubilization
		Burkholderiaceae	*Burkholderia sp.*	MK612762.1	100	N-fixing P solubilizing
		Chromobacteriaceae	*Chromobacterium piscinae*	LR634122.1	99.90	N-cycling
		Pseudomonadaceae	*Pseudomonas nitroreducens*	KY038284.1	99.60	N-cycling
	Post-harvest	*Burkholderiaceae*	*Burkholderia diffusa*	MG270179.1	100	N-cycling
		Burkholderiaceae	*Burkholderia ambifaria*	MN509179.1	100	N-cycling
		Burkholderiaceae	*Caballeronia sp.*	OR363922.1	88.82	N-fixing N-cycling

Gingindlovu	Pre-planting	*Pseudomonadaceae*	*Pseudomonas frederiksbergensis*	MT378522.1	99.80	N-fixing N-cycling
		Bacillaceae	*Priestia megaterium*	CP127877.1	100	N-fixing
	Post-harvest	*Pseudomonadaceae*	*Pseudomonas sp.*	MT354507.1	99.85	N-fixing P solubilizing
		Paenibacillaceae	*Paenibacillus sp.*	EU571197.1	99.69	N-fixing
Umzinto	Pre-planting	*Micrococcaceae*	*Arthrobacter sp.*	MN081011.1	100	N-fixing P solubilizing
		Pseudomonadaceae	*Pseudomonas sp*	MT354507.1	99.90	N-fixing P solubilizing
	Post-harvest	*Pseudomonadaceae*	*Pseudomonas umsongensis*	MT628350.1	100	N-cycling
		Pseudomonadaceae	*Pseudomonas sp.*	MT354507.1	100	N-fixing P solubilizing
		Rhizobiaceae	*Rhizobiaceae bacterium*	MT354281.1	99.54	N-fixing
Hibberdene	Pre-planting	*Pseudomonadaceae*	*Pseudomonas sp.*	MT354507.1	99.30	N-fixing P solubilizing
		Pseudomonadaceae	*Pseudomonas poae*	OR122193.1	99.90	N-fixing P solubilizing

	Post-harvest	*Pseudomonadaceae*	*Pseudomonas sp.*	MT354507.1	100	N-fixing P solubilizing
		Pseudomonadaceae	*Pseudomonas frederiksbergensis*	MT378522.1	100	N-fixing N-cycling
Empembeni	Pre-planting	*Burkholderiaceae*	*Burkholderia cepacia*	MN691121.1	99.40	P solubilisation
		Burkholderiaceae	*Burkholderia sp.*	KT390908.1	98.30	N-fixing P solubilizing
		Pseudomonadaceae	*Pseudomonas protegens*	MT505104.1	100	P solubilizing
		Burkholderiaceae	*Burkholderia sp.*	MT001454.1	99.40	P solubilizing
	Post-harvest	*Pseudomonadaceae*	*Pseudomonas koreensis*	MT629852.1	100	N-fixing N-cycling
		Pseudomonadaceae	*Paraburkholderia caledonica*	MN595030.1	99.54	P solubilizing
		Burkholderiaceae	*Burkholderia diffusa*	MG270179.1	100	N-cycling
		Pseudomonadaceae	*Pseudomonas sp.*	MH883997.1	99.54	N-fixing
		Pseudomonadaceae	*Pseudomonas sp.*	FJ786058.1	97.85	N-fixing

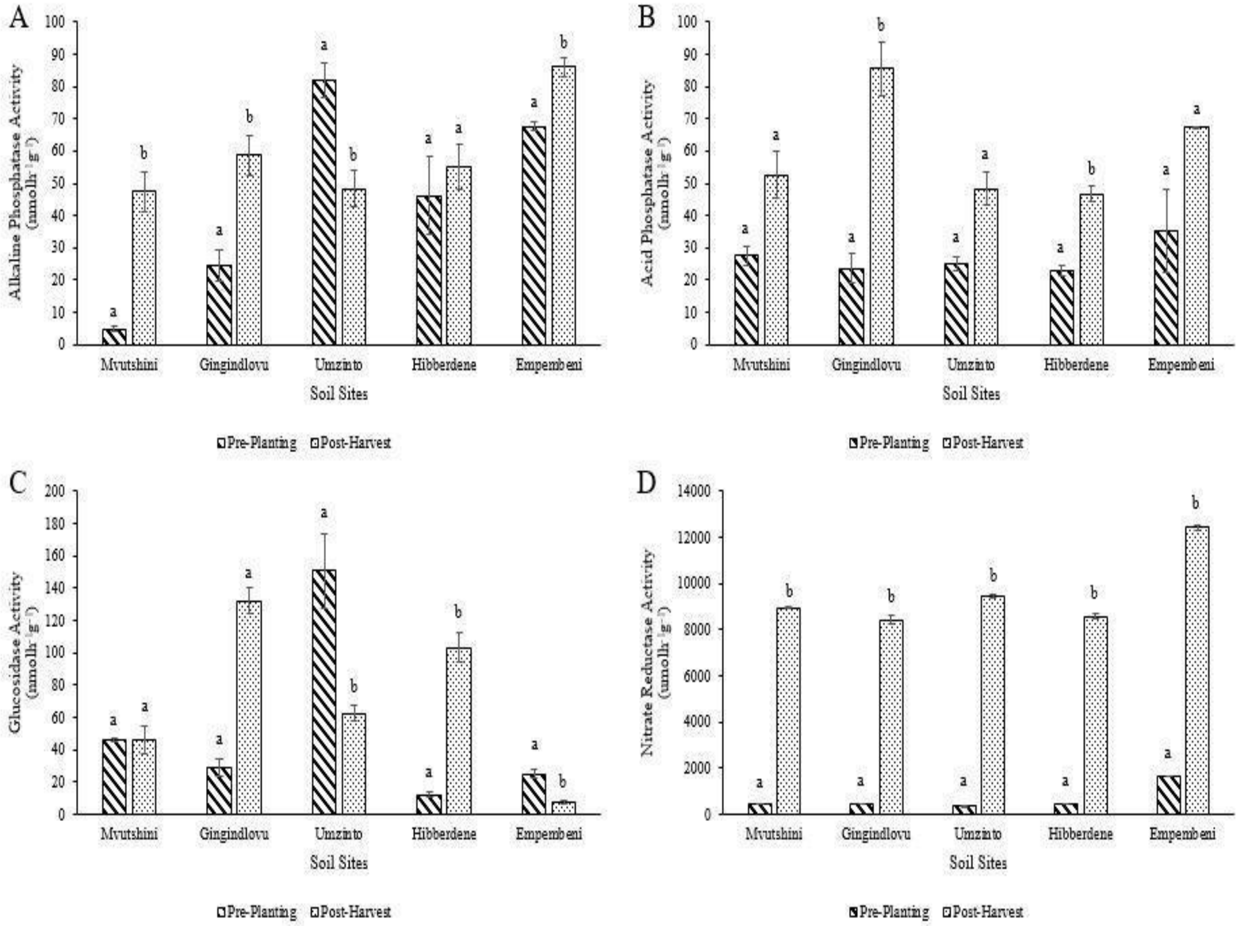

Figure 3.1: Soil enzyme activities of soils collected from Mvutshini, Gingindlovu, Umzinto, Hibberdene, and Empembeni pre-planting and post-harvest of *M. pruriens*: A. Alkaline phosphatase activity, B. Acid phosphatase activity, C. Glucosidase activity, D. Nitrate reductase activity. Different values represent the mean±SE, with significant differences ($p < 0.05$) within each treatment denoted by different letters.

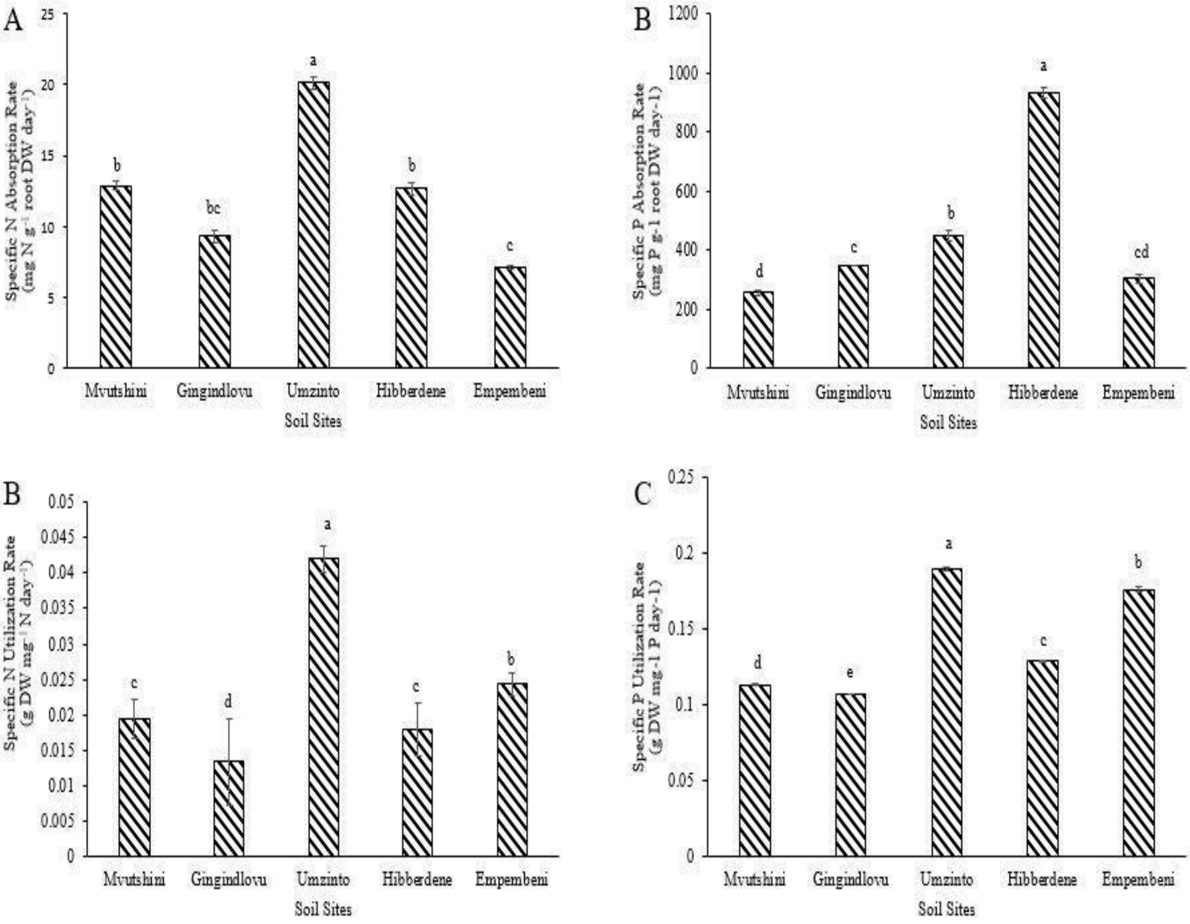

Figure 3.2: A. Specific N absorption rate, B. Specific P absorption rate, C. Specific N utilization rate, and D. Specific P utilization rate of *M. pruriens* plants grown in soils collected from Mvutshini, Gingindlovu, Umzinto, Hibberdene, and Empembeni for 54 days. Different values represent the mean±SE, with significant differences ($p < 0.05$) within each treatment denoted by different letters.

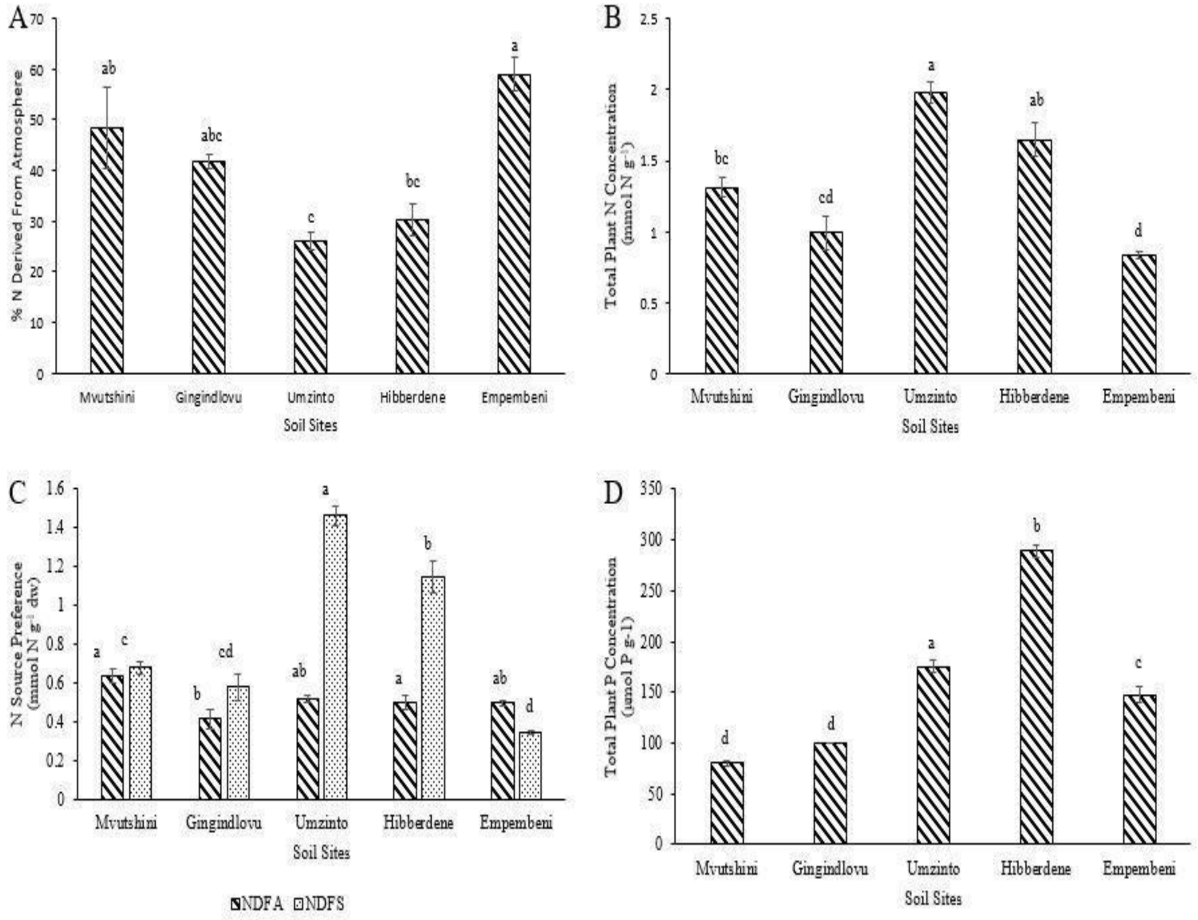

Figure 3.3: A. Percentage N derived from the atmosphere, B. Total plant N concentration, C. N source preference, and D. Total plant P concentration of *M. pruriens* plants grown in soils collected from Mvutshini, Gingindlovu, Umzinto, Hibberdene, and Empembeni for 54 days. Different values represent the mean±SE, with significant differences ($p < 0.05$) within each treatment denoted by different letters.

Table 3.3: Growth kinetics of *M. pruriens* grown in soils collected from Mvutshini, Gingindlovu, Umzinto, Hibberdene, and Empembeni for 54 days. Different values represent the mean±SE, with significant differences ($p < 0.05$) within each treatment denoted by different letters.

	Mvutshini	Gingindlovu	Umzinto	Hibberdene	Empembeni
Root RGR (g/day)	0.04±0.00^{b}	0.02±0.0^{c}	0.04±0.01^{b}	0.06±0.00^{a}	0.04±0.00^{b}
Shoot RGR (g/day)	0.03±0.00^{c}	0.04±0.00^{bc}	0.05±0.00^{ab}	0.06±0.00^{a}	0.04±0.00^{c}
RGR (g/day)	0.04±0.00^{c}	0.04±0.00^{bc}	0.05±0.00^{ab}	0.06±0.01^{a}	0.04±0.00^{bc}
Root: Shoot ratio (g/day)	0.17±0.02^{ab}	0.17±0.04^{ab}	0.15±0.01^{b}	0.26±0.02^{a}	0.19±0.02^{ab}

Chapter 4

Plant-associated bacteria and extracellular enzyme activity support the growth of *Arachis hypogaea* L. in nutrient-deficient small-scale sugarcane soils

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Abstract

Sugarcane monoculture coupled with fertilizers by small-scale growers in KwaZulu-Natal has led to poor soil health, decreased productivity and yield. The inclusion of legume cover crops during the bare fallow period represents a sustainable alternative that can increase sugarcane yield and reduce overreliance on fertilizers. This study investigated the effects of on soil chemical parameters, soil bacterial communities, and their associated enzyme activities in soils collected from five different sugarcane plantations in KwaZulu-Natal, South Africa. Post-harvesting, soil pH increased across all study sites while N and P concentrations decreased. Umzinto soils had the highest nitrate reductase and β-glucosidase activity and Umzinto-grown *A. hypogaea* accumulated the highest total plant N and P concentration. Acid phosphatase activity was the highest in Mvutshini while alkaline phosphatase activity was highest in Gingindlovu soils. There was an increase in bacterial diversity in Gingindlovu, Umzinto, Hibberdene, and Empembeni with the isolated strains belonging in the *Achromobacter*, *Azospirillum*, *Burkholderia*, *Caballeronia Chromobacterium*, *Pseudomonas*, *Paenibacillus*, *Rhizobium*, and *Sphingomonas* genera. Overall, these results show that plant-associated bacteria with N-fixing, N-cycling, and P solubilizing functions and their associated enzyme activities are key to the nutrient mineralisation that allows *A. hypogaea* to thrive in nutrient-deficient sugarcane soils.

Keywords: Crop rotation, groundnut, *Arachis hypogaea*, cover crop, nitrogen fixation

4.1 Introduction

Sugarcane farming is a key component of South Africa's agricultural sector, with KwaZulu-Natal (KZN) and Mpumalanga producing the majority of the country's total sugarcane production (SASA, 2024). However, the industry's small-scale growers have been facing productivity and yield declines due to degraded soils caused by long-term monoculture and chemical fertiliser overuse. According to Dubb. (2016), there has been a decline in small-scale sugarcane growers in recent decades from a peak of 50 000 in the early 2000s to 25 000 in 2024 (SASA, 2024). Small-scale sugarcane growers produce 20% of the total sugarcane milled in South Africa and depend on sugarcane revenue for their livelihoods (Dubb, 2016). As such, the declines in soil fertility and yield underscore the need to develop sustainable agricultural practices to enhance crop productivity and improve soil health (Mueller et al., 2012). Cover cropping has emerged as a practice that can synergistically boost crop production and soil health while reducing reliance on chemical fertilisers (Nouri et al., 2019; Jian et al., 2020).

Cover cropping has been reported to provide benefits such as soil erosion control, increased soil organic C and N retention, improved biodiversity, control of pests and weeds, and enhanced soil structure (Kocira et al., 2020; Quintarelli et al., 2022). Incorporating cover crops into the soil allows for the release of essential nutrients including N, P, and K, revitalizing the soil (da Silva et al., 2022). Leguminous cover crops play a key role in nutrient cycling and enhancing soil N availability for the following cash crop through biological N fixation (McCauley et al., 2012). According to Blanco-Canqui et al. (2011), soil N levels in a wheat rotation system increased by 258 kg N ha^{-1} and 279 kg N ha^{-1} under soybean and sunn hemp cover cropping, respectively. Alfalfa has been shown to increase the total availability of N and P concentrations in the soil (Gao et al., 2015; Yang et al., 2023). Cover crop rotation reinforces soil health by promoting the persistence of beneficial plant growth-promoting bacteria, and concurrently reduces the presence of detrimental microorganisms (Wang et al., 2022; LeBlanc, 2023).

Soil microorganisms are considered an important indicator of soil health, and play a crucial role in global nutrient cycling (Crecchio et al., 2004; Kanté et al., 2021). These microorganisms exude extracellular enzymes that enhance soil fertility by catalysing biochemical processes that decompose crop residues, nutrient cycling, and inorganic release of nutrients for plant growth (Henry 2013; Zuccarini et al., 2023). Microbial activities and their associated enzymes are shaped by soil properties and abiotic factors (Motsomane et al., 2023). As such, soil health can be considered a result of the interaction between soil properties, microbial and enzyme activity (Makoi and Ndakidemi, 2008; Das and Varma, 2011; Kotroczo et al., 2014). Plants can influence the composition of soil microorganisms by secreting root exudates that form the basis for plant-microbe host specificity based on the plants' requirements (Kumar et al., 2007). Legume cover crops have been shown to alter soil pH, modulating soil biological and enzymatic activities (Balota and Chaves, 2011; Zhou et al., 2011; Wang et al., 2021). A study by Qin et al. (2017) showed that legume cover crops increased soil microbial populations, soil enzyme activities, and tuber yield in a potato cropping system.

Groundnut (*Arachis hypogaea* L.) is an agro-economically important oilseed crop that contributes significantly to the nutrition of subsistence farmers in KZN and can be utilised in cropping systems to maximise N input into the soil (Ncube et al., 2010; Mokgehle et al., 2014; Ojiem et al., 2014). According to Witcombe and Tiemann. (2022), *A. hypogaea* residues can contribute up to 139 kg N ha^{-1} to cropping systems and improve soil fertility through the provision of multiple ecosystem services. Despite the importance of N fertilization to sugarcane production (van Heerden et al., 2010), *A. hypogaea* remains an underutilized rotation crop in sugarcane plantations. Furthermore, the influence of *A. hypogaea* on soil bacterial communities, soil extracellular enzyme activities, and soil nutrition on KZN sugarcane plantation soils remains unknown. Thus, this study aims to investigate the biological and chemical contributions of *A. hypogaea* growing in acidic and nutrient-poor sugarcane plantation soils. Understanding the growth physiology of *A. hypogaea* in these sugarcane soils will aid in determining its suitability as a cover crop and govern its biomass management post-harvesting. The objectives of this study include 1) Determining the soil chemical characteristics of the five different sugarcane soils pre-planting and post-harvesting *A. hypogaea* 2) Identifying the nutrient cycling bacteria present in these soils pre-planting and post-harvesting *A. hypogaea* 3) Quantifying the N, P and C cycling enzymes pre-planting and post-harvesting *A. hypogaea*.

4.2 Materials and Methods

4.2.1 Soil Collection

Soil samples were collected from five small-scale sugarcane plantations located at different geographical locations in KZN, South Africa. Northern KZN included three sites Mvutshini (28° 50' 52.9" S 32° 00' 09.0" E), Gingindlovu (28° 56' 29.3" S 31° 34' 58.9" E), and

Empembeni (28° 51’ 25.7” S 31° 58’ 19.4” E). The remaining two sites were from southern KZN, which included Umzinto (30°16’ 41.03” S, 30° 39’ 47.97” E) and Hibberdene (30° 35’ 37.12” S, 30° 31’ 30.29” E). From each site, experimental soil was collected from 10 random points at a depth of 0-30 cm, maintaining a distance of 2 m apart. Subsequently, the ten soil samples were homogenised to create a composite sample for a more accurate representation of the soil microbial community composition. Pre-planting soil samples for microbial identification and enzymatic assays were placed in sterile zip-lock bags, stored on ice in the field, and then kept at 4°C until further analysis.

4.2.2 Plant growth trials

Seeds of *A. hypogea* were sourced from AGT Foods Africa, Marji Mizuri farm, KZN. The seeds were soaked in warm water overnight to initiate seed germination. Thereafter, the seeds were transferred into petri dishes containing moist, sterile filter paper and kept in the dark for four days at room temperature. After germination, the seeds were transferred into 10 cm diameter pots filled with soils from the different plantations. Each plantation had 10 labelled pots in a completely randomized design, resulting in 50 experimental pots. The growth trials were conducted in greenhouse conditions at the University of KwaZulu Natal, Westville campus, School of Life Science Building, South Africa. Average day and night temperatures ranged from 30-35 °C and 11-15 °C, respectively. Two sets of harvests were conducted at 15- and 54 days post-seedling emergence. Five plants per plantation (initial and final harvests) were rinsed with distilled H_2O and then separated into leaves, stems, and roots, and dried at 65 ^{o}C until constant weight was maintained. The dried plant material was weighed, ground into powder and sent for C, N, and P analysis. $\delta^{15}N$ isotope analysis was conducted using a LECO-N analyzer (LECO Corporation, St. Joseph, MI, USA) at the Archaeometry Department, University of Cape Town, South Africa, while C and P concentrations were measured using inductively coupled mass spectrometry (ICP-MS) at the Central Analytical Facilities, Stellenbosch University, South Africa.

4.2.3 Soil nutrition analysis

Post-harvest soils were homogenised to create a composite sample and a portion of the soil was stored in the fridge at 4 °C for bacterial extraction and identification and enzyme activities. The remaining soils were air-dried, milled, weighed at 1 kg per sample, and sent to the Fertilizer Advisory Service (FAS) at the South African Sugarcane Research Institute (SASRI), South Africa for full nutrient, pH, total cation, and exchangeable acidity analysis.

4.2.4 Soil Bacterial Extraction and Identification

A three-fold serial dilution was conducted for 10 g of soil samples from each plantation. Briefly, each 10 g soil sample was diluted in 100 ml of autoclaved distilled H_2O and mixed thoroughly. The resulting dilutions were then transferred to selective media plates by inoculating each plate with 100 µl of the dilution. Tricalcium phosphate plates were used for P solubilizing bacteria, while Simmon's citrate and Jensen plates were used for N-cycling and N-fixing bacteria, respectively. The inoculated plates were incubated at 30 °C for 4 to 7 days. After incubation, pure colonies were obtained by repeated streaking into clean plates.

Pure bacterial colonies were amplified using a portion of the 16S rRNA gene primers: 63F (5′-CAG GCCTAACACATGCAAGTC-3′) and 1387R (5′-GGGCGGTGTGTACAA GGC-3′). The PCR reaction mixture (25 µl) consisted of; 12.5 µl EmeraldAmpGT Master mix (Separations, South Africa), 8.5 µl milliQ distilled H_2O, 0.5 µl forward primer, 0.5 µl reverse primer, and 3 µl diluted pure colony. The PCR amplification was carried out as follows: initial denaturation at 94 °C for 5 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and final elongation at 72 °C for 10 min using a T1000 Thermal Cycler (Biorad Laboratories Inc., USA). Thereafter, the resulting PCR products were resolved on a 1% (w/v) agarose gel (Seakem, Lonza, USA) stained with SafeView™ Classic (Applied Biological Materials Inc., Canada) and viewed in a ChemiDoc (Bio-Rad Laboratories Inc., USA). Positive amplicons were sent to Inqaba Biotech Inc, Pretoria, South Africa. The resultant chromatograms were cleaned and subjected to BLASTN using the National Center for Biotechnology Information database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (accessed 28/04/2024)

4.2.5 Extracellular Enzyme Activity

The enzyme activities of β-glucosidase, acid phosphatase, and alkaline phosphatase were assayed using a fluorescence-based method adapted from (Jackson et al., 2013). Briefly, 10 g of soil samples (3 soil samples per plantation) were homogenized at low speed in 100 ml dH_2O for 2 h and stored at 4 °C overnight. Thereafter, the resulting supernatants were transferred into 96-well black microplates before adding their respective 4-methylumbeliferyl (MUB)-linked substrates. The sample run consisted of 200 µl soil aliquot and 50 µl substrate, incubated alongside reference standards (200 µl buffer + 50 µl standard), quench standard (200 µl soil aliquot + 50 µl standard), sample control (200 µl soil aliquot + 50 µl buffer), negative controls (200 µl buffer + 50 µl substrate) and blanks (250 µl buffer). Following a 1 h incubation period at 30 °C, the reaction was stopped using 0,5 M NaOH. The fluorescence was measured at 450 nm using a Glomax Multi Plus microplate reader (BioTek, USA) and the activity was expressed as nmol h^{-1} g^{-1}. The buffer and standard were adjusted to pH 5 before determining acid phosphatase activity.

Nitrate reductase activity was determined according to a modified protocol described by (Bruckner et al., 1995). Briefly, 5 g of soil (3 soil samples per plantation) was added to a light-sealed volumetric flask containing a solution of 1 ml of 25 mM KNO_3, 4 ml of 0,9 mM 2,4-dinitrophenol and 5 ml of milliQ distilled H_2O. Thereafter, the mixture was thoroughly mixed before being incubated in the dark at 30 °C for 24 h. Following incubation, 10 ml of 4 M KCl was added to each sample, mixed thoroughly and the filtered using Whatman number 1 filter paper. The enzymatic reaction was initiated by adding 2 ml of the filtrate to 0.19 M of ammonium chloride (pH 8.5) and 0.8 ml of the colour reagent (1% sulphanilamide, 1 N HCl and 0.2% N-(1-naphthyl) ethylenediamine dihydrochloride (NEDD). The solution was then incubated at 30 °C for 30 min. The absorbance was measured at 520 nm using an Agilent Cary 60UV-Vis spectrophotometer (Agilent, Santa Clara, CA, USA). The nitrite released into the medium was extrapolated from a prepared KNO_3 standard curve and the enzyme activity was expressed as 0.1 μmol h^{-1} g^{-1}.

4.2.6 Growth rate calculations

4.2.6.1 Specific N/P absorption rate (SNAR/SPAR)

Total plant N/P was used to calculate the specific N/P absorption rate according to (Nielsen et al., 2001).

SNAR = (N2 – N1 / t2 – t1)*[(loge R2 – loge R1) / (R2 – R1)]

SPAR = (P2-P1/t2-t1)*[(logeR2-logeR1)/ (R2-R1)]

Where N, P, t and R represent total N content, total P content, duration of plant growth and root dry weight, respectively.

4.2.6.2 Specific N/P utilization rate (SNUR/SPUR)

Total plant N/P was used to calculate the specific N/P utilization rate according to (Nielsen et al., 2001).

SNUR = (W2 – W1 / t2 –t1)*[(loge N2 – loge N1) / (N2 – N1)]

SPUR = (W2 – W1 / t2 –t1)*[(loge M2 – loge M1) / (M2 – M1)]

Where W, N, M and t represent the plant DW, total N content, total P content and the duration of plant growth, respectively.

4.2.6.3 Relative growth rate (RGR)

The relative growth rate was calculated using the (ÅGREN and Franklin 2003) method.

RGR = [(ln W2 – W1)/t2 – t1]

Where W represents the plant DW accumulated from the initial (W_1) to final (W_2) and t is the time for plant growth.

4.2.6.4 Root: shoot ratio

The root: shoot ratio was calculated using the (ÅGREN and Franklin 2003) method.

Root: shoot= D_R/D_S

Where D_R is the root dry weight while D_S is the shoot dry weight.

4.2.6.5 Carbon construction cost (Cw)

Carbon construction cost was calculated using a formula modified by (Peng et al., 1993) and further verified by (Mortimer et al., 2005).

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

Where Cw is the total carbon construction cost in the tissues (mmol C g^{-1} DW), C is the total carbon concentration (mmol C g^{-1}), k is the reduced state of the N substrate (i.e. NH_3 = -3) and N is the total organic nitrogen content of the tissue (Williams et al., 1987).

The number 14 is the atomic mass of nitrogen, 180 is a conversion factor from mol to g of glucose. The number 24 represents the number of electrons in a glucose molecule; while 0.89 is an estimation of growth efficiency (Williams et al., 1987), and (6000/180) is a conversion factor for glucose that converts g dw^{-1} to mmol C g^{-1} dw.

4.2.6.6 Percentage N derived from the atmosphere (%NDFA)

The N isotope analyses was conducted at the University of Cape Town Archaeometry Department. The isotopic ratio of N was calculated as δ= 1000 (Rsample/ Rstandard), where R is the molar ratio of ^{15}N and ^{14}N of the samples and standards. Between 2.100 and 2.200 mg of each milled sample were weighed into 8 mm x 5 mm tin capsules (Elemental Micro-analysis, Devon, UK) on a Sartorius microbalance (Goettingen, Germany). The samples were then combusted in a Fisons NA 1500 (Series 2) CHN analyzer (Fisons Instruments SpA, Milan, Italy). The N isotope values for the N gas released were determined on a Finnigan Matt 252 mass spectrometer (Finnigan MAT GmbH, Bremen, Germany), which was connected to a CHN analyzer 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 $(NH_4)_2SO_4$.

Percent of N derived from the atmosphere was calculated according to (Shearer and Kohl 1986);

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

Where NFDA is the N derived from the atmosphere and β is the $\delta^{15}N$ natural abundance of N derived exclusively from the biological N fixation of *Pisum sativum* grown in N-free culture. The β value of *M. pruriens* for this study was -2.58%.

4.2.7 Statistical Analysis

Statistical analyses were conducted using RStudio version 4.3.2 (R Core Team, 2024). A paired samples T-test was conducted to compare the pre-planting and post-harvesting means of soil nutrients and enzyme activities. Data on growth kinetics, plant biomass, and plant mineral nutrition were analysed using a one-way analysis of variance (ANOVA). Where significant differences were detected ($p < 0.05$), mean values were further separated using Tukey’s multiple comparisons post hoc test. Assumptions of normality and homogeneity of variances were assessed with the Shapiro-Wilk and Levene’s tests, respectively.

4.3 Results

4.3.1 Soil Nutrient Analysis

Soil N concentrations post-harvesting were insignificantly lower than pre-planting soils across all study sites (Table 4.1). Contrastingly, soil P and K concentrations were significantly lower across all study sites post harvesting (Table 4.1). Calcium and magnesium showed significant increases in Mvutshini, Gingindlovu, Hibberdene and Empembeni, while a non-significant decrease was noted in Umzinto soils. The soils from all the sites remained acidic with a pH ranging from 5.02-6.21, with Gingindlovu, Umzinto, and Hibberdene increasing significantly, while Mvutshini and Empembeni increased non-significantly (Table 4.1). Exchangeable acidity and acid saturation showed significant decreases in Gingindlovu, Empembeni and Hibberdene soils (Table 4.1). The total cations showed slightly significant increases in Mvutshini, Empembeni and Hibberdene soils post harvesting (Table 4.1).

4.3.2 Bacterial identification

The molecular identification of N-fixing, P solubilizing and N-cycling bacteria in pre-planting and post-harvesting soils revealed a total of 16 and 19 bacterial strains, respectively. In Mvutshini, pre-planting soils were enriched with six bacterial strains that included two N-fixing bacteria (*Paraburkholderia phymatum* and *Paraburkholderia sabiae*), two N-fixing and P solubilizing (*Pseudomonas koreensis* and *Burkholderia sp.*), and two N-cycling bacteria (*Chromobacterium piscinae* and *Pseudomonas nitroreducens*). Post-harvesting, Mvutshini soils had two N-cycling bacterial strains (*Pseudomonas umsongensis* and *Achromobacter sp.*). Gingindlovu pre-planting soils had two bacterial strains, *Pseudomonas frederiksbergensis* (N-fixing and N-cycling) and *Priestia megaterium* (N-fixing), whereas post-harvesting soils were composed of N-fixing bacteria from the *Pseudomonadaceae* and *Paenibacillaceae* families. Pre-planting soils in Umzinto had two N-fixing and P solubilizing bacterial strains (Achromobacter sp. and *Pseudomonas* sp.). Post harvesting, Umzinto soils were completely enriched in *Pseudomonas sp.* that were N-fixing and P solubilizing (*Pseudomonas sp.*, and *Pseudomonas vancouverensis*) and *Pseudomonas umsongensis* involved in N-cycling. Pre-planting, Empembeni soils had four bacterial: three P solubilizing strains (*Burkholderia cepacia*, *Pseudomonas protegens*, and *Burkholderia cenocepacia*) and an N-fixing and P solubilizing strain (*Burkholderia sp.*). Post-harvesting, Empembeni soils had four strains consisting of *Caballeronia sp.* (N-fixing and N-cycling, *Caballeronia calidae* (N-cycling and P solubilisation), *Pseudomonas sp.* (N-cycling), and *Azospirillum rugosum* (N-fixing and P solubilizing). In Hibberdene pre-planting soils, there were two N-fixing and P solubilizing bacterial strains (*Pseudomonas sp.* and *Pseudomonas poae*), while post-harvesting soils had four strains; *Burkholderia ambifaria* (N-cycling and P solubilisation), *Rhizobium mesosinicum* (N-fixing), *Pseudomonas frederiksbergensis* (N-fixing and N-cycling) and *Sphingomonas sp.* (N-fixing).

4.3.3 Soil extracellular enzyme activities

Alkaline phosphatase activity increased significantly in all sites with the exception of Hibberdene soils (Figure 4.1A). Mvutshini, Gingindlovu, and Umzinto had significant increases in acid phosphatase activity (Figure 4.1B), while glucosidase activity increased significantly in Gingindlovu, Empembeni and Hibberdene soils (Figure 4.1C). Nitrate reductase activity was significantly higher across all the plantation soils (Figure 4.1D).

4.3.4 Plant nutrition and growth kinetics

Arachis hypogaea grown in Umzinto soils had the highest total plant biomass, while Mvutshini soils had the lowest total dry weight (Table 4.3). Similar trends were observed in root and shoot biomass, however, *A. hypogaea* cultivated in Empembeni soils had the lowest shoot biomass (Table 4.3). Plants grown in Gingindlovu soils had the highest specific nitrogen absorption rate (SNAR), followed by plants grown in Umzinto, whereas plants grown in Mvutshini soils had the lowest SNAR (Figure 4.2A). Umzinto had a slightly higher N utilization rate, followed by Hibberdene, while Mvusthini distinctively had the lowest N utilization rate (Figure 4.2C). The specific P absorption rate (SPAR) was slightly higher in Hibberdene soils, followed by Umzinto, while Mvutshini had the lowest P absorption rate (Figure 4.2B). Umzinto and Hibberdene had the highest specific P utilization rates (SPUR), whereas Mvutshini had the lowest SPUR (Figure 4.2D). Plants grown in Umzinto and Hibberdene relied on mineral N derived from the soil, while Mvutshini, Gingindlovu, and Empembeni plants obtained most of their N from the atmosphere (Figure 4.3C). Total plant N concentration was the highest in Umzinto, and lowest in Mvutshini (Figure 4.3B). Total plant P was significantly higher in Hibberdene-grown plants (Figure 4.3D).

4.4 Discussion

Soil microbes and their associated extracellular enzyme activities play an important role in nutrient mineralization and are likely responsible for the growth of *A. hypogaea* in these acidic and nutrient-deficient sugarcane plantation soils. In nutrient-deficient soils, plants produce root exudates that shape the function and composition of rhizosphere bacteria to enhance nutrient availability (Hinsinger et al., 2009). These root exudates act as an energy source for metabolically dormant or inactive rhizobacteria, leading to increased bacterial diversity (Baudoin et al., 2005; van Vliet., 2015). In the present study, the experimental soils were N and P deficient, which may prompted the proliferation of the identified N-cycling, N-fixing and P-solubilizing bacteria in the genus *Achromobacter*, *Azospirillum*, *Burkholderia*, *Caballeronia Chromobacterium*, *Pseudomonas*, *Paenibacillus*, *Rhizobium*, and *Sphingomonas.* The abovementioned findings coincide with Mula-Michel et al. (2023), who reported that soybean cover cropping increased bacterial diversity compared to sugarcane monoculture, which was attributed to the introduction of new types root exudates and rhizodeposits into the soil. The introduction of new plant species constitutes a breakage in long-term monoculture systems and drives changes in microbial community diversity and composition (Maul and Drinkwater, 2010). Therefore, the increased bacterial diversity supports the notion that *A. hypogaea* had a significant impact on the bacterial community composition.

The cultivation of *A. hypogaea* also influenced the soil pH by reducing the exchangeable acidity across all study sites. These results are in tandem with the findings of Balota and Chaves. (2011) and Gao et al. (2022), who observed increases in soil pH following cover crop treatments. Soil acidity is associated with impaired plant development and poor nutrient availability (Kopittke et al., 2015; van Antwerpen et al., 2023). Under acidic conditions, P forms insoluble complexes with Aluminium (Al) and Iron (Fe), making it unavailable for plant assimilation (Adnan et al., 2003). Moreover, P is required for effective biological N fixation, and its deficiency can decrease N fixation rates in legumes (Magadlela et al., 2016). It is surprising that *A. hypogaea* grown in Mvutshini, Gingindlovu, and Empembeni soils relied more on NDFA, considering the deficiency of P in these soils. Increased acid and alkaline phosphatase enzyme activities across these soil sites might have assisted with the P demands of BNF, since phosphatase enzymes are involved in the mineralization of organic P compounds. This concurs with Liu et al. (2004), Makoi et al. (2010), and Maseko and Dakora. (2013) who observed higher phosphatase enzyme activity in legumes compared to non-legumes, which is often attributed to the high P demand of BNF legumes (Makoi and Ndakidemi, 2008).

Despite the relatively high P concentrations in Hibberdene and Umzinto pre-planting soils, *A. hypogaea* growing in these soils showed decreased %NDFA, and relied more on soil N. Legumes switch N sources under nutrient-deficient conditions to reserve energy (Sithole et al., 2021). Biological N fixation (BNF) is an energetically costly process, requiring 16 molecules of adenosine triphosphate (ATP) to fix one molecule of N_2 into NH_3 (Vance et al., 2003). The increased reliance on soil N likely reduced carbon costs since less energy is required to obtain soil N (Valentine et al., 2010). As a result, the available P was likely used to maintain growth and functioning, which explains the high total plant biomass recorded in these sites. Furthermore, *A. hypogaea* grown in Hibberdene and Umzinto had the highest total plant N concentration, which could be due to the legume utilizing both sources of N. The high nitrate reductase activity recorded in Umzinto and Hibberdene soils post-harvesting likely increased N cycling in these sites, further justifying the reliance on soil N.

High total plant P corresponded with high SPAR, and this was generally highest in Hibberdene- and Umzinto-grown *A. hypogaea*, which could indicate high P solubilizing efficiency from the strains identified in these sites. Phosphate solubilizing bacteria contribute to P cycling by releasing organic acids, phosphatases, phytases, mineralization, chelation, and lowering soil pH (Kalayu 2019; Xiao et al., 2020). Bacterial species from the *Burkholderia* and *Pseudomonas* genera have been reported to be efficient at P solubilization (Rodriguez and Fraga, 1999; Saeid et al., 2018). Our results are consistent with previous studies that have reported increased P content in wheat, following inoculation with P solubilizing bacteria (Turan et al., 2012; Liu et al., 2019; Elhaissoufi et al., 2020; Li et al., 2023). In addition, the increased root biomass plausibly contributed to improved P acquisition leading to increased P assimilation rates and total plant P concentration. Root system architecture modification is an important survival strategy for legumes under nutrient-deficient conditions (Vance et al., 2003, Vardien et al., 2016), since high belowground biomass provides a larger surface area and increases contact with bacteria for nutrient uptake (Ndzwanana et al., 2019). A study by Magadlela et al. (2014) noted increased root biomass in *Virgilia oroboides* growing in P-deficient soils, thus demonstrating that legumes use root architecture as a strategy to scavenge for P.

Post-harvesting, N and P concentrations decreased across all study sites, and this can be linked to *A. hypogaea* utilizing these nutrients for growth and functioning (Weerasekara et al., 2017). Similar findings were reported by Zhou et al. (2011) and Gao et al. (2017), who observed decreases in soil nutrient concentrations following cover crop treatments. Legumes have a low C/N ratio, which results in faster decomposition and mineralisation of stored nutrients (Li et al., 2016). Therefore, the nutrients assimilated by *A. hypogaea* during growth can be made available to the subsequent sugarcane crop by incorporating the legume biomass into the soil as green manure. Groundnut residues have been reported to contribute up to 139 kg N ha^{-1} (Ojiem et al., 2014), minimizing reliance on chemical fertilizers for resource constrained smallholder farmers (Mokgehle et al., 2014). Moreover, groundnut residues can improve long term N and soil fertility by facilitating microbial biomass production, nutrient cycling and soil organic matter productivity (Witcombe and Tiemann, 2022).

4.5 Conclusion

This study demonstrates that *A. hypogaea* contributes to soil health improvement by reducing soil acidity, promoting beneficial plant growth promoting bacteria and their associated enzyme activities in different sugarcane plantation soils. Furthermore, the survival of *A. hypogaea* in these nutrient-deficient soils highlights the role played by bacterial communities and their associated enzyme activities in nutrient mineralization, accumulation, and subsequent plant growth. However, cultivating *A. hypogaea* for longer than 2 months could provide more insight into how these microbial associations and enzyme activities govern nutrient mineralization and support the growth of *A. hypogaea* in these soils.

Author contributions

A.M conceived the research idea; S.K performed the growth trials, data analysis and draft manuscript; A.M conducted the review, editing and supervision.

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Conflict of interest

We declare no known conflict of interest.

Data availability

The data can be made available upon request.

4.6 References

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Tables and figures

Table 4.1: Soil nutrient concentrations (N: Nitrogen, P: Phosphorus, K: Potassium, Ca: Calcium, and Mg: Magnesium) and relative acidity (pH, Exchangeable acidity, Total cations, and Acid saturation) in soils collected from Mvutshini, Gingindlovu, Umzinto, Hibberdene, and Empembeni pre- and post-*A. hypogaea* planting. Different values represent the mean±SE, with significant differences ($p < 0.05$) within each treatment denoted by different letters.

	Soil Sites					
		Mvutshini	Gingindlovu	Umzinto	Hibberdene	Empembeni
N (ppm)	Pre	0.09±0.00^{a}	0.06±0.00^{a}	0.07±0.00^{a}	0.06±0.00^{a}	0.06±0.00^{a}
	Post	0.06±0.00^{a}	0.04±0.00^{a}	0.04±0.00^{a}	0.03±0.00^{a}	0.05±0.00^{a}
P (mg/L)	Pre	4.29±0.14^{a}	3.81±0.05^{a}	10.69±0.23^{a}	9.41±0.05^{a}	3.41±0.09^{a}
	Post	2.53±0.14^{b}	2.69±0.32^{a}	7.41±0.46^{b}	6.77±0.00^{b}	2.69±0.05^{b}
K (mg/L)	Pre	33.05±0.19^{a}	55.17±1.79^{a}	61.01±1.78^{a}	100.56±2.47^{a}	18.43±0.36^{a}
	Post	16.06±1.30^{b}	44.67±0.39^{b}	34.64±3.33^{b}	36.31±0.61^{b}	11.57±1.48^{b}
Ca (mg/L)	Pre	238.78±4.45^{a}	219.92±2.2^{a}	717.85±18.0^{a}	531.38±6.80^{a}	143.87±6.1^{a}
	Post	319.48±11.6^{b}	292.70±4.5^{b}	663.24±12.5^{a}	553.51±6.84^{b}	236.12±2.7^{b}
Mg (mg/L)	Pre	78.79±2.37^{a}	132.49±2.1^{a}	166.47±7.42^{a}	141.83±1.39^{a}	39.19±0.12^{a}
	Post	86.89±6.95^{a}	141.69±3.2^{b}	141.66±3.04^{a}	152.65±0.16^{b}	73.72±1.30^{b}
pH (CaCl)	Pre	4.67±0.02^{a}	4.80±0.03^{a}	4.88±0.19^{a}	5.13±0.03^{a}	4.94±0.17^{a}
	Post	5.02±0.28^{a}	5.62±0.03^{b}	6.21±0.02^{b}	5.99±0.04^{b}	5.59±0.18^{a}
Exchangeable acidity (cmol/L)	Pre	0.54±0.01^{a}	0.32±0.01^{a}	0.06±0.01^{a}	0.06±0.01^{a}	0.19±0.01^{a}
	Post	0.49±0.01^{a}	0.13±0.02^{b}	0.05±0.00^{a}	0.05±0.00^{b}	0.07±0.01^{b}
Total cation (cmol/L)	Pre	2.59±0.03^{a}	2.88±0.01^{a}	5.26±0.16^{a}	4.21±0.03^{a}	1.30±0.03^{a}

	Post	3.03±0.11^{b}	3.08±0.08^{a}	4.74±0.10^{a}	4.30±0.04^{b}	2.02±0.00^{b}
Acid saturation (%)	Pre	20.85±0.03^{a}	11.13±0.37^{a}	11.14±0.07^{a}	1.43±0.01^{a}	14.32±0.51^{a}
	Post	16.14±0.89^{a}	4.02±0.55^{b}	1.06±0.02^{a}	1.16±0.01^{b}	3.23±0.43^{b}

Table 4.2: Pre-planting and post-*A. hypogaea* harvesting nutrient cycling bacteria isolated from soils collected in Mvutshini. Gingindlovu, Umzinto, Hibberdene, Empembeni.

Soil site		**Family**	***Scientific name***	**Accession number**	**Similarity (%)**	**Function(s)**
Mvutshini	Pre-planting	*Burkholderiaceae*	*Paraburkholderia phymatum*	HE864336.1	100	N-fixing
		Burkholderiaceae	*Paraburkolderia sabiae*	MK139731.1	99.80	N-fixing
		Pseudomonadaceae	*Pseudomonas koreensis*	ON428965.1	100	N-fixing P solubilisation
		Burkholderiaceae	*Burkholderia sp.*	MK612762.1	100	N-fixing P solubilisation
		Chromobacteriaceae	*Chromobacterium piscinae*	LR634122.1	99.90	N-cycling
		Pseudomonadaceae	*Pseudomonas nitroreducens*	KY038284.1	99.60	N-cycling
	Post-harvest	*Pseudomonadaceae*	*Pseudomonas umsongensis*	MT628350.1	100	N-cycling
		Alcaligenaceae	*Achromobacter sp.*	LC529441.1	100	N-cycling
Gingindlovu	Pre-planting	*Pseudomonadaceae*	*Pseudomonas frederiksbergensis*	MT378522.1	99.80	N-fixing N-cycling
		Bacillaceae	*Priestia megaterium*	CP127877.1	100	N-fixing

	Post-harvest	*Pseudomonadaceae*	*Pseudomonas sp.*	JQ316360.1	96.71	N-fixing
		Pseudomonadaceae	*Pseudomonas fluorescens*	MT509632.1	100	N-fixing N-cycling
		Paenibacillaceae	*Paenibacillus sp.*	OM060633.1	94.33	N-fixing
		Pseudomonadaceae	*Pseudomonas fluorescens*	MH518305.1	100	N-fixing
		Paenibacillaceae	*Paenibacillus sp.*	OM060633.1	94.54	N-fixing
Umzinto	Pre-planting	*Micrococcaceae*	*Arthrobacter sp.*	MN081011.1	100	N-fixing P solubilisation
		Pseudomonadaceae	*Pseudomonas sp.*	MT354507.1	99.90	N-fixing P solubilisation
	Post-harvest	*Pseudomonadaceae*	*Pseudomonas sp.*	MT354507.1	100	N-fixing P solubilisation
		Pseudomonadaceae	*Pseudomonas umsongensis*	MT065779.1	100	N-cycling
		Pseudomonadaceae	*Pseudomonas vancouverensis*	MT409572.1	100	N-fixing P solubilisation N-cycling
Hibberdene	Pre-planting	*Pseudomonadaceae*	*Pseudomonas sp.*	MT354507.1	99.30	N-fixing P solubilisation

		Pseudomonadaceae	*Pseudomonas poae*	OR122193.1	99.90	N-fixing P solubilisation
	Post-harvest	*Burkholderiaceae*	*Burkholderia ambifaria*	MN691467.1	99.85	N-cycling P solubilisation
		Rhizobiaceae	*Rhizobium mesosinicum*	JQ659535.1	100	N-fixing
		Pseudomonadaceae	*Pseudomonas frederiksbergensis*	MT378522.1	100	N-fixing N-cycling
		Sphingomonadaceae	*uncultured Sphingomonas sp.*	FJ037347.1	89.02	N-fixing
Empembeni	Pre-planting	*Burkholderiaceae*	*Burkholderia cepacian*	MN691121.1	99.40	P solubilisation
		Burkholderiaceae	*Burkholderia sp.*	KT390908.1	98.30	N-fixing P solubilisation
		Pseudomonadaceae	*Pseudomonas protegens*	MT505104.1	100	P solubilisation
		Burkholderiaceae	*Burkholderia cenocepacia*	MT001454.1	99.40	P solubilisation
	Post-harvest	*Burkholderiaceae*	*Caballeronia sp.*	OR363922.1	100	N-fixing N-cycling
		Burkholderiaceae	*Caballeronia calidae*	MW665205.1	100	N-cycling P solubilisation

	Pseudomonadaceae	*Pseudomonas sp.*	MT507079.1	100	N-cycling
	Azospirillaceae	*Azospirillum rugosum*	NR_042582.1	99.39	N-fixing P solubilisation

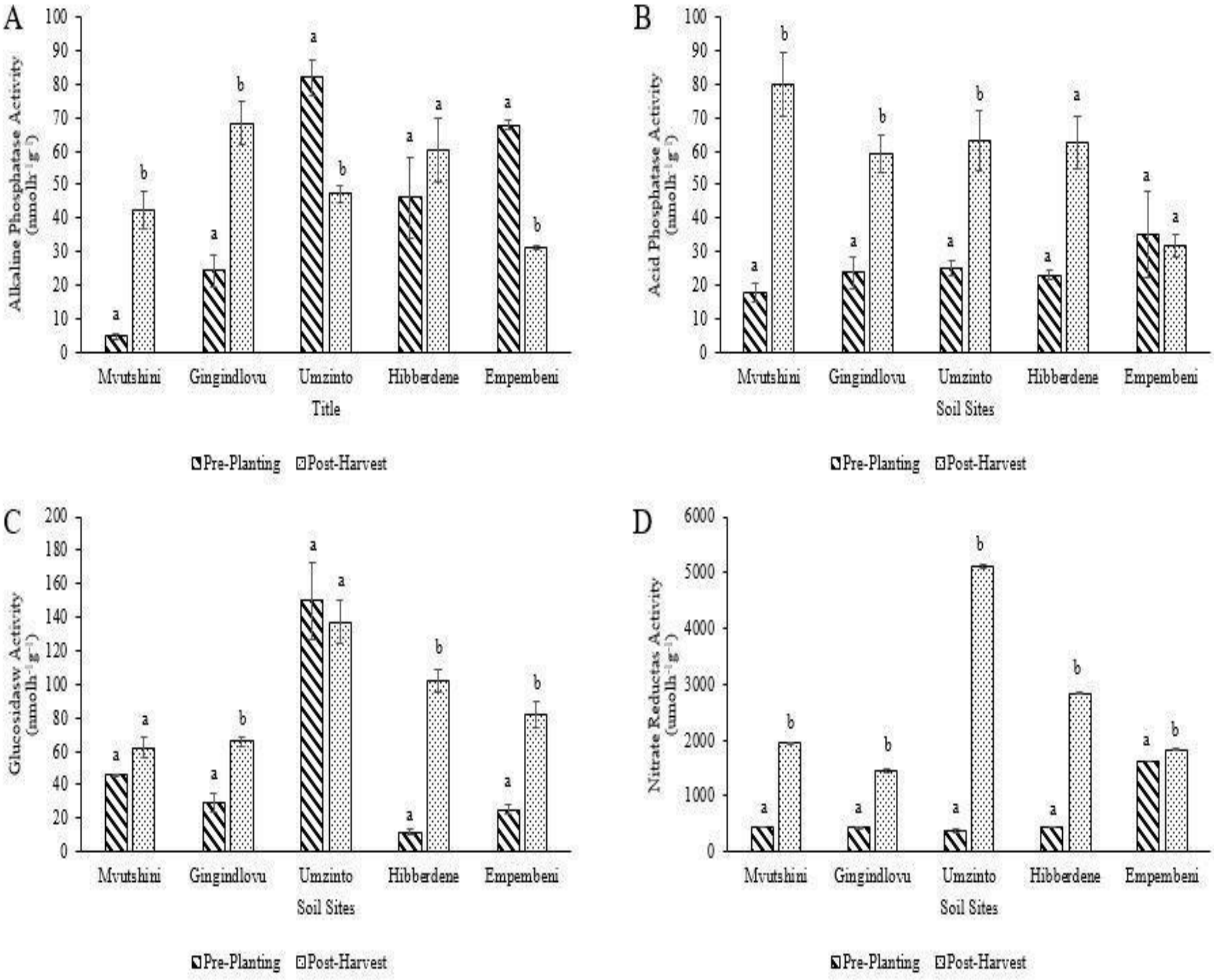

Figure 4.1: Soil enzyme activities of soils collected from Mvutshini, Gingindlovu, Umzinto, Hibberdene, and Empembeni pre-planting and post-harvest of *A. hypogaea*: A. Alkaline phosphatase activity, B. Acid phosphatase activity, C. Glucosidase activity, D. Nitrate reductase activity. Different values represent the mean±SE, with significant differences ($p < 0.05$) within each treatment denoted by different letters.

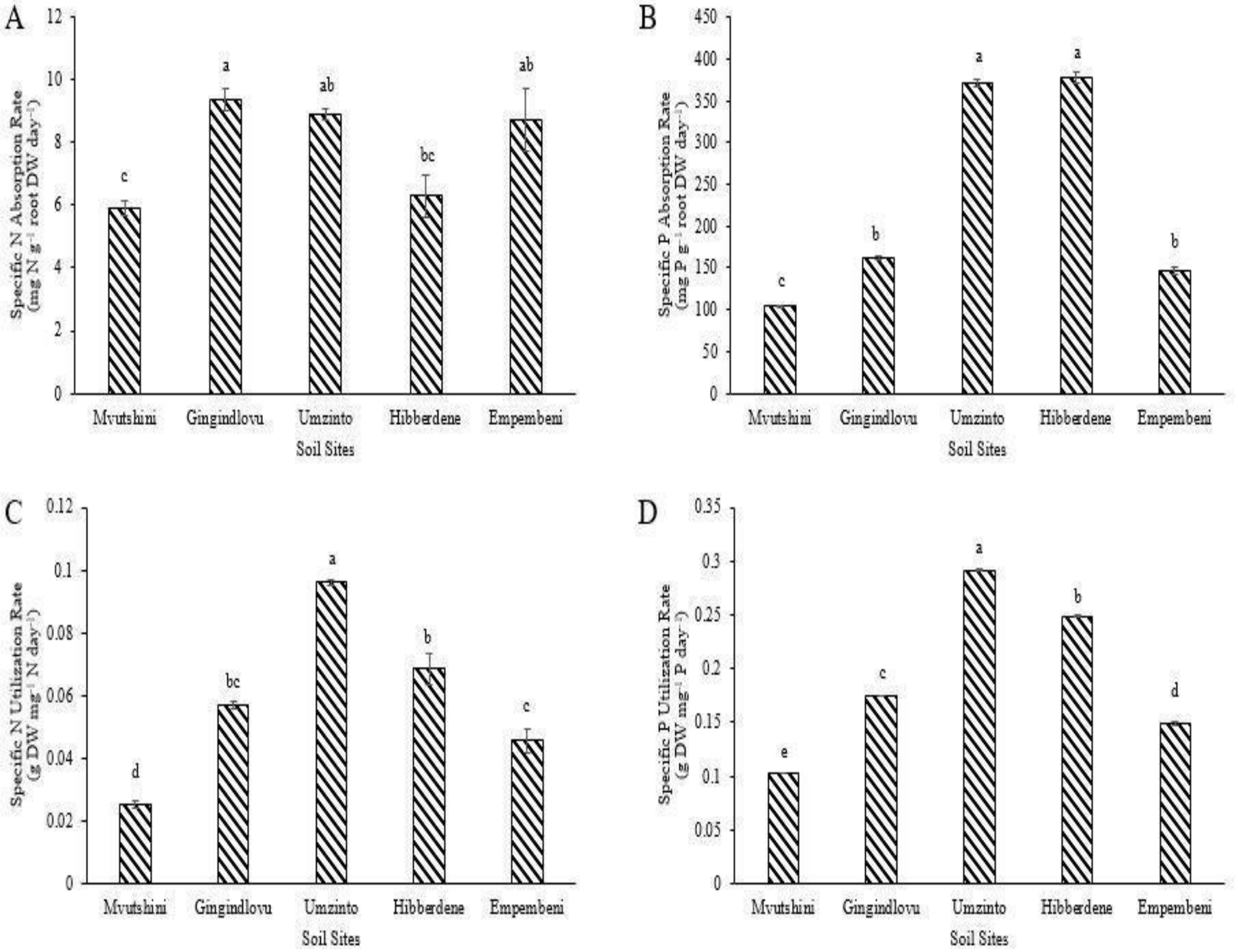

Figure 4.2: A. Specific N absorption rate, B. Specific P absorption rate, C. Specific N utilization rate, and D. Specific P utilization rate of *A. hypogaea* plants grown in soils collected from Mvutshini, Gingindlovu, Umzinto, Hibberdene, and Empembeni for 54 days. Different values represent the mean±SE, with significant differences ($p < 0.05$) within each treatment denoted by different letters.

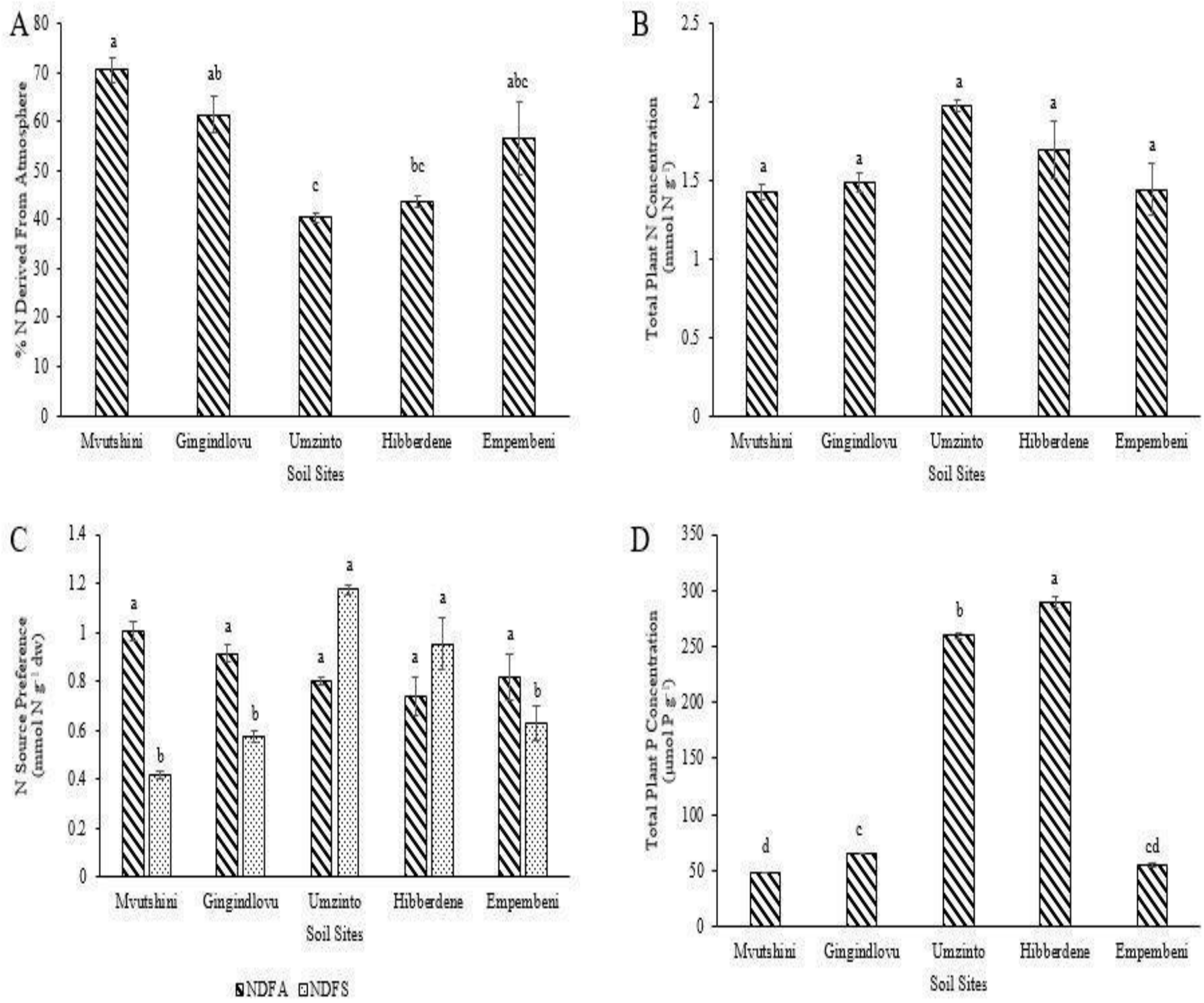

Figure 4.3: A. Percentage N derived from the atmosphere, B. Total plant N concentration, C. N source preference, and D. Total plant P concentration of *A. hypogaea* plants grown in soils collected from Mvutshini, Gingindlovu, Umzinto, Hibberdene, and Empembeni for 54 days. Different values represent the mean±SE, with significant differences ($p < 0.05$) within each treatment denoted by different letters.

Table 4.3: Growth kinetics of *A. hypogaea* grown in soils collected from Mvutshini, Gingindlovu, Umzinto, Hibberdene, and Empembeni for 54 days. Different values represent the mean±SE, with significant differences ($p < 0.05$) within each treatment denoted by different letters.

	Mvutshini	Gingindlovu	Umzinto	Hibberdene	Empembeni
Root RGR (g/day)	0.03 ± 0.01^{a}	0.03 ± 0.00^{a}	0.05 ± 0.01^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}
Shoot RGR (g/day)	0.03 ± 0.00^{b}	0.05 ± 0.00^{a}	0.04 ± 0.01^{ab}	0.03 ± 0.00^{ab}	0.04 ± 0.00^{a}
RGR (g/day)	0.03 ± 0.00^{b}	0.04 ± 0.00^{a}	0.04 ± 0.01^{ab}	0.03 ± 0.00^{ab}	0.04 ± 0.00^{ab}
Root: Shoot ratio (g/day)	0.51 ± 0.25^{a}	0.25 ± 0.03^{a}	0.40 ± 0.06^{a}	0.49 ± 0.04^{a}	0.31 ± 0.08^{a}

Chapter 5

5.1 General conclusions

The rapidly increasing human population confronts agricultural production with the challenge of improving crop production while minimizing external inputs and reducing detrimental environmental effects. While conventional intensive agriculture has produced significant yield increases, there have been growing concerns about the negative impacts on nutrient-use efficiency, biodiversity, and soil quality. Thus, it is important to focus on strategies that can sustainably improve the productivity of soils. The integration of cover crops into cropping systems has been shown to provide benefits such as soil erosion control, weed suppression, increased soil organic C, and modification of the nutrients and soil microorganisms within the soil (Blanco-Canqui et al., 2015; Qin et al., 2021). Amongst cover crops, legumes can increase soil N through their symbiotic with rhizobia, which increases the nutrients' availability for the subsequent crop (Herridge et al., 2008; Kaye et al., 2019). This can reduce N fertilizer requirements, and in some cases, exceed the subsequent crop's needs (Tonitto et al., 2006; Allito et al., 2015). Therefore, this study focused on elucidating the biological and chemical contributions, and survival strategies of *Mucuna pruriens* and *Arachis hypogaea* growing in acidic and nutrient-deficient sugarcane soils.

Post-harvesting, both legume species increased soil pH across all sugarcane plantation soils. The increased acid phosphatase activities coupled with the presence of P solubilizing bacteria across all study sites may have led to the mineralization of organic P, resulting in increased P assimilation rates and high total plant P concentration. Increased availability of P might have assisted with the P demands of BNF, allowing both legumes to effectively fix atmospheric N. It is important to note that both legume species were able to switch N sources, with *A. hypogaea* showing greater N fixing efficiency, further highlighting their adaptations to nutrient-deficient soils. The decreased soil N and P concentrations were likely assimilated by the legume species to support their growth and functioning. However, the depleted nutrients can be replenished by incorporating the legume residues into the soil as green manure. These findings demonstrate that *M. pruriens* and *A. hypogaea* were able to thrive in these acidic and nutrient-deficient soils and contribute to soil health by reducing soil acidity and increasing the diversity of nutrient-cycling bacteria and their associated enzyme activities.

5.2 Future recommendations

The influence exerted by legumes on soil fertility generally becomes apparent after a prolonged period of cultivation (Sánchez-Navarro et al., 2019). Therefore, despite the potential shown by these legume species, experimental field trials over an extended period might provide more insight into the plant-microbe interactions and nutrient contributions. Further to this, field trials should be conducted across varying climatic conditions, soil types, and management practices as these biophysical constraints play a vital role in the sustainable improvements provided by leguminous crops. Future research should focus on using shotgun sequencing to identify all the soil microorganisms within the soil, providing a better estimation of the influence of these species on the soil microbial community composition. Concurrently, researchers should also look to identify and quantify the root exudates secreted by *M. pruriens* and *A. hypogaea* to better understand how these legume species regulate their plant-microbe interactions.

5.3 References

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