Effects of mulching on water use efficiency and nutritional composition of three underutilized crops in Mpumalanga Province

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DECLARATION

I, Welile Nokwanda Malambe, hereby proclaim that this dissertation under the topic “effects of mulching on water use efficiency and nutritional composition of three underutilized crops in Mpumalanga Province”, is my original work that has never been submitted elsewhere previously. I have acknowledged and referenced all the work of others that has been used in this thesis. As such, this dissertation has been submitted to fufill the requirements of the awarding of a Master of Science in Agriculture degree at the University of Mpumalanga.

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DEDICATION

I dedicate this degree to my father, Justice, and I for always having confidence in me. Thank you for believing in me. May your soul continue to rest in peace.

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GLOSSARY

ANOVA	Analysis of variance
ARA	Acetylene reduction assay
ARC-SCW	Agricultural Research Council's Soil, Climate and Water
ARC-VIMP	Agriculture Research Council: Vegetable, Industrial, and Medicinal plants
BNF	Biological nitrogen fixation
C_3	Photosynthetic pathway (C3 plants)
cm	Centimetres
DAP	Days after planting
DMRT	Duncan's Multiple Range Test
Δ	Carbon isotope discrimination
G	grams
IKS	Indigenous Knowledge Systems
kg	kilograms
mg	Milligrams
N_2	Molecular Nitrogen
N_{ref}	Nitrogen reference
N_{leg}	Nitrogen Legume
$^{13}CO_2$	Carbon-13 Labelled Carbon Dioxide
^{15}N	Nitrogen 15
NRF	National Research Foundation
%Ndfa	Percent Nitrogen derived from atmospheric
RCBD	Randomized complete block design
RH	Relative humidity

SADC	Southern African Development Community
WHO	World Health Organization
WUE	Water Use-Efficiency

ABSTRACT

African agricultural systems are particularly vulnerable to changing climatic conditions, with water scarcity posing a major constraint in Sourthen Africa. Within this context, water scarcity in Sourthen Africa is projected to intensify, resulting in more financial and physical limitations as early as 2025. Projected climatic shifts are expected to intensity drought conditions and resource scarcity, particularly in regions with existing limitations, such as South Africa. In addition, as Africa's population grows, food demand is increasing daily. Underutilized legume crops play an essential role in climate resilience, thriving in extreme conditions (low soil fertility, drought, high temperature), and they have the capability to enhance soil health and boost food production. It is therefore vital to cultivate underutilized legume crops that survive under harsh conditions. In addition, animal protein is getting expensive for livelihoods with low incomes. The adoption of underutilized legume crops provides a highly important contribution strengthening the resilience of global food systems under increasing environmental and nutritional pressures.

Therefore, legumes such as those that incorporate cowpea, Bambara groundnut, and common bean are one of the food sources that can help fulfil the sustainable goal of offering enough essential nutrients. Despite their high nutrient density, they have also been reported to have the capacity to improve soil fertility via the fixation of atmospheric nitrogen. However, these crops are not widely cultivated, and information remains vacant regarding their cultivation implementation. Previous literature demonstrates significant gaps: the impacts of various mulching treatments on the growth performance of underutilized legume crops are not well documented; furthermore, it is unclear how mulching affects symbiotic nitrogen fixation. Therefore, this study assessed the effects of mulching on the growth, symbiotic

fixation of nitrogen, and nutritional composition of the previously mentioned crops above. To conduct the experiment, three underutilized legume crops such as bambara groundnut, common bean and cowpea were assessed under three mulching regimes (no mulch, plastic mulch, and pine bark mulch) using a randomized complete block design with four replications. The study set up was performed at the University of Mpumalanga, and standard cultivation practices (such as irrigation and weeding) were followed. Data was subjected to ANOVA using STATISTICA version 12.0. Regardless of the mulching material employed, data on cowpeas had the maximum plant height and chlorophyll content, common bean and Bambara groundnut under plastic mulch and pine bark, respectively, followed next.

Collectively as a whole, the results of this study showed a strong association between $\delta^{13}C$, WUE, and dry matter, which are closely linked, as they are all regulated by carbon assimilation and stomatal control, this regulates water loss as well as CO_2. Pine bark mulch substantially influences plant growth. In addition, cowpea-pine bark mulch (623.3 mg/100g) and common bean-plastic mulch (604.6 mg/100g) had a strong positive effect on potassium levels. Pine bark mulch (PBM) significantly improved the nutritional composition, particularly in minerals and vitamins. Interestingly, no mulch treatment resulted in the highest B-carotene content of Bambara groundnut (53.5 mg/100g) and cowpea (49.4 mg/100g), suggesting that some nutritional traits may benefit from un-mulched conditions.

The overall results demonstrate that all three legumes actively fixed atmospheric nitrogen and accumulated significant amounts of nitrogen in their tissues, despite variations in total N content due to differences in biomass and specific unit variations. Mulching significantly improved plant growth, nutritional composition of the underutilised crops investigated, and nitrogen fixation. This implies that by enhancing soil conditions, mulching promotes growth

and nutrient uptake. This leads to higher yields of more nutritious, protein-rich legumes that strengthen food security, particularly for communities facing challenging environmental conditions.

The findings of this study demonstrate that mulching positively influenced plant growth and nutritional composition of the underutilized crops investigated. This implies that by enhancing soil conditions, mulching promotes growth and nutrient uptake. Overall, the results demonstrate that mulching treatments played a vital role in promoting plant growth by improving soil microclimatic conditions, which in turn promoted greater carbon assimilation and stimulated biological nitrogen fixation, thereby contributing to improved nitrogen accumulation and biomass production in the legume crops.

Key words: Carbon assimilation, fixation, mulch, nitrogen, legume, underutilized legume, water use-efficiency.

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1. INTRODUCTION

1.1 INTRODUCTION

This chapter provides a general background of the study and outlines the problem statement. It further presents the study’s aims, objectives, and hypotheses of the study, and provides an overview of the structure of the dissertation.

1.2 BACKGROUND

Human activities and agricultural practices depend on water as a significant resource (for instance, food production, livestock grazing, pesticides and fertilizer application, and intensive farming practices). Nhamo *et al.* (2019), stated that water availability is a threat in many parts of the world, and its scarcity is exacerbated by population growth and competition for agricultural markets. The growth rate of the global population is estimated to increase, and this increase will affect the demand for food and agricultural water usage (Boretti and Rosa, 2019). By 2050, water demand is anticipated to outpace supply, and the impact of change in climate conditions are resumed to intensify water shortages as well as droughts, especially in areas already experiencing water scarcity (Stringer *et al.*, 2021).

According to reports by Mupedziswa and Kubanga (2017) and Ziervogel (2018), the Southern African Development Community (SADC) has experienced increased temperatures and reduced rainfall due to climate change. Rainfall trends vary within SADC countries, including South Africa, with increased inter-annual variability, exceptionally rainy intervals, and droughts (Archibald *et al.*, 2009). Underutilized crops and novel crop-growing methods are urgently needed to reduce irrigation water use, improve agricultural water use efficiency, and improve food production (Boutraa, 2010). Various water-saving strategies have been suggested, including mulching and irrigation techniques (Zhou *et al.*, 2011).

Mulching is the most popular water-saving technique for crop species, including cereals and vegetables, especially in regions with low temperatures and heavy rainfall (Bhardwaj, 2012). Contrary to conventional methods, mulch application lowers soil water losses through evaporation (Yin *et al.*, 2016). Crop water production efficiency can be improved through mulching, which also helps control and conserve soil moisture (Kader *et al.*, 2017). Mulch is an impermeable covering of either inorganic or organic material spread over topsoil to improve soil quality, reduce soil compaction, impede weed growth, and block evaporation and desiccating winds. Mulch reduces soil moisture loss by preventing weed growth and evaporation of light and desiccating winds (Metwally *et al*., 2022). Mulching using plastic film can significantly increase crop production and quality (Gao *et al.*, 2019). Film mulching is commonly utilized in vegetable cultivation, particularly for tomatoes and peppers (Zhang *et al.*, 2022). Liquid mulch can be diluted with water and sprayed onto the soil surface to create a black, cured layer that effectively prevents moisture from evaporating from the soil and increases soil temperature (Ahmad *et al.*, 2021). Although the value of mulching has been established, its impact on various underutilized and neglected crops remains understudied.

The neglected and underutilized crops include cowpea (*Vigna unguiculata* [L.] Walp), are fundamental, including grain and legume crop which are highly adapted to dry and semi-dry regions of sub-Saharan Africa (Gerrano *et al.*, 2019). Other underutilized crop species include the edible dry seeds of common beans (*Phaseolus vulgaris* L.), commonly referred to as navy and kidney beans, which are grown as annual plants in temperate and semitropical climates. Another one is the Bambara groundnut (*Vigna* *subterranean* (L.) Verdc), regarded as a promising crop to mitigate food insecurity due to its antioxidant potential, drought resistance, practical qualities, and nutritional composition, (Murevanhema and Jideani, 2013). Legumes are important for human nutrition, and they provide economic benefits, environmental, and agronomic traits that can help small-scale farmers improve their standard of living (Gonçalves *et al.*, 2016). In South Africa, these crops receive insufficient attention and are not cultivated commercially.

In contrast to major crops involving common known crops such as potatoes, rice, maize, and wheat, the Bambara groundnut, common bean, and cowpea have been overlooked and neglected because the scientific community, international research organizations, and industry consider that these crops lack economic relevance (Muhammad *et al.*, 2020), even though they might significantly reduce malnutrition and help secure the country's food supply. In various agroclimatic and soil conditions, it was found that neglected crops responded differently to irrigation and differed in their efficient use of water (Uniyal *et al.*, 2019). Inspired by this unique agronomic characteristic, this study chose Bambara groundnut, common beans, and cowpeas to promote sustainable agriculture and increase food security. Therefore, it was imperative to examine the effectiveness of mulch and water usage efficiency in underutilized crops. This study will evaluate the effect of mulching of various treatments on water use-efficiency, carbon assimilation, as well as nutritional composition of three underutilized crops.

1.2. PROBLEM STATEMENT

Africa has experienced rapid human population growth, accompanied by significant urbanization. By 2030, an estimated 8.6 billion people are projected to inhabit the planet, and by 2050, this number is likely to expand to 9.8 billion (Breiling, 2019). This means that more food needs to be cultivated to ensure an adequate supply for everyone. Unfortunately, South Africa is prone to experience water stress. The limited water availability in southern Africa is also predicted to cause nations to endure increased physical and financial water constraints as early as 2025 (Mabhaudhi *et al*., 2018). In South Africa, food insecurity primarily affects rural communities (Wayas *et al*., 2021); consequently, water scarcity exacerbates the rate of food insecurity in the country. Therefore, it is essential to cultivate underutilized and neglected crops, as they are well-suited for thriving in areas frequently affected by water scarcity. Mulching is a common practice that serves the purpose of conserving soil and water through the alteration of the physical soil environment to preserve soil moisture. Therefore, cultivating these crops can also be done in conjunction with mulching (Goel *et al*., 2019).

Mulching helps maximize water use efficiency while encouraging crop growth and production (Yu *et al.*, 2018). Mulches strengthen soil structure, improve aeration, regulate temperature, retain moisture in situ, increase organic matter, support microbial flora, control weeds, reduce nutrient loss, and mitigate soil erosion (Manoj *et al*., 2015). According to research, mulching crops has several advantages for crop production by enhancing the physical characteristics, biological, and soil's chemical (Abdelraouf *et al.*, 2020). Results also indicated that mulching interfere with the capacity of soil to contain water within itself i.e., soil water holding capacity, enhances the moisture within the soil by boosting infiltration, and lowers soil evaporation (Kader *et al.*, 2019). In contrast, mulch decomposition and humus formation increased the soil's water retention ability (Teame *et al*., 2017).

Applying mulch to cowpeas, common beans, and Bambara groundnut offers a sustainable and practical approach to enhance their growth, conserve water, and sustain food and nutritional security worldwide. Cowpeas, common beans, and Bambara groundnut are valuable food and nutritional legume (Nkoana, 2018). The mildly flavoured leaves are genuine sources of nutrient-rich, edible vegetables that can combat hunger and provide vital nutrients for human consumption (Enyiukwu *et al.,* 2018). Evaluating water-use efficiency and carbon assimilation is therefore crucial to assess the growth potential and nutritional quality of underutilized crops in Mpumalanga, supporting the advancement of sustainable farming practices (Mabhaudhi *et al.,* 2017). Several underused crops have also been found to be water-efficient and drought-tolerant, allowing them to be suitable for production in Mpumalanga. The eastern escarpment regions of the province, which make up most of the province, have humid or dry sub-humid climates. Therefore, this research was critical because it provided data to ensure that farmers cultivate the right crop, which is more water-use efficient and can be recommended for cultivation in water-limited regions.

1.3. RATIONALE

There is a significant dispute on the use of water resources for industry, farming, direct human usage, and other activities owing to the limited availability of water (Molden, 2013). The efficient use of agricultural water is a significant goal in agriculture and food security. Rural households often produce and eat most of their food, making household agricultural production essential to enhancing food security (Hadebe *et al*., 2017). Underutilized crops would be suitable to be promoted during droughts seasons, extensive research has revealed that they need less water (Hadebe *et al*., 2017). Most underutilized crops have qualities making them best suitable for cultivation in low-input agricultural production systems, and marginalised production areas, which characterize South Africa's rural landscape(Kunene *et al.,* 2022). With the current trends in climate change in South Africa, underutilized crops have developed into a crucial resource for adaptation (Mabhaudhi *et al*., 2017).

1.4 AIM

This study sought to assess the mulching treatments effects on the efficient use of water, carbon assimilation, and nutritional composition of three underutilized crops.

1.5 OBJECTIVES

- To evaluate how mulching treatments influence the growth and yield of three underutilized crop species.
- To assess the mulching treatment effects on photosynthetic efficiency and carbon assimilation in the three underutilized crops.
- To examine variations in the nutritional composition and nitrogen fixation capacity of the three underutilized crops under different mulching treatments.

1.6 HYPOTHESES

- Growth and yield of the three underutilized crops will differ across mulching treatments.
- Mulching treatments will significantly influence photosynthetic efficiency and carbon assimilation rates in three underutilized crops.
- The nutritional composition and nitrogen fixation of the three underutilized crops will vary in response to mulching treatments.

1.7 DISSERTATION STRUCTURE

The dissertation is structured as per the following fashion:

Chapter 1: Presents an overview of the study, including research aim and specific objectives.

Chapter 2: Reviews the literature relevant to the study, including the significance of underutilized crops, the origin, distribution of selected underutilized crops in Africa, and the taxonomic classification and botanical relationships of legume species.

Chapter 3: This chapter investigates the effects of mulching and water management practices on the growth and yield of the selected underutilized crops.

Chapter 4: Provides an analysis of the nutritional profiles of the three underutilized legumes crops in relation to mulching practices.

Chapter 5: Examines the effects of mulching on symbiotic nitrogen fixation in the selected legumes crops.

Chapter 6: General conclusions, recommendations, and references.

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

This chapter reviewed previous literature on topics associated with the importance of underutilized crops, the origin and distribution of the three underutilized crops mentioned in Chapter 1 under the African context, taxonomic classification and botanical relationships of selected legume, production levels and constraints of underutilized crops, carbon assimilation and water use efficiency of underutilized crops, and the importance of mulching and their symbiotic nitrogen fixation.

2.2 Importance of underutilized crops

The phrase "underutilized crops" refers to plant genera that have been traditionally utilized for fuel, fodder, medicinal purposes, and food. Underutilized crops in the African context can mitigate several constraints such as those related to nutrition, food security, and environmental sustainability (Popoola *et al*., 2023). In South Africa, underutilized crops are primarily produced by smallholder farmers and integrated into their cropping systems (Mabhaudhi *et al.,* 2017). These plant genera can survive in drought and severe ecological conditions (Chandra *et al*., 2020). Despite their importance in combating food insecurity and malnutrition in marginalized communities, underutilized and neglected crop species have been overlooked by researchers and users for many years (Mabhaudhi *et al*., 2017a, 2017b). Although underutilized crops are rich in protein, minerals, and vitamins, their potential remains underutilized due to limited agro-processing and market channels. The identified literature gap in the study points to numerous factors involving their inadequate utilization as previously mentioned, poor consumption, and neglect, particularly in rural communities where dietary diversity is limited (Asiwe, 2023). These underutilized crops are beneficial for preventing malnutrition and boosting health, as they contain bioactive substances, vital amino acids, vitamins, minerals, and proteins in abundance (Ebert, 2014; Popoola *et al*., 2023).

Additionally, they contain phytochemicals with antibacterial and antioxidant properties, which offer health benefits (Ramatsetse *et al*., 2023). According to Botai *et al*. (2018), South Africa is ranked as a semiarid country, particularly in the central and western regions, where rainfall is erratic. Crop yield is drastically decreased by this, especially for smallholder farmers who rely on rain-fed agriculture (Lembede, 2017). Given the recent surge in scientific and economic interest in underutilized cultivated crops, a review of their status is imperative to close the knowledge gaps and contribute to the advancement of this field. These crops hold considerable potential to directly reduce hunger by enhancing food production in marginal environments where the cultivation of major staple crops is severely constrained (Kafi, 2018). In this section, we discuss some underutilized crops, with a special focus on their production levels, utilization, taxonomy, and agricultural practices.

2.3 Taxonomic classification and botanical relationships of selected legumes

Legume crops are crucial for food security, and sustainable agriculture, providing multiple benefits to agroecosystems (Stagnari *et al*., 2017). They reduce greenhouse gas emissions, increase soil fertility, and fix atmospheric nitrogen (Stagnari *et al*., 2017). The classification of underutilized crops is essential for increasing the use of legume crops and realizing their full potential and expanding the use of legume crops. In this study, the three legume crops of focus were cowpea, common bean, and Bambara groundnut, all of which belong to the Leguminosae family (Jideani and Diedericks, 2014; Ramatsetse *et al*., 2023). Bambara groundnut and Cowpea belong to the Faboideae subfamily (Jideani and Diedericks, 2014; Ramatsetse *et al*., 2022). The Bambara groundnut (*Vigna subterranea*) and Cowpea (*Vigna unguiculata*) belong to the genus *Vigna*, whereas common beans (*Phaseolus vulgaris*) belong to the genus *Phaseolus* and the subfamily Papilionoideae. Several studies on the genetic connections between these legumes have revealed a significant diversity of taxonomic groups (Catarino *et al*., 2021).

Leguminosae plants are rich in a variety of phytochemicals, including flavonoids, alkaloids, terpenoids, and phenolics (Noviany *et al*., 2023). The above compounds positively influence the family's ethnomedicinal significance, with many species being utilized in traditional medicine by rural communities (Shreya *et al*., 2023). The family is also recognized for its nutritional value, containing various nutrients and bioactive compounds that may prevent diseases (Benevides *et al*., 2018).

Additionally, Leguminosae plants are essential for agriculture, as they fix nitrogen and can be utilized as green manure, crops, and fodder (Noviany *et al*., 2023). The anti-cancer potential of phytochemicals from the Leguminosae family has been the subject of recent research; these compounds may inhibit cancer through various pathways (Usman *et al*., 2022). The production of cowpea is primarily carried out in tropical and subtropical regions for fodder, soil improvement, and food (Horn *et al*., 2022). Common beans are widely cultivated globally, with significant production in Latin America and Africa (Tigist, 2020). Bambara groundnut thrives in poor soils and is drought-tolerant, making it valuable for food security in Africa (Mohammed, 2014).

According to Maroyi (2016), plant species that the locals do not use are hardly ever named. The names of the plant species are determined by traits like size,habitat and taste that are related to the species' interests (Adebayo *et al*., 2021). Local varieties exhibit significant variation in nutritional composition and morphological traits (Karuwal *et al*., 2021). In South Africa, cowpea is called timbaweni (isiSwati) or morogo wa dinawa (Sepedi). Cowpea is also known as southern pea and black-eyed pea in the United States of America, niébé in Francophone Africa, and frijole in Venezuela (Horn *et al.*, 2022).

According to a study by Alemu (2019), the naming scheme is determined by morphological traits, appearance, seed colour, seed size, growth habit, adaptation, the crop's multipurpose nature, and source locality. In South Africa, Bambara groundnut in the Northwest is called "Mankon", "Izindlubu" among the Zulus or Jugo beans, Ntoyo Cibemba (Zambia), and Nyimo beans (Zimbabwe) (Bamshaiye *et al*., 2011; Mabhaudhi *et al*., 2013; and FAO, 2017). It is also known as "tindlubu" in Swaziland. Mohammed (2014) reported that in the Limpopo region, Bambara groundnut is known as "Ditloo-marapo (Sepedi) ''Phonda'' (Venda) and ''Tindhluwa '' (Tsonga) and "Jugoboon'' (Afrikaans), "Jugo" (Xhosa), "Indlubu" (Ndebele).

Bareke *et al*. (2018) emphasized that a valuable plant species or variety with numerous vernacular names typically indicates its importance, widespread usage, and popularity. In South Africa, common beans are known as "ubhontshisi" in isiZulu, "emabotjisi" in siSwati, and "dinawa" in Sepedi. In Nigeria, a common bean is known as "Akidi" (Igobo) (Ikezu *et al*., 2015). This crop is also known as a kidney bean in India, a haricot bean in France, and a French bean in Uganda. In Ethiopian languages, the most common is Amharic. In Zambia, the large-seeded red type (non-climber, locally known as Lundazi beans). In Tanzania, common beans are known as "Mwasipenjele" (Ndimbo *et al*., 2022).

2.4 Origin and geographical distribution of three underutilized crops

2.4.1 Bambara groundnut

Although the Bambara groundnut (*Vigna subterranea* (L.) Verdc.) originated in West Africa, it is now widely found throughout the semiarid regions of Sub-Saharan Africa

(Majola *et al*., 2022). Outside of Africa, it is rarely cultivated, although it is occasionally produced in Asia and other regions (Mkandawire, 2007). The centre of origin is North–Eastern Nigeria and Northern Cameroon in West Africa (Effa and Uko, 2017). In Southern Africa, Bambara groundnuts are widely grown and sold as they are known as a balanced diet and a source of complete nutrition. Zimbabwe, Zambia, Swaziland, Botswana, Uganda, and South Africa have the best-growing conditions, characterized by semiarid to sub-humid tropical climates, for producing Bambara groundnuts in the region (Majola *et al*., 2021). Bambara groundnut cultivation in South Africa is largely found in KwaZulu-Natal, Gauteng, Limpopo, North West, and Mpumalanga. The crop is mainly produced by smallholder farmers, serving both as a source of food and a means to earn income (Hillocks *et al.*, 2012).

2.4.2 Cowpea

Cowpea is a dicotyledonous plant which belongs to the order Fabaceae, family Leguminosae (Sariah, 2010). In tropical regions, cowpea is a versatile food legume and serves as an essential source of nutrition for rural and tribal populations across Africa and Asia (Tripathi *et al.*, 2019). It is reported that the Gauteng region and the south-eastern African region in the Republic of South Africa are centres of diversity for wild *Vigna spp* owing to the presence of many wild species in the area (Ringo, 2017). Across sub-Saharan Africa, cowpea stands out as a key grain and vegetable legume, valued for its ability to thrive under arid and semi-arid climatic conditions (Gerrano *et al*., 2019). Cowpea and other pulses are commonly consumed in these areas to supplement the daily diet.

2.4.3 Common bean

Mesoamerica is the principal location of origin for the common bean, with the highest number of *Phaseolus vulgaris* species. It is believed that *the Phaseolus vulgaris* genus has its origin in this region, dating back between 4 and 6 million years ago (Smýkal *et al.*, 2015). Originating in what now is known as Mexico, wild varieties of the common bean were primarily found in northwest Argentina and northern Mexico, encompassing the Andean, Mesoamerican, and Peru-Ecuador regions (Nadeem *et al.*, 2021). The two main gene pools of common beans are thought to be nutrient content and drought tolerance, and their cultivation forms clearly reflect their geographic organization (Bellucci *et al.*, 2014). Throughout time, the common bean was spread to various nations. Nowadays, millions of people rely on the cultivation of common beans as a primary source of food worldwide (Nadeem *et al.*, 2021). Kenya, Tanzania, and Uganda are the three East African countries that are leading in common bean production (Akibode and Maredia, 2012). About 75% of the Common beans consumed annually in South Africa are produced domestically (DAFF, 2012).

2.5 Botany and Ecology of Underutilized Legume Crops

2.5.1 Bambara groundnut

The legumes in the family Leguminosae and the subfamily Faboideae include the drought-tolerant Bambara groundnut (*Vigna subterranean*) (Jideani and Diedericks, 2014; Ramatsetse *et al*., 2023). The genus *Vigna* replaced *Voandzeia subterranean* as the previous name (Panzeri *et al*., 2022). *V. subterranean var. spontaneous* (wild) and *V. subterranea var. subterranea* (cultivated) are two existing botanical varieties (Jideani and Diedericks, 2014). Bambara groundnut is an annual herbaceous plant that grows in an intermediate form, characterized by creeping branches at ground level (Gerrano *et al*., 2021).

2.5.2 Cowpea

One of the most economically significant native African legume crops is the cowpea, and the cowpea is an annual self-pollinating diploid (Moussa *et al*., 2011; Sindhu *et* *al*., 2019). Cowpeas are a member of the Faboideae subfamily and the family Leguminosae (Horn *et al*., 2022). As reported by Horn *et al*. (2022), cowpea is mainly cultivated in the arid savannahs of the Sahel, which are located on the edge of the Sahara Desert and receive 300 mm or less rainfall yearly. While other main crops struggle to thrive in challenging environmental conditions, cowpeas can flourish well (Pereira *et al.*, 2020). Cowpea, also known as timbaweni (siSwati) or Monawa (Sepedi), dishes are prepared differently in many tribes. Some of the familiar dishes include akara, commonly known as deep-fried cowpea paste, cowpea soup, cowpea pudding, and fortified cereal-based meals (Asiwe, 2023).

2.5.3 Common bean

The common bean (*Phaseolus vulgaris* L.) has a long history of cultivation, having been domesticated in the Americas approximately 8,000 years ago, and continues to be a major food crop worldwide (Guerrero *et al*., 2016). It provides essential nutrients, including carbohydrates, protein, vitamins, minerals, and fibre, which makes it more valuable (Bhide *et al*., 2022). Common bean consists of a variety of cultivars and preparation techniques, and the plant exhibits great diversity and adaptability (Abera *et al*., 2020). Common beans may help control weight, diabetes, and cholesterol (Abera *et al*., 2020), and they may possess anti-urolithiasis properties (Bhide *et al*., 2022). Since it fixes nitrogen, it is a legume that supports sustainable agriculture (Guerrero *et al*., 2016). Human nutrition may be significantly impacted by creating cultivars that flourish in nutrient-limited environments, particularly in nations where beans are a staple food (Guerrero *et al*., 2016). Common beans are also known as emabotjisi (siSwati). Common beans are incorporated into various recipes, such as samp with beans, which have a lower glycaemic index than samp alone. Moreover, the glycaemic index of dried bean stew is relatively low, at 68 (Jackson *et al*., 2022).

2.6 Production levels of underutilized legume crops in South Africa

2.6.1 Bambara groundnut

Bambara groundnut ranks third among cultivated legume crops, after peanuts and cowpeas. Worldwide, the production of Bambara groundnu in 2018 was estimated to be 0.2 million tonnes (FAOSTAT, 2009). West African countries including Nigeria, Mali, Rwanda, Togo, the Democratic Republic of Congo, Cameroon, and Burkina Faso, contributed significantly to global Bambara groundnut production, yielding 0.14 million tonnes in 2018 from an estimated production area of 0.18 million hectares (FAOSTAT, 2009). Majola *et al*. (2022) reported that "in South Africa, productivity is low at 0.62 t/ha in relative to potential yields of 3 t/ha, primarily due to a lack of improved cultivars". According to Cook (2017), the following provinces viz.Limpopo, Gauteng, KwaZulu-Natal, Mpumalanga, and North West are considered as primary proction areas for Bambara groundnut in South Africa. Still, yields are generally low due to the presence of pests and diseases. In the Limpopo province, Vhembe, Capricorn, Mopani, and Waterberg districts represent the key areas for Bambara groundnut production (DAFF, 2016). The large-scale, pure stand cultivation of Bambara groundnut remains uncommon (Effa and Uko, 2017). This translates to its production mostly carried out by Smallholder farmers, constrained by numerous diseases and pests, which constitute a significant barrier to increased production (Cook, 2017).

2.6.2 Cowpea

This leguminous crop contributes importantly to the agricultural systems of smallholder farmers in the country (Gerrano *et al*., 2019). South African provinces where cowpeas are grown are KwaZulu-Natal, Limpopo, Mpumalanga, the Eastern Cape, and the Northwest. Cowpea production is dominated by small-scale farmers practicing dryland farming, typically achieving yields of 0.5 t.ha^{-1}, considerably below the potential yield of 3.0 t/ha (Asiwe, 2009). According to Aboki and Yuguda (2013), about six million, nine hundred ninety-one thousand, one hundred seventy-four tons of dry cowpea grains are produced yearly across roughly twelve million, three hundred sixteen thousand, eight hundred seventy-eight hectares of land. On another note, the yield of cowpea plants in South Africa is limited owing to the use of poor agronomic practices and unimproved varieties during crop production (Mofokeng and Gerrano, 2021). Cowpea's productivity is constrained by various abiotic (temperature, soil fertility) and biotic (bacteria and fungi) stress (Horn and Shimelis, 2020). Thus, it is imperative to encourage farmers to apply mulch, especially on sandy or degraded soil. Mulching is also known to be effective at controlling pests and improving moisture retention and yields.

2.6.3 Common bean

The common bean is recognized as the most widely consumed legume on a global scale and is primarily cultivated in Southern Africa during the summer months, coinciding with the region’s rainy season (Bhandari *et al.*, 2017). For this reason, it is commonly cultivated throughout South Africa on both small and commercial scales. In the 2016-2017 season, the Mpumalanga, Limpopo, and Free State were among the central-producing provinces; the Gauteng, Northern Cape, and Western Cape provinces reported the lowest yields (Grain South Africa, 2017). Approximately 75% of the common beans consumed nationally each year in South Africa are produced domestically (DAFF, 2012), resulting in a 25% deficit, which is met by imports from other countries. Free State and Mpumalanga are amidst the nine central-producing provinces, in South Africa (DAFF, 2014). Despite its importance, the crop’s yield falls short of its potential owing to various abiotic and biotic stresses.

2.7 Importance of mulching to underutilized crops

The application of materials on soil surface to create a protective layer that preserves soil health, saves resources, and encourages crop growth is known as mulching (Pavlů *et al*., 2016). The application of mulch in farming systems leads to several positive outcomes, such as controlling soil erosion, limiting weed germination, reducing the impact energy of raindrops, and conserving soil moisture (Liu *et al*., 2022; Kasirajan and Ngoujio, 2012). Mulch application has been shown to enhance soil structure and facilitate earthworm movement remain available after it breaks down *(*Kader *et al.,* 2017).

Mulches made of plastic can significantly enhance soil health and pest control. This makes fertilizer less likely to drain and retain nutrients near the roots of the plants, where they may be used most efficiently. The mulched landscape appears more uniformly pleasing (Chen *et al.*, 2018). Notably, Gu *et al*. (2018) stated that plastic film mulch is an effective agricultural technique that can help maintain food security by increasing the amount of water use for crop transpiration and promoting physiological activities in crops. This boosts crop output and water usage efficiency. In addition, Marble *et al*. (2019) reported that plastic mulch reduced weeding time and weed weight by over 90%, while pine bark and other wood-derived mulches achieved reductions of 64-91%. According to Mohtisham *et al.* (2013), both plastic mulch and sesbania reduced weed density relative to the untreated control, with plastic mulch providing superior weed suppression compared to sesbania In contrast, Muhammad *et al.* (2011) revealed that after 30 days of sowing, mulched treatment resulted in a reducing weed intensity relative to the un-mulched treatment of approximately 100% for broad-leaved weeds and 95.8% for narrow-leaved weeds. On the same note, Qin *et al*. (2015) reported that wheat yields increased between plastic mulch and straw mulch, with plastic mulch being more effective than straw mulch. According to Cisneros *et al*. (2024), pine-bark mulch significantly increased the nitrogen-fixing legume *Lupinus elegans'* height and survival in challenging conditions, indicating that it might be used in restoration techniques.

Furthermore, during a crop's growth cycle, the ideal temperature and soil moisture content can vary. The decomposition of organic mulch contributes to a rapid enrichment of soil organic matter, thereby improving the soil's capacity to retain water (Yang *et al.*, 2015). By acting as a protective layer, mulches reduce surface evaporation, maintaining higher moisture levels in the rhizosphere and extending the duration over which plant roots can effectively uptake water (Kader *et al.*, 2017). Organic and biodegradable plastic mulches breakdown overtime, supplying nutrients to the soil, enhancing water retention, and enriching the humus layer. They also moderate root-zone temperatures, cooling the soil in summer and warming it in winter (Wang *et al.*, 2016).

2.8 Water Use-Efficiency (WUE)

According to Munoz *et al*. (2018), water use efficiency measures carbon uptake relative to transpirational water loss. Climate change has exacerbated water stress, which remains the leading constraint to crop production in arid areas, rendering it an urgent challenge (Medrano *et al*., 2015). Wateruse- efficiency reflects the response to phenological, physiological, and morphological changes resulting from water stress. When water is scarce, it is critical to comprehend the level of water usage (Babalola *et al*., 2017). Given that crop water use efficiency is a physiological metric essential for assessing the trade-off between CO_2 fixation and water loss, It is crucial to select crop types and agronomic techniques that maximize efficient use of water while minimizing yield reductions (Kang *et al*., 2021). The water consumption efficiency formula is shown below.

$WUE = Biomass$ /actual evapotranspiration ……………………………………Equation 1

Increasing the assimilation of carbon while maintaining transpiration or decreasing transpiration while maintaning carbon assimilation are two ways to improve plants' water-use efficiency (Yoo *et al*., 2009). A better understanding of water use-efficiency in response to changing climate improves climate projections. This is because agricultural productivity in regions with scarce water supplies is primarily dependent on drought resistance and efficient water usage (Ayalew *et al*., 2022). It has been determined that water consumption efficiency is a crucial characteristic that determines how effectively plants utilize available water for carbon sequestration.

Blessing *et al*. (2018), reported that while water usage is comparable across species, water-use efficiency in legume crops differs markedly due to differences in biomass and yield. According to Adams *et al*. (2016), legumes can have a 25% higher water use efficiency than wheat, but they often consume 10-25% more water. Low stomatal conductance and other conservative water-use techniques can lower yield potential, while temporal adaptations to environmental conditions maximize water loss and carbon gain (Blessing *et al*., 2018). To increase water efficiency in grain legumes, improved photosynthetic capacity and root length variability are advantageous characteristics (Blessing *et al*., 2018). In general, as soil temperature rises, water use increases and water use efficiency decreases, underscoring the intricate connection between environmental factors, water use, and legume development (Mbava *et al*., 2020).

2.9 Carbon assimilation

The process that converts inorganic carbon into organic molecules is known as carbon assimilation. According to Mininni *et al*. (2022), carbon isotope discrimination is associated with how water use efficiency responds to drought. In cowpea, a highly significant negative correlation between carbon isotope discrimination and water-use efficiency has been found, indicating the possibility of employing carbon isotope discrimination as an indirect way of assessing water use-efficiency (Ayalew *et al*., 2022).

An effective and scientific method for choosing plant species with greater water-use efficiency is isotope discrimination (Li *et al*., 2019). Overall, carbon isotope discrimination ($\delta^{13}C$) offers valuable information for assessing water use efficiency (Barbour, 2007). Interestingly, Basu et *al*. (2007) demonstrated that carbon isotope discrimination on the genetic regulation of Bambara groundnut involves multiple loci, making it a reliable species for assessing plant water status. In C_3 plants such as Bambara groundnut, $\delta^{13}C$ values have been shown to provide insights into their water-use efficiency (Farquhar *et al*., 1989).

Plants discriminate against the heavier $^{13}CO_2$ in favour of $^{12}CO_2$ during photosynthesis, causing ^{13}C to accumulate relatively more in plant tissues compared to atmospheric to ^{12}C (Mohale *et al*., 2014). However, under soil water deficit, partial stomatal closure may occur, resulting in intercellular CO_2 concentrations that are sufficient to saturate photosynthesis. In such circumstances, less ^{13}C discriminated against, and water is minimized, providing the basis for using $\delta^{13}C$ to assess water use efficiency in C_3 plants like Bambara groundnut (Mohale *et al*., 2014). Blum (2009), reported that high continuous stomatal conductance is necessary to facilitate CO_2

fixation, resulting in the maximum use of soil water for transpiration under water and high-temperature stress conditions.

Carbon assimilation in cowpea (*Vigna unguiculata*) is determined by multiple factors, including water availability, rhizobial inoculation, and potassium fertilization. Bradyrhizobium inoculation can improve carbon assimilation and water-use efficiency, particularly under water-limited conditions (Ayalew *et al*., 2022). Carbon isotope discrimination (Δ) has been studied in relation to gas exchange efficiency, showing agreement with the theory for drought-induced effects but not for genotypic variations (Ma *et al*., 2023). Temperature regimes also affect carbon metabolism and nitrogen assimilation in cowpea. Under adverse temperature conditions (warm days, cool nights), non-nodulated plants supplied with inorganic nitrogen outperform nodulated plants in terms of dry weight and seed yield. Nodulated plants rely more on current nitrogen assimilation during later fruit growth stages and allocate a more significant proportion of photosynthates to below-ground organs (Valentine *et al.*, 2017).

Research on carbon assimilation in cowpea with mulching has shown various benefits. Mulching improves soil moisture conservation, especially in the top 15 cm of soil during drier periods (He *et al*., 2023). It also increases chlorophyll content, stem diameter, and plant height in cowpea (Masete *et al*., 2022). However, mulching may reduce biological nitrogen fixation in cowpea compared to un-mulched controls (Masete *et al*., 2022). Water use efficiency and carbon assimilation in cowpea can be enhanced by Bradyrhizobium inoculation, which increases the carbon and nitrogen content in plant shoots (Ayalew *et al*., 2022). The planting date also affects cowpea growth and yield, with September planting showing better results in Sierra Leone, regardless of whether mulching is used (Mansaray *et al*., 2020). Overall, mulching and proper management practices can enhance cowpea growth and carbon assimilation in various environmental conditions. Carbon assimilation in common bean (*Phaseolus vulgaris L.*) is affected by various factors, including water stress, phosphorus availability, mulching, and nutrient management. Under low phosphorus conditions, plants allocate more biomass to root growth, which can increase phosphorus acquisition but may reduce overall growth rates (Karavidas *et al*., 2022). Nutrient availability affects carbon assimilation, with reduced nutrient supply decreasing photosynthesis and yield, although pod nutrient content remains stable (Smith *et al*., 2022).

2.10 Symbiotic Nitrogen Fixation: A case of three legume crops

Symbiotic nitrogen fixation occurs when atmospheric nitrogen is converted into a usable form for the plant and when rhizobia bacteria form nodules on the roots of plants (Bruijn, 2019). This symbiosis is mediated by Nod factors, which trigger nodule development and are recognized by plant receptors (Drevon *et al*., 2015). Over 200 plant genes, including those involved in nod factor signalling and early signalling, have been identified as essential for symbiotic nitrogen fixation, regulating processes such as microbial infection, nodule development, and metabolism (Roy *et al*., 2019). The nitrogen fixation rate can range from 15 to 145 kg $N.ha^{-1}$, depending on soil fertility (Abdullahi *et al*., 2020).

Table 2.1 presents a comparison of nitrogen fixation rates in three underutilized legume crops, cowpea, Bambara groundnut, and common bean, using two primary quantification methods: the ^{15}N natural abundance method and the acetylene reduction assay. Cowpea was reported to fix between 42.68 kg $N.ha^{-1}$ and 182 kg $N.ha^{-1}$, meanwhile Bambara groundnut showed a broad range from 4 to 200 kg $N.ha^{-1}$ in one study and 32 to 82 kg $N.ha^{-1}$ in another study. Common bean demonstrated nitrogen fixation rates ranging from 30 to 33 kg N/ha and 20 to 60 kg $N.ha^{-1}$. In view of its accuracy in measuring total nitrogen fixed, the ^{15}N natural abundance approach was used for all three crops. In contrast, the acetylene reduction assay was primarily used to assess nitrogenase activity in cowpea and common beans. These differences in nitrogen fixation values demonstrate the impact of crop type, environmental factors, and methodological techniques.

Table 2-1: Amount of N fixed by the three underutilized legume crops

Crop	**Amount of N-fixed**	**Methods used**	**References**
Cowpea	42.68 kg $N.ha^{-1}$ 182 kg $N.ha^{-1}$	Acetylene reduction assay method ^{15}N natural abundance method	Yakubu *et al*., 2010 Belane *et al*., 2011
Bambara groundnut	4-200 kg $N.ha^{-1}$ 32 to 81 kg $N.ha^{-1}$	^{15}N natural abundance method ^{15}N natural abundance method	Mohale *et al*., 2014 Musa *et al*., 2016
Common bean	30–33 kg $N.ha^{-1}$ 20 to 60 kg $N.ha^{-1}$	Acetylene reduction assay method ^{15}N natural abundance method	Karavidas *et al*., 2022 Chalk *et al*., 2014

Acetylene reduction assay (ARA) is the most used method to measure biological nitrogen fixation in legumes, including cowpeas. Studies reveal a strong correlation between ARA results and other techniques, such as ureide concentration measurements in stem tissue and xylem sap (Palmero *et al*., 2022). Cowpea shows two ARA maxima over 24 hours, indicating daily cycles. Rhizobia injection enhanced N fixation by 46% in cowpea and Bambara groundnut. According to Yakubu *et al*. (2010), cowpea fixed the highest amount of nitrogen, at 42.68 kg $N.ha^{-1}$. Plant growth stage, soil fertility, and intercropping all affect cowpea nitrogen fixation. In low-fertility soils, nitrogenase activity and the ratio of root nodule to shoot biomass can both increase (Wetzman and Dawson, 2015). When compared to mixed cropping systems, cowpea monocultures often exhibit higher nitrogenase activity (Wortman and Dawson, 2015). According to Pradhan *et al*. (2018), cowpea also exhibits early nitrogen fixation establishment during seedling development, as shown by the detection of ARA in young plants. Lindström and Mousavi (2020) reported that field beans exhibited maximum N-fixing rates of over 600 kg/ha/year. However, there is still a noticeable gap in Bambara groundnut N-fixing rates using the acetylene reduction assay method. Furthermore, another typical technique for evaluating nitrogen fixation in legume crops like common beans and cowpeas is the nitrogen-15 (^{15}N) isotope dilution method. Nitrogen from biological fixation, soil, and fertilizer can be quantitatively estimated using this method (Brito *et al*., 2009; Senthilkumar *et al*., 2020). Previous studies, for instance, Brito *et al*. (2009), revealed that the primary sources of nitrogen buildup in these symbiotic fixations are fertilizer and soil. Common bean can fix from 20 to 60 kg $N.ha^{-1}$; however, this depends on the environment and plant management techniques (Chalk *et al*., 2014).

The protein digestibility of legumes has also been evaluated using the ^{15}N technique, which has yielded greater digestibility values than true digestibility (Kashyap *et al*., 2023). Non-nodulating soybeans are suitable after the initial growth stage, and the technique's accuracy depends on selecting appropriate non-fixing reference plants (Brito *et al*., 2009; Senthilkumar *et al.,* 2020). Scholars have employed ^{15}N isotope methods to investigate nitrogen fixation in Bambara groundnut (*Vigna subterranea*). Mohale *et al*. (2014) found that the Bambara groundnut added 4-200 kg $N.ha^{-1}$ of N-fixed, with 33-98% of its nitrogen coming from symbiotic fixation. They also found that plant growth, N_2 fixation, and water use-efficiency were positively correlated. In addition, according to Musa *et al*. (2016), nitrogen fixation varied between 32 to 81 kg N/ha. On another note, Belane *et al*. (2011) revealed that cowpea genotypes can fix large amounts of nitrogen. In field conditions, certain varieties have the potential to contribute up to 182 kg $N.ha^{-1}$, as measured using the ^{15}N natural abundance method to assess the contribution of atmospheric nitrogen to the total nitrogen content through biological fixation.

Conversely, Egbe *et al*. (2013) reported that intercropping in Nigeria decreased nitrogen fixation and nodulation in Bambara groundnuts, with fixation rates varying depending on the intercropping system, ranging from 11.27 kg $N.ha^{-1}$ to 34.90 kg $N.ha^{-1}$. These results emphasize the necessity of accounting for a range of criteria when assessing the efficiency of nitrogen fixation in leguminous crops. Symbiotic nitrogen fixation is essential to ecosystem health and sustainable agriculture. Rhizobia bacteria and legumes form symbiotic interactions that enable both organisms to fix atmospheric nitrogen into a usable form (Bruijn, 2019; Chellem *et al*., 2024). By using this method, less synthetic fertilizer is required, which reduces greenhouse gas emissions and minimizes environmental pollution (Goyal *et al*., 2021). Symbiotic nitrogen fixation increases crop yields, enrich soil fertility, and promote sustainable farming (Martyniuk *et al*., 2008; Chellem *et al*., 2024). Legumes and rhizobia have developed highly effective symbioses through intricate genetic interactions (Chellem *et al*., 2024). Addressing global food security and ensuring sustainable crop nutrition demands a thorough understanding and strong commitment to improving the microbial nitrogen cycle in soil (Hirsch and Mauchline, 2015).

CHAPTER 3

GROWTH AND YIELD RESPONSE OF THREE UNDERUTILIZED CROPS TO MULCHING TREATMENTS AND WATER USE

ABSTRACT

Africa ranks as one of the regions where agricultural systems are most negatively affected by climate change. The need for food is predicted to increase due to projected population expansion, which will influence agricultural water use. Projected growth in the global population is expected to raise food demand, which will in turn impact the use of water in agriculture. Enhancing food yield, increasing agricultural water efficiency, and reducing irrigation all depend on underutilized crops and creative crop-growing methods. The current study evaluated the impact of several mulch treatments on growth, yield, and water-use efficiency. Four replications of each treatment were employed in this study's randomized complete block design (RCBD). Three underused legumes (common bean, cowpea and Bambara groundnut) and three mulches (plastic, pine bark mulch, and no mulch) were treatments. The study was carried out at the University of Mpumalanga farm, and cultivation practices (such as irrigation and weeding) were followed. ANOVA was performed on the data using STATISTICA version 12.0. Regardless of the mulching treatments used, the findings revealed a significant increase in plant growth for the underutilized cowpea legume. Similarly, the chlorophyll content was highest for the cowpea legume crop compared to the other two legumes (Common bean and Bambara groundnut). Pods were recorded at their highest level in 91 DAP and at a moderate level in 78 DAP. Cowpea with plastic mulch (C-PM) had the greatest number of leaves per plant (17.31), with cowpea with pine bark ranking close (17.08). Throughout the growth season, stomatal conductance was higher; however, Bambara groundnut with plastic mulch showed a decrease.

Furthermore, cowpea with pine bark mulch treatment (168.55 g.plant^{-1}) had the highest increase in shoot dry matter, followed by cowpea with plastic mulch (168.35 g.plant^{-1}), contrasting to the two legume crops (Bambara groundnut and common bean), irrespective of the mulching treatments. Cowpea exhibited the highest WUE values, also accumulated the most significant shoot dry matter, and showed moderately negative $\delta^{13}C$ values in its roots and shoots. The common bean had the lowest WUE, the lowest biomass, and the most negative $\delta^{13}C$ signatures, confirming poor wateruse- efficiency. This study noted that pine bark mulch promotes plant development.The study shows a strong relationship between $\delta^{13}C$, WUE, and dry matter, which are closely linked, as they are all regulated by stomatal control and carbon assimilation.

Keywords: Carbon isotope, Chlorophyll, Mulching, Sustainable agriculture, and Underutilized.

3.1 INTRODUCTION

Underutilized legume crops are commonly known as “climate-smart crops” because of their ability to enhance agricultural resilience owing to the results of climate change, offering substantial advantages for use in sub-Saharan African farming systems (Dutta *et al*., 2022). In Africa, underutilized crops like cowpea and amaranth offer potential solutions for improving food security, enhancing nutrition, and promoting environmental sustainability (Popoola *et al*., 2023). Underutilized crops are primarily integrated into cropping systems by smallholder farmers in South Africa (Mabhaudhi *et al.,* 2017). Smallholder farmers often produce underutilized crops because the inclusion of these crops addresses issues of nutrition security, enhances the resilience and accessibility of existing agricultural food systems, and promotes community development objectives and livelihood initiatives (Mabhaudhi *et al*., 2017; Mugiyo *et al*., 2021).

The majority of underutilized crops, such as Bambara groundnut (*Vigna subterranea*) and cowpea (*Vigna unguiculata*), are rich in protein, essential minerals, vitamins, and fibre (Snapp *et al*., 2019). Underutilized crops have enormous ability to address global issues in sustainable agriculture, food security, and nutrition; however, they are frequently overlooked in conventional agriculture (Kaur *et al*., 2025). Underutilized legumes, such as Bambara groundnut (*Vigna subterranea*), are protein-dense and contain secondary metabolites that possess health-promoting properties and reduce disease risk (Odeku *et al*., 2024). Additionally, since they can fix nitrogen and have a beneficial effect on soil health, they are sustainable contributors to biodiversity conservation and environmental balance (Odeku *et al*., 2024). However, in South Africa, production levels of underutilized crops, such as Bambara groundnut, remain very low, and their status has been reduced. Their potential as herbal medicinal crops is of interest, and food generates new value chains involving marginalized groups, motivating the need to move toward sustainable food systems that are more resilient (Mudau *et al*., 2022). Previous research found that mulching improves water use-efficiency (WUE), thereby increasing yields. For instance, Abdelghany *et al.* (2025) revealed that "mulched plots showed better WUE (3.53 kg m^{-3}) compared to non-mulched plots (3.17 kg m^{-3}), with potassium bicarbonate application yielding the highest WUE (3.80 kg m^{-3})".

Approximately 70% of the freshwater used worldwide is utilized in agriculture, making it a key industry (Mishra *et al*., 2023). Livestock, irrigation, and agricultural product processing are among its uses. Climate change and its effects on water supply will make irrigation increasingly more crucial for food production in the future. Other minor but expanding agricultural water uses include freshwater aquaculture and livestock watering (Ringler *et al*., 2020). A significant obstacle is the escalating competition for water resources, increasing the potential hazards of floods and drought, and demand for food consumption (Biswas *et al*., 2025). To mitigate water-related risks to and from food systems, it is thus crucial to implement effective water management methods including mulching, deficit irrigation, and drip irrigation.

Additionally, an effective method for decreasing water stress in agriculture is the combined use of mulching and drip irrigation in arid crop cultivation (Bhargavi and Anusha, 2023). Applying materials to the surface of the soil to create a protective layer that preserves soil health, saves resources, and encourages crop growth is known as mulching (Pavlů *et al*., 2016). Mulches are used in agriculture for various purposes, but the two most crucial ones, mostly in dry and semi-arid locations, are water conservation and erosion prevention (Verma *et al*., 2024). Mulching is also used for weed control, soil conservation, adjusting soil temperature, improving soil structure, improving crop production and quality and, adding plant nutrients after organic mulch breaks down (Verma *et al*., 2024). However, a noticeable gap remains in research on the relationship between mulching practices and water conservation in underutilized crops, which limits their potential to contribute to sustainable food systems. For these crops to be repositioned within mainstream agricultural systems, consumers, smallholder farmers, and policymakers must become more aware of their advantages. The aim of the current study was to evaluate how different mulching treatments affected the three neglected crops growth, production, and wateruse -efficiency.

3.2 Materials and methods

3.2.1 Description of the study site

The University of Mpumalanga's experimental farm served as the site of the experiment, situated in Mbombela (Figure 3.1), Mpumalanga Province, at a latitude of 25.4371° S and longitude of 30.9818° E, during the 2024/2025 cropping season. The farm consists of loamy sand soil, where the experiment was performed. The average yearly temperature in Mbombela ranges from 19 to 29 ^{o}C, indicating a subtropical environment (South African Weather Service, 2024). Rainfall flows from October to March during summer, with an average of 800 mm of precipitation annually (South African Weather Service, 2024).

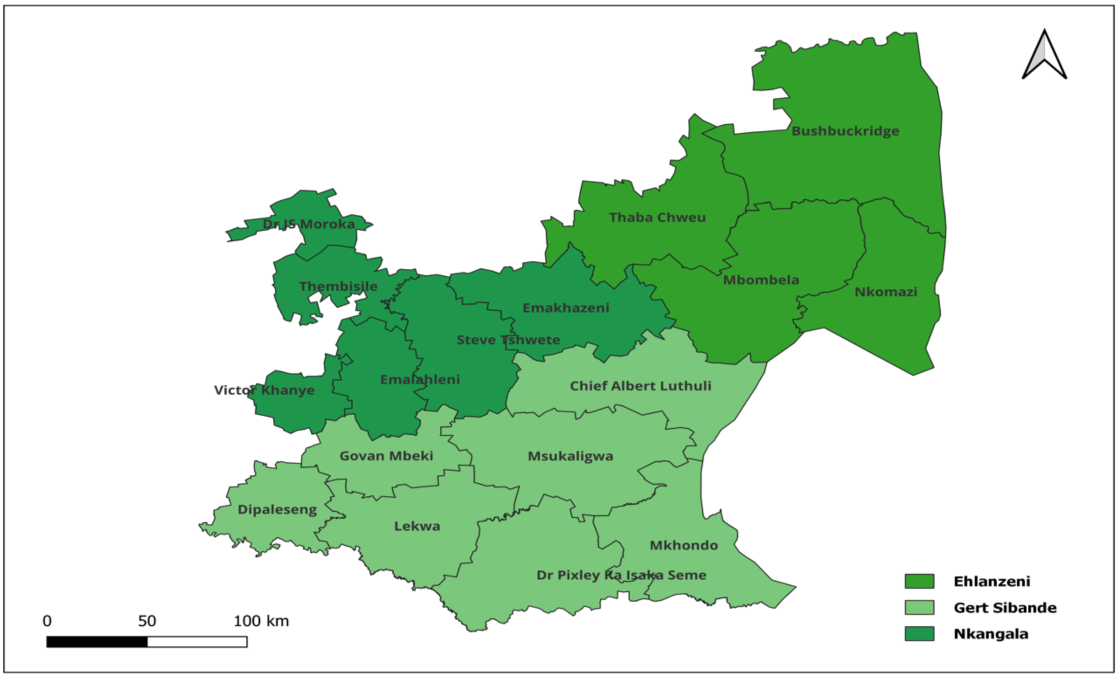

Figure 3-1: Map of Mpumalanga Province, highlighting Mbombela and surrounding municipalities.

3.2.2 Experimental design

A randomized complete block design (RCBD) with four replications was used in this investigation. The field was previously cropped with maize. The three crops (Cowpea, Bambara groundnut, and Common bean) and combined treatments used in this experiment are shown in Figures 3.2 and 3.3, respectively. The field was demarcated using the RCBD experimental design with four replications. The total plot size was 3 m × 1.5 m with a space of 30 cm between the plants and 60 cm between rows. Cultivation practices, including weeding and irrigation, were implemented throughout the cropping season. Prior to the mulch treatments were applied, small weeds were removed using a hand hoe and a hand fork. The mulching treatments were applied after the seedlings had fully emerged from the soil.

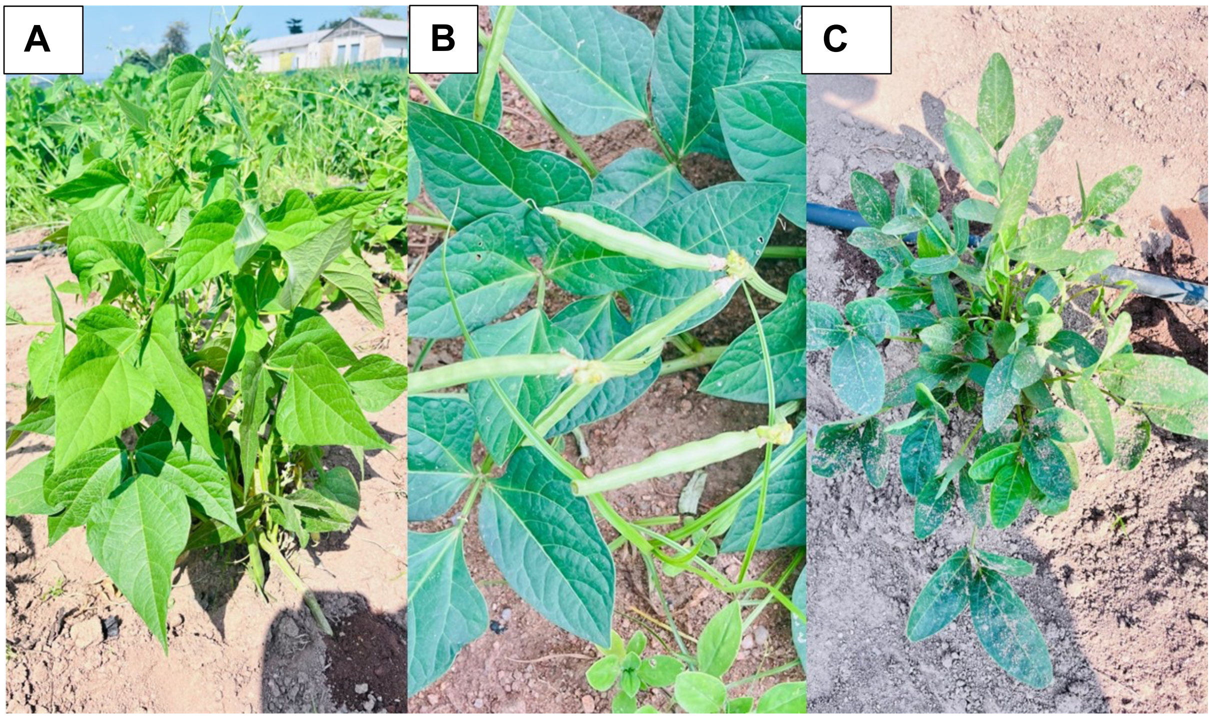

Figure 3-2: Underutilized legume crops (A: common bean, B: Cowpea, C: Bambara groundnut) used in the study in response to mulching treatments.

1. B-NM: Bambara groundnut-No mulch	2. B-PM: Bambara groundnut-Plastic mulch	3. B-PBM: Bambara groundnut-Pine bark mulch
4. C-NM: Cowpea-No mulch	5. C-PM: Cowpea-Plastic mulch	6. C-PBM: Cowpea-Pine bark mulch
7. CB-NM: Common bean-No mulch	8. CB-PM: Common bean-Plastic mulch	9. CB-PBM: Common bean-Pine bark mulch

Figure 3-3:Treatment combinations of underutilized legumes with mulching types used in the field experiment

3.2.3 Soil sampling

Using a soil auger (Volume ≈ 703.72 cm^3), to evaluate the physical and chemical properties, samples were taken from each plot at two depths: the topsoil layer (0–30 cm) and the subsoil layer (30–60 cm). The soil samples were collected both before and after the experiment to ascertain. Samples were analysed at the beginning and end of the experiment to establish a baseline and assess the effect of the different treatments. The collected soil samples were air-dried and sieved at room temperature in the Indigenous Knowledge Systems (IKS) science laboratory at the University of Mpumalanga to facilitate analysis. Atomic absorption spectroscopy and ammonium acetate extraction (1 N NH40Ac) were used in the analysis of the samples at the Agricultural Research Council's Soil, Climate and Water (ARC-SCW, South Africa).

3.2.4 Data collection

Plant growth parameters, such as plant height and the number of leaves per plant, were recorded during the 2024/2025 cropping season. The physiological data that was collected included stomatal conductance and chlorophyll content.

3.2.4.1 Growth parameters

The height of each plant, from the crown to the flag leaf tip, was determined using a measuring tape, and the total number of leaves was manually recorded twice monthly.

3.2.4.2 Leaf chlorophyll content

Chlorophyll Content Meter (CCM-200 plus Opti-Sciences, Inc., Hudson, NY, USA) was used twice a month to quantify the amount chlorophyll in the fully expanded flag leaves. Measurements were taken from six sampled plants per plot across the four replicates.

3.2.4.3 Stomatal conductance

A leaf porometer (SC-1 LEAF POROMETER, Model SC-1, METER Group, Inc., Pullman, WA, USA) was used to measure stomatal conductance once a month. At midday, when the environmental conditions were at their best, mature leaves that had fully expanded for each treatment were measured on the abaxial (bottom) side. For this measurement, six data plants from each of the four replicates were utilized. The minimum amount of time needed for the readings to stabilized before recorded was about four minutes for each measurement.

3.2.4.4 Carbon isotope analysis

Plant sampling for isotopic analysis

Typically, ten plant species near physiological maturity were carefully sampled from each plot's middle row. Prior to ^{13}C analysis, the shoots were weighed, ground (0.45 mm), oven dried at (60 ^{o}C) and then stored in vials. The reference plants underwent the same ^{13}C analysis procedures as the cowpea, Bambara groundnut, and common bean.

Measurement of ^{13}C and %C

A Carlo Erba NA Elemental Analyzer connected to a Finnigan MAT 252 Mass Spectrometer (Finnigan MAT GmbH, Bremen, Germany) via Conflo II Open-Plit Device was loaded with approximately 2.0 mg of the underutilized samples, 2.0 mg of the reference plant samples, and standards (Acacia, valine, sucrose, and ammonium chloride) in tin capsules. The analyzer was then run to find the plant materials' $^{13}C/^{12}C$ ratio. The machine errors associated with isotopic fractionation were addressed using an internal standard of Nasturtium spp. It was added following every five plant sample runs. The ^{13}C natural abundance was calculated as follows, as described by Unkovich *et al*. (2008). The widely recognized benchmark from the Belemnite Pee Dee limestone formation was the isotopic ratio of Belemnite Pee Dee (BPD). Mass spectrometric analysis was used to determine the content (%C) in the plant samples. The product of the C concentration and dry matter weight of the leaves was used to determine the C content per plant.

The ^{13}C natural abundance, or $\delta^{13}C$ (‰), was calculated as (Farquhar, Hubick, Condon and Richards, 1989):

$$\delta^{13}\mathrm{C} = \left[\frac{(^{13}\mathrm{C}/^{12}\mathrm{C})_{\mathrm{sample}}}{(^{13}\mathrm{C}/^{12}\mathrm{C})_{\mathrm{standard}}} - 1\right] \times 1000$$

where $^{13}C/^{12}C_{sample}$ is the isotopic ratio of the sample and $^{13}C/^{12}C_{standard}$ is the isotopic ratio of PDB, a universally accepted standard from the Belemnite Pee Dee limestone formation (Craig, 1957). While the carbon content per plant was computed as the product of the carbon concentration and the dry matter weight of the leaves, the carbon concentration (%C) in the plant samples was directly determined by mass spectrometric analysis.

3.2.6. Data analysis

Prior being subjected to statistical analysis using the STATISTICA software program version 12.0., all obtained data were examined and determined to have a normal distribution. The data were analyzed using Statistica software, version 12.0 (StatSoft, Inc., Tulsa, OK, USA). All data were subjected to One-way ANOVA to compare the growth parameters of three underutilized legume crops (Cowpea, Bambara groundnut, and Common bean), and Duncan's Multiple Range Test (DMRT) was used to distinguish the means at $p \leq 0.05$. Bar graphs and tables were constructed using Microsoft Excel.

3.3 Results and Discussion

3.3.1 Soil analysis results

The soil analysis results collected before planting showed increased values for elements calcium, magnesium, sodium, and phosphorus, as presented in Table 3.1. The increase in the reported elements in the soil before planting implies that the concentration of these elements was high. The concentration of components such as calcium, magnesium, sodium, and phosphorus is supported by the finding that for the soil to remain fertile, it requires the presence of these essential elements (White and Brown, 2010). Jakovljević *et al*. (2003) reported that deficiencies in calcium, magnesium, sodium, and potassium are not expected to constitute a problem for plant nutrition, as the soils under investigation are well to moderately supplied with these elements. For growth and nourishment, plants need macronutrients from the soil, such as calcium, magnesium, and phosphorus (White and Brown, 2010). Nguyen *et al*. (2017), revealed that the ratio and quantity of nutrients in the soil determine how well plants absorb nutrients. Integrating organic materials like organic fertilizers, manure, and mulch can increase the concentration of specific macronutrients and enhance productivity.

The soil analysis data at the end of the experiment indicated high levels of calcium (217.0 $mg.kg^{-1}$), magnesium (53.9 $mg.kg^{-1}$), potassium (18.2 $mg.kg^{-1}$), and phosphorus (16.9 $mg.kg^{-1}$) as presented in Table 3.1. The findings of present study found a significant decrease in the pH level from 6.7 before planting to 4.2 after planting. Previous studies support these results; according to Wang *et al.* (2023), intercropping tea with legumes resulted in a much lower pH value than monocropping. Shen *et al*. (2024) also noted a significant reduction in the pH level of *C. esculentus* when intercropped with legumes. The decrease in pH indicates soil acidification, which is likely driven by legume nitrogen fixation. Legumes improve nitrogen fixation and lower pH via increasing nitrogen build up, which explains these findings (*Chen et al*., 2023; Liu *et al*., 2023).

The study noted a decline in the phosphorus level (34.0 mg.kg^{-1}) before planting to (16.9 mg.kg^{-1}) after planting. These results indicate that legumes, such as cowpea, common bean, and Bambara groundnut, are highly effective in acquiring phosphorus. The results of the study are consistent with comparable patterns described by He *et al*. (2024), who reported similar trends in wheat-faba bean systems, where the amount of phosphorus in the soil decreased after the growth cycle. These results suggest that the availability of macronutrients is increased by the presence of a greater amount of organic matter in the soil. The concentration of mineral elements in edible crops can be increased using traditional agronomic strategies, such as the use of mulch and cultivating underutilized legume crops.

Table 3-1: Soil samples collected prior to planting and after harvest during the 2023/2024 and 2024/2025 seasons were analyzed.

Elements	Before planting (30-60 cm)	After planting (30-60 cm)
	mg.kg^{-1}	
C	0.41	0.28
Ca	278.8	217.0
Cu	25.8	13.7
Fe	11.3	11.3
K	28.0	18.18
Mg	87.9	53.9
Mn	20.9	14.2
Na	45.4	8.2
P	34.0	16.9
pH	6.7	4.2
Zn	27.0	12.5

3.3.2 Growth parameters

3.3.2.1 Plant height in response to treatments

The data present two-way ANOVA that is highly significant for the plant height and number of leaves per plant (Table 3.2). This section documents the plant height of the three underutilized crops evaluated in response to mulching treatments, as presented in Figure 3.4. The treatment combination of common bean with pine bark (CB-PBM) showed the highest plant height (47.07 cm), followed by the treatment combination of cowpea with pine bark mulch (44.29 cm). The findings indicate that pine bark mulch significantly increased the height of both cowpea and common bean plants. This is attributed to improved water retention, moderating soil temperature, improving soil structure, and providing nutrients. These combined effects lead to increased plant height, biomass accumulation, and overall growth in the two legume crops (Rossi *et al*., 2024). Evidence from this study agree with those of Ramadhani *et al*. (2024), who confirmed that mulching significantly improved common bean growth, yield, and economic feasibility. The results of the study further exhibited increased plant height for the treatment combination of cowpea with no mulch (41.84 cm) and cowpea with plastic mulch (41.79 cm). This was most likely caused by moisture held beneath the plastic mulch, which provided the root zone with ideal temperatures for nutrient absorption. The plants utilised the available nutrients for vegetative development; more nutrients were available for uptake by the plants for most of the time, as there was likely less nutrient volatilization in the plastic mulch (Kwambe *et al*., 2015). These results are consistent with those of Tarara (2009), who found that warm roots promoted faster plant development. The results of the current study are correspond with those of Zairani *et al.* (2023), whom stated that black plastic mulch produced increased plant height in chilli (*C. annuum*) plants. Mulching the soil improves the microclimate and boosts crop production, according to Demo and Asefa (2024). This study also noted that all the legume crops under plastic mulch had no weeds (Figure 3.4). These results concur with those of Marble *et al*. (2019), who reported that over 90% fewer weeds were observed when plastic mulch was used, which indirectly promotes improved plant growth by reducing competition for resources. These results match those of Kwambe *et al*. (2015), who claimed that plastic mulch served as a barrier against weeds. The variations in plant height under different mulching treatments demonstrated the crucial role of mulch type in maximising plant growth.

The results of this study align with those of Maseta *et al*. (2023), who found that regardless of the type and rate of application, mulches increased the amount of chlorophyll in cowpeas and improved agronomic traits, including plant height and stem diameter. The treatment combination of common bean with plastic mulch (39.35 cm) and without mulch (35.03 cm) resulted in similar plant height regardless of the mulching treatment presented in Figure 3.4. These results were followed by the treatment combination of Bambara groundnut with pine bark mulch (16.96 cm) and without mulch (16.50 cm). Bambara groundnut with plastic mulch had the lowest plant height (11.48 cm); in this case, the plastic mulch hindered plant growth in Bambara groundnut. These findings suggest that the low Bambara groundnut height is due to root zone overheating and reduced gas exchange, as Bambara groundnut is a drought-tolerant crop; however, it is a heat-sensitive legume. Whereas mulching using plastic mulch can significantly increase crop yield and aboveground biomass, particularly in arid and semi-arid areas, its effectiveness is contingent upon various factors, including crop type and growing season precipitation (Zhang *et al.,* 2019). The results of this investigation correspond with those of previous studies; for instance, Gu *et al*. (2024) reported that plastic film mulch treatment in faba beans was more sensitive, leading to decreased crop growth and above-ground biomass. The results of this study revealed that pine bark mulch had no significant effect on plant height for either common bean or cowpea.

Table 3-2: Two-way ANOVA of plant height and number of leaves for three underutilized crops and different mulch treatments

	Degree of freedom	Plant height				Number of leaves			
		SS	MS	F	P	SS	MS	F	P
Intercept	1	2180123	2180123	7649.54	0.000	283213.7	283213.7	18667.77	0.000
Mulch type	2	8992	4496	15.77	0.000	1078.9	539.4	35.56	0.000
Crop	2	316331	158165	554.96	0.000	25817.5	12908.8	850.87	0.000
Mulch type × crop	4	8604	2151	7.54	0.000	794.7	198.7	13.10	0.000
Error	1935	551476	285			29356.4	15.2		
Total	1943	885880				57104.9			

Bars followed by dissimilar letters are significantly different at $p \leq 0.05$. Vertical lines on bars represent S.E. (n=9).

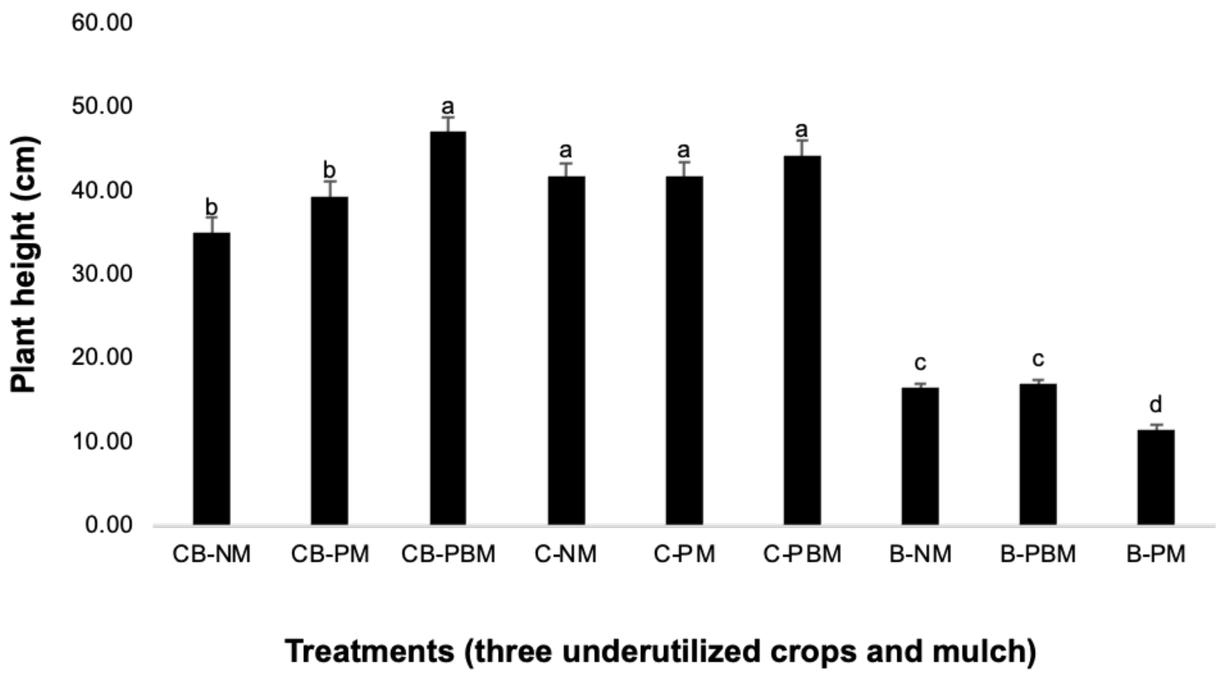

Figure 3-4: Plant height responses to the different treatments, comprising three underutilized crops and mulching practices. Error bars indicate standard error (n=9), and bars with different letters differ significantly at p≤0.05.

3.3.2.2 Plant height of three underutilized crops

Figure 3.5 indicates the impacts of different cropspecies on the plant height of the three underutilized crops. Figure 3.5 recorded the highest plant height for cowpea compared to the other two legume crops. Significant variation in plant height was observed across the three underutilized crops. Plant height ranged from 15.41 cm for Bambara groundnut to 44.35 cm for cowpea (Figure 3.5). The findings show that plant height gradually increase from one crop species reaching its highest peak of 44.35 cm, compared to the heights of the other crops. Onyeneke *et al*. (2022) observed similar findings, noting a noticeable increase in cowpea plant height during the vegetative stage from the first two weeks to the fifth week. However, there was a slight decline in plant height at 130 DAP. This decline in plant height might be due to the plants' ageing and reaching maturity.

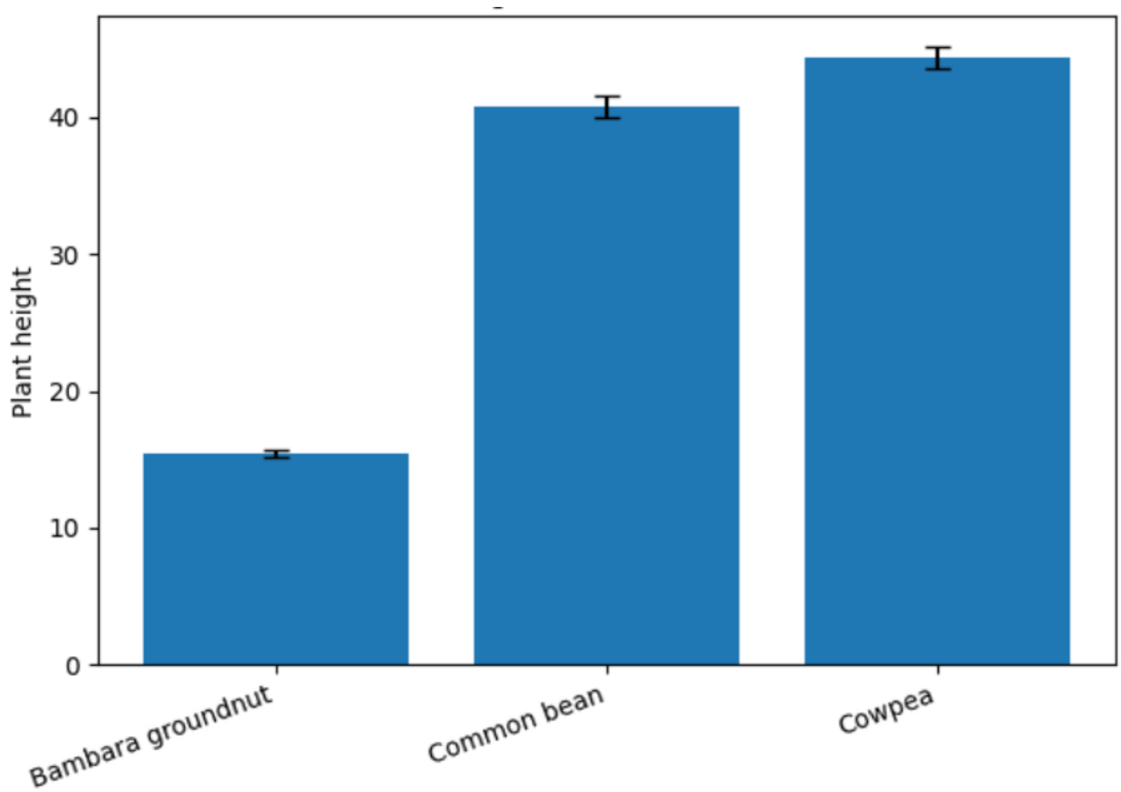

Figure 3-5: Plant height (cm) of three underutilized crops. Error bars indicate the standard error of the mean (n=9), and bars with different letters differ significantly at p≤0.05.

3.3.2.3 Number of leaves of three underutilized legumes in response to mulching

The research assessed how leaf production throughout the growth season was influenced by the different treatments, as illustrated in Figure 3.6. Among the treatments, cowpea grown with plastic mulch (C-PM) produced the most leaves per plant (17.31), with the cowpea planted with pine bark mulch following closely (17.08). Similar trends were observed by Orji *et al*. (2018), who found that plastic mulch significantly increased leaf numbers compared to untreated plants. In this study, however, cowpea plants under plastic mulch (17.31 leaves) and pine bark mulch (17.08 leaves) exhibited comparable leaf counts. Chi *et al.* (2022) found that mulching treatments, such as plastic mulch, compared to sawdust and elephant grass mulch, enhanced growth metrics, including plant height, leaf area, and pod yield, of green beans (*Phaseolus vulgaris* L). Cowpea exhibited a high number of leaves per plant across all treatments. Regardless of the mulching treatment, the high leaf number across all treatments was attributable to cowpea being one of the most drought-tolerant crops, capable of maintaining leaf area and biomass production even under limited conditions (Souza *et al*., 2017). Pine bark significantly increased the number of leaves per plant in all three underutilized legume crops. Legume crops, common bean (9.18) and Bambara groundnut (8.04), without mulch, showed a moderate number of leaves per plant. However, the number of leaves per plant in Bambara groundnut were decreases by plastic mulch. The number of leaves generated by a plant can be interpreted in relation to the physiological roles that each leaf plays, along with temperature and moisture gradients. These results suggest that both plastic and pine bark mulches improve agronomic parameters by maintaining moisture retention, suppressing weeds, and enhancing nutrient availability. These conditions enable legume crops to produce more leaves, resulting in higher crop yields and more sustainable agricultural practices.

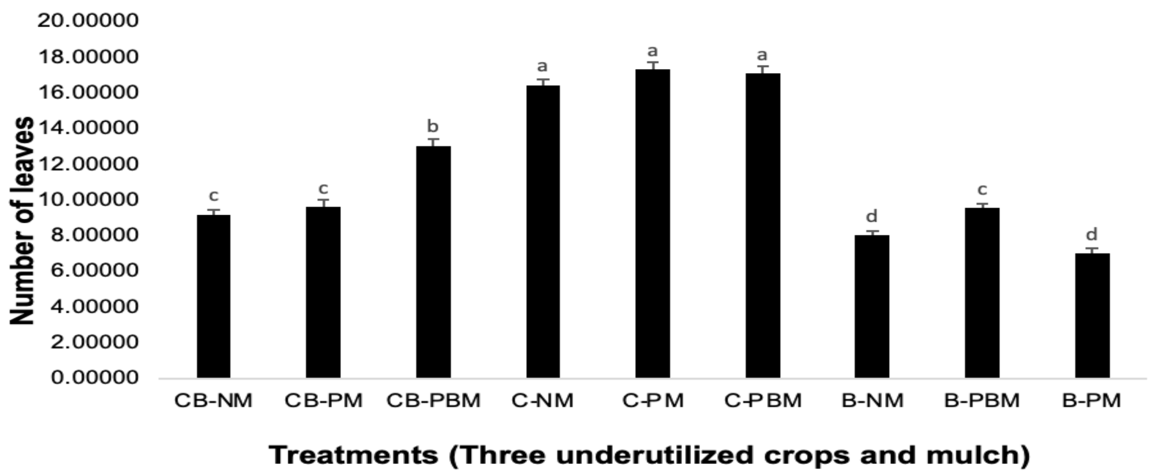

Figure 3-6: Number of leaves as influenced by the different treatments. Error bars represent the standard error (n=9), and bars with different letters indicate significant differences at p≤0.05.

3.3.2.3 Number of pods for different mulch treatments

The number of pods of the three underutilized crops has been reported in this study (data not shown). Pods were recorded at their highest level in 91 DAP and at a moderate level in 78 DAP. Pod count was markedly influenced by the days of the trial, as there was a considerable decline in the number of pods from 114 DAP to 130 DAP. In our study, the common bean with pine bark mulch (CB-PBM) had the highest number of pods in the growing season. A valid reason for the success of pine bark mulch is the higher rate of decomposition. Bhattarai *et al.* (2023) reported that rice husk mulch (organic mulch) yielded a noticeably larger number of pods, 29 per plant, with the highest benefit-to-cost ratio. These results confirm that organic mulch can significantly increase the number of pods. Comparable findings were reported by Junior *et al*. (2020), who demonstrated that mulching cowpea plants enhances their shoot dry weight, pod length, and seed quality per pod. Abdelrahma *et al*. (2016) confirmed that plastic mulch in cowpea contributed to increased pod formation per plant and improved grain output, corresponding to 17%, 30%, and 10% increases, respectively.

3.3.3 Chlorophyll content parameters

3.3.3.1 Chlorophyll content in response to mulching treatments

The effects of different mulching treatments on the chlorophyll content of three underutilized crops are shown in Table 3.3. The treatments with the highest chlorophyll content (44.39) were recorded for the treatment cowpea without mulch (C-NM), followed by cowpea with plastic mulch (C-PM) at (43.39) and pine bark mulch (C-PBM) at (43.19). The findings suggest that cowpea can naturally maintain high chlorophyll levels, regardless of whether mulch is applied. This was attributed to the fact that cowpea has adapted leaf morphology that optimizes light capture and minimizes water loss (Digrado *et al*., 2020). Similar trends were observed in previous studies; for instance, Poudel *et al*. (2025) reported that in cowpea plants under drought stress, stomatal conductance decreased by 79%, which was inversely correlated with a 2.9°C rise in canopy temperature, indicating a robust water conservation response. The findings of this study further support our speculation that cowpea, an underutilised legume crop, possesses mechanisms that can improve food security in Africa. Studies revealed that cowpea possesses physiological characteristics that contribute to its high chlorophyll content (Boukar *et al*., 2019). The resilient chlorophyll content across several genetic lines was demonstrated, this is supported by Dong *et al*. (2019), which assessed 155 cowpea genotypes under non-stressful conditions. The study found that SPAD chlorophyll values ranged from 31.27 to 60.68, with a mean of 44.10. Plant height and fresh shoot biomass were considerably decreased by salt stress. In contrast, chlorophyll content was slightly enhanced (Dong *et al*., 2019). These results demonstrate how breeding programs can enhance cowpea resilience to saline conditions by leveraging physiological features associated with salt tolerance (Akter *et al*., 2021).

In contrast, the common bean without mulch application had the lowest chlorophyll content (16.16), while the use of pine bark mulch (CB-PBM) improved its chlorophyll content (17.72). The findings align with previous studies, for instance, Khalifa *et al*. (2024) reported that in both growing seasons, different types of organic mulch (Rice straw and sawdust) demonstrated noticeably increased levels of chlorophyll and plant height when compared to the un-mulched treatment in common bean. The effect of mulching on chlorophyll content was more pronounced in common bean, where pine bark mulch was more beneficial than plastic mulch, which slightly reduced chlorophyll content (15.87). This study demonstrate that organic mulch enhances greenness and photosynthetic performance in common beans. Masete *et al.* (2022) reported that regardless of the type and rate of application, mulching increased the amount of chlorophyll in cowpeas and improved agronomic characteristics, including plant height and stem diameter. In comparison to plastic mulch, the absence of mulch increased the levels of chlorophyll in Bambara groundnuts (21.14). The reported values for Bambara groundnut ranged from (15.81) to (19.58) for treatments Bambara groundnut with plastic mulch and Bambara groundnut with pine bark mulch, as illustrated in Table 3.2.

Table 3-3: Effects of mulching practices on the chlorophyll content of three underutilized crops. Mean values ± standard error are shown, and differences are significant at p≤0.05.

Treatments	Chlorophyll content
CB-NM	16.16±0.71[c]
CB-PM	15.87±0.61[c]
CB-PBM	17.72 ±0.61[c]
C-NM	44.39± 1.78[a]
C-PM	43.19 ±1.69[a]
C-PBM	43.52 ±1.97[a]
B-NM	21.14± 1.13[b]
B-PBM	19.58 ±0.88[b]
B-PM	15.81± 1.14[c]

CB-NM: Common bean-no mulch, CB-PM: Common bean-plastic mulch, CB-PBM: Common bean-pine bark mulch, C-NM: Cowpea-no mulch, C-PM: Cowpea-plastic mulch, C-PBM: Cowpea-pine bark mulch, B-NM: Bambara groundnut-no mulch, B-PBM: Bambara groundnut-pine bark, and B-PM: Bambara groundnut-plastic mulch.

3.3.3.2 Chlorophyll content at different Days After Planting (DAP)

The results in Table 3.4 illustrate the change in chlorophyll content in plant leaves over the growing season. Chlorophyll content can be used to evaluate photosynthetic capacity and is a sensitive indicator of crop physiological state (Kancheva *et al*., 2014). The concentration was moderate at 59 DAP (25.11), indicating that the leaves were in their early stage of leaf growth and were actively engaged in photosynthesis. At 72 DAP, the chlorophyll content increased slightly (25.56), and at 78 DAP, it showed a notable peak (32.37), indicating peak photosynthetic activity. Supporting evidence is provided by Hendriyani *et al*. (2018), who observed that the highest concentration of chlorophyll in cowpeas was observed during the vegetative phase, four weeks after planting. The findings of this study concur with those of Olorunwa *et al*. (2022), who highlighted that cowpea is the most sensitive crop to waterlogging. Despite this, it remained tolerant during the vegetative stage, which demonstrates resilience in its photosynthetic traits. 91 DAP showed a slight drop (25.05) after this high, which could be related to ageing leaves, environmental stress, or a decrease in nutritional availability. 114 DAP showed a minor recovery in chlorophyll content (27.81), which may have been attributed to plant adaptability. However, this recovery was temporary, as 130 DA had the lowest levels of chlorophyll (22.36). This view of these results is supported by Sheikh *et al*. (2017), who reported that chlorophyll content in leaves declines rapidly when plants are under stress and during leaf senescence. These findings indicate that a range of variables cause plants' chlorophyll concentration to fluctuate significantly over time. These results also emphasize the crucial role of chlorophyll concentration as an indicator of crop yield and vigour.

Table 3-4: Chlorophyll content of three underutilized crops measured at different days after planting (DAP) under various mulching treatments. Data are presented as mean±standard error, with significance at p≤0.05.

DAYS AFTER PLANTING (DAP)	CHLOROPHYLL cONTENT
59 DAP	25.11±1.15[c]
72 DAP	25.56±1.05[c]
78 DAP	32.37±1.59[a]
91 DAP	25.05±1.00[c]
114 DAP	27.81±1.73[b]
130 DAP	22.36 ±1.23[d]

3.3.4 Stomatal conductance of three underutilized legumes in response to mulching

Stomatal conductance of the three underutilized legume crops was evaluated in response to mulching treatments as presented in Table 3.5. Cowpea with plastic mulch (C-PM) showed the highest stomatal conductance (447.74 mol m^{-2} s^{-1}), followed by cowpea without mulch (C-NM) with (435.65 mol m^{-2} s^{-1}), and the cowpea with pine bark mulch (C-PBM). Cowpea had the highest stomatal conductance among the three legume crops, regardless of the mulching treatment. The higher stomatal conductance concentration observed in the cowpea legume crop is associated with stomatal opening and water availability (Costa *et al*., 2020). Farzi and Gholami (2018) reported that different types of mulch, including plastic and organic materials, can enhance soil moisture retention and regulate soil temperature, resulting in increased stomatal conductance in olive plants. Bambara groundnut without mulch (B-NM) had the lowest stomatal conductance (277.41 mol m^{-2} s^{-1}), which may be due to limited gas exchange. Similar trends were documented by Zhao *et al*. (2014), who noted that the closure of stomata, which restricts water loss through transpiration and lowers intracellular carbon dioxide (CO_2) availability, is associated with the decreased stomatal conductance observed for Bambara groundnut with no mulch (B-NM). However, plastic mulch increased the stomatal conductance of Bambara groundnut (423.03 mol m^{-2} s^{-1}), the same as pine bark mulch in Bambara groundnut (405.76 mol m^{-2} s^{-1}). Similar results were reported by Li *et al*. (2023), who found that plastic film mulch improves temperature regulation and water retention, altering biophysical conditions and increasing stomatal conductance in maize. Plastic mulch is 12-38.9% more effective than organic mulch at lowering evaporation rates and improving water use efficiency (Saputra *et al*., 2025). The results showed significant variations. The common bean with pine bark mulch (383.49 mol m^{-2} s^{-1}) and the common bean without mulch (384.49 mol m^{-2} s^{-1}) had slightly similar results, but this is unlike the observations by Liu *et al*. (2023) ,who documented that straw mulch significantly increased stomatal conductance up to 44.0% in soybean and maize compared to no mulch. However, plastic mulch in common bean reduced the stomatal conductance to (351.37 mol m^{-2} s^{-1}). Mulching increases soil moisture, enhancing photosynthetic efficiency and gas exchange. However, compared to non-mulched environments, it may reduce biological nitrogen fixation in legumes (Masete *et al*., 2022).

The results in Table 3.5 indicate the effect of temperature variation on soil condition under different mulch and crop treatments. Bambara groundnut without mulch (B-NM) had the highest temperature (36.79 °C). Soils without mulch are directly exposed to sunlight, resulting in higher temperatures in the crops without mulch, followed by both

Bambara groundnut with pine bark mulch (B-PBM) and Bambara groundnut with plastic mulch (B-PM). Bambara groundnuts had the highest temperature regardless of the mulching treatments. Crops with pine bark mulch, both common bean and cowpea, had moderate temperature CB-PBM (33.70 °C) and C-PBM (33.23 °C). However, plastic mulch maintains a mild temperature in both crops, common bean (32.83°C) and cowpea (32.96°C). In cowpea, cowpea with plastic mulch (C-PM) had the highest temperature compared to cowpea without mulch (C-NM). These results are similar to those of a study conducted by Singh and Kamal (2012), who reported that the black polyethylene mulch had the highest soil temperature, which was 2.2-3.4 °C warmer than that of bare soil. Hong *et al*. (2023) reported that solarization with plastic mulch increased soil temperatures compared to no mulch, particularly in the 0- to 20-cm soil layer. The temperature of the soil under plastic mulch is determined by the material's thermal characteristics, such as its conductivity, reflectance, and absorptivity, in proportion to the incoming solar radiation. The soil temperature under plastic mulch is typically high, and it also depends on the colour of the plastic mulch (Bahadur *et al*., 2018). In the present study, black mulch increased the soil temperature. Komariah *et al*. (2021) documented that mulching increases soil temperature, with the results verifying the order from highest to lowest: transparent plastic mulch, straw, black plastic mulch, control, and biodegradable plastic mulch.

Table 3.5 evaluated the effect of treatments (mulch and three underutilized crops) on the leaf sensor. Cowpea with plastic mulch (C-PM) resulted in higher (66.32%) relative humidity. Plastic mulch has the highest value, attributed to its ability to enhance soil organic matter and nitrogen levels, reduce soil erosion and compaction, moderate soil temperature, and improve soil moisture retention (Demo and Bogale, 2024). The results show that plastic mulch increases the relative humidity of all the crops, with cowpea recording the highest increase in relative humidity. These results were followed by cowpea with pine bark mulch (C-PBM), with a moderate (64.60%) relative humidity around the leaf surface, and moderate relative humidity was also recorded for common bean with pine bark mulch (CB-PBM). Bambara groundnut without mulch (B-NM) showed the lowest relative humidity (57.31%), indicating that the absence of mulching leads to moisture loss around the leaf surface. These observations correspond with those of Zribi *et al*. (2011), who noted that, compared to bare soil, organic mulches such as straw, grass, pine bark, and wood chips can successfully regulate temperature variations in the soil and sustain higher humidity levels.

Table 3-5: Stomatal conductance of three underutilized crop species as influenced by different mulching treatments.

Treatments	Conductance (mol/m^{-2} s^{-1})	Temperature (°C)	Leaf sensor (% RH)
CB-NM	384.49±32.41^{b}	32.91± 0.76^{b}	62.34 ±1.43^{a}
CB-PM	351.37±27.74^{b}	32.83± 0.65^{b}	61.47± 1.54^{a}
CB-PBM	383.49± 29.67^{b}	33.70 ±0.61^{b}	63.11± 1.29^{a}
C-NM	435.65± 30.78^{a}	32.84 ±0.90^{b}	64.33± 1.36^{a}
C-PM	447.74± 25.56^{a}	32.96 ±0.78^{b}	66.32± 0.80^{a}
C-PBM	434.23± 28.56^{a}	33.23 ±0.90^{b}	64.60± 1.38^{a}
B-NM	277.41± 24.82^{c}	36.79± 0.42^{a}	57.31± 1.88^{b}
B-PBM	405.76± 36.36^{a}	36.61 ±0.44^{a}	62.73± 1.58^{a}
B-PM	423.03 ±42.64^{a}	36.62 ±0.71^{a}	63.61± 1.37^{a}

CB-NM: Common bean-no mulch, CB-PM: Common bean-plastic mulch, CB-PBM: Common bean-pine bark mulch, C-NM: Cowpea-no mulch, C-PM: Cowpea-plastic mulch, C-PBM: Cowpea-pine bark mulch, B-NM: Bambara groundnut-no mulch, B-PBM: Bambara groundnut-pine bark, and B-PM: Bambara groundnut-plastic mulch. Mean values and ±standard error, p≤0.05.

3.3.5 Shoot dry matter of three underutilized legumes in response to mulch treatments

Mulching type had a significant effect on the shoot dry matter of the three underutilized crops. Cowpea with pine bark mulch treatment (168.55 g.plant^{-1}) had the highest increase in shoot dry matter, followed by cowpea with plastic mulch (168.35 $g.plant^{-1}$), compared to the two other legume crops (Common bean and Bambara groundnut), irrespective of the mulching treatments. Common beans were moderate, ranging from CB-PBM (24.85 $g.plant^{-1}$) to CB-PM (16.22 $g.plant^{-1}$); however, common beans without mulch were the lowest (14.01 $g.plant^{-1}$). In addition to this, Bambara groundnut pine bark was (25.21 $g.plant^{-1}$), and a decline was noted in the treatment of Bambara groundnut with plastic mulch (13.16 $g.plant^{-1}$). The decline can be related to the senescence of the lower leaves (Graça *et al*., 2010). The total fresh weight and shoot dry matter of common beans showed minimal differences. The findings indicate that using mulching increased the dry matter of the three underutilized crops; however, a decline was noted in treatments without mulch in all three legume crops. These results corroborate the findings of Selamolela *et al*. (2020), who demonstrated that mulching influenced shoot dry matter yield, grain yield, and water use efficiency of cowpea compared to un-mulched plots. The increase in shoot dry matter of treatments covered with mulch was attributed to the general knowledge that organic mulch adds nutrients to the soil through decomposition and that plastic mulch conserves soil moisture and increases soil temperature, which gives plants the most favourable conditions to grow, plastic mulch also minimized the competition from weeds (Mahmoud *et al*., 2021). This study emphasises the importance of using mulching to enhance the productivity of underutilized crops, thereby contributing to food security.

Table 3-6: Shoot dry matter accumulation in three underutilized legume species under different mulching treatments.

Treatments	Shoot dry matter ($g.plant^{-1}$)
CB-NM	14.01±0.37[e]
CB-PM	16.22±0.57[d]
CB-PBM	24.85±0.46[c]
C-NM	47.30±1.23[b]
C-PM	168.35±1.77[a]
C-PBM	168.55±1.88[a]
B-NM	10.71±0.43[f]
B-PBM	25.21±1.00[c]
B-PM	13.16±0.65[e]

CB-NM: Common bean-no mulch, CB-PM: Common bean-plastic mulch, CB-PBM: Common bean-pine bark mulch, C-NM: Cowpea-no mulch, C-PM: Cowpea-plastic mulch, C-PBM: Cowpea-pine bark mulch, B-NM: Bambara groundnut-no mulch, B-PBM: Bambara groundnut-pine bark, and B-PM: Bambara groundnut-plastic mulch. Mean values and ±standard error, p≤0.05.

3.3.6 Water use-efficiency of three underutilized legumes in response to mulch treatments

Table 3.6 illustrates three underutilized crops in response to water use-efficiency water use. Cowpea significantly had the highest water use regardless of the mulching treatments; nonetheless, no meaningful differences were recorded among the cowpea treatments. This was attributed to the fact that cowpea had the highest stomatal conductance across all treatments. These results were followed by the treatment of Bambara groundnut without mulch (0.08 μmol CO_2 $mmol^{-1}$ H_2O). Despite having a large canopy and high biomass, Bambara groundnuts still require a significant amount of water. Under water-limited conditions, Bambara groundnut exhibited a favourable trait; Bambara groundnut without mulch had the lowest stomatal conductance. This may have detrimental effects on biomass yield, but it is a positive feature because it saves water. The reduced canopy concealed the water conservation caused by low stomatal conductance (Chibarabada *et al*., 2019). It has been demonstrated that several drought tolerance mechanisms support Bambara groundnut's high water use-efficiency (WUE) (Chibarabada *et al*., 2015).

Additionally, the treatments for common beans and Bambara groundnuts did not vary. Common bean pine bark treatments and plastic mulch were significantly the same (0.05 µmol CO_2 $mmol^{-1}$ H_2O). This was attributed to the fact that both growth conditions were favourable for the common bean, due to fewer weeds, reduced evaporation, and the fact that mulching conserved soil moisture (Iqbal *et al*., 2020). These outcomes are in line with those documented by Barros *et al*. (1993), who indicated that mulched plots had higher water use-efficiency compared to no mulch plots.

Table 3-7: Effects of water management on three underutilized legume species.

Treatments	WUE (µMOL CO_2 $MMOL^{-1}$ H_2O)
CB-NM	0.04c
CB-PM	0.05^{b}
CB-PBM	0.05^{b}
C-NM	0.10^{d}
C-PM	0.10^{d}
C-PBM	0.10^{d}
B-NM	0.08^{a}
B-PBM	0.05^{b}
B-PM	0.04^{c}

CB-NM: Common bean-no mulch, CB-PM: Common bean-plastic mulch, CB-PBM: Common bean-pine bark mulch, C-NM: Cowpea-no mulch, C-PM: Cowpea-plastic mulch, C-PBM: Cowpea-pine bark mulch, B-NM: Bambara groundnut-no mulch, B-PBM: Bambara groundnut-pine bark, and B-PM: Bambara groundnut-plastic mulch.

3.3.7 Carbon isotope

The study reveals a clear physiological connection between biomass production, water-use efficiency, and carbon isotope signatures in three underutilized legumes. Cowpea, which exhibited the highest WUE values, also accumulated the most significant dry matter and showed moderately negative $\delta^{13}C$ values in its roots and shoots (Table 3.7 and 3.8), indicating efficient water conservation during carbon fixation. The common bean had the lowest WUE, the lowest biomass, and the most negative $\delta^{13}C$ signatures, confirming poor water-use efficiency. Bambara groundnut held an intermediate position, meaning that across all measured parameters, water use-efficiency, shoot dry matter accumulation, and carbon isotope signatures, it consistently performed between the highest-ranking cowpea and the lowest-ranking common bean. Its WUE values were moderate, indicating that it was neither highly water-efficient, like cowpea, nor inefficient, like common bean. This was reflected in its $\delta^{13}C$ values, which were slightly less negative than those of common bean, suggesting somewhat better stomatal regulation and improved water conservation, but still not to the level observed in cowpea. Similarly, Bambara groundnut produced more biomass than common bean under mulched conditions but substantially less than cowpea, demonstrating a moderate growth response to mulching. Overall, the crop exhibited a balanced yet not exceptional physiological capability, able to utilize water reasonably well, maintain moderate carbon assimilation patterns, and respond positively to mulch, albeit without reaching the superior efficiency and productivity of cowpea.

Mulching increased shoot dry matter in all crops, and its impact on $\delta^{13}C$ showed alterations in WUE and stomatal behaviour, particularly in shoots. Shoots more accurately represented water-use physiology, whereas roots represented carbon storage capacity and C:N nutritional dynamics. The findings collectively demonstrate a strong relationship between $\delta^{13}C$, shoot dry matter, and WUE, and how mulching affects these relationships varies among underutilized legume species. The close relationship between $\delta^{13}C$, shoot dry matter, and WUE may be explained by the fact that stomatal conductance simultaneously limits CO_2 uptake and water loss, so any alteration in stomatal behaviour impacts both transpiration (T) and photosynthetic carbon assimilation (A) (Farquhar and Richards, 1984; Roche, 2015). As carbon isotope discrimination is controlled by the internal to ambient CO_2 ratio (c_i/c_a), which directly reflects this balance, $\delta^{13}C$ serves as a time-integrated record of the balance between A and stomatal conductance (Cernusak *et al*., 2013; Farquhar *et al*., 1989). Integrated WUE and the long-term A/g_s ratio that $\delta^{13}C$ captures have a significant impact on shoot dry matter accumulation, which represents cumulative carbon gain over the growing season (Condon *et al*., 2002; Blum, 2009). Mulching influences soil moisture availability, soil temperature, and nutrient cycling, thereby altering root water supply and the leaf microclimate, thereby affecting stomatal behaviour and influencing $\delta^{13}C$, instantaneous WUE, and final biomass accumulation (Ramakrishna *et al*., 2006; Zhang *et al*., 2020). While plastic mulches may raise canopy temperature and vapour pressure deficit, organic mulches often improve water retention and moderate temperature, resulting in species- and mulch-specific variations in WUE and $\delta^{13}C$ patterns (Li *et al.,* 2020).

In the current study, cowpea maintained the highest integrated WUE (as indicated by reduced negative $\delta^{13}C$ values) and accumulated the driest matter under mulch, which is in line with previous studies showing cowpeas superior drought tolerance and effective stomatal control (Singh and Reddy, 2011). Common beans, known to be sensitive to heat and water stress, showed lower shoot dry matter and more negative

$\delta^{13}C$ values, especially under plastic mulch (Polania *et al*., 2016). According to previous studies of its moderate WUE and drought avoidance tactics, the Bambara groundnut displayed intermediate physiological and growth responses (Modi and Mabhaudhi, 2013; Chibarabada *et al*., 2015).

Table 3-8: Effects of mulching treatments on the carbon isotope values of roots in three underutilized crop species.

Treatment	%C	C-Total mg kg^{-1}	C:N	δ 13C‰	C content%	C-fixed/ Total-N mg mg^{-1}
B-NMR	40.60[c]	406.00[i]	19.41[e]	-25.64[a]	0.41[f]	135.33[a]
B-PBMR	42.35[b]	1270.64[h]	18.65[e]	-26.13[b]	1.27[e]	28.24[c]
B-PMR	43.84[a]	2191.82[g]	21.33[d]	-25.90[a]	2.19[d]	48.71[b]
CB-NMR	43.46[a]	3042.14[f]	21.20[d]	-26.79[b]	3.04[c]	5.95[d]
CB-PBMR	41.23[c]	3710.53[d]	24.77[c]	-27.08[c]	3.71[c]	4.25[e]
CB-PMR	30.02[d]	3302.40[e]	17.76[f]	-27.89[c]	3.30[c]	1.78[f]
C-NMR	41.28[c]	5366.09[c]	25.56[b]	-26.87[b]	5.37[b]	1.95[f]
C-PBMR	42.30[b]	6344.89[b]	28.56[a]	-27.61[c]	6.34[b]	1.98[f]
C-PMR	42.44[b]	7214.94[a]	26.58[b]	-26.74[b]	7.21[a]	1.93[f]

B-NMR: Bambara groundnut-no mulch roots, B-PBMR: Bambara groundnut-pine bark roots, B-PMR: Bambara groundnut-plastic mulch roots, CB-NMR: Common bean-no mulch roots, CB-PBMR: Common bean-pine bark mulch roots, CB-PMR: Common bean-plastic mulch roots, C-NMR: Cowpea-no mulch roots, C-PBMR: Cowpea-pine bark mulch roots, C-PMR: Cowpea-plastic mulch roots.

Table 3-9: Response of shoot carbon isotope parameters in three underutilized crops to different mulching regimes.

Sample	C%	C-Total mg kg^{-1}	C:N	δ ^{13}C‰	C content %	C-fixed/ Total-N mg mg^{-1}
B-NMS	41.74^{d}	834.74^{h}	15.01^{c}	-25.70^{a}	0.83^{g}	32.11^{a}
B-PBMS	48.34^{a}	1933.66^{g}	14.26^{d}	-25.84^{a}	1.93^{f}	10.74^{c}
B-PMS	46.69^{b}	2801.23^{f}	13.94^{e}	-25.89^{a}	2.80^{e}	18.67^{b}
CB-NMS	45.12^{b}	3609.39^{e}	13.23^{e}	-25.30^{a}	3.61^{d}	1.09^{d}
CB-PBMS	45.82^{b}	4582.49^{d}	24.64^{b}	-27.96^{c}	4.58^{c}	1.25^{d}
CB-PMS	44.86^{c}	5382.87^{c}	30.42^{a}	-28.91^{d}	5.38^{c}	0.82^{e}
C-NMS	43.04^{c}	6025.45^{b}	15.50^{c}	-26.36^{b}	6.03^{b}	0.29^{g}
C-PBMS	45.44^{b}	7270.57^{a}	24.99^{b}	-27.61^{c}	7.27^{a}	0.38^{f}
C-PMS	34.61^{e}	6230.20^{b}	15.37^{c}	-27.09^{c}	6.23^{b}	0.25^{g}

B-NMS: Bambara groundnut-no mulch shoots, B-PBMS: Bambara groundnut-pine bark mulch shoots, B-PMS: Bambara groundnut-plastic mulch shoots, CB-NMS: Common bean-no mulch shoots, CB-PBMS: Common bean-pine bark mulch shoots, CB-PMS: Common bean-plastic mulch shoots, C-NMS: Cowpea-no mulch shoots, C-PBMS: Cowpea-pine bark mulch shoots, C-PMS: Cowpea-plastic mulch shoots.

The strength of the relationship differs across treatments, indicating that fertilizer-mulch combinations play a significant role in regulating how stomatal conductance is translated into chlorophyll accumulation (Figure 3.7). The PBM-C treatment exhibited a very strong relationship (r=1.00, R^2=1.00), suggesting a highly consistent physiological response; however, this should be interpreted with caution due to the limited number of observations. In contrast, both CB-NM (r≈0.67, R^2 ≈ 0.45) and B-PM (r≈0.69, R^2≈0.47) demonstrated moderate positive relationships, reflecting greater variability within these treatments. This variability implies that additional physiological or environmental factors such as nutrient availability, soil moisture, and microbial interactions may be influencing the relationship between stomatal conductance and chlorophyll content.

These findings are consistent with studies on legumes such as Cowpea and Bambara groundnut, where interactions between nutrient supply and soil management practices have been shown to affect photosynthetic traits, including stomatal behaviour and chlorophyll concentration (Kermah *et al.,* 2018).

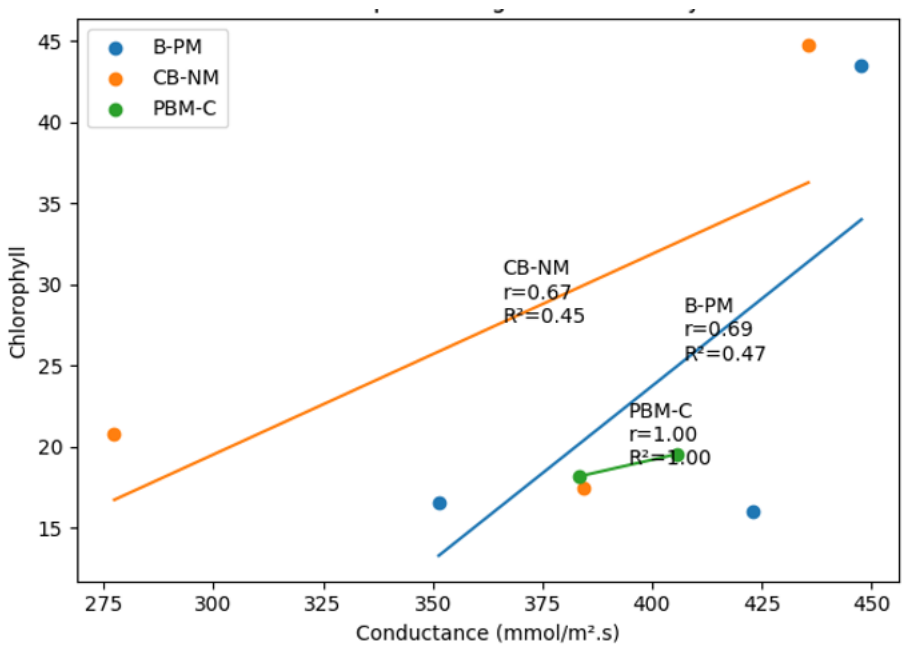

Figure 3-7: Correlation analysis presented in separate regression lines for each treatment group.

3.4 Conclusion

The data revealed marked differences in plant height among the three underutilized legume crops, irrespective of the mulching treatment (no mulch, plastic mulch, and pine bark mulch). Cowpea still recorded the highest plant height. Similarly, the chlorophyll content was highest for the cowpea legume crop compared to the other two legumes (Common bean and Bambara groundnut). This study noted that pine bark mulch has a positive effect on plant growth. Stomatal conductance was higher throughout the growing season; however, a decline was noted in Bambara groundnut with plastic mulch. The higher stomatal conductance was associated with stomatal opening, increased water availability, and a higher air vapour pressure deficit. The study revealed that using mulching increases carbon accumulation.

In conclusion, the study reveals a strong relationship among $\delta^{13}C$, WUE, and dry matter, as they are closely linked through stomatal control and carbon assimilation, which regulate both CO_2 and water loss. Mulching altered these interactions by altering the microclimate and soil moisture, leading the three legumes to respond physiologically differently. Common beans performed poorly, particularly when covered with plastic mulch; cowpeas had the highest WUE and biomass. Bambara groundnuts had poor findings. Mulching enhanced plant performance overall, but its efficacy varied by species, underscoring the significance of choosing the right mulch types for every crop that best support each crop's carbon-use efficiency and water use strategy.

CHAPTER 4

THE NUTRITIONAL COMPOSITION OF THE THREE UNDERUTILIZED LEGUME CROPS IN RESPONSE TO MULCHING

ABSTRACT

In sub-Saharan Africa, approximately 239 million individuals experience protein-calorie malnutrition, and food and nutritional insecurity continue to be significant issues. Low-income households are unable to meet the cost of animal protein; therefore, leafy vegetables and seed legumes are used to meet food/nutritional security, as well as address micronutrient deficiencies, in rural Africa. Weed control, soil moisture retention, and enhanced soil fertility are all advantages of mulching. One of the most essential traditional practices is mulching in the short term since its boots crop yield. Organic mulch boosts yield and enhances grain nutritional quality. Inductively coupled plasma optical emission spectrometry (ICP-OES) for mineral element analysis was used in triplicate to assess the nutritional composition of the three legume crops in response to mulching treatments. The mineral elements analyzed were calcium, magnesium, sodium, iron, zinc, copper, potassium, and phosphorus. High performance liquid chromotography (HPLC) was used to measure vitamin C and +β-Carotene. In this study, the calcium concentration was highest in B-PM (520.7 mg.100 g), followed by B-NM (513.3 mg.100 g). C-PBM (623.2 mg.100 g) and CB-PM (604.6 mg.100 g) showed higher potassium concentrations; mulching treatments significantly influenced potassium concentration. Copper and Iron showed no significant differences across all nine treatments. Phosphorus content was highest in CB-PM (302.7 mg.100 g) and B-PM (223.0 mg.100 g). B-carotene concentration differed among treatments, ranging from 17.5 mg.100 g (CB-PM) to 53.5 mg.100 g (B-NM). B-NM also showed a higher concentration of vitamin C (239.4 mg.100 g), and plastic mulch had a negative influence on C-PM, with the lowest (13.1 mg.100 g). Therefore, identifying the mulching treatment that can enhance the nutritional composition (including minerals and vitamins) of underutilised crops can improve malnutrition and micronutrient deficiencies in rural Africa.

Keywords: Nutritional, malnutrition, micronutrient, organic mulch, food security

4.1 INTRODUCTION

Neglected legume crops are a broad category of leguminous plants that despite their potential nutritional, environmental, and economical advantages, have historically been disregarded in conventional agricultural and food systems (Samal *et al*., 2023). Approximately 850 million people worldwide suffer from severe hunger, which hinders economic progress and makes achieving the Sustainable Development Goal (SDG) of ending hunger more challenging (Kousar *et al*., 2021). As the world's population is expected to grow by 70% by 2050, the global food system will confront significant challenges in guaranteeing a food safety and security (Omomowo and Babalola, 2021).

Underutilized legumes represent an important category of food sources that can help meet the sustainable goal of providing sufficient quantities of essential nutrients, especially inexpensive protein. Legumes, like the common bean, are an important source of minerals, vitamins, and carbohydrates, in a safe and healthy food supply, particularly for African countries (Celmeli *et al*., 2018). Additionally, they contain a high amount of unsaturated fatty acids, including oleic and linoleic acids. Moreover, three Sustainable Development Goals can be achieved by utilising underutilized crops, such as cowpeas, which have a short growth period, high protein content, and a low carbon footprint (Padhi *et al*., 2022).

Several variables impact food security, including economic factors, climate change, environmental issues, soil nutrients status, seed quality, the underutilization of legumes with critical qualities, and the lack of processing facilities and storage, which result in postharvest loss (Setsoafia *et al*., 2022). Making use of underutilized crops particularly legumes in Africa, will significantly reduce food insecurity due to their inherent qualities, which include their high nutrient status, resistance to drought, and above all, their high potential for nitrogen fixation, which in turn influence plant growth, crop yield, and crop production (Bano *et al*., 2016; Olanrewaju *et al*., 2022).

Limited demand has led to lower cultivation and distribution of underutilized legumes, owing to a limited knowledge and recognition of their nutritional and health contributions (Samal *et al*., 2023). To combat malnutrition and enhance health, it is essential to understand the nutritional composition of legumes. Legumes benefit low-income groups. After all, they are rich in dietary fibre, carbs, protein, and minerals (Banti and Bajo, 2020). Moreover, Gebrelibanos *et al*. (2013) reported that legumes help prevent and improve several illnesses, including diabetes mellitus, heart disease, cancer (prostate and breast cancer), and blood cholesterol levels.

Mulching is known to reduce soil erosion, inhibits weed growth in crops, and improves soil nutritional status, retains soil moisture (Iqbal *et al*., 2020). Mulching enhances soil biological health by stimulating microbial activity and enzyme function. Collectively, these advantages improve crop yields and quality in both fruit and field crops (Kaur and Bons, 2017), as mulching is recognised for its benefits to soil health and production yield.

Mulching has been demonstrated to enhance a variety of crops' nutritional composition. According to Kannaujia *et al*. (2024), organic mulch had the highest protein content (28.63%), followed by silver mulch (27.01%), while no mulch treatment had the lowest values (24.16%). In addition to this, Irshad *et al*. (2022) evaluated mulching wheat with *Jatropha curcas* leaves under simulated drought. Their findings revealed that plants had a positive increase in the leaf carotenoid content compared to un-mulched treatments. The report stated that the maximum decrease (26.5%) in sugar content was observed when mulch was applied at 5 $mg.ha^{-1}$. Awopegba *et al*.

(2017) also reported that Acio pruning's mulch increased potassium and Calcium by 59% and 92% respectively, over the control in maize. Most research focuses on major crops, such as maize and tomatoes, leaving a noticeable gap in understanding the influence of mulching on the nutritional composition of underutilised crops. Therefore, the purpose of this chapter is to ascertain the nutritional composition of three underutilised crops (cowpea, common bean, and Bambara groundnut) under different mulching treatments (plastic, pine bark mulch, and no mulch).

4.2 Materials and methods

4.2.1 Study site

The field experiment was undertaken at the University of Mpumalanga farming field, located at 25.4371° S, 30.9818° E, in Mbombela, South Africa. The University of Mpumalanga is in the Lowveld region of Mpumalanga Province, which has an average temperature of 30 °C and a tropical environment.

4.2.2 Experimental design

In this present study a randomized complete block design (RCBD) was implemented**.** Three underutilised legume crops (Common beans, cowpea, and Bambara groundnut) and three mulch treatments (plastic mulch, pine bark mulch, and no mulch) were treatments utilized in this study. The field was demarcated according to the RCBD experimental design. A hand hoe and rope were used to make rows and to achieve the desired 30 cm inter-row spacing. Thinning, weeding, and irrigation are cultivation practices followed. Before applying mulch, tiny weeds were removed using a hand hoe and a hand fork.

4.2.3 Harvesting and Sample Preparation

Bambara groundnut, cowpea, and common bean leaves were freeze-dried, ground using a pestle and mortar, and stored in an 80 °C freezer until the nutritional composition analysis was performed. The nutritional composition was determined using inductively coupled plasma optical emission spectrometry (ICP-OES) for mineral element analysis in triplicate. The mineral elements analysed were potassium, phosphorus, calcium, magnesium, sodium, iron, zinc, and copper. High-performance liquid chromatography (HPLC) was used to measure Vitamin C and β-Carotene.

4.2.4 Data analysis

Statistica software, version 12.0 (StatSoft, Inc., Tulsa, OK, USA) was used to analyze the data. To examine the nutritional composition of the three underutilized legume crops (Cowpea, Bambara groundnut, and Common bean), all data were subjected to one-way ANOVA. Duncan's multiple range test (DMRT) was employed to differentiate means at $p \leq 0.05$.

4.3 Results and discussion

Table 4.1 shows the nutritional content of three underutilized crops (cowpea, common bean, and Bambara groundnut). The nutritional composition reported in this study was derived from leaves collected from three underutilized legume crops. The macronutrients assessed in this study include calcium, magnesium, phosphorus, and potassium. Calcium was highest in B-PM (520.7 mg.100 g), followed by B-NM (513.3 mg.100 g). This study found that Ca was the lowest in common bean among the three legumes, regardless of the mulching treatments. Chibarabada *et al*. (2017) reported that Bambara groundnut demonstrated a high calcium content in the 2016-2017 cropping season, exceeding that of common beans (~100 mg.kg) across all treatments. Moreover, plastic mulch was reported to have a strong, positive influence on the Ca content of Bambara groundnut and cowpea. Ca content was (446.7 mg. 100 g) in C-PBM, the highest in pine bark mulch treatment across the three legumes (common bean, cowpea, and Bambara groundnut).

The micronutrient deficiency affecting approximately 232 million people in Africa can be addressed through research on nodulated legumes, which are able to store micronutrients in their organs (Andrea and Rose 2015). The micronutrients investigated in this study were zinc, copper, and iron (as shown in Table 4.1). Copper showed no significant differences across all treatments, regardless of the mulching treatment. Evidence from the present study suggests that mulching treatment did not affect Cu content, as levels remained constant across all treatments. These results align with a study carried out by Nazir *et al*. (2022), which demonstrated that copper exhibited extremely low coefficients of variation, indicating stability across all growing environments and common bean genotypes.

Anaemia is most frequently caused by iron deficiency, especially in underdeveloped countries and have a higher proportion of anaemic individuals than developed countries, such as the USA and Europe. The same researchers found that cereals and legumes accounted for 40% of iron consumption (Celmeli *et al*., 2018). Out of the three micronutrients (Fe, Cu, Zn) that were investigated in this study, iron had the highest concentrations. Iron was recorded as the highest in B-NM (121.1 mg.100 g); however, this study also noted that these results were relatively consistent across all nine treatments.

Zinc was the highest in C-NM (97.5 mg/100g), and no significant difference was observed across all treatments. The findings are consistent with the findings of Dakora and Belane (2019), who confirmed that cowpea seeds and leaves are inherently rich in zinc and protein, with Zn concentrations ranging from 33.9 to 69.2 µg. More importantly, these values remained relatively stable across environments, indicating that Zn content is predominantly genotype-dependent rather than influenced by agronomic treatments, such as mulching. This supports our observation that the high

Zn levels recorded in C-NM (97.5 mg.100 g) did not differ significantly from those in the other treatments. Micronutrients Zn and Fe have daily dietary intake recommendations of 8 and 11 mg, respectively (Hong, 2025). The estimated amounts required to achieve the everyday intakes of 8 and 11 mg per day for Zn and Fe, respectively, varied across mulching treatments, assuming that the three underutilised legume crop leaves are the only dietary sources of Fe and Zn (on a dry weight basis). Findings of this study revealed no marked differences among the three micronutrients.

Table 4-1: Nutritional content for three legume crops in response to mulching treatments

Parameter	Unit	Calcium	Copper	Iron	Potassium	Magnesium	Sodium	Phosphorus	Zinc
CB-NM	mg.100 g	266.3^{d}	94.9^{a}	100.6^{a}	556.7^{ab}	195.3^{b}	184.7^{a}	196.3^{bc}	96.7^{a}
CB-PM	mg.100 g	399.3^{c}	96.6^{a}	100.9^{a}	604.6^{a}	241.0^{a}	77.3b^{c}	302.7^{a}	95.5^{a}
CB-PBM	mg.100 g	307.6^{c}	95.5^{a}	101.9^{a}	452.6^{b}	188.5^{b}	79.5b^{c}	174.8^{c}	97.0^{a}
C-NM	mg.100 g	500.6^{a}	94.8^{a}	109.4^{a}	471.3^{b}	245.3^{a}	85.2^{b}	173.3^{c}	97.5^{a}
C-PM	mg.100 g	452.0^{ab}	94.8^{a}	100.7^{a}	444.7^{b}	230.7^{a}	87.8^{b}	178.9^{c}	96.4^{a}
C-PBM	mg.100 g	446.7^{ab}	95.1^{a}	105.2^{a}	623.3^{a}	223.3^{a}	79.2b^{c}	193.4^{bc}	96.7^{a}
B-NM	mg.100 g	513.3^{a}	94.9^{a}	121.1^{a}	341.3^{c}	238.0^{a}	56.1^{d}	154.0^{e}	96.1^{a}
B-PBM	mg.100 g	434.0^{ab}	95.2^{a}	98.1^{ab}	287.3^{cd}	216.0^{a}	19.3^{e}	172.4^{d}	96.2^{a}
B-PM	mg.100 g	520.7^{a}	94.9^{a}	105.5^{a}	470.7^{b}	288.0^{a}	51.1^{d}	223.0^{b}	97.1^{a}

CB-PM: Common Bean-Plastic mulch; CB-PBM: Common bean-Pine bark mulch; CB-NM: Common bean-No mulch; C-NM: Cowpea-No mulch; C-PM: Cowpea-Plastic mulch; C-PBM: Cowpea-Pine bark mulch; CB NM: Common bean-No mulch; B-NM: Bambara groundnut-No mulch; B-PM: Bambara Groundnut-Plastic mulch; B-PBM: Bambara groundnut-Pine bark mulch.

In addition, C-PBM (623.3 mg.100 g) and CB-PM (604.6 mg.100 g) were observed to have a strong and positive influence on potassium levels. A study conducted by Sabre *et al*. (2024) on cowpea grown in arid environments supports our findings, which revealed that adding foliar bio-stimulants and organic mulch considerably increased the potassium content in cowpea. The maximum K content in cowpea was observed in the sawdust mulch treatment, which was followed by rice straw and palm frond treatment. This confirms our findings that CB-PM and C-PBM treatments produced higher potassium content. The results of this study also concur with those Tswanya *et al*. (2017), who noted that mulching increased potassium content in tomatoes compared with unmulched plots. Our findings suggest that nutritional composition can be enhanced through appropriate traditional innovations, in our case, by using mulch. The lowest potassium level was recorded in B-PBM (287.3 mg.100 g). The study indicates that mulching enhances crop yield, reduces water use, and improves the nutritional composition of legume crops. Magnesium (Mg) is a cofactor in more than 300 enzyme activity and is known as the fourth most prevalent element in the human body (Baloch *et al*., 2022). Magnesium was the highest in B-PM (288.0 mg.100 g), followed by C-NM (245.3 mg.100 g), and lowest in CB-PBM (188.5 mg.100 g). Cowpea and Bambara groundnut had no significant difference across all the treatments. These results are in agreement with a study conducted by Baloch *et al*. (2022), that found magnesium levels in legume seeds ranged from 0.33 to 1.52 mg.kg with a non-significant genotype × environment interaction. This suggests that magnesium accumulation in legume seeds is permanent regardless of environmental change or agronomic interventions. In line with Mg's insensitivity to mulching intervention, this confirms our finding that cowpea and Bambara groundnut did not significantly differ across treatments, even though specific treatments (B-PM, CB-PBM) showed variations.

Phosphorus content was highest in CB-PM (302.7 mg.100 g), followed by B-PM (223.0 mg.100 g), but lowest in B-NM (154.0 mg.100 g). This study noted that PM (plastic mulch) in common bean and Bambara groundnut had a positive influence on phosphorus content; however, a decline was observed in B-NM. The results of this study are in agreements with the observations by Tswanya *et al*. (2017), who confirmed that mulching significantly increased both fruit yield and the nutritional quality of phosphorus (6.6 mg.100 g) content compared to un-mulched plots (3.8 mg.100 g). These findings align with our results, which show that plastic mulch treatments in legumes (CB-PM and B-PM) resulted in higher phosphorus levels compared to no mulch treatments.

In this study, sodium was recorded as the highest in CB-NM (184.7 mg.100 g), followed by C-PM (87.8 mg.100 g). No mulch had a positive influence on the sodium content of CB-NM. The pine bark negatively influenced the sodium content of B-PBM, with the lowest (19.3 mg.100 g). Straw and plastic mulch have been reported to significantly decrease the amount of salt that accumulates in the root zone by reducing the transport of Na toward plant roots and inhibiting soil evaporation and improving leaching, which can reduce soil salinity by up to 35% (Wang et al., 2022: Hou *et al*., 2016). Our results revealed that the sodium content was highest in the no-mulch treatment and considerably lower under plastic and organic mulch are consistent with these mechanisms.

Figure 4.1 shows the vitamin C content of three underutilized crops (cowpea, common bean, and Bambara groundnut). Vitamin C was the highest in Bambara groundnut with

B-NM (239.4 mg.100 g), followed by B-PBM (226.3 mg.100 g). These results align with those of Tosic *et al*. (2019) and Jahan *et al*. (2018), which showed that the addition of soil organic mulch increased the vitamin C content. On the same note, a study by Gonzalez *et al*. (2025) also noted that the highest total phenolic compounds and vitamin C were recorded in dry alfalfa organic mulch in lettuce. The lowest vitamin C content was recorded in C-PM (13.1 mg.100 g). The present study indicates that organic mulch PBM (Pine bark mulch) can significantly improve the vitamin content.

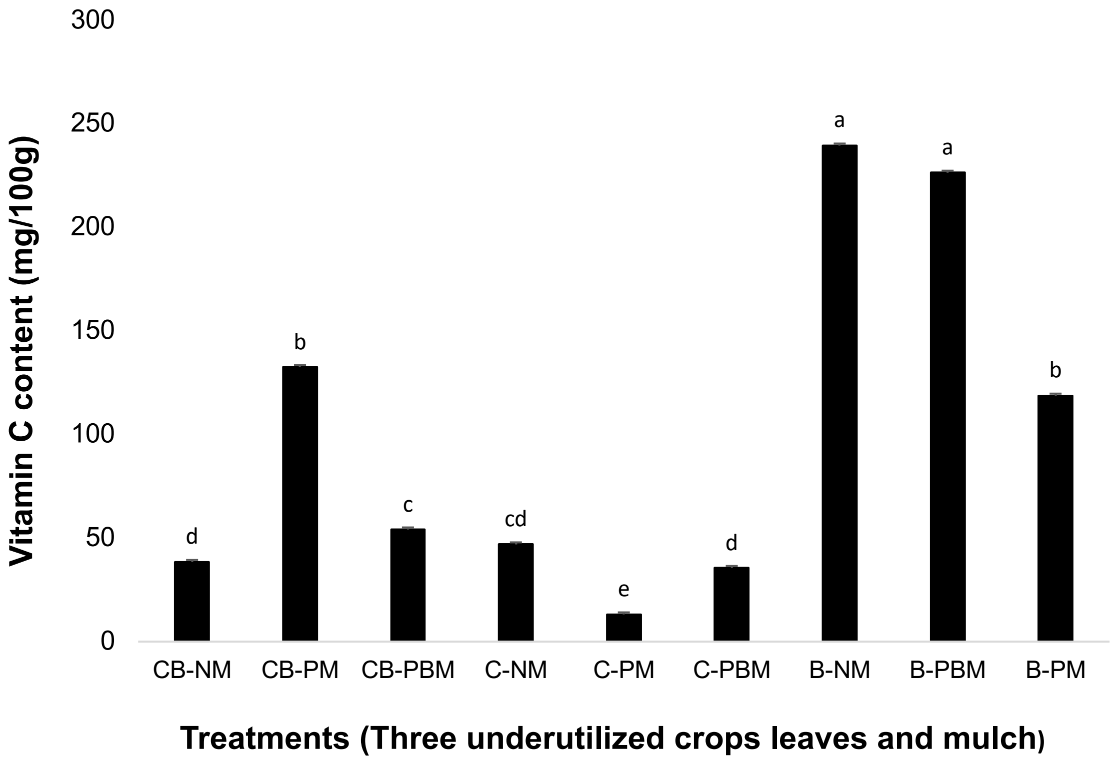

Figure 4-1: Vitamin C content of three underutilized crops leaves and mulch). Vertical lines on bars represent S.E. (n=9), bars followed by dissimilar letters.

Figure 4.2 shows the β-carotene content of the three underutilized crops. The β-carotene content was recorded as the highest in B-NM (53.5 mg.100 g) and C-NM (49.4 mg.100 g); these results indicate that no mulch treatment has a positive influence on the β-carotene content of most crops. However, this study noted that common beans had the lowest beta-carotene content, regardless of the treatment, with the lowest recorded in CB-PBM (16.9 mg.100 g). This study also indicated that natural legumes have low β-carotene content, recording the lowest reading across all elements. Consistent with these findings, Tee *et al*. (1995) discovered that the carotenoid content of legumes, tubers, and roots was significantly lower than that of fruits and vegetables. Futhermore, Gebregziabher *et al*. (2021) stated that all the chosen legumes contained lutein, with lutein contents ranging from 0.54 to 31.94 g. However, he stated that the amount of β-carotene found in three legumes ranged from 0.92 g to 9.9 g. These results confirm that β-carotene is naturally available in trace amounts in legumes.

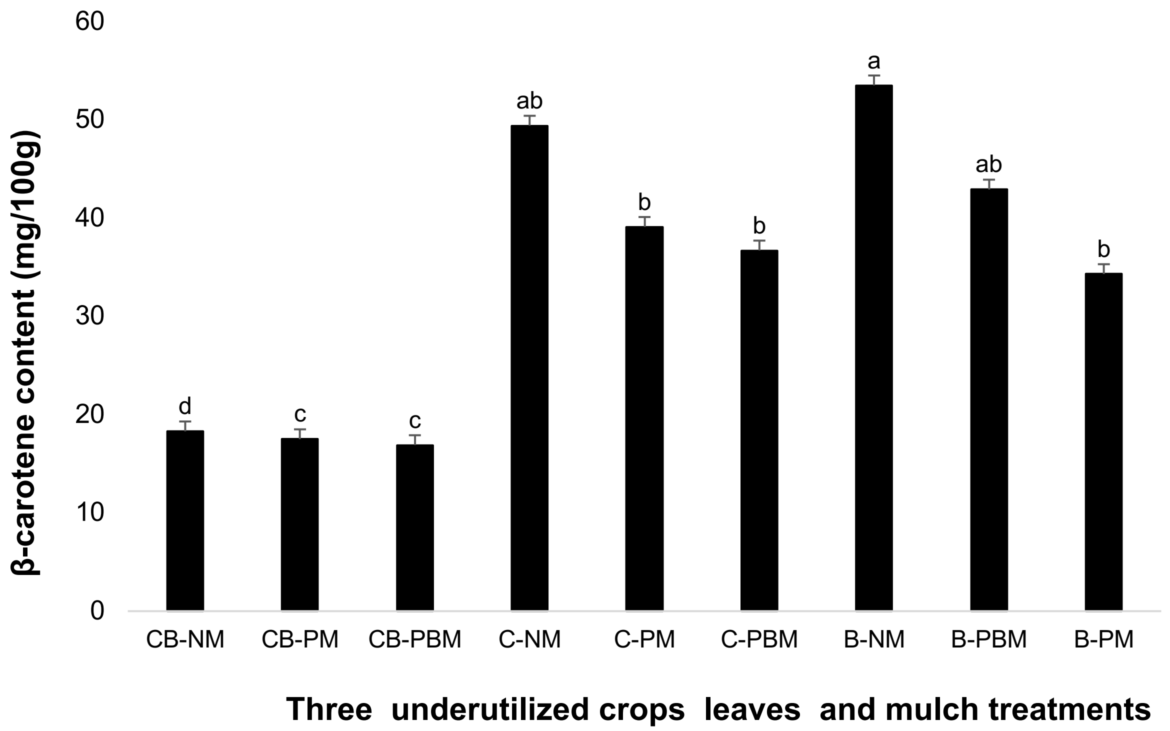

Figure 4-2: β-carotene content of three underutilized crop leaves and mulch. Vertical lines on bars represent S.E. (n=9), bars followed by dissimilar letters.

4.4 CONCLUSION

In summary, this chapter sought to assess how different mulching treatments influenced the nutritional composition of three underused legumes. This study found that plastic and pine bark mulch significantly influenced the content of phosphorus, calcium, and potassium; however, no mulch treatment resulted in the highest sodium levels. Additionally, the results for both Zn and Fe were relatively consistent across all nine treatments. The findings indicated that no mulch treatment has a positive influence on the +β-carotene content of most crops. This study suggests a potential nutritional benefit of agronomic practices that involve mulching, as they not only increase yield but also enhance micronutrient concentration in legumes. Therefore, identifying the mulching treatment that can improve the nutritional composition (including minerals and vitamins) of underutilized crops has the potential to overcome micronutrient deficiencies and malnutrition in rural Africa. It could be concluded that mulch is beneficial in legume production.

CHAPTER 5

SYMBIOTIC NITROGEN FIXATION OF THREE UNDERUTILIZED LEGUME CROPS IN RESPONSE TO MULCHING

ABSTRACT

A crucial ecological process, biological nitrogen fixation (BNF), happens when specific microorganisms, Bacteria and archaea, among others, convert atmospheric nitrogen gas into ammonia, which plants can utilze as nitrogen source. Legumes are essential in a cropping system, since they are not only add to soil fertility through their nitrogen-fixing ability but also have a considerable capacity to fix atmospheric nitrogen. However, nitrogen fixation efficiency is influenced by soil management practices. The study assessed how mulching practices affected the biological nitrogen fixation of three underutilized legume crops. The three underutilized legume crops (Common bean, Cowpea, and Bambara groundnut) have a considerable capacity to fix atmospheric nitrogen. In this study, this is indicated by their high %Ndfa values and low negative $\delta^{15}N$ values in the shoots. Shoots typically have higher nitrogen concentrations than roots, which is consistent with normal physiological N allocation. The overall trends clearly showed a significant reliance on biological nitrogen fixation across treatments. Thereby enhancing soil moisture and microclimate conditions that promote nodulation and N_2 fixation, mulching further improved nitrogen dynamics. Overall, the findings demonstrate the potential of underutilized legumes to significantly enhance soil nitrogen fertility and support more sustainable, low-input agricultural systems, particularly when combined with proper mulching management.

Keywords: Biological nitrogen fixation, mulching, nodulation, soil fertility, sustainable agriculture

5.1 Introduction

Growing energy demand, reducing the risk of climate change, and ensuring food security represent significant, interconnected challenges that require sustained attention and coordinated efforts across multiple sectors. As a result, sustainable production is becoming increasingly vital for food systems and agriculture, since it encourages the effective utilization of natural resources, enhances resilience to environmental stresses, minimizes negative environmental impacts, and supports long-term productivity (Stagnari *et al*., 2017). In this context, legume crops are vital, as they provide a wide variety of agronomic, nutritional, and environmental benefits that align closely with sustainability principles, including biological nitrogen fixation, enhanced soil fertility, less need for artificial fertilizers, and contributions to diversified and resilient cropping systems (Voisin *et al.,* 2013).

First and foremost, a significant source of plant proteins are legumes that are used by both humans and animals. Legumes are vital to the food chain and have a major influence in enhancing human health (Stagnari *et al*., 2017). Secondly, legumes are essential at the production system level because of their efficiency in fixing atmospheric nitrogen and the extent of their contributing in reducing greenhouse gas emissions make them potentially ideal for use in low-input cropping systems (Praharaj and Maitra, 2020). Additionally, as diversification crops in agroecosystems based on a few key species, legumes serve a critical function at the cropping system level. Legume crops can disrupt disease and pest cycles, helping to balance the deficit in plant protein production in various parts of the world (Jensen, 2010). When incorporated into cropping systems, legumes contribute more than nitrogen through their nitrogen-fixing capacity; they also enhance soil organic matter, improve soil porosity and structure, and promote efficient nutrient recycling (Praharaj and Maitra, 2020). Biological nitrogen fixation (BNF) is a crucial ecological process as some microorganisms, including bacteria and archaea, transform ambient nitrogen gas into ammonia so that plants can use it as a fertilizer (Roy *et al.,* 2024). Among the BNF processes, leguminous plants and the symbiotic association among rhizobia, has received the most attention due to its significant and well-quantified contribution to the nitrogen cycle (Mohammadi *et al.,* 2012). This conversion is catalysed by the nitrogenase enzyme system, which comprises two metalloproteins: the F^{e-}protein, responsible for coupling Adenosine Triphosphate hydrolysis to electron transfer, and the MoF^{e-}protein, which contains the FeMo-cofactor active site where nitrogen reduction occurs (Newton, 2015). Considering that symbiotic connections with nitrogen-fixing bacteria allow leguminous crops to absorb atmospheric nitrogen, BNF is an eco-friendly approach to addressing agricultural nitrogen shortages and is also crucial in reducing reliance on artificial fertilizers (Sharma *et al*., 2019). Several obstacles limit the effectiveness of biological nitrogen fixation in legumes within agricultural systems. Environmental conditions such as drought, salt, temperature extremes, and soil acidity have a substantial impact on rhizobia survival, nodule growth, and nitrogenase activity (Yeremko *et al*., 2025). Addressing these gaps requires integrated approaches, such as moisture conservation strategies, including mulching and the use of underutilized legume crops that are drought resistant. Therefore, this study investigated the influence of mulching on the biological nitrogen fixation of three underutilized legume crops.

The underutilized crops used in this study are, namely, *Vigna unguiculata* (L.) Walp. commonly known as cowpea, *Vigna subterranea* (L). Verdc, also known as Bambara groundnut and *Phaseolus vulgaris,* commonly referred to as common bean, is a grain legume that belongs to the family Fabaceae (Onuminya *et al*., 2023). These legumes' remarkable ability to fix nitrogen can greatly improve soil nutrient fertility and agricultural productivity (Babalola *et al*., 2017; Ayilara *et al*., 2022). Legume-rhizobia interaction enables the fixation of significant amounts of nitrogen, ranging from 15 to 145 kg.N^{-1}, which is essential for the legumes (Abdullahi *et al*., 2020). Cultivation of underutilized crops can play a crucial role in strengthening food security and enhancing dietary nutrition in developing regions. They may also offer beneficial features for enhancing essential crops given the effects of climate change (Cullis and Kunert, 2017). However, symbiotic nitrogen fixation remains poorly understood in underutilized legume crops, which are often better suited to harsh environmental conditions.

Mulching has a positive influence on soil conditions and microbial activity, which can influence nodulation and the presence of rhizobia. It alters the temperature of the soil, raising the minimum and lowering the maximum by two to eight degrees (Bhagat *et al*., 2016). Additionally, mulching increases the quantity of organic matter and controls soil moisture. These modifications enhance the conditions for soil microorganisms, thereby increasing the number of rhizobacteria that promote plant growth, as well as fungi and bacteria (Bhagat *et al*., 2016). Mulching alters microbial metabolic profiles and enhances extracellular enzyme activity, impacting the availability and cycle of nutrients (Tian *et al*., 2023). In general, mulching enhances soil fertility, microbial diversity, and enzyme activity by creating more favourable soil conditions and promoting increased microbial interactions. This may also help rhizobia activity and nodulation.

Different types of mulch, such as plastic and pine bark, might affect soil and plant results differently. Recent research highlights the importance of legumes' symbiotic nitrogen fixation in environmental conservation and sustainable agriculture (Goyal *et* *al*., 2021). However, a noticeable gap remains, as research on the interaction between mulching and biological nitrogen fixation in underutilized legumes, particularly in smallholder systems, is still limited. Therefore, this chapter investigated the symbiotic nitrogen fixation performance of three underutilized legume crops in response to mulching treatments.

5.2 Materials and methods

5.2.1 Study site

The field trials were performed at the University of Mpumalanga, located at 25.4371° S, 30.9818° E, in Mbombela, Mpumalanga, South Africa, under field conditions during the 2023/2024 and 2024/2025 cropping seasons.

5.2.2 Sample preparation

Plant samples of cowpea, common bean, Bambara groundnut, and six reference plants were sampled and then oven-dried at 50 °C for a period of 48 hours and subsequently weighed. Shoots and roots were partitioned from the harvested plants for subsequent analysis. Samples of the three legume crops were ground into fine powder using a miller and a blender. The pestle and mortar were used to grind the six reference plants into a fine powder. The grounded samples were then stored in vials in the -80 degrees Celsius freezer until the N-fixation analysis.

5.2.3 Percent Nitrogen derived from atmospheric fixation (%Ndfa)

The fraction of nitrogen originating from N_2 fixation was calculated using (Unkovich *et al*., 2008):

$$\%Ndfa = \frac{\delta^{15}N_{ref} - \delta^{15}N_{leg}}{\delta^{15}N_{ref} - \mathrm{B}} \times 100$$

where $\delta^{15}N_{ref}$ is the ^{15}N natural abundance of the reference plant; $\delta^{15}N_{leg}$, the natural abundance of the legume, and B, the ^{15}N natural abundance of underutilized crops (cowpea, common bean, and Bambara groundnut) that rely only on N_2 fixation for their N nutrition. Since the B value accounts for isotopic fractionation associated with N_2 fixation, it substitutes the value for atmospheric N_2 (Unkovich *et al*., 2008). For this study, the B values used to determine the percentage of Ndfa were (2.2‰) for cowpea, which was obtained from Nyemba and Dakora (2010); (1.5‰) for common bean, obtained from Unkovich *et al*. (2008), and (1.4‰) for Bambara groundnut, which was obtained from Mohale *et al*. (2014). The species collected as reference plants at the experimental site included wild desert spurge, Bengal dayflower, tall nutgrass, southern cone marigold, Mexican clover, hairy croton, and blackjack (Table 5.1). All reference plants sampled had a mean $\delta^{15}N$ value of (3.06‰) for desert spurge, (3.60‰) for Benghal day flower, (4.07‰) for tall nutgrass, (2.93‰) for Southern Cone Marigold, (2.94‰) for Mexican clover, and (3.96‰) for blackjack (Table 5.1). To determine the %Ndfa derived from fixation of atmospheric N_2, the mean $\delta^{15}N$ values were utilized.

The amount of N-fixed was calculated as (Shearer and Kohl, 1986):

$$N\text{ - fixed} = \%Ndfa \times \text{legume biomass N}$$

where legume biomass N was the N content of cowpea, common bean, and Bambara groundnut shoots and roots.

Table 5-1: δ^{15}N of annual herbaceous and non-herbaceous plant species used as reference plants to estimate soil N uptake by the three underutilized legumes.

Reference plant	Botanical names	δ^{15}N (‰)
Desert spurge	*Euphorbia granulata*	3.06
Benghal day flower	*Commelina benghalensis*	3.60
Tall nutgrass	*Cyperu rotundus*	4.07
Southern Cone Marigold	*Tagetes minuta*	2.93
Mexican clover/Florida pusley	*Richardia Scabia*	2.94
Blackjack	*Bidens pilosa*	3.96
		Mean: 3.43

5.2.4 Statistical analysis

Normality tests were conducted on all collected data, confirming that they followed a normal distribution, which justified the application of parametric statistical procedures. The data were then subjected to analysis of variance (ANOVA) using the STATISTICA software package (version 12.0). Dry matter yield and symbiotic parameters, including δ^{15}N, percentage nitrogen derived from the atmosphere (%Ndfa), and total nitrogen fixed by the three underutilized legume crops, were analysed using one-way ANOVA to compare species performance. When significant differences among treatment means were detected, means were separated using Duncan's Multiple Range Test

(DMRT) at p≤0.05. In addition, the coefficient of variation was calculated to assess the reliability and consistency of the data.

5.3 Results and Discussion

5.3.2 Plant growth

The dry matter data was analyzed using one-way ANOVA for the three underutilized legume crops and revealed significant differences, with cowpea dry matter (DM) ranging from 21.20 g.plant^{-1} to 146.90 g/plant (Table 5.2). The lowest was recorded in treatment (C-NMR), and the highest was in (C-NMS). Shoots resulted in larger biomass across all crops. The dry matter of the common bean ranged from 7.30 g (CB-NMR) to 63.90 g for the treatment (CB-PBMS). Bambra groundnut dry matter ranged from 0.30 g (B-NMR) to 13.00 g (B-NMS). Cowpea yielded the highest biomass, followed by common bean; the least biomass was observed in Bambara groundnut.

The significant differences in dry matter production observed among cowpea, common bean, and Bambara groundnut are well supported by earlier findings in the literature (Table 5.2). The superior biomass accumulation recorded for cowpea in this study is consistent with numerous reports identifying cowpea as one of the most vigorous grain legumes in terms of vegetative growth and shoot dry matter production. Cowpea's high dry matter is often attributed to its rapid canopy development, efficient photosynthetic capacity, and strong responsiveness to soil fertility and microbial associations, particularly under improved management conditions (Singh *et al.,* 2003; Timko and Singh, 2008).

The intermediate dry matter production recorded for common bean aligns with earlier research indicating that common bean generally produces lower biomass than cowpea but higher than Bambara groundnut. Common bean is known to be more sensitive to soil nutrient limitations and environmental stress, which can constrain biomass accumulation despite management interventions (Graham and Ranalli, 1997). The relatively low dry matter observed in Bambara groundnut corroborates previous findings characterising this crop as slow-growing, particularly during the early vegetative stages. Several studies have reported that Bambara groundnut allocates a greater proportion of assimilates to reproductive structures and belowground biomass rather than aboveground shoot growth, resulting in comparatively low shoot dry matter (Azam-Ali *et al.,* 2001). Despite its lower biomass, Bambara groundnut is widely recognized for its resilience to poor soils and drought, traits that often come at the expense of rapid vegetative growth.

Table 5-2: Dry matter data of three underutilized crops for cowpea (A), common bean (B), and bambara groundnut (C).

A: Cowpea

Treatments	**Dry matter (g)**
C-NMR	21.20[d]
C-NMS	146.90[a]
C-PBMR	21.40[d]
C-PBMS	120.20[c]
C-PMR	22.00[d]
C-PMS	137.30[b]

B: Common bean

Treatments	Dry matter (g)
CB-NMR	7.30[f]
CB-NMS	41.40[c]
CB-PBMR	9.70[e]
CB-PBMS	63.90[a]
CB-PMR	16.90[d]
CB-PMS	54.60[b]

C: Bambara groundnut

Treatments	Dry matter (g)
B-NMR	0.30[e]
B-NMS	1.30[c]
B-PBMR	1.50[c]
B-PBMS	4.50[a]
B-PMR	0.90[d]
B-PMS	2.50[b]

5.3.3 N concentration and content

The N concentration and N content varied significantly among the three different crops. The N concentration was higher in shoots compared to roots, as shown in Tables 5.3, 5.4, and 5.5. The shoots' N concentration ranged from 1.50% to 3.40%. The N concentration of the roots ranged from 1.50% to 2.30%. As a result of those differences in N concentration, the N content of shoots and roots also differed among the three underutilized crops, ranging from 6.30 g to 4113.20 g. Fageria *et al.* (2007) revealed that since nitrogen is necessary for photosynthetic proteins, shoot tissues always have greater N levels. Crops with a high percentage of N also showed a high N content, particularly in treatments such as C-NMS, CB-PMS, and B-PBMS. This suggests that mulching, particularly with plastic and pine bark mulch, enhances mineral uptake and nitrogen (N) accumulation by improving soil moisture and microbial activity. Mulching increases soil moisture and stimulates N uptake. Similarly, Liu *et al.* (2023) reported that straw mulch mostly controlled the rhizosphere's microbial ecosystem and significantly increased labile nitrogen fractions in the soil, indicating enhanced N-cycling under mulch cover. Additionally, research on legumes demonstrates that legumes effectively absorb and use nutrients, including nitrogen, under optimal soil conditions, supporting their effectiveness in enhanced soil environments (Fageria *et al.,* 2007).

Table 5-3: Nitrogen dynamics in Bambara Groundnut: A comparison of %N, and N content

Sample	%N	N content (g)
B-NMR	2.10^{e}	6.30^{e}
B-NMS	2.80^{c}	36.40^{c}
B-PBMR	2.30^{d}	34.50^{c}
B-PBMS	3.40^{a}	153.00^{a}
B-PMR	2.10^{e}	18.90^{d}
B-PMS	3.30^{b}	82.50^{b}

Table 5-4: Nitrogen dynamics in Common bean: A comparison of %N and N content

Sample	N%	N content g plant^{-1}
CB-NMR	2.00^{b}	146.00^{e}
CB-NMS	3.40^{a}	1407.60^{a}
CB-PBMR	1.70^{d}	164.90^{d}
CB-PBMS	1.90^{c}	878.43^{b}
CB-PMR	1.70^{d}	287.30^{c}
CB-PMS	1.50^{e}	819.00^{b}

Table 5-5: Nitrogen dynamics in Cowpea: A comparison of %N and N content.

Sample	N%	N content g plant^{-1}
C-NMR	1.60^{d}	339.20^{e}
C-NMS	2.80^{a}	4113.20^{a}
C-PBMR	1.50^{e}	321.00^{f}
C-PBMS	1.80^{c}	2163.60^{c}
C-PMR	1.60^{d}	352.00^{d}
C-PMS	2.30^{b}	3157.90^{b}

5.3.4 δ^{15}N values of three underutilized crops

The δ^{15}N values of the six reference plants varied between 2.94‰ to 4.07‰, with a combined δ^{15}N mean value of 3.43‰ (Figure 5.1), being used to calculate %Ndfa. The difference between the combined mean δ^{15}N value of the reference plant and the highest δ^{15}N value of Bambara groundnut was 1.95‰, 1.58‰ for cowpea, and 1.53‰ for common bean. The difference was high in both cowpea and common bean. The precision of the ^{15}N natural abundance method is often higher when the difference between the mean δ^{15}N of the reference and the δ^{15}N of the legume is larger (Unkovich *et al.,* 1994). The interpretation of δ^{15}N values from this present study is supported by Shearer and Kohl (1986), who reported that legumes that primarily rely on atmospheric N_2 fixation often exhibit whole plant δ^{15}N values close to the δ^{15}N of atmospheric N_2 (0 ‰), which supports the interpretation of δ^{15}N values from the current study.

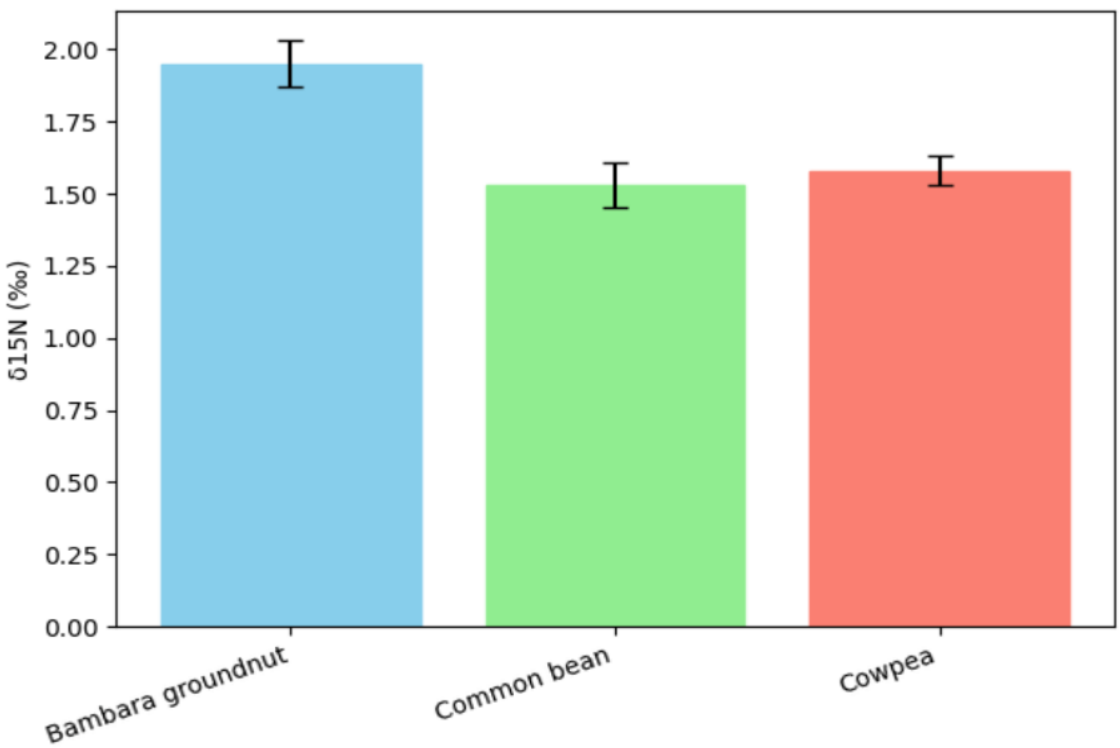

Figure 5-1: δ^{15}N values of three underutilized crops. Vertical lines on bars represent S.E. (n=9), bars followed by dissimilar letters.

5.3.5 Two-Way ANOVA for $\delta^{15}N$ and %Ndfa

A two-way analysis of variance (ANOVA) was conducted to evaluate the effects of crop type, mulch treatment, and their interaction on $\delta^{15}N$ and %Ndfa values. (Table 5.6). For $\delta^{15}N$, the results showed that both crop type ($F=25.9$, $p<0.001$) and mulch treatment ($F=29.1$, $p<0.001$) had significant effects on isotopic composition. In addition, the interaction between crops and mulch ($F=33.0$, $p<0.001$)was also highly significant, indicating that the influence of mulch on $\delta^{15}N$ varied depending on the crop species. This suggests that nitrogen source partitioning differs among crops and is strongly influenced by mulch practices.

Similarly, for %Ndfa, there were highly significant effects of crop type ($F=176.3$, $p< 0.001$) and mulch treatment ($F=47.5$, $p<0.001$). The crop × mulch interaction ($F= 57.1$, $p<0.001$) was also significant, demonstrating that the proportion of nitrogen derived from atmospheric fixation is not only crop-dependent but also influenced by the type of mulch applied. The large F-values for crops indicate that species differences play a dominant role in determining nitrogen fixation efficiency.

Overall, the ANOVA results confirm that both crop species and mulch management significantly influence biological nitrogen fixation, as reflected by $\delta^{15}N$ and %Ndfa values. The significant interaction effects further highlight that optimal nitrogen fixation depends on specific combinations of crops and mulch treatments rather than single factors alone.

The %Ndfa differed significantly among the three underutilized legume crops, varying from 72.82% for Bambara groundnut, followed by Cowpea at 110.65% and highest for Common bean 132.83% (Figure 5.2). Legumes have a well-established regulatory mechanism that allows them to balance nitrogen acquisition techniques based on soil nitrogen availability, shifting between soil nitrogen uptake and nitrogen fixation (Salvagiotti *et al*., 2008). The highest %Ndfa value obtained for Common bean indicating that %Ndfa values above 100% occur due to isotope fractionation and are seen in strong N-fixing legumes in very low $\delta^{15}N$ environments. Mulches such as pine bark and plastic maintained %Ndfa close to 100% (data not shown) for the three legume crops, showing consistent and efficient nitrogen fixation. The higher %Ndfa values under the mulch treatments are in line with ACIAR standards, which state that %Ndfa increases as legume $\delta^{15}N$ values approach or fall below the B-value (%Ndfa may exceed 100%) (Unkovich, 2013).

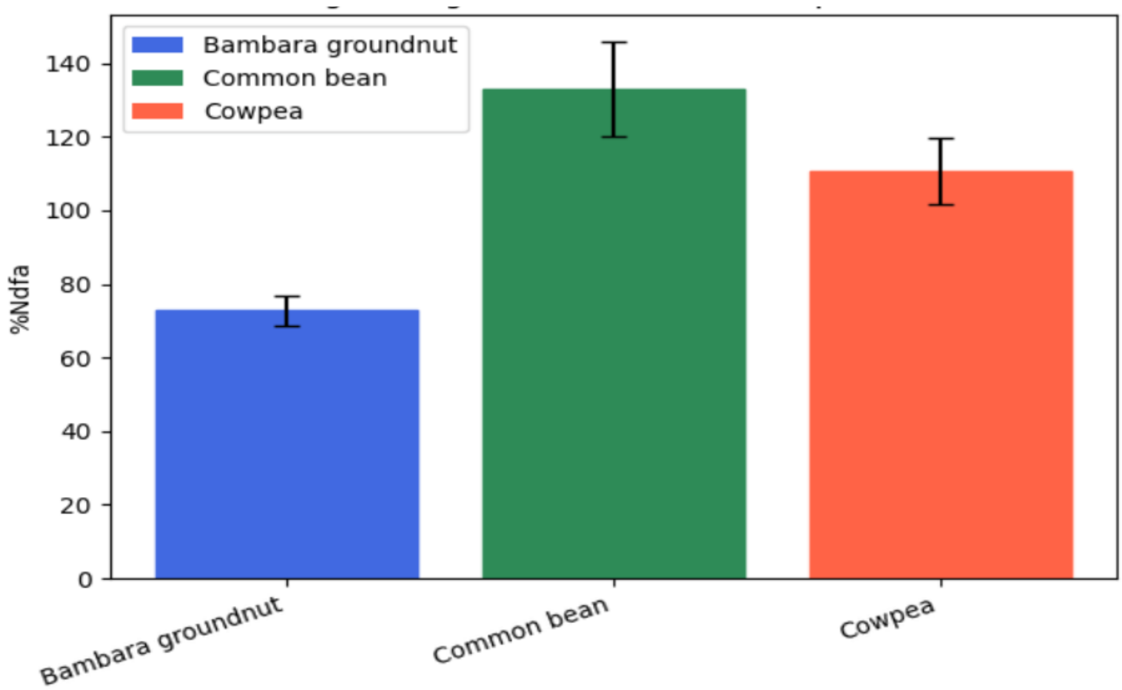

Figure 5-2: %Ndfa of three underutilized crops. Vertical lines on bars represent S.E. (n=9), bars followed by dissimilar letters.

5.3.6 Amount of N-fixed

The N-fixation differed significantly among the three underutilized legume crops (Figure 5.3). The results demonstrate clear differences among the three legumes in terms of nitrogen fixation capacity, as reflected by N-fixed (kg N ha^{-1}), %Ndfa, and $\delta^{15}N$ values, which together provide a comprehensive understanding of biological nitrogen fixation (BNF). Cowpea consistently exhibited the highest nitrogen fixation performance (irrespective of the mulching treatments applied), with N-fixed (88.78 kg N ha^{-1}) and a high %Ndfa (110.65%), indicating a strong reliance on atmospheric nitrogen. This is further supported by its relatively low $\delta^{15}N$ value (1.58‰), which reflects greater dilution of soil-derived nitrogen and a higher contribution from atmospheric fixation. The existence of samples with high N-fixation values is consistent with previous studies, which indicate that legumes can contribute 10-200 kg N ha^{-1} (Abdalla *et al*., 2019). These findings suggest that cowpea has a highly efficient symbiotic relationship with rhizobia, enabling enhanced nitrogen assimilation and overall productivity.

Common bean showed intermediate performance, with N-fixed (47.79 kg N ha^{-1}) and the highest %Ndfa (132.83%) among the three crops. Although the %Ndfa value is comparatively high, its $\delta^{15}N$ value (1.53‰) remains low, consistent with substantial biological nitrogen fixation. The discrepancy between %Ndfa and absolute N-fixed values may indicate that, while common bean relies heavily on atmospheric nitrogen, its total biomass or nitrogen accumulation is lower than that of cowpea, resulting in moderate total nitrogen fixation. In contrast, Bambara groundnut recorded the lowest nitrogen fixation, with N-fixed (2.38 kg N ha^{-1}) and %Ndfa (72.82%), alongside the highest $\delta^{15}N$ value (1.95‰). Since Bambara groundnut treatments recorded lower %Ndfa values, interestingly, higher N fixation was observed in treatments with shoots. This can be ascribed to the beneficial effects of mulching on plant growth and development, thereby increasing total N fixed. This study aligns with previous studies; for instance, Masete *et al*. (2022) outlined that mulched plots resulted in improved physiological responses and greater biomass, yet the nitrogen percentage fixed from the atmosphere (%Ndfa) was slightly lower than in un-mulched plots. Anglade *et al*.

(2015) also reported that increased biomass (shoot N) significantly increased the amount of N fixed. The elevated $\delta^{15}N$ suggests a greater dependence on soil nitrogen sources rather than atmospheric fixation. This may be attributed to less efficient nodulation, suboptimal rhizobial compatibility, or environmental constraints affecting symbiotic performance. Overall, an inverse relationship is evident between $\delta^{15}N$ and both %Ndfa and N-fixed, confirming that lower $\delta^{15}N$ values are associated with higher levels of biological nitrogen fixation. Among the crops studied, cowpea emerged as the most effective nitrogen fixer, followed by common bean, while Bambara groundnut showed comparatively limited fixation under the given conditions. These findings highlight the importance of species selection and symbiotic efficiency in optimizing nitrogen inputs in sustainable cropping systems, particularly for underutilized legumes such as Cowpea and Bambara groundnut.

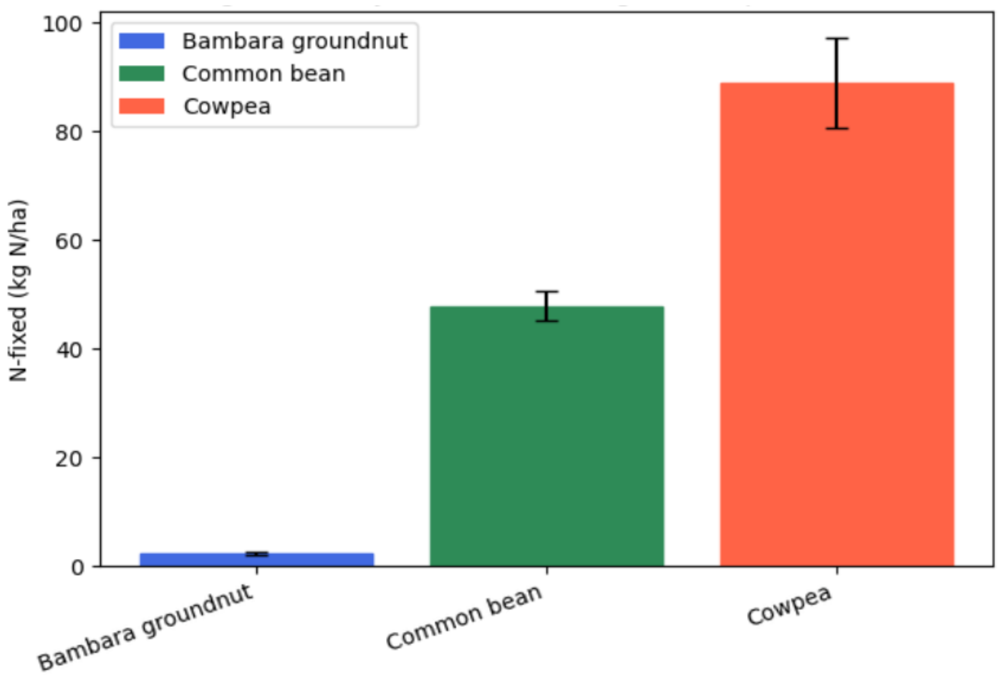

Figure 5-3: Amount of nitrogen fixed (kg N ha^{-1}) by underutilized legume crops. Vertical lines on bars represent S.E. (n=9), bars followed by dissimilar letters.

Table 5-6: Two-way ANOVA of $\delta^{15}N$, %Ndfa and N-fixed.

	Degree of freedom	$\delta^{15}N$				%Ndfa				mg.Nfixed			
		SS	MS	F	p	SS	MS	F	p	SS	MS	F	p
Intercept	1	153.7	153.7	4079	0.00	600280	600280	6385	0.00	375314	37531	1134	0.00
Crops	2	1.9	0.9	25.9	0.00	33145	16572	176.3	0.00	217883	10894	329.2	0.00
Mulch	5	5.5	1.1	29.1	0.00	22344	4468	47.5	0.00	247479	49495	149.5	0.00
Crops×Mulch	10	12.4	1.2	33.0	0.00	53685	5368	57.1	0.00	204068	20406	61.6	0.00
Error	36	1.3	0.03			3384	94.0			119123	33090		
Total	53	21.2				112560				681343			

5.3.7 Conclusion

The findings of this study confirm that all three legume species actively fixed atmospheric nitrogen and therefore have substantial potential to enhance soil nitrogen fertility. Nitrogen allocation patterns showed consistently higher nitrogen concentrations in shoot tissues than in roots, indicating preferential partitioning toward photosynthetically active organs that support biomass production. Although total nitrogen accumulation varied among species and treatments, these differences were primarily attributable to variations in biomass production and species-specific growth characteristics rather than limitations in nitrogen fixation capacity.

The $\delta^{15}N$ natural abundance results clearly demonstrated that atmospheric N_2 was the primary nitrogen source for all three legumes, underscoring their effectiveness as biological nitrogen fixers. Notably, treatment-related differences revealed that mulching practices likely influenced soil moisture retention and nitrogen availability, thereby affecting nitrogen fixation dynamics and overall nitrogen accumulation. Mulched treatments generally promoted greater nitrogen uptake and fixation, highlighting the importance of soil management practices for optimising legume performance. Overall, the results suggest that underutilized legumes represent a dependable and sustainable source of biologically fixed nitrogen, particularly when integrated with appropriate mulching strategies. By incorpating them into cropping systems, agricultural systems could become more resilient, resource efficient, and environmentally sustainable while also increasing soil fertility and decreasing reliance on artificial nitrogen fertilizers. These findings support the broader adoption of underutilized legumes as a viable component of climate-smart and low-input farming practices, especially in regions were soil fertility constraints and input costs limit crop productivity.

CHAPTER 6

GENERAL CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

South Africa has experienced rapid population growth alongside increasing urbanisation, intensifying pressure on already limited water resources. Water scarcity remains a persistent challenge in many regions of the country, exacerbated by climate variability and rising agricultural water demands. Due to these limitations, the agricultural industry is under tremendous pressure to produce enough food in increasing water constrained environment. These constraints place significant pressure on the agricultural sector to produce sufficient food under increasingly water-limited conditions. At the same time, food insecurity continues to disproportionately affect rural and peri-urban communities, where livelihoods are closely linked to rain-fed agriculture and access to water is often unreliable. Due to their high nutritional value, resistance to drought, capacity to increase soil fertility through biological nitrogen fixation, underutilized legume crops present a significant opportunity to solve these issues. In the context of South Africa's water-scarce, climate-vulnerable environments, cultivating underutilised legumes well-adapted to drought-prone conditions is increasingly important. Building on this premise, the present research evaluated how mulching impacts vegetative development and productivity in plants, water-use efficiency, and the nutritional composition of three underutilised legume crops, to support sustainable food production and improve the resilience of smallholder farming systems.

This study was conducted to contribute valuable knowledge and raise awareness, thereby enhancing the adoption of underutilized legume crops. The primary objective of this study was to investigate the effects of different mulching regimes on the growth dynamics, yield attributes, photosynthetic efficiency, symbiotic nitrogen fixation capacity, carbon assimilation rates, and proximate nutritional composition of three underutilized crop species.

In this study, the growth parameters, chlorophyll concentration, and stomatal conductance of the three legume species (cowpea, Bambara groundnut, and common bean) were evaluated in response to mulching treatments (plastic mulch, pine bark mulch, and no mulch). Regardless of the mulching treatments, the results showed variations among the three legume crops. The highest plant growth was observed in cowpea; cowpea consistently recorded the highest values. This was followed by common bean and Bambara groundnut. The results of this study indicate that cowpea possesses a superior photosynthetic capacity and vegetative growth compared to the other two legumes. Plant growth was significantly improved by pine bark mulch. This may be attributed to the fact that mulching improves soil moisture retention, reduces evaporation, and enhances soil fertility, which in turn improves plant growth. The present study revealed that using mulching increases carbon accumulation. The findings demonstrated a strong relationship among $\delta^{13}C$, WUE, and dry matter, all of which are closely linked and regulated by stomatal control and carbon assimilation, which control both CO_2 and water loss. The findings of this study indicate that the three legume crops could serve as a potential source to combat food insecurity, encourage cultivation across various growing conditions, and increase their adoption and utilisation. Comparative assessment of these crops under various mulching regimes is essential to elucidate their relative performance and agronomic potential.

The present study aimed to investigate the impact of the nutritional composition of three underutilized crops in response to mulching treatments. According to this study, the content of phosphorus, calcium, and potassium was positively affected by both plastic and pine bark mulch; however, the highest sodium levels were observed with no mulch treatment. Furthermore, the outcomes for Zn and Fe were comparatively constant throughout the nine treatments. Findings from this study revealed that the no-mulch treatment enhanced the concentration of several measured attributes across the majority of the crops, including β-carotene. This study suggests a potential nutritional benefit of agronomic practices that involve mulching, as they not only increase yield but also enhance micronutrient concentration in legumes. Therefore, identifying the mulching treatment that can improve the nutritional composition (including minerals and vitamins) of underutilized crops possesses the capacity to alleviate malnutrition and address micronutrient deficiencies in rural populations. Moreover, this study assessed symbiotic nitrogen fixation in the three legumes in response to mulching treatments. The overall findings indicate that, despite variations in total N content resulting from changes in biomass and specific components, all three legumes accumulated significant quantities of nitrogen in their tissues. The present study's findings revealed that the three legumes can significantly increase soil nitrogen fertility by actively fixing atmospheric nitrogen.

6.2 Recommendations

This study aims to assess the response of underutilized legume crops to increased organic mulching, with a particular emphasis on growth performance and nitrogen fixation. It also aims to enhance farmer awareness and inform training programs by generating evidence-based insights on the benefits of mulching practices. Additionally, the study examines changes in phytochemical profiles in response to various mulching treatments and evaluates the physiological responses of legume plants to abiotic stresses, including salinity. Ultimately, the findings aim to promote the adoption of mulching practices in underutilised legume production systems, thereby enhancing biological nitrogen fixation, improving soil health, and supporting sustainable agricultural management for smallholder farmers who can benefit from the study's outcomes. The research also underscored the need for future investigations into how different mulching strategies impact soil health and the management of soil nutrient dynamics.

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