Evaluating rhizosphere soil microbes, grain yield and nutritional composition of two legume species in farmers' fields in four Municipalities of Vhembe district.

By

Matidze A

Student number: 21008832

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Department of Soil and Plant Science

Faculty of Science, Engineering and Agriculture

University of Venda

Thohoyandou, Limpopo Province, South Africa

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SUPERVISOR: Prof J.B.O. Ogola

CO-SUPERVISOR: Dr S.N. Mokgehle

CO-SUPERVISOR: Dr H.T. Araya

DECLARATION

Except where otherwise stated, I, Avhakholwi Matidze, certify that the material reported in this dissertation is my original work. I also declare that these results have not been submitted to any University in any form for any degree. Unless acknowledged as found or sourced from other researchers, the work does not contain other people's information or data, such as graphs, tables, or pictures.

Student : A Matidze …… …. …23/February 2024………….

Signature Date

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DEDICATION

This dissertation is dedicated to my father, Ntshengedzeni Ronald Matidze, and my precious Son, Dakalo Matidze.

ABSTRACT

Despite the nutritional value and the amounts of N that legumes contribute to the soil, few studies have evaluated rhizosphere soil microbes, grain yield and nutritional composition of indigenous legumes in the fields of South African farmers. To explore the variations in grain yield, rhizospheres soil microbes and nutritional composition in Bambara groundnut and cowpea species, this study evaluated two legume species under five farmer's fields conditions at four locations (Thulamela, Musina, Makhado and Collin Chabane) in the Vhembe district regions of Limpopo province, South Africa during the 2021/2022 summer cropping season. Ten of each Bambara groundnut and cowpea were sampled at 75% physiological maturity from each farmers' field to determine growth yield and yield components, nutritional composition, and mineral content variations. Ca, K, Mg, Na, B, Cu, Fe, Zn, and protein levels were determined in ground pods and leaves. The rhizosphere soil samples were from 15 cm soil depth. Soil microbial diversity was determined using Carbon Source Utilisation Profiles (CSUP) BIOLOG™ GN2 plates. The abundance and richness of the soil microbes were also determined using the Shannon-Weaver and Evenness diversity indices. The collected data showed significant ($p \leq 0.05$) differences in plant dry matter (DM) yield, number of pods, pods dry weight and number of nodules from different farmer's fields. The high temperature reported in Musina, which exhibited higher growth and yield for Bambara groundnut compared to the other municipalities, indicates the different sites' climatic conditions. A positive correlation was observed between monthly maximum temperature and dry matter for Bambara groundnut ($r^2 = 0.33$) and cowpea ($r^2 = 0.30$). In Makhado municipality, the data showed increased dry matter (144.3 g/plant) and the least in Musina (68.3 g/plant).

The legume species studied exhibited differences in the grain protein fractions' profile and grain mineral content. The two species grains showed considerable significant variation for the following nine mineral contents (mg/g dry matter) obtained for the micro minerals of Bambara groundnut Na 897 mg/kg, Fe 534 mg/kg and Zn 35 mg/kg. Cowpea Na 219 mg/kg, Zn 45 mg/kg, and B 31 mg/kg, and for the macro minerals: Bambara groundnut K 1.55 mg/kg, Ca 0.16 mg/kg, Mg 0.21 mg/kg, P 0.26 mg/kg. Cowpea K 1.30 mg/kg, Ca 0.21 mg/kg, Mg 0.25 mg/kg, and P 0.36 mg/kg.

The significant difference in Shannon Weaver Diversity Index (H') (i.e., the ability of the microbial community to degrade more or fewer types of carbon sources at a threshold ODi value ≥ 0.25) wasobserved for samples in cowpea species under Makhado location, which could degrade more types of carbon sources. Statistically significant utilisation was detected for all five groups of carbon sources.

The microbial communities detected the highest AWCD for utilising all carbon sources (carbohydrates, carboxylic acids, amino acids, polymers and amines). This soil diversity and richness is an indicator of the quality of the soil to increase crop yields and agricultural production. Additional research is needed to determine the microbial diversity and activity yield variations, nutritional composition, and mineral elements of Bambara groundnut and cowpea species.

Keywords: Protein, Macronutrients, Micronutrients, Pods, Nodulation, Municipalities, Food Security.

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ANOVA - Analysis of variance

ARC-SCW – Agricultural Research Council Soil, Climate and Water

AWCD – Average well-colour development

AWS – Automatic weather station

B – Boron

Ca – Calcium

CSUP – Carbon source utilisation profiles

DM - Dry matter

DMRT- Duncan's multiple-range test

ET_o – Evapotranspiration

FAO - Food and Agriculture Organisation

FAOSTAT - Food and Agriculture Organisation Corporate Statistical Database

Fe - Iron

K - Potassium

LSD – Least significance difference

Mg - Magnesium

MO – Molybdenum

Na – Sodium

OP – Optical density

r^2 – Coefficient determination

RHn – Minimum Relative Humidity

RHx – Maximum Relative Humidity

SDW – Sterile distilled water

sSA – sub-Saharan Africa

Tn – Minimum Temperature

Tx – Maximum Temperature

Zn – Zinc

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CHAPTER 1: GENERAL INTRODUCTION

Bambara groundnut (*Vigna subterranean* (L.) Verdc) and cowpea (*Vigna unguiculata* L. Walp) are dual-purpose grain legumes widely cultivated in sub-Saharan Africa (SSA), Asia, South America, the United States and part of Southern Europe (Singh et al.,1997; Timko and Singh, 2008). In Limpopo province, particularly in Vhembe district municipalities, Bambara groundnut (*Vigna subterranean* (L.) *Verdc)* and cowpea *(Vigna unguiculata* L. *Walp*) are common in cropping systems (DAFF, 2016).

The use of legumes in agricultural systems and utilisation of associated Biological Nitrogen Fixation systems provide economically feasible and environmentally sound ways of decreasing external inputs and improving the soil nutrient content and, hence, can be suggested for the nutrition of sustainable agriculture (Postgate, 1998; Kebede, 2020). Grain legumes such as Bambara groundnut and cowpea can improve overall soil nutrient status due to their ability to fix atmospheric N_2 in association with rhizobia in the soil (Mohale et al., 2014; Nyemba and Dakora, 2010).

Bambara groundnut and cowpea's N_2-fixing trait, like that of most legumes, confer adaptation to low-nutrient soils, with some genotypes deriving up to 96% of their nitrogen (N) requirements from symbiosis, which often leads to significant N contribution to cropping systems and grain yield increases (Kermah et al., 2018; Marandu et al., 2013; Belane, 2010).

Bambara groundnut and cowpea help solubilise insoluble phosphorus (P) in soil, improve the soil's physical environment, increase soil microbial activity, and smother weeds (Shah et al., 2021). Due to their crucial roles in improving soil quality and health and their exceptional adaptability to marginal environments, legumes are now considered one of the most important components of a cropping system (Gogoi et al., 2018). Cropping systems involving legume species have been observed to stimulate the microbial community due to the association between the soil microbes and the rhizosphere (Igiehon and Babalola, 2018; Dang et al., 2020). This leads to the release of exudates that improve the cycling of plant nutrients, their availability to the plants, plant growth promotion, diseases, and pest resistance (McNear, 2013; Olanrewaju et al., 2017; Saeed et al., 2021). Legumes such as chickpeas and cowpeas have been shown to increase phosphatase activities compared with non-legumes (Maseko and Dakora, 2013).

Cowpea is widely cultivated in Africa for its edible grains and leaves, which are high in protein (22%), carbohydrates, carotenoids, and micronutrients (Belane and Dakora, 2012). Nigeria and Niger, both in sub-Saharan Africa, are the world's major cowpea-producing countries (FAO, 2017). However, in South Africa, cowpea is considered an indigenous leguminous crop and is cultivated in Limpopo, Mpumalanga, North-West and KwaZulu-Natal provinces (DAFF, 2015). Cowpea's wider adaptation to growth in marginal soils and drought makes it a preferred crop in most rural households, serving as a food security crop and an income source (Hall, 2012). Bambara groundnut is a popular legume among resource-constrained farmers in rural areas (Azam-Ali et al., 2014). Bambara groundnut seeds contain 63% carbohydrates, 19% protein, and 6.5% fat in oil on average (Yao et al., 2015). According to Nishinari and Philips (2014), the protein is of high quality in terms of human nutrition and has a good balance of essential amino acids, including high quantities of lysine (6.8%) and methionine (1.3%). Limpopo, Mpumalanga, North West, Gauteng, and KwaZulu-Natal are the main Bambara groundnut-producing provinces in South Africa. Bambara groundnut (*V. subterranean*) is a native African crop underutilized and understudied for human and animal consumption (Mabhaudhi et al., 2018). Bambara groundnut may be cultivated in many developing countries where other essential crops are challenging to grow because of its drought tolerance and low disease-insect infestation (Majola, 2021). Most rural communities may benefit from the innovative processing of legume crops to improve their nutritional status and combat diseases (Kamwamba et al., 2016). The ability of Bambara groundnut to grow in these diverse agro-climatic conditions (Jorgensen et al., 2010), with soils varying in pH, mineral nutrients, soil moisture, rhizosphere temperature, and N_2-fixing micro-symbionts, is intriguing.

As part of the intervention to increase the consumption and adoption of indigenous species, appropriate agricultural practices for smallholder agriculture and small-scale farming are crucially vital as innovative activities (Kamwamba et al., 2016). The relationship between agricultural practices and the nutritional quality of food is critical to ensuring food security. Crop yields and nutritional security depend mainly on climatic conditions (Burritt, 2019). The indigenous species can also increase food base diversity to address nutrition security and income generation for rural communities, especially women and children.

Increasing agricultural productivity, improving food security, nutrition, and income while increasing climate resilience and achieving major developmental goals can all be accomplished by accessing applicable technologies and sustainable productivity-boosting methods (SciDev.Net, 2014; Brohm et al., 2020). Climate-smart agriculture can be used to address food security and climate change jointly (Lipper et al., 2014; Brohm et al., 2020; Chandra et al., 2018).

In a world that at this time depends on few staple species for food and nutritional security (Mohale et al., 2014), it is essential to conserve and expand the potential yield of underutilized legumes such as Bambara groundnut and cowpea, which could serve as suitable species in sustainable farming systems. Therefore, this study aims to assess the rhizosphere soil microbes, grain yield and nutritional composition of two legume species in farmers' fields in four Municipalities of Vhembe district. Despite these crops being part of South Africa’s cultural heritage, little has been done to cultivate them. Study gaps are critical for effectively transferring BNF technologies to farmers, enhancing soil fertility, improving crop yields, and promoting sustainable agricultural practices.

1.2. Problem statement

Several studies have described the rhizospheres, and soil microbes of legume species under field environments and glasshouse experiments in Africa (Mahmud et al., 2021). However, in the Vhembe district of Limpopo province, there is a lack of information on appropriate rhizobia populations and varying environmental conditions that affect legume nodulation and rhizosphere microbial activities. A clear understanding of legume association and the response of agro-ecological zones of legume species is needed for promoting sustainable agriculture and enhancing food security. Despite this importance, few studies have been conducted on rhizosphere soil microbes, grain yield, and nutritional composition of legumes in smallholder farmers' fields in Limpopo province.

1.3 Justification

Information on rhizosphere soil microbes and grain yield will contribute to knowledge generation for smallholder farmers by providing strategies which smallholder farmers in the selected farmer's fields can use to improve agricultural productivity and sustainability. In Africa, food grain legume species such as cowpea (*Vigna unguiculata* L. Walp.) and Bambara groundnut (*Vigna subterranean L. Verdc.*) form an integral part of the traditional cropping systems and thus offer a great opportunity for increasing N supply in the fields of resource-poor farmers. According to Barnard and du Preez (2004); Strauss, 2021) the soil fertility level in South Africa is typically low.

1.4 Hypotheses

The following hypotheses were tested in the study:

- There is no significant difference in grain yield and yield components between the two legume species in the farmers' fields.
- There is no significant difference in diversity and abundance of rhizosphere soil microbes between the two legume species.
- The nutritional composition of the two legume species does not vary among farmers’ fields.

1.5. Objectives

The study assesses the rhizosphere soil microbes, grain yield and nutritional composition of two legume species in farmers' fields in four Municipalities of Vhembe district.

The specific objectives of the study were to:

- Evaluate the variations in soil microbes within the rhizosphere of two legume species in farmers’ fields in Vhembe district's four Municipalities.
- Assess grain yield and yield components of two legume species grown in farmers’ fields in Vhembe district's four Municipalities.
- Determine the nutritional composition and mineral elements of two legume species collected from farmers’ fields in four Municipalities in Vhembe district.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Indigenous legumes are a valuable plant protein source and the primary dietary protein source for most people in Sub-Saharan Africa (Popoola et al., 2023). The residues, as well as herbaceous and fodder tree legumes, are excellent sources of quality feed for livestock (Inaizumi, 2019), and their ability to fix atmospheric nitrogen (N_2) makes them desirable components of farming systems, as they provide residual nitrogen and reduce the need for mineral fertilizers by associated non-legumes (Quinn, 2009; Tzec-Gamboa et al., 2023 and Mucheru-Muna et al., 2010). Therefore, the study assesses the rhizosphere soil microbes, grain yield and nutritional composition of two legume species in farmers' fields in four Municipalities of the Vhembe district.

2.2 Origin of Bambara groundnut (*Vigna subterranean* (L.) and production in Africa

The centre of origin of Bambara groundnut (*Vigna subterranean* (L.) is believed to be somewhere between West and Central Africa by early researchers (Figure 2-1) (Temegne et al., 2018). All investigations on the origins of Bambara groundnut concluded that the plant came from the continent of Africa (Begemann, 1988; Pasquet and Fotso (1997) and Pasquet (2004). The species spread across all of Africa because of the migration of indigenous people. Other continents, like Asia and North America, also grow this crop. But despite being a native legume of Africa, Groundnuts (*Arachis hypogaea*) have recently overshadowed its popularity (Pasquet et al., 1999; Bamshaiye et al., 2011).

In South Africa, the Bambara groundnut is grown in KwaZulu-Natal, the Eastern Cape, Mpumalanga, Limpopo, and the Northern Province of South Africa (DAFF, 2016). Furthermore, the crop remained cultivated in Sub-Saharan Africa, primarily in semi-arid regions and has contributed to food security in the past (Mwale et al., 2007). The Bambara groundnut was initially discovered in West Africa (Hillocks et al., 2012) and appears to have spread over Sub-Saharan Africa (FAO, 2017). –Smallholder farmers in the Vhembe district, who often lack access to irrigation and other inputs on best practices traditionally grow legumes under harsh environmental conditions and tropical climates (Mpandeli, 2014). The Bambara groundnut is grown from West Africa to South Africa through Central Africa, including Ghana, Senegal, Cameroon, Mali, Burkina Faso, Niger, and Nigeria (Begemann, 1988).

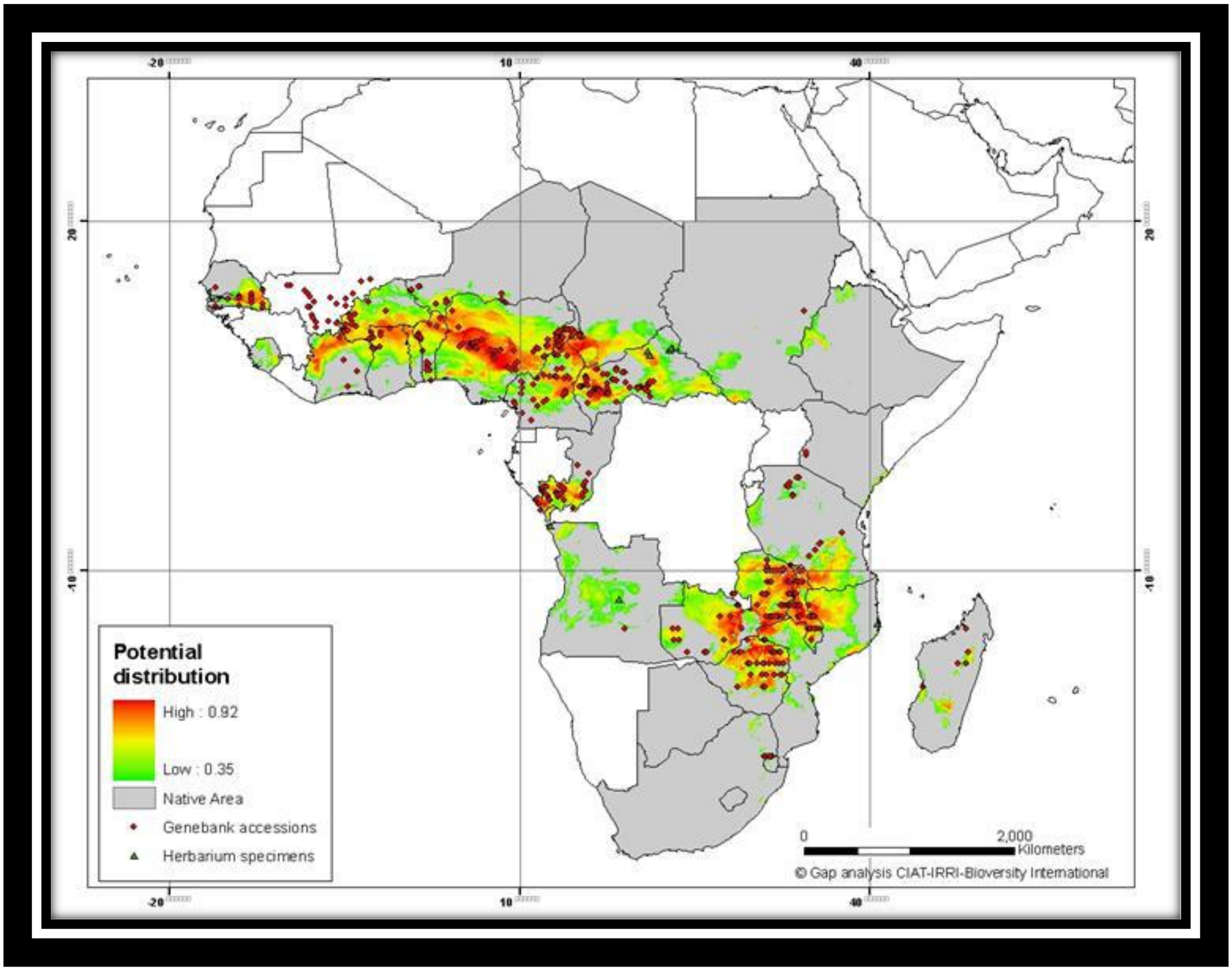


Figure 2-1: Distribution of Bambara groundnut (*Vigna subterranea* (L.) Verdc.) In Africa (Center for International Tropical Agriculture-International Rice Research Institute-Biodiversity International) (Temegne et al., 2018).

In Sub-Saharan Africa, the annual Bambara groundnut yield is estimated to range between 0.3 and 3 tons per hectare (FAO, 2016). Figure 2-2 shows that Nigeria, Chad, Togo, Ghana, Niger, and Benin produce 74% of Bambara groundnuts globally (FAO, 2020). Local smallholder farmers produce most of the Bambara groundnut, which is generally consumed at home. In the recent years, people started selling it in local markets (e.g., boiled groundnuts) (DAFF, 2011; Hillocks et al., 2012; Ojiewo et al., 2018; Mahlangu et al., 2020). In South Africa, Mpumalanga, Limpopo, and KwaZulu-Natal are the main producers of the crop (DAFF, 2016; Gerrno et al., 2019).

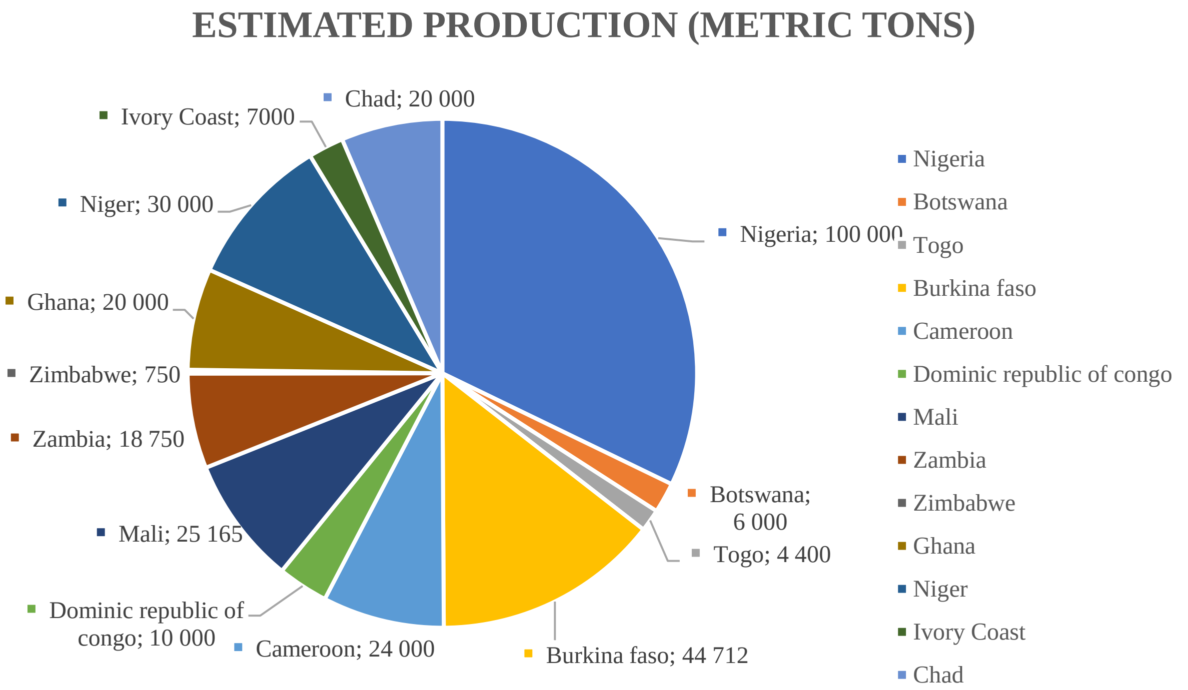


Figure 2-2: Major Bambara groundnut-producing countries in Africa and their contributions (FAOSTAT, 2017).

2.2.1 Agronomic importance of Bambara groundnut

Bambara groundnut is intercropped with maize and other major crops in most African countries. It is, however, commonly grown in rotation to increase the soil's nitrogen status (Berchie et al., 2012). Farmers primarily grow Bambara groundnut because of its considerable agronomic benefits. Due to its high yield under drought stress conditions, local and marginal farmers still grow Bambara groundnut (Olayide et al., 2018). Moreover, it offers more nutritional content, drought tolerance, and the ability to grow on poor soils than popular species such as common beans, peanuts, and groundnuts (Azam-Ali et al., 2001).

Bambara groundnut is an important component of traditional cropping systems and is a year-round growing plant that can improve soil physical qualities (Damfani, 2020). It fixes atmospheric nitrogen and enhances soil fertility, particularly in smallholder farming systems where fertilizer is used sparingly or not at all (Kyei-Boahen et al., 2017). Bambara groundnut yield, however, is heavily influenced by the sowing date. Early November is the ideal time to sow in KwaZulu-Natal and many parts of the country when yields are highest, rather than late December to January when yields are lowest (Damfani, 2021).

2.2.2 Nutritional compositions of Bambara groundnut

The nutritional content of Bambara groundnuts may help reduce malnutrition and increase food security. The Bambara groundnut seed contains 63% carbohydrates, 19% proteins, and 6.5% lipids (Chude et al., 2018). Furthermore, the Bambara groundnut contains vital elements, including potassium, calcium, iron, and zinc, making it a well-balanced and nutritious meal (Murevanhema and Jideani, 2013). This legume crop can improve the nutrition of weaning infants by supplementing cereals, flour for bread, and biscuits (James et al., 2018). Bambara groundnuts are rich in protein, which helps avoid protein shortage, common in undernourished children and kwashiorkor (Hoa et al., 2001). Bambara can be used to augment cereal-based meals as a protein source.

Bambara groundnut can be consumed as samp, roasted, or steamed (Bamshaiye et al., 2011). This legume crop can improve the nutrition of weaning infants by supplementing cereals, flour for bread, and biscuits (James et al., 2017). Bambara groundnut seeds are very caloric and therefore can be used to make a thick porridge (Adeleke et al., 2018). Due to their toughness, dehydrated seeds are difficult to crush into powder, but when crushed, delicious bread and fat cakes can be made (Harouna et al., 2018). Immature seeds can also be cooked or eaten while fresh (Jideani et al., 2021). The legume can be utilized as fodder to feed livestock (Belel et al., 2014).

2.2.4 Production constraints of Bambara groundnut

Despite its long history, it is still grown from local landraces rather than established cultivars with a specific breeding purpose suited to a certain agroecological location or production method, as several studies have indicated (Atoyebi, 2017; Gbaguidi et al., 2018). However, the Bambara groundnut breeding system has not yet been fully exploited until recently, as no true variety was produced. Like other indigenous African crops, researchers and funding bodies have almost ignored Bambara groundnut due to its position as a poor man's crop, cultivated for sustenance instead of cash (Khan et al., 2021). The Bambara groundnut has been ignored and underutilised because industry, international research groups, and the scientific community believe it has no economic worth (Cullis, 2017). Due to underutilisation, limited achievements are made on genetic advancement, reduced agricultural yield and quality may be one of the reasons Bambara groundnut has remained neglected and underutilized (Latincrop, 2019). This is attributable to various challenges such as a lack of adequate genetic variation, poorly coordinated or non-existent seed systems, complex flowering biology and pod formation, a lack of value additions, and poor market systems of the crop (Hardy and Jideani, 2020).

2.3 Origin of Cowpea (*Vigna unguiculata* (L.) and production levels in Africa

Although the exact place of domestication is unknown, Cowpea (*Vigna unguiculata* (L.) Walp) is considered to have originated in Africa. Ethiopia, Central Africa, Central and Southern Africa, and West Africa have suspected origin zones (Da Silva et al., 2018). Several authors have reported West Africa as the centre of maximum genetic diversity and local farmer's highest domestication of the cultivated cowpea (Padulosi et al., 1990; Herniter, 2020). Asia has been reported as the second centre of maximum cowpea diversity from which the crop was distributed to other world regions (Boukar et al., 2018). A study by Huynh et al. (2013) to assess the distribution of the cowpea species reported a small genetic difference occurring between cowpeas from different regions in Africa and outside, suggesting that Africa is the primary source of worldwide distribution of cowpeas (Figure, 2-3).

Figure 2.3: A Single Nucleotide Polymorphism-based association analysis for plant growth habit in worldwide Cowpea (*Vigna unguiculata* (L.) Walp) Germplasm (Huynh et al., 2013).

Africa as a whole accounts for 22% of the global production of grain legumes (Kebede, 2020). According to FAOSTAT (2020), cowpea was cultivated on an estimated 11 million hectares in

Africa in 2020, with most of the production concentrated in West Africa (10.6 million ha), especially in Niger, Nigeria, Burkina Faso, Mali, and Senegal. Cowpeas production is approximately 7.4 million tons worldwide, with Africa generating nearly 5.2 million tons. FAOSTAT (2017) states that Africa produces approximately 87% of cowpeas. Nigeria is the world's leading producer and consumer of cowpeas, accounting for 61% of global production (Baysah, 2013). About 52% of Africa's cowpea crop is used for food, 13% for animal feed, 10% for seeds, and 9% for other purposes, such as green manure to improve soil fertility (Baysah, 2013; Boukar et al., 2019; Kebede and Bekeko, 2020).

Niger, Burkina Faso, and Tanzania are the leading cowpea producer both in terms of area coverage (ha) and production (tons), following Nigeria (Table 2.1). According to the Food and Agriculture Organization, the world production of cowpeas in 2021 was estimated to be about 8.99 million metric tons from a harvested area of about 14.91 million ha (FAOSTAT, 2021).

Table 2-1: Cowpea production output and productivity by a few chosen nations worldwide (adapted from FAOSTAT 2020)

S/N	**Country**	**Production in tons**	**Yield per hectare**	**Area harvested**	**Inference on production**	**Inference on productivity**
1	Nigeria	2,606,912	9,137	2,853,097	1st	7th
2	Niger	2,376,727	4,035	5,889,677	2nd	18th
3	Burkina Faso	630,965	4,826	1,307,336	3rd	12th
4	Ghana	215,350	19,862	11,898	4th	2nd
5	Tanzania	202,865	4,096	30,366	5th	6th
6	Cameroon	185,832	4,043	258,896	6th	9th
7	Kenya	179,399	4,367	11,154	7th	10th
8	Mali	157,739	3,767	160,412	8th	11th
9	Myanmar	136,411	11,425	119,398	9th	4th
10	Sudan	104,667	2,678	333,638	10th	17th
11	Mozambique	89,356	5,545	284,451	11th	20th
12	Democratic Republic of Congo	72,726	4,432	95,803	12th	15th
13	Senegal	60,422	6,889	260,408	13th	19th
14	Malawi	42,456	13,515	159,345	14th	13th
15	United States	23,632	4,296	169,279	15th	1st
16	China	15,652	8,876	209,371	16th	5th
17	Madagascar	13,000	8,907	14,596	17th	8th
18	Uganda	12,439	9,750	208,059	18th	16th
19	Sri Lanka	11,180	11,770	9,499	19th	3rd
20	South Africa	4,871	10,360	15,108	20th	14th

Source: adapted from (FAOSTAT, 2020).

Cowpea is intercropped with cereal crops such as maize, sorghum, and pearl millet by smallholder farmers in South Africa (DAFF, 2016), and just 6% is grown as a single crop (Asiwe, 2009). Cowpea production in South Africa is still poor compared to other primary crops like maize, this could be because smallholder farmers commonly cultivate the species in South Africa and plant the crop on drylands. Furthermore, the crop's production area and output are unknown. (DAFF, 2016).

2.3.1 Agronomic Importance of Cowpea

Cowpea's wider adaptation to growth in marginal soils and drought makes it a preferred crop in most rural households, where it serves as a food security crop and a source of income (Hall, 2012). For its various attributes, cowpea is one of the most preferred crops and a valuable component in the farming systems of most resource-poor rural households in sub-Saharan Africa (Molosiwa et al., 2016). Furthermore, cowpeas can restore soil fertility through nitrogen fixation, making it an excellent crop for rotation with major cereal crops (Daryanto et al., 2015).

2.3.2 Nutritional compositions and utilization of Cowpea

Cowpea is an essential source of dietary protein for millions of people in developing countries, complementing low-protein cereal and tuber crops nutritionally (Tinko, 2008). According to Hall (2012), Cowpea has various applications, including animal feed and human consumption (IITA, 2009). Cowpea is a highly nutritious legume that contains 24% protein, 62% carbohydrate, and small amounts of other nutrients (depending on variety), (Chathuni et al., 2018). It also has a low-fat content, which is beneficial in preventing various metabolic and cardiovascular diseases (Goncalves et al., 2021). According to Hall (2012), cowpea leaves and seeds are a valuable source of protein in smallholder agricultural places where farming is the main activity. In semi-arid places, forage is used as animal feed throughout the long dry season and fatten small ruminants prepared for various celebrations (Hall et al., 2012).

2.3.4 Production Constraints of Cowpea

Cowpea legumes have historically received little attention from research and development programs (Ojiewo et al., 2018). According to Kamara et al. (2018), cowpea cultivation is primarily based on traditional systems, and cowpea grain yields in farmers' fields are low, particularly in the African region (0.025-0.3 t ha^{-1}). This is due to severe pest complexes, diseases, low soil fertility and drought. The lack of information and sustained production indicates that cowpea production is dominated by small-scale farming. Cowpea production and productivity are limited due to a lack of access to current technology, such as improved varieties and associated crop and pest management strategies, inputs such as fertilizers (both mineral and biofertilizers), improved variety seeds, and low input and output market access (Simion, 2018). Furthermore, because of a lack of enthusiasm and funding, there is relatively little knowledge about cowpea production and agronomy in South Africa. Research shows cowpea is still an underutilised crop (Mabhudhi, 2017). In Sub-Saharan Africa, many biotic and abiotic factors, such as insect pests, diseases (fungal, viral, and bacterial), poor soil fertility, heavy metal toxicity, and drought, reduce cowpea yield (Boukar et al., 2018). Other reasons contributing to low yields in Sub-Saharan Africa include a shortage of improved varieties that can tolerate abiotic and biotic stress and insufficient production guidelines and inputs required for higher productivity and profitability (Kamara et al., 2018). This yield gap can be closed if farmers can access improved varieties and production practices (Ajeigbe et al., 2010). Among the abiotic factors, drought has been identified as a major limitation restricting cowpea production in Southern Africa (Boukar et al., 2019).

Field, storage pests (aphids, leaf beetles, pod borers, and bruchids), and low soil fertility are all constraints to cowpea production in South Africa. Cowpea production is hampered by parasitic weeds such as *Striga gesnerioides* (Willd.) Vatke and *Alectra vogelii* (Benth) (Horn et al., 2015). Other constraints included a lack of a market for their produce, poor pricing, pilfering, and a lack of storage space (Horn et al., 2015).

2.4 Biological N_2 fixation by legumes

Cowpea (*V. unguiculata* L. Walp) and Bambara groundnut (*V. subterranean* L. Verdc) are leguminous crops that are noted for their ability to fix nitrogen from the atmosphere and improve nutritional balances in low-income populations (Abudulai et al., 2017). Ibny et al. (2019) and Pulido-Suárez et al. (2021) contend that the nitrogen fixed by legumes can be as high as the nitrogen fertilisers used in conventional farming practices. According to some estimates, Bambara groundnuts can fix up to 28.42 kg N/ha in Nigeria's Sudano-Sahelian zone (Yakubu et al., 2010). Like most legumes, the N_2-fixing trait of cowpea confers adaptation to low-nutrient soils, with some genotypes deriving up to 96% of their nitrogen (N) requirements from symbiosis, which often leads to significant N contribution to cropping systems and grain yield increases (Kermah et al., 2018; Belane, 2010; Marandu et al., 2013).

Biological nitrogen fixation by leguminous crops is a cost-effective and long-term biological strategy for improving soil fertility that South African smallholder farmers utilize to boost crop productivity according to Bloem et al (2019). Legume crops provide accessible rhizobia in the soil with organic resources such as carbon, while the rhizobia fixes atmospheric N_2 (Giller et al., 2013). As mono-crops, intercrops, and double-ups, legume crops are also used in cropping systems (Snapp et al., 2010). Incorporating legume crops into cropping systems can provide more economical and long-term nitrogen than synthetic fertilizers. Thus, there is a need to understand the biological nitrogen fixation components of Bambara groundnut and cowpea in farmer's fields. With a changing climate and significant heterogeneity in African soils, crop species adapted to different agro-ecologies are needed. Despite extensive research on legumes, little progress has been made in transferring Biological Nitrogen Fixation (BNF) technologies to farmers because the N_2 fixation processes are poorly understood in the farmer's environment. For instance, despite the importance of BNF in improving low soil fertility, particularly the N status of the soil, little is known about how much N_2 is fixed in farmers' fields and the differences in legume and associated crop yields in researcher-managed and farmer-managed plots.

Summary of literature review

This chapter showed that Bambara groundnut (*Vigna. subterranean* L. Verdc) and Cowpea (*Vigna. unguiculata* L. Walp) are important indigenous legumes. These species have a high nutritional value and drought tolerance, making them a potential multi-use crop in agriculturally marginal areas. Furthermore, the review found that Bambara groundnut and cowpea, as species, have a high potential to contribute to food and nutritional security in Africa, especially considering predicted climate change. However, despite having potential, the literature review showed that the species are still underutilized. In this regard, this study will seek to contribute to efforts on the benefits of on-farm trials toward the capacity building of the farmers from different locations, improving the management practices of the beneficial nutritional composition of two leguminous species, and rhizospheres soil microbes of selected two legume species.

There is a rising trend towards the consumption of plant-based diets which has led to a demand for more plant-based protein foods. Bambara groundnut is a suitable crop to consider in this regard as it provides essential nutrients and is an important source of protein in areas where animal protein is scarce (Boye et al., 2009). The nutritional composition of Bambara groundnut has earned it the reputation of being a complete food and maintaining soil fertility through BNF (Gogoi et al., 2018). Bambara groundnut and cowpea help solubilise insoluble phosphorus (P) in soil. Due to their crucial their exceptional adaptability to marginal environments, legumes are now considered one of the most important components of a cropping system (Gogoi et al., 2018).

CHAPTER 3: GENERAL MATERIALS AND METHODS

3.1 Experimental sites

The study was conducted at four municipalities (Collin Chabane, Makhado, Musina, and Thulamela), each location has five farmer's fields per site. in the Limpopo Province of South Africa during the 2021/22 cropping season. The Thulamela, Makhado, and Collins Chabane locations are in the cooler region of Limpopo Province, while Musina is in the warmer (Figure 3.1). The area has an unimodal rainy season that starts at the end of October each year and ends in April the following year.

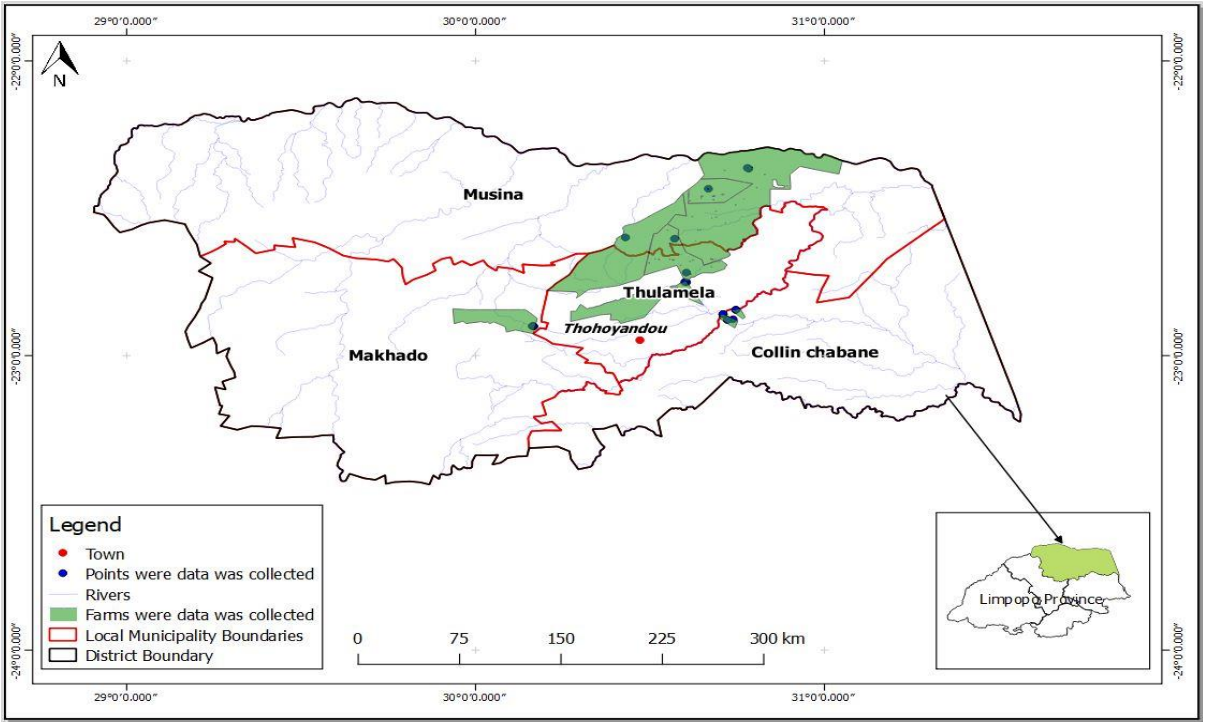


Figure 3-1: Map of Vhembe district indicating the study sites (Collin Chabane, Makhado, Musina, and Thulamela) in Limpopo Province.

3.2 Weather data

Seasonal weather data were obtained from an automatic weather station 30km from each experimental sites. Weather conditions varied over the experimental locations of 2021 and 2022 (Figures 3-2a-d). The Thulamela, Makhado, and Collins Chabane sites are located in the cooler region of Limpopo Province, while Musina is in the warmer. These variations have implications on the yield components of the two legume and soil rhizosphere microbial activity.

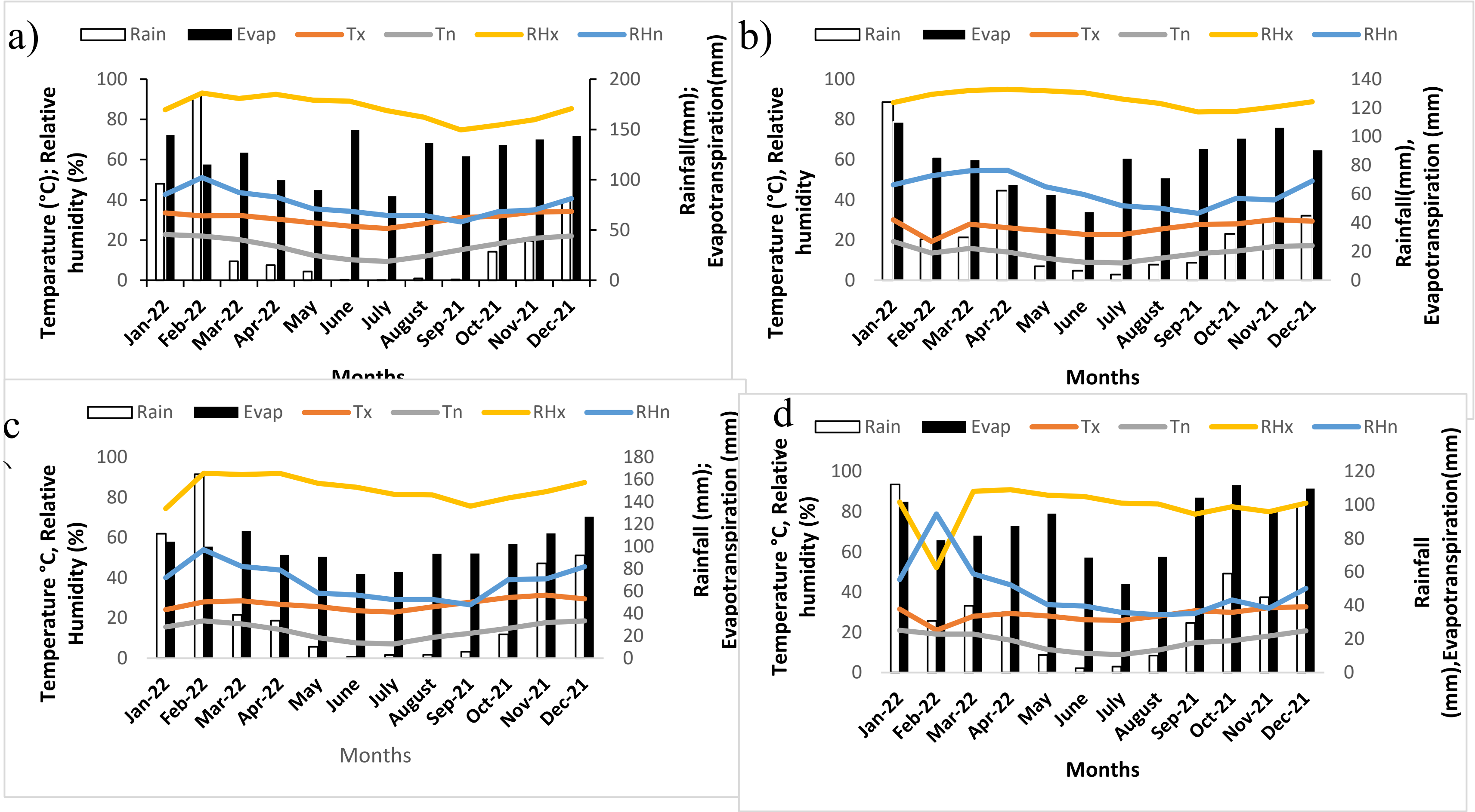


Figure 3-2: Total rainfall (mm), Evapotranspiration, average maximum (Rhx), minimum (Rhn) relative humidity, average maximum (Tx) and minimum (Tn) temperatures collected from the nearest weather station at four municipalities during the production season. Thulamela (a), Musina (b), Makhado (c) and Collin chabane (d). Seasonal weather data were obtained from an automatic weather station 30km from each experimental site.

3.3 Experimental Design

The experimental design consisted of four treatments (Thulamela, Makhado, Musina Collin Chabane). The experiment was laid out with five replicates (farmers' fields) per treatment. With the help of their local agricultural advisors, 20 farmer fields were chosen based on their willingness to participate in the legume species research study project. In each treatment and replicate, two legume species, Bambara groundnut and cowpea, were cultivated.

3.4 Cultural practice and field management

Seeds were weighed and then distributed equally to farmers in their fields for planting. The experimental plot size of 2 m × 4 m was prepared with a tractor-mounted disc plough and harrow to enhance a good seedbed for good germination and seedling emergence. Farmers were allowed to adapt their farming experience and methods to their local conditions. These management were considered to help explain any possible differences in the measured parameters. Conventional tillage was practised most in the dryland farmer's fields of Collin Chabane. Round up with an active ingredient of glyphosate, N-(phosphonomethyl) glycine, in the form of its isopropylamine salt, and dual gold with an active ingredient of S-Metolachlor (chloro-acetanilide) (30 mL/15 L water knapsack = 0.5 L/ha) were applied to control weeds at planting across all the municipalities. Manual weeding was done regularly in all the farmer's fields.

3.5 Plant and soil sampling

Soil physiochemical properties

The trials were established during the 2021/2022 cropping season. Four seeds were planted and thinned out to 1 plant per cavity. Supplemental irrigation was applied when necessary to obtain optimum growth and yield. Two soil samples were randomly collected by collecting samples from different locations within the plot to ensure representative data. from each farmer's field area of 2 m × 4 m to make one composite sample collected from each experimental farmer's field (0–30 cm depth) before planting. The soil samples were taken to the Agricultural Research Council–Natural Resources and Engineering (ARC-NRE) laboratory–, sieved (2 mm), and analyzed for chemical properties (such as s pH (H_2O), P using Bray-2 (Bray, 1945), K, Na, Ca, Mg, Na, B, Mo, Fe and Z) and physical (Sand, Silt, and Clay) properties of the soil (Table 3.1). Both leguminous species were planted at each location.

Table 3. 1: Physio-chemical properties of soils sampled from farmers' fields before planting 2021/2022.

Location	**Sand**	**Textural Clay**	**Silt**	**Field**	**pH (H_2O)**	**P-Bray 1**	**Resistance**			**Exchangeable cations**					
		%							(mg/kg)						
						(mg/kg)	(ohm)	K	Ca	Mg	Na	B	Mo	Fe	Zn
Musina	68	10	22	1	8.4	43.2	370	312	4360	433	191	0.03	0.02	0	0
	60	10	22	2	8.4	45.6	420	312	4340	469	185	0.03	0.02	0	0
	72	10	18	3	8.6	54.2	350	401	4900	1200	263	0.12	0.02	0	0.5
	76	8	16	4	8.7	54.6	380	335	4590	1390	219	0.11	0.02	0	0.5
	90	0	10	5	8.3	78.4	680	80	2710	472	24.9	0.17	0.02	0.3	2.0
Average	73.2	7.6	17.6	3	8.48	55.2	440	287	4180	793	176	0.092	0.02	0.06	0.6
Thulamela	90	0	10	6	5.6	3.4	120	48	146	48.2	5.44	0.01	0.02	13.3	2.1
	68	4	10	7	5.0	4.4	260	78.7	360	82.6	7.28	0.02	0.02	4.1	0.4
	78	4	18	8	5.0	19.2	1592	27.5	49.7	16	3.72	0.00	0.02	3.5	0.06
	68	6	26	9	4.7	4.8	1205	32	146	56	4.31	0.01	0.02	24.3	0.0
	88	0	12	10	6.4	25.4	4340	81	259	76.5	4.24	0.15	0.02	2.5	0.1
Average	78.4	2.8	15.2	8	5.34	11.4	1503	53	192	56	4.998	0.038	0.02	9.54	0.532
Makhado	54	10	36	11	7.4	8.4	930	132	1930	728	108	0.01	0.02	0.9	0.6
	46	10	44	12	6.7	12.8	820	85	1460	540	61.2	0.29	0.35	2.9	82.9
	63	9	28	13	7.2	3.8	650	138	1930	882	130	0.01	0.02	0.9	0.6
	66	10	24	14	7.3	37.5	680	147	2330	862	87.6	0.03	0.02	1.0	1.7
	46	12	44	15	7.1	12.7	1070	81	1270	564	60.1	0.01	0.02	4.9	0.4
Average	55	10.2	35.2	13	7.14	15.04	830	116	1784	715	89.38	0.07	0.086	2.12	17.24
Collin Chabane	24	20	56	16	6.2	4.1	280	164	2690	796	28.4	0.02	0.02	0.5	1.1
	26	20	56	17	6.1	3.4	230	157	2700	804	27.4	0.02	0.02	0.5	1.1
	63	9	28	18	6.6	8.2	990	140	1410	448	18.6	0.01	0.02	13.1	1.0
	60	12	28	19	6.6	0.8	960	130	2480	838	23.9	0.01	0.02	3.2	0.5
	28	16	56	20	6.4	0.2	550	132	2260	840	18.6	0.04	0.02	5.0	0.6
Average	40.2	15.4	44.8	18	6.38	3.34	602	144	2308	745	23.38	0.02	0.02	4.46	0.86

3.6 Plant sampling

Ten plants of Bambara groundnut and cowpea were randomly sampled from each farmer's fields at 75% physiological maturity. The sampling was done in a zigzag manner across the field's length of 2 m × 4 m (Nyemba and Dakora, 2005). The plants were dug up and then separated into roots~~,~~ and shoots (plus pods). The shoots and pods were oven-dried at 50 °C for 72 hours in a labelled bag and weighed to determine the shoot + pods' dry matter (shoot+pods DM) complete drying was obtained when reaching a constant weight.

3.7 Rhizospheres sampling and preparation

The soil rhizosphere samples were collected from all the plots of Bambara groundnut and cowpea. A total of twenty (20) rhizosphere samples were taken, each at a depth of 15 cm. The samples were immediately stored in a cooler box filled with ice (0 °C) before being transported to the laboratory for analysis. Ten (10) g of each soil sample was then passed through an 8 mm sieve, and the soil retained in a 4 mm sieve was used. The samples were measured and added to 90 ml of sterile distilled water (SDW). The resultant solutions were then shaken on a rotary shaker for 45-60 minutes at 160 rpm. After shaking, the solutions were allowed to settle for 45 minutes to ensure no solid particles were transferred during dilution. To perform serial dilution on all samples, 1 mL of the resultant supernatant was mixed with 2 mL of sterile distilled water (SDW). 0.1 ml of the previous supernatant was added to 9.9 ml of SDW, and finally, 2 mL of this supernatant was added to 18 mL of SDW (Arana et al., 2013).

3.8 Determination of Carbon Source Utilization

Rhizosphere samples were suspended in saline buffer and homogenised. 150 µL of the aqueous suspension was inoculated on 96 well Biolog Microplates, which were then incubated at 26 °C for one week. The rate of colour change (pattern development) in each well was recorded, and the optical density (OD) of each well was read on Biolog MicroLog™ 3E or Micro Station™ Systems every 24 hours for seven days until the readings became constant (Wang et al., 2007; Habig and Swanepol, 2015; Mofokeng et al., 2020). Before analysis, the data of each plate reading was normalized by subtracting the blank well (control) values from each plate well. The total carbon source utilization (microbial response) was quantified by the average well-colour development (AWCD). Similarly, the specific utilization of all carbon sources for the group substrates (carbohydrates, amino acids, amines, polymers, and carboxylic acids) was determined. Using the OD of 0.25 as a threshold for a positive response, the Shannon Weaver index (H) value, i.e., the microbial communities' richness and evenness of response, was calculated (Habig and Swanepoel, 2015).

3.9 Determination of Enzyme Activities

The ability of the soil microbial population to obtain carbon, phosphorus, and nitrogen was determined by measuring the activities of enzymes such as ß-glucosidase, alkaline phosphatase, acid phosphatase, and urease. The activities of ß-glucosidase and phosphatase were calculated by measuring the release of p-nitrophenol after the soil is incubated with p-nitrophenyl glucoside and p-nitrophenyl phosphate, respectively (Tabatabai and Bremner, 1969). The overall enzyme activity is calculated as the sum of all enzyme activities, divided by four (expressed as the µg/g/h).

3.10 Total microbial count

The total microbial count was determined by preparing the suspense of soil samples in saline buffers., These suspensions were then serially diluted, and a 100 µL aliquot from each dilution was spread on Nutrient Agar media. Plates were then subsequently incubated for 24-48 hours to allow microbial growth. The resulting colonies were counted to determine the colony-forming units/g soil (Wang et al., 2007; Gryta et al., 2014).

3.11 Grain and leaves minerals elements determination

Bambara groundnut and cowpea leaves and pods were harvested from 10 plants per plot at the 75% physiological stage. Before the minerals elements analysis, the Bambara groundnut and cowpea pod samples were oven-dried (50 °C) until a constant weight was reached and ground to a fine powder (0.85 mm). A 0.1-0.5 g ground sample was taken from the milled pods for analysis. The mineral content was determined at the (ARC-ISCW) analytical laboratory in Pretoria, South Africa. The concentrations of nine mineral elements (Ca, Cu, Fe, K, Mg, Mn, Na, B, and Zn) were measured in mg.100 g^{-1} using an Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-9820, Shimadzu).

3.12 Measurements of nutrient composition in species leaves

Fully emerged trifoliate leaves were harvested from 10 plants per plot during the physiological stage before the onset of flowering. The percent N in cowpea leaves was determined using mass spectrometry, as described by Belane and Dakora (2010). The leaf protein was estimated as %N of organ × 6.25 (Jones, 1941; Mariotti et al., 2008).

3.13 Statistical analysis

Data on the number of pods per plant, number of nodules, mineral elements, dry matter, and grain yield were subjected to ANOVA using STATISTICA software program version 10.0 (Statistica, 2011). The data were expressed as mean ± standard error of the mean to show variation. One-way ANOVA was used to analyse yield component parameters such as dry matter and the number of nodules and pods to compare species performance within each farmer's field where treatment means were different, the Duncan's multiple range test (DMRT) was used to separate means at $p \leq 0.05$. The two-way ANOVA was used to compare the means of species × farmers field interaction and revealed significant ($p \leq 0.05$) differences; the means were separated using a Duncan

Multiple Range Test. Raw data on carbon source utilization from the spectrophotometer reading were transferred into Excel. Data was analyzed using non-parametric statistical analyses in STATISTICA 12 (StatSoft, Inc.©). This program has four blocks. Average Well Color Development (AWCD) was calculated from the fourth block at Optical Density 0.25 nm. Soil microbial diversity was statistically analyzed using Principal Component Analysis (PCA) and cluster analyses (vertical hierarchical tree plots).

CHAPTER 4: YIELD RESPONSE OF TWO LEGUMES FROM DIFFERENT AGRO-CLIMATIC CONDITIONS IN FARMER'S FIELDS IN THE VHEMBE DISTRICT, SOUTH AFRICA

ABSTRACT

Grain legumes such as Bambara groundnuts (*Vigna subterranean*) and Cowpea (*Vigna unguiculata*) are rich in protein and mineral elements and are nutrient-dense sources of food for most smallholder subsistence and resource-poor farmers in South Africa. However, there is a need to evaluate the variability in the yield component of these legume species may vary with the environment. Therefore, the objective is to evaluate the yield of two legume species in the farmer's fields of the Vhembe district, a geographic area that has not been extensively studied considering how they respond to different management practices and environmental factors-. We hypothesized significant variations in the grain yield component of the two-legume species under different locations caused by management practices and environmental factors. Bambara groundnut and cowpea were grown during the 2021/2022 summer cropping season in South Africa. Farmers field in four different locations (Thulamela, Makhado, Musina Collin Chabane). Ten plants were sampled from 20 farmers' fields and used to determine growth, grain and yield components and nutritional composition. For minerals analysis, a sample of 0.1 to 0.5 g flour was taken from the milled pods. Mineral element analysis determination using inductively coupled plasma optical emission spectrometry (ICP-OES), beta-carotene and vitamins).

The legume species studied exhibited differences in the grain protein fractions' profile and grain mineral content. The two species grains showed considerable variation for the following nine mineral contents: calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (B), and zinc (Zn). The ranges (mg/ g dry matter) obtained for the micro minerals are Bambara groundnut with Na 889 mg/kg, Fe 87 mg/kg, Mn 2 mg/kg, Zn 1 mg/kg, and B 1 mg/kg. Cowpea Na 22 mg/kg, Fe 23 mg/kg, Mn 5 mg/kg, Zn 2 mg/kg and for the macro minerals: Bambara groundnut K 1.55 mg/kg, Ca 0.16 mg/kg, Mg 0.21 mg/kg, P 0.26 mg/kg. Cowpea K 1.30 mg/kg, Ca 0.21 mg/kg, Mg 0.25 mg/kg, P 0.36 mg/kg. The collected data showed significant ($p\leq0.05$) differences in plant dry matter (DM) yield, number of pods, pods dry weight and number of nodules from different farmer's fields. A positive correlation was observed between monthly maximum temperature and dry matter for Bambara groundnut (r^2 =0.33) and cowpea (r^2=0.30). Although the results converge on the best-performing legume species across all study sites, some specific differences were discovered that should be investigated further. The results may indicate the suitability of certain agro-climatic conditions within Vhembe district for the cultivation of specific legumes. Farmers can use this information to make informed decisions about crop selection based on their local environmental conditions.

Keywords: Pods, nodulation, municipalities, food security.

4.1 Introduction

Bambara groundnuts (*Vigna subterranean*), cowpea (*Vigna unguiculata*) and groundnuts (*Arachis hypogaea*) are leguminous species endemic to many parts of Limpopo province (Mohale et al., 2014). Bambara groundnut is a popular legume among resource-constrained rural farmers because It is drought-resistant and can be grown in low-fertility soils where other crops fail (Azam-Ali et al., 2014; Obidiedube et al., 2020). Most rural communities may benefit from the innovative processing of legume crops to improve their nutritional status and combat diseases (Kamwamba et al., 2016). Because it can withstand drought and grow in low-fertility soils where other crops fail, Bambara groundnut is Africa's third most common legume after groundnut and cowpea (Obidiebube et al., 2020.).

Bambara groundnut (*V. subterranean (L.) Verdc*) is a native African crop that has been underutilized and understudied for human and animal consumption (Mabhaudhi et al., 2018). Bambara groundnut may be cultivated in many developing countries where other essential crops are challenging to grow because of its drought tolerance and low disease-insect infestation (Majola, 2021). The ability of Bambara groundnut to grow in these diverse agro-climatic conditions (Jorgensen et al., 2010), with soils varying in pH, mineral nutrients, soil moisture, rhizosphere temperature, and N_2-fixing microsymbionts, is intriguing.

In Limpopo province, particularly in Vhembe district municipalities, Bambara groundnut (*Vigna subterranea* (L.) *Verdc)* and cowpea *(Vigna unguiculata* L. *Walp*) are common in cropping systems (DAFF, 2016). Cowpea (*Vigna unguiculata*) is grown and consumed by subsistence farmers in Africa's semi-arid and sub-humid regions (DAFF, 2015). Cowpea is an indigenous leguminous crop cultivated in South Africa in Limpopo, Mpumalanga, North-West and KwaZulu-Natal provinces. Cowpeas are grown not just for dried seeds but also for the leaves and young pods used as vegetables by these farmers (IITA, 2009). Smallholder farmers in rural communities in sub-Saharan Africa (SSA) grow the crop as a cash crop for its grains and leaves (Anderman et al., 2014). It's a resilient crop identified as a good source of nutrients when food is scarce (Mbosso, 2020).

Bambara groundnut and cowpea are still underutilised species due to a shortage of improved proper cultivars that can tolerate different agroclimatic conditions and insufficient production procedures and inputs required for higher productivity and profitability (Mabhudhi et al., 2017; Kamara et al., 2018). The yield gap can be closed if farmers can access improved varieties and production practices (Ajeigbe et al., 2010). Numerous studies have described the N_2 fixation of legume species under field environments and glasshouse experiments (Mokgehle et al., 2014; Karane et al., 2013; Barbieri et al., 2014). Despite the nutritional value and amounts of N that legumes contribute to the soil, few studies have assessed indigenous legume species growth and grain yield parameters in farmers' fields in South Africa at different agro-climatic conditions (Mohale et al., 2014). Crop yields and nutritional quality are primarily determined by climatic conditions and soils (Burritt, 2019). Therefore, the study assessed the grain and yield components of two legume species grown from farmers' fields in the four municipalities (Collins Chabane, Makhado, Musina, and Thulamela) in Vhembe district. With a continuous changing agro-climatic conditions (temperature,rainfall) combined with a marked heterogeneity in African soils, there is a need to identify crop species that are adapted todifferent agro-ecologies.

4.2. Materials and methods

4.2.1 Description of the trial sites

The study was conducted in farmers' fields at four municipalities (Collin Chabane, Makhado, Musina, and Thulamela) in the Limpopo Province of South Africa during the 2021/2022 cropping season. The area has an unimodal rainy season that starts at the end of mid-November each year and ends in April the following year. The seasonal rainfall pattern of the study area is indicated in Chapter Three (Figures 3.2 a-d). The geographical coordinates and elevation are also described in Chapter Three.

4.2.2 Plant and soil sampling

Soil physiochemical properties

One composite soil sample was randomly collected at (0 - 30 cm soil depths). From each farmer's field. Each municipality had five farmers' fields. The collected soil samples were processed as described in Chapter Three.

4.2.3 Plant sampling

Ten plants were randomly sampled at 75% physiological maturity from each farmer's field following the zigzag manner across the field's length (Nyemba and Dakora, 2005). The plants were dug up and separated into roots and shoots (plus pods). In all farm fields, the data was collected from five farmer's fields per municipality. The nodules were removed from the roots, weighed, and stored in vials. The shoots and pods were oven-dried at 50 °C for 72 hours in a labelled bag and weighed to determine the shoot + pods' dry matter (shoot + pods DM).

4.2.4 Statistical analysis

Data on the number of pods per plant, number of nodules, mineral elements, dry matter, and grain yield were subjected to ANOVA using STATISTICA software programme version 10.0

(Statistica, 2011). The data were expressed as mean ± standard error of the mean. One-way ANOVA was used to analyse growth and yield component parameters such as dry matter and the number of nodules to compare species performance within each farmer's field. The two-way ANOVA was used to compare the means of species × farmers fields interaction and revealed significant ($p \leq 0.05$) differences; the means were separated using a Duncan Multiple Range Test.

To ascertain the effect of the agro-climatic conditions on plant growth of Bambara groundnut and cowpea at farmer's fields, correlation analyses were done between daily maximum temperatures and yield/yield components. Linear correlation was used to evaluate the relationships among shoot + pods, soil pH, and micro-minerals using Excel to compute Pearson correlation coefficients (r) between pairs of variable. Subsequently, all relationships between soil properties were evaluated using Pearson’s correlation tests.

4.3. Results

4.3.1 Soil physical and chemical properties

The results of the soil analysis taken before planting for the 2021/22 cropping season are presented in Table 3.1. The soils at Musina farmer's fields were alkaline. Soils at 0-30 cm depth indicate a high percentage of sand over clay and silt texture. Alkaline soils in Musina farmers’ fields promoted the abundance of cations such as magnesium (Mg), calcium (Ca) and sodium (Na), Table 3.1. Boron (B), molybdenum (Mo), iron (Fe) and copper (Cu) are in fewer quantities compared to macronutrients like Calcium and Magnesium.

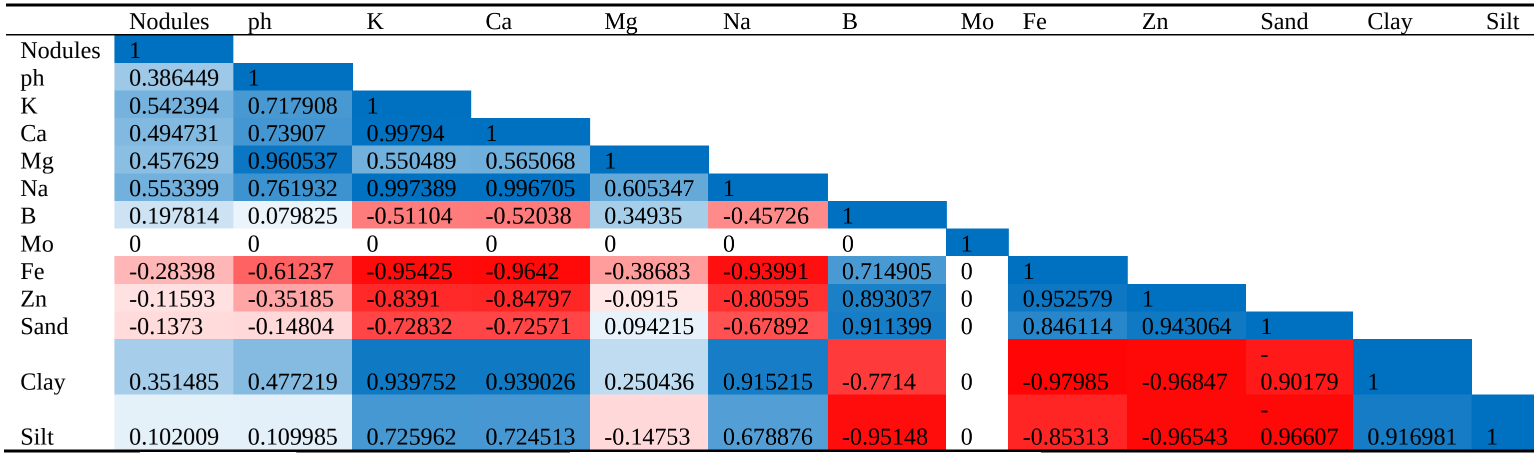
Thulamela farmer's fields had moderately acidic soils with a high quantity of sandy over clay and silt textures. Calcium was in high quantity over magnesium (Mg), potassium (K), and sodium (Na) Table 3.1.

Makhado farmer's fields were characterised by sandy clay loam and sandy clay textures, with neutral pH ranging from 7.4 to 7.1. The soil's chemical properties regarding nutrient content also varied across the locations. For example, Musina farmers' fields had greater K, Ca, Mg, Na, B, Mo, Fe, and Zn levels than Thulamela and Makhado farmers' fields Table 3.1. Collin Chabane also had greater K, Ca, Mg, Na, and B levels than Thulamela and Makhado.

4.3.2 Correlation analysis between nodulations and soil physio-chemical properties across farmer’s fields

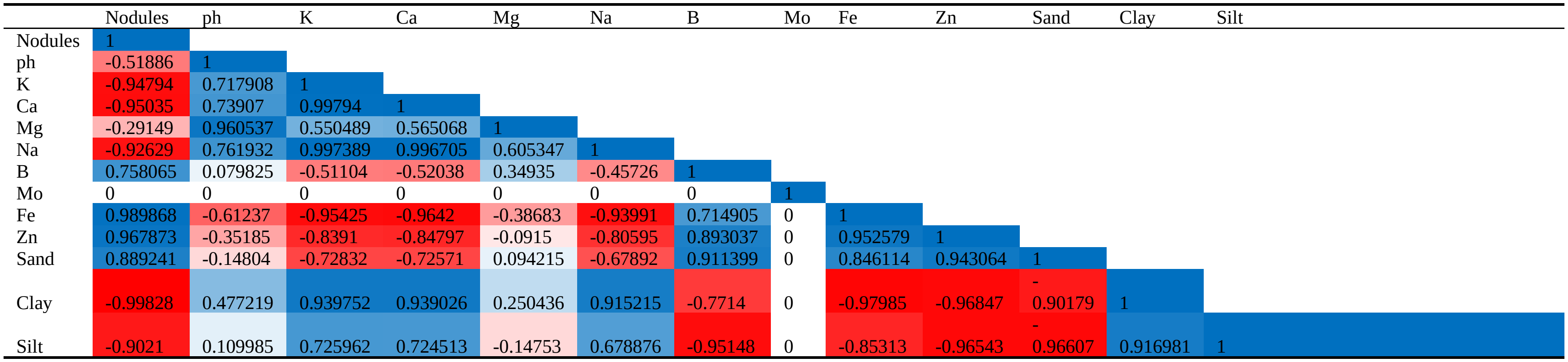
The data showed that molybdenum and iron significantly affected the number of nodules plant^{-1} in cowpea and Bambara groundnut compared with other micro-nutrients. The analysis showed positive correlations of nodules with macro-nutrients, such as K, Ca, Mg, Na, and pH. Similar, slightly positive correlations were observed between available Silt and Clay. However, there was a negative correlation between Fe, Zn, Sand, and Mo in Musina Bambara groundnut farmers' fields

(Figure 4-1). Furthermore, positive correlations of nodules with soil physio-chemical properties, B, Mo, Fe, Zn, and sand.



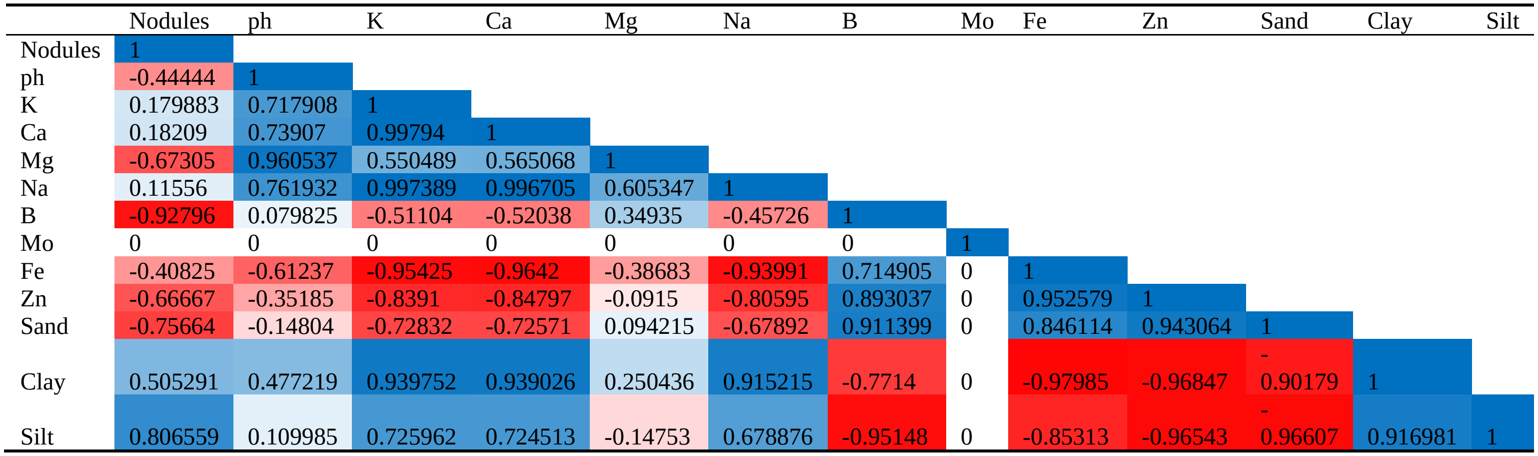
	Nodules	ph	K	Ca	Mg	Na	B	Mo	Fe	Zn	Sand	Clay	Silt
Nodules	1												
ph	0.386449	1											
K	0.542394	0.717908	1										
Ca	0.494731	0.73907	0.99794	1									
Mg	0.457629	0.960537	0.550489	0.565068	1								
Na	0.553399	0.761932	0.997389	0.996705	0.605347	1							
B	0.197814	0.079825	-0.51104	-0.52038	0.34935	-0.45726	1						
Mo	0	0	0	0	0	0	0	1					
Fe	-0.28398	-0.61237	-0.95425	-0.9642	-0.38683	-0.93991	0.714905	0	1				
Zn	-0.11593	-0.35185	-0.8391	-0.84797	-0.0915	-0.80595	0.893037	0	0.952579	1			
Sand	-0.1373	-0.14804	-0.72832	-0.72571	0.094215	-0.67892	0.911399	0	0.846114	0.943064	1		
Clay	0.351485	0.477219	0.939752	0.939026	0.250436	0.915215	-0.7714	0	-0.97985	-0.96847	-0.90179	1	
Silt	0.102009	0.109985	0.725962	0.724513	-0.14753	0.678876	-0.95148	0	-0.85313	-0.96543	-0.96607	0.916981	1

Figure 4-1: Makhado (Bambara groundnut) Correlation matrix of soil properties.



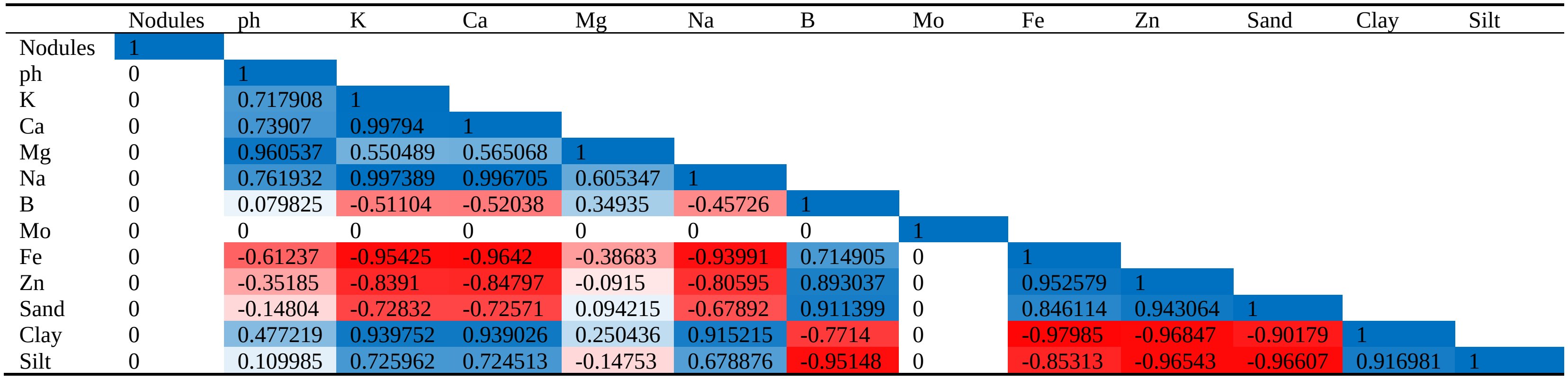
	Nodules	ph	K	Ca	Mg	Na	B	Mo	Fe	Zn	Sand	Clay	Silt
Nodules	1												
ph	-0.51886	1											
K	-0.94794	0.717908	1										
Ca	-0.95035	0.73907	0.99794	1									
Mg	-0.29149	0.960537	0.550489	0.565068	1								
Na	-0.92629	0.761932	0.997389	0.996705	0.605347	1							
B	0.758065	0.079825	-0.51104	-0.52038	0.34935	-0.45726	1						
Mo	0	0	0	0	0	0	0	1					
Fe	0.989868	-0.61237	-0.95425	-0.9642	-0.38683	-0.93991	0.714905	0	1				
Zn	0.967873	-0.35185	-0.8391	-0.84797	-0.0915	-0.80595	0.893037	0	0.952579	1			
Sand	0.889241	-0.14804	-0.72832	-0.72571	0.094215	-0.67892	0.911399	0	0.846114	0.943064	1		
Clay	-0.99828	0.477219	0.939752	0.939026	0.250436	0.915215	-0.7714	0	-0.97985	-0.96847	-0.90179	1	
Silt	-0.9021	0.109985	0.725962	0.724513	-0.14753	0.678876	-0.95148	0	-0.85313	-0.96543	-0.96607	0.916981	1

Figure 4-2: Makhado (cowpea) Correlation matrix of soil properties.



	Nodules	ph	K	Ca	Mg	Na	B	Mo	Fe	Zn	Sand	Clay	Silt
Nodules	1												
ph	-0.44444	1											
K	0.179883	0.717908	1										
Ca	0.18209	0.73907	0.99794	1									
Mg	-0.67305	0.960537	0.550489	0.565068	1								
Na	0.11556	0.761932	0.997389	0.996705	0.605347	1							
B	-0.92796	0.079825	-0.51104	-0.52038	0.34935	-0.45726	1						
Mo	0	0	0	0	0	0	0	1					
Fe	-0.40825	-0.61237	-0.95425	-0.9642	-0.38683	-0.93991	0.714905	0	1				
Zn	-0.66667	-0.35185	-0.8391	-0.84797	-0.0915	-0.80595	0.893037	0	0.952579	1			
Sand	-0.75664	-0.14804	-0.72832	-0.72571	0.094215	-0.67892	0.911399	0	0.846114	0.943064	1		
Clay	0.505291	0.477219	0.939752	0.939026	0.250436	0.915215	-0.7714	0	-0.97985	-0.96847	-0.90179	1	
Silt	0.806559	0.109985	0.725962	0.724513	-0.14753	0.678876	-0.95148	0	-0.85313	-0.96543	-0.96607	0.916981	1

Figure 4-3: Thulamela (Bambara groundnut) Correlation matrix of soil properties.



	Nodules	ph	K	Ca	Mg	Na	B	Mo	Fe	Zn	Sand	Clay	Silt
Nodules	1												
ph	0	1											
K	0	0.717908	1										
Ca	0	0.73907	0.99794	1									
Mg	0	0.960537	0.550489	0.565068	1								
Na	0	0.761932	0.997389	0.996705	0.605347	1							
B	0	0.079825	-0.51104	-0.52038	0.34935	-0.45726	1						
Mo	0	0	0	0	0	0	0	1					
Fe	0	-0.61237	-0.95425	-0.9642	-0.38683	-0.93991	0.714905	0	1				
Zn	0	-0.35185	-0.8391	-0.84797	-0.0915	-0.80595	0.893037	0	0.952579	1			
Sand	0	-0.14804	-0.72832	-0.72571	0.094215	-0.67892	0.911399	0	0.846114	0.943064	1		
Clay	0	0.477219	0.939752	0.939026	0.250436	0.915215	-0.7714	0	-0.97985	-0.96847	-0.90179	1	
Silt	0	0.109985	0.725962	0.724513	-0.14753	0.678876	-0.95148	0	-0.85313	-0.96543	-0.96607	0.916981	1

Figure 4-4: Thulamela (cowpea) Correlation matrix of soil properties.

4.3.3 Weather data

The total rainfall received during the growing season in Thulamela was 363.3 mm (Figure 3-2a-d). Temperatures at the trial sites measured at farmers fields within the study sites varied during the cropping season. The average maximum temperatures in Makhado and Collin Chabane during the reproductive stage of growth were 31 °C and 32 °C monthly temperature, with a minimum of 14 °C and 16 °C respectively (Figures 3-3 and 3-4). The overall rainfall received during the 21/22 growing season was 450 mm in Collin Chabane. Meanwhile, in Makhado, maximum temperatures were 31 °C with a minimum of 16 °C. Thus, Thulamela was regarded as a cooler environment than Makhado and Collin Chabane. Maximum temperatures in Musina are 36 °C, with a minimum temperature of 18 °C.

4.3.4 Plant growth

Using the dry matter (DM) of pods and shoots as components of Bambara groundnut and Cowpea yields, a substantial significant interaction between the factors (legume species, agro-climatic condition and location) was found between and among farmers across all the municipalities. Yield differed across sites. The yield over four agro-climatic conditions ranged from 258.7 g/plant (field 1) to 248.1 g/plant (field 5) in Musina farmers' fields (Table 4.1). In addition, Thulamela produced the 1.9 g/plant lowest pod DM yields over the other farmer's fields, respectively to other farmers fields. Makhado and Collins Chabane municipalities produced similar yields from 1.6 g/plant and 2.4 g/plant. Cowpea pod DM was the highest-yielding species in Musina municipality (Table 4.2). Across trials including locations and species, the combined shoot plus pods DM was significant ($p \leq 0.001$) for grain yield for both species in Musina and Thulamela. The data recorded from Musina and Thulamela showed increased dry matter for cowpeas compared to Collin Chabane and Makhado (Table 4.2). Nodules were present in this research study on all Bambara groundnut farmer fields (Tables 4.2 and 4.1). However, the nodule number showed that the Bambara groundnut in Thulamela and Makhado municipalities had the best nodulation, had higher number relatively to other locations (Table 4.2).

Table 4. 1: Comparison of fresh weight, nodule number, pod number, and dry matter yields of Bambara groundnut and cowpea sampled from 20 farmers' fields in the Vhembe district.

Location	Field no.	Fresh weight (Whole plant g/plant)	Dry-weight Whole Plant (Shoots+Pods g/plant)	Number of Pods (g/plant)	Pod-fresh weight (g/plant)	Pod-dry weight (g/plant)	Number of nodules
Musina	1	258.7 ± 59.5^{a}	68.3 ± 14.9^{a}	18.6 ± 2.9^{b}	11.1 ± 1.6^{C}	7.9 ± 1.7^{b}	3.8 ± 1.1^{c}
	2	196.3 ± 38.4^{b}	67.8 ± 14.1^{ab}	22.0 ± 3.6^{a}	41.7 ± 11.3^{a}	25.4 ± 7.7^{c}	27.5 ± 7.9^{c}
	3	251.6 ± 31.7^{c}	86.1 ± 22.3^{a}	19.5 ± 3.5^{b}	36.8 ± 8.8^{ab}	34.5 ± 10.4^{c}	0.0 ± 0.0^{a}
	4	199.4 ± 51.6^{d}	68.5 ± 26.7^{ab}	25.8 ± 6.1^{a}	34.0 ± 11.2^{b}	22.1 ± 10.0^{c}	1.6 ± 0.8^{a}
	5	248.1 ± 30.3^{e}	80.9 ± 21.8^{b}	40.9 ± 7.4^{C}	65.9 ± 11.6^{d}	37.6 ± 6.3^{c}	0.3 ± 0.1^{ab}
Thulamela	1	74.1 ± 9.7	16.8 ± 3.3^{c}	9.0 ± 1.5^{d}	11.2 ± 2.1^{b}	5.0 ± 1.4^{e}	2.6 ± 0.9^{d}
	2	76.9 ± 10.7^{a}	15.2 ± 2.2^{c}	12.6 ± 2.8^{c}	11.9 ± 1.6^{a}	4.5 ± 0.9^{d}	2.5 ± 1.0^{d}
	3	124.5 ± 21.8^{c}	24.4 ± 4.4^{a}	7.5 ± 1.9^{d}	9.3 ± 1.8^{b}	2.7 ± 0.5^{d}	4.7 ± 1.2^{e}
	4	79.6 ± 14.6^{a}	15.1 ± 2.7^{c}	10.1 ± 2.6^{c}	11.0 ± 2.7^{a}	3.8 ± 0.7^{d}	4.1 ± 1.1^{e}
	5	111.1 ± 31.3^{c}	20.6 ± 5.3^{c}	5.1 ± 2.4^{d}	6.5 ± 2.2^{b}	1.9 ± 0.6^{e}	2.6 ± 0.7^{d}
Makhado	1	330.6 ± 104.1^{e}	144.3 ± 53.3^{a}	9.9 ± 2.5^{a}	14.8 ± 3.75^{e}	5.4 ± 1.2^{d}	3.2 ± 1.4^{d}
	2	55.0 ± 7.5^{a}	20.9 ± 2.7^{d}	7.5 ± 2.1^{c}	7.2 ± 2.15^{c}	2.9 ± 0.9^{c}	2.1 ± 0.9^{d}
	3	89.8 ± 23.6^{a}	29.8 ± 7.4^{d}	4.3 ± 1.5^{c}	2.3 ± 0.61^{c}	1.6 ± 0.6^{c}	1.4 ± 0.5^{e}
	4	193.5 ± 66.2^{e}	56.6 ± 20.3^{d}	13.8 ± 3.5^{a}	9.7 ± 2.04^{c}	3.3 ± 0.7^{d}	1.9 ± 1.0^{e}
	5	135.3 ± 37.1^{a}	38.8 ± 8.7^{d}	5.3 ± 1.1^{ac}	11.7 ± 4.32^{ec}	3.4 ± 1.3^{dc}	0.7 ± 0.4^{ed}
Collin chabane	1	247.7 ± 64.57^{a}	56.7 ± 14.6^{d}	7.5 ± 1.4^{e}	16.1 ± 4.26^{c}	5.3 ± 1.4^{b}	0.0 ± 0.0^{b}
	2	80.05 ± 16.8^{d}	26.2 ± 5.3^{a}	4.0 ± 0.5^{a}	4.9 ± 1.0^{e}	2.4 ± 0.7^{c}	1.5 ± 1.0^{bd}
	3	129.10 ± 27.9^{a}	34.6 ± 6.2^{a}	7.7 ± 0.8^{e}	10.4 ± 1.4^{c}	4.2 ± 0.7^{b}	14.3 ± 5.8^{b}
	4	150.20 ± 27.4^{a}	41.5 ± 7.3^{a}	8.0 ± 1.3^{e}	10.7 ± 2.7^{c}	4.1 ± 0.6^{b}	20.1 ± 6.2^{b}
	5	156.30 ± 28.9^{a}	44.1 ± 8.1^{a}	13.0 ± 3.0^{a}	18.7 ± 4.8^{ce}	7.3 ± 1.7^{bc}	$21.3\pm5.9b^{d}$

Values (mean± SE) with dissimilar letters in a column significantly differ at $p\leq0.05$.

Table 4. 2: Comparison of two legume species yield components across different locations.

Location	**Legume species**	**Fresh weight (Whole plant g/plant)**	**Dry weight Whole Plant (Shoots+Pods g/plant)**	**Number of Pods (g/plant)**	**Pod-dry weight (g/plant)**	**Number of nodules**
Musina	Bambara groundnut	198.2 ± 20.1^{a}	79.6 ± 13.1^{A}	24.2 ± 3.7^{a}	7.7 ± 1.9^{c}	2.9 ± 1.3^{b}
	Cowpea	263.4 ± 32.7^{a}	69.0 ± 12.4^{b}	26.5 ± 2.9^{a}	43.2 ± 6.0^{ab}	10.4 ± 3.5^{d}
Thulamela	Bambara groundnut	75.0 ± 9.0^{C}	35.2 ± 12.9^{d}	13.1 ± 1.8^{c}	3.8 ± 0.5^{c}	3.7 ± 0.7^{a}
	Cowpea	246.6 ± 51.7^{a}	81.0 ± 19.6^{c}	3.2 ± 0.5^{b}	2.8 ± 0.7^{c}	0.0 ± 0.0^{c}
Makhado	Bambara groundnut	104.9 ± 9.9^{B}	28.8 ± 2.5^{E}	10.1 ± 1.4^{d}	3.7 ± 0.8^{e}	20.4 ± 4.0^{d}
	Cowpea	200.3 ± 31.4^{e}	52.4 ± 7.4^{d}	6.0 ± 0.5^{e}	5.6 ± 0.6^{d}	2.4 ± 0.7^{e}
Collin Chabane	Bambara groundnut	74.8 ± 7.4^{C}	15.8 ± 1.8^{A}	13.2 ± 1.8^{c}	2.2 ± 0.3^{c}	2.4 ± 0.4^{a}
	Cowpea	111.7 ± 15.5^{c}	21.0 ± 2.8^{c}	4.5 ± 0.4^{a}	4.9 ± 0.6^{c}	4.2 ± 0.7^{c}

Values (mean± SE) with dissimilar letters in a column are significantly different at p≤0.05.

4.4 Discussion

The soil analysis, conducted prior to the trial establishment showed high potassium, calcium, and magnesium content at all sites. Nguyen et al. (2017) attributed the uptake of plants' nutrients depending on the soil's nutrient concentration and ratio. Soil pH has a dominant effect on solubility and therefore availability of cations (Clark and Baligar, 2000). Whereas low pH shifts the equilibrium toward free metal cations and protonated anions, higher pH favours carbonate or hydroxyl complexes. Therefore, the availability of the micronutrient and toxic ions present in soil solution as cations (e.g. Al^{3+}, $Mo^{2+}$and Fe^{2+}) increases with increasing soil acidity (Porter et al., 2004; Khabaz-Saberi and Rengel, 2010), whereas availability of those present as anions increase with increasing pH (Rengel, 2002; 2011). The study found that Thulamela municipality farmers' fields had moderately acidic soil, while Collin Chabane's and Makhado farmers' fields had optimal pH for legume production (Kyomuhendo et al., 2018)**.** The soil pH of the farmer's fields in Thulamela was below the critical level for cowpea and Bambara groundnut growth, ranging from 4.7 to 5.0 (Table 4.2) (Edmeades et al., 2012).

In rainfed farming systems, where our two-grain legume species were typically cultivated, growth and yield are highly dependent on total seasonal rainfall and distribution. This was evident in the present study, in which two-grain legume species were grown under different weather conditions. Almost all the traits evaluated showed considerable variation, which was attributed primarily to seasonal differences in rainfall and distribution availability. However, the degree to which each legume responded to environmental changes factors such as (rainfall and temperatures were monitotred using weather stations) varied, with cowpeas maintaining acceptable yield in Collin Chabane and Makhado locations, even in the year with the least rainfall, as evidenced by the presence of the highest Dry weight (pods+shoots 81 g plant^{-1}) when compared to the other species such as groundnut and drybean. These findings support previous studies that reported environmental-induced differences in grain legume yield patterns, with more pods and shoots developing in wetter seasons than in drier seasons (Hossain et al., 2017).

In addition to long-term average rainfall, maximum and minimum temperatures varied between the four sites, which could account for differences in species × location performance. The optimal temperature range for both reproductive and vegetative growth of the species is between 21-34 ^{o}C (Majola et al., 2021; Azam-Ali et al., 2001). The crop was planted when temperature was above the base temperature (18 ^{o}C) (Azim-Alli., 2001), providing favourable conditions for successful growth for two planted legume species. The total rainfall received during the growing season was 363.3 mm (Makhado) and 450 mm in Collin Chabane.

Correlation analysis

Maximum daily temperatures at four municipal study sites were significantly correlated with the dry matter yield of Bambara groundnuts and cowpeas, as shown in Tables 4.3-4.6. However, there was no positive correlation between monthly rainfall and plant growth or dry matter (data not shown).

Table 4. 3 Correlation coefficients analysis between temperature and plant growth and shoot dry matter in Musina farmers' fields.

Legume species	**Parameter**	**Significance**	
		r-Value	***p*-Value**
Bambara groundnut	Max. Temp. vs dry matter	0.33	*
Cowpea	Max. Temp. vs dry matter	0.21	**

Table 4. 4 Correlation coefficients analysis between temperature and plant growth and shoot dry matter in Makhado farmers' fields.

Legume species	**Parameter**	**Significance**	
		r-Value	***p*-Value**
Bambara groundnut	Max. Temp. vs dry matter	0.007	*
Cowpea	Max. Temp. vs dry matter	0.13	**

Table 4. 5. Correlation coefficients analysis between plant growth and shoot dry matter in Collins Chabane farmer's fields.

Legume species	**Parameter**	**Significance**	
		r-Value	***p*-Value**
Bambara groundnut	Max. Temp. vs dry matter	0.07	*
Cowpea	Max. Temp. vs dry matter	0.02	**

Table 4. 6. Correlation coefficients analysis between plant growth and shoot dry matter in Thulamela farmer's fields.

Legume species	**Parameter**	**Significance**	
		r-Value	***p*-Value**
Bambara groundnut	Max. Temp. vs dry matter	0.10	*
Cowpea	Max. Temp. vs dry matter	0.30	**

The nodulation of Bambara groundnut indicates the presence of native populations of bacteria capable of colonising the roots in the soils of the two sites' farm fields. Ngo Nkot et al. (2015)

emphasized the Bambara groundnut's great nodulation potential in Cameroon's Center and Littoral areas. Furthermore, Puozaa et al. (2017) revealed that Bambara groundnut nodulation was associated with much more diverse bradyrhizobial populations in South Africa's drier soils (where rainfall was deficient) than in moist Ghanaian soils. These findings are consistent with the findings of more nodules in soils from Africa's drier or less humid environments than in wetter regions (Law et al., 2007; Krasova-Wade et al., 2003; Grönemeyer et al., 2014). Cowpea nodules in Thulamela were not present due to low soil pH. It has been reported that the reproductive stage in cowpeas is the most sensitive to temperature increase, resulting in the loss of flower buds, pods, and seed production (Singh et al. 2010; Ahmed et al. 2010). The low nodulation observed in Makhado and Collin Chabane may have been caused by soil pH and mineral content in those areas. On the other hand, when a legume species is planted outside of its native zone, nodulation usually fails (Bala et al., 2003; Costa et al., 2014), and an inoculant is often recommended.

4.5 Conclusion

Based on the results discussed, it can be concluded that there are significant differences in Bambara groundnut and cowpea growth in farmer's fields in the Vhembe district agro-climatic conditions. Bambara groundnuts grown in all fields are an excellent source of macro and micro minerals, mainly Na, Fe, Mn, Zn, B, K, Ca, Mg, and P. The significantly positive correlation obtained in this study were between average maximum temperature during growing season and dry matter yield (r =0.33*) and between maximum temperature and grain yield (r =0.30**) suggests that maximum temperatures affected the growth and grain yield performance of Bambara groundnut and Cowpea sampled from Musina and Thulamela farmers' fields in study sites. The production environments of Bambara groundnut and cowpea species had significant on their yield components, and Musina was more suitable for Bambara groundnut production because Bambara groundnut performed better than cowpea.

CHAPTER 5: MINERAL ELEMENTS AND NUTRITIONAL COMPOSITION OF TWO LEGUME SPECIES GROWN IN FARMER'S FIELDS IN VHEMBE DISTRICT, LIMPOPO PROVINCE.

ABSTRACT

This study determined the mineral content and nutritional composition of two underutilized legume species in the Vhembe district. Ca, K, Mg, Na, B, Cu, Fe, Zn, and protein content were determined from trifoliate leaves and ground pods. There were similarities and differences in the components determined in the legume species grown in a specific farm's fields and between the same species grown in four different municipalities within the same district. It is hypothesized that there is no variability in the concentrations of essential minerals and the nutritional composition of two legume species.

On the other hand, the concentration ranges within each municipality's farm fields differed significantly ($p \leq 0.05$). The two species pod showed considerable variation for the following nine mineral contents: calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (B), and zinc (Zn). The ranges (mg/ g dry matter) obtained for the micro minerals are Bambara groundnut Fe 87 to 534 and Zn 1 to 35. Cowpea Zn 2 to 45 and B 0 to 31, and for the macro minerals: Bambara groundnut K 1.55, Ca 0.16, Mg 0.21, P 0.26. Cowpea K 1.30, Ca 0.21, Mg 0.25, P 0.36. The study's findings revealed that the protein content of Bambara groundnut and cowpea leaves is 49.95% to 52.57%. These legumes' mineral content and nutritional composition indicate that they could be promoted to improve legume consumption by farmers in the study area.

Key words: Protein, minerals, Bambara groundnut and Cowpea.

5.1 Introduction

Several indigenous legume species can potentially improve food security and nutrition while combating the scourge of micronutrient deficiencies (Chivenge et al., 2015; Tadele,2018). For example, Bambara groundnuts (*Vigna subterranean* L. *Verdc*) and Cowpea (*Vigna unguiculata* L. *Verdc*) are leguminous species endemic to many Southern African regions (Mohale et al., 2014). Bambara groundnut is a cheap source of protein and minerals for many households in Africa, where animal protein is expensive (Amarteifio et al., 2006).

This legume can help to improve food security and alleviate nutritional problems. However, it has been designated an underutilized species and has only recently received increased attention (Mahazib et al., 2013; Tadele,2018). This legume species is drought-resistant, tolerates poor soils, and is relatively resistant to pests and diseases. Bambara groundnut is widely cultivated on a subsistence basis in Africa and is the third most important legume crop after cowpea and groundnut (Kay, 1979), and it is widely consumed in Southern Africa.

The high carbohydrate (65%), protein (23.6%), and 5.5% fibre contents and the grain quantity of fat (6.5%) make Bambara groundnut a complete meal that people can rely on for all their dietary needs (Azman et al., 2019). Furthermore, the Bambara groundnut contains vital elements, including potassium, calcium, iron, and zinc, making it a well-balanced and nutritious meal (Murevanhema and Jideani, 2013).

Cowpea is an essential source of dietary protein for millions of people in developing countries, complementing low-protein cereal and tuber crop mainstays nutritionally (Tinko, 2008). Cowpea is a highly nutritious legume that contains 24% protein, 62% carbohydrate, and small amounts of other nutrients (depending on variety), making it a well-balanced African diet (Chathuni et al., 2018). It also has a low fat content, which is beneficial in preventing various metabolic and cardiovascular illnesses (Goncalves et al., 2021). The grain's vitamin and amino acid composition complement that of cereals. According to Hall (2012), the least expensive source of protein in smallholder agricultural places where farming is the main activity is cowpea.

According to studies, the relative content of protein fractions and mineral composition of cowpea grains vary greatly depending on the cultivar and environment (Kachare et al., 1988; Gonçalves et al., 2016). So far, there is little information on the nutritional composition and mineral elements concentration in the leaves and pods of field-grown Bambara groundnut and cowpea in Vhembe district, Limpopo Province. Furthermore, this study aims to determine the nutritional composition (protein) and mineral elements Ca, K, Mg, Na, P, Cu, Fe, and Zn in two legume species grown in farmer fields in Vhembe district of South Africa influenced by varying agro-climatic conditions.

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5.2. Materials and methods

5.2.1 Description of the trial sites

The study was conducted at four municipalities (Collin Chabane, Makhado, Musina, and Thulamela) in the Limpopo Province of South Africa during the summer 2021/22 cropping season. The areas of the study have an unimodal rainy season that starts at the end of October each year and ends in April the following year. The details of the trial sites are also described in Chapter Three.

5.2.2 Plant and soil sampling

Soil sampling and analysis

One composite sample collected from each experimental farmer's field (0–30 cm depth) before planting and stored in the fridge (4 °C) in labelled plastic bags. The soil samples were taken to the analytical laboratory (ARC-Natural Resources and Engineering (ARC-NRE), sieved (2 mm), and analysed for chemical properties and soil micro-nutrients.

5.2.3 Grain mineral elements determinations

Bambara groundnut and cowpea pods were harvested per replicate when they reached physiological maturity. Before the minerals elements analysis, the Bambara groundnut and cowpea pod samples were oven-dried (50 °C) until a constant weight was reached and ground to a fine powder (0.85 mm). A sample of 0.1-0.5 g pod flour was taken from the milled pods for analysis. The mineral content was determined at the (ARC-ISCW) analytical laboratory in Pretoria, South Africa. The concentrations of nine mineral elements (Ca, Cu, Fe, K, Mg, Mn, Na, B, and Zn) were measured in mg.100 g^{-1} using an Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-9820, Shimadzu).

5.2.4 Measurements of nutrient composition in species leaves and pods.

Leaf and pod material were harvested from each plot's three inner rows 2 m × 4m plot size and stored for nutritional analysis. The nutritional composition included mineral element analysis of green leaves from the farmer's field from each plant of the two legume species using inductively coupled plasma optical emission spectrometry (ICP-OES and vitamins) and quantified using High-performance liquid chromatography (HPLC) 20 samples. The total nitrogen content in the different cowpea plant parts was determined using the Kjeldahl digestion procedure (Page et al., 1982) while the percent protein content was thereafter estimated from the Kjeldahl Total nitrogen determination (crude protein % = Total N % x 6.25).

5.2.5 Statistical analysis

The data on minerals elements in Bambara groundnut and cowpea leaves and pods were analyzed using the STATISTICA 8.0 analytical software programme (Statsoft Inc., Tulsa, OK). A One-way ANOVA was used to compare the means of Bambara groundnut and cowpea leaves and pods, and a 2-way ANOVA was used to compare mineral nutrient levels between the two legume species. The Duncan Multiple Range Test (DMRT) was used to separate treatment means where significant differences were found at $p \leq 0.05$.

5.3 Results

5.3.1 Mineral concentrations in Bambara groundnut and cowpea pods.

The tested two legume species are rich in K and Fe, which varied from 897 mg/g (K) of Bambara groundnut to 218 mg/g (K) of cowpea. The highest concentration of Fe was found in Bambara groundnut (534.08 mg/g). Cowpea showed a high concentration of Mn, B and Zn relative to Bambara groundnut.

Table 5. 1. A comparison of micronutrient content among species and between edible grains grown in farmers' fields in four municipalities.

Species	Na (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	B (mg/kg)
Bambara groundnut	897.00±124^{b}	534.08±87.9^{e}	26.19±2.0^{a}	35.59±1.59^{f}	29.49±1.1^{b}
Cowpea	219.71±22.^{a}	169.17±23.^{c}	43.39±5.67^{d}	45.81±2.03^{ab}	31.13±0.98^{d}

Values (mean± SE) with dissimilar letters in a column are significantly different at $p\leq0.05$

5.3.2 **Nutritional content concentrations in Bambara groundnut and cowpea leaves sampled in the farmers' fields.**

The protein content of harvested Bambara groundnut and cowpea leaves is presented in Table 5.2. The mean leaf protein content of the two legume species, Bambara groundnut and Cowpea, differed significantly ($p\leq0.05$), with Bambara groundnut having (49.95%) and cowpea a (52.57%). The Bambara groundnut species were characterized by high levels of potassium (1.55 mg/g) relative to cowpea (1.30 mg/g). Calcium and manganese were low in all the species evaluated.

Table 5. 2: A comparison of the accumulation (%) of macro minerals contents of Bambara groundnut and cowpeas grown in farmers' fields in Limpopo province.

Species	**K (mg/g)**	**Ca (mg/g)**	**Mg (mg/g)**	**P (mg/g)**	**Protein (%)**
Bambara groundnut	1.55±0.08^{b}	0.16±0.01^{a}	0.21±0.01^{a}	0.26±0.01^{c}	49.95 ± 4.19^{a}
Cowpea	1.30±0.03^{a}	0.21±0.01^{b}	0.25±0.01^{a}	0.36±0.02^{ab}	52.57 ± 5.59^{b}

Values (mean± SE) with dissimilar letters in a column are significantly different at p≤0.05

5.4 Discussion

Bambara groundnut and cowpea are two important legume species that are often overlooked and underutilised, despite their significant contribution to food and nutritional security in Sub-Saharan Africa. This study aimed to evaluate the variability of these two legume species in terms of their mineral elements and protein content under four different environmental conditions. The cowpea species has been found to exhibit a range of variability in terms of mineral elements and protein content. Our study found lower values of Ca compared to those reported by Belane and Dakora in 2012 and Mamiro et al. in 2011 for cowpea genotypes in Ghana and Tanzania, respectively. This difference could be attributed to environmental variations that occurred during the growth and development period of the species.

The protein content of cowpea leaves reported in this study is comparable to 28% for Swiss chard but way higher than the 15.4% reported by Srisangnam et al. (2007) for cabbage. In rural Limpopo, Bambara groundnut and cowpea are the most important sources of plant protein and mineral nutrients for human nutrition and health. Although a few studies (Toomer, 2018; Nielsen et al., 1997) have assessed the protein levels of edible cowpea leaves and minerals grain, as well as minerals of Bambara groundnut, few (if any) have determined the minerals of these organs as a nutrient source. Bambara groundnut grains are an excellent source of calcium (Ca), iron (Fe), and potassium (K), which are highly desirable from a nutritional perspective. However, these mineral elements may also result in undesirable characteristics such as increased grain hardness and longer cooking time (Bamshaiye et al., 2011). This study shows a wide range of concentrations, which is consistent with previous research. However, the iron and zinc values in this study are similar to those reported by Carvalho et al. (2012), who reported higher iron values and lower zinc values.

The Bambara groundnut concentration of Ca (37-128 g/100 g), K (1545-2200 g/100 g), Mg (159-332 g/100 g), and Na (16-25 g/100 g) obtained in this study was marginally more than the Ca (0.16-0.01 %), K (1.55-0.08 %), Mg (0.21-0.01%) and Na (897.00-124.06 mg/kg) determined by Amarteifio et al., (2002). The results obtained for Fe (mg/kg) (534.08-87.99) are only somewhat different from the 174-613 results from Amarteifio et al. (2002) and the 515-642 results from Kemo (2000). However, The Mn concentrations (26.19-2.03 mg/kg) are consistent with previously published data, including ranges of 75–122 (Kemo, 2000), 41.9 (Aganga et al., 2000), and 49–99. The variations could be due to the genetic capability of the cowpea variety to absorb more calcium from the soil, which was partitioned in the seeds better than other species (Boukar et al., 2011; Seetharama et al., 1987).

The Cowpea concentration of Ca (0.21 mg/g) obtained in this study was slightly similar to the Ca (0.18 mg/kg^{-1} and 0.13 mg/kg^{-1}) determined by (Gerrano et al., 2019). Ye et al. (2006) reported species-by-location interactions and proposed the presence of various genetic association patterns in various contexts. Similar findings were made by Allard and Allard (1964), who noted the complexity of mineral and protein composition and how species-by-location interactions affected it. In addition, Vandemark et al. (2018) studied the impact of species by location interactions on the concentration of mineral elements in lentils and chickpeas grown in Washington and Idaho, USA.

5.5 Conclusion

From the study, it can be concluded that both Bambara groundnut and cowpea were found to be rich in essential nutrients, with each legume species studied exhibiting a different nutritional composition. Bambara groundnut in particular is high in potassium (K) and iron (Fe), relative to cowpea, which is rich in protein, manganese (Mn), boron (B), and zinc (Zn). These results highlight the potential of the studied legume species to continue contributing to the nutritional needs of the communities in the Vhembe district.

CHAPTER 6: EVALUATION OF RHIZOSPHERE SOIL MICROBES OF TWO-LEGUME SPECIES GROWN IN FARMERS' FIELDS IN FOUR MUNICIPALITIES OF VHEMBE DISTRICT

ABSTRACT

Plant-beneficial microorganisms are determinants of plant health and productivity. However, the effects and functional diversity of rhizosphere soil microbes associated with two different legume species grown in farmers' fields are unclear. This study assessed the functional diversity of soil microbes, variations in the total microbial count, carbon source utilisation profile, and soil-plant interactions in the rhizosphere of Bambara groundnut and cowpea. We hypothesized that the rhizosphere of the two-legume species would not host distinct microbial communities with variations in composition and functional diversity under different locations.

Bambara groundnut and cowpea were cultivated in five farmers’ fields each in Thulamela, Makhado, Musina, and Collin Chabane during the 2021/2022 summer cropping season in South Africa. Soil samples were taken from the two-rhizosphere soil at a depth of 15 cm during the 50% flowering stage. Microbial Carbon Source Utilisation Profiles (CSUP) were used to determine the diversity of microorganisms in the soil, and the carbon source utilization pattern of the soil microbial communities was determined using the average well-color development (AWCD) of the BIOLOG microplate. The abundance and richness of the soil microbes were determined using the Shannon-Weaver and Evenness diversity indices, respectively. Soil microbial enzymatic activities were assessed by measuring β-glucosidase and alkaline phosphatase in the soil microbes. Principal component analysis showed differences in carbon source utilisation profiles between the two legume species samples and replicates (farmer's fields). The microbial abundance (richness index) in this study ranged from 18 to 31, with cowpea at Collin chabane farm fields being the highest, while Bambara groundnut in Thulamela and Collin chabane farmer’s fields ranged from 11 to 28 being the lowest. The Shannon-Weaver index varied between 2.49 and 3.41 across all the farmer’s fields. Carbon sources utilized by bacterial communities spread across the 31 carbon sources. The highest utilization of carboxylic acids, amino acids, amines, polymers, and carbohydrates was found in the bacterial communities of cowpea species in (Makhado and Collin chabane), and Bambara groundnut species in (Makhado). This soil diversity and richness is an indicator of the quality of the soil to increase crop yields and agricultural production. The study provides insights into the functional diversity of soil microbes associated with Bambara groundnut and cowpea, highlighting the importance of considering the specific microbial communities present in the rhizosphere of different legume species. Furthermore, the variation in microbial abundance, richness, and carbon source utilization profiles across different municipalities and farmers' fields suggests that environmental factors and farming practices influence microbial communities. This emphasizes the importance of site-specific management approaches tailored to the local agroecological conditions. We hypothesized that the rhizosphere of the two-legume species would host distinct microbial communities with variations in composition and functional diversity under different locations.

Keywords: Enzyme and Soil microbial activity, Shannon Weaver Diversity Index.

6.1 Introduction

Agriculture in arid and semi-arid areas, such as the Vhembe District, is characterised by declining soil fertility and poor microbial count (Ramaru et al., 2000). Smallholder farmers in the Vhembe District commonly grow leguminous crops such as groundnuts, Bambara groundnuts, and cowpeas (Mpandeli and Maponya, 2014) due to their yield stability and source of cheap protein (Akinyele, 1991; Sanginga et al., 2001).

Bambara groundnuts and cowpeas are drought-tolerant and adapted to nutrient-poor soils where they can still produce reasonably good yields relative to other legume species (Massawe et al., 2005). Due to their crucial roles in improving soil quality and health and their exceptional adaptability to marginal environments, legumes are now considered one of the most important components of a cropping system (Gogoi et al., 2018). The legume species impact the nearby soil by exuding compounds known as rhizodeposits, primarily comprising carbohydrates, secondary metabolites, organic acids, and amino acids (Ahkami et al., 2017). Furthermore, soil enzyme activities (particularly those in the rhizosphere) can be effective indicators of changes in microbial functioning in the soil as influenced by a variety of variables (Dirk, 1997; Gianfreda, 2015; Egamberdieva et al., 2011; Yin et al., 2014).

Cropping systems that involve legumes promote the growth of microbes due to the association between soil microbes and the rhizosphere (Yan et al., 2023). This results in the release of exudates that enhance the cycling of plant nutrients, making them more available for plants. This process also promotes plant growth, improves resistance to pests and diseases, and boosts soil health and quality. As a result, it contributes to improved crop health, crop yield, and food security. Several studies have highlighted the benefits of this process (Igiehon and Babalola, 2018; Dang et al., 2020; McNear, 2013; Olanrewaju et al., 2017; Saeed et al., 2021; Ajilogba and Babalola, 2019; Ajilogba et al., 2022).

Soil microbes also regulate plant growth by producing phytohormones and other phytoactive compounds. They also improve plant adaptability by promoting antioxidant defence, inducing systemic resistance, and protecting from pathogens and organic and inorganic toxicants (Igiehon and Babalola, 2018; Dang et al., 2020). The rhizosphere is the narrow zone of the soil in direct proximity to plant roots and the hotspot of various microbes (Igiehon and Babalola, 2017). Studies show that different plant species and types have slightly distinct microbiomes (Cong et al., 2015; Lebeis, 2015; Gałazka et al., 2022). Therefore, studying rhizosphere microbial communities of legume species growing under different environmental conditions is important for understanding the habitat-dependent formation of rhizosphere microbiomes. Both culture-based (Moreno-Espíndola, 2018) and culture-independent methods are used to study rhizosphere microbial communities, which benefits plant health, yields, and the effectiveness of using plants to remediate disturbed soils (phytoremediation) (Mohanram 2019; Kumar and Dubey, 2020).

Rhizosphere biology research has increased interest over the past ten years (Fan et al., 2018; Mohanram, 2019; Bais et al., 2008 and Srivastava, 2021). This interest exists because the plant root zone is a special niche saturated with physical, chemical, and biological interactions between macro- and microorganisms and between organisms and their environment. However, literature has limited information on rhizosphere soil microbes' functional diversity associated with two legume species grown in diverse farmers' fields. The study assessed the functional diversity of soil microbes, variations in the total microbial count, carbon source utilization profile, and soil–plant interactions in the rhizosphere of Bambara groundnut and cowpea.

6.2 Materials and Methods

6.2.1 Experimental sites

The study was conducted at four municipalities (Collin Chabane, Makhado, Musina, and Thulamela) in the Limpopo Province of South Africa during the 2021/2022 cropping season. The area has an unimodal rainy season that starts at the end of October each year and ends in April the following year. The rainfall pattern of the study area is indicated in Chapter Three (Figure 3.2-3.5). The geographical coordinates and elevation are also described in Chapter Three.

6.2.2 Experimental Design and field management

Bambara groundnut and cowpea were grown in four municipalities (Thulamela, Makhado, Musina Collin chabane). Five farmers' fields from each municipality were selected with assistance from their local agricultural advisors and based on their interest in becoming part of the legume species project.

6.2.3 Preparation of Rhizospheres sampling

The rhizosphere samples were destructively obtained from all the plots of Bambara groundnut and cowpea. Ten (10) g of each soil sample were measured into 90 ml of sterile distilled water (SDW). The resultant solutions were shaken on the rotary shaker for 45– 60 min at 160 rpm and were allowed to settle for another 45 min so that solid particles were not transferred during dilution. Serial dilution was performed on all samples by taking 1 ml from the resultant supernatant and adding 2 ml of SDW. To 9.9 ml of SDW, 0.1 ml of the previous supernatant was added, and finally, 2 ml of this supernatant was added to 18 ml of SDW as described in Chapter Three.

6.2.4 Determination of Carbon Source Utilization

Rhizospheres samples were suspended in saline buffer and homogenised. 150 µl of the aqueous suspension was inoculated on 96 well Biolog Microplates, which were then incubated at 26 ^{o}C for 7 days. The rate of colour change (pattern development) in each well was recorded, and the optical density (OD) of each well was read on Biolog MicroLog™ 3E or Micro Station™ Systems every 24 hours for seven days until the readings became constant (Wang et al., 2007; Habig and Swanepol, 2015; Mofokeng et al., 2020), as described in Chapter Three.

6.2.5 Determination of Enzyme Activities

The soil microbial population's ability to obtain carbon, phosphorus, and nitrogen was assayed by measuring the activities of ß-glucosidase, alkaline phosphatase, and urease as described in Chapter Three.

6.2.6 Total microbial count

The total microbial count was determined by preparing the suspense of soil samples in saline buffers., These suspensions were then serial diluted, and a 100µl aliquot from each dilution was spread on Nutrient Agar media. Plates were then subsequently incubated for 24-48 hours to allow microbial growth. The resulting colonies were counted to determine the colony-forming units/g soil (Wang et al., 2007; Gryta et al., 2014).

6.3.7 Statistical analysis

Data was analysed using non-parametric statistical analyses in STATISTICA 12 (StatSoft, Inc.©). Average Well Colour Development was calculated from the fourth block at an Optical Density of 0.25 nm. Soil microbial diversity was statistically analysed using Principal Component Analysis (PCA) and cluster analyses (vertical hierarchical tree plots). Fisher Least Significant Difference (LSD, 0.05) was applied to determine homogenous grouping.

6.4 Results

6.4.1 Soil physical and chemical properties

The initial soil physio-chemical properties varied across the four locations (Table 3.1). The soils at Musina farmer's fields were alkaline, with sandy clay loam texture and pH levels ranging from 8.32 to 8.72 (Table 3.1). Thulamela farmer's fields had moderately acidic and acidic soils with loamy sand and sandy clay loam textures and pH values of 4.7 to 6.4. Collin Chabane had sandy clay loam textured soils that were slightly acidic, with pH values ranging from 6.1 to 6.6 across five fields (Table 3.1).

Makhado farmer's fields were characterized by sandy clay loam and sandy clay textures, with neutral pH. The nutrient content of soil also varied across the locations. For example, Musina farmers' fields had greater K, Ca, Mg, Na, B, Mo, Fe, and Zn levels than Thulamela and Makhado farmers' fields (Table 3.1). Collin Chabane also had greater K, Ca, Mg, Na, and B levels than Thulamela and Makhado.

6.4.2 Carbon Utilisation Profiles (CSU)

Biolog carbon sources of two legume species utilised by soil microbial communities in the farmer’s fields are summarised in (Table 6.2). The highest C-source utilisation by the microbial communities was recorded for soil microbe cowpea samples in Makhado farmer's fields (Table 6.1). The microbial communities' lowest C-source utilisation was detected for Soil microbe’s cowpea samples in Musina farmers' fields and Bambara groundnut in Makhado farmers' fields (Table 6.1). The Shannon–Weaver diversity index was used to quantify the functional diversity of soil microbial populations based on the number of different carbon sources utilised by soil microbial populations in Biolog EcoPlatesTM, an index comparable to species richness in the soil.

The significant difference in Shannon Weaver Diversity Index (H’) (i.e. the ability of the microbial community to degrade more or fewer types of carbon sources at a threshold ODi value ≥ 0.25) was observed for samples of cowpea in Makhado farmer's fields which could degrade more types of carbon sources. In the rhizospheres soil samples from the two species of Bambara groundnut and cowpea sampled, varying percentages of carbon sources were utilised, with values ranging from 2.5712 to 3.3085, as seen in Table 6.1. These values show a higher diversity.

Substrate evenness is assumed to have a value between 0 and 1, with 1 denoting a circumstance in which all species of microbes present in the samples were equally prevalent. There will be less dominance where substrate evenness is close to 1 and more diversity because of reduced interspecies variation in microbial communities. In this analysis, Substrate evenness indices were high and ranged significantly between 0.9723 in Musina farmers' fields on Cowpea species and 1.0658 in Makhado farmers' fields on Bambara groundnut (Table 6.1). Carbon source utilization results showed the potential of soil microbes to degrade or convert substrates from an organic form into plant-available nutrients. By implication, the higher the microbial activity, the faster the nutrients released from organic substrates will be made available to be taken up by the plant.

Table 6-1: Biolog Carbon-source utilization and enzyme activities of microbial communities of the soil samples.

			Biolog 31 Carbon Source Utilization (Functional diversity)			
Treatment	**Farmers' fields**	**Legume species**	***AWCD*** ^{a}	***Shannon(H')*** ^{b}	***Richness(S)*** ^{c}	***Evenness(E)*** ^{d}
Musina	1	Cowpea	1.150^{a}	3.167^{a}	24	1.000^{a}
	2	Cowpea	0.811^{ab}	2.907^{b}	18	1.006^{a}
	3	Cowpea	1.374^{ab}	3.272^{a}	28	0.982^{ab}
	4	Cowpea	0.926^{ac}	2.968^{a}	20	0.986^{ab}
	5	Cowpea	1.021^{a}	3.109^{a}	24	0.974^{ab}
Thulamela	6	Cowpea	1.315^{a}	3.197^{a}	25	0.993^{ab}
	7	Cowpea	0.620^{a}	2.926^{a}	20	0.979^{ab}
	8	Cowpea	0.966^{b}	3.005^{a}	21	0.985^{ab}
	9	Cowpea	0.401^{ab}	2.495^{a}	11	1.014^{a}
	10	Cowpea	1.050^{ac}	3.150^{a}	26	0.972^{ab}
Collin Chabane	11	Cowpea	1.288^{ab}	3.231^{a}	27	0.977^{ab}
	12	Cowpea	1.042^{ac}	3.146^{a}	25	0.982^{ac}
	13	Cowpea	0.657^{b}	2.781^{a}	15	1.032^{ab}
	14	Cowpea	0.701^{bc}	2.873^{a}	18	1.002^{ab}
	15	Cowpea	1.290^{ac}	3.417^{a}	31	0.995^{a}
Makhado	16	Cowpea	1.672^{ab}	3.359^{a}	29	0.994^{a}
	17	Cowpea	1.220^{ac}	3.189^{a}	24	1.003^{ab}
	18	Cowpea	1.095^{c}	3.134^{ab}	23	0.996^{a}
	19	Cowpea	1.186^{c}	3.221^{b}	25	0.996^{a}
	20	Cowpea	1.275^{c}	3.295^{a}	28	0.992^{a}
Collin Chabane	1	Bgnut	0.591^{ac}	2.668^{ab}	16	0.994^{ac}
	2	Bgnut	1.329^{c}	3.182^{a}	25	0.988^{a}
	3	Bgnut	1.324^{c}	3.214^{a}	25	0.999^{a}
	4	Bgnut	0.994^{ab}	3.048^{b}	22	0.992^{a}
	5	Bgnut	0.418^{a}	2.571^{a}	11	1.065^{ab}
Makhado	6	Bgnut	0.875^{ab}	2.867^{a}	18	0.999^{b}
	7	Bgnut	1.089^{ac}	3.102^{b}	23	0.990^{a}
	8	Bgnut	0.955^{ac}	2.996^{a}	20	0.992^{a}
	9	Bgnut	1.401^{ac}	3.308^{ab}	28	0.989^{a}

	10	Bgnut	1.079^{c}	3.173^{a}	26	0.978^{a}
Musina	11	Bgnut	1.079^{c}	3.173^{a}	26	0.978^{a}
	12	Bgnut	1.349^{ac}	3.243^{b}	26	0.996^{a}
	13	Bgnut	1.236^{c}	3.199^{a}	26	0.986^{a}
	14	Bgnut	1.093^{ac}	3.127^{a}	23	1.002^{ab}
	15	Bgnut	1.152^{bc}	3.182^{a}	24	1.002^{ab}
Thulamela	16	Bgnut	1.565^{bc}	3.265^{b}	27	0.994^{a}
	17	Bgnut	1.321^{cb}	3.203^{b}	25	0.995^{a}
	18	Bgnut	1.284^{bc}	3.162^{a}	24	0.999^{a}
	19	Bgnut	1.402^{bc}	3.280^{b}	28	0.988^{a}
	20	Bgnut	1.060^{ac}	3.142^{a}	23	1.002^{ab}
		CV	7.3	2.2	7.5	1.3
		LSD0.05	0.3	0.2	5.7	0.04
		Pr>F	<.0.0001	<.0001	<.0001	<.0001
		R^2	0.10	0.9	0.9	0.7

6.4.3 Soil Microbial Enzymatic Activity

This study analysed two soil microbial enzymes′ activities for ß-glucosidase and alkaline phosphatase (Table 6.2). Of the two enzyme activities tested, variation between the soil microbial communities of the different samples was observed, rhizospheres soil sample from Cowpea in Collin chabane, Bambara groundnut in Collin Chabane, and Bambara groundnut in Makhado expressed the highest β-glucosidase enzyme activity. Cowpea soil microbe samples in Makhado had the lowest expression of β-glucosidase. In terms of Phosphatase enzyme activity (phosphomonoisomerase), microbial communities of all soil samples expressed high phosphatase activity (expressed in µg *p*-nitrophenol/g soil) in almost all treatments. Cowpea soil microbe samples in Musina had the lowest expression of alkaline phosphatase enzyme activity (Table 6.2). Microbial population and activities of soil enzymes are important parameters to measure the quality and health of soil. The higher the enzyme microbial activities, the faster the nutrients released from organic substrates will be made available to be taken up by the plant.

Table 6-2: Soil microbial enzymatic activities between different samples. Two soil microbial enzyme activities analyzed in this study for β-glucosidase and Phosphatase Alkaline.

			Enzyme Activities	
Treatment	**Farmers' fields**	**Legume species**	***β-glucosidase*** **(µg *p* –nitrophenol/ g soil)**	***Phosphatase* Alkaline (µg *p* –nitrophenol/ g soil)**
Musina	1	Cowpea	9.31^{a}	91.80^{b}
	2	Cowpea	8.11^{b}	77.69^{b}
	3	Cowpea	11.70^{a}	89.25^{b}
	4	Cowpea	7.46^{b}	87.06^{b}
	5	Cowpea	14.10^{a}	88.09^{b}
Thulamela	6	Cowpea	9.63^{a}	86.96^{b}
	7	Cowpea	10.68^{b}	88.60^{b}
	8	Cowpea	8.65^{a}	90.15^{b}
	9	Cowpea	6.83^{b}	81.58^{b}
	10	Cowpea	7.69^{a}	75.72^{ac}
Collin Chabane	11	Cowpea	8.22^{b}	84.10^{b}
	12	Cowpea	9.27^{a}	89.04^{b}
	13	Cowpea	3.98^{ac}	84.77^{b}
	14	Cowpea	8.18^{b}	85.60^{b}
	15	Cowpea	9.54^{a}	85.53^{b}
Makhado	16	Cowpea	9.18^{a}	86.01^{b}
	17	Cowpea	6.90^{ac}	79.98^{ac}
	18	Cowpea	7.81^{c}	85.54^{b}
	19	Cowpea	7.57^{c}	88.75^{b}
	20	Cowpea	9.12^{a}	88.27^{b}
Collin Chabane	1	Bgnut	12.29^{a}	93.09^{b}
	2	Bgnut	12.28^{a}	93.00^{b}
	3	Bgnut	10.47^{a}	92.56^{b}
	4	Bgnut	11.45^{a}	86.36^{b}
	5	Bgnut	11.56^{a}	87.22^{b}
Makhado	6	Bgnut	8.32^{b}	80.36^{b}
	7	Bgnut	8.72^{b}	91.25^{b}
	8	Bgnut	12.79^{a}	93.59^{b}
	9	Bgnut	8.66^{b}	90.45^{b}
	10	Bgnut	6.03^{ac}	85.24^{b}
Musina	11	Bgnut	9.59^{a}	88.86^{b}
	12	Bgnut	12.24^{a}	86.99^{b}
	13	Bgnut	7.18^{ac}	96.84^{b}

Thulamela	14	Bgnut	8.59^{ab}	100.20^{c}
	15	Bgnut	7.96^{c}	102.84^{c}
	16	Bgnut	9.34^{a}	99.05^{c}
	17	Bgnut	7.44^{c}	94.44^{b}
	18	Bgnut	9.99^{a}	91.41^{b}
	19	Bgnut	8.01^{b}	97.78^{b}
	20	Bgnut	7.98^{ac}	90.44^{b}

6.4.4 AWCD analysis

Significant differences in carbon source utilisation by soil microbial communities in the two legume species are evident, in Table 6.3. Cowpea soil microbes sample in Musina and Collin Chabane, and Bambara groundnut in Makhado had the lowest utilisation rates of all carbon sources (carbohydrates, carboxylic acids, amino acids, polymers, and amines). On the other hand, the three-soil microbes sample Musina had the lowest utilisation of polymers and amines. One rhizosphere soil microbe sample in Bambara groundnut farmers' fields had the highest utilisation of amines, Table 6.3.

On average, carbohydrates, amino acids, carboxylic acids, polymers, and amines in the Bambara groundnut species' most utilized carbon sources, whereas amines were the least utilised by cowpeas across all treatments (Table 6.3). AWCD in the cowpea in Musina's few farmer's fields demonstrated the lowest utilisation of amino acids and polymers, while Bambara's groundnut fields were responsible for the highest utilisation of carbohydrates. The number of microbial species in the soil microbes sample able to utilise specific carbon sources is indicated in the intensity of the colour change.

Table 6-3: AWCD values show the extent of utilising five major groups of carbon sources across the farmer's field sites (carbohydrates, amino acids, polymers, amines, and carboxylic acids) by microbial communities of soil samples.

Treatment	**Farmers fields**	**Crop**	***Aminoacids***	***Carbohydrates***	***Carboxyl icacids***	***Polymers***	***Amines***
Musina	1	Cowpea	1.105^{a}	1.180^{ab}	0.495^{a}	0.541^{a}	0.045^{a}
	2	Cowpea	1.378^{ab}	1.497^{ab}	1.305^{ab}	1.036^{a}	1.732^{a}
	3	Cowpea	0.784^{a}	0.259^{a}	0.347^{a}	0.473^{a}	0.053^{a}
	4	Cowpea	1.259^{a}	1.407^{ab}	0.979^{a}	1.285^{ac}	0.392^{a}
	5	Cowpea	1.036^{a}	1.246^{a}	0.959^{a}	0.768^{a}	1.780^{abc}
Thulamela	6	Cowpea	1.374^{a}	1.098^{a}	1.410^{ab}	1.288^{a}	1.432^{ab}
	7	Cowpea	1.472^{a}	0.913^{a}	1.120^{abc}	0.831^{a}	0.472^{a}
	8	Cowpea	0.890^{a}	0.563^{a}	0.777^{a}	0.330^{a}	0.535^{a}
	9	Cowpea	0.808^{a}	0.717^{a}	0.622^{a}	0.634^{a}	0.794^{a}
	10	Cowpea	1.502^{a}	1.209^{a}	1.216^{ac}	0.763^{a}	1.355^{ab}
Collin Chabane	11	Cowpea	1.376^{a}	1.402^{ab}	1.025^{a}	1.442^{a}	1.753^{ab}
	12	Cowpea	0.783^{a}	0.608^{a}	0.579^{a}	0.777^{a}	0.058^{a}
	13	Cowpea	0.991^{a}	1.006^{a}	0.711^{a}	0.882^{a}	0.2878^{a}
	14	Cowpea	1.381^{ab}	1.147^{a}	0.733^{a}	0.987^{a}	0.6742^{a}
	15	Cowpea	1.260^{a}	1.258^{a}	1.028^{a}	0.927^{a}	1.271^{a}
Makhado	16	Cowpea	1.370^{a}	1.256^{a}	1.268^{ac}	1.251^{a}	1.400^{abc}
	17	Cowpea	1.796^{a}	1.794^{ac}	1.490^{abc}	1.474^{abc}	1.911^{abcd}
	18	Cowpea	0.948^{a}	1.131^{a}	0.598^{a}	0.755^{a}	1.657^{a}
	19	Cowpea	1.214^{a}	1.167^{a}	1.166^{a}	1.118^{a}	1.420^{a}

	20	Cowpea	1.332^{a}	1.461^{ac}	1.168^{a}	0.994^{a}	1.212^{a}
Collin Chabane	1	Bambara groundnut	0.887^{a}	0.504^{a}	0.594^{a}	0.825^{a}	0.374^{a}
	2	Bambara groundnut	1.546^{a}	1.478^{a}	1.166^{a}	1.177^{a}	0.978^{a}
	3	Bambara groundnut	1.290^{a}	1.359^{ac}	1.213^{a}	1.420^{a}	1.555^{a}
	4	Bambara groundnut	1.154^{a}	1.047^{a}	0.878^{a}	0.983^{a}	0.787^{a}
	5	Bambara groundnut	0.965^{a}	1.430^{ac}	0.777^{a}	1.096^{a}	1.002^{a}
Makhado	6	Bambara groundnut	1.477^{a}	1.021^{a}	0.875^{a}	1.023^{a}	1.364^{a}
	7	Bambara groundnut	0.944^{a}	1.271^{a}	0.750^{a}	0.930^{a}	0.385^{a}
	8	Bambara groundnut	1.443^{a}	1.381^{ac}	1.292^{a}	1.490^{ac}	1.685^{abc}
	9	Bamara groundnut	0.433^{a}	0.387^{a}	0.342^{a}	0.645^{a}	0.421^{a}
	10	Bambara groundnut	1.540^{a}	1.632^{ab}	0.950^{a}	1.062^{a}	1.732^{a}
Musina	11	Bambara groundnut	1.215^{a}	1.438^{ab}	1.043^{a}	0.933^{a}	1.764^{abc}
	12	Bambara groundnut	0.812^{a}	1.375^{ab}	0.625^{a}	0.707^{a}	0.025^{a}
	13	Bambara groundnut	0.964^{a}	1.430^{ac}	0.776^{a}	1.095^{a}	1.002^{a}
	14	Bambara groundnut	1.481^{a}	1.422^{ac}	1.361^{abc}	1.162^{a}	1.732^{abc}
	15	Bambara groundnut	1.092^{a}	1.095^{a}	1.029^{ab}	0.842^{a}	1.370^{ab}

Thulamela	16	Bambara groundnut	1.396^{ac}	1.096^{a}	1.180^{a}	0.731^{a}	0.511^{a}
	17	Bambara groundnut	1.329^{ac}	1.156^{a}	1.134^{a}	1.162^{a}	0.668^{a}
	18	Bambara groundnut	1.782^{ac}	1.795^{ac}	1.449^{ac}	0.781^{a}	1.851^{abc}
	19	Bambara groundnut	1.389^{a}	1.475^{ab}	1.245^{ab}	0.877^{a}	1.579^{ab}
	20	Bambara groundnut	1.651^{6ab}	1.223^{a}	1.364^{ab}	0.831^{a}	1.045^{a}
		Cv	15.063	11.551	11.184	20.300	37.827
		$LSD_{0.05}$	0.607	0.451	2.891	0.642	1.313
		Pr>F	<.0001	<.0001	<.0001	<.0001	<.0001
		R^2	0.796	0.906	0.919	0.741	0.769

6.4.5 Total soil microbial count

Principal component analysis (PCA1 and PCA2) was used to identify the variations among the treatment and replicates grouped or clustered together. The soil microbes samples that had similar C-source utilization patterns were into three major groups in which soil microbes samples Bambara groundnut and cowpea in Makhado, Bambara groundnut and Cowpea in Musina, Musina had the lowest scores for the attributes measured group, as compared to the rest of samples soil microbes (Figure 6.1). This is also indicated in the hierarchical clustering dissimilarity chart (Figure 6.2).

Most samples had the lowest microbial count, below the normal expected total microbial load of soil. However, cowpea soil microbe samples in Musina, Makhado, and Colin Chabane fields have a total bacterial count of $> 10^{9}$ CFU/g. The normal optimal pH range for most microbial activity is between 5.5 and 7.0. Soil pH is the main driving factor of microbial community structure in many cultivated soils, the samples tested were found to be within the normal optimal pH range. However, the sample Collin chabane (Bambara groundnut) field was more acidic, while cowpea in Musina fields and Bambara groundnuts were more alkaline.

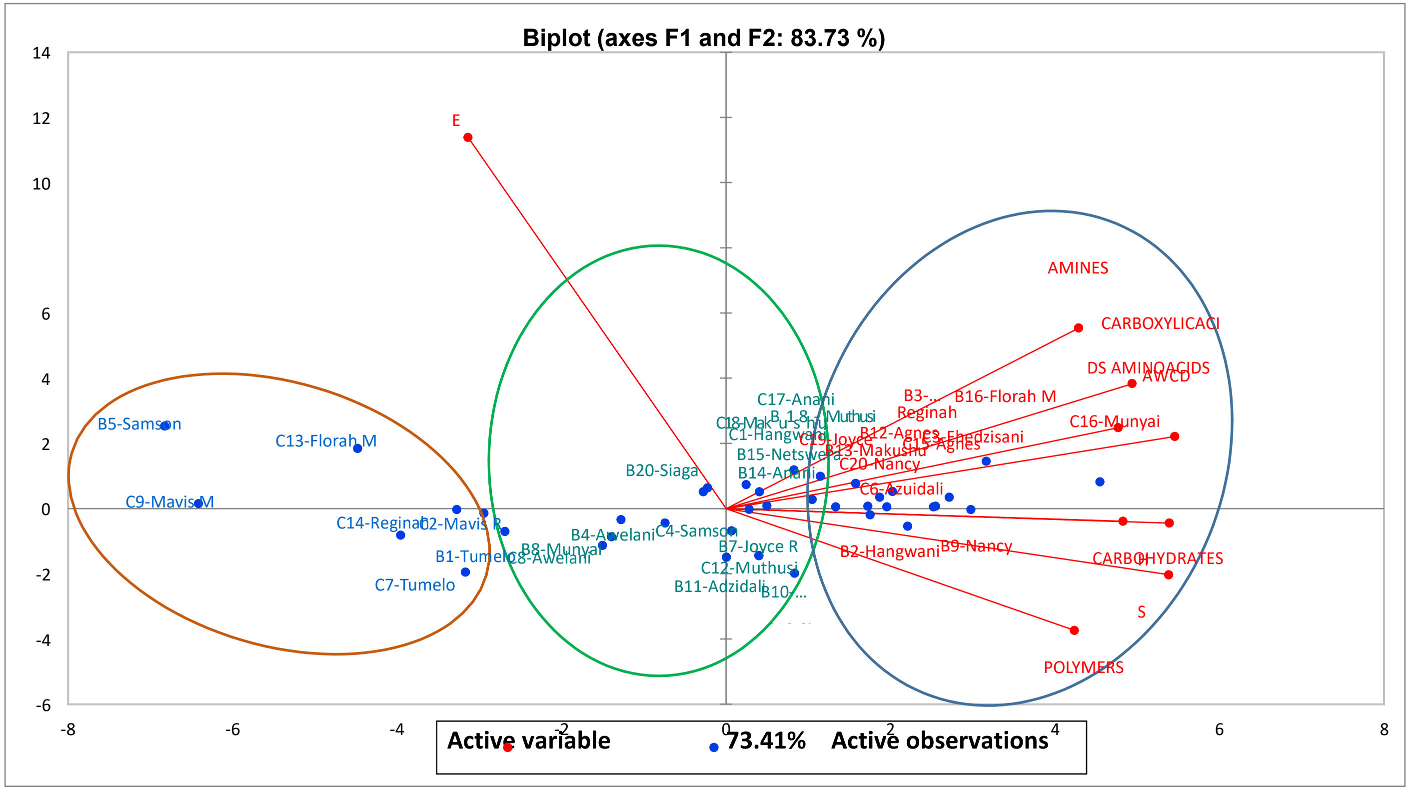

Figure 6. 1. Principal component analysis of the soil samples based on using 31 carbon sources (AWCD) on Biolog microplates to illustrate their similarity and differences. Samples B5- Samson, C9- Mavis M, C13- Florah M, C14- Reginah, C7 Tumelo, B6- Mavis M, C2 Mavis R had the lowest PCA scores, located in opposite directions to all tested variables and are grouped.

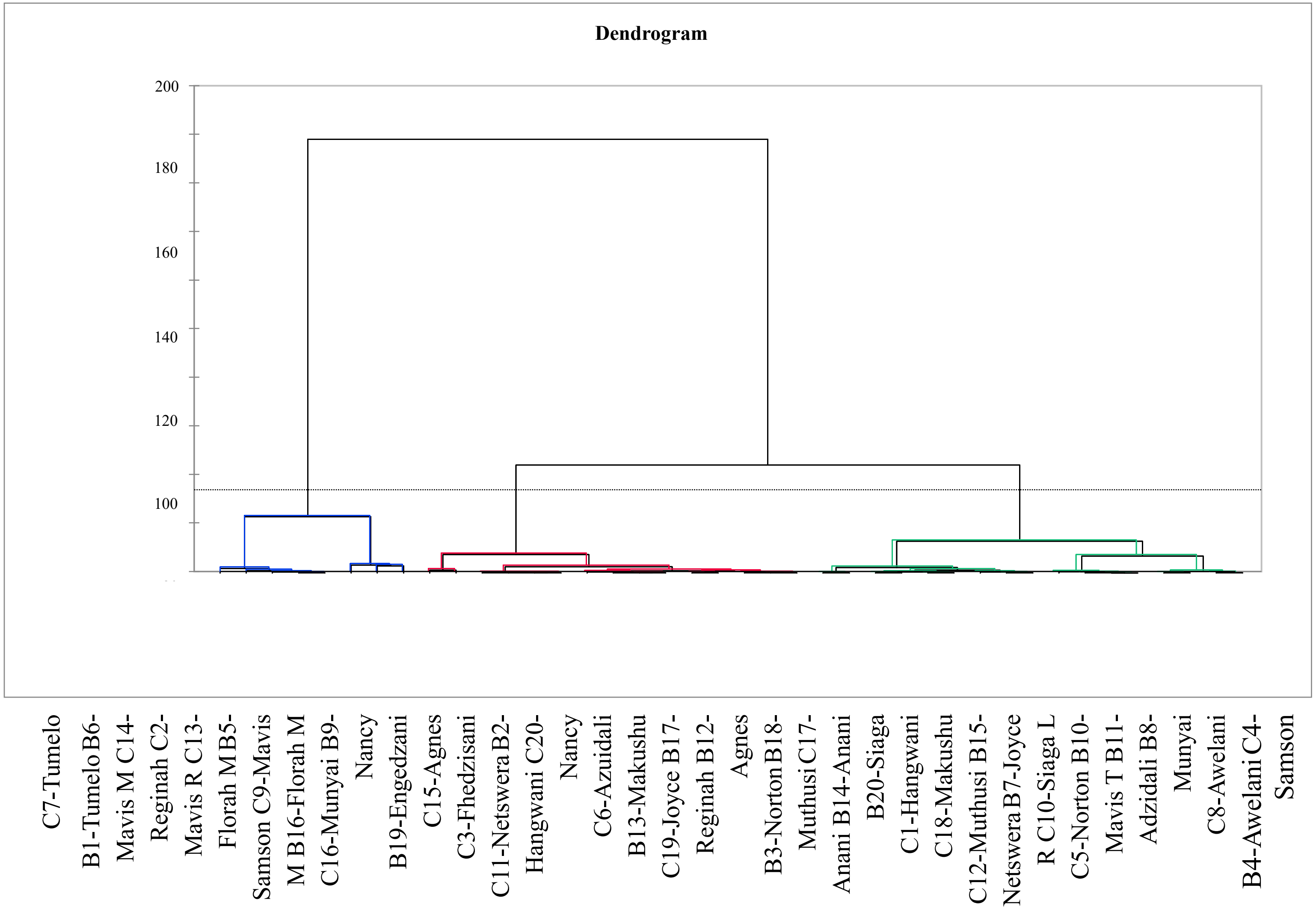

Figure 6. 2. Dissimilarity Hierarchical Clustering of the soil samples using 31 carbon sources on Biolog microplates. The soil samples were grouped into three major clusters with different colours. Generally, samples with the lowest C-source utilization are clustered together (blue), and those with the highest C-source utilization are grouped into their cluster (red).

Table 6-4: The total microbial count of the soil samples was determined in colony-forming units per g (CFU/g0 of soil.

Treatment	Farmers fields	**Legume species**	**pH**	**No. of colonies** (Average of triplicateplates)	**Colony forming units** (CFU g -1)
Musina	1	Cowpea	7.83	24(10^{-7})	2.4x10^{9}
	2	Cowpea	7.19	91(10^{-7})	9.1x10^{9}
	3	Cowpea	8.03	43(10^{-6})	4.3x10^{8}
	4	Cowpea	7.35	24(10^{-5})	2.4x10^{7}
	5	Cowpea	7.99	20(10^{-4})	2.0x10^{6}
Collin Chabane	6	Cowpea	6.56	142(10^{-7})	1.42x10^{10}
	7	Cowpea	6.52	27(10^{-5})	2.7x10^{7}
	8	Cowpea	6.21	190(10^{-7})	1.90x10^{10}
	9	Cowpea	6.11	40(10^{-4})	4.0x10^{6}
	10	Cowpea	6.48	45(10^{-5})	4.5x10^{7}
Thulamela	11	Cowpea	5.68	36(10^{-5})	3.6x10^{7}
	12	Cowpea	5.46	75(10^{-6})	7.5x10^{8}
	13	Cowpea	5.87	20(10^{-6})	2.0x10^{8}
	14	Cowpea	5.89	35(10^{-5})	3.5x10^{7}
	15	Cowpea	6.05	25(10^{-4})	2.5x10^{6}
Makhado	16	Cowpea	6.73	20(10^{-5})	2.0x10^{7}
	17	Cowpea	6.62	60(10^{-6})	6.0x1^{0}
	18	Cowpea	6.75	300(10^{-7})	3.0x10^{10}
	19	Cowpea	6.60	23(10^{-4})	2.3x10^{6}
	20	Cowpea	6.45	41(10^{-5})	4.1x10^{7}
Collin Chabane	1	Bgnut	6.50	35(10^{-5})	3.5x10^{7}
	2	Bgnut	6.72	20(10^{-5})	2.0x10^{7}
	3	Bgnut	6.63	50(10^{-4})	5.0x10^{6}
	4	Bgnut	5.96	22(10^{-5})	2.2x10^{7}
	5	Bgnut	5.98	22(10^{-5})	2.2x10^{7}
Makhado	6	Bgnut	6.77	90(10^{-6})	9.0x10^{8}
	7	Bgnut	6.47	23(10^{-5})	2.3x10^{7}
	8	Bgnut	6.87	23(10^{-4})	2.3x10^{6}
	9	Bgnut	6.66	35(10^{-4})	3.5x10^{4}
	10	Bgnut	7.08	26(10^{-5})	2.6x10^{7}
Musina	11	Bgnut	8.34	32(10^{-6})	3.2x10^{8}
	12	Bgnut	7.2	21(10^{-4})	2.1x10^{6}
	13	Bgnut	8.29	24(10^{-4})	2.4x10^{6}
	14	Bgnut	8.24	42(10^{-5})	4.2x10^{7}
	15	Bgnut	6.52	44(10^{-5})	4.4x10^{5}
Thulamela	16	Bgnut	4.81	37(10^{-6})	3.7x10^{8}
	17	Bgnut	5.42	22(10^{-4})	2.2x10^{6}
	18	Bgnut	5.54	25(10^{-4})	2.5x10^{6}
	19	Bgnut	6.23	21(10^{-4})	2.1x10^{6}
	20	Bgnut	5.49	28(10^{-5})	2.7x10^{7}

6.5 Discussion

Soil pH and extractable Fe, an essential micronutrients for organisms because they influence the availability of the nutrients availability, carbon source utilization and development of two legume species (Moreno-Jiménez et al., 2019). Many factors influence the Rhizosphere functional diversity, including soil physio-chemical properties and agro-climatic conditions. Legume species growing in acidic soil usually have a denser and more diverse root microbial community than in alkaline areas (Santos et al., 2021).

Average well colour development observed in carbon sources with soil samples reflects the ability of the microbes to utilise them, and it is directly related to the number of species present in the soil, as observed in this study and previous studies (Chen et al., 2008; Yang et al., 2013; Koner et al., 2021). These variations are most frequently observed in the rhizosphere of plant roots (Cong et al., 2015; Lebeis, 2015; Gaazka et al., 2022). This is necessary because microorganisms are both a fundamental component of soil and the basis of its operations. Microorganisms are essential for preserving soil fertility and quality (Jezierska-Tys et al., 2020). The highest C-source utilisation by the microbial communities was recorded for soil samples of cowpea in Makhado and Musina. The rhizosphere is a hotspot for microbial–plant interactions, with a lot of exchange of energy and nutrients between them (Kong and Liu, 2022; Wang et al., 2022). The microbial abundance (richness index) in this study ranged from 11 to 28 for both species, the abundance of microbial species was high, which shows that the different microbial species were reaching a point where they were all present in almost equal amounts—there is a very low level of dominance (equally amounts to the utilization of carbon source) (Habig and Swanepoel, 2015). The significant difference in Shannon Weaver Diversity Index) (i.e., the ability of the microbial community to degrade more or fewer types of carbon sources at a threshold ODi value ≥ 0.25) was observed for samples. According to Thompson and Kao-Kniffin (2016), healthy and fertile soil should have a high diversity of microbial species and a balanced distribution of species among them (high Shannon-Weaver and Evenness indices). Plant species or cultivars influence the soil community's microbes composition (Berg and Smalla, 2009; Eisenhauer et al., 2010; Reese et al., 2018; Liu et al., 2020). Additionally, the diversity of influences on soil nutrients and root exudation mechanisms can be used to determine a plant species' richness (Berg and Smalla, 2009; Eisenhauer et al., 2017). Statistically significant utilisation was detected for all the four groups of carbon sources (AWCD, Shannon (H'), richness (S) and evenness (E). This microenvironment is rich in carboxylic acid, carbohydrates and amino acids because of the diverse exudates from plants (Uroz et al., 2010; Eisenhauer et al., 2017). The lowest expression of ß-glucosidase activity was shown in cowpeas under Makhado municipality. This may have been because of the varying substrate and organic matter composition associated with each soil. Other studies have shown that increased soil salinity has led to an exponential and linear decline in β-glucosidase activity, respectively (Rietz and Haynes, 2003).

Healthy soils are characterized by abundant soil microorganisms and high levels of microbial activity, indicating extensive interaction among microbial species. Phosphatases are typically more abundant in the rhizosphere and have been, likely, the most studied rhizosphere enzymes (Spohn et al., 2014).

In the study, the soil pH was strongly acidic (5.5) in one Bambara groundnut sample. Whereas two others were found to be alkaline. Additionally, plants play a significant role in determining the nutrients produced by decomposing plant matter as well as the content of plant root exudates (Canarini et al., 2019; Girvan et al., 2003). The differences in soil microbial populations are partly due to the composition of the nutrients produced during decomposition or the exudates from plant roots, which draw microbial populations that are extremely well suited to use the chemicals quickly; this is proven in the dendrogram (Figure 6.2).

6.6 Conclusion

Farmers can improve their soil enzyme activity and microbial communities by cultivating legumes in cropping systems. The inclusion of legume species promotes soil processes that make nutrient availability accessible to crops. Both soil pH and soil minerals' element content were observed to influence the distribution of carbon source utilisation patterns of the soil microbial communities and microbial diversity. It is also vital for the farming field in Thulamela Municipality, which was found to be more acidic, and samples farmers field 3 and field 5 were more alkaline in Musina Municipality to monitor the pH levels of their soils because it has a direct impact on enzyme activity and microbial communities. Although carbon source utilisation profiles can be used as indicators of soil health and quality, these indicators are subjective to changes in different environmental conditions, the historical crops grown, and soil and agricultural management practices. To better understand the effect of cultivation management practices such as cropping systems, for instance, crop rotation and tillage, on the biological component of soils. However, future research studies should investigate how changes in carbon source utilisation soil microbial communities under different management practices influence the ecological functions of rhizosphere soil microbes of two legume species.

7. GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATION

7.1 General discussion

Bambara groundnut (*Vigna subterranean (L). Verdc)* and cowpea (*Vigna unguiculata (L). Walp)* are grain legumes grown mainly by subsistence farmers in sub-Saharan Africa. Bambara groundnut is cultivated for its underground pods, is extremely hardy, and produces reasonable yields even under drought conditions and low soil fertility. Many households consume the crop for its rich nutrients in the grain (Bamashiye et al., 2011).

The high carbohydrate (65%), high protein (18%) contents and the grain quantity of fat (6.5%) make Bambara groundnut and cowpea a complete meal that people can rely on for all their dietary needs (Mahazib et al., 2013). Given the high rates of malnutrition in the world, including sub-Saharan Africa, coupled with the fact that the world currently depends on few improved crop species for food and nutritional security (Azam-Ali et al., 2001), Bambara groundnut could be further researched and exploited to improve nutritional security. In Vhembe district, Bambara groundnut and cowpea are common in cropping systems as they fix atmospheric nitrogen and, therefore, an advantage in intercropping as well as subsequent crops when excess nitrogen is left in the soil (Majola et al., 2021).

In rural Limpopo, Bambara groundnut and cowpea are the most important sources of plant protein and mineral nutrients for human nutrition and health. Although a few studies (Toomer, 2018; Nielsen et al., 1997) have assessed the protein levels of edible cowpea leaves and minerals grain, as well as minerals of Bambara groundnut, few (if any) have determined the mineral density of these organs as a nutrient source.

Nguyen et al. (2017) attributed the uptake of plants' nutrients depending on the soil's nutrient concentration and ratio. The concentration of different micronutrient levels will be corrected with organic matter, including organic fertilizers and manure, for improved productivity. The slight acidity, observed with a deficient Ca level, suggests lime before planting to correct the imbalances. Most of the study areas' soil has good water-holding capacity, which creates a good growing media for the species. The soils in Vhembe district are dominated by a loamy texture (Molepo et al., 2017). The pH of the soil also plays a crucial role in plant growth and nutrient availability.

Plant species grown in locations with high temperatures and low rainfall regimes tend to experience low soil moisture and drought, leading to variation in dry matter (Norton et al., 2016). However, in locations with low rainfall and high average temperatures during the crop growth period in the growing season, the Bambara groundnut and cowpea plants grown by farmers are likely to respond differently. Agriculture in arid and semi-arid areas such as Vhembe district is characterised by low and poor microbial counts (Mpandeli et al., 2014; Kom et al., 2020). The total bacterial count is a sensitive microbial indicator of soil health (Schloter et al., 2018). One of the study hypotheses was that the rhizospheres soil microbes do not influence two legume species collected from farmers' fields. However, Soil samples of cowpea in farmer fields 1 and 2 under Musina, farmer field 3 in Makhado, and farmer fields 1 and 3 in Collin Chabane have a total bacterial count of > 10^9 CFU/g, which is considered high and desirable for cultivation.

Average well colour development observed in carbon sources with soil samples reflects the ability of the microbes to utilize them, and it is directly related to the number of species present in the soil, as observed in this study and previous studies (Chen et al., 2008; Yang et al., 2013; Koner et al., 2021). Different species of legumes have slightly different microbiomes, according to research (Muema et al., 2022; Han et al., 2020). These variations are most frequently observed in the rhizosphere of plant roots (Cong et al., 2015; Lebeis, 2015; Gaazka et al., 2022). The microbial abundance (richness index) in this study ranged from 11 to 28 for both species, the abundance of microbial species was high, which shows that the different microbial species were reaching a point where they were all present in almost equal amounts—there is a very low level of dominance (Habig and Swanepoel, 2015).

7.2 General conclusion

The rhizosphere soil microbes positively influenced the growth and yield of two legume species. Both soil pH and soil minerals' element content were observed to influence the distribution of carbon source utilization patterns of the soil microbial communities and microbial diversity. The selected physicochemical properties of soil affected the rhizosphere in most farmer's fields. Low pH and calcium concentrations displayed a low microbial diversity among the bacterial communities and populations characterised in this study. Cowpea plant parts had a higher nutritional content than Bambara groundnut.

Additionally, the high nutritional content observed in cowpea plant parts highlights their potential to address dietary deficiencies more effectively than Bambara groundnut. The findings of these legumes highlight not just their nutritional value but also their potential to create a well-balanced diet. It can also be beneficial and promoted in areas where these minerals and nutrients are deficient in the diet. Further research is recommended to understand microbial diversity and symbiotic functioning in response to various agro-climatic for sustainable production.

7.3 Recommendations

The study was conducted for one season, which may not be long enough to make conclusive recommendations, especially on rhizosphere soil microbes, soil health, and quality. Furthermore, more research is required to evaluate the rhizosphere's soil microbes, grain yield and nutritional compositions in different locations under different agro-climatic conditions at farmer's fields over a long period because different farmer's fields had different soil health that could be due to management differences, type of soil, cropping history.

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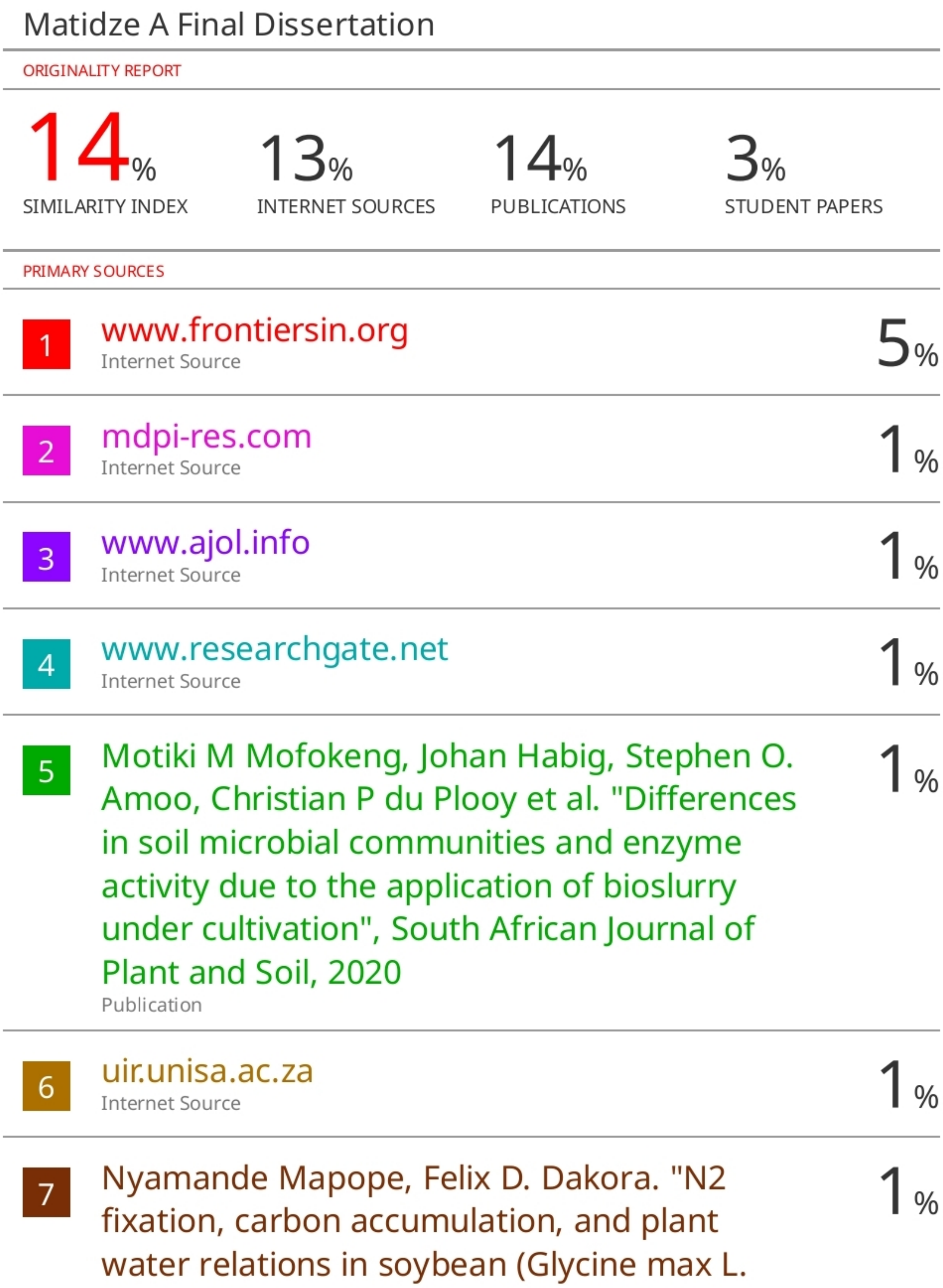

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