# ROLE OF FERTILISATION REGIMES ON THE YIELD AND NUTRITIONAL BENEFITS OF COWPEA-AMARANTH INTERCROPPING SYSTEMS

**by**

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Submitted in fulfillment of the requirements for the Degree of Doctor of Philosophy in Crop Science

Centre for Transformative Agricultural and Food Systems
School of Agricultural, Earth and Environmental Sciences
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South Africa

**October 2020**

# STUDENT DECLARATION

**Role of Fertilisation Regimes on the Yield and Nutritional Benefits of Cowpea-Amaranth Intercropping Systems**

I, **Buhlebelive Melusi Mgcini Phiwayinkosi Mndzebele**, student number: **215067642** declare that:

a. The research reported in this thesis, except where otherwise indicated, is the result of my own endeavours in the Centre for Transformative Agricultural and Food Systems, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

b. This thesis has not been submitted for any degree or examination at any other university.

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Signed by __[redacted]__________________________________

# DECLARATION BY SUPERVISORS

We hereby declare that we acted as supervisors of this PhD student:

Student full name: **Buhlebelive Melusi Mgcini Phiwayinkosi Mndzebele**

Student number: **215067642**

Thesis title: Yield and nutritional benefits of cowpea-amaranth intercropping in varying fertilisation levels

Regular consultation took place between the student and us throughout the investigation. We advised the student to the best of our ability and approved the final document for submission to the College of Agriculture, Engineering and Science Higher Degrees Office for examination by the University appointed examiners.

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# DECLARATION 1 - PLAGIARISM

I, Buhlebelive Melusi Mgcini Phiwayinkosi Mndzebele, student number: 215067642, declare that:

The research reported in this thesis, except where otherwise indicated, is my original research.

This thesis has not been submitted for any degree or examination at any other university.

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## DECLARATION – PUBLICATIONS

Details of contribution to publications that form part of and/or include research presented in this thesis (include publications in preparation, submitted, in the press and published and give details of the contributions of each author to the experimental work and writing of each publication).

Publication 1 (Published):

**Buhlebelive Mndzebele, Bhekumthetho Ncube, Melake Fessehazion, Tafadzwanashe Mabhaudhi, Stephen Amoo, Christian du Plooy, Sonja Venter and Albert Modi**. **2020**. Effects of Cowpea-Amaranth Intercropping and Fertiliser Application on Soil Phosphatase Activities, Available Soil Phosphorus, and Crop Growth Response. *Agronomy*, 10(1), 79; https://doi.org/10.3390/agronomy10010079

Contributions: The first author under the guidance of supervisors prepared field trials, collected data, analysed data and drafted the manuscript.

Publication 2 (Published):

**Buhlebelive Mndzebele, Bhekumthetho Ncube, Melvin Nyathi, Sheku Alfred Kanu, Melake Fessehazion, Tafadzwanashe Mabhaudhi, Stephen Amoo and Albert Thembinkosi Modi. 2020.** Nitrogen Fixation and Nutritional Yield of Cowpea-Amaranth Intercrop. *Agronomy*, 10(4), 565; https://doi.org/10.3390/agronomy10040565

Contributions: Field trials, data collection, analysis and manuscript preparation were performed by the first author under the supervision of the three supervisors.

Publication 3 (Under review):

**Buhlebelive Mndzebele, Bhekumthetho Ncube, Melake Fessehazion, Tafadzwanashe Mabhaudhi, and Albert Thembinkosi Modi.** An assessment of NPK fertiliser on the edible yield and agro-biological parameters in a cowpea-amaranth intercrop. *Communications in Soil Science and Plant Analysis - Manuscript ID LCSS-2020-0613*

Contributions: Field trials, data collection, analysis and manuscript preparation were performed by the first author under the supervision of the three supervisors.

Publication 4 (Under review):

**Buhlebelive Mndzebele, Bhekumthetho Ncube, Melake Fessehazion, Tafadzwanashe Mabhaudhi, and Albert Thembinkosi Modi.** Potential of NPK fertilisation and intercropping amaranth and cowpea in meeting nutritional requirements of children under five years. *Archives of Agronomy and Soil Science.* Manuscript submitted on 23 Oct 2020 (ID: 202414183)

Contributions: Field trials, data collection, analysis and manuscript preparation were performed by the first author under the supervision of the three supervisors.

Signed ________________________________________

Buhlebelive Melusi Mgcini Phiwayinkosi Mndzebele

# ABSTRACT

Several African leafy vegetables (ALVs) contribute to food and nutritional security of rural communities, particularly due to their ability to grow in marginal soils. These ALVs such as amaranth and cowpea among others provide valuable macro- and micronutrients that are key to rural household dietary needs. The aim of this study was to assess the effect of fertiliser application on the symbiotic nitrogen fixation, enzymatic phosphatase activity, agro biological properties, nutrition as well as recommended daily allowance in an intercropped *Amaranthus cruentus* (amaranth) and *Vigna unguiculata* (cowpea) farming system. The nitrogen fixation and nutritional yield of cowpea-amaranth intercrop study was motivated by limited information relating symbiotic nitrogen fixation and fertilisation of ALVs, such as cowpea and amaranth grown under intercropping system, in addition to nutritional yield. Field trials were conducted at the Agricultural Research Council (ARC), Vegetables and Ornamental Plants campus situated in Roodeplaat, Pretoria, South Africa, during 2014/15 and 2015/16 summer seasons from November to January. The 2 x 4 factorial experiments were laid out in a completely randomized design (CRD) with four replications. The factors evaluated were intercropping (amaranth and cowpea) and fertiliser (control, 25%, 50%, and 100% of the recommended NPK levels). Soil sampling was done before land preparation and soil nutrient analysis was done at the Agricultural Research Council–Soil, Climate and Water (ARC–SCW). The application of nitrogen, phosphorus and potassium were guided by the soil analyses results and recommendations on both seasons. *Vigna unguiculata* was sown directly in the soils and amaranth was transplanted approximately four weeks after planting amaranth in the nursery. Irrigation was done based on reference evapotranspiration (ET) and a crop factor for each crop. Collected data included acid and alkaline phosphatase activity, phosphorus in the soils, phosphorus in the cowpea and amaranth plants, as well as biomass of cowpea and amaranth at physiological maturity. In the rhizosphere of cowpea and amaranth grown as sole crops, there was a higher acid and alkaline phosphatase activity as compared to those on intercropping. The highest rhizospheric phosphatase activity occurred when both crops were grown without fertilizer or 25% NPK. Applying NPK activates soil-bound phosphorus (P) using root exudates, which is important for the production of ALVs. The results showed a reduction in symbiotic $N_2$ fixation of cowpea with the increase fertiliser addition.

The above ground and above ground edible biomass of amaranth increased proportionately to the rate of fertiliser application up to 100% NPK, but in cowpea it only increased up to 50% NPK. Nutritional yield of iron and zinc increased with the increase in fertiliser application amounts on cowpea and amaranth. The land utilisation values were greater than one, hence an advantage of intercropping. Cowpea was more aggressive, showed high actual yield losses and high competitive ratio relative to amaranth. More income could be obtained from intercropping cowpea and amaranth compared to the respective sole crops at 100% NPK. In the experiment on the potential of intercropped amaranth and cowpea to meet nutritional requirements, the seasonal above ground and above ground edible biomass of amaranth and cowpea increased with fertiliser application up to 100% NPK. More above ground and above ground edible biomass on amaranth and cowpea were obtained in sole cropping when compared to intercropping. Macro and trace nutritional element contents were highest at 100% NPK fertiliser level. The lowest nutritional contents of macro and trace elements was recorded at the control. Overall, amaranth and cowpea contributed to the recommended daily allowance of calcium, magnesium, iron, and zinc, where there was more at the 100% NPK fertiliser level. The research demonstrates the benefits of grain leguminous crops in soil nutrient fertility enhancement and inorganic fertilization with intercropping in managing micronutrient deficiency to meet the nutritional needs of rural communities. Moreover, the study demonstrated the benefit of applying 25%NPK to 50%NPK fertiliser on the above ground and above ground edible biomass of amaranth and cowpea. In sum, macro and trace elements that are crucial for the nutritional health of rural communities were improved, thus contributing more to the recommended daily allowance, which limits food and nutrition insecurity, and fosters sustainable development.

# ACKNOWLEDGEMENTS

First, the glory goes to God, the Almighty who is the beginning and the end of everything in life!!! Through his mercies and grace this doctoral degree (PhD) was possible. The end of this journey acknowledges where it all started, with Dr Melake Fessehazion, in the Agricultural Research Council, Vegetable and Ornamental Plants in 2015. Since I was researching African leafy vegetables on a project funded by the Department of Rural Development and Land Reform (now Department of Agriculture, Land Reform and Rural Development), under the Pipeline Technology, he encouraged me to do a PhD. The biggest motivations for doing this doctoral degree was the availability of support to cover all the research costs. When the necessary financial arrangements were availed, Prof Albert Thembinkosi Modi was contacted to supervise my studies. He guided me through the application process to enrol for the doctoral degree.

The Agricultural Research Council is acknowledged for providing the space to run the trial, temporary workers, technical expertise as well as all the resources to pay for the laboratory analyses. The University of Kwa Zulu Natal (UKZN) is acknowledged for providing the opportunity to enrol for the doctoral degree. Through the UKZN, I met Prof AT Modi. It has been a great privilege to be supervised by Prof Albert Thembinkosi Modi through shaping my career and character as a future scientist. Through his guidance and beautiful heart, I have obtained many traits of becoming a better human being in the scientific community. The good relations formulated when he was supervising me have opened more opportunities for future collaborations. Prof Albert Thembinkosi Modi encouraged me to work hard and complete the degree within the stipulated time.

To my co-supervisor, a key player in the doctoral degree journey was Prof Tafadzwanashe Mabhaudhi who played a key role in the initial stages through ensuring guiding conceptualisation of the research. He introduced me to Dr Bhekumthetho Ncube who was instrumental in drafting publications and the thesis. The guidance of Dr. Ncube enabled me to publish two papers in accredited journals and also drafted additional two papers which are under review in accredited journals. His guidance and exposure, managed to get me opportunities to become a reviewer for some internationally recognised journals. Overall, Dr Bhekumthetho Ncube was instrumental in the completion of the degree.

I am delighted to express my gratitude to the African Leafy Vegetables team particularly Mr Samuel Molikoe, Mr William Maluleke, Mrs Sidah Mokale, Ms Ndivhuwo Mphaphuli as well as Mrs Tshegofatsho Matseding in their role on data collection and entry. Special gratitude goes to Dr CP du Plooy for his role in the research project. His guidance and dedication to the project were invaluable. Gratitude Dr Sonja Venter for her support on the project.

Professor Felix Dapare Dakora made this degree possible with the expertise who granted me access to his laboratory to do the symbiotic nitrogen fixation analyses. The ARC- Plant Protection Research did the enzyme activity analyses and the ARC-Soil Climate and Water did the nutrient analyses in the plant and soil samples. Dr ST Maseko is acknowledged in guiding the phosphatase activity analyses. Gratitude goes to the ARC-Biometry department, especially Frikkie Calitz, Mr Eric Mathebula and Liesl Morey, who assisted with the analyses of the data.

This journey would not have been possible without emotional and spiritual support. In this regard, my appreciation goes to Dr. Innocent Maseko, Boy Mashaba, Jerry Vusi Nkambule, Lindani Mtshali and Musa Khuzwayo for the emotional support. The Tshwane East Seventh Day Church, Seventh Day Adventist Student Movement at the University of Pretoria as well as Akasia Seventh Day Adventist church for providing spiritual support.

Last but certainly not least, my deepest gratitude goes to the source of my life, family. First, my gratitude goes to my wife, Mrs Tlamelo Tshepiso Mndzebele and children, Siphosenkosi Acimah Arefah Mndzebele and Nkosingizwile Akim Rafiwa Mndzebele. The whole family supported me throughout the studies even in difficult times. Within the PhD journey, we got married and had two children when she hoped that I was going to complete sooner. She even accepted that she was married to a student, and better still supported me throughout. Thank you for your support. To everyone who played a role in my studies, thank you!!, dankie!!, ngiyabonga!!, enkosi!!, ke aleboga!!

# DEDICATION

This thesis is dedicated to my wife (Tlamelo Tshepiso Mndzebele), children (Siphosenkosi Acimah Arefah Mndzebele and Nkosingizwile Akim Rafiwa Mndzebele) who supported me since the beginning of my studies.

Also, this thesis is dedicated to my late father, Canada Enock Mndzebele and my late mother Nomusa Queen Mndzebele who passed away during the last year of my studies.

Finally, the thesis is dedicated to all my siblings and extended family members as a motivation to reach for greater heights in learning.

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# INTRODUCTION TO THE STUDY AND LITERATURE REVIEW

## 1.1 Contribution of indigenous African leafy vegetables to food and nutritional security

African leafy vegetables (ALVs) are defined as plant species, introduced to an area or particular region through natural processes and/or selection by the farming community (Jansen Van Rensburg et al., 2007). These ALVs are commonly referred to as indigenous leafy vegetables (Neugart et al., 2017), wild vegetables (Nesamvuni et al., 2001), or traditional leafy vegetables (Odhav et al., 2007; Vorster et al., 2008). The Plant Resources of Tropical Africa – PROTA, have documented a list of approximately 6,376 indigenous African plants commonly utilised for variable purposes, with 397 known as vegetables. In the list of vegetables there are about 280 indigenous African Leafy Vegetables (Grubben and Denton, (eds) 2004). Within the South African context, ALVs have variable names such as imfino, morogo and miroho by Ngunis, Sothos and Vhendas respectively (Maunder and Meaker, 2007). Common ALVs, normally cited in different studies in South Africa include amongst others *Abelmoschus esculentus* Moench, *Amaranthus* spp., *Bidens spinosa* L., *Brassica rapa* L. subsp. *Chinensis*, *Chenopodium album* L., *Citrillus lanatus*, *Cleome gynandra* L., *Corchorus olitorius* L., *Cucumis melo* L., *Cucurbita spp.*, *Galinsoga parviflora* Cav., *Momordica balsamina* L., *Portulaca oleracea* L., *Solanum retroflexum* Dun., *Vigna unguiculata* (L.) Walp (van Averbeke et al., 2012, Mavengahama, 2013).

### 1.1.1 Amaranth

*Amaranthus* is a vegetable plant that originated from South America, belonging to the genus Amaranthaceae (Janovská et al., 2012). The plant has about 70 species (Espitia-Rangel, 1994; Ebert et al., 2011) and is mostly cultivated for its leaves and grains. *Amaranthus* shows a wide diversity in growth habit, leaf shape, colour and size, plant size and inflorescence characteristics (Shukla et al., 2010). The vegetable grows in a vast range of agroecological zones (Sauer, 1967; Katiyar et al., 2000), and is tolerant to stresses such as heat, drought, diseases, and pests (Shukla et al., 2010). It grows well in soils with a high nitrogen content and pH of about 6.4 (Sarker et al., 2020).

In South Africa, amaranth is mostly harvested from the wild, by the rural population. However, some few farmers cultivate (van Rensburg et al., 2007) the plant commercially (Greve, 2015). The leaves of amaranth are widely consumed in the country compared to the grain, mostly among the rural population. Species that are harvested from the wild includes *Amaranthus thunbergii* Moq., *A. graecizans* L., *A. spinosus* L., *A. deflexus* L., *A. hypochondriacus* L., *A. viridus* L., *A. cruentus* L. and *A. hybridus* L. The vegetable thrives well during the summer rainfall in Limpopo, Mpumalanga, KwaZulu Natal and in the North West provinces due to the high temperatures observed in these provinces (Oelofse and Van Averbeke, 2012). Even though this vegetable is mostly harvested from the wild by the rural population, strong potentials exist to cultivate it, as it can play an important role in combating nutritional insecurity in the country, especially among the rural poor. Research in cultivated fields has shown that amaranth can produce fresh leaves of up to 40 $t.ha^{-1}$ (Schippers, 2004). Additionally, it is a nutritionally dense vegetable, containing several nutrients such as proteins, amino acids (methionine and lysine), dietary fibre and minerals, such as magnesium, calcium, potassium, copper, phosphorus, zinc, iron, and manganese (Sarker and Oba, 2019). Amaranth also contains antioxidants, such as vitamin C, betacarotene, flavonoids, and phenolic acids (Sarker et al., 2018; Sarker and Oba, 2019). Consuming this vegetable can therefore provide an affordable source of nutrients, and cultivation should be encouraged among smallholder farmers.

*Amaranthus cruentus* is taxonomically classified as shown below;

- Kingdom: Plantae
- Subkingdom: Tracheobionta
- Superdivision: Spermatophyta
- Division: Magnoliophyta
- Class: Magnoliopsida
- Subclass: Caryophyllidae
- Order: Caryophyllales
- Family: Amaranthaceae
- Genus: Amaranthus L.

• Species: *Amaranthus cruentus* L.

Morphologically, *Amaranthus cruentus* is an annual herbaceous plant propagating only through seeds (Makinde et al., 2010). It has predominantly a tap root with stems that are either straight or branched growing to height of up to 2.0 m. amaranth leaves are spiral, devoid of stipules, coupled with ovate to rhombic-ovate shape. The leaves and stems have hairy surfaces with unisexual flowers which are green (Grubben, 2004; Śmigerska, 2016). It has large and complex inflorescence with concentrated cymes with racemes and spikes (Grubben, 2004) with variable colours. On average each *Amaranthus cruentus* plant has approximately 50 000 shiny, and dark brown seeds, which are round or lenticular in shape (Robertson et al., 2003; Grubben, 2004). Suitable temperatures for irrigation ranges between 20°C and 35°C (Weaver, 1984).

### 1.1.2 Cowpea

Cowpea (*Vigna unguiculata* (L.) Walp.) is a grain legume belonging to the family Leguminosae that is cultivated globally across Asia, Europe, Africa, and America (Carvalho et al., 2017; De Souza, et al., 2017). In South Africa, the main cowpea producing areas are KwaZulu-Natal, Limpopo, Mpumalanga and North West (DAFF–Department of Agriculture, Forestry and Fisheries, 2011), due to its adaptability to different environments with both high and low rainfall (Kay, 1979) as well as tolerance to drought and low phosphorus (Boukar et al., 2015). The common cowpea genotypes planted in South Africa include Bechuana white, Ngoji, Encore, Bensogla, and CH14, for which leaves, immature pods, and grains are consumed as vegetables (Singh et al., 2002).

In South Africa, cowpea is listed among indigenous African leafy vegetables, with production dominated by smallholder farmers. This crop has the potential to curb food and nutritional insecurity as both the leaves and the grain are consumed. Additionally, the leaves are used as fodder for livestock (Kay, 1979; Ano and Ubochi, 2008; Saidi et al., 2010; Edeh and Igberi, 2012). Cowpea is a grain legume that fixes atmospheric nitrogen, therefore, its cultivation requires no or little fertiliser inputs (Bisikwa et al., 2014; Ddamulira et al., 2015), an important trait that can reduce cost of production for farmers, while contributing to sound ecosystem function. Due to its nitrogen fixing potential, cowpea is often intercropped with a wide range of crops including major cereal crops such as maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L. Moench), pearl millet (*Pennisetum glaucum* L.R.Br) (Namatsheve et al., 2020), and fruits and vegetables such as pineapple and pepper (Ajayi et al., 2020). Symbiotic nitrogen fixation contribution by cowpea on average has been reported to be 201 kg N $ha^{-l}$ globally (Dakora et al., 1987), and between 77 to 557 kg N $ha^{-1}$ in South Africa (Mndzebele et al., 2020b). The cowpea crop is nutritionally rich in proteins, ranging from 23 to 28% in the grain (Gerrano et al., 2019) and 23 to 40% in the leaves (Dakora and Belane, 2019). Also, cowpea is a rich source of macro and micro nutrients such as N, P, Ca, Mg, K, S, Na, Fe, Zn (Maseko, 2019), nutrients key to human health. Other nutrients contained by cowpea are essential amino acids such as lysine, leucine, and phenylalanine (Venkidasamy et al., 2019), fat and carbohydrates (Mafokoane et al., 2019). Through the consumption of cowpea, the dietary requirements of several communities are met, therefore farmers are encouraged to grow the crop for its food and nutritional benefits. Even though cowpea is a much cheaper source of nutrients for many households, it is still considered an under-researched crop compared to other grain legumes such as soybean and groundnut in South Africa.

*Vigna unguiculata* (L.) Walp. is taxonomically classified as shown below;

- Domain: Eukaryota
- Kingdom: Plantae
- Phylum: Spermatophyta
- Subphylum: Angiospermae
- Class: Dicotyledonae
- Division: Magnoliophyta
- Class: Magnoliopsida
- Order: Fabales
- Family: Leguminosae
- Tribe: Phaseoleae
- Genus: *Vigna*

The genus *Vigna* includes more than 80 species and is subdivided into six sections, namely, *Vigna*, *Comosae*, *Macrodontae*, *Reticulatae*, *Liebrechtsia* and *Catiang* (Maxted et al., 2004). Cowpea' is commonly referred to as *V. unguiculata* globally (Coulibaly et al., 2002).

Morphologically, cowpea is an annual herbaceous plant with commonly planted in summer (Fery, 1985; Badiane et al., 2014). This crop has variable genotypic traits with growth habits ranging from semi-prostate, semi-erect, erect or climbing. Cowpea has a determinate or indeterminate growth habit and adaptable to an extensive soil types ranging from sands to heavy, particularly nutrient poor soils (IBPGR, 1983; Timko et al., 2007). Cowpea is able to grow in a wider range of temperatures with 28ºC considered as optimum (Timko et al., 2007). The crop can take between 60 and 150 days for the early and late flowering genotypes respectively, depending on the photoperiod. Cowpea leaves could be categorised into four classes namely sub-globose, sub-hastate, globose and hastate/lanceolate (IBPGR, 1983). The flowers of cowpea have alternate pairs on racemes at the distal ends of long peduncles, commonly having two flowers in each inflorescence. The colour of cowpea flowers vary from yellow to brown or dark purple. Cowpea seeds vary in size and can be either kidney, ovoid, crowder, globose or rhomboid (IBPGR, *Descriptors for Cowpea*. IBPGR Secretariat, Rome (1983). Colour of cowpea also varies from white, cream, green, buff, red, brown or black. Cowpea rooting system is thick and well-built (Pandey et al., 1984).

## 1.2 Soil fertility in Africa

One of the major challenge faced by smallholder farmers in relation to increased productivity in Africa is the issue of low soil fertility. Over 60% of cultivated lands are nutrient deficient coupled with acidity, with nitrogen, phosphorus and potassium being the major limiting nutrients (Sanchez, 2002; Nanganoa et al., 2020). For example, in south western Uganda, soils dominated by Haplic ferralsols were found to deficient in N (80%), P (40%) and K (50%). Additionally, in eastern Uganda, soils dominated by light textured ferralsols had 90% of the soils limiting in N, 50% by P and 10% by K (Bekunda et al., 2002). Factors such as inadequate use of inputs or the lack of it, volatilization, leaching and nutrient mining have contributed tremendously to the poor state of African soils. For example, low P in soils is exacerbated by the high P-fixation capacity (Nalivata et al., 2017). In addition, repeated weathering, erosion, leaching, inadequate fertilizer use, complete removal of crop residues, continuous cropping systems, lack of proper cropping systems, soil erosion and continuous cultivation causes low amounts of nutrients (Voortman et al., 2003; Aleminew and Alemayehu, 2020). These factors independently or in combination deplete soil nutrients as a result of loss of organic matter. The low fertility of these soils have resulted in considerable decline in yields (Tan et al., 2005). These challenges highlight the need to devise more sustainable soil fertility management strategies that are cost-effective and adapted to the specific needs of smallholder farmers.

## 1.3 Management of soil fertility to improve crop production

In South African rural communities, smallholder farmers are currently growing crops, such as indigenous vegetables under nutrient-poor and acidic soils. However, crop production even in these marginal conditions is still important due to the need to satisfy food and nutrition security. Therefore, the adoption of strategies that will improve soil fertility is the key to optimise productivity. These strategies include conventional and non-conventional cropping systems. The conventional strategy entails the common usage of inorganic fertilisers, which is unsustainable, due to limited access and high costs. Alternatively, there are non-conventional strategies including intercropping with leguminous crops, in order to harness the benefits of biological nitrogen fixation by the non-legume.

### 1.3.1 Biological Nitrogen Fixation (BNF)

Legumes such as cowpea have the ability to convert unavailable $N_2$ from the atmosphere to available forms such as ammonia for plant utilization (Bado et al., 2018). Biological nitrogen-fixation in legume is initiated by the N-fixing bacteria in the soil which enters the root hair and reproduce. In turn, the leguminous crops develop tumour-like structure known as nodules on the surface of plant roots (Kakraliya et al., 2018). The rhizobia in the nodule absorb $N_2$ from soil air and transform it into ammonia (Kakraliya et al., 2018). The symbiotic association between the legume host plant and the nodule bacteria mutually benefit each other (Meena et al., 2014; Meen 2014; Skorupska et al., 2017). Estimates indicate that legumes can contribute about 80% of N to the pool of soil N (Galloway et al., 2004). In general, grain legumes in Africa seasonally fix approximately 15–210 kg N $ha^{-1}$ (Dakora and Keya, 1997). Cowpea symbiotic N contribution in African soils has been estimated to be at the rate of 56 kg $ha^{-1}$ (Timko et al, 2007). In South Africa, symbiotic contribution by cowpea is reported to be at the rate of 24-186 kg N $ha^{-1}$, resulting in yield increases of 2000 to 3500 kg $ha^{-1}$ (Belane et al., 2011). Benefits of legume N fixation is often realized when the crop is grown as a sole, intercropped or as a succeeding crop (Bado et al., 2006; Bado et al., 2012).

### 1.3.2 Phosphatase Enzymatic Activity

Another key process of harnessing nutrients to manage soil fertility by leguminous crops, such as cowpea, is phosphatase enzymatic activity, which is key in phosphorus nutrition. Legumes have inherent mechanisms to enhance the acquisition and exploitation of phosphorus (Vance, 2001; Touhami et al., 2020), due to phosphatase enzymes that hydrolyse organic-P (George et al., 2008). This is made possible by legumes that solubilize P through phosphate solubilizing microorganisms, thereby availing phosphorus in the soil. Some studies have demonstrated a correlation between soil phosphatase activity and organic-P (Tate and Salcedo, 1988; Rojo et al., 1990; Trasar-Cepeda et al., 1991; Richardson et al., 2009). It is important to note that inorganic fertiliser application stimulates the activity of phosphatase enzymes, which can subsequently liberate soil-bound phosphorus that is key to additional and sustainable yields, hence improved food and nutrition security (Mndzebele et al., 2020a).

It has been indicated that legumes could contribute approximately 15% of N to a non-legume (cereal) in an intercropping (Li et al., 2009), therefore contributing to additional biomass (Pappa et al., 2012; Ram and Meena, 2014). When intercropping is practised in the presence of a legume, there is improved soil enzyme activity (Chai and Huang, 2004). Other studies such as Mafongoya et al. (2006) have shown the importance of decreasing the dependence on inorganic fertilisers in legume intercropping systems. Overall, there are advantages in intercropping relative to sole cropping, for example, in cereals with grain legumes (Mekuanint, 2020).

### 1.3.3 Utilization of Inorganic Fertilisers

In intercropping and sole cropping systems, the application of inputs such as inorganic NPK fertiliser is a common nutrient management conventional strategy (Crews and Peoples, 2004). Commonly available and applied inorganic fertilisers in South African soils are N-P-K-based, and are mostly single and compound, with different formulations. Their usage is limited, mainly by high cost, transport costs, limited access and little understanding of fertiliser usage (Zapata and Roy, 2004; Odhiambo and Mag, 2008). Even though there are constraints, an important goal is to optimize yield (Ciampitti and Vyn, 2014). Fertilisers are applied to supplement, for example, nitrogen (N), and phosphorous (P) that are generally low in SSA soils, for improved fertility that eventually leads to enhanced nutrition and higher yields (Sanchez, 2002; Gikonyo and Simpson, 2004; Bado et al., 2010). Several studies have shown that inorganic fertilisers can improve crop production, for example, indigenous leafy vegetables (Bvenura and Afolayan, 2014; van Jaarsveld et al., 2014; Zikalala et al., 2016). A study by Mndzebele et al. (2020a), on cowpea showed that application of inorganic fertilisers even below recommended rates improved growth and yield.

Each of the processes, namely BNF and phosphatase enzymatic activity, independently, are not sufficient to enhance the performance of legumes to meet food and nutritional requirements, hence the need of a mechanism which integrates several techniques. This has led to the integrated soil fertility management (ISFM) paradigm, which is a holistic systems approach that is defined as a set of soil fertility management strategies constituting optimum agronomic principles that utilise organic and inorganic inputs to increase nutrient use efficiency and improve crop productivity (Vanlauwe et al., 2010). An important fact to note is that neither organic nor inorganic fertilizer inputs could be substituted as they benefit the soil differently; therefore, both are required for sustainable crop production (Buresh and Tian, 1998; Vanlauwe and Giller, 2006). The major driver of ISFM is ensuring a good balance between these nutrient resources (Vanlauwe et al., 2001), particularly in smallholder farming systems, where resources are insufficient due to affordability and/or accessibility constraints (Chianu et al., 2012). These nutrient resources can curb the inherently variable soil fertility constraints when utilised in combination (Vanlauwe et al., 2015). There is increasing need for alternative and sustainable nutrient sources as substitutes for expensive fertilisers and a combination of these different sources. ISFM in these scenarios utilises locally available soil amendment resources and mineral fertilisers to increase the productivity of arable land while maintaining, sustaining and/or enhancing soil fertility (Vanlauwe, 2010). Some key aspects in ISFM include legume-intercropping, microbial inoculation, seed systems biotechnology, organic and inorganic fertilisers, as well as micro-doses of fertiliser application.

## 1.4 Statement of Problem

Access to nutritious food to meet dietary requirements remains a challenge in most rural African households (Tumushabe, 2018). The intake or consumption of indigenous vegetables may offer a low-cost option to mitigate micronutrient deficiencies, therefore contributing to food and nutritional security, especially amongst rural poor communities. Many of these vegetables are rich in vitamins, micronutrients, and protein (Ochieng et al., 2018). However, crop production efforts are compromised by acidic and nutrient-poor soils (Materechera, 2018). As a basis for this study, the researcher identified the problem to be four-fold. First, studies have shown that phosphorus (P) is low in South African soils (Nongqwenga, et al., 2017). The challenges of low P are associated with its limited mobility in the soil, due to high adsorption, and hence its unavailability (Nziguheba, et al., 2016), thus contributing to its deficiency. Various mechanisms exist to supplement P in the soil and can be implemented to mitigate P deficiency and/or stress (Bulmer et al., 2018). Phosphorus can either be applied through organic (cattle manures, etc.) or inorganic sources (fertilisers). These sources could potentially contribute to improved P supply in a non-legume crop through species interaction and P-acquisition (Bargaz et al., 2017), in an intercropping system (Adigbo, 2009). *Vigna unguiculata* L. Walp (cowpea), through its leaves and seeds, is an important legume crop nutritionally as it provides proteins and minerals that can be easily harnessed by resource-poor rural communities (Aworh, 2018). It is therefore important to embark on studies that will further enhance our understanding of P nutrition in relation to yield, quality and different environments where cowpea is grown (Sing and Singh, 2017) in an effort to ensure food and nutritional security for resource-constrained communities. Second, nutrient-poor soils and micro-nutrient deficiency (also known as ‘‘hidden hunger’’) are two interrelated challenges, affecting rural resource-poor farming communities in sub Saharan Africa (Jones, et al., 2013; Laurie et al., 2017). Low soil fertility affects crop production in rural resource-poor farming communities, therefore reduce nutritional food security, leading to malnutrition (Laurie et al., 2017; Stewart et al., 2019). Lack of nitrogen (N), phosphorus (P), and potassium (K) in sub Saharan African soils tends to reduce micro-nutrient accumulation in plant tissues (Marschner and Marschner, 2012). There is a synergistic relationship between macro-mineral elements such as NPK in soils and trace elements accumulation in plant tissue (Rietra et al., 2017), hence the relatedness of the two factors. For example, N and P plays an important role in the development of roots, which lead to acquisition, and subsequent translocation of nutrients to edible plant parts and those that constitutes the economic yield (Prasad,2014). It is therefore, important to address these factors in combination due to an escalating rate of population growth which is estimated at 3.09% per annum in sub Saharan Africa (Asongu and Odhiambo, 2019) and hence the need for additional micro-nutrients in human diets. Third, vegetable production yields are estimated to be significantly reduced in nutrient-poor soils (Pastori, 2019), which are often low in, for example, nitrogen (N), phosphorus (P) and potassium (K). Farmers usually mitigate low soil nutrients by using exogenous inorganic fertilisers constituting N, P and K (Marschner, 2012; Steward et al., 2020). Inorganic fertilisers are commonly applied in sole cropped as well as intercropped vegetables, some of which are sequentially harvested. If sufficient inorganic fertiliser amounts are applied together with proper management, it results in increased biomass and hence better yields (Ojeniyi, 2002). One of the sustainable crop production mechanisms is intercropping, which is mainly, practised to optimize efficient utilisation of resources, eventually increasing yield (taking into consideration both crops). Common intercropping practices entail the inclusion of a legume in combination with a non-legume crop (Ahamefule and Peter, 2014), for example, cowpea and amaranth. Inter-and intraspecific competition, as well as facilitation, also referred to as agro-biological parameters, are key components of intercropping (Vandermeer, 1989; Zhang and Li, 2003). Inter and intra-specific competition relate to the development of two crops in which there is a variation between them while facilitation describes the improvement in yield of companion crops in an intercropping (Fan et al., 2006; Mei et al., 2012). There are several ratios used to determine the agro-biological parameters. Fourth, in Sub Saharan Africa for example, in South Africa, chronic malnutrition is reported as one of the major challenges, particularly to children (Faber and Wenhold, 2010; FAO, IFAD and WFP, 2015). This has been exacerbated by the consumption of carbohydrates-based diets which are low in both micro and macro nutrients (Weinheimer et al., 2012). This challenge is mainly prominent among the vulnerable groups, which include children under the age of five, as they are considered to be at high risk (Arimond et al., 2010; Black et al., 2013). Lack of availability and accessibility to nutritious and balanced diets is a major challenge that causes malnutrition in rural communities (Govender et al., 2017). The commonly cultivated indigenous vegetables and usually consumed by most communities in South Africa are *Amaranthus cruentus* (amaranth) and *Vigna unguiculata* L. Walp (cowpea) (van Rensburg et al., 2007; Oelofse and van Averbeke, 2012; Mavengahama, 2013).

## 1.5 Objective of the study

To provide insights into the cultivation of amaranth and enhance knowledge on the potential of cowpea-amaranth intercropping system fertilized with different levels of NPK to improve soil fertility and crop productivity. Therefore, the objectives of the study were;

1. To quantify the acid and alkaline phosphatase activity as an indicator of P supply and availability under varying levels of NPK and intercropping of *Amaranthus cruentus* (amaranth) and *Vigna unguiculata* L. Walp (cowpea) as test crops.
2. To assess the effect of fertiliser application and intercropping on the nodulation, nitrogen accumulation and symbiotic nitrogen fixation of cowpea. Also, the effect of fertiliser application and intercropping on iron and zinc concentration, as well as nutritional yield of cowpea and amaranth were assessed.
3. To investigate the effect of different fertiliser application rates on the yield as well as agro-biological parameters in an amaranth-cowpea intercrop.
4. To investigate the yield, nutritional compositions of intercropped amaranth and cowpea indigenous field-grown indigenous vegetables.

## 1.6 The rationale of the study

Poor soil fertility, especially N and P, is one of the major factors contributing to food and nutritional insecurity in Africa. Cowpea and amaranth are two indigenous African leafy vegetables that have great potential to contribute additional income and cheaper sources of nutrients to African households, contributing to nutritional security. Frequent consumption of these vegetables can provide the daily nutritional requirements as they are nutritionally dense. However, there is little research attention on these vegetables, resulting in them often being referred to as neglected and under-researched. Moreover, production of these vegetables is often hampered by low soil fertility, resulting in yield losses and produce of poor nutritional quality. In addition, because amaranth is often harvested from the wild, the findings of this study would encourage their cultivation by smallholder farmers. Nonetheless, this will require greater understanding of its production strategies, especially under poor nutrient conditions. Overcoming this problem to increase productivity is often addressed through the application of inputs such as organic and inorganic fertilisers, intercropping non-legumes with legumes, inoculating beneficial microbes, rotational practices, among others. Several studies have assessed the effects of these conventional soil fertility methods on the growth and yield of amaranth and cowpea as sole crops. This study attempts to achieve this by assessing the benefits of intercropping cowpea and amaranth, at varying levels of organic fertiliser. Furthermore, the study provides valuable insights on the contribution of cowpea to soil N and P through the process of BNF and acid phosphatase, respectively.

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# CHAPTER 2 :
# PHOSPHATASE ENZYME ACTIVITY AND PHOSPHORUS CONCENTRATION IN THE RHIZOSPHERE OF COWPEA AND AMARANTH INTERCROP

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Received: 4 November 2019; Accepted: 24 December 2019; Published: 6 January 2020

## 2.1 Abstract

Low available soil phosphorus is associated with its immobility and hence, unavailable for crops. In addition to inorganic fertilization, farmers often grow legumes, to activate soil-bound phosphorus (P) using root exudates. Sufficient soil nutrition is key to sustainable crop production and hence food and nutritional security. The aim of this study was to quantify the acid and alkaline phosphatase activity as an indicator of P supply and availability under varying levels of NPK fertilization. An intercropping (amaranth and cowpea) and fertilizer (control, 25%, 50% and 100% of the recommended NPK levels) field trial was laid out in a 2 x 4 factorial treatment structure in a completely randomized design with four replications. Shoot phosphorus concentration of cowpea and amaranth plants increased proportionately to the increase in fertiliser application up to 50% of the recommended NPK level. The land equivalent ratio (LER) was greater than 1 indicating that there were benefits in intercropping cowpea and amaranth as opposed to planting them as sole crops. There was a higher acid and alkaline phosphatase activity in the rhizosphere of cowpea and amaranth grown as sole crops compared to those from intercropping. The cowpea and amaranth plants grown without fertiliser or 25% NPK had the highest rhizospheric phosphatase activity, while 100% NPK application exhibited the least. The markedly higher phosphatase activity from the low fertiliser application treatments indicates possible stimulation of microbial activity, which resulted in a favorable environment for enzyme synthesis and accumulation of phosphate. The study revealed that the application of inorganic fertilisers is important in the enhancement of a legume to activate soil-bound phosphorus (P) using root exudates, which is important for vegetable production. In addition, the study highlighted benefits of intercropping in vegetable production.

**Keywords:** Acid phosphatase; alkaline phosphatase; Land Equivalent Ratio, amaranth; cowpea; intercropping.

## 2.2 Introduction

Access to nutritious food to meet dietary requirements remains a challenge in most rural African households [1]. The intake or consumption of indigenous vegetables may o er a low-cost option to mitigate micronutrient deficiencies and contribute to food and nutritional security, especially amongst rural poor communities. Many of these vegetables are rich in vitamins, micronutrients, and protein [2]. To ensure sustainable food and nutritional security, interventions are needed to capture and utilize nutrients [3]. Fertilisers are key inputs in the production of su cient vegetable supplies to the African population. However, production e orts are compromised by acidic [4] and nutrient-poor soils, such as low phosphorus (P) levels [5].

Studies have shown that P is low in South African soils [6]. The challenges of low P are associated with its limited mobility in the soil, due to high adsorption, and hence its unavailability [7] and fixation [8], thus contributing to its deficiency. Various mechanisms exist to supplement P in the soil and can be implemented to mitigate P deficiency and/or stress [9]. Phosphorus can either be applied through organic (cattle manures, etc.) or inorganic sources (fertilisers). These sources could potentially contribute to improved P supply in a non-legume crop through species interaction and P-acquisition [10], in an intercropping system [11]. *Vigna unguiculata* L. Walp (cowpea), through its leaves and seeds, is an important legume crop nutritionally as it provides proteins and minerals that can be easily harnessed by resource-poor rural communities [12]. It is therefore important to embark on studies that will further enhance our understanding of P nutrition in relation to yield, quality and different environments where cowpea is grown [13] in an e ort to ensure food and nutritional security for resource-constrained communities.

With respect to P solubilisation, the key enzymes involved are acid and alkaline phosphatase [14]. Acid phosphatases are of plant origin [15], whilst bacteria, fungi, and earthworms secrete alkaline phosphatase enzymes [16]. Both enzymes, in association, facilitate the liberation of organic phosphate esters in both acid and alkaline conditions in phosphorus-deficient soils [17]. These mechanisms ensure the release of P for legumes and/or other plants to utilize [18]. Even though the acid and alkaline phosphatase enzymes are perfectly functional, in natural ecosystems [19], research evidence also suggests that they are responsive in fertilized soils and cropping systems such as intercropping [20].

Several studies have reported the acid and alkaline phosphatase activity in legumes [21–24]. However, few studies have reported the performance of these in relation to intercropping and inorganic fertilization [25, 26]. It was therefore hypothesized that phosphatase activity, phosphorus (soil and plant), as well as and crop yield will be affected by fertiliser application and intercropping. The objective of this study, therefore, was to quantify the acid and alkaline phosphatase activity as an indicator of P supply and availability under varying levels of NPK and intercropping of *Amaranthus cruentus* (amaranth) and cowpea as test crops.

## 2.3 Materials and Methods

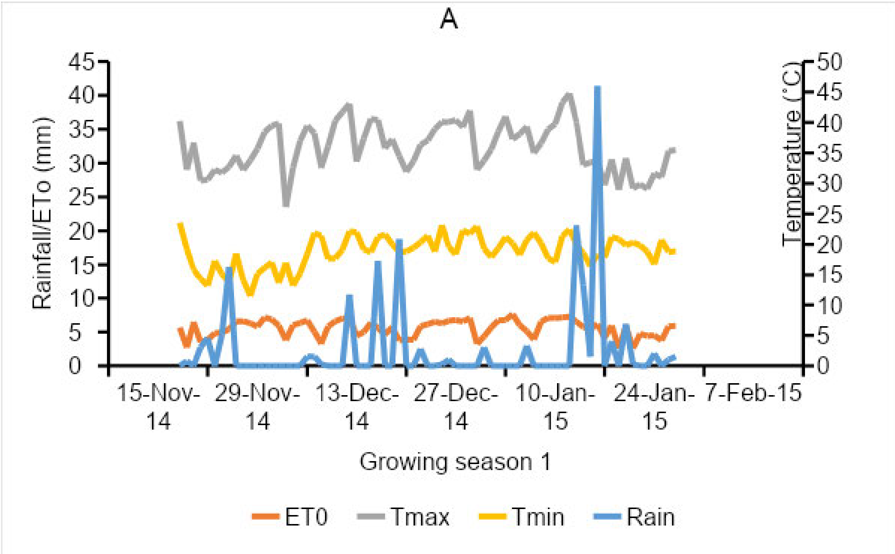
### 2.3.1 Site description

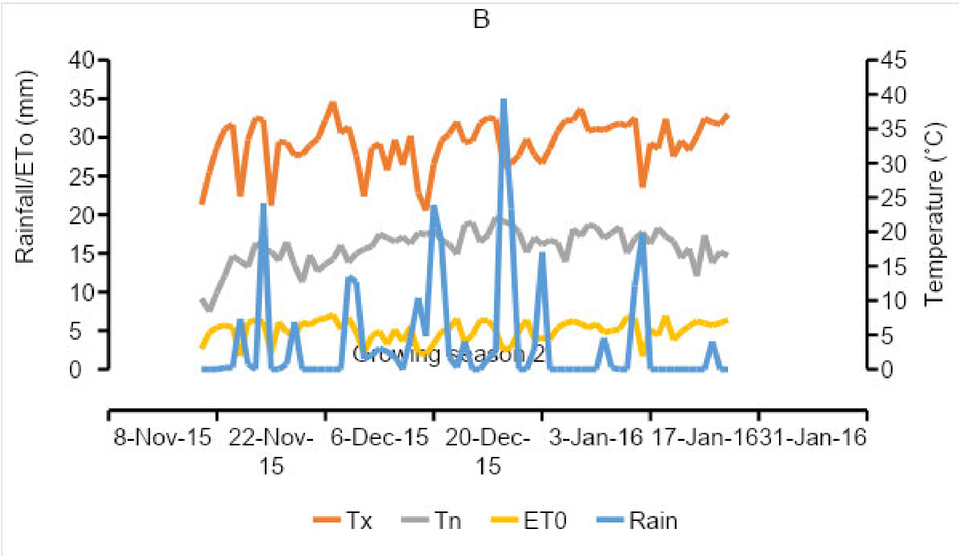
The trial was conducted at the Agricultural Research Council (ARC), Vegetables and Ornamental Plants campus situated in Roodeplaat, Pretoria, South Africa (25°35’ S, 28°21’ E, 1165 masl) during 2014/15 and 2015/16 summer seasons from November to January. Soils in which the experiments were carried out are classified as Hutton clay loam (25–32% clay percentage) with red pedal, composing P, potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), nitrate-nitrogen $NO_3$-N and ammonium-nitrogen ($NH_4$-N) in low to moderate fertility status and a pH ($H_2O$) range of 6.17 to 7.26 as indicated in the South African soil taxonomic classification (Table 2.1). The area has a long-term summer rainfall of approximately 635 mm annually. The highest precipitation is normally experienced during December and January, although it is highly variable. The growing summer seasons in 2014/15 and 2015/16 experienced variations in the weather conditions. On average, in the first season, maximum temperatures ranged between 27.9 °C to 30.2 °C. The second season maximum temperatures ranged from 30.8 °C to 33.9 °C. Daily minimum temperatures for the first season ranged from 13.2 °C to 16.5 °C, and for the second season were 14.2 °C and 18.1 °C (Figure 2.1). There was less rainfall (193 mm) in the first season compared to the second which received 274 mm (Figure 2.1).

Table 2.1: Chemical properties of the topsoil layer (0.3 m) for the experimental sites.

| **Chemical Properties** | **2014/15** | | **2015/16** | |
|---|---|---|---|---|
| | **Before** | **After** | **Before** | **After** |
| pH ($H_2O$) | 6.2 ± 0.4 | 6.2 ± 0.2 | 7.3 ± 0.4 | 7.2 ± 0.5 |
| P(Bray 1) (mg $kg^{-1}$) | 20 ± 0.6 | 18 ± 1.6 | 57 ± 3 | 55 ± 2 |
| K (mg $kg^{-1}$) | 218 ± 3.9 | 203 ± 14 | 158 ± 14 | 155 ± 14 |
| Na (mg $kg^{-1}$) | 18 ± 0.9 | 15 ± 1 | 56 ± 1.2 | 56 ± 1 |
| Ca (mg $kg^{-1}$) | 635 ± 3.3 | 602 ± 40 | 857 ± 49 | 847 ± 5 |
| Mg (mg $kg^{-1}$) | 198 ± 1.3 | 190 ± 13 | 174 ± 11 | 170 ± 1.5 |
| $NO_3$-N (mg $kg^{-1}$) | 7.8 ± 0.6 | 7.7 ± 0.6 | 2.6 ± 0.3 | 2.4 ± 0.8 |
| $NH_4$-N (mg $kg^{-1}$) | 2.4 ± 0.2 | 2.2 ± 0.1 | 3.6 ± 0.4 | 3.9 ± 0.1 |
| Clay % | 25 ± 2 | 25 ± 2 | 32 ± 2 | 32 ± 2 |

Values (Mean ± SE) are averages of three duplicate runs.


A
Rainfall/ETo (mm)
Temperature (˚C)
15-Nov-14
29-Nov-14
13-Dec-14
27-Dec-14
10-Jan-15
24-Jan-15
7-Feb-15
Growing season 1
ET0
Tmax
Tmin
Rain


B
Rainfall/ETo (mm)
Temperature (˚C)
Growing season 2
8-Nov-15
22-Nov-15
6-Dec-15
20-Dec-15
3-Jan-16
17-Jan-16
31-Jan-16
Tx
Tn
ET0
Rain

### 2.3.2 Experimental treatments, layout and plot management

Fertiliser (NPK) was applied based on the recommended cowpea requirement, taking into consideration the lower fertiliser requirements of the legume. In this study, cowpea was the main crop. Recommended fertiliser rates for cowpea in 2014/15 season based on soil analysis results were 135 kg N $ha^{-1}$, 31 kg P $ha^{-1}$ and 18 K $ha^{-1}$ and for 2015/2016 recommendations were 135 kg N $ha^{-1}$, 20 kg P $ha^{-1}$ and 250 kg K $ha^{-1}$. The soil K was adequate for the 25% treatment in the first season and as such was not applied for that treatment level. The NPK fertiliser forms used were limestone ammonium nitrate (28%N) for N, single superphosphate (12%P) for P, and potassium chloride (50%K) for K. In each season N, P, and K, were broadcasted just before planting for cowpea and just before transplanting for amaranth. In the intercropping treatments, fertiliser was applied at the planting stage of cowpea to cover nutrition for amaranth which was transplanted after three weeks. Nitrogen application was split to 40% at planting with two top dressings of 30% applied at 40 and 60 days after planting. Due to its small seed size, the soil type (high clay percentage), and to improve on growth uniformity, amaranth seedlings were first raised in polystyrene trays. Approximately three weeks (19–21 days) after planting on 200-hole polystyrene trays, amaranth seedlings were transplanted into the field plots on 10 December 2014 in the first season and 02 December 2015 in the second season. A week after planting of the amaranth in trays, cowpea seeds were planted directly in the field at a rate of 2 seeds per station at a depth of approximately 10 mm. Prior to direct planting and/or transplanting, irrigation was done to minimize the transplanting shock and to ensure uniform crop establishment.

The trial was laid out in a 2 × 4 factorial treatment structure in a completely randomized design (CRD) with four replications. The field trial comprised of cropping system (2 sole crops and an intercrop) and fertiliser (control, 25%, 50% and 100% of the NPK fertiliser recommendation) as the two factors. The test crops were amaranth and cowpea. Sole cropped amaranth was spaced at 0.30 m between rows by 0.30 m between plants while cowpea plants were spaced at 0.60 m between rows and 0.30 m between plants. Intercropped amaranth plots were spaced at 0.6 m between rows and 0.60 m between plants. Intercropped plots had alternate rows of amaranth spaced at 0.60 m placed in-between cowpea rows which were 0.60 m apart. The fertiliser was applied to cowpea and amaranth based on requirements [27]. Thus, the trial had 12 treatments and 48 plots of 3 m by 3 m, amounting to 9 $m^2$ each. In order to circumvent plot to plot cross contamination, a distance of 1.5 m was maintained between plots.

Compensating non-leaking (CNL) Urinam dripper lines, with a discharge dripper rate of 2.3 l $h^{-1}$ were used for irrigation. Irrigation scheduling was based on crop water requirement ($ET_c$) of each crop, either in a sole cropping or intercropping. For the 2014/15 season, $ET_0$ ranged from 2.7 to 7.27 mm. In the 2015/16 season, reference evapotranspiration ($ET_o$) ranged from 1.71 to 7.05 mm. The crop factors ($K_c$) used were 0.85 for cowpea and 0.9 for amaranth. The crop water requirement ($ET_c$) was calculated based on the product of, $ET_0$ and ($K_c$)

### 2.3.3 Data collection and statistical analysis

***Preparation of Plant and Soil Samples***

Rhizosphere soil samples were collected from the roots of cowpea and amaranth plants for determination of acid and alkaline phosphatase activity when cowpea reached its physiological maturity at 71 and 69 days after planting in the first season and second season respectively. Twelve plants were randomly sampled per treatment (three plants per plot). Soil samples were collected by carefully digging up each plant with its roots intact. The loose soil around the roots was gently shaken off and the soil adhering to the roots (hereafter referred to as rhizosphere soil) was collected into a pre-labelled plastic bag. The rhizosphere soil samples were immediately frozen and kept frozen (−20 °C) in the laboratory before being analyzed for phosphatase activity.

For biomass determination, plants were carefully dug out with intact root system, washed with distilled water, and separated into roots, and shoots for both crops. The separated plant parts were oven-dried at 60 °C for 48 h, ground into fine powder (2-mm sieve), and stored at room temperature, before analysis for tissue P concentrations.

### *Bioassay for acid and alkaline phosphatase activity in rhizosphere soil*

The acid and alkaline phosphatase activities in the rhizosphere soil were assayed following the method of Eivazi and Tabatabai [28] as modified by Hedley et al. [29]. The *p*-nitrophenyl phosphate tetrahydrate method was used in the colourimetric assay for acid and alkaline phosphatase activities. One mL *p*-nitrophenyl phosphate tetrahydrate was dissolved in acetate buffer (pH 6.5) adjusted with 0.1 M HCl and to pH 11.0 with 0.1 M NaOH for acid and alkaline phosphatases, respectively. For each enzyme activity, 1 g of fresh rhizosphere soil in duplicates was transferred to a 50 mL Erlenmeyer flask and each treated separately with 0.2 mL of toluene and 4 mL of modified universal buffer (MUB) at pH 6.5 or 11 for acid or alkaline phosphatases, respectively. For each soil sample, controls were included where *p*-nitrophenyl phosphate tetrahydrate was added after halting the reaction by adding 1 mL of 0.5 M NaOH and 4 mL of 0.5 M $CaCl_2$ immediately before filtration. Samples were mixed thoroughly and incubated at 37 °C for 1 h. Following incubation, the enzyme activity was halted by adding 1 mL of 0.5 M NaOH and 4 mL of 0.5 M $CaCl_2$. The contents were mixed and filtered through Whatman No. 2 filter paper. The supernatant was transferred to pill vials and the absorbance of the supernatant read at 420 nm using a UV-visible spectrophotometer (Pharmacia LKB, Ultrospec II E). In order to account for non-enzymatic substrate hydrolysis, soils sampled outside the rhizosphere of roots were used to obtain values for control, where these were subtracted from sample replicates. The control samples were prepared the same way as in the rhizosphere soils. After correction for soil moisture content, the enzyme activity was expressed on soil dry weight basis as mg *p*-nitrophenol. $g^{-1}$ soil dry weight $h^{-1}$. One unit of acid phosphatase activity was defined as the activity per gram of soil which produced 1 mmol *p*-nitrophenol $h^{-1}$.

### *Determination of soil-P in the rhizosphere soils*

Phosphorus was determined using the Bray 1 method as developed by Dyer [30] and modified by Division of Chemical Services [31] and Du Plessis and Burger [32]. This was done by measuring and recording soil weight, which was further mixed for not more than 60 s to get extractable P. These inorganic minerals in the extract were measured by colometric analysis through conversion of phosphates to orthophosphate by hydrolysis with sulphuric acid at 90 °C. Phosphate concentration is measured by reducing phosphomolybdic acid using a 1-amino-2-naphthol-4-sulfonic acid to yield an intense blue colour sufficient for detection at 660 nm. A 6.67 g mass of soil was placed in an extraction bottle with a volume of 50 mL Bray solution at 20 °C. This was closed using a stopper and shaken for 60 s. Thereafter, two drops of flocculant were added followed by filtering using a Whatman no. 2 filter paper into an empty bottle. Analysis was done using the MS spectrometry (IRIS/AP HR DUO Thermo Electron Corporation, Franklin, MA, USA).

***Measurement of P concentration in plant tissues***

Phosphorus concertation in plant tissues were determined by ashing 1 g ground sample in a porcelain crucible at 500 °C overnight. This was followed by dissolving the ash in 5 mL of 6 M HCl and placing it in an oven at 50 °C for 30 min, before adding 35 mL deionised water. The mixture was then filtered through Whatman No. 1 filter paper, and the concentration of P in plant extracts determined using the Inductively Coupled Plasma (ICP) [33].

***Yield***

Crops were harvested 71 and 69 days after cowpea seeding corresponding to 49 and 50 days after transplanting in case of amaranth during first and second growing season, respectively. Cowpea was harvested at physiological maturity. Twelve plants were sampled per plot on sole cropping on amaranth amounting to an area of 1.08 $m^2$ on sole cropping. In cowpea nine plants were harvested per plot amounting to a harvested area of 1.62 $m^{2.}$ on both sole cropping and intercropping. From cowpea, the root nodules were detached, and counted. The aboveground material was weighed to determined fresh biomass, and thereafter oven-dried to determine shoot dry weight. Grain yield was determined by removing the dry pods from plants and air-drying them. The seeds were removed from the dry pods (12% moisture content determined using a moisture meter) and their mass recorded to obtain grain yield.

***Land Equivalent Ratio (LER)***

Land equivalent ratio (LER) was determined using the formula as shown below;

$$LER = I_A/S_A + I_C/S_C \quad (1)$$

where LER is the land equivalent ratio; $I_A$ is amaranth above ground biomass in intercropping; $I_c$ is cowpea above-ground biomass in intercropping; $S_A$ is amaranth above ground biomass as sole crop; $S_C$ is cowpea above ground biomass as sole crop.

***Statistical Analysis***

A two-way ANOVA involving fertiliser levels and cropping systems between cowpea and amaranth to analyse above ground dry biomass, plant and soil phosphorus concentration, rhizosphere acid, and alkaline phosphatase activities was performed. Data analysis was performed using GENSTAT version 18. Where treatment means were significant, Duncan multiple range test (DMRT) was used to separate them at $p \leq 0.05$.

## 2.4 Results

### 2.4.1 Phosphatase activity in the rhizosphere of cowpea and amaranth

There was generally a significant characteristic increase in acid and alkaline phosphatase activity of the rhizosphere of cowpea and amaranth in sole cropping at control NPK fertiliser level before dropping down again at 100% fertiliser level in both seasons (Table 2.2 and Figures 2.2 and 2.3). In all cases, the lowest enzymatic activity was recorded in an intercropping system with 100% fertilization level. The rhizosphere phosphatase (acid and alkaline) enzymatic activity significantly decreased inversely proportional to the increase in fertiliser (NPK) application level from the highest in the control to the lowest in 100% NPK in the rhizosphere of both amaranth and cowpea sole cropping in both seasons (Table 2.2). In rhizosphere of cowpea, acid phosphatase decreased from 1394 μg p-nitrophenol $g^{-1}$ DWt. soil $h^{-1}$ (control) to 978 μg p-nitrophenol $g^{-1}$ DWt. soil $h^{-1}$ (100% NPK) in the first season and from 1246 μg p-nitrophenol $g^{-1}$ DWt. soil $h^{-1}$ (control) to 998 μg p-nitrophenol $g^{-1}$ DWt. soil $h^{-1}$ (100% NPK) in the second season (Figure 2.2).

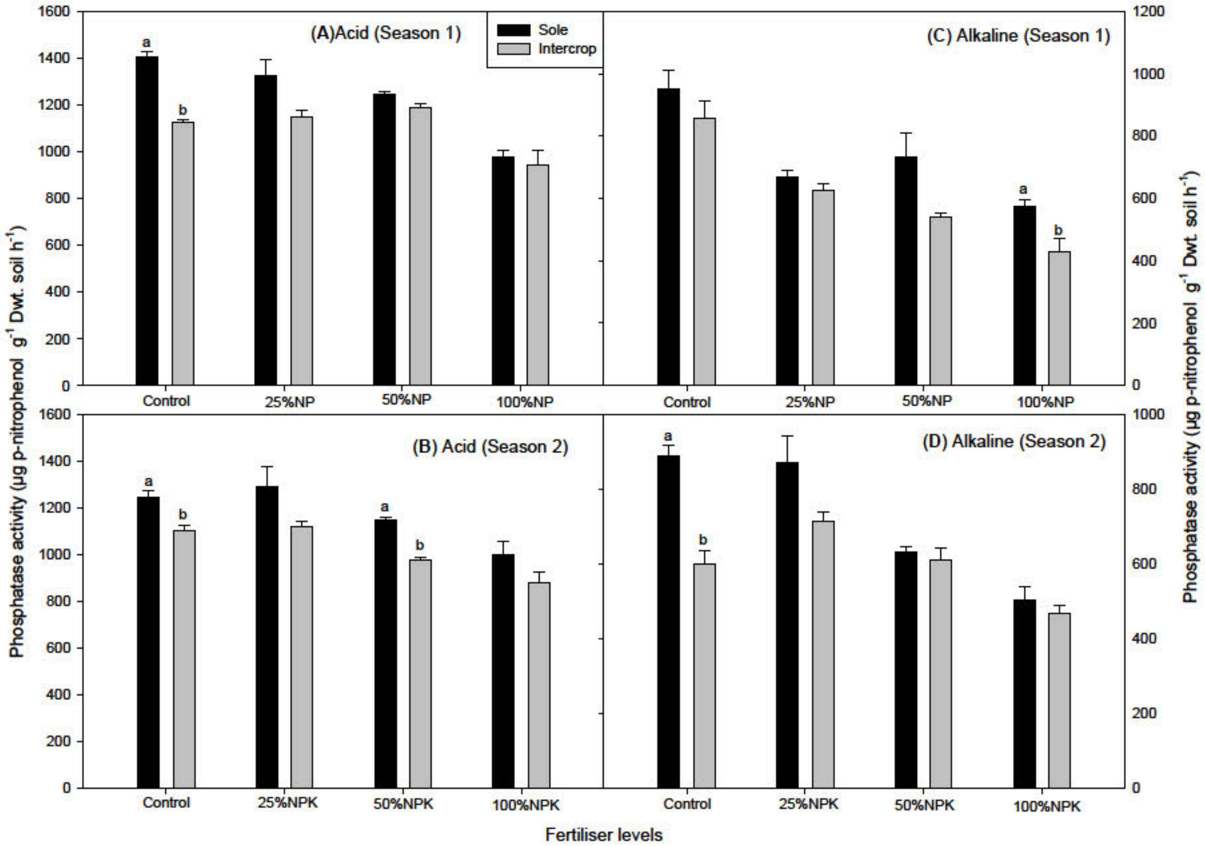



Figure 2.2: Two-way analysis showing the effects of fertiliser levels and cropping systems on acid and alkaline phosphatase activity on rhizosphere of cowpea at four NPK levels in (**A**,**B**) 2014/15 and (**C**,**D**) 2015/16 seasons. Each bar represents the mean ± SE (n = 4). Different letters in each fertiliser treatment shows significant differences at $p \leq 0.05$ in sole or intercropping.

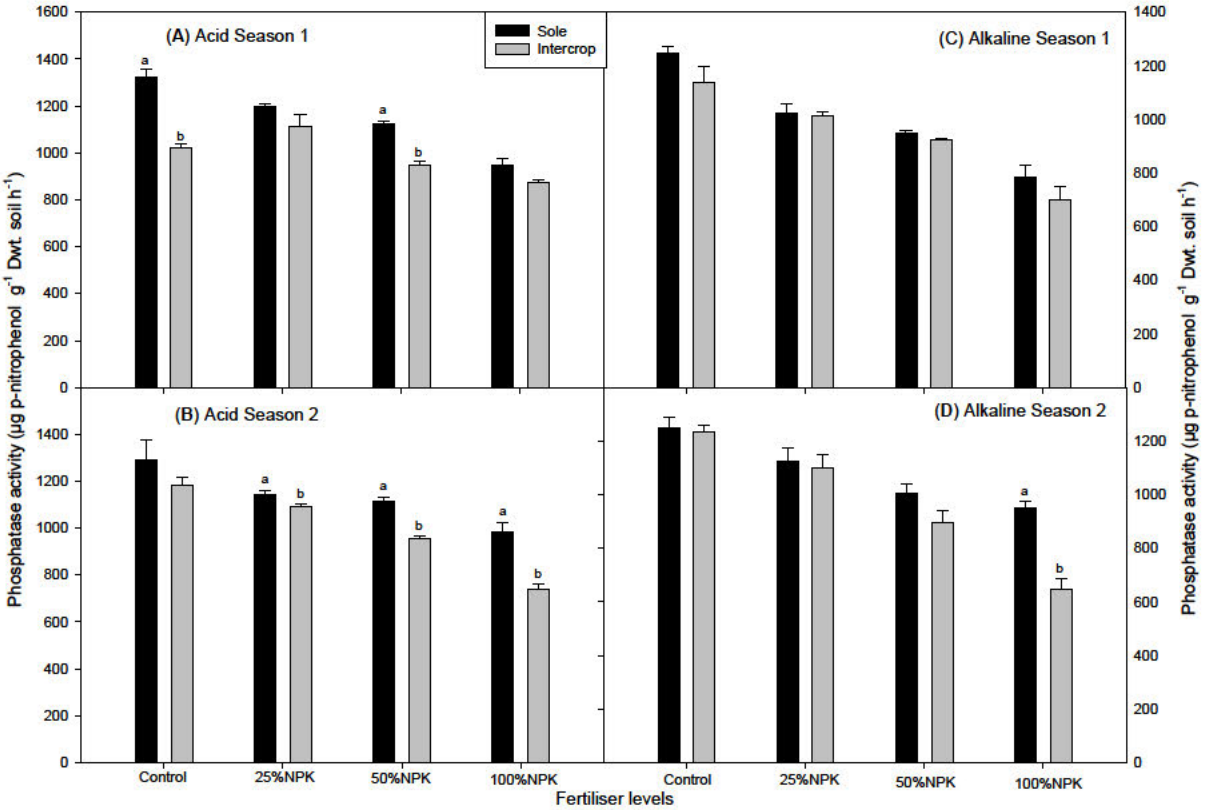



Figure 2.3: Two-way analysis showing the effects of four NPK fertiliser levels and cropping systems on acid and alkaline phosphatase activity on rhizosphere of amaranth in (2.3A,B) 2014/15 and (2.3C,D) 2015/16 seasons. Each bar represents the mean ± SE (n = 4). Different letters in each fertiliser treatment show significant differences at $p \leq 0.05$ in sole or intercropping.

A similar trend to that observed in sole cropping was recorded for the enzymatic activity (acid and alkaline) in the rhizosphere of amaranth intercrop where the activity increased with the decrease in fertiliser application from 100% to 0% in both seasons (Table 2.2). Similarly, the alkaline phosphatase activity in the rhizosphere of cowpea intercrop in season one followed a similar trend of increasing activity with decrease in levels of fertiliser applied. The acid phosphatase activity in the rhizosphere of cowpea intercropping (both seasons) and alkaline phosphatase activity (season two) however, increased only up to an optimum 25% level of fertiliser application, beyond which there was a drop at 100% fertiliser level. Comparatively, in both seasons and across all fertiliser levels, the phosphatase (acid and alkaline) activity was consistently higher in the rhizosphere of sole cropping than intercropping in rhizosphere of both crops (Figures 2.2 and 2.3).

Table 2.2: Soil acid and alkaline phosphatase activities in the rhizospher of cowpea and amaranth plants that are fertilised with four levels of NPK in 2014/15 (season 1) and 2015/16 (season 2) seasons.

| Cropping System | Fertiliser Levels | Cowpea | | | | Amaranth | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Season 1 | Season 2 | Season 1 | Season 2 | Season 1 | Season 2 | Season 1 | Season 2 |
| | | Acid Phosphatase Activity (µg *p*-nitrophenol g$^{-1}$ DWt. soil h$^{-1}$) | | Alkaline Phosphatase Activity | | Acid Phosphatase Activity | | Alkaline Phosphatase Activity | |
| Sole | CONTROL | 1394 ± 7a[1] | 1246 ± 9ab[1] | 950 ± 4a[1] | 888 ± 6a[1] | 1325 ± 8a[1] | 1292 ± 8a[1] | 1246 ± 8a[1] | 1471 ± 4a[1] |
| | 25%NPK | 1325 ± 4ab[1,2] | 1292 ± 6a[1] | 766 ± 4b[2] | 868 ± 7a[1] | 1199 ± 8b[2] | 1144 ± 7b[12] | 1021 ± 7c[2] | 1126 ± 4bc[12] |
| | 50%NPK | 1244 ± 8bc[2] | 1144 ± 7bc[12] | 667 ± 3bc[2] | 630 ± 4b[2] | 1122 ± 9bc[2] | 1116 ± 7b[2] | 948 ± 7c[2] | 1004 ± 8bc[2] |
| | 100%NPK | 978 ± 7d[3] | 998 ± 6cde[2] | 575 ± 4cd[2] | 501 ± 3cd[2] | 946 ± 7de[3] | 982 ± 8c[3] | 784 ± 5d[3] | 949 ± 7c[2] |
| Intercrop | CONTROL | 1144 ± 5c[1] | 1099 ± 7bcd[1] | 892 ± 5a[1] | 597 ± 4bc[2] | 1021 ± 8d[1] | 1179 ± 7b[1] | 1137 ± 8b[1] | 1236 ± 9ab[1] |
| | 25%NPK | 1177 ± 6c[1] | 1119 ± 8bcd[1] | 626 ± 3cd[2] | 712 ± 5b[1] | 1113 ± 6c[12] | 1094 ± 8b[2] | 1015 ± 8c[2] | 1098 ± 6bc[1] |
| | 50%NPK | 1150 ± 7c[1] | 977 ± 8de[2] | 542 ± 3d[23] | 610 ± 4bc[12] | 945 ± 9de[23] | 956 ± 6c[3] | 922 ± 7c[2] | 894 ± 5cd[2] |
| | 100%NPK | 946 ± 6d[2] | 879 ± 6e[2] | 395 ± 2e[3] | 468 ± 2d[3] | 875 ± 6e[3] | 740 ± 4d[4] | 698 ± 5d[3] | 649 ± 5d[3] |
| LSD(*p*-value) | Cropping | 56 (<0.001) | 69 (<0.001) | 53 (<0.001) | 55 (<0.001) | 39 (<0.001) | 55 (<0.001) | 49 (0.025) | 124 (0.011) |
| LSD(*p*-value) | Fertiliser Level | 79 (<0.001) | 97 (<0.001) | 75 (<0.001) | 78 (<0.001) | 55 (<0.001) | 78 (<0.001) | 69 (<0.001) | 176 (<0.001) |
| LSD(*p*-value) | Cropping X Fertiliser Level | 111 (0.017) | 138 (0.935) | 107 (0.404) | 110 (0.007) | 77 (0.001) | 110 (0.101) | 97 (0.366) | 248 (0.375) |

Mean ± SE (n = 4) in each column followed by different letters indicate significant differences between treatments.

Numerical values that have been superscripted compare means of each cropping system at different fertiliser levels ($p \leq 0.05$).

### 2.4.2 Soil P concentration in cowpea and amaranth

There were significant increases in soil P concentration on amaranth with the increase in NPK fertiliser application from the control (0%) to 100% fertiliser level in both seasons (Table 2.3 and Figure 2.4 C, D). A similar pattern to that of cowpea was obtained on amaranth in the first season. However, soil P concentration on cowpea in the second season of cowpea increased from control (0%) until 50% NPK fertiliser level, beyond which there was a decline at 100% NPK (Table 2.3 and Figure 2.4 C, D). In all the fertiliser levels, soil P concentration was lower in the intercropping system than with the sole cropping on both cowpea and amaranth (Table 2.3 and Figures 2.4 C, D and 2.5 C, D).

Table 2.3: Soil P concentration in cowpea and amaranth fertilised with four levels of nitrogen, phosphorus and potassium (NPK) in 2014/15 (season 1) and 2015/16 (season 2) seasons.

| Cropping System | Fertiliser Levels | Cowpea | | Amaranth | |
|---|---|---|---|---|---|
| | | Season 1 | Season 2 | Season 1 | Season 2 |
| | | mg.kg$^{-1}$ | | | |
| Sole | CONTROL | 53.7 ± 2.5d$^{3}$ | 37.0 ± 3.2c$^{3}$ | 78.7 ± 2.2bc$^{2}$ | 50.1 ± 3.0c$^{3}$ |
| | 25%NPK | 84.8 ± 1.9c$^{2}$ | 65.4 ± 3.6a$^{12}$ | 97.9 ± 3.1abc$^{12}$ | 64.1 ± 4.2bc$^{23}$ |
| | 50%NPK | 97.2± 2.9bc$^{12}$ | 75.1 ± 5.4a$^{1}$ | 102.1 ± 2.4ab$^{12}$ | 81.4 ± 4.7a$^{12}$ |
| | 100%NPK | 131.2 ± 9.5a$^{1}$ | 52.3 ± b$^{23}$ | 142.4 ± 10.8a$^{1}$ | 92.0 ± 8a$^{1}$ |
| Intercrop | CONTROL | 51.4 ± 2.8d$^{2}$ | 36.5 ± 2.9c$^{3}$ | 48.9 ± 3c$^{2}$ | 34.1 ± 2.1d$^{2}$ |
| | 25%NPK | 63.9 ± 3.9d$^{2}$ | 52.1 ± 2.6b$^{2}$ | 77.7 ± 4.7bc$^{12}$ | 51.0 ± 3.4c$^{12}$ |
| | 50%NPK | 88.0 ± 5.3c$^{1}$ | 66.6 ± 3.9a$^{1}$ | 97.2 ± 3.5abc$^{1}$ | 56.0 ± 4.5c$^{12}$ |
| | 100%NPK | 101.4 ± 9.1b$^{1}$ | 46.7 ± 2.8bc$^{23}$ | 104.1 ± 9.8ab$^{1}$ | 79.0 ± 6.9ab$^{1}$ |
| LSD(*p*-value) | Cropping | 6.1 (<0.001) | 6.2 (0.030) | 23.3 (0.050) | 7.9 (<0.001) |
| LSD(*p*-value) | Fertiliser Level | 8.7 (<0.001) | 8.8 (<0.001) | 33.0 (0.013) | 11.2 (<0.001) |
| LSD(*p*-value) | Cropping X Fertiliser Level | 12.2 (0.019) | 12.5 (0.492) | 46.6 (0.732) | 15.9 (0.609) |

Mean ± SE (n = 4) values in each column followed by different letters indicate significant differences between treatments. Numerical values following different letters have been superscripted to compare means of each cropping system at different fertiliser levels ($p \leq 0.05$).

### 2.4.3 Plant P concentration in cowpea and amaranth

There was generally a significant characteristic decrease in plant P concentration in cowpea proportional to the increase in fertiliser application levels in season 1 (sole cropping) and season 2 (intercropping) (Table 2.4). Plant P concentration in cowpea was also observed to be similar in the second season on sole cropping with the first season of intercropping except for increases up to 25% fertiliser application and decrease in the control treatment (0%). Conversely there was generally a significant characteristic increase in plant P concentration amaranth (in both sole and intercrop) with the increase in fertiliser application from control (0%) to 100% NPK in both seasons (Table 2.4 and Figure 2.4 A, B). A comparison between the two cropping systems at each fertiliser level in amaranth, indicated plant P to be consistently higher (though not significantly in some cases) in sole cropping than in intercropping in both seasons (Figure 2.4 A, B). Sole cropping in cowpea like in amaranth had consistently higher plant P than in an intercrop (Figure 2.5 A, B).

Table 2.4: Plant phosphorus concentration in cowpea and amaranth fertilized with four levels of NPK in 2014/15 (season 1) and 2015/16 (season 2) seasons.

| Cropping System | Fertiliser Levels | Cowpea | | Amaranth | |
|---|---|---|---|---|---|
| | | Season 1 | Season 2 | Season 1 | Season 2 |
| | | mg $kg^{-1}$ | | | |
| Sole | CONTROL | 4935 ± 23a$^{1}$ | 4249 ± 22a$^{1}$ | 4514 ± 14cd$^{2}$ | 3401 ± 15cd$^{3}$ |
| | 25%NPK | 4586 ± 34a$^{1}$ | 4569 ± 24a$^{1}$ | 5220 ± 9b$^{1}$ | 3732 ± 18bc$^{23}$ |
| | 50%NPK | 4401 ± 35a$^{1}$ | 4224 ± 21a$^{1}$ | 5340 ± 37ab$^{1}$ | 3896 ± 19ab$^{12}$ |
| | 100%NPK | 2675 ± 16c$^{2}$ | 3517 ± 19b$^{2}$ | 5671 ± 32a$^{1}$ | 4114 ± 3a$^{1}$ |
| Intercrop | CONTROL | 2793 ± 22c$^{2}$ | 3744 ± 20b$^{1}$ | 4174 ± 28d$^{3}$ | 3166 ± 19d$^{2}$ |
| | 25%NPK | 3673 ± 27b$^{1}$ | 3651 ± 18b$^{1}$ | 4629 ± 31c$^{2}$ | 3399 ± 22cd$^{12}$ |
| | 50%NPK | 3710 ± 22b$^{1}$ | 3560 ± 19b$^{1}$ | 4653 ± 38c$^{2}$ | 3427 ± 28cd$^{12}$ |
| | 100%NPK | 2396 ± 24c$^{2}$ | 3086 ± 21c$^{2}$ | 5120 ± 48b$^{1}$ | 3851 ± 26ab$^{1}$ |
| LSD(*p*-value) | Cropping | 256 (<0.001) | 215 (<0.001) | 184 (<0.001) | 163.2 (<0.001) |
| LSD(*p*-value) | Fertiliser Level | 363 (<0.001) | 304 (<0.001) | 260 (<0.001) | 230.9 (<0.001) |
| LSD(*p*-value) | Cropping X Fertiliser Level | 513 (<0.001) | 430 (0.362) | 368 (0.552) | 326.5 (0.707) |

Mean ± SE (n = 4) values in each column followed by different letters indicate significant differences between treatments. Numerical values following different letters have been superscripted to compare means of each cropping system at different fertiliser levels ($p \leq 0.05$).

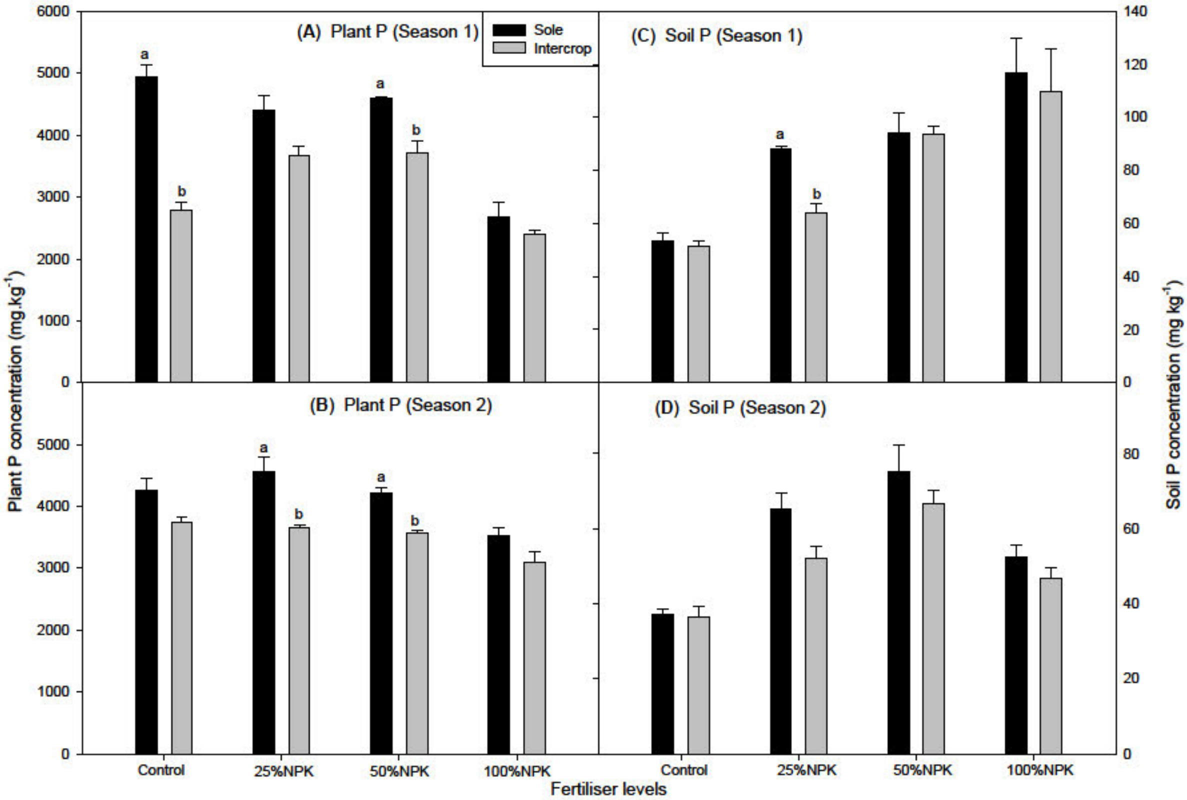



Figure 2.4: Two-way analysis of variance showing the effects of four NPK fertiliser levels and cropping systems on plant (**A,B**) and soil (**C,D**) P concentration on rhizosphere of cowpea in 2014/15 and 2015/16 seasons. Each bar represents the mean (mean ± SE). Different letters in each fertiliser treatment shows significant differences at $p \leq 0.05$ in sole or intercropping.

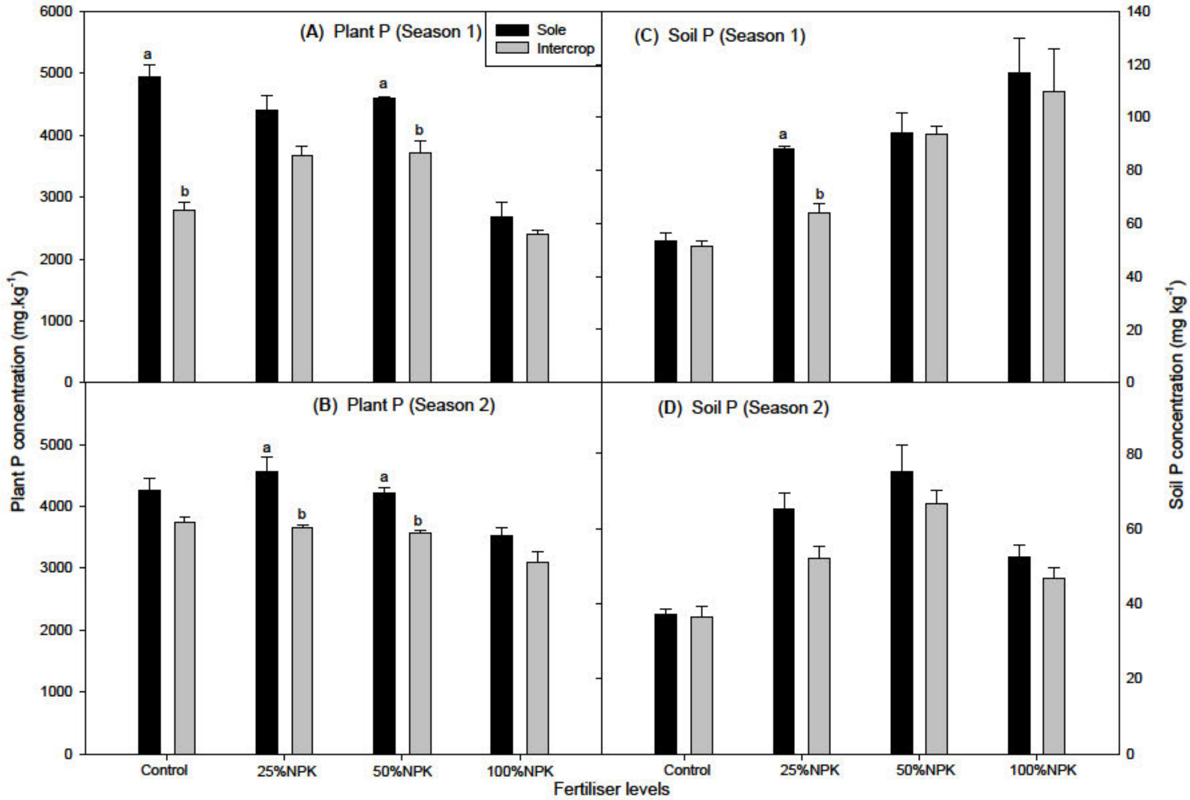



Figure 2.5: Two-way analysis showing the effects of four NPK fertiliser levels and cropping systems on plant (**A**,**B**) and soil (**C**,**D**) P concentration on rhizosphere of amaranth in 2014/15 and 2015/16 seasons. Each bar represents the mean ± SE (n = 4). Different letters in each fertiliser treatment shows significant differences at $p \leq 0.05$ in sole or intercropping.

### 2.4.4 Above ground, above ground edible on dry weight in cowpea and amaranth

There was a general characteristic increase in the above ground and above ground edible biomass of cowpea in sole cropping as fertilization increased from control up to 50% NPK fertiliser level before dropping slightly at 100% fertiliser level in both seasons (Table 2.5). The lowest above ground and above ground edible biomass in cowpea sole cropping (2731 kg ha$^{-1}$) for first season and for second season (2922 kg ha$^{-1}$), were observed in the control and 100% NPK fertilization levels respectively. Sole cropping in combination with 50% NPK fertilization gave the highest above ground and above ground edible dry biomass in cowpea, in both seasons. On the other hand, intercropping showed an increase in above ground and above ground edible dry mass from the control, but only up to 25% NPK, which was the highest (4009 kg ha$^{-1}$) in the first season before declining from 50% NPK to the lowest (1485 kg ha$^{-1}$) at 100% NPK fertilization. In the second season, although 100% NPK fertilization remained the lowest above ground and above ground edible accumulation in intercropping, 50% NPK recorded the highest (4354 kg $ha^{-1}$) above ground and above ground edible. A comparison between cropping systems with regard to above ground and above ground edible dry matter revealed that at all fertiliser levels, shoot dry biomass was higher in sole cropping compared to intercropping in both seasons, with significant differences being recorded in some fertiliser level treatments (Figure 2.6).

There was a marked increase in grain yield of cowpea in sole cropping as fertilization increased from control (0%) up to 50% NPK and 25% fertiliser levels for first and second season respectively (Table 2.5). The lowest grain yield in cowpea was obtained 100%NPK in both seasons (Table 2.5). Sole and intercropping in combination with 50% fertilization gave the highest grain yield in the first year (Table 2.5).

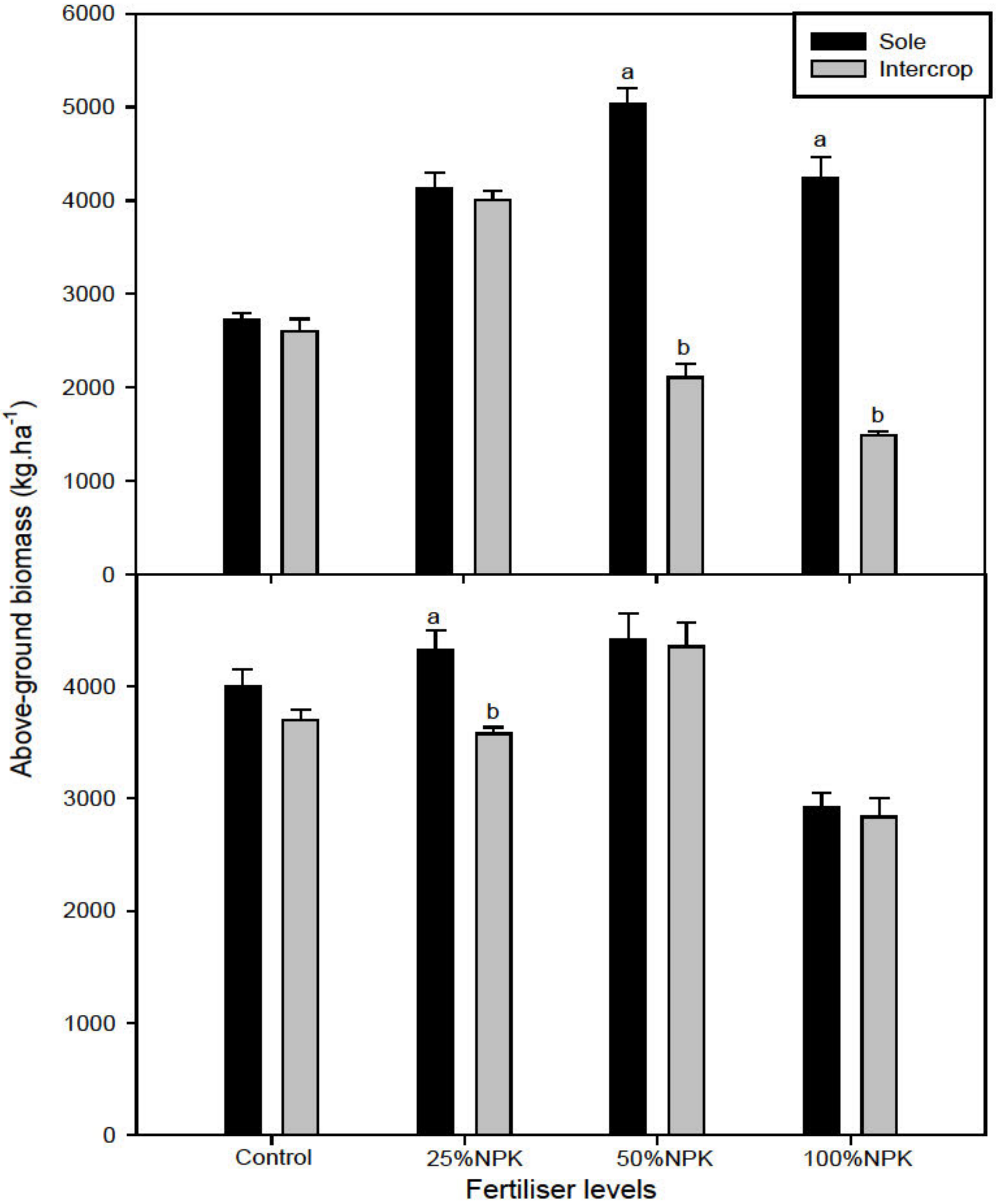



Figure 2.6: Two-way analysis showing the effects of four NPK fertiliser levels and cropping systems on plant (**A**,**B**) and soil (**C**,**D**) P concentration on rhizosphere of above ground biomass of cowpea in 2014/15 and 2015/16 seasons. Each bar represents the mean ± SE (n = 4). Different letters in each fertiliser treatment shows significant differences at $p \leq 0.05$ in sole or intercropping.

In amaranth, above ground and above ground edible biomass increased significantly proportional to the increase in fertiliser levels from the control (0%), right up to the 100% NP fertilization in the first season of both sole and intercropping systems (Table 2.5 and Figure 2.7). A similar trend to that of the first season was also observed in the second season with the exception of sole cropping where a slight decrease in above ground and above ground edible biomass (though not significant) was recorded at 100% NPK from that of 50% NPK fertilization. Similar to the trend observed in the cowpea, a comparison between the two cropping systems showed that above ground and above ground edible biomass accumulation was consistently higher in sole cropping compared to intercropping across all fertiliser levels in both seasons (Figure 2.7). These differences were significant in all fertiliser levels, except the control in the first season. However, in the second season only 50% NPK fertilization had sole cropping yielding significantly higher above ground and above ground edible biomass than that of intercropping (Figure 2.7).

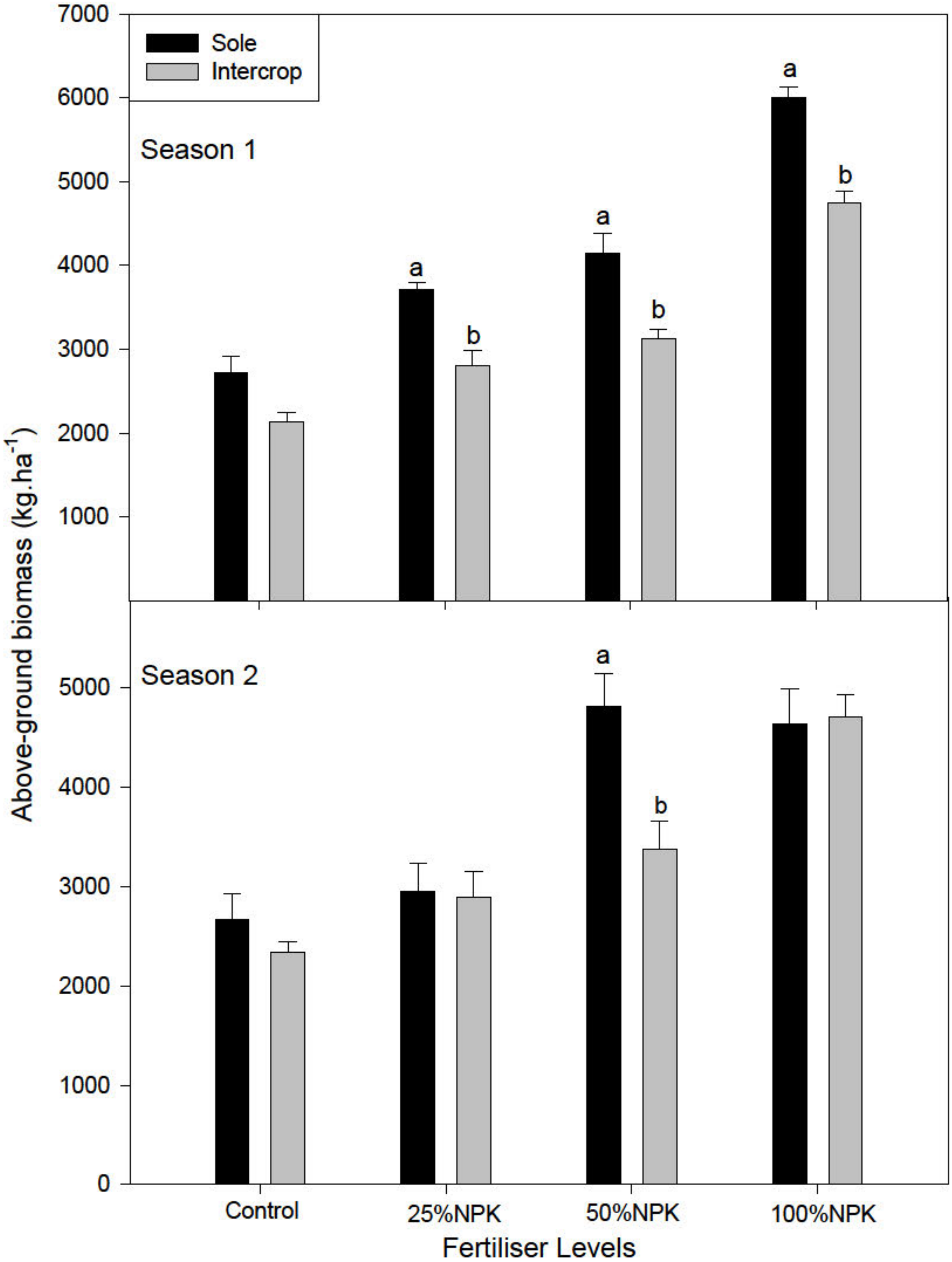



Figure 2.7: Two-way analysis showing the effects of four NPK fertiliser levels and cropping systems on plant (**A**,**B**) and soil (**C**,**D**) P concentration on rhizosphere of above ground biomass of amaranth in 2014/15 and 2015/16 seasons. Each bar represents the mean ± SE (n = 4). Different letters in each fertiliser treatment shows significant differences at $p \leq 0.05$ in sole or intercropping.

The results of this study showed LER values greater than one (LER > 1) (Table 2.5), indicating intercropping of cowpea and amaranth to be more beneficial than sole cropping of each. There was an indication of proportionately high land equivalent ratios ranging from 1.08 to 1.81 for the different fertiliser levels for the first year as well as ratios ranging from 1.59 to 1.8 for the second year. The LERs of more than one (>1), could be interpreted to mean that the land area planted for cowpea and amaranth as a sole cropping would need more land (8–81% for first season), for it to match the equivalent land area if the same crops were planted in an intercropping.

Table 2.5: Above ground biomass (AGB), above ground edible biomass (AGEB), in cowpea and amaranth under sole and intercropping systems as well as Land Equivalent Ratio (LER) and grain yield of cowpea at four fertiliser levels (F. level) (NPK) in 2014/15 (season 1) and 2015/16 (season 2) seasons.

| **2014/15** | **F. Level** | **Cowpea** | | | **Amaranth** | | | |
|---|---|---|---|---|---|---|---|---|
| | | **AGB** | **AGEB** | **GRAIN YIELD** | **AGB** | **AGEB** | **F. level** | **LER** |
| | | kg.ha$^{-1}$ | kg.ha$^{-1}$ | kg.ha$^{-1}$ | kg.ha$^{-1}$ | kg.ha$^{-1}$ | | |
| Sole | CONTROL | 2731 ± 137c$^{3}$ | 573 ± 29b$^{2}$ | 3577 ± 179b$^{2}$ | 2389 ± 119c$^{1}$ | 116 ± 6de$^{2}$ | | |
| | 25%NPK | 4126 ± 165b$^{2}$ | 760 ± 30a$^{1}$ | 5678 ± 227a$^{1}$ | 3519 ± 141abc$^{12}$ | 177 ± 7bcd$^{2}$ | | |
| | 50%NPK | 5034 ± 151a$^{1}$ | 869 ± 26a$^{1}$ | 6016 ± 180a$^{1}$ | 4370 ± 131ab$^{23}$ | 206 ± 6bc$^{2}$ | | |
| | 100%NPK | 4238 ± 254b$^{2}$ | 816 ± 49a$^{1}$ | 3259 ± 196bc$^{2}$ | 5278 ± 317a$^{3}$ | 388 ± 23a$^{1}$ | | |
| Intercrop | CONTROL | 2605 ± 130c$^{2}$ | 507 ± 25bc$^{1}$ | 2415 ± 121bc$^{23}$ | 2056 ± 103c$^{1}$ | 103 ± 5e$^{3}$ | CONTROL | 1.81 |
| | 25%NPK | 4009 ± 160b$^{1}$ | 546 ± 22bc$^{1}$ | 5709 ± 228a$^{1}$ | 2481 ± 99bc$^{1}$ | 126 ± 5de$^{23}$ | 25%NPK | 1.68 |
| | 50%NPK | 2108 ± 63d$^{3}$ | 406 ± 12bc$^{1}$ | 3642 ± 109b$^{2}$ | 3074 ± 92bc$^{1}$ | 146 ± 4cde$^{2}$ | 50%NPK | 1.12 |
| | 100%NPK | 1485 ± 89e$^{4}$ | 393 ± 24c$^{2}$ | 1989 ± 119c$^{3}$ | 3870 ± 232abc$^{1}$ | 211 ± 13b$^{1}$ | 100%NPK | 1.08 |
| LSD($p$-value) | Cropping | 230(<0.001) | 106 (0.157) | 578 (<0.001) | 862 (0.024) | 29 (<0.001) | | |
| LSD($p$-value) | Fertiliser Level | 325(<0.001) | 75 (<0.001) | 817 (<0.001) | 1220 (0.007) | 41 (<0.001) | | |
| LSD($p$-value) | Cropping X Fertiliser Level | 460(<0.001) | 151 (0.003) | 1156 (0.050) | 1725 (0.783) | 58 (0.004) | | |
| **2015/16** | | | | | | | | |
| Sole | CONTROL | 3996 ± 216ab$^{1}$ | 1796 ± 90bc$^{2}$ | 3430 ± 172c$^{2}$ | 2667 ± 133b$^{2}$ | 107 ± 5c$^{3}$ | | |
| | 25%NPK | 4319 ± 177a$^{1}$ | 2749 ± 110a$^{1}$ | 5230 ± 209a$^{1}$ | 2944 ± 118b$^{2}$ | 118 ± 5c$^{3}$ | | |
| | 50%NPK | 4419 ± 88a$^{1}$ | 1945 ± 58b$^{2}$ | 4275 ± 128 bc$^{12}$ | 4815 ± 144a$^{1}$ | 187 ± 6b$^{2}$ | | |
| | 100%NPK | 2922 ± 222c$^{2}$ | 1060 ± 64b$^{2}$ | 2035 ± 122d$^{3}$ | 4630 ± 278a$^{1}$ | 270 ± 16a$^{1}$ | | |
| Intercrop | CONTROL | 3702 ± 179b$^{2}$ | 1574 ± 79d$^{3}$ | 1993 ± 100d$^{2}$ | 2333 ± 117b$^{1}$ | 117 ± 6c$^{2}$ | CONTROL | 1.80 |
| | 25%NPK | 3576 ± 174b$^{2}$ | 1710 ± 68c$^{1}$ | 4843 ± 194ab$^{1}$ | 2889 ± 116b$^{1}$ | 126 ± 5c$^{2}$ | 25%NPK | 1.81 |
| | 50%NPK | 4354 ± 85a$^{1}$ | 1555 ± 47bc$^{1}$ | 4226 ± 127bc$^{1}$ | 2889 ± 87 b$^{1}$ | 141 ± 4bc$^{12}$ | 50%NPK | 1.59 |
| | 100%NPK | 2835 ± 85c$^{3}$ | 1134 ± 60cd$^{2}$ | 1553 ± 93d$^{2}$ | 3407 ± 204ab$^{1}$ | 190 ± 11b$^{1}$ | 100%NPK | 1.71 |
| LSD($p$-value) | Cropping | 205(0.008) | 155(<0.001) | 429(0.011) | 684(0.015) | 25(0.033) | | |
| LSD($p$-value) | Fertiliser Level | 290(<0.001) | 219(<0.001) | 607(<0.001) | 967(0.012) | 35(<0.001) | | |
| LSD($p$-value) | Cropping X Fertiliser Level | 410(0.085) | 309(<0.001) | 859 (0.132) | 1368(0.194) | 49(0.042) | | |

Mean ± SE (n=4) values in each column followed by different letters indicate significant differences between treatments. Numerical values following different letters have been superscripted to compare means of each cropping system at different fertiliser levels ($p \leq 0.05$).

## 2.5 Discussion

The higher enzymatic phosphatase activity in the rhizosphere of cowpea grown as a sole crop (Table 2.2 and Figure 2.2 and Figure 2.3) could be attributed to the greater demand for phosphorus from soil (Table 2.3), by cowpea for its growth and symbiotic functioning [34]. These enzymes (acid and alkaline phosphatases) are housed in the roots of plants and soil microbes [35]. Acid phosphatase enzymes are located in root exudates and in some instances in the rhizospheric soil of plants roots [36]. On the other hand, alkaline phosphatases are formed mainly by soil microorganisms [37]. Collectively these enzymes are key in the harnessing of P from the soil as well as its accessibility in soils [36]. In combination, acid and alkaline phosphatases enzymes play an important role in the organic phosphate mineralisation as well as, release of inorganic P by dephosphorylation of organic P into soils.

Even though theory would support the assumption that intercropping would result in more activity due to the interaction of roots, the proximity between companion crops could have resulted to less activity based on the results obtained in this study. An increase in the phosphatase activity on sole cropping indicated the high soil phosphorus in the rhizosphere of the cowpea crop (Table 2.2), which could be required for the cellular biosynthesis of adenosine triphosphate, necessary for the reduction of $N_2$ to $NH_3$ by the nitrogenase in the cowpea root nodules [38].

The results also indicated that amaranth as a non-legume crop has more enzymatic activity on sole cropping as opposed to intercropping. The ability of a non-legume crop such as amaranth to have phosphatase activity in sole cropping is an indication of the diversity of enzymatic activity across variable crops [39]. Soil phosphatase activity of the non-legumes are affected by crop production practices, which could be either sole or intercropping [40]. Despite such notable phosphatase activity, previous indications have shown the ability of non-legumes such as Leucadendron strictum, Tetraria bromoides, and Zea mays subsp. mays to have rhizosphere phosphatase activity lower than those of legumes [41]. A similar trend was also observed in the results of this study, in which there was a lower acid phosphatase activity in amaranth relative to cowpea (Table 2.2; Figure 2.3). In other studies, for example, elevations in arbuscular mycorrhizal fungi spores in a non-legume crops such as maize [42], cluster roots in *L. strictum* [43] and higher organic matter [44] prompted the phosphatase activity. In the case of this study, for amaranth to be able to have enzymatic activity, root hairs may have played a key role. Part of the mechanisms contributing to lower phosphatase activity in non-legume crops could be linked to their low phosphorus demand as exhibited by the low shoot P concentration since these crops do not fix atmospheric nitrogen [45]. Some studies have also shown the comparable capability of non-legumes to secrete acid phosphatase [46] although their levels are lower than those of legumes such as chickpea and cowpea [23]. This could be particularly attributed in part to the phosphorus requirements for symbiotic nitrogen fixation relative to non-legumes that lacks this metabolic function [47]. The high phosphatase activity in the legume roots and soils results in a substantial increase in plant available P [23]. The secretion of phosphatases is an indicator of soil quality, since their activity mirrors the soil P characteristics (Table 2.1). Phosphatases are affected by crop management practices and therefore are an indicator of soil quality [47]. In this case, the higher enzymatic activity at sole cropping of cowpea could be an indication of P crop demand under this cropping system, with the possibility of benefiting a non-legume crop in an intercropping system. If phosphatase enzyme activity could be higher in intercropping relative to sole cropping [48], the roots of the legumes would spread to accommodate the non-legume, in a complementary relationship [49]. It could be established that different crops on variable cropping systems differ in their phosphatase activity. Intercropping is able to benefit the non-legume through a leguminous crop over its high phosphatase activity and thus liberation of P [50]. Combining legumes with non-legumes crops in and intercrop can, therefore, exploit of the activity of phosphatase enzymes in liberating P for crop utilisation. Enzymatic activity, such as alkaline phosphatase, is lowered in soils cultivated with non-legumes such as corn and wheat [51].

The significantly higher phosphatase activity from the control plants (without supplementary fertiliser application) of the tested crops in this study (Table 2.2; Fig. 2.2 and Figure 2.3), was evidence that the application of NPK fertiliser increased the soil available P, thus reducing the phosphatase activity (Table 3 and Figure 2.4A,B and Figure 2.5A,B). This is because low P content in soil and/or plant triggers the mechanisms that increase P solubility in the soil, or its remobilization within tissues [52]. Just as in the control, when NPK fertiliser applied was low (25%NPK), there could have been stimulation of microbial activity, which resulted in a favourable environment for enzymatic synthesis and accumulation of P [53]. The decrease in enzyme activity with the increase in fertiliser levels in this study corroborate with this logic. For example, alkaline phosphate decreased with increased fertilization on both cowpea and amaranth. Studies have also shown that acid phosphatase is sensitive to increasing NPK fertilization in legumes and non-legumes, hence the least acid and alkaline rhizospheric phosphatase activity in the recommended rate of 100% NPK (Table 2.2) in both crops in this study. Therefore, phosphatase activity could be used as a good indicator of soil quality with regard to P.

In this study, shoot P concentration was higher in crops planted in sole cropping when compared to the intercrop. Some studies have shown that legumes and non-legumes planted as sole crops exhibit comparably high shoot P concentration, relative to the intercropped counterparts when similarly fertilized [54]. These higher concentrations of P on sole cropping are in line with the theory that legumes (e.g., chickpea) and non-legumes (e.g., durum wheat) demonstrate higher affinity mechanisms of P acquisition on sole stands relative to intercrops.

There was an increase in shoot P with the increase in fertiliser application up to 50% NPK in both cropping systems. Increased shoot P at NPK fertiliser concentration levels below the 100% NPK can be attributed to the fact that higher amounts have been found to negatively affect soil P, which in turn affects shoot mineral composition [55]. Just as shoot P concentrations increased with fertilization (Table 2.4), yield correspondingly increased in non-legumes, for example in amaranth, proportional to plant biomass [56]. The uptake of P by plants contributes to crop growth and increased yield on both cowpea and amaranth.

The lower individual above ground and above ground edible biomass in an intercropping system of both crops, i.e., cowpea and amaranth, could be attributed to competition for resources between the two crops [44]. On the other hand, the higher yields in sole cropping were as a result of high plant density (only in amaranth) as well as absence of competition for resources such as light, nutrients, water and solar radiation [57]. The results of this study show similar patterns to those obtained in other studies on the yields of cowpea and maize where low yields were recorded in intercropping due to low plant densities of individual crops than those in sole cropping [58]. Moisture also played a key role in plant growth as shown by increased biomass in the second season corresponding to more rainfall amounts as opposed to lower amounts on the first season (Figure 2.1).

In order to determine land utilisation efficiency, this study also looked into the land equivalent ratio (LER), defined as the comparative land space necessary in sole cropping to match production of similar yield in an intercropping system [59]. It is the sum of the fractions of the intercropped yields divided by the sole crop yields. If the LER is 1, the same acreage would be sufficient to get a specified yield of each of the individual crops irrespective of whether they are grown as sole or intercrops. LER values greater than 1 (>1) indicate that more land area would be required to produce the same yield in each crop in a sole cropping as in intercropping [59]. The results of this study showed LER values greater than one (LER > 1) (Table 2.5), indicating intercropping of cowpea and amaranth to be more beneficial than sole cropping of each. These results corroborated with those from other authors such as [59] on corn-bean intercropping.

The LER of more than one (Table 5), shows that, sole cropping may need more land area to be cultivated to get the similar yield as that of intercropping. These results represent the role of fertilisers to increasing yield therefore, LER and merits of intercropping when compared to sole cropping in terms of the utilisation of resources for improved plant growth and efficient land utilization. In this context, there were higher LER values, irrespective of the fertiliser level applied as well as the improvement in intercropping relative to sole cropping [60].

## 2.6 Conclusions

In conclusion, in an intercropping system, interactions between companion crops improved their survival and growth, [61]. This was confirmed by a higher LER indicating the merits of intercropping, which might have contributed to improved nutrition and enzyme activity [53]. Intercropping thus enhances soil fertility, which could lead to increased yields [62]. In more detail, phosphatase activity provided inorganic P through enzymatic activity in both cowpea and amaranth, mainly in limited NPK fertilization levels of up to 25%. However, higher fertiliser application levels tend to reduce the phosphatase enzyme activity. There was more shoot phosphorus concentration on sole crops compared to intercrops up to 50% NPK. Moderate fertiliser application enhances legume ability to harness phosphorus key mineral nutrient for biomass production. Overall, the application of NPK fertiliser to amounts of up to 50%, based on the results of this study, appear to be better than 100% in terms of biomass accumulation and phosphate activity. Farmers can thus benefit from applying less than the recommended doses of fertilization in combination with intercropping and thus economically contributing to cost saving measures for resource-constrained smallholder farming communities.

## 2.7 Acknowledgments

We are grateful to the assistance of J. Habig and Agricultural Research Council-Soil Climate and Water for the analyses of the soil and plant samples. We are further grateful to M. Nyathi for access to most of the journal articles.

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# CHAPTER 3 :

# NITROGEN FIXATION AND NUTRITIONAL YIELD OF COWPEA-AMARANTH INTERCROP

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## 3.1 Abstract

Sub-Saharan soils are nutrient-poor coupled with rural resource poor communities who are deficient in micronutrients. Nutrient poor soils can be managed through various soil amendment or fertilisation strategies. Micronutrients can be supplied through plants. The study was aimed at determining the symbiotic nitrogen fixation of cowpea as well as the contribution of intercropping under varying levels of nitrogen, phosphorus and potassium (NPK) fertilisation. In addition, the amount of micronutrients supplied by cowpea and amaranth were determined. The experiment was laid out in a 2 x 4 factorial treatment structure in a completely randomized design, with intercropping (cowpea and amaranth) and fertiliser (control, 25%, 50%, and 100% of the recommended NPK levels) as treatment factors with four replications. Symbiotic $N_2$ fixation of cowpea decreased from (341-448 kgN.ha$^{-1}$ to 77-91 kgN.ha$^{-1}$) for year one and (557-227 kgN.ha$^{-1}$ to 92-164 kgN.ha$^{-1}$) for the second year), with fertilisation while biomass increased up to 50% of the NPK fertiliser level. Amaranth biomass increased with fertiliser application of up to 100% NPK level. The iron and zinc nutritional yield increased (61-210 g.ha$^{-1}$ for year one and 304-867 g.ha$^{-1}$, for second year), proportional to fertiliser application on both crops. The research shows the benefit of leguminous crops in soil nutrient fertility and that inorganic fertilisation with intercropping are key in managing micronutrient deficiency to meet the nutritional needs of rural communities.

**Keywords:** Cowpea; amaranth; intercropping; $N_2$ fixation; hidden hunger; nutritional yield

## 3.2 Introduction

Nutrient-poor soils and micro-nutrient deficiency (also known as ‘‘hidden hunger’’) are two interrelated challenges, affecting rural resource-poor farming communities in sub Saharan Africa [1, 2]. Hidden hunger is defined as a scenario where intake and absorption of minerals (such as zinc, and iron) are below amounts to maintain good health and development [3]. Poor soil fertility can be addressed through exogenous sources of fertilisation such as the application of organic/inorganic fertilisers and sometimes the use of leguminous crops through their symbiotic $N_2$ fixation, which provide bio-fertilisers in cropping systems [4]. Low soil fertility affects crop production in rural resource-poor farming communities, therefore reduce nutritional food security, leading to malnutrition [2, 5]. Lack of nitrogen (N), phosphorus (P), and potassium (K) in sub Saharan African soils tends to reduce micro-nutrient accumulation in plant tissues [6]. There is a synergistic relationship between macro-mineral elements such as NPK in soils and trace elements accumulation in plant tissue [7], hence the relatedness of the two factors. For example, N and P plays an important role in the development of roots, which lead to acquisition, and subsequent translocation of nutrients to edible plant parts and those that constitutes the economic yield [8]. It is therefore, important to address these factors in combination due to an escalating rate of population growth which is estimated at 3.09% per annum in sub Saharan Africa [9] and hence the need for additional micro-nutrients in human diets. When soils are low in macro-mineral nutrient elements, it translates to reduced trace element accumulation in plant tissues, thus escalating “hidden hunger” in humans.

These measures are, however, inadequate to match the exploding population’s nutritional food needs, largely because of unaffordability [10]. In the quest to address nutritional food insecurity, food-based approaches involving vegetable production in smallholder farming systems, are now employed as alternative solutions as they have been proven sustainable [11]. In addition, crop production approaches have also been confirmed as effective in addressing household nutritional security [12].

Crop production, involves the growing of nitrogen-fixing leguminous crops, such as cowpea (*Vigna unguiculata* L. Walp), that are able to fix up to 337 kg N. $ha^{-1}$ [13]. In addition, cowpea can supply essential micro-nutrients [14], therefore, improved nutritional yield. When growing cowpea under subsistence farming, some rural communities add, for example, inorganic fertiliser in addition to intercropping in order to optimize on limited agricultural productive land [15]. Intercropping by rural resource-poor farmers' dates back to ancient civilization and is today, still being practiced [16]. Some common intercropping systems entail a combination of a legume and non-legume crop [17]; for example, cowpea and amaranth (*Amaranthus cruentus* L.), both of which are utilised as indigenous vegetables. These indigenous vegetable crops are nutrient-dense particularly in crucial elements like iron and zinc [18]. Through intercropping, the physical, chemical and biological properties of the soil are improved, leading to increased crop growth and yield [19]. In order to measure the benefit of yield several ratios are commonly used such as land equivalent ratio (LER) are used. Others entail the Competition Ratio, Aggressivity, Relative Crowding Coefficient, Actual Yield Loss, Actual Yield Loss, Relative yield, Over-yielding, The area-time equivalent ratio (ATER) and Monetary Advantage Index [20]. In addition, it has been shown that through intercropping (in consideration of benefit from two companion crops), there is more bioavailability of micro-nutrients in terms of concentration, when compared to sole cropping [21]. Micro-nutrient concentration becomes valuable to rural resource-poor communities if they are related to available land and hence the concept of nutritional yield (NY). Nutritional yield is defined as a unit of measure, which is a function of edible biomass (for example leaves) and mineral nutritional composition in plant tissues [22].

There is paucity of information on the link between symbiotic nitrogen fixation of mostly neglected indigenous vegetables such as cowpea and amaranth grown under intercropping. Furthermore, the information on the effect of different fertilisation levels on biomass accumulation and nutritional yield is also limited. The objectives of the study were therefore to assess the effect of different fertiliser levels and intercropping on the nodulation, symbiotic nitrogen fixation, and nitrogen accumulation potential of cowpea. In addition, biomass accumulation (above ground biomass and aboveground edible biomass), iron and zinc concentration, nutritional yield of cowpea and amaranth were also assessed. This study therefore hypothesized that, (1) the biomass of cowpea and amaranth will increase in response to fertiliser application (2), different fertiliser levels will affect the nitrogen fixation by cowpea (3), cowpea-amaranth intercrop will contribute to improved NY compared to sole cropping of each crop.

## 3.3 Materials and Methods

### 3.3.1 Site description and environmental conditions

The experiment was conducted at the Agricultural Research Council (ARC), Vegetables and Ornamental Plants campus situated in Roodeplaat, Pretoria, South Africa (25°35’ S, 28°21’ E, 1165 masl) during 2014/15 and 2015/16 summer seasons, which runs from November to January as described by Mndzebele et al. [23]. The soils in which the experimental sites were carried out are classified as Hutton clay loam (25-32% clay percentage) with red pedal, composing P, K, Na, Ca, Mg and $NO_3$-N and $NH_4$-N in low to moderate fertility status and a pH ($H_2O$) range of 6.17 to 7.26 as indicated in the South African soil taxonomic classification [23]. The area has a long-term summer rainfall of approximately 635 mm annually. The highest precipitation is normally experienced during December and January, although it is highly variable. The growing summer seasons in 2014/15 and 2015/16 experienced variations in the weather conditions. On average, in the first season, maximum temperatures ranged between 27.9°C to 30.2°C. The second season maximum temperatures ranged from 30.8°C to 33.9°C. Daily minimum temperatures for the first season ranged from 13.2°C to 16.5°C, and for the second season were 14.2°C and 18.1°C [23]. There was less rainfall (193 mm) in the first season compared to the second, which received 274 mm [23].

### 3.3.2 Experimental treatments, layout and plot management

The experimental treatments, layout and the management of the experiment were as described by Mndzebele et al. [23]. These entailed the amount of fertiliser applied, seedling preparation, transplanting, planting, inter-row spacing, intra-row spacing as well as irrigation amounts applied.

Fertiliser (NPK) was applied based on the recommended cowpea requirement taking into consideration the lower fertiliser requirements of the legume. In this study, cowpea was the main crop. Recommended fertiliser rates for cowpea in 2014/15 season based on soil analysis results were 135 kg N $ha^{-1}$, 31 kg P $ha^{-1}$ and 18 K $ha^{-1}$ and for 2015/2016 recommendations were 135 kg N $ha^{-1}$, 20 kg P $ha^{-1}$ and 250 kg K $ha^{-1}$. The soil K was adequate for the 25% treatment in the first season and as such was not applied for that treatment level. The NPK fertiliser forms used were limestone ammonium nitrate (28%N) for N, single superphosphate (12%P) for P and potassium chloride (50%K) for K. In each season N, P, and K, were broadcasted just before planting for cowpea (cultivar Vigna ONB) and just before transplanting for amaranth (cultivar Arusha). In the intercropping treatments, fertiliser was applied at the planting stage of cowpea to cover nutrition for amaranth, which was transplanted after three weeks. Nitrogen application was split to 40% at planting with two top dressings of 30% each applied at 40 and 60 days after planting. Due to its small seed size, the soil type (high clay percentage), and to improve on growth uniformity, amaranth seedlings were first raised in polystyrene trays. Approximately three weeks (19-21 days) after planting on 200-hole polystyrene trays, amaranth seedlings were transplanted into the field plots on 10 December 2014 in the first season and 02 December 2015 in the second season. A week after planting of the amaranth in trays, cowpea seeds were planted directly in the field at a rate of 2 seeds per station at a depth of approximately 10 mm. Prior to direct planting and/or transplanting, irrigation was done to minimize the transplanting shock and to ensure uniform crop establishment. The experiment was laid out in a 2 x 4 factorial treatment structure in a completely randomized design (CRD) with four replications. The field experiment comprised of cropping system (2 sole crops and an intercrop) and fertiliser (control, 25%, 50% and 100% of the NPK fertiliser recommendation) as the two factors. Sole cropped amaranth was spaced at 0.30 m between rows by 0.30 m between plants while cowpea plants were spaced at 0.60 m between rows and 0.30 m between plants. Intercropped amaranth plots were spaced at 0.6 m between rows and 0.60 m between plants. Intercropped plots had alternate rows of amaranth spaced at 0.60 m placed in-between cowpea rows, which were 0.60 m apart. Thus, the trial had 12 treatments and 48 plots of 3 m by 3 m, amounting to 9 $m^2$ each. In order to circumvent plot-to-plot cross contamination, a distance of 1.5 m was maintained between plots.

Compensating non-leaking (CNL) Urinam dripper lines, with a discharge dripper rate of 2.3 l $h^{-1}$ were used for irrigation. Irrigation scheduling was based on crop water requirement ($ET_c$) of each crop, either in a sole cropping or in intercropping. For the 2014/15 season, $ET_o$ ranged from 2.7 to 7.27mm. In the 2015/16 season, reference evapotranspiration ($ET_o$) ranged from 1.71 to 7.05mm. The crop factors ($K_c$) used were 0.85 for cowpea and 0.9 for amaranth. Crop coefficient ($K_c$) values for amaranth and cowpea obtained from Bhavya et al. [24]. The crop water requirement ($ET_c$) was calculated based on the product of, $ET_o$ and ($K_c$). During the 2014/15 season, the total amount applied ($ET_c$) for cowpea was 266 mm and amaranth was 153 mm in both cropping systems. For the 2015/16 season, $ET_c$ was 289 for cowpea and 174 mm for amaranth in both sole cropping and intercropping.

### 3.3.3 Data collection

***Determination of symbiotic $N_2$ fixation in cowpea***

To determine symbiotic $N_2$ fixation, nodulation, relative ureide-N (%RU-N), percentage N derived from $N_2$ fixation (%Ndfa), N-fixed and soil N uptake of cowpea were determined. The choice of the method was informed by the simplicity in the analyses and less costly equipment. Nodulation was done by carefully up-rooting cowpea from which the nodule mass and the number of nodules were determined. Sampling was done for the ureides in the xylem sap, in which ureide N and nitrate N concentrations, were obtained to calculate %RU-N, %Ndfa, N-fixed and soil N uptake in cowpea. The %Ndfa was determined using the calibration equation based on root-bleeding sap by Herridge and Peoples [26]. The amount of N-fixed was calculated as [25], as shown in Table 3.2. The total soil N uptake was calculated as indicated in Table 3.2.

### *N-accumulation, ureide-and tissue Nitrate-N of cowpea at physiological maturity*

Shoots were washed with distilled water to remove debris and analysed for mineral concentration. The dried samples were digested using perchloric + nitric acid methods. A mass of 0.5 g of dry sample was digested in a 100mL volumetric flask containing 7mL $HNO_3$ (conc. nitric acid) and 3mL $HClO_4$ (perchloric acid) at 180°C [27]. The digested samples were analysed using an Inductively Coupled Plasma-Optical Emission Spectrometric (ICP-OES) to determine the concentrations of the nitrogen element. The N content, indicating N-accumulation of cowpea was calculated as a product of N% and sample mass [28]. The ureide-N and nitrate-N were analysed using the Rimini-Schryver reaction as described by Young and Conway [29], in Unkovich et al. [30]. Nitrates in cowpea was quantified using the salicylic acid method of Cataldo et al. [31], as outlined by Unkovich et al. [30]. To determine the relative ureide-N abundance, equation 1 was used as shown in Table 2.

### *Measurement of mineral concentrations in plant tissue*

Leaf samples were washed with distilled water to remove debris and analyzed for mineral concentration at the Agricultural Research Council-Soil Climate and Water (ARC-SCW) laboratory. The dried samples were digested using perchloric + nitric acid methodologies. A mass of 0.5 g of dry sample was digested in a 100mL volumetric flask with 7mL $HNO_3$ (concentrated nitric acid) and 3mL $HClO_4$ (perchloric acid) at 180°C [27]. The digested samples were analysed using an Inductively Coupled Plasma-Optical Emission Spectrometric (ICP-OES) to determine the concentrations of the nutrient (Fe and Zn) elements.

***Above ground biomass (AGB), above ground edible biomass (AGEB), land equivalent ratio (LER) and nutritional yields (NYs)***

Crops were harvested 69 and 71 days after cowpea seeding corresponding to 49 and 50 days after transplanting in case of amaranth during first and second growing season, respectively, to determine AGB as described by Mndzebele et al., [32]. Above ground edible biomass (AGEB) was further determined through separating edible leaves from cowpea and amaranth. Grain yield was determined by removing the dry pods from plants and air-drying them. The land equivalent ratio (LER) was calculated using equations 3 (Table 3.1). It is defined as the relative land area necessary in a sole crop to produce the same yields in an intercrop [33]. Nutritional yield was calculated for cowpea and amaranth using equations 5 to 7, respectively (Table 3.1). The computations included the concentrations of Fe and Zn and the AGEB of each crop. The total NY factoring both amaranth and cowpea were also determined as shown in equations 5 to 7 in Table 3. 1. To obtain the total NY, the NY of cowpea and amaranth were added.

### 3.3.4 Statistical analysis

A two-way analysis of variance involving fertiliser levels and cropping system between cowpea and amaranth to analyse symbiotic $N_2$ fixation, N-accumulation, AGB, AGEB, grain yield and the nutritional yield. Data analysis was performed using GENSTAT version 19. Where treatment means were significant, Duncan Multiple Range Test (DMRT) was used to separate them at $p \leq 0.05$.

Table 3.1: Equations utilised to compute selected factors.

| Equations | Description | Number |
|---|---|---|
| Relative ureide abundance (%) | [ureide-N /ureide-N + nitrate-N] x 100 | 1 |
| N-fixed | %Ndfa x legume biomass-N; where, legume biomass N was the N content of AGB. AGB is the above ground biomass (t $ha^{-1}$). | 2 |
| The total soil N uptake | It was calculated by computing the difference between plant total N and N-fixed. | 3 |
| LER= $I_C/S_C + I_A/S_A$ | LER is the land equivalent ratio; $I_c$ is cowpea above ground biomass in intercropping; $I_A$ is amaranth above ground biomass in intercropping; $S_C$ is cowpea above ground biomass as sole crop; $S_A$ is amaranth above ground biomass as sole crop. | 4 |
| $NY_{cowpea\ (Fe\ and\ Zn)}$ = [(MC × AGEB× 10] | $NY_{cowpea\ (Fe\ and\ Zn)}$ is the above ground edible biomass nutritional yield (NY) in g $ha^{-1}$) for iron (Fe) and zinc (Zn); MC is mass concentrations of Fe and Zn (mg 100 $g^{-1}$); AGEB is the above ground edible biomass (t $ha^{-1}$). | 5 |
| $NY_{amaranth\ (Fe\ and\ Zn)}$ = [(MC × AGEB× 10] | $NY_{AGEB\ amaranth\ (Fe\ and\ Zn)}$ is the above ground edible biomass nutritional yield (NY) g $ha^{-1}$) for iron (Fe) and zinc (Zn); MC is mass concentrations of Fe and Zn (mg 100 $g^{-1}$); AGEB is the above ground edible biomass (t $ha^{-1}$). | 6 |
| $NY_{Total} = NY_{cowpea\ (Fe\ and\ Zn)} + NY_{amaranth\ (Fe\ and\ Zn)}$ | $NY_{Total}$ (g $ha^{-1}$) is total nutritional yield for iron (Fe) and zinc (Zn); $NY_{cowpea\ (Fe\ and\ Zn)}$ is the above ground edible biomass nutritional yield (NY. g $ha^{-1}$) for iron (Fe) and zinc (Zn); $NY_{amaranth\ (Fe\ and\ Zn)}$ is the above ground edible biomass nutritional yield (NY) g $ha^{-1}$) for iron (Fe) and zinc (Zn). | 7 |

## 3.4 Results

### 3.4.1 Above-Ground Biomass (AGB), Above Ground Edible Biomass (AGEB) and Grain Yield (Cowpea) of cowpea and amaranth

There was a significant interaction between cropping system and fertiliser level with regard to AGB, AGEB and grain yield of both crops in both seasons. With the exception of AGEB and grain yield from intercropping at 50% NPK in the first season, the highest AGB, AGEB and yield were obtained at 25% NPK in both sole and intercropping in cowpea. The lowest AGB and AGEB were obtained at 100% NPK in both cropping systems except for the intercropping control treatment in the first season for AGEB. There was a general characteristic increase in all three parameters from 0% NPK to 25% NPK in both cropping seasons before declining again as fertiliser level increased beyond this level. The only exception was, however, AGEB and grain yield, which increased up to 50% NPK level in the intercrop treatment. Amaranth, on the other hand showed a significant proportional increase in AGB and AGEB with the increase in fertiliser level from 0-100%NPK in both cropping systems and in both seasons.

### 3.4.2 Land Equivalent Ratio (LER) in cowpea and amaranth

The results of this study showed LER values greater than one (LER>1), indicating intercropping of cowpea and amaranth was more beneficial than sole cropping of each crop. The ratios ranged from 1.2 to 1.8, with significant differences recorded among different fertiliser levels in the first season while no significant differences were reported in the second season (ratios ranging from 1.63 to 1.84). The highest ratios (1.8 and 1.84) in the first and second seasons were in obtained from 50%NPK and 25% NPK, respectively.

### 3.4.3 N-Accumulation, Ureide-and Tissue Nitrate-Nitrogen of cowpea at physiological maturity

There was a significant interaction between cropping systems and fertiliser levels with regard to N%, shoot N-content, ureide and tissue $NO_3$ concentration in cowpea tissues (Table 3.2). The highest N% and shoot N-content were recorded at 25% NPK fertilisation in both cropping systems, with the lowest obtained at 100% NPK (Table 3.2). Nitrogen accumulation (N% and N-content) increased up to 50% NPK fertiliser level in the first season, on both cropping systems. The lowest N-accumulation was observed in the control as well as 100% NPK fertilisation (Table 3.2). The highest ureide concentration was shown in the interaction of the control fertiliser level with intercropping. Ureide concentration showed an inverse proportion in response to fertilisation from control (0%) right up to 100% NPK (Table 3.2) in both seasons. There were significant ($p < 0.05$) interactions between cropping system and fertiliser level on tissue nitrate concentration in both seasons, with a characteristic increase as the fertiliser level increased up to 50% NPK and a sharp decline at 100% NPK although the concentrations were not significantly different from those of the control (Table 3.2).

Table 3.2: Percentage N, shoot N-content, ureides and tissue $NO_3$-concentration of cowpea fertilised with four different fertiliser (NPK) levels in 2014/15 (S1) and 2015/16 (S2) seasons.

| **Cropping System [2014/15(S1)]** | **Fertiliser level** | **N** | **Shoot N-content** | **Ureide** | **Tissue $NO_3$-Concentration** |
|---|---|---|---|---|---|
| | | **%** | **g.plant$^{-1}$** | **mM** | **%** |
| | Fertiliser Levels | | | | |
| Sole | CONTROL | 5.3±0.1a$^{1}$ | 2.6±0.18b$^{2}$ | 0.05±0.025b$^{1}$ | 1.5±0.17cd$^{3}$ |
| | 25%NPK | 5.4±0.2a$^{1}$ | 4.0±0.30a$^{1}$ | 0.02±0.002d$^{2}$ | 2.9±0.09b$^{2}$ |
| | 50%NPK | 4.7±0.2a$^{1}$ | 4.3±0.28a$^{1}$ | 0.02±0.003d$^{2}$ | 3.9±0.34a$^{1}$ |
| | 100%NPK | 4.7±0.3a$^{1}$ | 3.6±0.29a$^{1}$ | 0.01±0.003d$^{2}$ | 1.2±0.04d$^{3}$ |
| Intercrop | CONTROL | 3.4±0.3b$^{2}$ | 1.6±0.24bc$^{2}$ | 0.06±0.013a$^{1}$ | 2.7±0.25b$^{1}$ |
| | 25%NPK | 4.9±0.3a$^{1}$ | 3.5±0.11a$^{1}$ | 0.05±0.011b$^{2}$ | 1.6±0.05cd$^{3}$ |
| | 50%NPK | 4.8±0.2a$^{1}$ | 1.8±0.24bc$^{2}$ | 0.03±0.005c$^{3}$ | 2.5±0.18b$^{12}$ |
| | 100%NPK | 3.9±0.2b$^{2}$ | 1.0±0.02c$^{3}$ | 0.03±0.013c$^{3}$ | 1.8±0.12c$^{2}$ |
| LSD (*p*-value) | Cropping System | 0.3(<.001) | 0.39(<.001) | 0.002(<.001) | 0.28(0.155) |
| LSD (*p*-value) | Fertiliser Levels | 0.5(0.007) | 0.55(<.001) | 0.003(<.001) | 0.40(<.001) |
| LSD (*p*-value) | Cropping System X Fertiliser Levels | 0.7(0.005) | 0.77(0.001) | 0.005(<.001) | 0.56(<.001) |
| Cropping System [2015/16(S2)] | Fertiliser Levels | | | | |
| Sole | CONTROL | 3.5±0.1b$^{2}$ | 2.5±0.02b$^{2}$ | 0.052±0.024b$^{1}$ | 1.8±0.12c$^{3}$ |
| | 25%NPK | 5.6±0.4a$^{1}$ | 4.4±0.30a$^{1}$ | 0.058±0.058b$^{1}$ | 3.5±0.54bc$^{2}$ |
| | 50%NPK | 3.6±0.1b$^{2}$ | 2.9±0.10b$^{2}$ | 0.045±0.012bc$^{1}$ | 5.1±0.36b$^{1}$ |
| | 100%NPK | 3.5±0.1b$^{2}$ | 1.8±0.08cd$^{3}$ | 0.046±0.004bc$^{1}$ | 1.3±0.14c$^{3}$ |
| Intercrop | CONTROL | 2.1±0.3c$^{2}$ | 1.4±0.20d$^{3}$ | 0.084±0.052a$^{3}$ | 3.9±0.31bc$^{2}$ |
| | 25%NPK | 3.5±0.2b$^{1}$ | 2.3±0.12bc$^{12}$ | 0.054±0.009b3 | 5.7±1.38b$^{12}$ |
| | 50%NPK | 3.6±0.3b$^{1}$ | 2.8±0.38b$^{1}$ | 0.034±0.036cd$^{2}$ | 8.7±0.23a$^{1}$ |
| | 100%NPK | 3.9±0.2b$^{1}$ | 2.0±0.09cd$^{23}$ | 0.025±0.029d$^{1}$ | 1.9±0.15c$^{2}$ |
| LSD (*p*-value) | Cropping System | 0.4(<.001) | 0.29(<.001) | 0.007(0.774) | 1.24(0.002) |
| LSD (*p*-value) | Fertiliser Levels | 0.6(<.001) | 0.40(<.001) | 0.010(<.001) | 1.76(<.001) |
| LSD (*p*-value) | Cropping System X Fertiliser Levels | 0.8(0.002) | 0.57(<.001) | 0.014(<.001) | 2.48(0.387) |

Mean ±SE (n=12) values in each column followed by different letters indicate significant differences between treatments (cropping system and fertiliser levels). Numerical values that have been superscripted compare means of each cropping system at different fertiliser levels ($p \leq 0.05$).

### 3.4.4 Symbiotic $N_2$ fixation in cowpea

There was a significant ($p \leq 0.05$) increase in nodulation (nodule biomass and number of nodules) of cowpea at physiological maturity in sole and intercropping when fertilisation was increased from control (0% NPK) up to 50% NPK, with an abrupt decline at 100% NPK (Table 3.3). The lowest nodulation was observed at the 100% NPK fertiliser level in both seasons on sole and intercropping although not significantly different from those obtained at 25% NPK (Table 3.3). Intercropping in combination with 50% NPK fertilisation gave the highest nodule mass, in both seasons. In addition, the highest number of nodules was shown in sole cropping in combination with 25% NPK fertilisation in both seasons. Cropping system comparison showed more nodule mass and the number of nodules on sole cropping relative to intercropping. The relative ureide-N (%RU-N) and the percentage N derived from $N_2$ fixation (%Ndfa) on cowpea responded inversely proportional to the amount of fertiliser applied, with a significant decrease from control (0%) to 100% NPK in both cropping systems (sole and intercropping) in both seasons (Table 3.3).

Table 3.3: Nodule mass, number of nodules, %RU-N, %Ndfa, N-fixed and soil N uptake of cowpea fertilized with four different fertiliser (NPK) levels in 2014/15 and 2015/16 seasons.

| **Cropping System [2014/15(S1)]** | Fertiliser Levels | **Nodule Mass** g.plant$^{-1}$ | **Nodule Number** per.plant$^{-1}$ | **RU-N** % | **%Ndfa** % | **N-fixed** kgN.ha$^{-1}$ | **Soil N-uptake** kg.ha$^{-1}$ |
|---|---|---|---|---|---|---|---|
| Sole | CONTROL | 6.0±0.2d$^{3}$ | 54±4bc$^{12}$ | 75±2a$^{1}$ | 91±3a$^{1}$ | 341±23b$^{1}$ | 34±2b$^{2}$ |
| | 25%NPK | 9.0±0.3c$^{2}$ | 99±5a$^{1}$ | 32±1c$^{2}$ | 24±2c$^{2}$ | 138±18de$^{2}$ | 438±32a$^{1}$ |
| | 50%NPK | 11.3±0.6ab$^{1}$ | 59±4b$^{12}$ | 28±2d$^{3}$ | 17±1d$^{3}$ | 104±5def$^{23}$ | 509±37a$^{1}$ |
| | 100%NPK | 6.0±0.4d$^{3}$ | 27±1c$^{2}$ | 26±3d$^{3}$ | 15±1d$^{4}$ | 77±2f$^{3}$ | 438±34a$^{1}$ |
| Intercrop | CONTROL | 6.0±0.4d$^{2}$ | 34±3bc$^{2}$ | 73±5a$^{1}$ | 88±4a$^{1}$ | 202±2c$^{2}$ | 28±3b$^{3}$ |
| | 25%NPK | 10.3±0.5bc$^{1}$ | 30±2bc$^{2}$ | 73±3a$^{1}$ | 88±2a$^{1}$ | 448±17a$^{1}$ | 61±3b$^{2}$ |
| | 50%NPK | 12.7±0.5a$^{1}$ | 43±2bc$^{1}$ | 54±3b$^{2}$ | 57±5b$^{2}$ | 149±2d$^{3}$ | 113±11b$^{1}$ |
| | 100%NPK | 5.0±0.4d$^{2}$ | 35±2bc$^{12}$ | 53±5b$^{2}$ | 61±1b$^{2}$ | 91±9ef$^{4}$ | 58±4b$^{2}$ |
| LSD (*p*-value) | C. System | 1.1 (0.426) | 14(0.003) | 2(<.001) | 3(<.001) | 23(<.001) | 45(<.001) |
| LSD (*p*-value) | F. Levels | 1.5(<.001) | 20(0.023) | 3(<.001) | 4(<.001) | 33(<.001) | 64(<.001) |
| LSD (*p*-value) | C. System X F. Levels | 2.2(0.330) | 28(0.008) | 4(<.001) | 6(<.001) | 47(<.001) | 90(<.001) |
| Cropping System [2015/16(S2)] | Fertiliser Levels | | | | | | |
| Sole | CONTROL | 3.7±0.3de$^{3}$ | 42±4bc$^{12}$ | 74±1a$^{1}$ | 88±2a$^{1}$ | 319±3bcd$^{2}$ | 43±2cd$^{2}$ |
| | 25%NPK | 6.0±0.4bd$^{2}$ | 76±4a$^{1}$ | 74±1a$^{1}$ | 89±2a$^{1}$ | 557±2a$^{1}$ | 69±5cd$^{2}$ |
| | 50%NPK | 8.3±0.7abc$^{1}$ | 45±2b$^{12}$ | 68±2ab$^{1}$ | 80±2ab$^{1}$ | 330±5b$^{2}$ | 82±3b$^{1}$ |
| | 100%NPK | 3.0±0.6e$^{3}$ | 21±1c$^{2}$ | 56±3cd$^{2}$ | 62±2cd$^{2}$ | 164±4bc$^{2}$ | 101±2b$^{1}$ |
| Intercrop | CONTROL | 4.0±0.5de$^{2}$ | 23±2bc$^{2}$ | 76±3a$^{1}$ | 92±4a$^{1}$ | 185±17cd$^{1}$ | 16±2d$^{3}$ |
| | 25%NPK | 8.3±0.6b$^{1}$ | 26±2bc$^{2}$ | 62±2bc$^{2}$ | 70±3bc$^{2}$ | 227±6bcd$^{1}$ | 97±6bc$^{23}$ |
| | 50%NPK | 10.7±0.5a$^{1}$ | 33±2bc$^{1}$ | 50±1d$^{3}$ | 51±2d$^{3}$ | 207±3d$^{1}$ | 199±3b$^{1}$ |
| | 100%NPK | 3.0±0.5e$^{2}$ | 27±2bc$^{12}$ | 37±1e$^{4}$ | 32±2e$^{4}$ | 92±6d$^{1}$ | 195±12a$^{2}$ |
| LSD (*p*-value) | C. System | 1.1(0.033) | 11(0.003) | 5(<.001) | 8(<.001) | 48(<.001) | 34( 0.015) |
| LSD (*p*-value) | F. Levels | 1.6(<.001) | 16(0.023) | 7(<.001) | 11(<.001) | 69(<.001) | 48(<.001) |
| LSD (*p*-value) | C. System X F. Levels | 2.3(0.279) | 22(0.008) | 10(0.006) | 16(0.006) | 97(0.059) | 68(0.007) |

Mean ±SE (n=12) values in each column followed by different letters indicate significant differences between treatments (cropping system and fertiliser levels). Numerical values that have been superscripted compare means of each cropping system at different fertiliser levels ($p≤0.05$). C. System and F. levels represent cropping system and fertiliser level, respectively.

N-fixed significantly decreased with the added amount of fertiliser application from the control (0% NPK) up to the 100% NPK in sole cropping system in the first season. However, there was an exception with other treatment where there a gradual increase from the control (0% NPK) until 25% NPK which was highest, with 100% NPK showing the lowest. There was a general characteristic increase in soil N-uptake with increase in fertilisation up to 50% NPK in both cropping systems and in both seasons. On the other hand, intercropping recorded the highest soil N-uptake in combination with 50% NPK in the second season (Table 3.4).

### 3.4.5 Nutrient concentration and nutritional yield in cowpea and amaranth

There was an overall significant characteristic increase in iron (Fe) and zinc (Zn) concentration of cowpea and amaranth leaves as the fertiliser level increased from control (0%) to 100%NPK in sole and intercropping in both seasons for both crops (Table 3.5). Similarly, nutritional yield (Fe-NY, Zn-NY and Fe+Zn-NY) (Table 3.6) followed the same trend to that of the nutritional concentration except for the intercropping treatment, which showed an increase from 0%NPK to 25%NPK followed by a decline beyond these fertiliser levels for both crops in both seasons.

Table 3.4: Moisture content, iron (Fe) and zinc (Zn) mass concentration of cowpea and amaranth under sole and intercropping systems at four NPK fertiliser levels in 2014/15 and 2015/16 summer seasons.

| **Cropping System [2014/15(S1)]** | | **Cowpea** | | | **Amaranth** | | |
|---|---|---|---|---|---|---|---|
| | | **Moisture content** | **Fe** | **Zn** | **Moisture content** | **Fe** | **Zn** |
| | **Fertiliser Levels** | | **mg.100g$^{-1}$** | **mg.100g$^{-1}$** | | **mg.100g$^{-1}$** | **mg.100g$^{-1}$** |
| Sole | CONTROL | 0.869±0.006d$^{3}$ | 25.7±1.5d$^{4}$ | 2.2±0.1e$^{3}$ | 0.908±0.006b$^{1}$ | 41.9±1.7c$^{2}$ | 3.1±0.03a$^{1}$ |
| | 25%NPK | 0.908±0.006b$^{2}$ | 60.4±1.0c$^{3}$ | 3.4±0.1cd$^{2}$ | 0.916±0.005ab$^{1}$ | 44.3±4.2bc$^{12}$ | 3.1±0.16a$^{1}$ |
| | 50%NPK | 0.919±0.006ab$^{12}$ | 75.1±5.3b$^{2}$ | 3.6±0.1bcd$^{2}$ | 0.918±0.005ab$^{1}$ | 45.7±1.5bc$^{12}$ | 3.5±0.33a$^{1}$ |
| | 100%NPK | 0.927±0.004a$^{1}$ | 92.3±4.1a$^{1}$ | 4.4±0.1a$^{1}$ | 0.931±0.013ab$^{1}$ | 54.8±4.0ab$^{1}$ | 3.7±0.34a$^{1}$ |
| Intercrop | CONTROL | 0.843±0.008e$^{3}$ | 23.7±1.4d$^{3}$ | 3.3±0.1d$^{2}$ | 0.912±0.008ab$^{1}$ | 41.9±1.7c$^{2}$ | 3.1±0.08a$^{1}$ |
| | 25%NPK | 0.847±0.008e$^{3}$ | 24.1±1.4d$^{3}$ | 3.3±0.1d$^{2}$ | 0.917±0.010ab$^{1}$ | 48.0±2.1bc$^{2}$ | 3.2±0.16a$^{1}$ |
| | 50%NPK | 0.890±0.008c$^{2}$ | 52.3±1.6c$^{2}$ | 3.8±0.1bc$^{1}$ | 0.930±0.005ab$^{1}$ | 54.8±4.0ab$^{12}$ | 3.5±0.33a$^{1}$ |
| | 100%NPK | 0.917±0.005ab$^{1}$ | 68.9±3.5b$^{1}$ | 4.0±0.1ab$^{1}$ | 0.939±0.011a$^{1}$ | 63.6±7.7a$^{1}$ | 3.7±0.34a$^{1}$ |
| LSD (*p*-value) | Cropping System | 0.02(<.001) | 4.90(<.001) | 0.270(0.056) | 0.02(0.319 | 6.18(0.060) | 0.1(0.922) |
| LSD (*p*-value) | Fertiliser Levels | 0.01(<.001) | 6.11(<.001) | 0.282(<.001) | 0.02(0.040 | 8.14(0.001) | 0.5(0.022) |
| LSD (*p*-value) | Cropping System X Fertiliser Levels | 0.01(0.001) | 8.07(<.001) | 0.385(<.001) | 0.02(0.896 | 10.96(0.594) | 0.6(1.000) |
| Cropping System [2015/16(S2)] | Fertiliser Levels | | | | | | |
| Sole | CONTROL | 0.816±0.005b$^{1}$ | 66.5±3.0c$^{3}$ | 3.1±0.1c$^{2}$ | 0.903±0.009ab$^{1}$ | 64.0±2.6e$^{4}$ | 4.0±0.,4bcd$^{2}$ |
| | 25%NPK | 0.806±0.006b$^{1}$ | 82.0±5.7b$^{23}$ | 3.1±0.2c$^{2}$ | 0.908±0.005ab$^{1}$ | 77.8±4.4d$^{3}$ | 4.1±0.2bcd$^{2}$ |
| | 50%NPK | 0.826±0.009ab$^{1}$ | 91.7±3.3b$^{2}$ | 3.4±0.1bc$^{12}$ | 0.911±0.006ab$^{1}$ | 90.9±2.3c$^{2}$ | 4.8±0.2ab$^{12}$ |
| | 100%NPK | 0.811±0.007b$^{1}$ | 141.5±10.4a$^{1}$ | 3.7±0.2ab$^{1}$ | 0.913±0.006ab$^{1}$ | 110.5±1.6b$^{1}$ | 5.6±0.5a$^{1}$ |
| Intercrop | CONTROL | 0.804±0.003bc$^{2}$ | 67.5±1.8c$^{3}$ | 3.2±0.3c$^{2}$ | 0.896±0.008b$^{2}$ | 79.5±2.7d$^{3}$ | 3.1±0.2d$^{3}$ |
| | 25%NPK | 0.841±0.007a$^{1}$ | 82.8±5.8b$^{2}$ | 3.2±0.1bc$^{2}$ | 0.911±0.003ab$^{12}$ | 85.1±2.3cd$^{3}$ | 3.5±0.3cd$^{23}$ |
| | 50%NPK | 0.784±0.009c$^{3}$ | 84.3±4.6b$^{2}$ | 3.5±0.2abc$^{12}$ | 0.913±0.009ab$^{12}$ | 106.2±2.6b$^{2}$ | 3.8±0.3bcd$^{2}$ |
| | 100%NPK | 0.752±0.016d$^{4}$ | 95.3±2.5b$^{1}$ | 3.9±0.1a$^{1}$ | 0.917±0.005a$^{1}$ | 129.5±3.7a$^{1}$ | 4.4±0.3bc$^{1}$ |
| LSD (*p*-value) | Cropping System | 0.02(0.918) | 9.78(<.001) | 0.20(0.001) | 0.02(0.918) | 9.33(<.001) | 1.6(0.001) |
| LSD (*p*-value) | Fertiliser Levels | 0.01(0.100) | 10.11(<.001) | 0.32(0.002) | 0.01(0.100) | 5.46(<.001) | 0.4(0.002) |
| LSD (*p*-value) | Cropping System X Fertiliser Levels | 0.02(0.792) | 13.86(0.258) | 0.41(0.871) | 0.02(0.792) | 9.47(0.258) | 1.5(0.871) |

Mean ±SE (n=12) values in each column followed by different letters indicate significant differences between treatments (cropping system and fertiliser levels). Numerical values that have been superscripted compare means of each cropping system at different fertiliser levels ($p \leq 0.05$).

Table 3.5. Iron nutritional yield (Fe-NY), Zinc nutritional yield (Zn-NY) and combined zinc and iron nutritional yield (Fe+Zn-NY) of cowpea and amaranth under sole and the combination at four fertiliser (NPK) levels in 2014/15 and 2015/16 summer seasons.

| **Crops (2014/15)** | **Fertiliser level** | **Fe-NY** | **Zn- NY** | **Zn+Fe-NY** |
|---|---|---|---|---|
| | | | **$g.ha^{-1}$** | |
| Amaranth | CONTROL | 48±2efg$^{2}$ | 3.5±0.5h$^{3}$ | 52±2ef$^{2}$ |
| | 25%NPK | 76±4ef$^{2}$ | 5.3±0.9gh$^{23}$ | 81±4ef$^{2}$ |
| | 50%NPK | 97±3e$^{2}$ | 7.7±0.8g$^{2}$ | 105±3e$^{2}$ |
| | 100%NPK | 197±1cd$^{1}$ | 13.3±1.3ef$^{1}$ | 210±1d$^{1}$ |
| Cowpea | CONTROL | 19±1g$^{2}$ | 12.4±0.3f$^{3}$ | 31±1f$^{2}$ |
| | 25%NPK | 31±3fg$^{1}$ | 21.4±1.6c$^{1}$ | 53±3ef$^{1}$ |
| | 50%NPK | 33±1fg$^{1}$ | 16.6±1.7de$^{2}$ | 50±1f$^{1}$ |
| | 100%NPK | 41±2fg$^{1}$ | 20.0±.8cd$^{12}$ | 61±2ef$^{1}$ |
| Amaranth and Cowpea | CONTROL | 172±14d$^{3}$ | 21.0±1.8c$^{3}$ | 193±13d$^{3}$ |
| | 25%NPK | 234±34c$^{3}$ | 28.2±1.7b$^{2}$ | 262±35c$^{3}$ |
| | 50%NPK | 514±22b$^{2}$ | 36.5±2.4a$^{1}$ | 550±21b$^{2}$ |
| | 100%NPK | 605±20a$^{1}$ | 35.5±2.1a$^{1}$ | 641±21a$^{1}$ |
| LSD (*p*-value) | Crops | 28(<.001) | 1.9 (<.001) | 29 (<.001) |
| LSD (*p*-value) | Fertiliser Level | 32(<.001) | 2.2 (0.003) | 33 (<.001) |
| LSD (*p*-value) | Cropping System X Fertiliser Level | 55(<.001) | 3.8 (0.588) | 58 (<.001) |
| **Crops (2015/16)** | | | | |
| Amaranth | CONTROL | 67±6h$^{3}$ | 4.2±g$^{3}$ | 71±6h$^{3}$ |
| | 25%NPK | 89±4h$^{3}$ | 4.7±g$^{3}$ | 94±4h$^{3}$ |
| | 50%NPK | 162±7gh$^{2}$ | 8.5±fg$^{2}$ | 170±7gh$^{2}$ |
| | 100%NPK | 290±3g$^{1}$ | 14.5±f$^{1}$ | 304±3g$^{1}$ |
| Cowpea | CONTROL | 857±12def$^{3}$ | 39.5±2.3cd$^{2}$ | 896±12def$^{3}$ |
| | 25%NPK | 1617±9a$^{1}$ | 61.4±3.2a$^{1}$ | 1678±9a$^{1}$ |
| | 50%NPK | 1298±16b$^{2}$ | 47.5±2.5b$^{2}$ | 1346±16b$^{2}$ |
| | 100%-NPK | 1084±13c$^{23}$ | 28.3±2.6e$^{3}$ | 1112±13c$^{23}$ |
| Amaranth and Cowpea | CONTROL | 770±16f$^{2}$ | 35.9±2.8cd$^{12}$ | 805±17f$^{2}$ |
| | 25%NPK | 1025±28cd$^{1}$ | 40.7±2.2c$^{1}$ | 1066±28cd$^{1}$ |
| | 50%NPK | 962±27cde$^{1}$ | 39.5±2.9cd$^{1}$ | 1001±28cde$^{1}$ |
| | 100%NPK | 835±12ef$^{2}$ | 32.8±2.5de$^{12}$ | 867±12ef$^{2}$ |
| LSD (*p*-value) | Crops | 78(<.001) | 4.1(<.001) | 78 (<.001) |
| LSD (*p*-value) | Fertiliser Levels | 90(<.001) | 4.7(<.001) | 90 (<.001) |
| LSD (*p*-value) | Crops X Fertiliser Levels | 155(<.001) | 8.2(<.001) | 156 (<.001) |

Mean ±SE (n=18) values in each column followed by different letters indicate significant differences between treatments (crops and fertiliser levels). Numerical values that have been superscripted compare means of each cropping system at different fertiliser levels ($p \leq 0.05$).

### 3.5 Discussion

This study hypothesized that, (1) cowpea and amaranth will increase in biomass in response to fertiliser application (2), nitrogen fixation in cowpea will be affected by different fertiliser levels, (3), cowpea-amaranth intercrop will contribute to improved NY compared to sole cropping of each crop. The objectives of the study were therefore to assess the effect of fertiliser application and intercropping on the nodulation, nitrogen accumulation as well as symbiotic nitrogen fixation of cowpea. In addition, biomass accumulation (above ground biomass and aboveground edible biomass), iron and zinc concentration, nutritional yield of cowpea and amaranth were also assessed.

Cowpea biomass at physiological maturity increased due to N-accumulation that was affected by fertiliser application as well as cropping system. In this study, cowpea biomass in the intercrop system in consideration of individual crops were lower compared to that in the sole cropping system [32], just as was recorded in maize-legume intercropping at full maturity due to low plant densities of individual crops relative to those in sole cropping [34]. The application of fertiliser up to 50% NPK resulted in increased biomass and grain yield in both seasons similar to results reported by Mndzebele et al. [32]. On the cowpea grain yield, our results corroborate with those of the previous studies, which reported that as the application of NPK fertiliser increases, though not at recommended rates, growth and grain yield of crops increases correspondingly [35]. In another related study, cowpea showed highest yields on lower fertiliser levels than the recommended rates [36]. Although the application of N, P and K fertilisers, in combination promoted increased biomass and grain yield, their application in higher concentrations tends to reduce growth and grain yield of both sole-cropped and intercropped legumes [37]. Intercropping, as a cropping system practice, is commonly adopted for the optimization of limited agricultural productive land. Together with cowpea, some rural households intercrop it with sorghum [38, 39]. However, amaranth, though largely growing naturally in cropping fields [40] and commercially [41] could be a suitable candidate for intercropping with cowpea as shown in this study.

Amaranth biomass on the other hand was increased by an addition in fertiliser application, in sole cropping, but reduced through intercropping as a result of competition for resources. The responses of amaranth could have been caused by enhanced acquisition, utilization and storage of energy, root formation and proliferation, division, enlargement and growth of plant cells, plant height and root growth, photosynthesis, and the number and size of the leaves, all which are associated with the application of optimum levels of N, P and K fertilisers [42]. This study, therefore, demonstrated that the application of NPK could proportionately increase the growth of amaranth. Hence, the application of NPK is important in the growing of amaranth. The current study results corroborated with others who found that the addition of fertiliser on amaranth increased yield [43]. It is noteworthy that cowpea and amaranth yields in an intercropping, showed increased biomass with NPK up to 50%. This is an important finding because smallholder farmers that largely practice intercropping can hardly afford large quantities of fertilisers. Reduced fertiliser application limits the risk associated with nutrient leaching and environmental pollution. Our results agree with the general assertion that the application of NPK fertiliser increases, the growth of crops though variable depending on the type and cultivar of crops [35]. Of note is the application of 100%NPK that decreased the biomass and grain yield of cowpea in the current study. Although the application of N, P and K fertilisers, in combination promoted increased biomass and grain yield, however it has been noted that their application in higher concentrations reduced growth and grain yield, of sole cropped and intercropped legumes [37]. This study confirms the hypothesis that cowpea and amaranth biomass will increase in response to fertiliser application as demonstrated by the results.

Intercropping benefits are normally measured through the land equivalent ratio, which addresses the efficiency of agricultural productive land usage. The LERs of more than one (>1), are interpreted to mean that the land area needed for cowpea and amaranth as sole crop would be more for it to match the equivalent land area if the same crops were planted in an intercrop. Given that all the results of this study demonstrated LER values above one (LER>1) (Table 3.3), the beneficial effects of intercropping cowpea with amaranth relative to sole cropping are evident. The LER value of greater than one (>1) obtained in this study could be attributed to the efficient use of resources such as fertiliser [44]. Interactions between companion crops in an intercropping system, tends to improve the survival and growth of crops [45] through enhanced soil fertility [46, 47]. It can thus be deduced that sole cropping of either cowpea or amaranth requires additional space to match yields obtained in intercropping on equivalent land area. Irrespective of fertilisation level, there were LER values of more than one demonstrating preference for intercropping compared to sole cropping [48]. Our results are in agreement with those obtained from studies such as Mahallati et al. [49] on corn-bean intercropping. In cowpea and amaranth, the leaves, shoots, seeds are harvested for household consumption and commercial purposes. Both crops play a significant role as sources of minerals, energy, vitamins, proteins, amino acids, dietary fibre and phytochemicals especially to low-income households [50] Therefore, these crops can provide more microelements to address micronutrient (iron and zinc) deficiency prevalent in resource-poor rural households.

Nitrogen accumulation indicators include among others the ureide-N and nitrate-N. The ureide-N was markedly higher in control treatments and decreased with increasing NPK fertiliser application treatments (Table 3.2). On the other hand, nitrate-N was significantly enhanced by an increased application of fertiliser treatment with the highest at 25%NPK and 50%NPK and lowest in cowpea grown with 100%NPK fertiliser (Table 3.2). The overall results of this study indicated that, both the ureide- and nitrate-N were decreased by the application of 100% NPK. This demonstrates that the application of the highest NPK suppressed ureide-N and nitrate-N concentrations in the cowpea, regardless of cropping system, sole crop or intercropped with amaranth. Studies have shown that the application of higher levels of NPK in legumes tends to suppress the ureide-N and nitrate-N concentrations [51]. All these N-accumulation processes lead to biomass accrual by cowpea, therefore, plant growth. In general, the co-application of these nutrient elements at optimum concentrations increased the uptake and accumulation of N in crops [52]. Overall, the application of NPK at optimum concentrations result in synergistic interaction and promote not only the growth but the uptake and accumulation of N in plant tissue [53], as found in this study.

Symbiotic performance of cowpea indicated by nodulation as well as $N_2$ fixation showed some degree of sensitivity to both fertilisation [54] and intercropping. The higher nodulation (number of nodules and nodule mass) at physiological maturity in cowpea in treatments with fertiliser levels below the recommended rate (Table 3) could be attributed to the application of the 25% of NPK, which probably served as starter

N, P, and K, and was therefore crucial in stimulating nodule formation for symbiotic functioning [55]. By contrast, the study showed the least nodulation in cowpea, occurring at 100%NPK (Table 3.3), which somewhat confirms the assertion that higher mineral NPK levels tends to inhibit nodule formation in legumes [56]. Other studies have actually shown sensitivity of nodulation to fertiliser application [57]. A comparison of cropping systems showed greater nodulation in the sole cropping [58] relative to intercropping which exhibited lower amounts [59]. The reduced nodulation observed in the intercropping system (in consideration of individual crops), could have been caused by cowpea competition with amaranth, especially for belowground resources. Intercropping results in competition between crops due to proximity with the companion crop [60] thus, resulting in low nodulation as obtained in this study. Higher nodulation in fertiliser treatments below the 100%NPK are perhaps an indication that the soil only required some level of NPK for the cowpea crop to kick start symbiotic performance (Table 3.3), in which root nodules function effectively. Indications through other studies have shown that increased fertiliser amounts inhibit nodulation [61]. This study satisfies the hypothesis that nitrogen fixation in cowpea will be higher at low fertiliser amounts as shown by the results.

The results also indicated the dependence on atmospheric $N_2$ (%Ndfa) and N-fixed in cowpea at physiological maturity showed inverse relation to additional fertilisation in both seasons (Table 3.3). According to Anglade et al. [62], legumes tend to increase their %Ndfa, whilst decreasing the biomass yield and N assimilation. The inverse relationship between biomass as well as %Ndfa, and N-fixed to fertiliser application level is caused by a competition for carbon use through sink-source assimilation partitioning. The carbon costs associated with %Ndfa and N-fixed are substantial and therefore the process occurs and increases at the expense of the biomass accumulation given that $N_2$ fixation is an energetically expensive process [63]. The reduced dependence on symbiotic $N_2$ fixation by intercropped cowpea for its N nutrition could have been caused by competition for soil N by the companion crop amaranth. This study, corroborates with earlier studies by [Fan et al., 2006] which showed lower %Ndfa by the intercropped legume due to competition from the non-legume crop In the current study, the control and/or 25%NPK treatments, though not significantly different from each other, exhibited greater %Ndfa, whereas the application of 100%NPK reduced %Ndfa. With legumes, normally the %Ndfa is usually enhanced when grown under low soil-N compared to that planted with the supply of high N [64]. Also, with the application of higher NPK, the legume reduced its symbiotic performance competitive ability. The reduced N accumulation in the intercropping could have been because, the non-legume crop is more competitive for soil N, especially inorganic N and the competition forces the legume to rely only $N_2$ fixation, which does not supply as much N [65]. Other studies have reported that the addition of higher N fertilisers results in a decrease in the richness, diversity, and composition of soil bacterial communities [66]. That being the case, the decreased microbial community, some of which are responsible for solubilization of soil N, could result in their inefficacy and therefore reduced N uptake. Understanding cowpea N-accumulation dynamics under varying levels of fertilisation is therefore, crucial in optimising crop production particularly for the resource-constrained farmers. The cowpea crop tends to dependent on atmospheric $N_2$ under limited N conditions and thus induces N-accumulation in the soil. The findings of this study corroborate with others, such as Singh and Usha [67].

Our results showed that micronutrient (Fe and Zn) concentrations increased with additional fertiliser application in both cowpea and amaranth across seasons [Table 3.5]. On a crop-by-crop basis, cowpea and amaranth bio-accumulate trace mineral nutrients more on sole cropping when compared to intercropping. Fertiliser application, as well as cropping system, plays a key role in micronutrient accumulation of legumes and non-legumes [68]. Cowpea had more micronutrients (Fe and Zn), at 25%NPK fertiliser application level compared to amaranth that increased at the recommended 100%NPK rate. The behaviour of cowpea agrees with the assumption that legumes have the ability of more nutrient bioaccumulation when compared to non-legumes [69], such as observed in this study. Higher mineral element concentrations on sole cropping is in line with the theory that non-legumes and legumes show capabilities of acquiring Fe and Zn in an environment with minimal competition from companion plant such as on sole stands relative to intercrops. Similar to others such as Dakora and Belane [70], our study found that there is more Fe concentration than Zn in both cowpea and amaranth. The reasons for more iron than zinc on earth generally, is due to phytates bioavailability [71].

Micronutrients becomes valuable to the vulnerable in society, which include women and children under the age of five, [72,73], if they are able to quantify amounts of vegetables to be planted in available land to meet sufficient nutrition, using a concept known as nutritional yield (NY). Nutritional yield is affected, amongst other factors, by fertiliser application as well as intercropping. In this study, the NY-values for both seasons and for both crops increased in response to fertiliser application (Table 3.6). Generally, cowpea had more Fe-NY and Zn-NY, relative to amaranth (Table 3.6). The NY findings of the combination of the crops in an intercrop showed more Fe-NY and Zn-NY. This shows the advantage of intercropping to NY. This study provided insights into the benefits related to NY that can be derived from intercropping. The farming community particularly the resource-constrained group could be encouraged to consider more than one crop in limited land area to optimize nutrition in plant tissue, primarily trace elements associated with hidden hunger. The value of the determination of NY with intercropping of indigenous vegetables (cowpea and amaranth) on various NPK fertility rates in the soil is to provide more benefit to optimize on limited productive land and eventually minimize trace element deficiency in plant tissue. The variable NY's from cowpea and amaranth especially when combined in an intercropping are key in expanding options for the vulnerable in society.

## 3.6 Conclusions

In conclusion, cowpea, through symbiotic nitrogen fixation and N-accumulation, was able to contribute N to the soil, however, the application of fertiliser at 100% NPK reduced the amounts. The biomass of amaranth increased with additional fertilisation. Land utilization efficiency showed benefits in an intercrop, which resulted to more yield, increased accumulation of iron and zinc in plant tissues, hence increased nutritional yield. The study alludes to the importance of minimum resource utilisation to provide more zinc and iron, especially for vulnerable populations in the rural poor communities. It is recommended therefore, that smallholder farmers, intercrop while reducing fertiliser application by up to 50% of the recommended dosages.

## 3.7 Acknowledgments

We are grateful to the assistance of FD Dakora for symbiotic nitrogen fixation and Agricultural Research Council-Soil Climate and Water for the analyses of the soil and plant samples.

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# CHAPTER 4 :
# ASSESSMENT OF NPK FERTILISER ON THE EDIBLE YIELD AND AGRO-BIOLOGICAL PARAMETERS IN A COWPEA-AMARANTH INTERCROP

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## 4.1 Abstract

Estimates indicate that food and nutritional demand in Sub Saharan African nutrient poor soils will exceed the high population demand. Vegetables such as amaranth and cowpea are key in contributing to food and nutritional security. Fertilisers are used to mitigate low nutrient levels in soils. The study aimed to investigate the effect of fertiliser application and intercropping on the yield and yield parameters of cowpea-amaranth vegetables. The experiment was laid out in a 2 x 4 factorial treatment structure in a completely randomized design, with intercropping (cowpea and amaranth) and fertiliser (control, 25%, 50%, and 100% of the recommended NPK levels) as treatment factors with four replications. Biomass of amaranth and cowpea increased with addition of fertiliser application up to 100% NPK fertiliser level, which was the recommended rate. Land utilisation showed values greater than one. Crop comparison showed that cowpea was more aggressive, had high actual yield loss and highly competitive compared to amaranth. More income could be obtained in intercropping relative to sole cropping of each crop at 100% NPK. The research shows the benefit of fertiliser application in improving the biomass of amaranth and cowpea. Also, the benefits of intercropping, measured through agro-biological parameters are key in the estimation of yields, which is crucial for rural communities. Overall, the application of NPK fertilizer to amounts of up to 100%, based on the results of this study, resulted to higher biomass accumulation and improved intercropping indices.

Keywords: amaranth; cowpea; intercropping; intercropping indices; yield

## 4.2 Introduction

The Food and Agriculture Organization of the United Nations (FAO) predicts that food and nutritional demand in Sub Saharan African (SSA) rural and resource-poor farming communities will likely double by 2050 (FAO, 2009), corresponding with the escalating human population growth at a rate of 3% per annum (Asongu and Odhiambo, 2019). Yet, about 57% of the population, particularly the resource-poor farming communities, is solely dependent on agriculture for livelihoods including for food and nutrition (Gashu et al., 2019). Meeting this inevitable demand requires an increase in crop production by approximately 70% to match yields sufficient for food and nutritional demands (FAO, 2009). Meanwhile, vegetables are the most common primary sources of food and nutrition (e.g. minerals) that are affordable and readily available in rural communities (Gupta et al., 2005; Schönfeldt, 2011; Schreinemachers, 2018). Unfortunately, the increase in vegetable production yields are estimated to be significantly reduced in nutrient-poor soils (Pastori, 2019), which are often low in, for example, nitrogen (N), phosphorus (P) and potassium (K). Farmers usually mitigate low soil nutrients by using exogenous inorganic fertilisers constituting N, P and K (Marschner, 2012; Steward et al., 2020). Inorganic fertilisers are commonly applied in sole cropped as well as intercropped vegetables, some of which are sequentially harvested. If sufficient inorganic fertiliser amounts are applied together with proper management, it results in increased biomass and hence better yields (Ojeniyi, 2002).

Among the list of vegetables available for rural resource-poor farming communities are African leafy vegetables (ALVs). African leafy vegetables are crop species that originated from specific agro-ecologies and over time have established themselves in new environments through choice by those communities or evolution (van Rensburg et al., 2007). These ALVs have been part of the human diet for centuries in Sub Saharan households (Odhav et al., 2007; Vorster et al., 2008). Some of the commonly cultivated vegetables and usually consumed all over South Africa are *Amaranthus cruentus* (amaranth) and *Vigna unguiculata* L. Walp (cowpea) (Van Rensburg et al., 2007; Oelofse and van Averbeke, 2012; Mavengahama, 2013). These vegetables are commonly harvested sequentially. The scale of production and yields from ALVs is insufficient to match the food and nutritional needs of the growing population.

Therefore, it is important to address food and nutritional security sustainably in consideration of limited resources such as agricultural productive land (Jayne and Muyanga, 2012). One of the sustainable crop production mechanisms is intercropping, which is mainly, practised to optimize efficient utilisation of resources, eventually increasing yield (taking into consideration both crops). Common intercropping practices entail the inclusion of a legume in combination with a non-legume crop (Ahamefule and Peter, 2014), for example, cowpea and amaranth.

Inter-and intraspecific competition, as well as facilitation, also referred to as agro-biological parameters, are key components of intercropping (Vandermeer, 1989; Zhang and Li, 2003). Inter and intra-specific competition relate to the development of two crops in which there is a variation between them while facilitation describes the improvement in yield of companion crops in an intercropping (Fan et al., 2006; Mei et al., 2012). There are several ratios used to determine the agro-biological parameters. These entail, the land equivalent ratio (LER) (De Wit and Van den Berg, 1965), land use efficiency (LUE), relative yield (RY), actual yield loss (AYL) (Banik, 1996), relative crowding coefficient (RCC) (De Wit, 1960), aggressivity (A) (McGilchrist, 1965), competition ratio (CR) (Willey and Rao, 1980). Others include over-yielding (OY), the area-time equivalent ratio (ATER) (Tan et al., 2020), intercropping advantage (IA) (Banik et al., 2000), and monetary advantage index (MAI) (Tan et al., 2020). In an intercropping system, the yield of one crop exceeds the other, therefore, reducing it (Li et al., 2011). In an intercrop, there is commonly an improvement in yield of companion crops in a concept known as facilitation. Facilitation promotes interactions among crops necessary in the complementarity.

In facilitation, if one crop is unable to harness available nutrients the other species take over (Brooker et al., 2008). These are manifested in the determination of factors such as the intercropping advantage and monetary advantage index. Therefore, the primary objective of this study was to investigate the effect of different fertiliser application rates on the yield as well as agro-biological parameters in an amaranth-cowpea intercrop. The study hypothesized that; (i) fertiliser application would increase the yield of amaranth and cowpea in an intercropping (in consideration of both crops),

(ii) there would be variable inter and intraspecific competition as well as facilitation in amaranth and cowpea. In testing, the hypothesis (i) amaranth and cowpea yield and (ii) agro-biological parameters in an amaranth-cowpea intercrop were determined.

### 4.2.1 Experimental treatments, layout and plot management

The experimental treatments, layout and the management of the experiment were as described by Mndzebele et al. (2020). These entailed the amount of fertiliser applied, seedling preparation, transplanting, planting, inter-row spacing, intra-row spacing as well as irrigation amounts applied (Mndzebele et al., 2020).

## 4.3 Materials and Methods

### 4.3.1 Site Description and Environmental Conditions

The experiment was conducted at the Agricultural Research Council (ARC), Vegetables and Ornamental Plants campus situated in Roodeplaat, Pretoria, South Africa (25°35' S, 28°21' E, 1165 masl) during 2014/15 and 2015/16 summer seasons, which runs from November to March. The soils in which the experimental sites were carried out are as described by Mndzebele et al. (2020). The area has a long-term summer rainfall of approximately 635 mm annually. The highest precipitation is normally experienced during December and January, although it is highly variable. The two growing seasons (2014/15 and 2015/16) experienced variations in the weather conditions. On average, in the first season, maximum temperatures ranged between 23.5°C to 40.3°C. The second season maximum temperatures ranged from 20.6°C to 34.8°C. Daily minimum temperatures for the first season ranged from 7.5°C to 19.7°C, and 10.3°C and 21.2°C for the second season (Fig 4.1). The seasonal rainfall in the first and second seasons were 369 mm and 390 mm, respectively.

Table 4.1: Chemical properties of the topsoil layer (0.3 m) for the experimental sites before and after harvesting

| Chemical properties | 2014/15 season | | 2015/16 season | |
|---|---|---|---|---|
| | Before Planting | After Planting | Before Planting | After Planting |
| pH ($H_2O$) | 6.2 ± 0.4 | 6.7± 0.2 | 7.3 ± 0.4 | 7.1± 0.5 |
| P(Bray 1) (mg $kg^{-1}$) | 20.1 ± 0.6 | 19.1± 3.2 | 57.4 ± 3.2 | 56.0± 2.1 |
| K (mg $kg^{-1}$) | 218.3 ± 3.9 | 177.2± 12.2 | 158.1 ± 14.4 | 104.0±14.0 |
| Na (mg $kg^{-1}$) | 18.4 ± 0.9 | 16.2± 0.6 | 56.2 ± 1.2 | 50.0± 1.4 |
| Ca (mg $kg^{-1}$) | 635.0 ± 3.3 | 613.1± 41.4 | 857.1 ± 49.3 | 731.0± 5.3 |
| Mg (mg $kg^{-1}$) | 198.2 ± 1.3 | 190.0± 14.1 | 174.1 ± 11.1 | 170.0± 1.5 |
| $NO_3$-N (mg $kg^{-1}$) | 7.8 ± 0.6 | 6.9± 0.7 | 2.6 ± 0.3 | 1.8± 0.8 |
| $NH_4$-N (mg $kg^{-1}$) | 2.4 ± 0.2 | 2.2± 0.2 | 3.6 ± 0.4 | 3.4± 0.1 |
| Clay % | 25.0 ± 2.0 | 25.0 ± 2.0 | 32.0 ± 2.0 | 32.0 ± 2.0 |

Values (Mean ± SE) are averages of three duplicate runs.

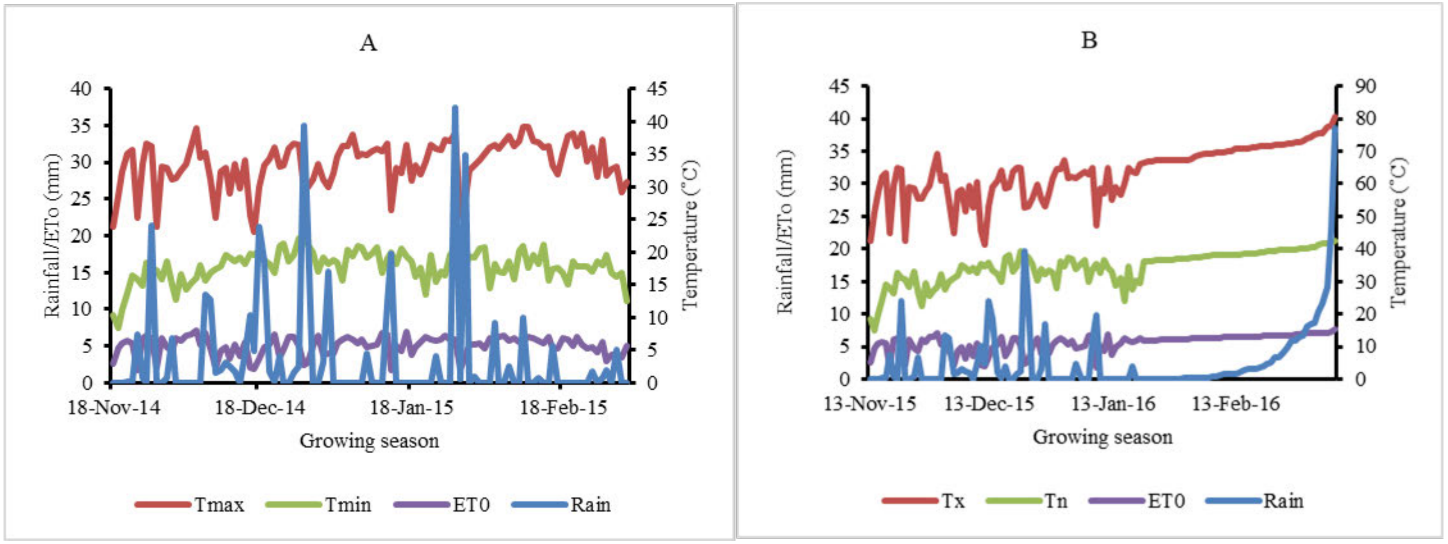


Figure 4.1: Weather data (monthly meteorological) for the 2014/15 season 1(**A**) and 2015/16 season 1(**B**) at Roodeplaat. Pretoria. South Africa. The reported values are daily climatic data during season 1 (S1) and season 2 (S2) from day of direct seeding of cowpea and transplanting of amaranth until the end of harvest. Legend: $T_{max}$: maximum temperature. (˚C); $T_{min}$: minimum temperature. (˚C); Rain: Rainfall. (mm); $ET_0$: Reference evapotranspiration. (mm)

### 4.3.2 Above-ground edible biomass (AGEB)

Vegetable crops were sequentially harvested at 8, 10, 12, 14 and 16 weeks after planting for cowpea and 4, 6, 8, 10 and 12 weeks after transplanting for amaranth during the first and second growing seasons. In the case of cowpea, only leaves were defoliated to determine above ground edible biomass (AGEB). Amaranth was cut above ground for their biomass to include leaves and stems. The stems were separated from the leaves to determine above ground edible biomass (AGEB). Twelve plants were sampled per plot on sole cropping in amaranth amounting to an area of 1.08m$^2$. In cowpea, nine plants were harvested per plot amounting to a harvested area of 1.62m$^2$ on both sole and intercropping. In amaranth intercropping, nine plants were sampled per plot. The aboveground material was weighed to determine fresh above-ground biomass and thereafter oven-dried at 50°C for 48 hours to determine dry above-ground biomass and above ground edible biomass.

### 4.3.3 Estimation of agro-biological parameters

***Land Equivalent Ratio***

Land equivalent ratio (LER) was calculated as shown in Mndzebele et al. (2020). A LER value of 1.0 indicates no difference in yield between the intercropping and the sole cropping systems. Meanwhile, any value >1.0 indicate a yield advantage while values <1 demonstrate a yield disadvantage for the intercropping system (Kurata, 1986).

***Land utilisation efficiency % (LUE)***

The land equivalent ratio was applied in this study to evaluate the utilisation efficiency of the land occupied by the crops known as land utilisation efficiency (LUE). It was defined as the total land area of sole crops required to achieve the same yields as intercrops (Willey, 1979). In an intercropping system, the partial land utilisation efficiency ratio (PLUE) of each component comprises the total land utilisation efficiency ratio (TLUE), they were calculated as:

$PLUE = Y_{inter} / Y_{sole}$

$TLUE = Y_{inter}/Y_{sole} + Y_{inter}/Y_{sole}$

$Y_{inter}$ = yield in an intercrop

$Y_{sole}$ = yield in a sole crop

***Actual Yield Loss (AYL)***

The actual yield loss (AYL) was calculated as the proportionate yield loss or gain of intercrops in comparison to the respective sole crop and calculated using the following formula as described by Banik (1996).

$AYL_{amaranth} = \{A_{intercropping}/ Z_{50\%}\}/\{(A_{sole\ cropping}/ Z_{100\%}) - 1\}$

$AYL_{cowpea} = \{C_{intercropping}/ Z_{50\%}\}/\{(C_{sole\ cropping}/Z_{100\%}) - 1\}$

$AYL_{total} = AYL_{cowpea} + AYL_{amaranth}$

$Z_{50\%}$= biomass proportion of amaranth or cowpea in intercropping

$Z_{100\%}$ = biomass proportion of amaranth or cowpea in sole cropping

$A_{sole\ cropping}$ yield of amaranth in sole cropping

$C_{sole\ cropping}$ = yield of cowpea in sole cropping

$A_{intercropping}$ = yield of amaranth in intercropping

$C_{intercropping}$ = yield of cowpea in intercropping

***Relative Crowding Coefficient (RCC)***

The total relative crowding coefficient ($RCC_{total}$) was used to estimate the relative dominance of one species over the other in intercropping by the following formula (De Wit, 1960)

$RCC_{total} = (RCC_{amaranth} \times RCC_{cowpea})$

$RCC_{amaranth} = \{A_{intercropping} \times Z_{50\%}\}/\{( A_{sole\ cropping} - A_{intercropping}) \times Z_{50\%}\}$

$RCC_{cowpea} = \{C_{intercropping} \times Z_{50\%}/\{(C_{sole\ cropping} - C_{intercropping}) \times Z_{50\%}\}$

$RCC_{amaranth}$= relative crowding coefficient of amaranth

$RCC_{cowpea}$ = relative crowding coefficient of cowpea

$Z_{50\%}$ = sown proportion of either amaranth or cowpea in intercropping

$A_{intercropping}$= yield of amaranth in intercropping

$C_{intercropping}$ = yield of cowpea in intercropping

$C_{sole\ cropping}$= yield of cowpea in sole cropping

$A_{sole\ cropping}$= yield of amaranth in sole cropping.

***Aggressivity (A)***

Aggressivity (A) was used to indicate if the relative yield increase in $A_{amaranth}$ crop is greater than that of $A_{cowpea}$ in an intercropping system and vice versa (McGilchrist, 1965) and expressed as follows;

$A_{amaranth} = \{Z_{50\%}/ (A_{sole\ cropping} \times Z_{50\%})\} - \{C_{intercropping}/ (C_{sole\ cropping} \times Z_{50\%})\}$

$A_{cowpea} = \{C_{intercropping}/ (C_{sole\ cropping} \times Z_{50\%})\} - \{Z_{50\%}/ (A_{sole\ cropping} \times Z_{50\%})\}$

$A_{sole\ cropping}$= the yield of amaranth in sole cropping

$C_{sole\ cropping}$= the yield of cowpea in sole cropping

$A_{intercropping}$ = the yield of amaranth in an intercropping

$C_{intercropping}$ = the yield of cowpea in an intercropping

$Z_{50\%}$ = sown proportion of amaranth or cowpea in intercropping

If the value of A is zero, both crops are equally competitive.

A positive or larger aggressivity means that the crop is dominant when compared to the companion crop and vice versa.

***Competition Ratio (CR)***

Competition ratio (CR) was computed through the measurement of the competitive ability of the amaranth and cowpea (Willey and Rao, 1980) and computed with the following formula CR = $(PLER_{cowpea}/PLER_{amaranth})(Z_{50\%}/Z_{50\%})$

$PLER_{amaranth}$ = partial land equivalent ratio of amaranth

$PLER_{cowpea}$ = partial land equivalent ratio of cowpea

$Z_{50\%}$= yield proportion of amaranth or cowpea in an intercropping

***Over-yielding (OY)***

Over-yielding of intercropped crops relative to sole crops was assessed by an increase or decrease in the intercropped crops over the corresponding mono-cropped crops according to Li et al. (2011), which was calculated as:

Over-yielding = $Y_{intercrop} - (P \times Y_{solecrop}) /(P \times Y_{solecrop}) \times 100\%$

Where;

$Y_{intercrop}$ and $Y_{solecrop}$ are the yields of either amaranth or cowpea in intercropping and solecropping, respectively and

P is the proportion of a given crop in the intercropping system

A positive overyielding value indicated a yield advantage and a negative value denoted a yield disadvantage.

***The area-time equivalent ratio (ATER)***

The area-time equivalent ratio (ATER) provided a more realistic comparison of the yield advantage of intercrops

$$ATER = \frac{(RYa \times Ta) + (RYb \times Tb)}{T}$$

Where

RYa = Relative yield of component A (cowpea) in intercrop

Ta and Tb = duration (in days) of components “a” and “b”

RYb = Relative yield of component “b” (amaranth) in the mixture

T = Total duration of the intercropping system in days

ATER > 1 implies yield advantage while

ATER < 1 indicates yield disadvantage

***Intercropping Advantage (IA)***

The intercropping advantage (IA) was calculated using the following formula (Banik et al., 2000):

$IA_{amaranth} = (AYL_{amaranth}) \times (P_{amaranth})$

$IA_{cowpea} = (AYL_{cowpea}) \times (P_{cowpea})$

$IA = IA_{cowpea} + IA_{amaranth}$

Where;

$IA_{amaranth}$ = is the intercropping advantage of amaranth

$IA_{cowpea}$ = is the intercropping advantage of cowpea

$AYL_{amaranth}$ = is the actual yield loss in amaranth

$AYL_{cowpea}$ = is the actual yield loss in cowpea

$P_{cowpea}$ is the average commercial value of cowpea (R5.50/kg) and $P_{amaranth}$ is the average commercial value of amaranth (R5.50/kg).

***Monetary Advantage Index (MAI)***

Monetary advantage index (MAI) was computed using the following formula:

MAI = (value of combined intercrops) (LER – 1)/LER

Where the value of combined intercrops entails the yields in amaranth in an intercrop with cowpea in an intercrop. Each was multiplied by the price. Thereafter these were added to come up with the value of the combined intercrops.

### 4.3.4 Statistical analysis

The fertiliser levels and cropping system between multiple harvested cowpea and amaranth were subjected to appropriate analysis of variance (ANOVA) to analyse AGEB and estimation of agro biological parameters. The Shapiro-Wilk's test was performed on the standardised residuals to test for deviations from normality (Shapiro and Wilk, 1965). In cases where significant deviation from normality was observed and due to skewness, outliers were removed until it was normal or symmetrically distributed (Glass et al., 1972). Least significant differences (LSDs) at 5% significance level were used to compare means of significant source effects (Snedecor and Cochran, 1967). The analyses were done using SAS (1999) (SAS version 9.3, SAS Institute Inc. Cary, NC, the United States of America) and Genstat Release 19 (Version 19, VSN International, Hemel Hempstead, UK).

## 4.4 Results

### 4.4.1 Above ground edible biomass (AGEB)

There were significant interactions ($p \leq 0.05$) between cropping systems and fertiliser levels regarding AGEB of amaranth and cowpea vegetables from the first to fifth harvests in both 2014/15 and 2015/16 seasons (Tables 4.2 to 4.6). Mean AGEB for amaranth and cowpea showed gradual increase from the first harvest until it reached the highest in the third harvests. This was followed by a gradual decrease at fourth, with the lowest at the fifth harvests, respectively (Tables 4.2 to 4.6). Mean AGEB values in both amaranth and cowpea were consistently higher in sole cropping relative to intercropping in all harvests (Figs. 4.2 and 4.3).

Table 4.2: Above ground edible biomass (AGEB), in amaranth and cowpea in the first harvest under sole and intercropping systems at four fertiliser (NPK) levels in 2014/15 (season 1) and 2015/16 (season 2) seasons.

| | | 2014/15 season | | 2015/16 season | |
|---|---|---|---|---|---|
| Cropping system | Fertiliser level | Amaranth-AGEB | Cowpea-AGEB | Amaranth-AGEB | Cowpea-AGEB |
| | | kg.ha$^{-1}$ | kg.ha$^{-1}$ | kg.ha$^{-1}$ | kg.ha$^{-1}$ |
| Sole cropping | Control | 34± 4e$^{2}$ | 142±3de$^{3}$ | 41±3cd$^{2}$ | 338±32c$^{3}$ |
| | 25%NPK | 62± 6d$^{2}$ | 207±21bc$^{23}$ | 60±8ab$^{2}$ | 449±38b$^{2}$ |
| | 50%NPK | 119±10b$^{1}$ | 241±20b$^{1}$ | 65±5a$^{12}$ | 470±16b$^{2}$ |
| | 100%NPK | 143±12 a$^{1}$ | 313±30a$^{1}$ | 69±5a$^{1}$ | 580±47a$^{1}$ |
| Intercropping | Control | 32± 4e$^{3}$ | 82±8f$^{3}$ | 29±3d$^{2}$ | 254±35d$^{3}$ |
| | 25%NPK | 59±2 d$^{2}$ | 126± 6ef$^{2}$ | 40±3cd$^{1}$ | 423±40b$^{2}$ |
| | 50%NPK | 84± 5c$^{1}$ | 147±5de$^{2}$ | 46±5bc$^{1}$ | 434±18b$^{2}$ |
| | 100%NPK | 96± 7c$^{1}$ | 187± 20cd$^{1}$ | 51±6bc$^{1}$ | 548±44a$^{1}$ |
| Cropping system | | <.001 | <.001 | <.001 | 0.015 |
| Fertiliser Level | | <.001 | <.001 | <.001 | <.001 |
| Cropping system x Fertiliser Level | | 0.011 | 0.242 | 0.805 | 0.592 |

Mean ± SE (n = 4) in each column followed by different letters indicate significant differences between treatments. Numerical values that have been superscripted compare means of each cropping system at different fertiliser levels ($p \leq 0.05$).

Table 4.3: Above ground edible biomass (AGEB), in amaranth and cowpea in the second harvest under sole and intercropping systems at four fertiliser (NPK) levels in 2014/15 (season 1) and 2015/16 (season 2) seasons.

| | | 2014/15 season | | 2015/16 season | |
|---|---|---|---|---|---|
| Cropping system | Fertiliser level | Amaranth-AGEB | Cowpea-AGEB | Amaranth-AGEB | Cowpea-AGEB |
| | | $kg.ha^{-1}$ | $kg.ha^{-1}$ | $kg.ha^{-1}$ | $kg.ha^{-1}$ |
| Sole cropping | Control | 445±32bc$^{4}$ | 637±54e$^{3}$ | 445±51bc$^{1}$ | 637±55e$^{4}$ |
| | 25%NPK | 492±33bc$^{3}$ | 888±29cd$^{2}$ | 492±25bc$^{1}$ | 888±71cd$^{3}$ |
| | 50%NPK | 572±60ab$^{2}$ | 1243±48b$^{12}$ | 572±17ab$^{1}$ | 1243±110b$^{2}$ |
| | 100%NPK | 732±42a$^{1}$ | 1724±63a$^{1}$ | 732±22a$^{1}$ | 1724±81a$^{1}$ |
| Intercropping | Control | 337±11c$^{4}$ | 533±28e$^{3}$ | 337±31c$^{1}$ | 533±43e$^{4}$ |
| | 25%NPK | 390±14bc$^{3}$ | 798±34d$^{2}$ | 390±28bc$^{1}$ | 798±78d$^{3}$ |
| | 50%NPK | 423±20bc$^{2}$ | 998±26c$^{12}$ | 423±42bc$^{1}$ | 998±94c$^{2}$ |
| | 100%NPK | 462±46bc$^{1}$ | 1294±86b$^{1}$ | 462±45bc$^{1}$ | 1294±115b$^{1}$ |
| Cropping system | | <.001 | <.001 | <.001 | <.001 |
| Fertiliser Level | | 0.012 | <.001 | <.001 | <.001 |
| Cropping system x Fertiliser Level | | 0.451 | 0.002 | 0.002 | 0.002 |

Mean ± SE (n = 4) in each column followed by different letters indicate significant differences between treatments. Numerical values that have been superscripted compare means of each cropping system at different fertiliser levels ($p \leq 0.05$).

Table 4.4: Above ground edible biomass (AGEB), in amaranth and cowpea in the third harvest under sole and intercropping systems at four fertiliser (NPK) levels in 2014/15 (season 1) and 2015/16 (season 2) seasons.

| | | 2014/15 season | | 2015/16 season | |
|---|---|---|---|---|---|
| Cropping system | Fertiliser level | Amaranth-AGEB | Cowpea-AGEB | Amaranth-AGEB | Cowpea-AGEB |
| | | kg.ha$^{-1}$ | kg.ha$^{-1}$ | kg.ha$^{-1}$ | kg.ha$^{-1}$ |
| Sole cropping | Control | 388d±35e$^{3}$ | 159±5d$^{3}$ | 539±54abc$^{1}$ | 915±77fg$^{3}$ |
| | 25%NPK | 645±69c$^{2}$ | 194±14d$^{2}$ | 707±76ab$^{1}$ | 1355±110cd$^{2}$ |
| | 50%NPK | 827±82b$^{2}$ | 271±20c$^{1}$ | 733±55ab$^{1}$ | 1397±86c$^{2}$ |
| | 100%NPK | 1415±101a$^{1}$ | 419±46a$^{1}$ | 781±61a$^{1}$ | 2165±204a$^{1}$ |
| Intercropping | Control | 148±14f$^{3}$ | 151±16d$^{2}$ | 351±27c$^{1}$ | 737±61g$^{3}$ |
| | 25%NPK | 198±12f$^{2}$ | 176±16d$^{2}$ | 405±41c$^{1}$ | 1099±104ef$^{2}$ |
| | 50%NPK | 282±17ef$^{12}$ | 249±11c$^{1}$ | 428±42c$^{1}$ | 1172±64de$^{2}$ |
| | 100%NPK | 532±51cd$^{1}$ | 327±37b$^{1}$ | 473±38bc$^{1}$ | 1745±107b$^{1}$ |
| Cropping system | | <.001 | 0.003 | <.001 | <.001 |
| Fertiliser Level | | <.001 | <.001 | 0.188 | <.001 |
| Cropping system x Fertiliser Level | | <.001 | 0.045 | 0.861 | 0.341 |

Mean ± SE (n = 4) in each column followed by different letters indicate significant differences between treatments. Numerical values that have been superscripted compare means of each cropping system at different fertiliser levels ($p \leq 0.05$).

Table 4.5: Above ground edible biomass (AGEB), in amaranth and cowpea in the fourth harvest under sole and intercropping systems at four fertiliser (NPK) levels in 2014/15 (season 1) and 2015/16 (season 2) seasons.

| | | 2014/15 season | | 2015/16 season | |
|---|---|---|---|---|---|
| Cropping system | Fertiliser level | Amaranth-AGEB | Cowpea-AGEB | Amaranth-AGEB | Cowpea-AGEB |
| | | kg.ha$^{-1}$ | kg.ha$^{-1}$ | kg.ha$^{-1}$ | kg.ha$^{-1}$ |
| Sole cropping | Control | 319±25b$^{2}$ | 366±36d$^{4}$ | 473±46bc$^{1}$ | 283±29f$^{3}$ |
| | 25%NPK | 362±37b$^{1}$ | 448±31c$^{3}$ | 620±59ab$^{1}$ | 600±69d$^{2}$ |
| | 50%NPK | 421±40b$^{1}$ | 551±33b$^{2}$ | 683±66a$^{1}$ | 796±80c$^{2}$ |
| | 100%NPK | 611±59a$^{1}$ | 675±47a$^{1}$ | 738±85a$^{1}$ | 1130±115a$^{1}$ |
| Intercropping | Control | 78±5c$^{4}$ | 243±12e$^{3}$ | 251±22d$^{1}$ | 227±20f$^{3}$ |
| | 25%NPK | 101±2c$^{3}$ | 308±11de$^{2}$ | 278±26d$^{1}$ | 435±36e$^{2}$ |
| | 50%NPK | 124±9c$^{2}$ | 370±38d$^{12}$ | 286±24d$^{1}$ | 621±61d$^{2}$ |
| | 100%NPK | 152±14c$^{1}$ | 478±50c$^{1}$ | 335±23cd$^{1}$ | 976±85b$^{1}$ |
| Cropping system | | <.001 | <.001 | <.001 | 0.001 |
| Fertiliser Level | | <.001 | <.001 | 0.024 | <.001 |
| Cropping system x Fertiliser Level | | 0.030 | 0.374 | 0.315 | 0.632 |

Mean ± SE (n = 4) in each column followed by different letters indicate significant differences between treatments. Numerical values that have been superscripted compare means of each cropping system at different fertiliser levels ($p \leq 0.05$).

Table 4.6 : Above ground edible biomass (AGEB), in amaranth and cowpea in the fifth harvest under sole and intercropping systems at four fertiliser (NPK) levels in 2014/15 (season 1) and 2015/16 (season 2) seasons.

| | | 2014/15 season | | 2015/16 season | |
|---|---|---|---|---|---|
| Cropping system | Fertiliser level | Amaranth-AGEB | Cowpea-AGEB | Amaranth-AGEB | Cowpea-AGEB |
| | | kg.ha$^{-1}$ | kg.ha$^{-1}$ | kg.ha$^{-1}$ | kg.ha$^{-1}$ |
| Sole cropping | Control | 234±13c$^{3}$ | 121±11cde$^{4}$ | 330±33ab$^{1}$ | 274±24b$^{2}$ |
| | 25%NPK | 272±9bc$^{2}$ | 188±19c$^{3}$ | 350±40ab$^{1}$ | 309±28b$^{1}$ |
| | 50%NPK | 316±1b$^{12}$ | 280±26b$^{2}$ | 399±39a$^{1}$ | 321±29b$^{1}$ |
| | 100%NPK | 419±74a$^{1}$ | 407±41a$^{1}$ | 416±41a$^{1}$ | 458±44a$^{1}$ |
| Intercropping | Control | 96±6e$^{3}$ | 51±11e$^{3}$ | 245±26b$^{1}$ | 181±13c$^{2}$ |
| | 25%NPK | 131±6de$^{2}$ | 96±8de$^{2}$ | 257±24b$^{1}$ | 260±26bc$^{12}$ |
| | 50%NPK | 136±9de$^{2}$ | 151±14cd$^{12}$ | 265±25b$^{1}$ | 287±29b$^{1}$ |
| | 100%NPK | 162±9d$^{1}$ | 287±22b$^{1}$ | 281±28b$^{1}$ | 339±35b$^{1}$ |
| Cropping system | | <.001 | <.001 | <.001 | 0.001 |
| Fertiliser Level | | <.001 | <.001 | 0.249 | <.001 |
| Cropping system x Fertiliser Level | | 0.004 | 0.674 | 0.796 | 0.412 |

Mean ± SE (n = 4) in each column followed by different letters indicate significant differences between treatments. Numerical values that have been superscripted compare means of each cropping system at different fertiliser levels ($p \leq 0.05$).

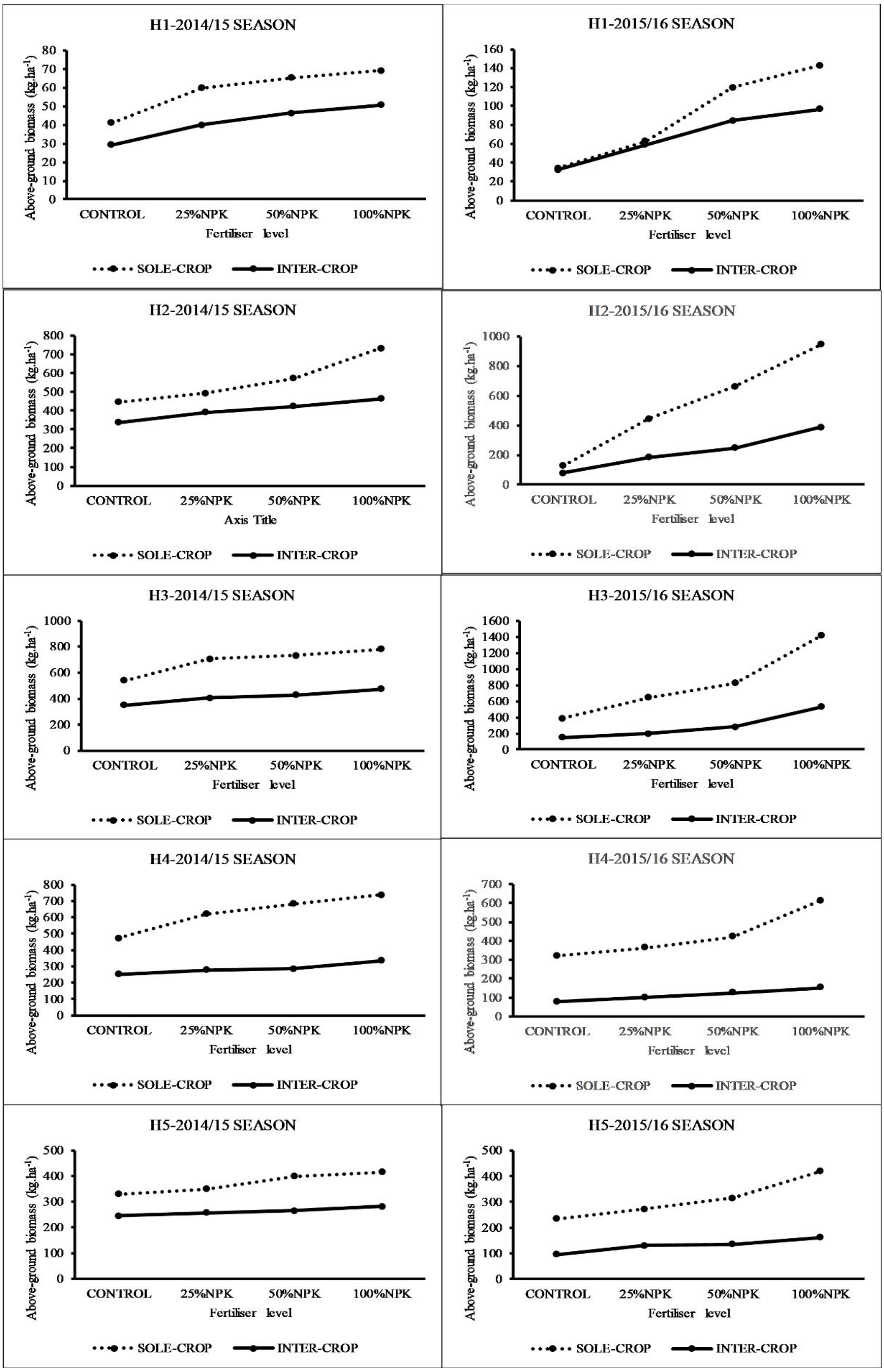


Figure 4.2: Comparison of sole and intercropping systems above ground edible biomass (AGEB), in amaranth from the first-fifth harvest grown under four different fertiliser (NPK) levels in 2014/15 (season 1) and 2015/16 (season 2) seasons. H1, 2,3,4,5 represents harvests 1, 2,3,4,5, respectively.

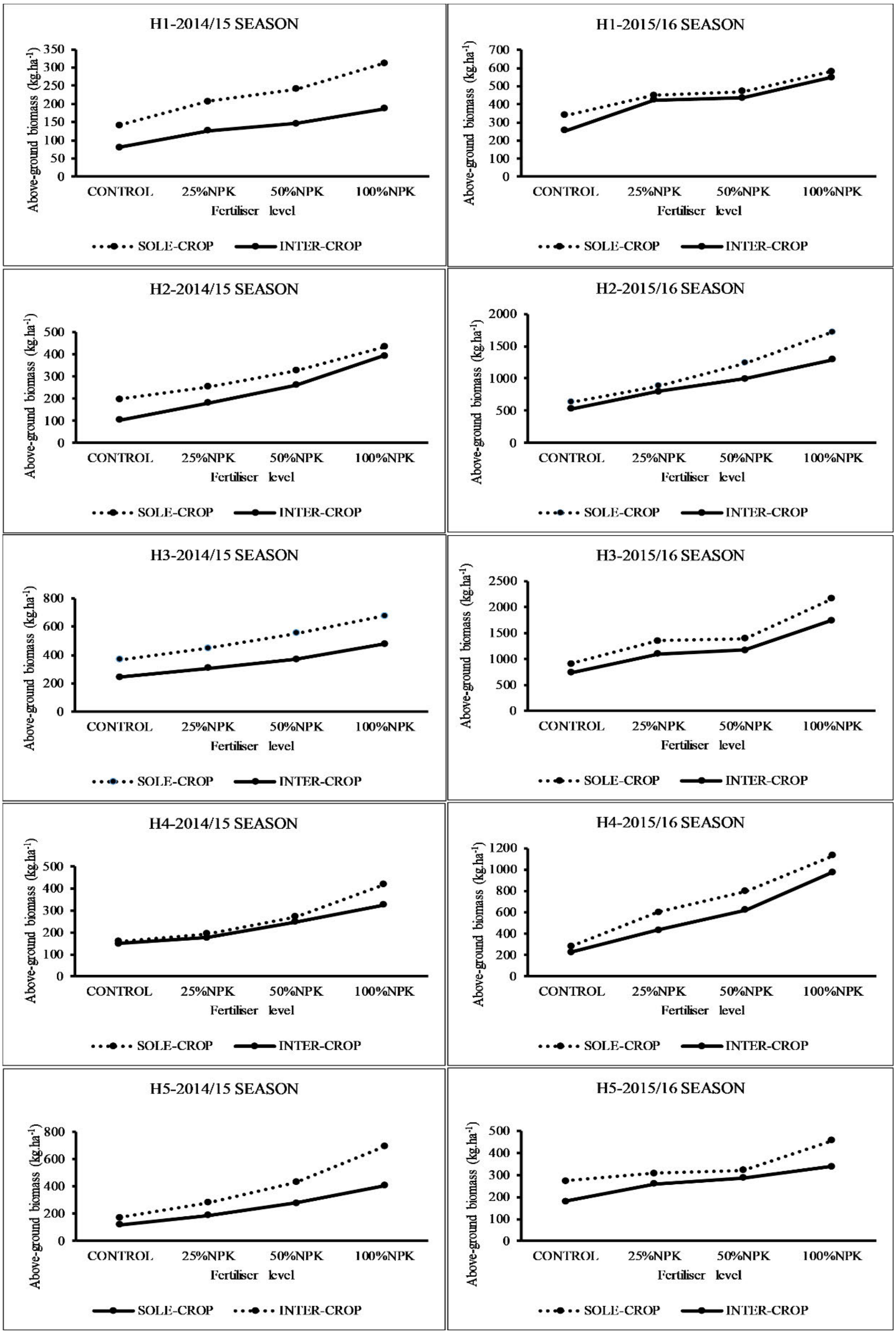


Figure 4.3: Comparison of sole and intercropping systems aboveground edible biomass (AGEB), on cowpea from the first-fifth harvest grown under four different fertiliser (NPK) levels in 2014/15 (season 1) and 2015/16 (season 2) seasons. H1, 2,3,4,5 represents harvests 1, 2,3,4,5, respectively.

In both 2014/15 and 2015/16 seasons, the highest AGEB were obtained at 100% NPK in combination with sole cropping on the third harvest on both crops. The lowest AGEB was obtained from the control (0% NPK) in combination with the intercropping system in the 2014/15 and 2015/16 seasons. The lowest AGEB in both crops was obtained in the first and fifth harvests (Tables 4.2 to 4.6). There was a significant increase in AGEB of amaranth and cowpea from 0% NPK to 100% NPK in all harvests for both cropping systems and seasons (Tables 4.2 to 4.6).

### 4.4.2 Land Equivalent Ratio (LER), and Land Utilisation Efficiency % (LUE)

The study showed mean LER values of greater than one (LER>1), except for the control treatments in the fourth and fifth harvests in the first season (2014/15) respectively for the rest of the fertiliser levels (Table 4.7). The LER ratios ranged from 0.8 to 1.6 for the first season and 1.2 to 1.7 in the second season. The LER mean values in the first and second harvests were higher from the control and 25% NPK treatments (1.6) and lower at 50% NPK (1.3) in the first season. In the third harvest, LER showed statistically similar values of 1.3 from the control until 50% NPK, which was followed by 100% NPK that was lowest, in the first season. In the fourth and fifth harvests, the LER mean values at 0.9 and 0.8 increased from control to 1.0 at 25% NPK which was also equal to 50% NPK and 100% NPK, respectively, in the first season (Table 4.7). The LER mean values in the second season, showed a gradual increase from control until 25% NPK, which was highest. This was followed by a drop at 50%NPK and 100%NPK which were lowest in the first, second and third harvests. The fourth harvest showed increased values at the control and 100% NPK. However, the fifth harvest increased at 25% NPK and 50% NPK, with the lowest at the control at 1.4 (Table 4.7). The land utilisation efficiency % (LUE), mean values expressed in percentages showed trends, similar to the LER in all the harvests in both seasons (Table 4.7).

Table 4.7: Land Equivalent Ratio (LER) and Land Use Efficiency (LUE) of the five harvests fertilised with four different NPK levels in 2014/15 (season 1) and 2015/16 (season) seasons

| HARVESTS | Fertiliser Level | 2014/15 season | 2015/16 season | 2014/15 season | 2015/16 season |
|---|---|---|---|---|---|
| | | LER | LER | LUE (%) | LUE (%) |
| Harvest 1 | Control | 1.6±0.1 | 1.6±0.06 | 159±13 | 155±8 |
| | 25%NPK | 1.6±0.1 | 1.7±0.07 | 159±13 | 170±3 |
| | 50%NPK | 1.3±0.1 | 1.4±0.06 | 134±13 | 143±5 |
| | 100%NPK | 1.4±0.1 | 1.4±0.05 | 133±13 | 138±6 |
| Harvest 2 | Control | 1.6±0.1 | 1.6±0.02 | 159±12 | 155±8 |
| | 25%NPK | 1.6±0.1 | 1.7±0.03 | 159±13 | 170±3 |
| | 50%NPK | 1.3±0.1 | 1.4±0.02 | 134±14 | 143±5 |
| | 100%NPK | 1.4±0.1 | 1.4±0.02 | 133±18 | 138±6 |
| Harvest 3 | Control | 1.3±0.07 | 1.6±0.13 | 134±13 | 155±8 |
| | 25%NPK | 1.3±0.07 | 1.7±0.14 | 122±9 | 170±3 |
| | 50%NPK | 1.3±0.07 | 1.4±0.14 | 127±6 | 143±5 |
| | 100%NPK | 1.2±0.07 | 1.4±0.14 | 116±12 | 138±6 |
| Harvest 4 | Control | 0.9±0.04 | 1.4±0.06 | 92±6 | 136±10 |
| | 25%NPK | 1.0±0.05 | 1.2±0.05 | 97±7 | 119±10 |
| | 50%NPK | 1.0±0.05 | 1.2±0.05 | 97±9 | 121±5 |
| | 100%NPK | 1.0±0.05 | 1.4±0.06 | 99±9 | 135±11 |
| Harvest 5 | Control | 0.8±0.02 | 1.4±0.1 | 83±5 | 142±14 |
| | 25%NPK | 1.0±0.03 | 1.6±0.1 | 99±6 | 158±9 |
| | 50%NPK | 1.0±0.03 | 1.6±0.1 | 97±4 | 164±20 |
| | 100%NPK | 1.0±0.03 | 1.5±0.1 | 92±2 | 147±2 |

Mean ± SE (n = 4) in each column indicate significant differences between different fertiliser levels ($p \leq 0.05$).

### 4.4.3 Actual Yield Loss (AYL), Relative Crowding Coefficient (RCC), Aggressivity (A) and Competition Ratio (CR)

The AYL mean values for amaranth in the four different fertiliser levels were positive for the first and second harvests (ranging from 0.41 to 1.01), in the first season indicating an increase in yield by 41% to 101% when intercropped with cowpea. There was a characteristic decrease in AYL mean values from control (1.01), until 100% NPK (0.41) in the first season. On the other hand, the second season showed a gradual decrease of AYL from 0.68 in the control to 100% NPK at 0.26, on the first to third harvests. The third, fourth and fifth harvests in the first season showed AYL mean values to be highest at the control (-0.21), 50% NPK (-0.41), and 25% NPK (-0.04), translating to yield decreases by 21%, 41% and 4%. The lowest were at control (-0.37), 50% NPK (-0.49) and 100% NPK (-0.20) (Table 4.8).

Actual Yield Loss values of cowpea in the first season showed characteristic increases from control until 100% NPK in the first (0.17 to 0.25), second (0.17 to 0.25) and fifth (-0.16 to 0.20) harvests. This translated to 17% to 25% increases in the first and second season. This was followed by a loss of 16%, which increased to 20% in the 100% NPK at the fifth harvest. The third harvest showed decreases from the control (0.89) to 100%NPK (0.45), which was lowest, indicating yield increases of 89% and 45%, respectively. The fourth harvest showed the lowest values at the control (0.34) and the highest at the 100% NPK (0.41). In the second season, there were characteristic increases from control at 0.66 to 0.80 on the first, second and third with an exception in the fifth increasing from control until 50% NPK. The lowest AYL was obtained at the 100% NPK fertiliser level, in the first to third harvests. The fourth and fifth harvests showed lowest values at 25% NPK (0.47) and control (0.33) (Table 4.8).

In the first season on the first to the second harvests, the total AYL increased from the control to 25% NPK where it was highest, thereafter it dropped until 100%NPK fertiliser level, which was lowest. The third harvest had the highest AYL mean values at the control and lowest at the 100% NPK. The fourth harvest was highest at the 100% NPK fertiliser level, and lowest at the control. The fifth harvest showed high AYL mean values at the 25% NPK and lowest at the control. In the second season, the total AYL increased from control until 25% NPK and thereafter decreased until 100% NPK which was lowest in the first, second, third and fifth harvests. The control and the 100% NPK showed high AYL mean values, with the lowest at the 25% NPK (Table 4.8).

The highest mean RCC values of amaranth showed increasing trends from the control to 100% NPK for the first and second harvests in the first season. The fifth harvests increased until 25% NPK and then dropped with the lowest at the 100% NPK. The fourth harvest showed highest mean values at 100% NPK with 25% NPK being the lowest in the first season. In the second season, amaranth gradually increased from control to 25% NPK after which there was a drop at 50%NPK. However, there was another increase at 100% NPK on the first to third harvests. The fifth harvest showed RCC mean values that increased from the control to the 50% NPK, with 100% NPK being the lowest in the second season. The fourth harvest was highest at the control with the lowest at the 50%NPK in the second season (Table 4.8).

With the RCC of cowpea in the first season, there were higher mean values on the 100% NPK in the first, second, fourth and fifth harvests, with 1.96, 1.96, 2.78 and 1.22. The lowest mean values were obtained in the control treatments for all harvests except for the fourth harvest in the first season. The fourth harvest showed high RCC mean values of cowpea at the control treatment and lowest at 100% NPK in the first season. In the second season, there was a gradual increase from the control to the 25% NPK fertiliser level, which was highest in the first to third harvests. The lowest RCC of cowpea were obtained at 100% NPK. The total RCC showed increasing trends from control to 100% NPK in the first, second and fifth harvests in the first season. The third and fourth harvests showed higher mean values in the control and 50% NPK treatments in the first season. The second season had higher total RCC mean values on 100%NPK, 25% NPK, 25% NPK, control and 50% NPK for the first to fifth harvests.

Table 4.8: Actual Yield Loss of amaranth (AYL-amaranth), Actual Yield Loss of cowpea (AYL-cowpea), Relative Crowding Coefficient of amaranth (RCC-amaranth), and Relative Crowding Coefficient of cowpea (RCC-cowpea) for harvests one to five grown under four NPK fertilisation levels in 2014/15 (season 1=S1) and 2015/16 (season 2=S2) seasons.

| HARVESTS | Fertiliser Level | 2014/15 season | | 2015/16 season | | 2014/15 season | 2015/16 season | 2014/15 season | | 2015/16 season | | 2014/15 season | 2015/16 season |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | AYL- | | | | | | RCC | | | | | |
| | | Amaranth | Cowpea | Amaranth | Cowpea | Total | Total | Amaranth | Cowpea | Amaranth | Cowpea | Total | Total |
| Harvest 1 | Control | 1.01±0.2 | 0.17±0.1 | 0.68±0.07 | 0.66± 0.08 | 1.18±0.25 | 1.10± 0.17 | -0.77±8.6 | 1.48±0.36 | 1.86±0.38 | 5.80±0.46 | -0.19±16.0 | -3.77±43.5 |
| | 25%NPK | 0.95±0.2 | 0.23±0.1 | 0.61±0.09 | 0.80± 0.04 | 1.19±0.25 | 1.40± 0.06 | 0.64±8.6 | 1.73±0.38 | 4.30±0.24 | 9.07±1.56 | -11.1±16.0 | 37.72±6.37 |
| | 50%NPK | 0.45±0.2 | 0.23±0.1 | 0.58±0.01 | 0.61± 0.06 | 0.67±0.25 | 0.85± 0.09 | 3.52±8.6 | 1.68±0.14 | 4.79±0.40 | 6.09±0.16 | 7.19± 1.6 | -9.28±34.34 |
| | 100%NPK | 0.41±0.2 | 0.25±0.1 | 0.26±0.09 | 0.50± 0.01 | 0.66±0.25 | 0.76± 0.02 | 8.31±8.6 | 1.96±0.06 | 1.72±0.35 | 3.14±0.31 | 17.68±1.0 | 5.34±1.59 |
| Harvest 2 | Control | 1.01±0.02 | 0.17±0.01 | 0.68±0.07 | 0.66± 0.18 | 1.18±0.04 | 1.10±0.07 | -0.77±0.48 | 1.48±0.13 | 1.86±0.38 | 5.80±1.16 | -0.19±7.19 | -3.77±43.5 |
| | 25%NPK | 0.95±0.03 | 0.23±0.01 | 0.61±0.09 | 0.80±0.04 | 1.19±0.03 | 1.40±0.06 | -12.96±8.00 | 1.73±0.18 | 4.30±0.24 | 9.07±1.16 | 14.58±28.47 | 37.72±6.37 |
| | 50%NPK | 0.45± 0.02 | 0.23±0.01 | 0.58±0.01 | 0.61±0.16 | 0.67±0.03 | 0.85±0.09 | 3.52±0.07 | 1.68±0.14 | 4.79±0.40 | 6.09±1.16 | 7.19±8.49 | -9.28±34.34 |
| | 100%NPK | 0.41± 0.03 | 0.25±0.02 | 0.26±0.09 | 0.50± 0.10 | 0.66±0.05 | 0.76±0.02 | 8.31±0.80 | 1.96±0.16 | 1.72±0.35 | 3.14±0.87 | 17.68±28.21 | 5.34±1.59 |
| Harvest 3 | Control | -0.21±0.12 | 0.89±0.08 | 0.68± 0.07 | 0.66±0.08 | 0.68±0.05 | 1.10±0.17 | 0.53±0.07 | 24.39±3.41 | 1.86±0.38 | 5.8±0.46 | 17.95±13.78 | -3.77±43.50 |
| | 25%NPK | -0.37±0.13 | 0.81±0.07 | 0.61±0.06 | 0.80±0.04 | 0.44±0.08 | 1.40±0.06 | 0.47±0.07 | 11.67±1.91. | 4.30±0.24 | 9.07±0.56 | 5.99±5.20 | 37.72±6.37 |
| | 50%NPK | -0.3±0.03 | 0.84±0.09 | 0.58±0.06 | 0.61±0.12 | 0.53±0.02 | 0.85±0.09 | 0.55±0.06 | 14.08±1.73 | 4.79±0.40 | 6.09±0.16 | 7.09±2.58 | -9.28±34.34 |
| | 100%NPK | -0.25±0.10 | 0.45±0.05 | 0.26±0.05 | 0.50±0.10 | 0.31±0.05 | 0.76±0.12 | 0.61±0.05 | 6.84±0.31 | 1.72±0.35 | 3.14±0.8 | 4.79±6.43 | 5.34±1.59 |
| Harvest 4 | Control | -0.49±0.08 | 0.34±0.01 | 0.11± 0.10 | 0.61± 0.08 | -0.15±0.15 | 0.72±0.14 | 0.35±0.06 | 2.24±0.6 | 1.29±0.14 | 4.34±1.19 | 0.81±0.28 | 5.75±2.83 |
| | 25%NPK | -0.43±0.07 | 0.38±0.13 | -0.08±0.11 | 0.47± 0.07 | -0.06±0.13 | 0.39±0.11 | 0.40±0.07 | 2.34±0.6 | 0.86±0.14 | 3.16±1.31 | 0.96±0.27 | 2.69±1.46 |
| | 50%NPK | -0.41±0.07 | 0.35±0.03 | -0.14±0.12 | 0.56±0.07 | -0.05±0.11 | 0.42± 0.12 | 0.42±0.07 | 2.43±0.7 | 0.77±0.10 | 4.15±1.00 | 0.98±0.24 | 3.30±1.90 |
| | 100%NPK | -0.44±0.06 | 0.41±0.08 | -0.02±0.13 | 0.73±0.09 | -0.02±0.12 | 0.71±0.13 | 0.43±0.07 | 2.78±0.6 | 1.14±0.14 | 7.09±1.58 | 0.66±0.21 | 7.10±4.01 |
| Harvest 5 | Control | -0.18±0.06 | -0.16±0.09 | 0.50±0.12 | 0.33±0.13 | -0.34±0.09 | 0.83±0.20 | 0.71±0.09 | 0.76±0.17 | 18.14±2.80 | 3.95±0.90 | 0.50±0.22 | 18.14±28.49 |
| | 25%NPK | -0.04±0.05 | 0.02±0.09 | 0.47±0.16 | 0.69±0.12 | -0.01±0.11 | 1.15±0.17 | 0.93± 0.08 | 1.06±0.20 | 20.27±2.40 | 7.05±0.73 | 0.99±0.19 | 20.27±15.24 |
| | 50%NPK | -0.14±0.07 | 0.08±0.09 | 0.49±0.15 | 0.79±0.04 | -0.07±0.08 | 1.27±0.24 | 0.76±0.08 | 1.18±0.22 | 63.86±6.23 | 8.65±1.94 | 0.88±0.16 | 63.86±66.23 |
| | 100%NPK | -0.20±0.05 | 0.20±0.11 | 0.44±0.14 | 0.51±0.13 | 0.24±0.12 | 0.95±0.24 | 0.71±0.09 | 1.22±0.17 | 16.97±4.5 | 5.15±0.75 | 1.18±0.22 | 16.97±16.45 |

Mean ± SE (n = 4) in each column indicate significant differences between different fertiliser levels ($p \leq 0.05$).

Crop comparison showed equal aggressivity between amaranth and cowpea. The aggressivity of amaranth and cowpea, increased with fertilisation from control to 100% NPK in all harvests, except third harvest in the aggressivity of cowpea (Table 4.9). The second season showed more aggressivity in the first to third harvests at 25% NPK, with the lowest at 100% NPK (Table 9). The fourth harvest as well and fifth harvests showed high aggressivity mean values at the control and 50% NPK (Table 4.9).

The CR of amaranth and cowpea showed comparably similar values due to the equal plant population on intercropping plots. There was an increased competitiveness as shown on the third (2.3 to 3.3) and fourth (2.3 to 2.7) harvests in both seasons. There was an increasing trend of CR of amaranth and cowpea from control to 100% NPK on the first, second and fifth harvests. The third and fourth harvests were highest at 25% NPK and control (Table 4.9).

Table 4.9: Aggressivity of amaranth (Aggressivity-amaranth), aggressivity of cowpea (aggressivity-cowpea), competition ratio of amaranth (CR-amaranth) and competition ratio of cowpea (CR-cowpea) of harvests one to five fertilised with four NPK levels in 2014/15 (season 1=S1) and 2015/16 (season 2=S2) seasons.

| HARVESTS | Fertiliser Level | 2014/15 season | | 2015/16 season | | 2014/15 season | | 2015/16 season | |
|---|---|---|---|---|---|---|---|---|---|
| | | Aggressivity | | | | CR | | | |
| | | amaranth | cowpea | amaranth | cowpea | amaranth | cowpea | amaranth | cowpea |
| Harvest 1 | Control | -1,84±0.5 | 0,28±0,02 | 0,37±0,002 | 0,38±0,002 | 0.63±0.13 | 0.63±0.13 | 1.00±0.10 | 1,1±0,10 |
| | 25%NPK | 0,02±0,02 | 0,30±0,04 | 0,47±0,005 | 0,47±0,005 | 0.66±0.11 | 0.66±0.11 | 1.12±0.10 | 1,41±0,02 |
| | 50%NPK | 0,03±0,00 | 0,30±0,03 | 0,46±0,005 | 0,46±0,005 | 0.85±0.08 | 0.85±0.08 | 0.90±0.11 | 1,47±0,11 |
| | 100%NPK | 0,06±0,02 | 0,31±0,02 | 0,47±0,003 | 0,47±0,003 | 0.98±0.10 | 0.98±0.10 | 1.20±0.10 | 1,34±0,12 |
| Harvest 2 | Control | -0,31±0,59 | 0,29±0,03 | 0,41±0,04 | 0,41±0,04 | 0.63±0.13 | 0.63±0.13 | 1.00±0.11 | 1,05±0,10 |
| | 25%NPK | 0,06±0,03 | 0,36±0,04 | 0,45±0,01 | 0,45±0,01 | 0.66±0.11 | 0.66±0.11 | 1.12±0.10 | 1,12±0,07 |
| | 50%NPK | 0,11±0,04 | 0,40±0,03 | 0,40±0,06 | 0,40±0,06 | 0.85±0.08 | 0.85±0.08 | 0.90±0.14 | 1,15±0,17 |
| | 100%NPK | 0,08±0,02 | 0,46±0,05 | 0,37±0,02 | 0,37±0,02 | 0.98±0.10 | 0.98±0.10 | 1.20±0.10 | 1,2±0,11 |
| Harvest 3 | Control | -0,03±0,05 | 0,47±0,02 | 0,40±0,02 | 0,40±0,02 | 2.53±0.28 | 2.53±0.28 | 1.00±0.31 | 1,41±0,34 |
| | 25%NPK | 0,14±0,04 | 0,45±0,01 | 0,41±0,02 | 0,41±0,02 | 3.34±0.26 | 3.34±0.26 | 1.12±0.30 | 1,42±0,35 |
| | 50%NPK | 0,14±0,03 | 0,46±0,02 | 0,42±0,01 | 0,42±0,01 | 2.81±0.25 | 2.81±0.25 | 0.90±0.24 | 1,64±0,37 |
| | 100%NPK | 0,11±0,03 | 0,39±0,01 | 0,41±0,02 | 0,41±0,02 | 2.32±0.23 | 2.32±0.23 | 1.20±0.13 | 1,39±0,27 |
| Harvest 4 | Control | -0,07±0,03 | 0,33±0,02 | 0,40±0,02 | 0,40±0,02 | 2.74±0.31 | 2.74±0.31 | 1.46±0.11 | 1,48±0,14 |
| | 25%NPK | 0,18±0,02 | 0,34±0,03 | 0,37±0,02 | 0,37±0,02 | 2.46±0.12 | 2.46±0.12 | 1.63±0.15 | 1,62±0,13 |
| | 50%NPK | 0,22±0,06 | 0,34±0,02 | 0,39±0,04 | 0,39±0,04 | 2.32±0.10 | 2.32±0.10 | 1.83±0.14 | 1,83±0,11 |
| | 100%NPK | 0,30±0,05 | 0,35±0,03 | 0,43±0,02 | 0,43±0,02 | 2.52±0.21 | 2.52±0.21 | 1.95±0.14 | 1,98±0,14 |
| Harvest 5 | Control | -0,26±0,03 | 0,21±0,02 | 0,33±0,03 | 0,33±0,03 | 1.06±0.13 | 1.06±0.13 | 0.89±0.11 | 0,89±0,15 |
| | 25%NPK | -0,01±0,01 | 0,25±0,02 | 0,42±0,03 | 0,42±0,03 | 1.06±0.13 | 1.06±0.13 | 1.17±0.14 | 1,17±0,16 |
| | 50%NPK | 0,02±0,02 | 0,27±0,02 | 0,45±0,01 | 0,45±0,01 | 1.26±0.11 | 1.26±0.11 | 1.30±0.15 | 1,3±0,13 |
| | 100%NPK | 0,09±0,03 | 0,36±0,02 | 0,38±0,03 | 0,38±0,03 | 1.63±0.12 | 1.63±0.12 | 1.07±0.12 | 1,07±0,12 |

Mean ± SE (n = 4) in each column indicate significant differences between different fertiliser levels ($p \leq 0.05$).

### 4.4.4 Over-Yielding (OY) and the Area-Time Equivalent Ratio (ATER)

Crop comparison showed more over-yielding on cowpea when compared to amaranth. The over-yielding ratio of amaranth in the first and second seasons increased from the control until 100% NPK in which it was highest. Similarly, OY of cowpea in both seasons revealed characteristic increases from control until 100% NPK fertiliser level, which was highest (Table 4.10).

The area-time equivalent ratios mean values increased from control until 25%NPK fertiliser level, with the lowest at 100% NPK for the first and second harvests (Table 10). The fourth and fifth harvests increased with fertiliser levels from control to 100% NPK that was highest. The third harvest showed similar patterns with the first and second harvests, in the second season. However, the first season in the third harvest showed highest values in the control with the lowest at the 100% NPK (Table 4.10).

Table 4.10: Overyield of amaranth (OY-amaranth), overyield of cowpea (OY-cowpea) and Area Time Equivalent Ratio (ATER) of harvests one to five fertilised with four NPK levels in 2014/15 (season 1=S1) and 2015/16 (season 2=S2) seasons.

| HARVESTS | Fertiliser Level | 2014/15 season | | 2015/16 season | | 2014/15 season | 2015/16 season |
|---|---|---|---|---|---|---|---|
| | | OY | | | | ATER | |
| | | amaranth | cowpea | amaranth | cowpea | | |
| Harvest 1 | Control | -68±4 | -19±7 | -71±7 | 154±16 | 1.72±0.13 | 1.73±0.07 |
| | 25%NPK | -41±2 | 26±6 | -60±5 | 323±24 | 1.73±0.11 | 1.90±0.03 |
| | 50%NPK | -16±2 | 47±5 | -54±20 | 334±16 | 1.47±0.10 | 1.59±0.02 |
| | 100%NPK | -4±3 | 87±7 | -49±17 | 448±38 | 1.47±0.13 | 1.54±0.07 |
| Harvest 2 | Control | -21±11 | 4±0.01 | 251±19 | 433±48 | 1.72±0.12 | 1.73±0.02 |
| | 25%NPK | 83±7 | 82±8 | 305±25 | 698±68 | 1.73±0.11 | 1.90±0.03 |
| | 50%NPK | 148±12 | 161±16 | 328±27 | 898±65 | 1.47±0.11 | 1.59±0.02 |
| | 100%NPK | 432±51 | 295±36 | 373±25 | 1194±99 | 1.47±0.10 | 1.54±0.03 |
| Harvest 3 | Control | 48±4 | 51±5 | 237±20 | 637±53 | 1.55±0.03 | 1.73±0.07 |
| | 25%NPK | 98±10 | 76±7 | 305±25 | 999±56 | 1.42±0.02 | 1.90±0.03 |
| | 50%NPK | 182±17 | 148±12 | 323±25 | 1072±55 | 1.47±0.07 | 1.59±0.02 |
| | 100%NPK | 432±41 | 227±21 | 362±19 | 1645±93 | 1.33±0.02 | 1.54±0.07 |
| Harvest 4 | Control | -22±5 | 143±12 | 145±16 | 127±11 | 1.07±0.06 | 1.54±0.06 |
| | 25%NPK | 1±2 | 208±11 | 157±14 | 335±31 | 1.12±0.07 | 1.35±0.06 |
| | 50%NPK | 24±5 | 270±18 | 165±15 | 521±50 | 1.12±0.07 | 1.38±0.04 |
| | 100%NPK | 52±6 | 378±30 | 181±17 | 876±84 | 1.14±0.07 | 1.54±0.04 |
| Harvest 5 | Control | -4±4 | -50±11 | 145±15 | 81±8 | 0.92±0.04 | 1.56±0.11 |
| | 25%NPK | 31±4 | -4±17 | 157±14 | 160±17 | 1.10±0.05 | 1.76±0.10 |
| | 50%NPK | 36±4 | 51±12 | 165±15 | 187±18 | 1.08±0.05 | 1.83±0.12 |
| | 100%NPK | 62±5 | 187±19 | 181±18 | 239±32 | 1.04±0.01 | 1.64±0.12 |

Mean ± SE (n = 4) in each column indicate significant differences between different fertiliser levels ($p \leq 0.05$).

### 4.4.5 Intercropping Advantage (IA) and Monetary Advantage Index (MAI)

The IA of cowpea was more than of amaranth in both seasons at all harvest in the different fertiliser levels. The IA of amaranth in the first and second seasons showed highest mean values in the control treatments, except the 50% NPK at the fourth harvest (Table 4.11). Lowest mean values on IA of amaranth were obtained at 100% NPK, apart from third and fourth harvests respectively at 25% NPK and control respectively in the first season (Table 4.11). In the second season the IA of amaranth showed lowest mean values at 100% NPK. The IA of cowpea on the other hand in the first season, gave higher mean values at 100% NPK, besides the third harvest at the control treatment (Table 4.11). The lowest were obtained at the control for all harvests except the third harvest. In the second season, the IA of cowpea showed highest values at 25% NPK, from the first to the third harvest, with 100% NPK fertiliser level and 50% NPK fertiliser level at the fourth and fifth harvests respectively (Table 4.11). Overall, the total IA was above one (1) for the first to the third harvest. However, the fourth and fifth harvests showed negative mean values in the first season. The highest IA was obtained at the 25% NPK from the first to the second harvests. In the third, fourth and fifth harvests the IA was highest at the control, 100% NPK and 25% NPK respectively. The IA in the second season showed values above one (1) for all the harvests (Table 4.11). In the first season, the highest IA was obtained at the 25% NPK from the first to the third harvests. In the fourth and fifth harvests, the IA was highest at the control and 50% NPK respectively (Table 4.11).

Monetary Advantage Index (MAI) as an indicator of the economic feasibility showed higher mean values on the second season relative to the first season. The MAI in the first to the fifth harvests on both seasons showed increases from the control, which was lowest until 100% NPK that was highest (Table 4.11).

Table 4.11: Intercropping Advantage for amaranth (IA-amaranth). Intercropping Advantage for cowpea (IA-cowpea). Intercropping Advantage (IA) as well as Monetary Advantage Index (MAI) in amaranth and cowpea of harvests one to five fertilised with four NPK levels in 2014/15 (season 1=S1) and 2015/16 (season 2=S2) seasons.

| HARVESTS | Fertiliser Level | 2014/15 season | S1 | 2015/16 season | S2 | 2014/15 season | 2015/16 season | 2014/15 season | 2015/16 season |
|---|---|---|---|---|---|---|---|---|---|
| | | | | IA | | | | MAI | |
| | | amaranth | cowpea | amaranth | cowpea | | | | |
| Harvest 1 | Control | 5.6±1.0 | 0.9±0.3 | 3.8±0.6 | 3.6±0.11 | 6.5±0.5 | 6.1±0.9 | 214±23 | 1249±94 |
| | 25%NPK | 5.2±1.1 | 1.3±0.6 | 3.3±0.5 | 4.4±0.08 | 6.5±0.9 | 7.7±0.3 | 374±40 | 1849±144 |
| | 50%NPK | 2.5±1.4 | 1.2±0.5 | 3.2±0.7 | 3.3±0.08 | 3.7±0.1 | 4.7±0.5 | 308±75 | 1508±139 |
| | 100%NPK | 2.3±1.2 | 1.4±0.7 | 1.4±0.5 | 2.8±0.08 | 3.6±0.1 | 4.2±0.7 | 404±24 | 1986±95 |
| Harvest 2 | Control | 5.6±0.6 | 0.9±0.01 | 3.8±0.6 | 3.6±0.5 | 6.5±1.3 | 6.1±0.9 | 214±23 | 1249±94 |
| | 25%NPK | 5.2±0.1 | 1.3±0.01 | 3.3±0.5 | 4.4±0.2 | 6.5±1.1 | 7.7±0.3 | 374±40 | 1849±144 |
| | 50%NPK | 2.5±0.4 | 1.2±0.8 | 3.2±0.4 | 3.3±0.4 | 3.7±0.1 | 4.7±0.5 | 308±37 | 1508±139 |
| | 100%NPK | 2.3±0.2 | 1.4±0.7 | 1.4±0.5 | 2.8±0.6 | 3.6±0.1 | 4.2±0.7 | 404±24 | 1986±395 |
| Harvest 3 | Control | -1.1±0.2 | 4.9±0.3 | 3.8±0.3 | 3.6±0.3 | 3.8±0.4 | 6.1±0.3 | 406±82 | 1249±423 |
| | 25%NPK | -2.0±0.4 | 4.5±0.4 | 3.3±0.5 | 4.4±0.2 | 2.4±0.5 | 7.7±0.3 | 496±46 | 1849±144 |
| | 50%NPK | -1.7±0.4 | 4.6±0.3 | 3.2±0.4 | 3.3±0.4 | 2.9±0.4 | 4.7±0.5 | 663±83 | 1508±139 |
| | 100%NPK | -1.4±0.4 | 2.5±0.3 | 1.4±0.5 | 2.8±0.3 | 1.7±.0.6 | 4.2±0.7 | 593±55 | 1986±95 |
| Harvest 4 | Control | -2.7±0.4 | 1.9±0.2 | 0.6±0.6 | 3.4±0.3 | -0.8±0.4 | 4.0±0.1 | -165±144 | 360±32 |
| | 25%NPK | -2.4±0.4 | 2.1±0.2 | -0.5±0.6 | 2.6±0.1 | -0.3±0.7 | 2.1±0.1 | -80±132 | 391±39 |
| | 50%NPK | -2.2±0.4 | 1.9±0.3 | -0.8±0.7 | 3.1±0.3 | -0.3±0.7 | 2.3±0.4 | -76±163 | 608±43 |
| | 100%NPK | -2.4±0.4 | 2.3±0.3 | -0.1±0.5 | 4.0±0.3 | -0.1±0.3 | 3.9±0.2 | -15±198 | 1388±135 |
| Harvest 5 | Control | -1.0±0.3 | -0.9±0.4 | 2.7±0.3 | 1.8±0.5 | -1.9±0.2 | 4.6±0.1 | -165±32 | 318±82 |
| | 25%NPK | -0.2±0.3 | 0.1±0.1 | 2.6±0.3 | 3.8±0.8 | -0.1±0.2 | 6.3±0.9 | -7±33 | 557±36 |
| | 50%NPK | -0.8±0.4 | 0.4±0.5 | 2.7±0.3 | 4.3±0.2 | -0.4±0.2 | 7.0±0.2 | -54±33 | 617±58 |
| | 100%NPK | -1.1±0.3 | 1.1±0.6 | 2.4±0.2 | 2.8±0.7 | -0.8±0.2 | 5.2±0.4 | -11±38 | 623±88 |

Mean ± SE (n = 4) in each column indicate significant differences between different fertiliser levels ($p \leq 0.05$).

## 4.5 Discussion

African leafy vegetable production can be improved through a number of different interventions, and among them is fertiliser application and intercropping, (Mndzebele et al., 2020). The combined effect of fertiliser and intercropping yielded variable biomass in amaranth and cowpea. The yield of amaranth and cowpea significantly increased in sole cropping up to 100% NPK (Tables 4.2 to 4.6) indicating a positive response to fertiliser application. The high biomass at 100% NPK was due to cowpea and amaranth response to additional nutrient supply. The increase is attributed to the fact that NPK fertilization as guided by the 4R principles is beneficial on soil physical properties, which eventually increases crop productivity (Haynes, and Naidu, 1998; Gellings and Parmenter, 2016). The efficient utilisation of NPK efficiently is key in growth (Addai, 2016; Tongos, 2016). Our study corroborates with others, such as spinach in sole cropping (El-Saady, 2016; Zikalala et al., 2017; Patel et al., 2021). In addition, there was more biomass on the sole cropping when compared to intercropping. The closer spacing between alternate rows of amaranth and cowpea in an intercropping led to competition between crops for resource utilization (Ndakidemi, 2006). On the other hand, the higher yields in sole cropping were as a result of high plant density (only in amaranth) as well as the absence of competition for resources such as nutrients (Eskandari, 2009). This study corroborates with other studies done on cowpea and maize where low yields were recorded in an intercropping due to low plant densities of individual crops than those in sole cropping (Manasa, 2018).

Agro-biological parameters or intercropping indices are used to quantify the effects of an intercropping system on biomass and/or yield (Dordas et al., 2019; Saeidi et al., 2019). Numerous intercropping indices such as land equivalent ratio (LER), land utilisation efficiency (LUE), relative crowding coefficient (RCC), aggressivity (A), competitive ratio (CR), actual yield loss (AYL) and intercropping advantage (IA) are used to explain competition between crops (Banik, et al., 2000; Ghosh, 2004; Dhima et al., 2007). The ultimate goal of an intercropping is for the economic benefit, which can be measured through the monetary advantage index (MAI) (Willey, 1979; Ghosh, 2004).

One of the ratios used to determine efficient resource utilisation in an intercropping system relative to sole cropping is LER (Tan et al., 2020). The LER ratios of above one (>1), in different fertiliser levels across all harvests, on both seasons showed an advantage, with a few exceptions. Higher LER (Table 4.7) indicated more biomass on intercropping relative to sole cropping of each due to efficient land utilisation (Banik et al., 2006). The response of LER to different fertiliser levels indicated that there was greater land utilisation efficiency at lower fertiliser levels up to the second harvest. Other harvests showed highest LER at 100% NPK. This study corroborates with LER values of higher than one in different fertiliser levels, therefore indicating an intercropping advantage (Singh et al., 2017; Raza et al., 2019). However, the fourth and fifth seasons in the first season on control were less than one (<1). Observations, irrespective of the fertiliser level, have shown that LERs below 1.00 demonstrated a disadvantage of intercropping vis a vis sole cropping (Banik et al., 2000; Ghosh, 2004; Midya et al., 2005). Similar to the LER, the land-use efficiency (LUE) (>100%), in both seasons in all harvests were an indication that the intercropping was beneficial hence increased biomass relative to sole-cropping as a result of efficient land utilisation (Table 7) (Banik et al., 2006). These are important findings for farmers to identify the optimum fertiliser level for the best land utilisation efficiency.

The actual yield loss (AYL) data provides accurate information on intercropping advantage or disadvantage (Banik et al., 2000). This study showed positive AYL mean values, which were highest at 25% NPK or 50% NPK fertiliser levels and lowest at 100% NPK fertiliser levels, on the first to the fifth harvests in both seasons. However, the fertiliser levels in the fourth and fifth harvests on the first season were negative (Table 4.8). Positive AYL mean values within the varying fertiliser levels in different harvests indicated advantages of the intercropping system, but the negative values showed the contrary in consideration of the yield of crops (Thorsted et al., 2006).

The relative crowding coefficient (RCC) was estimated to calculate the relative dominance of one species over the other in an intercropping (De Wit, 1960). In our study, the RCC of amaranth showed lower mean values in both seasons for all the harvests relative RCC of cowpea that was higher (Table 4.8). In general, the 100% NPK fertiliser level showed the highest RCC in both crops, except for the fifth harvest at 25% NPK. The total RCC showed positive mean values on both crops, in the fourth and fifth harvests on both seasons with an increase from the control to 25% NPK or 50% NPK or 100% NPK fertiliser levels, indicating a yield advantage. However, the first to the third harvest had negative RCC mean values, from control to 50% NPK indicating yield disadvantages within an intercropping (Willey et al., 1980; Ghosh, 2004), if no or low fertiliser was applied.

Aggressivity indicated the relative yield increase in one crop in relation to the other one in an intercropping system (McGilchrist, 1965). This study showed more aggressivity on cowpea was more aggressive when compared to amaranth in the first season (Table 4.9). However, there were similarities in the aggressivity on both amaranth and cowpea in the second season. Generally, there was characteristically more aggressivity at the 100% NPK and 50% NPK fertiliser levels on all harvests in the first and second seasons respectively, with a few exceptions, with the lowest at the control fertiliser level. This could be explained by increased growth in the legume as a result of the plants being able to harness more nutrients, (Jat et al., 2012; da Silva et al., 2020), especially at higher levels such as 50% NPK or 100% NPK, in an intercropping (Tables 4.2 to 4.6). Our study corroborates with others in terms of aggressivity, as shown by Li et al. (2009); Hu et al. (2016), on maize-legume strip intercropping in which the legume was more aggressive relative to the non-legume. While studies (Banik et al., 2000; Xu et al., 2006a; Xu et al., 2006b) have reported the greater competitive ability, irrespective of fertiliser level of non-leguminous crops, our study demonstrated higher competitiveness in cowpea.

The CR provides better competitive ability determination between crops (Dhima et al., 2007). Our study showed relatively equal CR on both amaranth and cowpea, with highest mean values at 100% NPK and 50% NPK fertiliser levels for the first and second seasons respectively (Table 9). Proportionately equal plant population of amaranth and cowpea in the intercropping treatments could have caused this; hence, their ratio becomes zero (Willey and Rao, 1980; Dhima et al., 2007). Over-yielding was assessed by an increase or decrease in the intercropped crops over the corresponding mono-cropped crops according to Li et al. (2011). Our study showed an increasing trend of OY (amaranth) and that of OY (cowpea) from control to 100% NPK fertiliser level. This could have been caused by the biomass corresponding the additional fertiliser application. This study showed higher OY mean values on cowpea, relative to amaranth (Table 4.10), which corroborated to a study on maize-cowpea intercropping (Masvaya et al., 2017), indicating more OY in a legume. The area-time equivalent ratio (ATER) provides a more realistic comparison of the yield advantage of intercrops. This study showed ATER values of more than one varying from the 25% NPK to 100% NPK fertiliser levels in different harvests in both seasons, which was highest (Table 4.10). The lowest ATER was obtained at the control fertiliser level treatment. If the ATER was, greater than one, irrespective of fertiliser level, it implied yield advantage, while mean values below one indicated yield disadvantages. Several studies (Takim, 2012; Olowolaju and Okunlola, 2017; El-Ghobashy et al., 2018), have proven that intercropping with a legume as a companion crop results to ATER's of above one. The intercropping advantage (IA) was worked out according to Banik et al. (2000). The overall IA showed mean values ranging from the control to the 100% NPK fertiliser level in both seasons (Table 4.11). The increased IA, could be attributed to the response to fertiliser application in which additional fertiliser application resulted to more biomass (Table 4.2 to 4.6). This study corroborates with previous work by Dhima et al. (2007) on common vetch and cereal intercropping, to measure the economic feasibility of intercropping systems, hence showing the advantage of intercropping systems.

The ultimate is the economic assessment, measured as the monetary advantage index (MAI), which is able to show the most profitable fertiliser level and harvest. There was an increasing trend on the MAI mean values from the control to 100% NPK on the first to the fourth harvest, with the fifth until 50% NPK fertiliser level. The lowest MAI was obtained at the control fertiliser level. This means that in terms of money it was economic to harvest the crop until the fourth harvest, after which it was wasteful (Table 4.11). Similar to our results, Banik et al. (2000) and Yang et al. (2015) reported intercropping advantage due to the positive values of monetary advantage in all fertiliser levels. This index is the indicator of the economic viability of the most profitable fertiliser level and harvest. This study addresses the yield on amaranth-cowpea intercropping, as well as its intercropping indices, which are indicators in resource utilisation to addresses low food security in rural resource-poor communities. However, more studies have to be done in addition to yield to determine the nutrition in plants and eventually relate the harvested biomass and nutrition to the daily-recommended allowance.

## 4.6 Conclusions

Fertiliser application increased yields of amaranth and cowpea, as well the improved the agro biological parameters. The application of 100% NPK fertiliser application rate contributed to increased biomass, hence more yields on amaranth and cowpea. However, the application of the control (0% NPK) showed the lowest plant growth. There increased yield on sole cropping relative to the intercropping on all fertiliser levels in each harvest on both seasons. There was more land utilisation efficiency in the amaranth-cowpea intercrop, in all the fertiliser levels in different harvests on both seasons. There was higher actual yield loss and relative competition on cowpea when compared to amaranth across different fertiliser levels in all harvests. Amaranth-cowpea intercrop showed an intercropping advantage at 100% NPK fertiliser level. Additional income was obtained on the 100% NPK fertiliser level on all harvests in both seasons. The study addresses the effect of fertiliser application and intercropping on yield and agro-biological parameters, which are determinants of intercropping. The study has implications for increased food and nutrition in rural communities, through amaranth and cowpea intercropping. Therefore, smallholder farmers should intercrop and also apply the recommended 100% NPK fertiliser dosage. The study recommends additional data on the nutritional concentration, nutritional yield in the amaranth and cowpea intercrop. In addition, the benefit of the amaranth and cowpea to human nutrition should be studied in relation to the daily nutrient requirements.

## 4.7 Acknowledgements

We are grateful to the Agriculture Research Council-Vegetable and Ornamental Plants for providing facilities and financial support to carry out the research. In addition, we are grateful to Mr Eric Mathebula for his support with statistical analyses and support.

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# CHAPTER 5 :

# POTENTIAL OF NPK FERTILISATION AND AMARANTH-COWPEA INTERCROPPING IN MEETING NUTRITIONAL REQUIREMENTS

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## 5.1 Abstract

African leafy vegetables are good sources of magnesium, calcium, iron and zinc, which are key to estimate nutritional demands amongst children under the age of five. Fertilisers play an important role in the production of these vegetables. Therefore, this study was conducted to investigate the yield, nutritional compositions of intercropped amaranth and cowpea grown in the field. The experiment was laid out in a 2 x 4 factorial treatment structure in a completely randomized design, with intercropping (cowpea and amaranth) and fertiliser (control, 25%, 50%, and 100% NPK levels) with four replications. Growing these vegetables in an intercropping improved the recommended daily nutrient dietary. Seasonal biomass of both crops increased with the addition of fertiliser up to 100% NPK recommendation. Biomass was higher in sole cropping compared to intercropping. Nutrient content showed significantly higher values in both crops at 100%NPK, with the lowest at the control on sole and intercropping. Both crops contributed to RDA of calcium, magnesium, iron and zinc with higher values recorded at 100%NPK fertiliser level. This study demonstrated the benefit of fertiliser application on the biomass of amaranth and cowpea. Macro and trace elements crucial for rural communities were improved, thus contributing to more RDA.

Keywords: amaranth; cowpea; intercropping, nutrient content; Recommended Daily Allowance

## 5.2 Introduction

African Leafy Vegetables (ALVs) are crop species that originated from specific agro-ecologies, and over time have established themselves in new environments through choice by those communities or evolution (van Rensburg et al., 2007). These ALVs are commonly accepted, amongst other reasons, due to the short growing season, high nutrition and fewer agronomic requirements (Faber et al., 2010; Kwenin et al., 2011; Schönfeldt and Pretorius, 2011; Oelofse and van Averbeke, 2012). They have been part of the human diet for centuries in Sub Saharan rural resource-poor households (Odhav et al., 2007; Vorster et al., 2008). In Sub Saharan Africa for example, in South Africa, chronic malnutrition is reported as one of the major challenges, particularly to children (Faber and Wenhold, 2010; FAO, IFAD and WFP, 2015). This has been exacerbated by the consumption of carbohydrates-based diets which are low in both micro and macro nutrients (Weinheimer et al., 2012). This challenge is mainly prominent among the vulnerable groups, which include children under the age of five, as they are considered to be at high risk (Arimond et al., 2010; Black et al., 2013). Nutrients contained in ALVs comprise, among others, magnesium, calcium, iron and zinc that are highly deficient in typical South African diets (Odhav et al., 2007; Vorster et al., 2008; Oelofse and van Averbeke, 2012; Maseko et al., 2019). Amounts of nutrient contained in ALVs are comparable, to commonly grown vegetables such as cabbage and Swiss chard (Oelofse and van Averbeke, 2012). Lack of availability and accessibility to nutritious and balanced diets is a major challenge that causes malnutrition in rural communities, resulting to under nutrition, which affects children under the age of five (Govender et al., 2017). For example, calcium deficiency causes osteopenia, osteoporosis, rickets and impaired growth (Kaludjerovic and Vieth, 2010). Zinc deficiency distresses mainly children aged under 5 years (Marra and Alaniz, 2000; Bi et al., 2020). Lack of iron causes a high prevalence of anaemia in children under the age of five (Kessy et al., 2019).

The common indigenous vegetables and usually consumed by most communities in South Africa are *Amaranthus cruentus* (amaranth) and *Vigna unguiculata* L. Walp (cowpea) (van Rensburg et al., 2007; Oelofse and van Averbeke, 2012; Mavengahama, 2013). These are normally grown as sole crops with inorganic fertiliser types such as those rich in nitrogen, phosphorus and potassium (Van Averbeke et al., 2007; Van Averbeke et al., 2012; Maseko et al., 2017). Inorganic fertilisers are necessary as the soils in sub Saharan Africa are inherently poor in nutrients, with reported low in N, P and K (Sanchez, 2002; Vanlauwe et al., 2015; Nanganoa et al., 2020). Additionally, these mineral nutrients are key for plant growth and nutrient content in these vegetables. To optimize land use, most rural farmers grow ALV's under intercropping. Intercropping is defined as the synchronized farming of two or more crop species in the same field for either the complete season or partially (Vandermeer, 1989). Intercropping would therefore assure efficient land utilization from both crops as opposed to having them cultivated as sole crops (Odhav et al., 2007). In addition to efficient land utilization, it offers the advantage of having a varied nutrient composition from the different component crops and thus collectively contributing to improved nutrition.

Vulnerable groups like women and children can benefit more from the intercropping practice through improved yield and nutrition, hence reducing nutritional insecurity among rural households (Vorster et al., 2008). Studies on nutrition, especially in vegetables are normally conducted alongside harvesting frequency to ascertain the best time to get more yield and/or nutrition (Maseko et al., 2018). This study was conducted to investigate the yield, nutritional compositions of field grown and intercropped indigenous vegetables amaranth and cowpea, which is expected to improve the recommended daily nutrient dietary requirements of rural households that would help alleviate food and nutritional security challenges. Hence, it was hypothesized that the interaction of fertiliser application and intercropping will enhance the growth and above ground biomass or above ground edible biomass of amaranth and cowpea crops.

## 5.3 Materials and Methods

### 5.3.1 Site description and environmental conditions

The experiment was conducted at the Agricultural Research Council (ARC), Vegetable and Ornamental Plants campus in Roodeplaat, Pretoria, South Africa (25°35' S, 28°21' E, 1165 masl) during 2014/15 and 2015/16 summer seasons, from November to March as described by Mndzebele et al. (2020). The description of the soils and weather conditions (e.g. rainfall and temperature) of the experimental sites during the trials are presented in Mndzebele et al. (2020).

### 5.3.2 Experimental treatments, layout and plot management

The experimental treatments, layout and the management were as described by Mndzebele et al. (2020). These entailed the amount of fertiliser applied, seedling preparation, planting, inter- and intra-row spacing, as well as irrigation amounts.

Fertiliser (NPK) was applied based on the recommended cowpea requirement taking into consideration the lower fertiliser requirements of the legume. The experiment was laid out in a 2 x 4 factorial treatment structure in a completely randomized design (CRD) with four replications.

Irrigation scheduling was based on crop water requirement (ETc) of each crop, either in a sole cropping or in intercropping. Crop coefficient (Kc) values for amaranth and cowpea were obtained from Beletse et al. (2012).

### 5.3.3 Data collection

***Above-ground edible biomass (AGEB)***

Vegetable crops were sequentially harvested at 4, 6, 8, 10 and 12 weeks after transplanting for amaranth and 8, 10, 12, 14 and 16 weeks after planting for cowpea during the first and second growing seasons as described by Mndzebele et al. (2020). Amaranth was cut above ground for their biomass to include leaves and stems. The stems were separated from the leaves to determine above ground edible biomass (AGEB). In the case of cowpea, only leaves were defoliated to determine AGEB. The sequential harvests from each crop were combined to obtain seasonal yields from both crops in all the seasons.

***Preparation of plant samples***

The separated healthy edible leaves in the shoots of amaranth from stems and the cowpea were soaked and thoroughly washed with tap water (three to four times) to remove soil debris, and this was followed by carefully washing with distilled water. The separated plant parts were oven-dried at 60ºC for 48 h, ground into fine powder (2-mm sieve) and stored at room temperature. All ground samples of both crops from both seasons were stored in airtight vials prior to analysis.

***Measurement of mineral element concentrations in plant tissue***

Dried, leaf samples were analysed for mineral element content at the Agricultural Research Council–Soil, Climate and Water (ARC-SCW) laboratory, Pretoria, South Africa. The dried samples were digested using perchloric + nitric acid methodology. A mass of 0.5 g of dry sample was digested in a 100 mL volumetric flask with 7 mL

$HNO_3$ (concentrated nitric acid) and 3 mL $HClO_4$ (perchloric acid) at 180°C (Giron, 1973). The digested samples were analysed using an Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) to determine the content of the macro and micronutrient elements (specifically: Total N, P, K, Ca, Mg, Cu, Zn, Mn, Fe, and B). Nutrient elements were determined sequentially at 4, 6, 8, 10 and 12 weeks after transplanting for amaranth and 8, 10, 12, 14 and 16 weeks after planting for cowpea during for seasons. The statistical analyses showed no significant differences in the sequential harvests, and therefore the results were pooled for each crop. To convert the mineral nutrient elements to wet mass basis, the moisture content of vegetables in each treatment were multiplied by the nutrient concentration (dry mass basis).

***Determination of amaranth and cowpea to meet the %RDA (Ca, Mg, Fe and Zn)***

The nutrient contribution (on wet mass basis) of an estimated serving of cooked leaves of amaranth and cowpea to the nutrient requirements was calculated and expressed as a percentage of the recommended dietary allowance (RDA) for children less than the age of five (Trumbo et al., 2001; Otten et al., 2006).

$$\%\ \text{mineral contribution to RDA} = \frac{\text{mineral content (mg/100mg)}}{\text{mineral requirements } (\frac{\text{mg}}{\text{day}})}$$

The daily-recommended nutrient intakes (DRNI) for calcium, Mg, Fe and Zn were sourced from Uusiku et al. (2010). Percentage contribution to the DRNI was calculated [nutrient concentrations (calcium, Mg, Fe and Zn in mg 100 $g^{-1}$) divided by nutrient requirements in mg $day^{-1}$ × 100].

### 5.3.4 Statistical analysis

The seasonal yield, nutrient content, as well as %RDA were subjected to analysis of variance (ANOVA). The Shapiro-Wilk's test was performed on the standardized residuals to test for deviations from normality (Shapiro and Wilk, 1965). In cases where significant deviation from normality was observed and due to skewness, outliers were removed until it was normal or symmetrically distributed. (Glass et al., 1972) Student's t-LSDs (Least significant differences) were calculated at a 5% significance level to compare means for significant source effects (Snedecor and Cochran, 1967). All the analyses were performed using SAS (1999) (SAS version 9.3, SAS Institute Inc. Cary, NC, United States of America).

## 5.4 Results

### 5.4.1 Above-ground edible biomass (AGEB)

There were significant ($p<0.05$) interactions between cropping systems and fertiliser levels with regard to the seasonal above ground edible biomass of amaranth and cowpea in both seasons (Figure 5.1). The AGEB, characteristically increased from 0% NPK to 100% NPK, in both cropping systems, on both seasons for both crops (Figure 1). In both seasons, the highest seasonal AGEB were obtained at 100%NPK in combination with sole cropping on both amaranth and cowpea (Figure 1). The lowest seasonal AGEB were obtained from the control (0% NPK) in combination with the intercropping system in the 2014/15 and 2015/16 seasons (Figure 5.1)

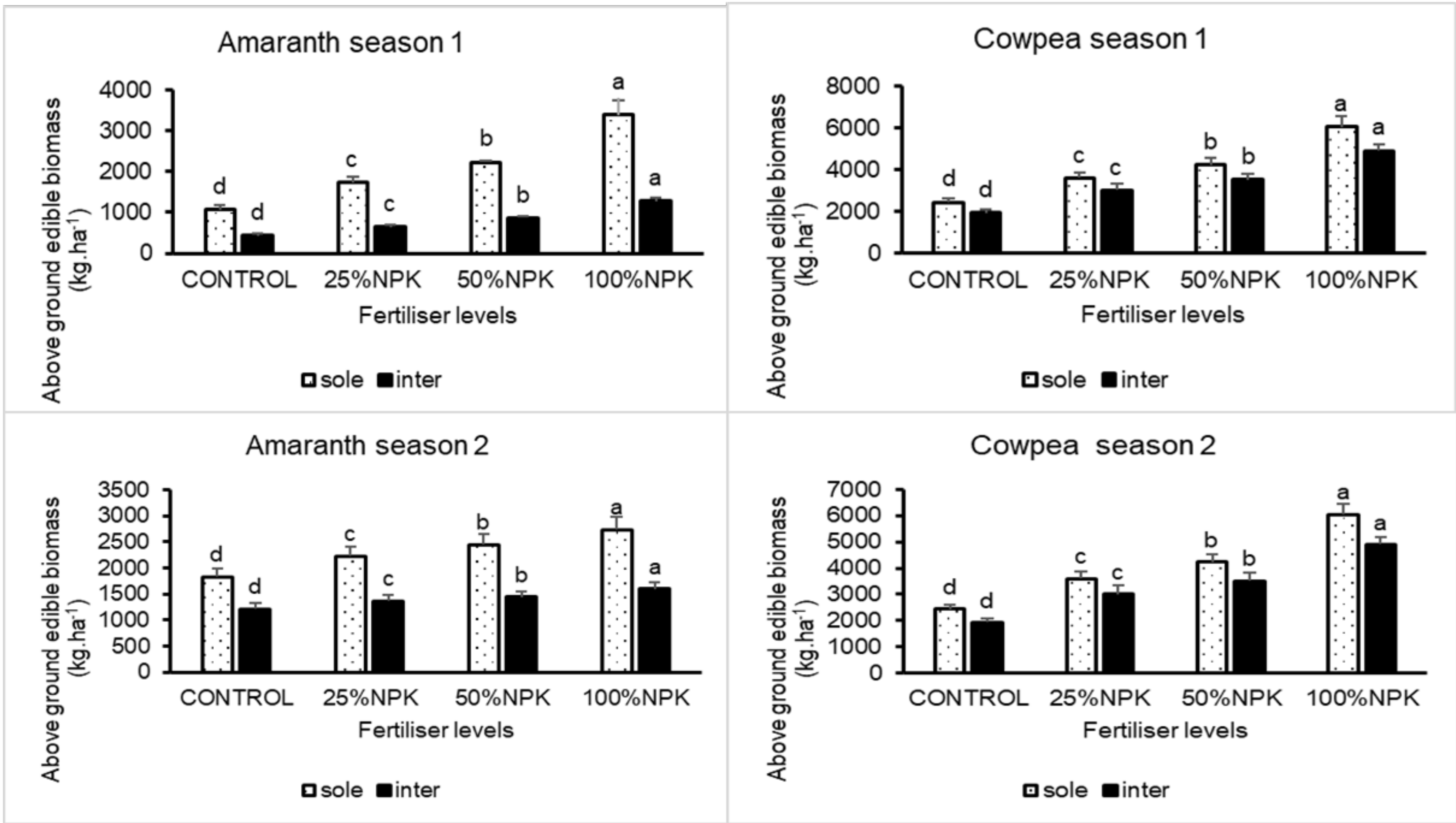


Figure 5.1: Above ground seasonal dry biomass of amaranth and cowpea at four NPK levels in 2014/15 and 2015/16 seasons. Each bar represents mean (Mean±SE). Letters in each fertiliser treatment shows significant differences at $p < 0.05$ in sole or intercropping.

### 5.4.2 Macro elements tissue mineral content

There were generally no significant interactions between cropping systems and fertiliser levels with regard to total N, P, K, Ca, Mg, Na and S macro nutrient content in amaranth and cowpea leaves in both seasons (Table 5.1 and 5.2). However, nutrient content on amaranth in the first season showed significantly higher N, P, K, Ca, Mg, Na and S mean values in the 100%NPK fertiliser levels on the intercrop. The lowest mean values were obtained at the control on the sole cropping system (Table 5.1). Fertiliser application (NPK) showed significantly higher mean values at 100% NPK fertiliser level relative to the 0%NPK in both sole and intercropping, which was lowest (Table 5.1).

Macro nutrient content of amaranth in the second season, showed no significant interaction effect between cropping systems and the different fertiliser levels although higher mean values were recorded for N, P, K, Ca, Mg, Na and S from the 100% NPK fertiliser level. In all cases, N, P, K, Ca, Mg, Na and S showed an increasing trend in response to an increase fertiliser level (Table 5.1).

A similar trend to that of amaranth was observed in the cowpea in both seasons (Table 5.2). However, nutrient content in cowpea in the first season showed higher N, P, K, Ca and S in the 100% NPK fertiliser levels from an intercrop, with the exception of Mg and Na on sole cropping (Table 5.2).

### 5.4.3 Micro element content in plant tissues

There were significant ($p<0.05$) interactions between cropping systems and fertiliser levels with regard to iron concentration in amaranth on both seasons in (Table 5.1). The first season showed higher mean values in the 100% NPK fertiliser level on intercropping, with the lowest values recorded at the control fertiliser level in sole cropping. The second season recorded higher iron nutrient content in amaranth in the 100% NPK fertiliser level of either sole cropping or intercropping. The lowest content was obtained at the control of either sole cropping or intercropping. A though the rest of the other nutrients did not show a significant ($p>0.05$) interaction between cropping system and fertiliser level, the trends of the results were somewhat similar to those of iron. There was a significant increase in all the micronutrient concentrations corresponding to the increase in fertiliser levels for amaranth in both seasons for both cropping systems. There were, however, no significant effect of intercropping on Mn, Zn and B concentrations, except for Zn in the first season (Table 5.1).

The nutrient content in cowpea showed significant ($p<0.05$) interactions between cropping systems and fertiliser levels with regard to Fe content in both seasons and for Mn in the second season (Table 5.2). The highest content in both elements were obtained at the combined effect of sole cropping in both seasons. The lowest iron content in cowpea on both seasons was obtained at the combined effect of the intercropping and the control fertiliser levels. With regard to the rest of the other measured micronutrients in this study, a similar characteristic trend to that recorded in amaranth was reported. Fertiliser application resulted in a significant ($p<0.05$) increase in the micronutrients with the corresponding increase in fertiliser levels (Table 5.2) in both seasons for both cropping systems.

Table 5.1: Average macro nutrient and micronutrient content in leaves of amaranth fertilised with four levels of NPK in 2014/15 (season 1) and 2015/16 (season 2) seasons.

| 2014/15 (season 1) | | N | P | K | Ca | Mg | Na | S | Fe | Mn | Zn | B |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cropping systems | Fertiliser Level | | | | | | mg/100g | | | | | |
| Sole cropping | CONTROL | 4546±444e$^{4}$ | 355±27e$^{4}$ | 1949±194f$^{4}$ | 1823±125de$^{4}$ | 403±40d$^{4}$ | 23±2e$^{4}$ | 273±23e$^{4}$ | 52±5e$^{4}$ | 13±1.8d$^{4}$ | 2.9±0.5e$^{4}$ | 3.17±0.28d$^{3}$ |
| | 25%NPK | 4831±408de$^{3}$ | 412±37d$^{3}$ | 2212±168de$^{3}$ | 2060±208c$^{3}$ | 475±48c$^{3}$ | 30±3cde$^{3}$ | 321±28d$^{3}$ | 72±7d$^{3}$ | 15±1.1c$^{3}$ | 3.5±0.4d$^{3}$ | 3.44±0.31d$^{3}$ |
| | 50%NPK | 5148±397bc$^{2}$ | 478±45c$^{3}$ | 2862±94c$^{2}$ | 2224±204b$^{2}$ | 535±24b$^{2}$ | 38±4c$^{2}$ | 421±42c$^{2}$ | 93±9c$^{2}$ | 17±1.2b$^{2}$ | 4.1±0.4bc$^{2}$ | 5.42±0.65bc$^{2}$ |
| | 100%NPK | 5446±368ab$^{1}$ | 528±48ab$^{1}$ | 3215±306ab$^{1}$ | 2584±209a$^{1}$ | 607±51a$^{1}$ | 60±6a$^{1}$ | 524±47b$^{1}$ | 116±12b$^{1}$ | 19±1.2a$^{1}$ | 4.9±0.6a$^{1}$ | 7.22±2.84a$^{1}$ |
| Intercropping | CONTROL | 4736±359e$^{4}$ | 336±30e$^{4}$ | 2030±161ef$^{4}$ | 1751±106e$^{4}$ | 425±38d$^{4}$ | 24±2de$^{4}$ | 273±11$^{4}$ | 50±4e$^{4}$ | 13±1.2d$^{4}$ | 2.7±0.7e$^{4}$ | 3.15±0.23d$^{3}$ |
| | 25%NPK | 5049±477cd$^{3}$ | 417±33d$^{3}$ | 2372±145d$^{3}$ | 1918±121cd$^{3}$ | 478±19c$^{3}$ | 34±3bcd$^{3}$ | 340±31d$^{3}$ | 68±7de$^{3}$ | 15±1.4c$^{3}$ | 3.5±0.4d$^{3}$ | 3.47±0.22d$^{3}$ |
| | 50%NPK | 5301±437abc$^{2}$ | 502±48bc$^{2}$ | 3109±112bc$^{2}$ | 2071±143bc$^{2}$ | 508±19b$^{2}$ | 44±4b$^{2}$ | 441±38c$^{2}$ | 91±28c$^{2}$ | 17±1.4b$^{2}$ | 3.9±0.4c$^{2}$ | 5.24±0.60c$^{2}$ |
| | 100%NPK | 5597±372a$^{1}$ | 547±45a$^{1}$ | 3429±287a$^{1}$ | 2459±239a$^{1}$ | 581±43a$^{1}$ | 54±5a$^{1}$ | 573±46a$^{1}$ | 144±14a$^{1}$ | 20±1.7a$^{1}$ | 4.4±0.7b$^{1}$ | 6.24±0.84b$^{1}$ |
| Cropping | | 0.0186 | 0.4870 | 0.0037 | 0.0036 | 0.3543 | 0.4870 | 0.4870 | 0.2128 | 0.8076 | 0.0038 | 0.2995 |
| Fertiliser Level | | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |
| Cropping* Fertiliser level | | 0.9882 | 0.4584 | 0.8126 | 0.8884 | 0.0355 | 0.4584 | 0.4584 | 0.0432 | 0.7626 | 0.1468 | 0.3614 |
| 2015/16 (season 2) | | | | | | | | | | | | |
| Cropping systems | Fertiliser Level | N | P | K | Ca | Mg | Na | S | Fe | Mn | Zn | B |
| Sole cropping | CONTROL | 2979±256de$^{4}$ | 349±30d$^{4}$ | 3003±305d$^{4}$ | 2261±174ef$^{4}$ | 1314±117de$^{4}$ | 21.6±2.5cd$^{4}$ | 372±35de$^{4}$ | 33±3e$^{4}$ | 6±0.5d$^{4}$ | 3.6±0.4ef$^{4}$ | 4.76±0.96d$^{4}$ |
| | 25%NPK | 3254±239cd$^{3}$ | 392±20c$^{3}$ | 3747±348c$^{3}$ | 2474±177cd$^{3}$ | 1446±137bc$^{3}$ | 24.7±2.8cd$^{3}$ | 417±35bc$^{3}$ | 54±5d$^{3}$ | 7±0.6c$^{3}$ | 3.9±0.4de$^{3}$ | 5.89±0.57bcd$^{3}$ |
| | 50%NPK | 3698±327b$^{2}$ | 430±20b$^{2}$ | 4321±366b$^{2}$ | 2640±208c$^{2}$ | 1546±127b$^{2}$ | 29.3±2.5b$^{2}$ | 442±48b$^{2}$ | 71±7b$^{2}$ | 9±0.9b$^{2}$ | 4.3±0.4cd$^{2}$ | 6.75±0.21b$^{2}$ |
| | 100%NPK | 4466±460a$^{1}$ | 484±43a$^{1}$ | 4999±367a$^{1}$ | 3054±308a$^{1}$ | 1765±179a$^{1}$ | 38.2±3.7a$^{1}$ | 506±49a$^{1}$ | 91±9a$^{1}$ | 11±1.1a$^{1}$ | 4.8±0.5b$^{1}$ | 8.18±0.81a$^{1}$ |
| Intercropping | CONTROL | 2903±141e$^{4}$ | 356±37d$^{4}$ | 3190±278d$^{4}$ | 2146±194f$^{4}$ | 1237±119e$^{4}$ | 20.7±1.6d$^{4}$ | 362±39e$^{4}$ | 31±3e$^{4}$ | 6±0.6d$^{4}$ | 3.3±0.5f$^{4}$ | 5.08±.0.20cd$^{4}$ |
| | 25%NPK | 3443±342bc$^{3}$ | 396±35c$^{3}$ | 3741±333c$^{3}$ | 2390±222de$^{3}$ | 1414±131cd$^{3}$ | 25.6±2.8bc$^{3}$ | 405±34cd$^{3}$ | 35±4e$^{3}$ | 7±0.1cd$^{3}$ | 4.0±0.5d$^{3}$ | 6.14±0.70bc$^{3}$ |
| | 50%NPK | 3631±310b$^{2}$ | 441±38b$^{2}$ | 4258±406b$^{2}$ | 2597±249c$^{2}$ | 1518±147bc$^{2}$ | 30.0±2.9b$^{2}$ | 434±43bc$^{2}$ | 60±6c$^{2}$ | 8±0.2bc$^{2}$ | 4.4±0.8bc$^{2}$ | 6.39±0.69b$^{2}$ |
| | 100%NPK | 4188±372a$^{1}$ | 510±48a$^{1}$ | 5022±487a$^{1}$ | 2835±241b$^{1}$ | 1824±134a$^{1}$ | 37.1±3.6a$^{1}$ | 509±41a$^{1}$ | 93±9a$^{1}$ | 10±0.3a$^{1}$ | 5.4±0.2a$^{1}$ | 8.45±0.76a$^{1}$ |
| Cropping | | 0.4681 | 0.1312 | 0.6033 | 0.0037 | 0.4438 | 0.859 | 0.4199 | <.0001 | 0.072 | 0.1528 | 0.7011 |
| Fertiliser Level | | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |
| Cropping* Fertiliser level | | 0.2349 | 0.7203 | 0.5884 | 0.5459 | 0.4689 | 0.8861 | 0.9396 | <.0001 | 0.8967 | 0.0163 | 0.8432 |

Mean values in each column followed by different letters indicate significant differences between treatments. Numerical values compare means of each cropping system at different fertiliser levels ($p<0.05$).

Table 5.2: Average macro nutrient and micro nutrient content in leaves of cowpea fertilised with four levels of NPK in 2014/15 (season 1) and 2015/16 (season 2) seasons.

| 2014/15 (Season 1) | | N | P | K | Ca | Mg | Na | S | Fe | Zn | Mn | B |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cropping systems | Fertiliser Level | | | | | | | | | | | |
| Sole cropping | CONTROL | 4529±335f$^{4}$ | 355±30e$^{4}$ | 1944±181f$^{4}$ | 1820±119de$^{4}$ | 403±47f$^{4}$ | 23±2e$^{4}$ | 276±25e$^{4}$ | 53±5de$^{4}$ | 3.0±0.5e$^{4}$ | 13.1±1.3d$^{4}$ | 3.2±0.3d$^{3}$ |
| | 25%NPK | 4862±400de$^{3}$ | 415±40d$^{3}$ | 2230±222de$^{3}$ | 2074±204b$^{3}$ | 475±48e$^{3}$ | 34±3cde$^{3}$ | 324±32de$^{3}$ | 72±7cd$^{3}$ | 3.5±0.4d$^{3}$ | 15.0±1.2c$^{3}$ | 3.5±0.3d$^{3}$ |
| | 50%NPK | 5148±397bcd$^{2}$ | 478±45c$^{2}$ | 2862±24c$^{2}$ | 2224±200b$^{2}$ | 535±24c$^{2}$ | 38±4cd$^{2}$ | 421±42c$^{2}$ | 93±9c$^{2}$ | 4.1±0.4bc$^{2}$ | 16.7±1.2b$^{2}$ | 5.4±0.7bc$^{2}$ |
| | 100%NPK | 5438±360ab$^{1}$ | 528±46ab$^{1}$ | 3208±308ab$^{1}$ | 2566±207a$^{1}$ | 606±50a$^{1}$ | 60±7a$^{1}$ | 498±46b$^{1}$ | 115±12b$^{1}$ | 4.9±0.6a$^{1}$ | 18.8±1.1a$^{1}$ | 7.2±0.8a$^{1}$ |
| Intercropping | CONTROL | 4736±359ef$^{4}$ | 336±30e$^{4}$ | 2030±201ef$^{4}$ | 1751±106e$^{4}$ | 425±38f$^{4}$ | 24±2e$^{4}$ | 273±26e$^{4}$ | 50±4e$^{4}$ | 2.7±0.7e$^{4}$ | 12.8±1.2d$^{4}$ | 3.2±0.2d$^{3}$ |
| | 25%NPK | 5049±507cd$^{3}$ | 417±33d$^{3}$ | 2372±205d$^{3}$ | 1918±121cd$^{3}$ | 478±19e$^{3}$ | 30±3de$^{3}$ | 340±31d$^{3}$ | 68±6de$^{3}$ | 3.5±0.4d$^{3}$ | 14.7±1.5c$^{3}$ | 3.5±0.2d$^{3}$ |
| | 50%NPK | 5301±437bc$^{2}$ | 502±48bc$^{2}$ | 3109±112bc$^{2}$ | 2071±143bc$^{2}$ | 508±19d$^{2}$ | 44±4bc$^{2}$ | 441±38c$^{2}$ | 91±8c$^{2}$ | 3.9±0.4c$^{2}$ | 16.8±1.4b$^{2}$ | 5.2±0.6c$^{2}$ |
| | 100%NPK | 5612±375a$^{1}$ | 549±46a$^{1}$ | 3447±282a$^{1}$ | 2471±245a$^{1}$ | 571±66b$^{1}$ | 54±5ab$^{1}$ | 573±57a$^{1}$ | 146±14a$^{1}$ | 4.4±0.7d$^{1}$ | 19.9±1.7a$^{1}$ | 6.2±1.9b$^{1}$ |
| Cropping | | 0.0214 | 0.5006 | 0.0058 | 0.0029 | 0.0731 | 0.7346 | 0.0641 | 0.2826 | 0.0017 | 0.5813 | 0.1962 |
| Fertiliser Level | | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |
| Cropping* Fertiliser Level | | 0.9958 | 0.4475 | 0.7698 | 0.8174 | 0.0111 | 0.3841 | 0.2155 | 0.0432 | 0.1593 | 0.3378 | 0.3397 |
| | | N | P | K | Ca | Mg | Na | S | Fe | Zn | Mn | B |
| 2015/16 (Season 2) | | | | | | | | | | | | |
| Sole cropping | CONTROL | 3930±312g$^{4}$ | 318±28d$^{4}$ | 1793±149d$^{4}$ | 1846±184c$^{4}$ | 368±36e$^{4}$ | 13±1d$^{4}$ | 4±0.1cd$^{3}$ | 102±10e$^{4}$ | 14.8±1.6d$^{4}$ | 52.5±4.4g$^{4}$ | 2.7±0.4e$^{4}$ |
| | 25%NPK | 4320±386ef$^{3}$ | 348±29c$^{3}$ | 1978±195c$^{3}$ | 2018±205b$^{3}$ | 407±39c$^{3}$ | 15±2cd$^{3}$ | 4±0.1bc$^{2}$ | 146±12c$^{3}$ | 16.8±1.4cd$^{3}$ | 70.0±6.4e$^{3}$ | 2.9±0.4de$^{3}$ |
| | 50%NPK | 4545±335de$^{2}$ | 366±28bc$^{2}$ | 2097±203bc$^{2}$ | 2153±208b$^{2}$ | 425±42bc$^{2}$ | 20±2b$^{2}$ | 4±0.1ab$^{2}$ | 189±19b$^{2}$ | 18.7±1.2bc$^{2}$ | 86.1±6.1bc$^{2}$ | 3.2±0.5cd$^{2}$ |
| | 100%NPK | 4863±337b$^{1}$ | 401±32a$^{1}$ | 2354±202a$^{1}$ | 2390±206a$^{1}$ | 476±43a$^{1}$ | 24±3a$^{1}$ | 5±0.1a$^{1}$ | 248±21a$^{1}$ | 20.9±1.5a$^{1}$ | 107.4±10.1a$^{1}$ | 3.5±0.6ab$^{1}$ |
| Intercropping | CONTROL | 4133±364fg$^{4}$ | 320±31d$^{4}$ | 1779±179d$^{4}$ | 1808±156c$^{4}$ | 376±32de$^{4}$ | 18±1bc$^{4}$ | 4±0.1d$^{3}$ | 74±7f$^{4}$ | 14.8±1.4d$^{4}$ | 61.8±6.5f$^{4}$ | 2.7±0.3e$^{4}$ |
| | 25%NPK | 4568±283cd$^{3}$ | 359±30bc$^{3}$ | 1970±138c$^{3}$ | 2026±192b$^{3}$ | 402±34cd$^{3}$ | 19±1b$^{3}$ | 4±0.1cd$^{2}$ | 109±10e$^{3}$ | 17.3±0.9c$^{3}$ | 74.1±7.2de$^{3}$ | 3.1±0.4cd$^{3}$ |
| | 50%NPK | 4790±355bc$^{2}$ | 372±25b$^{2}$ | 2065±148c$^{2}$ | 2169±208b$^{2}$ | 435±40b$^{2}$ | 21±2b$^{2}$ | 4±0.1bc$^{2}$ | 127±12d$^{2}$ | 18.6±1.5bc$^{2}$ | 79.2±7.1cd$^{2}$ | 3.3±0.4bc$^{2}$ |
| | 100%NPK | 5188±502a$^{1}$ | 394±23a$^{1}$ | 2203±146b$^{1}$ | 2370±219a$^{1}$ | 472±46a$^{1}$ | 24±2a$^{1}$ | 5±0.1ab$^{1}$ | 172±15b$^{1}$ | 20.3±2.0ab$^{1}$ | 88.7±6.6b$^{1}$ | 3.6±0.5a$^{1}$ |
| Cropping | | <.0001 | 0.4897 | 0.1154 | 0.8165 | 0.7440 | 0.2773 | 0.0999 | <.0001 | 0.9263 | 0.1372 | 0.1476 |
| Fertiliser Level | | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |
| Cropping* Fertiliser Level | | 0.9184 | 0.5839 | 0.3563 | 0.9587 | 0.8253 | 0.5073 | 0.9969 | 0.0003 | 0.9135 | <.0001 | 0.9823 |

Mean values in each column followed by different letters indicate significant differences between treatments. Numerical values compare means of each cropping system at different fertiliser levels ($p<0.05$).

### 5.4.4 Estimation of %RDA

There were no significant ($p>0.05$) interactions between cropping system and fertiliser level with regard to the %RDA of magnesium, iron and zinc except calcium in the second season for amaranth (Table 5.3). The highest %RDA-Ca was recorded in 100%NPK in both seasons. With the exception of %RDA-Mg, there was a significant ($p<0.05$) interaction between cropping system and fertiliser level with respect to rest of the measured %RDA for amaranth in the second season. In terms of fertiliser application levels there was significant increase in %RDA with the increase in fertiliser application levels in both seasons and in both sole and intercropping systems. There were also significant ($p<0.05$) differences on all the measured %RDA of micronutrient due to cropping systems in both seasons.

With regard to cowpea, a characteristically similar trend to that of amaranth was observed in both seasons (Table 5.4). A significant interaction effect between cropping system and fertiliser level was recorded in %RDA-Ca in the second season, with the highest percentage (122%) in 100%NPK under sole cropping. A significant ($P<0.05$) and proportional increase in %RDA of micronutrients with the increase levels of application was observed in both seasons for both cropping systems (Table 5.4).

Table 5.3: The estimated potential of amaranth fertilised with four levels of NPK in 2014/15 (season 1) and 2015/16 (season 2) seasons to provide the recommended daily allowance of calcium, magnesium, iron, and zinc.

| 2014/15 (season 1) Cropping systems | Fertiliser Level | %RDA-Ca | %RDA-Mg | %RDA-Fe | %RDA-Zn |
|---|---|---|---|---|---|
| Sole cropping | CONTROL | 44±4a$^{3}$ | 156±12a$^{2}$ | 150±15c$^{4}$ | 11±0.3bc$^{3}$ |
| | 25%NPK | 44±4a$^{23}$ | 158±13a$^{12}$ | 168±17b$^{3}$ | 11±0.2bc$^{23}$ |
| | 50%NPK | 47±5a$^{2}$ | 167±14a$^{12}$ | 184±12b$^{2}$ | 12±0.3ab$^{2}$ |
| | 100%NPK | 47±5a$^{1}$ | 169±13a$^{1}$ | 214±21a$^{1}$ | 13±0.3a$^{1}$ |
| Intercropping | CONTROL | 33±3c$^{3}$ | 118±12c$^{2}$ | 94±6e$^{4}$ | 7±0.2d$^{3}$ |
| | 25%NPK | 35±4c$^{23}$ | 126±13cb$^{12}$ | 120±13d$^{3}$ | 8±0.2d$^{23}$ |
| | 50%NPK | 36±3bc$^{2}$ | 129±13cb$^{12}$ | 135±12cd$^{2}$ | 9±0.2d$^{2}$ |
| | 100%NPK | 39±4b$^{1}$ | 139±12b$^{1}$ | 142±12c$^{1}$ | 11±0.3c$^{1}$ |
| | Cropping | <.0001 | <.0001 | <.0001 | <.0001 |
| | Fertiliser Level | 0.0065 | 0.0334 | <.0001 | <.0001 |
| | Cropping* Fertiliser Level | 0.6005 | 0.8922 | 0.5775 | 0.8365 |
| 2015/16 (season 2) | | | | | |
| Sole cropping | CONTROL | 68±6b$^{3}$ | 246±24bc$^{2}$ | 88±5d$^{2}$ | 18±0.4c$^{3}$ |
| | 25%NPK | 73±7b$^{2}$ | 247±25bc$^{2}$ | 106±10c$^{2}$ | 26±0.6a$^{2}$ |
| | 50%NPK | 82±5a$^{2}$ | 254±25ab$^{2}$ | 122±11b$^{1}$ | 27±0.5a$^{2}$ |
| | 100%NPK | 84±8a$^{1}$ | 280±28a$^{1}$ | 134±12a$^{1}$ | 27±3a$^{1}$ |
| Intercropping | CONTROL | 39±4d$^{3}$ | 150±12e$^{2}$ | 61±6f$^{2}$ | 12±1d$^{3}$ |
| | 25%NPK | 39±4d$^{2}$ | 153±13e$^{2}$ | 68±7ef$^{2}$ | 14±0.3d$^{2}$ |
| | 50%NPK | 47±5c$^{2}$ | 192±18d$^{2}$ | 71±7ef$^{1}$ | 19±2c$^{2}$ |
| | 100%NPK | 73±7b$^{1}$ | 217±21cd$^{1}$ | 73±7e$^{1}$ | 23±2b$^{1}$ |
| | Cropping | <.0001 | <.0001 | <.0001 | <.0001 |
| | Fertiliser Level | <.0001 | <.0001 | <.0001 | <.0001 |
| | Cropping* Fertiliser Level | <.0001 | 0.0881 | <.0001 | 0.0022 |

Mean values in each column followed by different letters indicate significant differences between treatments. Numerical values compare means of each cropping system at different fertiliser levels ($p<0.05$).

Table 5.4: The estimated potential of cowpea fertilised with four levels of NPK in 2014/15 (season 1) and 2015/16 (season 2) seasons to provide the recommended daily allowance of calcium, magnesium, iron, and zinc.

| 2014/15 (season 1) | Fertiliser Level | %RDA-Ca | %RDA-Mg | %RDA-Fe | %RDA-Zn |
|---|---|---|---|---|---|
| Sole cropping | CONTROL | 32±3cd[3] | 45±4de[4] | 68±7de[4] | 8±0.2de[4] |
| | 25%NPK | 34±3bc[3] | 50±5c[3] | 89±6c[3] | 10±0.2c[3] |
| | 50%NPK | 37±4b[2] | 55±5b[2] | 100±8bc[2] | 11±0.2b[2] |
| | 100%NPK | 41±4a[1] | 60±7a[1] | 114±11b[1] | 13±1a[1] |
| Intercropping | CONTROL | 24±2f[3] | 37±4g[4] | 51±3e[4] | 6±0.2f[4] |
| | 25%NPK | 25±2ef[3] | 39±4fg[3] | 65±3de[3] | 8±0.2e[3] |
| | 50%NPK | 27±2e[2] | 41±4ef[2] | 85±3cd[2] | 8±0.2de[2] |
| | 100%NPK | 31±3d[1] | 47±5cd[1] | 134±8a[1] | 9±0.2cd[1] |
| Cropping | | <.0001 | <.0001 | 0.0933 | <.0001 |
| Fertiliser Level | | <.0001 | <.0001 | <.0001 | <.0001 |
| Cropping* Fertiliser Level | | 0.8179 | 0.2185 | 0.0121 | 0.1883 |
| 2015/16 (season 2) | | | | | |
| Sole cropping | CONTROL | 106±10bcd[1] | 116±11c[2] | 392±32cd[3] | 132±12cd[2] |
| | 25%NPK | 97±9def[1] | 135±11a[1] | 430±40bc[2] | 150±12ab[2] |
| | 50%NPK | 118±11ab[1] | 135±13a[1] | 475±40b[2] | 151±14ab[12] |
| | 100%NPK | 122±11a[1] | 134±13ab[1] | 550±46a[1] | 153±16a[1] |
| Intercropping | CONTROL | 101±9cde[1] | 117±12c[2] | 128±12e[3] | 133±13cd[2] |
| | 25%NPK | 111±10ab[1] | 121±12bc[1] | 411±32c[2] | 138±12bc[2] |
| | 50%NPK | 85±7f[1] | 127±13abc[1] | 355±33d[2] | 113±11e[12] |
| | 100%NPK | 91±8ef[1] | 120±12c[1] | 430±43bc[1] | 120±12de[1] |
| Cropping | | <.0001 | 0.0114 | <.0001 | <.0001 |
| Fertiliser Level | | 0.7256 | 0.0254 | <.0001 | 0.1087 |
| Cropping* Fertiliser Level | | <.0001 | 0.4051 | <.0001 | 0.0011 |

Mean values in each column followed by different letters indicate significant differences between treatments. Numerical values compare means of each cropping system at different fertiliser levels ($p<0.05$).

## 5.5 Discussion

African leafy vegetable production could be improved through fertiliser application and intercropping (Mndzebele et al., 2020). The combined effect of fertiliser and intercropping yielded variable biomass in amaranth and cowpea. This was shown by a positive response of amaranth and cowpea in sole cropping to NPK fertiliser application (Figure 1). The high biomass at 100% NPK was due to amaranth and cowpea response to additional nutrient supply. The increase is attributed to the fact that NPK fertilisation increases crop productivity (Gellings and Parmenter, 2016), which is key in plant growth (Tongos, 2016). Our study corroborates with studies showing increased growth due to fertilisation such as sole-cropped spinach and lettuce (El-Saady, 2016; Mahlangu et al., 2016; Machado et al., 2021). There was more biomass on the sole cropping relative to intercropping. The lower yield was caused by high plant population as a result of close spacing in between amaranth and cowpea in an intercropping, therefore leading to competition for growth resources/factors amongst crops (Ndakidemi, 2006). Higher yields in sole cropping on the other hand can be attributed to high plant density (only in amaranth) and minimal competition for resources such as nutrients, moisture and sunlight (Eskandari et al., 2009). This study recorded comparable results with other studies done on cowpea and maize in which low component crop yields were recorded in an intercropping system due to low plant densities of individual crops than those in sole cropping (Manasa et al., 2018).

In addition to the role of fertiliser application in biomass production in ALVs, fertiliser also play key roles in the accumulation of nutrients in plant tissues, which is beneficial to the resource-poor rural households. For examples increased N led to increased uptake and thus accumulation in the leaves (Chaichi et al., 2015). These in turn reduced chronic malnutrition upon consumption, especially for the vulnerable groups of the society (Faber and Wenhold 2010; FAO, IFAD and WFP, 2015).

The higher mean values of macro elements total N, P, K, Ca, Mg, Na and S macro nutrient content in amaranth tissues at 100% NPK fertiliser levels in either sole or intercropping system, showed the benefits of NPK fertilization in the accumulation of nutrients in plant tissues (Table 5.1). Since nutrient elements co-function with several others (Marshner and Rengel, 2012), it was not surprising that NPK application increased tissue levels of N, P, K, Ca, Mg and Na at 100% NPK fertiliser application. Nutrients are known to synergistically interact with other mineral elements once taken up by plants. This study corroborates with other research findings that showed the synergistic response of macro nutrients to fertiliser application (Rietra et al., 2017; Adekiya et al., 2020; Barłóg et al., 2020). This was evidenced by the enhanced uptake of N, P, K, Mg, Ca and Na when NPK fertiliser application was applied to both crops in this study. The lowest nutrient accumulation in the control fertiliser application (Table 5.1) was an indication of the necessity of macro elements to induce nutrient accumulation. Comparing nutrient content of leaves from different data sources is however, challenging, due to sampling procedures, sample preparation as well as analytical methodologies. Observations, irrespective of the fertiliser level, have shown that N, P, K, Ca, Mg, Na, S values are within range in terms of nutrient content of amaranth and cowpea (Maseko et al., 2019). The increased nutrient content corresponded to more biomass accumulation. The significant differences in the concentration of macro elements between sole and intercropping has implications for improved nutrition in rural households with the use of intercropping technique. This means that in consideration of both crops in an intercrop, there would be collectively more nutrients for the rural households when compared to sole cropping. This could be explained by the corresponding higher biomass in sole cropping as opposed to intercropping (Banik et al., 2006). These are important findings in the context of farmers to identify the optimum fertiliser level for optimum nutrient accumulation as well as the best cropping system to attain these.

The iron, manganese, zinc and boron nutrient content in the 100% NPK of either sole cropping or intercropping in amaranth and cowpea leaves (Table 5.1 and 5.2). It is known that some nutrients can synergistically promote the accumulation of trace elements in plant organs. As observed in this study, NPK application though with other nutrients has been shown to improve micronutrient absorption by plants (Elayaraja, 2016; Fouda, 2016; Mandal et al., 2020; Wolde and Tomas, 2020). These results are consistent with the findings of Fageria and Baligar (1999), which showed strong interactions between NPK application and the micronutrients Fe, Zn and Mn in dry bean plants. As reported in this study, NPK application improved trace element concentrations in non-legume plant species. For example, NPK application was found to increase Zn concentration in corn, pearl millet and sorghum (Dev and Shukla, 1982; Aulakh and Malhi, 2005; Hekmat et al., 2019), just as it also synergistically affected the level of manganese in the same plants (Fageria, 2001; Aulakh and Malhi, 2005). Considered together, amaranth and cowpea have shown that supplementation with NPK can synergistically cause increased uptake and accumulation of trace elements such as Fe, Zn, Cu and B in leaves. From the point of view of health benefits, the greater accumulation of trace elements in amaranth and cowpea is likely to increase the antioxidant properties and thus raise rural household’s livelihoods. Given the high NPK nutrition, more micronutrient accumulation could increase vegetable yields in the long term. Under intercropping scenarios, there is efficient nutrient resources utilisation emanating from the interspecific interactions between leguminous and non-leguminous plants (Li et al., 2007).

The nutritional importance of dark-green leafy vegetables such as amaranth and cowpea are their contribution to the %RDA of Ca, Mg, Fe and Zn. The ability of amaranth and cowpea to meet the recommended daily allowance of calcium, magnesium and iron were considerably higher. For both vegetables, in terms of RDA of zinc supplied lower amounts for children under the age of five. However, there was a general increase %RDA from 0%NPK to 100% NPK fertiliser level. Sole cropped vegetables generally had more %RDA relative to intercropping. From a public health perspective, dietary sources of calcium, magnesium, iron, and zinc are important. Amaranth and cowpea provide more than 20%, 37%, 28%, and 6% of the RDA for children under the age of five for calcium, magnesium, iron and zinc respectively. The presence of oxalates, phytates and polyphenols in dark green leafy vegetables (Gupta et al., 2005) may, inhibit the absorption of iron (Zimmermann et al., 2005). Agricultural interventions to increase the supply and intake of calcium, magnesium, iron and zinc to meet RDA should be promoted. The results of this study showed that amaranth and cowpea were nutritionally diverse, hence provided variable RDA for calcium, magnesium, iron and zinc. Among the two, cowpea are the best sources of calcium, magnesium, iron and zinc especially in the second season. The results of the recommended daily allowance of amaranth and cowpea are comparable to others from literature e.g., results from Afolayan and Jimoh (2009) research, indicating that ALVs could contribute towards the nutrient requirements of the general population of the vulnerable. Therefore, cultivating them in either sole or intercropping together with fertiliser application and ultimately consuming them should be encouraged. Intercropping ALVs will optimise nutrient bioavailability to meet the RDA for calcium, magnesium, iron and zinc.

## 5.6 Conclusions

Fertiliser application increased yields of amaranth and cowpea, improved nutrient content as well the provided the %RDA of Ca, Mg, Fe and Zn. The application of 100% NPK fertiliser level increased biomass, hence more yields on amaranth and cowpea over the season. The lowest yield was obtained at the control (0% NPK) with the least seasonal biomass. More season biomass was obtained at the sole cropping when compared to the intercropping on all fertiliser levels on both seasons. There was more N, P, K, Ca, Mg, Na and S content with the addition of fertiliser on both seasons. There was increased iron, manganese, zinc and boron nutrient content at the 100% NPK fertiliser level of either sole cropping or intercropping in amaranth and cowpea leaves. The ability of amaranth and cowpea to meet the recommended daily allowance of calcium, magnesium and iron were considerably higher. More ability to meet the %RDA was obtained on the 100% NPK fertiliser level in both seasons. The study addresses the effect of fertiliser application improved nutrient content as well the provided the %RDA of Ca, Mg, Fe and Zn which are key in rural households. The study has implications for improved nutritional security in rural communities, using amaranth and cowpea as test crops. It is recommended that rural farmers should practice intercropping together the recommended 100% NPK fertiliser level to meet nutritional needs.

## 5.7 Acknowledgements

We are grateful to the Agriculture Research Council-Vegetable and Ornamental Plants for providing facilities and financial support to carry out the research. In addition, we are grateful to Mr Eric Mathebula for his support with statistical analyses.

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# CHAPTER 6 :

# SYNTHESIS

African leafy vegetables (ALVs) contribute to food and nutritional security in Africa, with cowpea and amaranth as some of the ALVs that are widely consumed by rural populations in South Africa. Cowpea is commonly cultivated, while amaranth is mostly harvested from the wild although sometimes cultivated. Recently, many smallholder farmers have started cultivating amaranth. However, poor soil nutrients especially N and P can significantly impair growth and thus significantly affect yields and nutritional quality of these vegetable crops. To address this challenge, sound cropping systems and adapted farm management practices including the use of appropriate doses of inorganic fertilisers, biological $N_2$ fixation crops, and increased phosphatase enzymatic activity may play key roles in increasing productivity. A combination of these mechanisms in an intercropping system promotes an integrated soil fertility management approach, which ensures that crop yields and their nutritional quality are improved. Legumes have the ability to contribute N to the soil through biological N-fixation, and promote the P-availability in soils through acid and alkaline phosphatase. Since the cultivation of ALVs by smallholders is dominated by cowpea and amaranth in most rural communities in South African, it is imperative to understand the contribution of legume based intercropping on the soil fertility and improving nutrient benefits of the non-legume crop. Two separate experiments were performed in this study to firstly (Chapters 2 and 3) address the effect of cropping systems and fertiliser application on the P and N nutrition of the tested crops, and to secondly (Chapters 4 and 5) assess the effect of fertiliser application on the edible biomass and nutritional quality of cowpea and amaranth leaves.

It was observed in chapter 2 that sole crops improved phosphatase enzyme activities as compared to their intercropped counterparts. In addition, the application of low levels of fertilisers between control (0% NPK) to 50% NPK significantly improved phosphatase activity, thereby, releasing/mobilising immobile P, and making it available for plant uptake. In some instances, fertiliser application at these rates resulted in significant differences between the vegetables in sole and intercropping systems. However, the application of NPK at higher rate of 100% NPK recorded lower available P, both in the soil and plant shoots. Although it demonstrated in this study that for the resource-poor farmers, cultivation of cowpea and amaranth can be carried out with minimal resources required, irrespective of cropping system, even though intercropping would be more sustainable.

Chapter 3 demonstrated higher N accumulation and fixation at zero or low fertiliser application rates (25% NPK and 50% NPK) for both sole and intercropped plants. However, nodulation was generally higher in sole cropping systems as compared to the intercropped plants. Furthermore, the study highlighted the role of cowpea and amaranth intercropping in improving the biomass yield of both crops at minimal fertilisation, through N contribution by the cowpea. For resource poor smallholder farmers, this implies that little or no N-input may be required when cowpea is intercropped with amaranth, hence, low production cost. The significantly higher micronutrient concentration (Fe and Zn) despite the minimal fertiliser application is an added advantage to reduce malnutrition among rural communities.

Chapter 4 demonstrated that irrespective of the cropping system or season, frequent harvesting consistently increased biomass of plants fertilised with higher NPK. Additionally, sole cropped plants accumulated higher biomass over the two seasons compared to their intercropped counterparts. Furthermore, fertiliser application rate of zero to medium also resulted in higher biomass accumulation, irrespective of the season and/or cropping system. These findings are particularly important because they relate to food availability for the communities that consume these vegetables, leading to food and nutritional security. For resource-poor farmers, frequent harvesting can ensure a continuous supply of nutritious vegetables. Overall, the land utilisation efficiency revealed benefits of intercropping relative to sole cropping systems.

Chapter 5 demonstrated higher macro- and micronutrient accumulation in plants fertilised with 50% NPK and 100% NPK, regardless of the cropping system. These findings have implications for both farmers who apply recommended or minimal fertilisers with regard to nutritional security. Malnutrition, especially among children under the age of five is an important health problem faced by many rural communities in South Africa, and the consumption of either cowpea, amaranth or both has a higher chance of reducing the incidence of malnutrition at lower cost.

## 6.1 Conclusions

This study has demonstrated the importance of intercropping cowpea and amaranth for sustainable integrated soil fertility management and enhancing the land utilisation efficiency to maximise productivity per unit area, since most smallholder farmers grow crops on smaller pieces of land. In addition, the production of cowpea and amaranth require little or no fertiliser application (0% NPK, 25% NPK and 50% NPK) to improve productivity, which is adapted to most smallholder farmers who often use little or no fertilisers that usually result in nutritionally poor produce. Overall, the production and consumption of cowpea and amaranth either grown solely or in an intercrop system has potential to improve the food and nutritional security among the rural poor communities at minimal cost.

## 6.2 Recommendations

The following recommendations were formulated based on results of this study:

- Cowpea and amaranth intercropping systems should be practiced to enhance integrated soil fertility management and increase crop productivity per unit area of cultivable land.
- Minimal fertiliser application should be practiced in sole of intercopped cowpea and amaranth system to increase productivity and nutritional quality of food produce.
- It is recommended that farmers should adopt intercropping to improve land utilisation efficinency as well as increased nutritional value
- To have a complete understanding of the benefits of legumes in an intercrop, it would be important to quantify the N-benefit to succeeding crops and the associated nutritional value. Additional research should be done on crop rotation as a follow up on the intercropping system to obtain a complete picture on the benefits over a long-term.
- To obtain more informative data, it would be key to conduct these experiments across different sites (multiple sites) and seasons where these crops are prevalent.
- It would be interesting to do studies and compare various symbiotic $N_2$ fixation methodologies
- Additional studies should be done to assess the other purposes of cowpea and amaranth in an intercropping to benefit a wider range of communities or population groups.
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## 6.3 Perspectives

- Since plants are exposed to multiple enzymes, there is need to do more studies on other enzymes such as β-glucosidase, ureases in the soils as well as microbial community to obtain a complete picture on the soil enzymatic activity.