UNIVERSITY OF BOTSWANA

BOTSWANA UNIVERSITY OF AGRICULTURE AND NATURAL RESOURCES



EFFECT OF NITROGEN FERTILIZER AND PLANTING DENSITY ON THE GROWTH AND YIELD OF INTERCROPPED SORGHUM AND COWPEA UNDER RAINFED CONDITIONS

A dissertation submitted in partial fulfillment of the requirements for the award of MSc

in Crop Science (Soil Sciences Stream)

By

Marang Mosupiemang (201000262)

November 2017

Supervisor: Prof. F. Pule-Meulenberg

Department of Crop Science and Production

Botswana University of Agriculture and Natural Resources

CERTIFICATION

Supervisor's name and signature	Date
Head of department's name and signature	Date

APPROVAL

Supervisor's name and signature	Date
Head of department's name and signature	Date
Dean of faculty's name and signature	Date

STATEMENT OF ORIGINALITY

The work contained in this dissertation was compiled by the author at the University of Botswana, Botswana University of Agriculture and Natural Resources between 2016 and 2017. To the best of my knowledge it contains no material previously published by another person or material which has been accepted by any other degree, certificate or diploma of any other University except where due acknowledgement and reference has been made in the text.

.....

Author's signature

Date

DEDICATION

I dedicate this dissertation to my father Kealogile Mosupiemang, my brothers, sister and friends who were always supportive to me. I also dedicate it to my late mother, may her soul rest in peace.

ACKNOWLEDGMENTS

Firstly I would like to thank my heavenly Father who has been my strength and inspiration. Through His grace and mercies I was able to win every challenge that came my way during my study period. I extend my sincere gratitude to my supervisor Prof. F. Pule-Meulenberg who has been my mother and mentor. Her advice, guidance, patience and constructive criticism made the completion of my research work and dissertation possible. I would like to acknowledge Professor Nnyaladzi Batisani whose project (African Union sponsorship) funded this research. SASSCAL, through project Task 316 awarded to Prof Pule-Meulenberg contributed some materials for this thesis. I acknowledge Prof. Munthali and Prof. Emongor who encouraged me to pursue my master's degree.

The hardwork of the Chief Technician Mr T. Mathowa during my field work is highly appreciated, not forgetting the efforts of Mr Patrick Mashiqa, Mr Mogomotsi Molefe and Miss Hilary Shoniwa for their assistance in taking photosynthesis measurements. I appreciate the work of my field assistants, I would not have managed the field work without their assistance. I would also like to thank BUAN lab technicians from Crop Science and Production, Food Science and Technology, Animal Science and Production and Basic Science Departments for their technical assistance. I give thanks to the Department of Agriculture Research for allowing me to use their machines and equipment. BUAN Crop Science staff and graduate students who gave me advice, I say thank you.

I give special thanks to my brother Mogomotsi Mosupiemang for always coming to school to check my progress. Finally I would like to give thanks to the rest of my family members and friends for their endless support and prayers during my study period.

TABLE OF CONTENTS

CERTIFICATION	i
APPROVAL	ii
STATEMENT OF ORIGINALITY	iii
DEDICATION	iv
ACKNOWLEDGMENTS	v
TABLE OF CONTENTS	vi
LIST OF TABLES	x
LIST OF FIGURES	xi
LIST OF SYMBOLS AND ABBREVIATIONS	xiii
ABSTRACT	xv
Chapter 1	1
1 Introduction	1
1.1 Justification	3
1.2 Objectives	4
1.3 Hypotheses	4
Chapter 2	5
2 Literature review	5
2.1 Plant nutrition and soil fertility	5
2.1.1 Photosynthesis assimilation in plants	5
2.1.2 Nitrogen application on increasing plant productivity	6
2.1.3 Legumes as a source of nitrogen	8
2.2 Crops and cropping system	9
2.2.1 Sorghum	9
2.2.1.1 Sorghum production	9
2.2.1.2 Nitrogen application in sorghum	10
2.2.1.3 Sorghum planting density	11
2.2.1.4 Sorghum under an intercrop system with cowpea and other legumes	12

2.2.2 Cowpea	13
2.2.2.1 Cowpea production	13
2.2.2.2 Cowpea in biological nitrogen fixation	13
2.2.2.3 Nitrogen application on cowpea	14
2.2.2.4 Cowpea planting densities	15
2.2.2.5 Cowpea under intercrop system	16
2.2.3 Intercropping	17
Chapter 3	19
3 Methodology	19
3.1 Experimental site	19
3.2 Soil sampling and analysis	19
3.3 Experimental setup	21
3.4 Land preparation and agronomic practices	21
3.5 Photosynthetic parameters measurements	22
3.6 Plant sampling and handling	22
3.7 Nutrient concentration	23
3.8 Nutrient uptake	23
3.9 Agronomic nitrogen use efficiency (NUE)	24
3.10 Yield and yield components	24
3.11 Land equivalent ratio (LER)	24
3.12 Statistical analysis	25
3.13 Soil Analysis results	25
Chapter 4	26
4 Results	26
4.1 Plant growth parameters	26
4.1.1 Effect of nitrogen application rate on photosynthetic parameters of cowpea	26
4.1.2 Effect of cropping system on photosynthetic parameters of cowpea	26
4.1.3 Effect of planting density on photosynthetic parameters of cowpea	27

4.1.4 Effect of nitrogen application rate on photosynthetic parameters of sorghum36
4.1.5 Effect of cropping system on photosynthetic parameters of sorghum
4.1.6 Effect of planting density on photosynthetic parameters of sorghum
4.1.7 Flowering in cowpea and sorghum42
4.2 Yield and yield components43
4.2.1 Effect of nitrogen fertilizer application rate on cowpea nodulation and biomass at
flowering43
4.2.2 Effect of cropping system on cowpea nodulation and biomass
4.2.3 Effect of planting density on cowpea nodulation and biomass
4.2.4 Effect of nitrogen fertilizer application rate on sorghum biomass yield47
4.2.5 Effect of cropping system on sorghum biomass yield
4.2.6 Effect of planting density on biomass yield
4.2.7 Sorghum grain yield and yield components
4.2.8 Cowpea grain yield and yield components
4.3 Nitrogen use efficiency and nutrients uptake
4.3.1.1 Nitrogen use efficiency in sorghum
4.3.2 Nitrogen use efficiency in cowpea
4.3.3 Effect of nitrogen fertilizer application rate on cowpea nutrient uptake55
4.3.4 Effect of cropping system on cowpea nutrient uptake
4.3.5 Effect of planting density on cowpea nutrient uptake
4.3.6 Effect of nitrogen fertilizer application rate on sorghum nutrient uptake
4.3.7 Effect of cropping system and planting density on sorghum nutrient uptake62
4.4 Efficiency of intercropping system
4.4.1 Land equivalent ratio (LER)
Chapter 5
5 Discussion
5.1 Effect of nitrogen application and planting density on the growth of intercropped sorghum
and cowpea69

5.1.1 Photosynthetic parameters of cowpea
5.1.2 Photosynthetic parameters in sorghum72
5.1.3 Days to flowering in sorghum and cowpea72
5.2 Effect of nitrogen application, cropping system and planting density on yield and yield
components
5.2.1 Cowpea nodulation and biomass yield73
5.2.2 Sorghum biomass yield75
5.2.3 Yield and yield components of sorghum and cowpea76
5.3 Effects of nitrogen application and planting density on the nutrient uptake of sorghum and
cowpea intercrop77
5.3.1 Nitrogen use efficiency in sorghum and cowpea77
5.3.2 Sorghum and cowpea shoot nutrient uptake78
5.3.2.1 Cowpea nutrient uptake78
5.3.2.2 Sorghum nutrient uptake
5.4 Efficiency of intercropping system
5.4.1 Land equivalent ratio (LER)
Conclusions
Recommendations
References

LIST OF TABLES

Table 3.1 Selected soil physical and chemical properties before planting	5
Table 4.1: Photosynthesis, stomatal conductance, internal CO ₂ , transpiration, WUE and	
chlorophyll of cowpea measured at the flowering stage	8
Table 4.2: Photosynthesis, stomatal conductance, internal CO ₂ , transpiration, WUE and	
chlorophyll of sorghum measured at the flowering stage	8
Table 4.3: Number of days to 50% flowering in cowpea and sorghum	2
Table 4.4: Cowpea nodulation and whole plant biomass at flowering stage	4
Table 4.5: Correlation between cowpea biomass at the flowering stage	7
Table 4.6: Below and above ground sorghum biomass yield measured at the flowering stage	
	8
Table 4.7: Sorghum yield and yield components	0
Table 4.8: Correlation of yield and yield components in sorghum	0
Table 4.9: Cowpea yield and yield components 51	1
Table 4.10: Correlation of yield and yield components in cowpea 52	2
Table 4.11: Nitrogen use efficiency (NUE) at flowering and at harvest in sorghum	3
Table 4.11: Nitrogen use efficiency (NUE) at flowering and at harvest in sorghum	3 4
Table 4.11: Nitrogen use efficiency (NUE) at flowering and at harvest in sorghum	3 4 6

LIST OF FIGURES

Figure 1.1: World fertilizer consumption during 2012 to 20141
Figure 4.1: Interactive effect of N fertilizer application rate and cropping system on: A)
photosynthetic rate, B) internal CO ₂ concentration, C) chlorophyll content, and D)
photosynthetic WUE in cowpea
Figure 4.2: The interaction of N fertilizer rate and planting density on: A) photosynthetic rate,
B) internal CO ₂ and C) transpiration rate on cowpea leaves
Figure 4.3: Interactive effect of N fertilizer application rate and cropping system on the
photosynthetic WUE in cowpea
Figure 4.4: The interaction of cropping system and planting density on internal CO ₂
concentration in cowpea plants, planted under varying N fertilizer application rates
Figure 4.5: Interactive effect of N fertilizer application rate, planting density and cropping
system on: A) photosynthetic rate, B) internal CO ₂ concentration and C) transpiration rate on
cowpea
Figure 4.6: The interactive effect of fertilizer rate, cropping system and plant density on
chlorophyll content in cowpea
Figure 4.7: Interactive effect of N fertilizer application rate and cropping system on: A)
stomatal conductance, B) internal CO ₂ concentration, C) transpiration rate and D)
photosynthetic WUE in sorghum leaves
Figure 4.8: Correlation analysis of: A) photosynthesis and stomatal conductance, B) stomatal
conductance and transpiration and C) photosynthesis and transpiration in sorghum at the
flowering stage
Figure 4.9: Interaction of fertilizer application rate and cropping system on nodule number
per plant in cowpea plants45

Figure 4.10: Interaction of fertilizer application rate and cropping system on cowpea shoot
biomass
Figure 4.11: Interaction of fertilizer application rate, planting density and cropping system on
cowpea shoot biomass per plant46
Figure 4.12: Interaction of fertilizer application rate and cropping system on sorghum shoot
biomass at the flowering stage
Figure 4.13: Interaction of fertilizer rate and cropping system on sorghum root biomass49
Figure 4.14: The interaction of cropping system and planting density on NUE of sorghum at
harvest
Figure 4.15: Interactive effect of N fertilizer application rate and cropping system on the
uptake of: A) N B) Ca, C) Mg, D) Fe, E) Cu and F) Zn in cowpea shoot57
Figure 4.16: The interactive effect of N fertilizer application rate, cropping system and
planting density on the uptake of: A) Ca, B) Mg and C) Na in cowpea shoot
Figure 4.17: The interactive effect of N fertilizer application rate, cropping system and
planting density on the uptake of: A) Fe and B) Zn in cowpea shoot61
Figure 4.18: The interaction of fertilizer application rate and cropping system on the uptake
of: A) N, B) P, C) K, D) Ca, E) Mg and F) Na in sorghum64
Figure 4.19: Interactive effect of N fertilizer application rate and cropping system on the
uptake of: A) Mn, B) Cu and C) Zn in sorghum shoot65
Figure 4.20: Interactive effect of planting density and cropping system on the uptake of: A) P,
B) K, C) Ca and D) Mg in sorghum shoot
Figure 4.21: Interactive effect of planting density and cropping system on the uptake of: A)
Na, B) Fe, C) Mn and D) Zn in sorghum shoot67

LIST OF SYMBOLS AND ABBREVIATIONS

Α	Photosynthetic rate
BNF	Biological nitrogen fixation
BUAN	Botswana University of Agriculture and Natural Resources
Ca	Calcium
CEC	Cation exchange capacity
Ci	Internal carbon dioxide gas
CO_2	Carbon dioxide
Cu	Copper
Ε	Transpiration rate
EC	Exchangeable acidity
FAO	Food agricultural organization
Fe	Iron
gs	Stomatal conductance
H_2O_4	Hydrogen peroxide
H_2SO_4	Sulphuric acid
ha	Hectare
HCl	Hydrochloric acid
IFAD	International Fund for Agricultural Development
К	Potassium
kg	Kilogram
LAN	Limestone ammonium nitrate
LER	Land equivalent ratio
LSD	Least significance difference

Mg	Magnesium
Mn	Manganese
Ν	Nitrogen
N ₂	Atmospheric nitrogen gas
Na	Sodium
Ndfa	Nitrogen derived from the air
NO ₃ -	Nitrate
NUE	Nitrogen use efficiency
Р	Phosphorus
рН	Potential of hydrogen
UNEP	United Nations Environment Programme
WUE	Water use efficiency
Zn	Zinc

ABSTRACT

Cereal-legume intercropping can be an alternative for smallholder farmers to mitigate the effects of climate change and to promote sustainable agricultural production. A field experiment was conducted at BUAN gardens during the 2015/2016 planting season to assess the effect of nitrogen fertilization, planting density and cropping system on the growth, yield and nutrient uptake of sorghum and cowpea. The experiment was arranged in a split-split plot design with five levels (0, 50, 75, 100, 125 kg N/ha) of lime ammonium nitrate (LAN) fertilizer as the main plot (with plants that received 0 kg N/ha being control plants), three cropping systems (sole cowpea, sole sorghum and intercrops) as sub plots and three planting densities (40 000, 53 333 and 66 667 plants/ha) as sub-sub plots. The results have shown that LAN application had little effect on the growth, yield and nutrient uptake of cowpea. The control cowpea plants had significantly ($p \le 0.01$) higher photosynthetic rates irrespective of planting density and cropping system. They also exhibited the highest stomatal conductance, WUE, nodule number, nodule biomass, root and shoot biomass and therefore higher nutrient uptake. Planting at a density of 53 333 plants/ha significantly ($p \le 0.01$) increased photosynthetic rate in cowpea. Intercropped cowpea had significantly lower nodule biomass, lower WUE and increased transpiration rates. Planting cowpea at a higher density of 66 667 plants/ha significantly ($p \le 0.05$) increased its WUE but decreased the uptake of some nutrients. In sorghum N application increased growth, yield and nutrient uptake. Nitrogen application rate of 50 kg/ha increased shoot and root biomass, NUE and nutrient uptake in sorghum. Intercropped sorghum had significantly higher photosynthetic rates and lower NUE than monocropped sorghum. The control sorghum plants showed significantly higher photosynthetic rates with intercropped plants exhibiting higher values. However, there were no significant differences in the yield of sorghum whether it is among the various levels of LAN applications, between the cropping systems or among different planting densities. Similar

to sorghum, all treatments did not lead to any increase in the yield of cowpea grain. In conclusion, the 2015/2016 cropping season was not a normal one due to the extreme soil temperature. Thus, increased growth, biomass yield and nutrient uptake in cowpea and sorghum caused by the interaction between LAN application and planting density did not translate to increased grain yields. Intercropping sorghum with cowpea was efficient than monocropping in resource utilization as shown by LER value that is greater than one. It is recommended that more leguminous plants with high nitrogen fixing capability should be included to fix more nitrogen and reduce fertilizer cost by smallholder farmers. Modelling should be included to help policy makers in planning to assist farmers on improving crop production to increase food security in a sustainable manner.

Chapter 1

1 Introduction

The use of mineral fertilizers in agricultural production is well known for increasing productivity and has contributed to one third of the increase in cereal production worldwide (Camara and Heinemann, 2006). Its success was quite remarkable in large farms of high potential irrigated areas in Latin America and Asia but it is not so in Africa, because of difficult environmental conditions and inability of farmers to invest in inputs (Muller-Samann and Kotschi, 1994). Therefore, Africa's fertilizer consumption remains low in the world (Camara and Heinemann, 2006), figure 1.1 shows fertilizer consumption in Africa as compared with other parts of the world. The smallholder farmers are mostly faced with challenges such as soil degradation which is promoted by continuous cropping, insufficient or no fertilizer use which account for low productivity (Hilhorst and Muchena, 2000). This forms part of the reason why smallholder farms in Sub-Saharan Africa are less productive. Despite being nonproductive, the world's smallholder farms provide more than 80 % of the food consumed in many developing countries greatly adding to poverty eradication and food nutrition security (IFAD and UNEP, 2013).





Figure 1.1: World fertilizer consumption during 2012 to 2014

Fertilizer use is necessary for sustainable agricultural production if smallholder farms are to be raised to levels that can sustain the growing population. History has shown that no region in the world has accomplished food security and significant increase in productivity without extensively expanding fertilizer use (African Union Fertlizer Summit, 2006). Haber's invention of industrial ammonia synthesis is one of the cornerstones of modern civilization and for many years agriculture has progressively come to rely on synthetic nitrogen fertilizers produced from ammonia, and is likely to continue to do so until there is an effective substitute (Cocking, 2005). It is possible to shift from synthetic nitrogen fertilizers to nitrogen fixing legumes only if increasing the role of legumes as a N source becomes a goal but this could be achieved in particular places by either increasing the amounts of N₂ fixed where legumes are already included in cropping systems, reducing the amount of N lost from legume-based cropping systems and/or increasing the amount of land planted under legumes (Crews and Peoples, 2004).

Cereal-legume intercropping is another way in which smallholder farmers can adopt to mitigate the effects of climate change and to promote sustainable agricultural production. Intercropping cereal with legumes is widespread among smallholder farmers due to the ability of the legume to cope with soil erosion and with declining levels of soil fertility (Matusso *et al.*, 2014). In general, cereal legume intercropping presents a solution to obtain higher yields per unit area, diversified food and reduced risk of crop failure under rainfed conditions (Khan *et al.*, 2012). A large part of the population in Sub-Saharan Africa earn their living from rainfed agriculture, and they mostly rely on smallholder subsistence agriculture for their source of income (Rockstrom, 2000). In cereal legume intercropping system, water availability determines productivity (Matusso *et al.*, 2014). However, smallholder farmers depend on rainfed rather than irrigated possibly because of high cost of providing supplementary irrigation system, and in Botswana, this is also due to shortage of surface water. Therefore sustainability of rainfed agriculture is a challenge in many agricultural production system. Increasing crop productivity with the available water and nutrients should be a major priority in order to improve food security.

1.1 Justification

Application of mineral fertilizers is the most popular method of increasing crop productivity and yield but mineral fertilizers are mostly out of reach for the majority of smallholder farmers. In addition if not applied judiciously, inorganic fertilizers may pollute the environment through greenhouse gas emission and nitrate pollution of groundwater resources. Thus, it is important to add optimal amounts of fertilizer under different environments. To minimize pollution risks, and to reduce costs, incorporation of legumes into cereal cropping system remains a viable option for the majority of smallholder farmers. Therefore information on the role legumes under intercropping system may help to assess the prospects of relying on legumes as a source of nitrogen fertilizer and ground cover. Previous studies have shown that often the benefit of incorporating legumes is not realized due to problems associated with planting population, for example, in Botswana the optimum density of sorghum/cowpea intercrop still remain unclear under Botswana conditions.

There are several studies made on levels of N that can increase the yield of sorghum, however, information on the level of nitrogen fertilizer application on sorghum/cowpea intercrop and their (fertilizer) effect on increasing crop productivity is still insufficient in Botswana. Knowledge about efficacies of different levels of nitrogen will enable the selection of the most suitable nitrogen application rate for sorghum and cowpea as intercrop and as monocrops. Using different plant densities will help to optimize plant populations. This study investigates the potential of intercropping cereals with legumes, the use of mineral fertilizers and plant

density optimization on increasing the productivity of smallholder farms in Southern Kgatleng and Southeast agricultural regions.

1.2 Objectives

The main objective of the study was to improve the yield of sole and intercropped sorghum and cowpea by use of nitrogen fertilizer and plant density optimization under rainfed conditions.

The specific objectives were:

- I. To evaluate the effect of nitrogen fertilizer and planting density on the growth and yield of sole and intercropped sorghum and cowpea.
- II. To determine the effect of planting density and nitrogen fertilizer on the nutrient uptake(N, P, K, Ca, Mg, Na, Fe, Mn, Zn and Cu) of intercropped sorghum and cowpea.

1.3 Hypotheses

- **1.3.1a Ho**: There are no significant differences in the growth and yield of N fertilized and intercropped sorghum and cowpea under different plant densities.
- **1.3.1b Ha**: There are significant differences in the growth and yield of N fertilized and intercropped sorghum and cowpea under different plant densities.
- **1.3.2a Ho**: There are no significant differences in nutrient uptake (N, P, K, Ca, Mg, Na, Fe, Mn, Zn and Cu) of intercropped sorghum and cowpea under different planting densities.
- **1.3.2b Ha**: There are significant differences in nutrient uptake (N, P, K, Ca, Mg, Na, Fe, Mn, Zn and Cu) of intercropped sorghum and cowpea under different planting densities.

Chapter 2

2 Literature review

2.1 Plant nutrition and soil fertility

2.1.1 Photosynthesis assimilation in plants

Photosynthesis is a metabolic process by which energy of sunlight is captured by chlorophyll to convert carbon dioxide (CO₂) and water into carbohydrates and oxygen gas (Sadava et al., 2011). In most situations photosynthesis is limited by available carbon dioxide and water (Hopkins and Huner, 2009). Besides water and carbon dioxide, nitrogen supply also has an influence on photosynthesis because deficiency of nitrogen leads to loss of green color in the leaves, decrease in leaf area and later decrease in the intensity of photosynthesis (Bojović and Marković, 2009). Accumulation of potassium cause stomatal opening and a dissipation of potassium may cause stomatal closing (Outlaw and Vlieghere-He, 2001). Stomatal movements which allow exchange of gases needed for photosynthesis are very sensitive to external environmental factors such as light, carbon dioxide, water status and temperature (Hopkins and Huner, 2009). In principle, increase in stomatal conductance which regulates gas exchange can allow plants under well-watered growth conditions to increase their CO₂ uptake and subsequently enhance photosynthesis (Kusumi et al., 2012). Reduced stomatal conductance results in greater water use efficiency and reduced evapotranspiration, and it may conserve soil water content and increased N mineralization (Drake et al., 1997). However reduced stomatal conductance at the leaf level does not necessarily mean that stand transpiration will be lower because there could be a compensatory increase in leaf area index and or a decrease in stomatal conductance is likely to increase leaf temperatures that would in turn, increase the driving force for transpiration (Drake et al., 1997; Leakey et al., 2009). In general reduction of stomatal conductance improves water balance, delays the onset of midday water stress and extends the period of most active photosynthesis (Drake et al., 1997). Various experiments has shown that stomatal responses are often more closely linked to soil water content than leaf water status (Prsa *et al.*, 2007). Water stress affect the plants in many different ways. An example is that as the plant becomes deficient in water the exchange rate of carbon dioxide and oxygen is slowed and as a result reduced exchange of these gases slows photosynthesis and plant growth is inhibited (Plaster, 2009). Intercropping using crops that are efficient at capturing water and exchanging it for CO_2 for biomass production can be a suitable water management strategy for resource poor farmers practicing agriculture under rainfed conditions (Chimonyo *et al.*, 2016b).

Under high temperature and high light intensity, C_4 plants exhibit higher photosynthetic and growth rates due to gains in the water, carbon and nitrogen use efficiency (Lara and Andreo, 2011). Therefore C_4 plants gains an advantage over C_3 plants by maintaining high rates of photosynthesis when the stomata are partially closed to conserve water during a period of water stress (Hopkins and Huner, 2009). The lower stomatal conductance of C_4 plants at any given CO_2 level means lower average transpiration and higher leaf temperatures in C_4 plants, which may increase heat related damage in C_4 plants compared with C_3 plants in the same habitat (Lara and Andreo, 2011).

2.1.2 Nitrogen application on increasing plant productivity

Nitrogen is an element key to soil fertility and the development of sustainable food production system. Although other minerals such as phosphorus, potassium and micronutrients are essential for plant growth, in most situations it is the N supply that determines crop yields (Noordwijk *et al.*, 2004). Plants usually depend upon combined and or fixed, forms of nitrogen, such as ammonia and nitrate because nitrogen is unavailable in its most prevalent form as atmospheric nitrogen (Al-Mujahidy s*et al.*, 2013). It is a yield limiting factor for most plants and hence controls growth thus making it an important element of plant productivity (Lea and

Morot-Gaudry, 2001). Plants require nitrogen as an essential part of protein and chlorophyll, so a plant well-supplied with N photosynthesizes much more efficient than a deficient plant (Plaster, 2009). Otieno *et al.* (2007) pointed that lack of adequate amounts of nitrogen in most soils puts a limitation on the farmers goals of increasing yield per unit area. The adoption of synthetic N fertilizers has increased the overall farm production of food crops by allowing farmers to grow cereals or other crops on land that would have otherwise been dedicated to fertility-generating legume rotations (Crews and Peoples, 2004). Currently, there is a high demand for maintenance and improvement of soil fertility thus making farming more fertilizer dependent for higher yields (Buah *et al.*, 2012). In Africa, average use of NPK fertilizers is 8 kg per hectare, which is only 10 percent of the world average (African Union Fertilizer Summit, 2006).

Low productivity in agriculture correlates with low quality of the soil resource base which is often caused by inherent or induced deficiencies of major nutrients, namely, nitrogen, phosphorus, and potassium, low nutrient holding capacities, high acidity and low organic matter (Bationo *et al.*, 2003). Strategic application of nitrogen improves the performance of most cropping system, for example, little amounts of available soil nitrogen or nitrogen fertilizer have shown to have a stimulatory effect on legume nodulation and nitrogen fixation (Giller and Cadisch, 1995). Most nitrogen is provided to cropping systems in the form of industrially produced nitrogen fertilizers (Al-Mujahidy *et al.*, 2013). In excess, applied N promotes lush vegetative growth, delays maturity, may reduce seed yield, and may suppress N-fixation (Ajeigbe *et al.*, 2010).

2.1.3 Legumes as a source of nitrogen

Legumes are very important both ecologically and agriculturally because they are responsible for a substantial part of the global flux of nitrogen from atmospheric N_2 to fixed forms such as ammonia, nitrate, and organic nitrogen (Zahran, 1999). Legumes are most widely recognized nitrogen fixing symbiosis because of their importance as a food source (Paul, 2007). The predominant nitrogen fixing species that have shown to make real contribution to cropping system are the rhizobia bacteria that form symbiotic associations with legumes (Giller, 2001).

It is now increasingly being realized that the intensive use of chemical fertilizers, so long promoted for higher productivity in agriculture, is harmful for soil and proves counterproductive in the long run (Ghosh, 2004). Therefore, research is shifting to replenishing the soil with inputs that are environmentally friendly such as biologically fixed N.

The world production of fixed nitrogen from dinitrogen for chemical fertilizer accounts for about 25%, of the earth's newly fixed N₂, and biological processes account for about 60% (Zahran, 1999). Including legumes in a cropping system can help to increase the level of soil nitrogen and consequently reducing the amount of soil N decline due to intensive cultivation (Dakora and Keya, 1997). The N contribution made by legumes in intercropping system is associated with the decay of crop residues, roots, nodules and fallen leaves (Noordwijk *et al.*, 2004). The use of nodulated legumes is often a more achievable and practical alternative for smallholder farming system as compared to expensive chemical fertilizers (Sanginga and Woomer 2009) because most smallholder farmers like to include legumes in their cropping system. Therefore, in areas where high costs of nitrogen fertilizer reduce its use, screening of legumes is essential for their potential effect in increasing productivity (Ghosh *et al.*, 2007).

Fixed nitrogen provided by biological nitrogen fixation is less likely to leach and volatilize because it is utilized in situ and therefore the biological process contributes an important and

sustainable input into agriculture (Dixon and Kahn, 2004). Significant decline in nitrogen fixation with soil fertility implies that the nitrogen advantage of legumes is likely to be small in smallholder systems characterized by poor soil fertility (Ojiem *et al.*, 2007). However in various parts of Africa yields are still very small such that there could be readily be doubled by only depending on biological nitrogen fixation as a nitrogen source (Giller and Cadisch, 1995).

2.2 Crops and cropping system

2.2.1 Sorghum

2.2.1.1 Sorghum production

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the most important cereal crops in Botswana commonly grown by smallholder farmers who rely on rainfall. Sorghum can perform very well even on drought conditions. Sorghum is the second most cultivated cereal by traditional sector and the top most cultivated cereal by the commercial sector in Botswana (Statistics Botswana, 2015). It is also a major crop in the warm low rainfall areas of tropics (Chantereneau and Nicou, 1994). It is tolerant to soil water deficits and efficient in soil nutrient uptake due to its fibrous root system (Abunyewa *et al.*, 2017). Grain sorghum approaches its genetic potential in seed number when growing conditions are favorable however unfavorable conditions like water stress or biotic stresses during the panicle development anthesis interval lower final seed numbers (Gerik *et al.*, 2004). During the growing season sorghum plant can survive periods of moisture stress by becoming dormant and then resuming growth when conditions become favorable (Sheaffer and Moncada, 2009). Smallholder farmers in Botswana mainly grow sorghum for its grain which is milled and consumed as porridge (bogobe) or as soft porridge (motogo). Its grain can be cooked as a rice (Mosutlhane). It can also be brewed to make traditional beer.

2.2.1.2 Nitrogen application in sorghum

Nitrogen is a major fertilizer required by sorghum. If the quantities of available P in soil are adequate applied N is usually completely available, thus deficiency in one nutrient results in reduced plant growth and less ability to make use of all other nutrients (Chantereneau and Nicou, 1994). Low usage of P in relation to N has been identified as one of the major factors limiting higher crop yields possibly because P acts to balance N in many ways for example, while N delays maturity P hastens it (Plaster, 2009; Ahmad, 2011). Several studies have shown that addition of fertilizer N to the soil led to yield increment in sorghum. According to Sibhatu et al. (2015), increasing amounts of N fertilizer resulted in increase in dry matter yield of intercropped sorghum. Thus, plots that received 61.5 kg N/ha over yielded the unfertilized treatments. They also found that not only did N application led to increase in yield, but also led to the vigorous growth of above ground parts of sorghum plants which enabled them to harvest ample solar radiation resulting in the corresponding increment of photosynthetic rate. Similarly Sawargaonkar et al. (2013) found that N fertilizer had profound linear effect on sorghum grain yield up to 90 kg/ha but, further increased N level did not improve grain yield proportionately. They also found significantly higher economic returns and benefit cost ratio (B:C) with the application of 90 kg N/ha, however increasing N level further to 120 and 150 kg/ha did not significantly affect productivity or income, but instead caused severe lodging at harvest in the plots with 150 kg N/ha. Sibhatu (2016) found that addition of N fertilizer resulted in significant variation in panicle length of sorghum intercropped with cowpea, hence application of N fertilizer improved panicle length as compared to nil N application probably due to the attributes of N fertilizers to increasing the vegetative growth of crops.

Turgut *et al.* (2005) also found that under irrigated conditions the lowest forage and dry matter yield of sorghum was associated with treatment which was not applied nitrogen but forage and dry matter yield increased as the amount of N increased but decline in yields occurred at

application rate of 200 kg N/ha, indicating that optimum N rates were reached. They also found that the highest seed yield was obtained in plots fertilized with 150 kg/ha N.

Buah and Mwinkaara (2009) found that plant density did not affect response of sorghum to applied nitrogen therefore grain yield in response to applied nitrogen was the same for a range of sorghum plant densities. Furthermore increasing N application in sorghum to 120 kg N/ha significantly increased chlorophyll content while the application of no N fertilizer resulted in the lowest chlorophyll content and consequently more sorghum biomass at 50% flowering was found on the nitrogen fertilized treatment as compared to the zero nitrogen treatment (Buah and Mwinkaara, 2009).

2.2.1.3 Sorghum planting density

Plant density is one of the most important cultural practices determining grain yield and it is therefore essential to consider when practicing intercropping (Nthabiseng *et al.*, 2015). In areas where crop growth is constrained by limited precipitation, optimizing planting density is critical as high population densities may deplete most of the available moisture before the crop matures while low density may leave moisture unutilized (Bayu *et al.*, 2005). Fernandez *et al.* (2012) highlighted that the responses to narrow-row spacing in grain sorghum have been varied and inconsistent mostly because of environmental conditions. High density is undesirable because it encourages inter plant competition for resources (Tajul *et al.*, 2013). Buah and Mwinkaara (2009) found that chlorophyll content in the leaves of sorghum was significantly affected by plant density, that is, the highest sorghum density of 133 300 plants/ha resulted in the lowest chlorophyll concentration and the highest chlorophyll content was found at lower densities. When cowpea was intercropped with sorghum, cowpea yield was found to be affected by the change in sorghum population, thus reducing sorghum plant population

improved cowpea yield by between 5.6 and 35.1% but increasing sorghum population increased its overall yield hence the results showed that this had a negative effect of cowpea yield (Chimonyo *et al.*, 2016a).

2.2.1.4 Sorghum under an intercrop system with cowpea and other legumes

Studies on sorghum planted as an intercrop with legumes are many and varied depending on factors such as the density of the intercropped legume and environmental conditions. Sorghum when intercropped with Bambara groundnut (*Vigna subterranea*) produced higher yields because the two combinations had a greater light interception over a larger surface area and higher and wider exposure to sunlight (Karikari *et al.*, 1999). The seed yield of intercropped sorghum compared to sole sorghum increased by about 100% while that of intercropped soybean increased by only 2.2% implying that soybean did not benefit from intercropping to the same degree as sorghum (Ghosh *et al.*, 2006).

Amedie *et al.* (2004) found that when sorghum was intercropped with different legumes such as soybean, groundnut, French bean and cowpea, significantly lower grain yield was obtained in intercropped cowpea while the other intercropped legumes had significantly higher grain yields compared with cowpea. Thus implying that sorghum produce different effect on different legumes when intercropped. Moreover intercropping of grain sorghum with cowpea increased sorghum grain yield per plant revealing the beneficial effect of intercropping of grain sorghum with cowpea (Refay *et al.*, 2013). On the contrary Karanja *et al.* (2014) found that sorghum produced the highest grain yields (of 2729 kg/ha and 3011 kg/ha) when grown as a monocrop than intercropped with cowpea in both the respective seasons. Intercropping has been found to affect nutrient concentrations. Musa *et al.* (2012) found that intercropping significantly increased Ca, Mg, Cu, Mn, and Fe contents of sorghum seeds, while it had no

effect on the concentration of P, K, Na, and Zn in the sorghum seeds. Makoi *et al.* (2010) found that intercropping sorghum with cowpea reduced the concentration of Fe, Ca, P, K, Mg, Cu and Zn in cowpea rhizosphere and thus leading to a decrease in their concentration in plant tissue.

2.2.2 Cowpea

2.2.2.1 Cowpea production

Cowpea (*Vigna unguiculata* (L.) Walp) is one of the highly grown pulses in Botswana and it is a source of income for many smallholder farmers. It is also a commonly grown food legume by traditional farmers in Sub-Saharan African countries possibly because of its relatively wide adaptation to drought and low-nutrient environments (Pule-Meulenberg *et al.*, 2010). Its shade tolerant characteristic makes it to be very compatible as an intercrop with a number of cereals (Ajeigbe *et al.*, 2010). Its quick growth and rapid ground cover has made it an essential component of sustainable subsistence agriculture (Ajeigbe *et al.*, 2010).

2.2.2.2 Cowpea in biological nitrogen fixation

The nitrogen fixing ability of cowpea is well known and has been found to fix N_2 even in low nutrient soils (Ajeigbe *et al.*, 2010). The total N contribution in cowpea varies with the amount of N_2 fixed and the proportion of the plant that is harvested (Thom *et al.*, 2008). According to Sprent *et al.* (2010) legumes are very good scavengers of soil nitrogen and do not necessarily enrich soil nitrogen so Ndfa (nitrogen derived from air) will depend not only on the efficiency of the nodules, but also on soil fertility and other factors. The variation in the amount of nitrogen fixed by various legumes could be attributed to the amount of plant biomass produced by the various legumes because the higher the biomass produced, the higher the amount of N fixed (Adeleke and Haruna, 2012). Nodulation has been found to be affected by the stage of growth of the plant for example at the flowering stage cowpea nodules are at the peak of N_2 fixation and nodules had not started senescing to release the N they contain (Marandu *et al.*, 2014).

When cowpea and groundnut were planted in rotation with sorghum, cowpea accumulated almost twice as much N than did groundnut and total N yields in the above ground part of groundnut and cowpea were atleast 31 and 59 kg N/ha, respectively thus they increased soil N availability and N uptake by succeeding sorghum but the N effect of cowpea was higher than that of groundnut (Bado *et al.*, 2006). In a similar experiment by Adeleke and Haruna (2012) when soybean, cowpea, lablab, and groundnut were planted in rotation with maize the result showed increase in the total soil N, after planting any of the four legumes however previous lablab plots had the highest total N of 0.49% followed by groundnut, cowpea, soybean and fallow plots with 0.42%, 0.38, 0.29 and 0.26%, respectively.

2.2.2.3 Nitrogen application on cowpea

Legumes are known to be less reliant on mineral nitrogen for their growth and productivity because they are able to fix their own nitrogen in symbiosis with rhizobia. Furthermore high nitrogen content in soil is known to reduce the nitrogen fixing ability of rhizobia. Sibhatu *et al.* (2015) found that zero N treatment had significantly higher number of nodules than any of the N levels while the lowest number was recorded from application of 61.5 kg N/ha therefore increasing rate of N reduced number of nodules per plant. Singh and Usha (2003) found that the number of nodules per plant varied from 1-20 and 1-14 when cowpea plants were fertilized with nitrogen at an application rate of 40 and 120 kg/ha respectively indicating the overall inhibition of nodule number due to high level of N. They further found that high N fertilizer

level of 120 kg/ha reduced nodule mass. Oroka (2010) found that nitrogen fertilizer application of 0, 15, 30 and 40 kg/ha had no significant effect on the number of nodules of cowpea when it was planted as either monocrop or intercropped with rice.

Application of N fertilizer was found to enhance the number of seed per pod thus application of zero fertilizer produced lower number of seeds pod^{-1} than the others but number of seed per pod of sole cowpea exceeded 37% of the intercropped (Sibhatu *et al.*, 2015). Abayomi *et al.* (2008) found that plant height, number of leaves per plant and dry matter at 50% flowering were highest with the highest level of fertilizer but nodulation was significantly reduced by successive application of NPK fertilizer from 0-0-0 to 60-30-30 NPK/ha.

2.2.2.4 Cowpea planting densities

Plant density is one of the factors to be considered in cowpea production. The optimization of planting density is paramount since it has been found that often N-fixed amounts were low due to sparse plants in the field (Naab *et al.*, 2009). Information on the effect of planting density on some growth parameters is sometimes contradictory for example; according to Makoi *et al.* (2009) increasing cowpea density reduced dry matter content of the shoots, roots, pods and nodules but it significantly increased grain yield. When cowpea was intercropped with maize, its dry matter production in sole cropping increased with increasing density (Moriri *et al.*, 2010). Omae *et al.* (2014) found out that the highest crop density of 29 412 to 32 418 plants/ha increased cowpea biomass by 132% and grain yield by 97%. Cowpea planting density was found not to significantly affect nodule number of intercropped cowpea (Sibhatu *et al.*, 2015). Makoi *et al.* (2009) found out that increasing plant density decreased percentage of N fixed in all organs of cowpea.

2.2.2.5 Cowpea under intercrop system

Reports on the performance of cowpea grown as sole/intercropped with cereals are many and most of them has shown that cowpea is suppressed by the companion cereal crop. According to Alhaji (2008) cowpea performed better when grown as a sole crop than when grown with maize in an intercrop system, that is, the number of pods per plant, pod weight and seed yield were significantly reduced when intercropped with maize. Similarly Ibrahim et al. (2014) found that cowpea performed better when grown as a sole crop than when grown in an intercrop with sorghum because the number of pods per plant, pod weight and seed yield were significantly reduced when intercropped with sorghum. Shata et al. (2007) also found that the highest yield of cowpea was obtained when cowpea was sown alone while lowest yield was obtained when cowpea was sown with millet and maize as intercrops. Karanja et al. (2014) found that in season I and II, cowpea intercropped with sorghum produced significantly lower grain yields ranging from 56%-64% than their corresponding sole crops. Ewansiha et al. (2014) found that intercropping influenced days to 50% flowering in cowpea that is intercropped cowpea took more days to flower than sole cowpea hence intercropping increased number of days to flowering by 8%. Intercropping has been found to influence the nodule number/plant of cowpea. Sole cowpea produced significantly higher nodule number as compared to intercropped cowpea in which the low nodule number in intercropped cowpea could be due to the shading effect of sorghum that hinders N₂-fixation (Sibhatu et al., 2015). Similarly cowpea when grown as monocrop it fixed greater N compared to when it was intercropped with sorghum and consequently grain yield of cowpea was significantly greater in cowpea monocrop relative to when it was intercropped with sorghum (Makoi et al., 2009).

2.2.3 Intercropping

Intercropping is the practice of growing more than one crop species together at the same time. In intercropping, there is usually one main crop and one or more added crop(s), with the main crop being of the most importance for economic or food production reasons and the two or more crops in an intercrop are usually from different species and different plant families, or less commonly they may be simply different varieties or cultivars of the same crop (Lithourgidis et al., 2011). In intercropping, there is an efficient utilization of resources because of different crop species which have different requirements of light, water and nutrients (Martin et al., 2006). Increased efficiency on the use of resources in intercropping may occur because the component crops use the resources either at different times, acquire resources from different parts of the soil or in different forms (Echarte et al., 2011). The differences in the depth of rooting, lateral root spread and root densities are some of the factors of competition between the component crops in an intercropping system for water and nutrients, and hence input use efficiency (Ghosh et al., 2006). The success of an intercrop system depends on understanding the physiology of the species to be grown together, their growth habits, canopy and root architecture, and water and nutrient use (Machado, 2009). Therefore selecting crops that differ in competitive ability in time or space is necessary for an efficient intercropping system as well as decisions on planting time, planting density, and in what arrangement (Lithourgidis et al., 2011). Competition for available resources is a major concern in intercropping because it determines the component crop yield and productivity of the system. According to Tsubo et al. (2005) in most cereal-legume intercropping, cereal crops form higher canopy structures than legume crops, and the roots of cereal crops grow to a greater depth than those of legume crops. Since lack of arable land is a constraint, optimizing intercropping performance can assist in effective use of space and nutrients (Nthabiseng et al., 2015). Nowadays intercropping is receiving attention because it offers potential advantages for resource utilization, decreased

inputs and increased sustainability in crop production (Ghosh *et al.*, 2006). It also offers the farmer with numerous options for returns from land and labour, often increases efficiency in which scarce resources are used, and reduces dependence upon a single crop that is susceptible to environmental and economic fluctuations (Bationo *et al.*, 2003). Traditionally, intercropping aimed to avoid dependence on a single crop, obtain a variety of products from the same piece of land, improve efficiency of the available resources and increase farm income from small holdings (Rashid *et al.*, 2004). Intercropping can provide many benefits through increased efficiency of land use, enhancing the capture and use of light, water and nutrients, controlling weeds, insects, diseases and increasing the length of production cycles (Alla *et al.*, 2014).

When intercropping is well managed, intercrops will usually yield more than the same crops grown separately (Martin *et al.*, 2006). During intercropping, cereal crops usually germinate and create an effective root system faster than legumes resulting in reduced concentration of soil N in a way that nodulation and nitrogen fixation is not inhibited (Giller and Cadisch, 1995).

Intercropping cereals with legumes is highly advantageous to the farmers since the cereal crop will benefit from the nitrogen fixed if the legume matures earlier than the cereal crop and residual N will also be left in the soil for use by a subsequent crop grown on the same portion of land during the next cropping season (Kombiok *et al.*, 2012). Nitrogen accumulated by root nodule bacteria may pass into the sap of the host plant and be used directly, it can also be stored in bacterial cell and be released when the nodules decay or diffuse into the soil and be absorbed by the roots of other plants (Miller, 2007). Moreover relatively dense roots system of a cereal such as sorghum may trap the nitrogen in soil thereby reducing losses by leaching (Chantereneau and Nicou, 1994).

Chapter 3

3 Methodology

3.1 Experimental site

The study was conducted at the Botswana University of Agriculture and Natural Resources (BUAN) gardens under rainfed conditions during the 2015/2016 growing season. This site is located at the latitude of 24⁰ 33' South and longitude of 25⁰ 54' East in Sebele, Gaborone in the southern part of Botswana. The climate is semi-arid, characterized by low precipitation (about 450 mm per annum). Botswana receives summer rainfall, with the rainy season commencing around October and ending in April. Rainfall in Botswana is limited and highly erratic. The 2015/2016 growing season was characterized by low rainfall and extremely high temperatures (with an average temperature of 29.2 °C from December 2015 to May 2016). During the planting season rainfall was erratic and there was dry spells for several weeks. The potential evapotranspiration of crops exceeded available rainfall during the rainy season therefore soil moisture was a limiting factor for crop production.

3.2 Soil sampling and analysis

The soil was sampled from five different spots in the field using a zigzag pattern and subsamples were mixed to make a composite sample. The soil was air dried and sieved with a 2 mm mesh sieve. Soil analysis were done for pH, EC, CEC, N, P, K, Ca, Na, Mg, texture and organic carbon.

Soil pH was determined in 0.01 N calcium chloride solution using 1:5 soil to calcium chloride ratio while soil EC was determined using portable EC meter in 1:5 soil to distilled water ratio as described by Reeuwijk (2002). Exchangeable cations (Mg, Na, Ca and K) were extracted in ammonium acetate as described by (Reeuwijk, 2002). After extraction the concentration of
ions were analyzed using Inductively Coupled Plasma -Optical Emission Spectrometer (Optima 2100 DV). Total cation exchange capacity (CEC) was measured after the soil was extracted with ammonium acetate and then distilled and titrated with 0.01N hydrochloric acid as described by Reeuwijk (2002). Available phosphorus in soil was extracted according to Bray and Kurtz method as described by (Reeuwijk, 2002). Organic carbon was determined by modified spectrophotometric Walkley-Black method as described by Souza *et al.* (2016). Soil texture was determined using bouyoucos hydrometer method as described by Estefan *et al.* (2013).

Percentage nitrogen was determined by the micro- Kjeldahl procedure which involve digesting in sulphuric acid-selenium mixture and hydrogen peroxide. The digest was distilled and ammonium was trapped into boric acid and titrated with hydrochloric acid as described by (Reeuwijk, 2002). Percentage nitrogen was calculated using the formula by Estefan *et al.* (2013) below:

% Nitrogen =
$$\frac{14.007 \text{ x} (\text{Va} - \text{Vb}) \text{x N x V}}{\text{W x t x 1 000}} \text{x 100}$$

Where:

14.007: atomic weight of nitrogen

Va: Volume of acid used for sample titration

Vb: Volume of acid used for blank titration

N: Normality of acid (0.01 N HCl)

V: Total volume of the digest (200 mL)

W: Sample weight in grams

t: volume of the digest sample used for distillation (25 mL)

3.3 Experimental setup

The experiment was a split-split block design with five levels of N fertilizer as the main plot. Nitrogen was added at levels of 0, 50, 75, 100 and 125 kg/ha from Lime ammonium nitrate (LAN). Plants that received 0 LAN were the control plants. Three levels of cropping system, that is, whether crops (sorghum var segaolane and cowpea var Inia 37) were planted as sole or intercrops was the sub-plots within the main plot. Within the subplots there were the sub-subplots which were made of three different planting densities (40 000, 53 333 and 66 667 plants/ha). Sorghum and cowpea were planted at an inter-row spacing of 0.75 m and the intra row spacing (of 0.4, 0.3 and 0.25 m) respectively to give different plant populations (of 40 000, 53 333 and 66 667 plants/ha respectively). The main plots were 11 m x 8.5 m, the subplots 2.5 x 11 m, the sub-sub plots 2.5 x 3 m in size making 45 treatment combinations replicated three times. The sub subplots were separated by 1 m in between while the blocks and the main plot were separated by 2 m in between. There was a total of five rows per sub sub-plot.

3.4 Land preparation and agronomic practices

The experimental site was disc ploughed using a tractor. A blanket application of single super phosphate (10.5%, P₂O₅, at a rate of 85 kg/ha) was done prior to planting in all the treatments at a rate recommended by Ministry of Agriculture. Three seeds were sown per hole and three weeks after emergence plants were thinned to one plant per hole. Supplementary irrigation was provided for emergence thereafter plants were not irrigated further. The nitrogen fertilizer treatment was applied at week five because it is the period when the nitrogen demand for the main crop sorghum is at peak. Side dressing of nitrogen fertilizer was applied in a row 5 cm away from roots. Weeds were controlled by hoeing. Sorghum stemborer and sorghum aphids outbreaks were controlled by foliar application of cypermethrin and dimeto 40 EC respectively. Birds were scared by field assistants from grain filling stage until harvest. Lodging in sorghum

due to strong winds was controlled by making the plant stand upright by supporting the plant with soil on the stem.

3.5 Photosynthetic parameters measurements

The number of days to flowering were recorded when 50% of the plants in a sub subplot had flowered. Photosynthesis rate (A), stomatal conductance (gs), transpiration rate (E) and internal carbon dioxide concentration (C_i): were measured using portable photosynthesis system (LI-6400/LI6400XT model). Measurements were taken from three plants from inner rows on each sub-subplot during the flowering stage. On sorghum, measurements were made on the flag leaf and on cowpea it was done on the 3 fresh photo leaves on each plant. Photosynthesis water use efficiency was calculated by dividing leaf photosynthesis by leaf transpiration (Wilson *et al.*, 2012). The chlorophyll content was measured from the flag leaf of sorghum and from fresh photo leaf in cowpea using chlorophyll meter (SPAD -502 plus, Konica Minoita) at the flowering stage from three plants selected from inner rows of each sub-sub plot.

3.6 Plant sampling and handling

Destructive sampling was done at the flowering stage on the same plant that measurements of photosynthesis, stomatal conductance, transpiration and carbon dioxide were taken. These plants were carefully uprooted with soil still attached to the roots, making an effort to dig out majority of the root system including the nodules, using a spade. Roots were washed gently with a clean water avoiding the root nodules from falling. Root nodules were detached and their number was recorded per cowpea plant. The uprooted plants (sorghum and cowpea) were separated into roots and shoots. After separation, shoots, nodules and roots were oven dried at 60 ^oC until constant weight for dry matter determination. The dried shoots were ground into fine powder for mineral analysis.

3.7 Nutrient concentration

Macro and micro nutrient (N, P, K, Ca, Mg, Fe, Cu, Na, Mn and Zn) concentration on sorghum and cowpea shoot were determined by the micro-Kjeldahl procedure which involve digestion in a mixture of concentrated H₂SO₄ and H₂O₂. The plant digests were analyzed using Inductively Coupled Plasma -Optical Emission Spectrometer (Optima 2100 DV) to find the concentration of these minerals (P, K, Ca, Mg, Fe, Na, Mn, Cu and Zn). To find total nitrogen the plant digests were distilled and titrated with 0.01 hydrochloric acid then % nitrogen was determined by calculation using the formula by Estefan *et al.* (2013) below:

% Nitrogen =
$$\frac{14.007 \text{ x} (\text{Va} - \text{Vb}) \text{x N x V}}{\text{W x t x 1 000}} \text{x 100}$$

Where:

14.007: atomic weight of nitrogen

Va: Volume of acid used for sample titration

Vb: Volume of acid used for blank titration

N: Normality of acid (0.01 N HCl)

V: Total volume of the digest (200 mL)

W: Sample weight in grams

t: volume of the digest sample used for distillation (25 mL)

3.8 Nutrient uptake

The uptakes of N, P, K, Ca, Mg, Fe, Na, Zn, Cu and Mn per plant were calculated by multiplying nutrient concentration in shoot by the shoot biomass as described by Malik *et al.* (2013).

3.9 Agronomic nitrogen use efficiency (NUE)

It was calculated from the shoot yield at flowering and from the total grain yield at harvest for both cowpea and sorghum using the formula by (Wortmann *et al.*, 2007); where agronomic nitrogen use efficiency $(kg/kg) = (Y_f - Y_C) / (Fappl)$ where Y_F and Y_C denote yields (kg/ha) in the treatment where nitrogen fertilizer have been applied and in the control plot where no nitrogen has been applied respectively, and Fappl is the amount of nitrogen fertilizer applied (kg/ha).

3.10 Yield and yield components

After sorghum plants reached physiological maturity, panicles were harvested from the two middle rows of each sub-sub plot, and panicle length and weight were measured. After threshing, 1000 seed weight measured and then total grain yield determined. For cowpea, at physiological maturity pods were harvested from the two middle rows from each sub-sub plots then pod length, pod weight and number of seeds per pod were measured from 10 pods selected randomly. After threshing 100 seed weight was measured and total grain yield was determined.

3.11 Land equivalent ratio (LER)

Land equivalent ratio which determines the efficiency and/or the advantage of an intercropped system was calculated as:

LER = (Yab/Yaa) + (Yba/Ybb)

Where Yaa and Ybb are yields of sole crops of a and b and Yab and Yba are yields as intercrops of a and b where values of LER greater than 1 are considered advantageous (Egbe, 2010).

3.12 Statistical analysis

All measured variables were analyzed using three Way analysis of variance (ANOVA) using STATISTICA programme version 13.0 after checking for normality. Treatment means and interaction effects were compared using the Fisher's Least significant difference (LSD) procedure at significance level of 5%. Pearson's correlation coefficient were used to test if there was any associations.

3.13 Soil Analysis results

The soil textural class of the experimental site was loamy sand with very low organic carbon and N content and higher P and K content and medium Ca content (Table 3.1).

Soil characteristic	Value
pH (CaCl ₂)	6.55
EC (µs/cm)	82.4
CEC (meq/100g)	1.73
Organic carbon (%)	0.25
Textural class	Loamy sand
Sand (%)	80.67
Silt (%)	11.69
Clay (%)	7.64
Total N (%)	0.13
Available P (ppm)	48.87
Exchangeable K (mg/kg)	212.79
Exchangeable Ca (mg/kg)	235.81
Exchangeable Mg (mg/kg)	57.84
Exchangeable Na (mg/kg)	19.24

Table 3.1 Selected soil physical and chemical properties before planting

Chapter 4

4 Results

4.1 Plant growth parameters

4.1.1 Effect of nitrogen application rate on photosynthetic parameters of cowpea

Photosynthetic parameters in cowpea were significantly affected by nitrogen application rate. Control plants had significantly higher rate of photosynthesis, stomatal conductance and WUE compared to plants that received N (Table 4.1). Internal carbon dioxide was high on the plants that received 0, 100 and 125 kg N/ha but lower on plants that received 50 and 75 kg N/ha (Table 4.1). Application of 75 kg N/ha increased the rate of transpiration while the application 100 kg N/ha reduced it. Cowpea plants applied with 50 kg N/ha had significantly higher chlorophyll content. Higher nitrogen application rates (100 and 125 kg/ha) reduced the chlorophyll content (Table 4.1).

4.1.2 Effect of cropping system on photosynthetic parameters of cowpea

Cropping system significantly ($p \le 0.001$) affected the rate of transpiration and WUE. Intercropped cowpea had higher rate of transpiration than monocropped cowpea, while monocropped cowpea had higher WUE than intercropped cowpea (Table 4.1). The rate of photosynthesis, stomatal conductance, internal CO₂ and chlorophyll content were not significantly affected by cropping system (Table 4.1).

4.1.3 Effect of planting density on photosynthetic parameters of cowpea

Planting density significantly affected the rate of photosynthesis and WUE in cowpea (Table 4.1). A significantly ($p \le 0.01$) higher photosynthetic rate was found on crops that were planted at a moderate planting density of 53 333 plants/ha. The WUE increased with increasing planting density. The lowest density of 40 000 plants/ha exhibited a lower WUE (Table 4.1). Planting density had no significant effect on the rate of stomatal conductance, transpiration, internal CO₂ and chlorophyll content in cowpea (Table 4.1).

Treatments	Photosynthesis (A) μmol CO ₂ m ⁻² s ⁻¹	Stomatal conductance (gs) mol m ⁻² s ⁻¹	Internal CO ₂ (C_i) µmol m ⁻² s ⁻¹	Transpiration (<i>E</i>) mmol H ₂ O m ⁻² s ⁻¹	Water use efficiency (WUE) (µmol CO ₂ / mmol H ₂ O)	Chlorophyll (SPAD reading)
Fertilizer rate (kg N/ha)						
0	28.54±0.39a	1.40±1.16a	251.46±7.74a	7.11±0.12d	4.23±0.07a	51.41±0.50b
50	23.23±0.47c	0.71±0.19b	225.12±8.98b	7.78±0.14c	3.45±0.08c	53.87±0.54a
75	23.29±0.47c	0.80±0.20b	227.55±9.24b	9.38±0.15a	2.22±0.08e	52.74±0.71ab
100	25.46±0.40b	0.73±0.18b	265.76±8.43a	6.56±0.13e	4.01±0.07b	48.95±0.44c
125	23.12±0.31c	1.01±0.16ab	266.79±7.54a	8.18±0.12b	2.99±0.07d	49.65±0.48c
Cropping system						
Monocrop cowpea	24.49±0.29	0.95±0.11	243.36±5.27	7.37±0.08b	3.50±0.05a	51.65±0.35
Intercrop cowpea	24.97±0.27	0.92±0.11	251.32±5.37	8.22±0.08a	3.26±0.05b	51.00±0.36
Plant density (plants/ha)						
66 667	24.29±0.35b	0.99±0.14	248.69±6.62	7.64±0.10	3.45±0.06a	50.73±0.43
53 333	25.46±0.33a	0.93±0.14	238.93±6.53	7.92±0.10	3.43±0.06a	51.82±0.43
40 000	24.44±0.34b	0.87 ± 0.14	254.40 ± 6.40	7.84±0.10	3.26±0.06b	51.42±0.44
F statistic						
Fertilizer rate	35.61***	2.84^{*}	5.517***	62.19***	119.22***	15.27***
Cropping system	1.81ns	0.03ns	1.121 ns	51.74***	13.87***	1.88 ns
Plant density	4.37**	0.22 ns	1.458 ns	1.98 ns	3.35*	1.83 ns
Fertilizer rate*cropping system	12.22***	0.16 ns	5.23***	1.45 ns	12.49***	2.89^{*}
Fertilizer rate*plant density	2.77^{**}	1.40 ns	6.15***	4.05^{***}	6.77***	0.96 ns
Cropping system*plant density	0.40ns	0.67 ns	8.29***	2.07 ns	1.06 ns	1.33 ns
Fertilizer rate*cropping system* plant density	2.60**	0.34 ns	3.33***	2.38*	1.00 ns	5.98***

Table 4.1: Photosynthesis, stomatal conductance, internal CO₂, transpiration, WUE and chlorophyll of cowpea measured at the flowering stage

Values followed by dissimilar letters in the same column in a treatment are significant at P \leq 0.05 according to Fischer LSD. *: P \leq 0.05; **: P \leq 0.01; ***: P \leq 0.001. ns=not significant. Values in the columns represent the means and their standard errors.



Figure 4.1: Interactive effect of N fertilizer application rate and cropping system on: A) photosynthetic rate, B) internal CO₂ concentration, C) chlorophyll content, and D) photosynthetic WUE in cowpea. Values followed by dissimilar letters in the same column within a treatment are significant at P \leq 0.05 according to Fischer LSD. Error bars represents standard error.

The control cowpea exhibited the greatest photosynthetic rates irrespective of cropping system. There were no significant differences in photosynthetic rates between the monocrop and intercrop cowpea on plants that received 0, 100 and 125 kg N/ha fertilizer (Figure 4.1A). Interestingly at fertilizer application rate of 75 kg N/ha, intercropped cowpea exhibited significantly higher photosynthetic rates (Figure 4.1A). At 0 and 100 kg N/ha monocropped cowpea had higher internal CO₂ concentration compared to intercropped plants (Figure 4.1B), while the reverse was true for 50 kg N/ha with intercropped cowpea showing significantly higher internal CO₂ (Figure 4.B). At 75 and 125 kg N/ha, there were no significant differences in the internal CO₂ concentration between monocropped and intercropped cowpea. Chlorophyll was measured using a hand held meter. Monocropped cowpea that received 0 and 125 kg N/ha had the greatest chlorophyll content compared to their intercrop counterparts. At 75 kg N/ha, the opposite was true, with intercropped plants exhibiting higher chlorophyll content (Figure 4.1C). At 50 kg N/ha, there were no differences in chlorophyll content between intercropped and monocropped cowpea (Figure 4.1C).

The photosynthetic water-use efficiency was obtained by dividing leaf photosynthesis by the leaf transpiration rate. At 0, 50, 100 and 125 kg N/ha rates, monocropped cowpea plants exhibited significantly higher water-use efficiency compared to intercropped plants (Figure 4.1D). The reverse was true for the 75 kg N/ha application rate with intercropped plants showing higher WUE. Water-use efficiency was significantly lower on plants that received 75 kg N/ha irrespective of cropping system (Figure 4.1D).



Figure 4.2: The interaction of N fertilizer rate and planting density on: A) photosynthetic rate, B) internal CO₂ and C) transpiration rate on cowpea leaves. Values followed by dissimilar letters in the same column within a treatment are significant at P \leq 0.05 according to Fischer LSD. Error bars represents standard error.



Figure 4.3: Interactive effect of N fertilizer application rate and cropping system on the photosynthetic WUE in cowpea. Values followed by dissimilar letters in the same column within a treatment are significant at P \leq 0.05 according to Fischer LSD. Error bars represents standard error.

There was a significant N fertilizer application and planting density interaction on photosynthesis rate, internal CO₂ and transpiration rate (Figure 4.2A – 4.2C). The control cowpea plants exhibited the greatest photosynthetic rate irrespective of planting density (Figure 4.2A). From 0 – 100 kg N/ha, the highest photosynthetic rate was shown by plants planted at 53 333 plants/ha (Figure 4.2A). At the highest fertilizer rate, plants from the highest density had the highest photosynthetic rate (Figure 4.2A).

At 0 kg N/ha cowpea plants which were most densely planted had higher internal CO₂ concentration compared to the other two densities (Figure 4.2B). At 50 kg N/ha plants from the lowest experimental density showed higher internal CO₂ concentration with the middle density showing the lowest concentration (Figure 4.2B). From 75 to 125 kg N/ha, the middle density (53 333 plants/ha) exhibited the highest internal CO₂ concentration (Figure 4.2B). For the 0 kg N/ha, the highest planting density exhibited the highest transpiration rate (Figure 4.2C). At 50 kg N/ha rate, cowpea plants, planted at the lowest density transpired significantly more than those planted at higher densities (Figure 4.2C). With respect to plants that received

75 kg N/ha, the cowpea plants at the middle density lost more water through their stomata. At the high N application rates (100 and 125 kg N/ha) the low density plants transpired significantly more than those planted in higher densities (Figure 4.2C). Decreasing planting density increased water-use efficiency of cowpea at 0 kg N/ha fertilizer application rate (Figure 4.3). At 50 kg N/ha, cowpea planted at a 53 33 plants/ha planting density exhibited the highest water use efficiency. Interestingly, at 75 kg N/ha, water-use efficiency of cowpea was generally low, with cowpea plants planted at 53 333 density once more exhibiting the highest water-use efficiency. At 100 and 125 kg N/ha fertilizer application rate, water-use efficiency increased with increasing planting density (Figure 4.3).



Figure 4.4: The interaction of cropping system and planting density on internal CO₂ concentration in cowpea plants, planted under varying N fertilizer application rates. Values followed by dissimilar letters in the same column within a treatment are significant at P \leq 0.05 according to Fischer LSD. Error bars represents standard error.

At the highest planting density, monocropped cowpea plants exhibited significantly higher internal CO_2 concentration relative to intercropped plants (Figure 4.4). Interestingly, at the middle planting density, the reverse was true whereas there were no significant differences in internal CO_2 concentration between monocropped and intercropped cowpea plants at 40 000 plants/ha (Figure 4.4).



Figure 4.5: Interactive effect of N fertilizer application rate, planting density and cropping system on: A) photosynthetic rate, B) internal CO₂ concentration and C) transpiration rate on

cowpea. Values followed by dissimilar letters in the same column within a treatment are significant at P \leq 0.05 according to Fischer LSD. Error bars represents standard error.



Figure 4.6: The interactive effect of fertilizer rate, cropping system and plant density on chlorophyll content in cowpea. Values followed by dissimilar letters in the same column within a treatment are significant at P \leq 0.05 according to Fischer LSD. Error bars represents standard error.

The control cowpea exhibited the greatest photosynthetic rates irrespective of planting density and cropping system (Figure 4.5A). Cowpea plants that received 75 kg N/ha photosynthesized more when grown as intercrop than monocrop irrespective of planting density (Figure 4.5A). On crops that received 50 kg N/ha, higher photosynthetic rates were found on monocropped plants irrespective of planting density (Figure 4.5A). Under monocropping, at the highest planting density, application of nitrogen fertilizer decreased the internal CO₂ concentration in cowpea plants (Figure 4.5B). For the next highest density of (53 333 plants/ha) the internal CO₂ concentration was significantly highest for the 100 and 125 kg N/ha application rates (Figure 4.5B). With respect to intercropping, at the highest planting density, the highest fertilizer application rate significantly increased the internal CO₂ concentration of cowpea (Figure 4.5B). At the moderate planting density, the three highest (75, 100 and 125 kg N/ha) fertilizer application rates induced the highest CO₂ concentration in cowpea plants. At the lowest planting density, the 100 kg N/ha fertilizer rate significantly increased the highest CO₂ concentration in cowpea (Figure 4.5B). Cowpea plants that received 75 kg N/ha had higher transpiration rates irrespective of planting density and cropping system (Figure 4.5C). Transpiration rate decreased with planting density on intercropped N unfertilized cowpea (Figure 4.5C). On cowpea plants that received higher fertilizer application rates (100 and 125 kg N/ha), spacious planting densities, increased the rate of transpiration irrespective of cropping system (Figure 4.5C).

Regarding monocropped plants, at the highest density, the 75 and 125 kg N/ha application rates of fertilizer induced the highest chlorophyll content (Figure 4.6). Interestingly at the moderate planting density, application of fertilizer reduced the chlorophyll content of cowpea plants. At the lowest planting density, the highest chlorophyll content was induced by the 50 kg N/ha application (Figure 4.6). With respect to intercropping, application of fertilizer also affected the chlorophyll content of cowpea. For plants grown at 66 667 plants/ha, the 50 kg N/ha application increased chlorophyll content the most (Figure 4.6). At the 53 333 and 40 000 plants/ha, the 75 kg N/ha induced the highest chlorophyll content. Overall, the lowest chlorophyll content was exhibited by plants which received 100 and 125 kg N/ha (Figure 4.6).

4.1.4 Effect of nitrogen application rate on photosynthetic parameters of sorghum

Nitrogen fertilizer application significantly affected the photosynthetic parameters in sorghum. (Table 4.2). The control sorghum plants had significantly higher rates of photosynthetic parameters compared to treatments with N application (Table 4.2). There were no significant differences in the internal CO_2 concentration of sorghum plants among all the nitrogen application rates except the 75 kg N/ha rate which exhibited the lowest value. Plants that received 0, 75 and 125 kg N/ha, exhibited the highest transpirational pull while those that received 50 and 100 kg N/ha had the lowest value. Using *A/E* as a proxy for water use-efficiency, significantly higher water- use efficiency was found for sorghum plants that received 0 and 100 kg N/ha while the lowest water- use efficiency was found in plants that received the highest fertilizer application rate of 125 kg N/ha. Nitrogen fertilizer application rate had no significant effect on chlorophyll content of sorghum (Table 4.2).

4.1.5 Effect of cropping system on photosynthetic parameters of sorghum

Cropping system significantly ($p \le 0.05$) affected the rate of photosynthesis in sorghum (Table 4.2). Intercropped sorghum plants exhibited the highest photosynthesis rate relative to monocropped plants. Cropping system had no significant effect on the rate of stomatal conductance, transpiration rate, internal CO₂ concentration, photosynthetic water-use efficiency and chlorophyll content in sorghum (Table 4.2).

4.1.6 Effect of planting density on photosynthetic parameters of sorghum

Planting density had no significant effect on the photosynthetic parameters (photosynthesis rate, stomatal conductance, transpiration, internal CO₂ concentration, photosynthetic wateruse efficiency and chlorophyll content of sorghum (Table 4.2).

Treatments	Photosynthesis (A) μmol CO ₂ m ⁻² s ⁻¹	Stomatal conductance (gs) mol m ⁻² s ⁻¹	Internal CO ₂ (C_i) µmol m ⁻² s ⁻¹	Transpiration (E) mmol H ₂ O m ⁻² s ⁻¹	Water use efficiency (WUE) (µmol CO ₂ / mmol H ₂ O)	Chlorophyll (SPAD reading)
Fertilizer rate (kg N /ha)						
0	31.25±0.94a	0.36±0.02a	139.43±10.53ab	5.28±0.23a	6.78±0.23a	55.05±0.69
50	22.18±0.68c	0.20±0.02c	156.21±10.53a	3.87±0.23b	5.62±0.23b	52.83±0.65
75	21.99±0.57c	0.20±0.02c	106.82±12.90b	5.09±0.28a	4.20±.0.28d	54.10±0.95
100	21.76±0.89c	0.26±0.02b	171.21±12.90a	3.92±0.28b	6.72±0.28a	54.17±0.60
125	26.01±0.54b	0.31±0.02b	158.91±10.53a	5.57±0.23a	4.93±0.23c	53.64±0.69
Cropping system						
Monocrop sorghum	23.95±0.55b	0.28 ± 0.01	146.97 ± 7.3	4.73±0.16	5.74±0.16	54.18 ± 0.44
Intercrop sorghum	25.33±0.57a	0.25 ± 0.01	146.06 ± 7.3	4.76±0.16	5.56±0.16	53.74 ± 0.48
Plant density (plants/ha)						
66 667	24.77±0.69	0.26 ± 0.02	141.14 ± 8.94	4.78±0.20	5.74 ± 1.20	54.08 ± 0.66
53 333	24.57±0.68	0.26 ± 0.02	151.99±8.94	4.42±0.20	5.75±1.20	54.19±0.52
40 000	24.57±0.70	0.28 ± 0.02	146.41 ± 8.94	5.04 ± 0.20	5.40 ± 1.20	53.61±0.50
F statistic						
Fertilizer rate	29.64***	15.87***	3.94**	10.63***	18.26***	1.22 ns
Cropping system	4.18^{*}	2.63 ns	0.01 ns	0.02 ns	0.58 ns	0.45 ns
Plant density	0.04ns	0.87 ns	0.37 ns	2.54 ns	0.70 ns	0.30 ns
Fertilizer rate*cropping system	0.62 ns	4.06**	10.55***	2.99*	12.56***	1.89 ns
Fertilizer rate*plant density	0.63 ns	0.69 ns	1.77 ns	0.63 ns	0.85 ns	0.81 ns
Cropping system*plant density	0.43 ns	1.12 ns	0.63 ns	1.26 ns	2.70 ns	0.02 ns
Fertilizer rate*cropping system* plant density	0.55 ns	0.56 ns	1.35 ns	1.00 ns	0.52 ns	0.44 ns

Table 4.2: Photosynthesis, stomatal conductance, internal CO₂, transpiration, WUE and chlorophyll of sorghum measured at the flowering stage

Values followed by dissimilar letters in the same column within a treatment are significant at P \leq 0.05 according to Fischer LSD. *: P \leq 0.05; **: P \leq 0.01; ***: P \leq 0.001. ns=not significant. Values in the columns represent the means and their standard errors.



Figure 4.7: Interactive effect of N fertilizer application rate and cropping system on: A) stomatal conductance, B) internal CO₂ concentration, C) transpiration rate and D) photosynthetic WUE in sorghum leaves. Values followed by dissimilar letters in the same column within a treatment are significant at P \leq 0.05 according to Fischer LSD. Error bars represents standard error.

At 0, 100 and 125 kg N/ha fertilizer application rate, monocropped sorghum exhibited higher stomatal conductance and internal CO_2 concentration compared to intercropped ones (Figure 4.7A and B). Interestingly the reverse was true for sorghum that received 75 kg N/ha, with intercropped sorghum exhibiting the highest stomatal conductance and internal CO_2

concentration (Figure 4.7A and B). Furthermore, the transpirational pull was significantly higher for monocropped sorghum at 0 and 125 kg N/ha application rate (Figure 4.7C). It is also noteworthy that for stomatal conductance, internal CO₂ concentration and WUE, intercropped sorghum had higher values relative to sole crops (Figure 4.7 A, B, C)

The sorghum plants that received 0 and 125 kg N/ha under monocropping showed higher transpirational water loss than intercropped plants (Figure 4.7C). At 50 kg N/ha application rate, intercropped sorghum plants transpired more than monocropped plants, whereas at 75 kg N/ha there were no significant differences in the transpirational water loss between monocropped and intercropped plants (Figure 4.7C).

There was a positive relationship between photosynthesis and stomatal conductance in sorghum (Figure 4.8A). An increase in stomatal conductance may increase photosynthetic rate (Figure 4.8A). There was a positive relationship between the rate of transpiration and stomatal conductance in sorghum (Figure 4.8B). Increasing stomatal conductance can increase the rate of transpiration (Figure 4.8B). There was a positive relationship between the rate of photosynthesis and transpiration in sorghum therefore increase in transpiration may lead to increase in the rate of photosynthesis (Figure 4.8C).



Figure 4.8: Correlation analysis of: A) stomatal conductance and photosynthesis, B) transpiration and stomatal conductance and C) transpiration and photosynthesis of sorghum at flowering stage.

4.1.7 Flowering in cowpea and sorghum

Flowering in cowpea was significantly ($p \le 0.05$) affected by fertilizer application rate. The lowest application rate of 0 kg N/ha showed increased number of days to flowering while application rate of 75 kg N/ha reduced the number of days it took cowpea to flower. Cropping system and planting density had no significant effect on the number of days to 50% flowering in cowpea (Table 4.3). In case of sorghum, fertilizer application rate, cropping system, planting density and their interactions had no significant effect on the number of days to flowering (Table 4.3).

	~	~ •
Treatments	Cowpea	Sorghum
Fertilizer rate (kg N/ha)		
0	45.9±0.8a	49.44±0.60
50	45.1±0.8ab	49.72±0.73
75	42.6±1.1c	47.83±1.06
100	42.9±0.7bc	47.16±1.01
125	44.7±0.5abc	47.55±1.28
Cropping system		
Monocrop	43.5±0.5	48.13±0.59
Intercrop	45.0±0.5	48.55±0.64
Planting density (plants/ha)		
66 667	44.0 ± 0.8	48.60±0.65
53 333	44.5±0.6	47.90±0.83
40 000	44.2 ± 0.6	48.53±0.79
F statistic		
Fertilizer rate	2.83^{*}	1.25 ns
Cropping system	3.92 ns	0.21 ns
Plant density	0.14 ns	0.23 ns
Fertilizer rate*cropping system	0.96 ns	2.17 ns
Fertilizer rate*plant density	0.46 ns	0.14 ns
Cropping system*plant density	0.49 ns	0.42 ns
Fertilizer rate*cropping system* plant	0.42 ns	0.30 ns
density		

Table 4.3: Number of days to 50% flowering in cowpea and sorghum

Values followed by dissimilar letters in the same column within a treatment are significant at P \leq 0.05 according to Fischer LSD. *: P \leq 0.05; **: P \leq 0.01; ***: P \leq 0.001. ns = not significant. Values in the columns represent the means and their standard errors.

4.2 Yield and yield components

4.2.1 Effect of nitrogen fertilizer application rate on cowpea nodulation and biomass at flowering

Nitrogen fertilizer application significantly affected cowpea biomass (Table 4.4). At a lower nitrogen application of 0 and 50 kg N/ha, nodulation (the number of nodules and nodule biomass) was significantly higher than at higher rates of nitrogen fertilizer application rates (Table 4.4). Fertilizer application resulted in reduced cowpea shoot and root biomass, while application rate of 75 kg N/ha led to lower shoot biomass (Table 4.4).

4.2.2 Effect of cropping system on cowpea nodulation and biomass

Cropping system had no significant effect on the number of nodules, shoot biomass and root biomass of cowpea (Table 4.4). However, it significantly affected the nodule biomass. Cowpea plants when planted as a monocrop had higher nodule biomass per plant compared to intercropped plants (Table 4.4).

4.2.3 Effect of planting density on cowpea nodulation and biomass

Planting density did not have any significant effect on cowpea nodulation and root biomass whereas cowpea shoot biomass was significantly affected by planting density (Table 4.4). At a higher density of 66 667 plants/ha the cowpea shoot biomass was significantly lower than at the other two densities (Table 4.4).

Treatments	Nodule no/plant	Nodule biomass (mg/plant)	Shoot biomass (g/plant)	Root biomass (g/plant)
Fertilizer rate (kg N/ha)				
0	$15.3 \pm 2.0a$	145.3±25.1a	29.5±1.9a	3.0±0.2a
50	15.4±2.4a	168.0±27.5a	25.5±1.3abc	2.9±0.1bc
75	7.4±1.5c	53.2±11.2b	21.5±1.4c	2.6±0.1c
100	8.4±1.4bc	58.6±14.2b	24.9±1.8bc	3.0±0.2a
125	12.2±1.6ab	62.8±10.8b	27.6±1.6ab	2.7±0.1c
Cropping system				
Monocrop cowpea	12.9 ± 1.2	118.6±14.5a	25.5 ± 1.1	2.9±0.1
Intercrop cowpea	10.6 ± 1.1	76.7±10.4b	26.1±1.0	2.8 ± 0.1
Planting density (plants/ha)				
66 667	11.3 ± 1.2	94.6±15.0	22.9±1.1b	2.8 ± 0.1
53 333	14.1 ± 1.7	107 ± 17.4	26.6±1.3a	2.8 ± 0.1
40 000	9.8±1.3	91.5±14.5	27.9±1.3a	2.9±0.1
F statistic				
Fertilizer rate	5.720^{**}	10.914***	4.740^{**}	3.199*
Cropping system	2.740 ns	8.135***	0.227 ns	0.235 ns
Planting density	2.983 ns	0.360 ns	5.847**	0.392 ns
Fertilizer rate*cropping system	3.111**	1.701 ns	3.901**	1.318 ns
Fertilizer rate*planting density	0.991 ns	1.048 ns	1.357 ns	1.785 ns
Cropping system*planting density	1.039 ns	0.087 ns	1.112 ns	1.128 ns
Fertilizer rate*cropping system* planting density	0.642 ns	0.515 ns	2.142*	0.830 ns

Table 4.4: Cowpea nodulation and whole plant biomass at flowering stage

Values followed by dissimilar letters in the same column within a treatment are significant at P \leq 0.05 according to Fischer LSD. *: P \leq 0.05; **: P \leq 0.01; ***: P \leq 0.001. NS= not significant. Values in the columns represent the means and their standard errors.



Figure 4.9: Interaction of fertilizer application rate and cropping system on nodule number per plant in cowpea plants. Values followed by dissimilar letters in the same column within a treatment are significant at P \leq 0.05 according to Fischer LSD. Error bars represents standard error.

Sole cowpea plants that received 0, 50 and 125 kg N/ha showed significantly higher number of nodules compared with intercropped plants. At 75 and 100 kg N/ha, intercropped plants significantly higher number of nodules (Figure 4.9).



Figure 4.10: Interaction of fertilizer application rate and cropping system on cowpea shoot biomass. Values followed by dissimilar letters in the same column within a treatment are significant at P \leq 0.05 according to Fischer LSD. Error bars represents standard error.

Interestingly, shoot biomass followed the same trend as number of nodules per plant (Figure 4.10). For instance, monocropped cowpea had significantly higher shoot biomass than intercropped on the plants that received 0, 50 and 125 kg N/ha. Intercropped plants that received 75 and 100 kg N/ha exhibited higher shoot biomass compared with monocropped plants (Figure 4.10).



Figure 4.11: Interaction of fertilizer application rate, planting density and cropping system on cowpea shoot biomass per plant. Values followed by dissimilar letters in the same column within a treatment are significant at P \leq 0.05 according to Fischer LSD. Error bars represents standard error.

For monocropped cowpea planted at 66 667 plants/ha, plants that received 125 kg N/ha, exhibited the highest shoot biomass followed by control plants, while those that received 75 kg N/ha had the lowest shoot dry weight (Figure 4.11). A similar trend was evident with respect to cowpea plants planted at the middle planting density. Interestingly at 40 000 plants/ha, control cowpea plants displayed the highest shoot dry matter followed by those that received 50 kg N/ha, while those that received 75 kg N/ha had the least shoot dry matter accumulation (Figure 4.11).

There was a strong positive correlation between nodule number and nodule weight of cowpea and between root weight and shoot weight (Table 4.5).

	Nodule no/plant	Nodule biomass (mg/plant)	Shoot biomass (g/plant)	Root biomass (g/plant)
Nodule no/plant	1			
Nodule biomass (mg/plant)	0.678^{***}	1		
Shoot biomass (g/plant)	0.170^{***}	0.033 ^{ns}	1	
Root biomass (g/plant)	0.186^{**}	0.096 ^{ns}	0.518^{***}	1

Table 4.5: Pearson correlation coefficient for cowpea biomass at the flowering stage.

Values for correlation coefficient are significant at P \leq 0.05 according to Pearson correlation. *: P \leq 0.05; **: P \leq 0.01; ***: P \leq 0.001. NS= not significant

4.2.4 Effect of nitrogen fertilizer application rate on sorghum biomass yield

Nitrogen fertilizer application significantly ($p \le 0.001$) affected the biomass yield of sorghum (Table 4.6). The application of 50 kg N/ha resulted in significantly higher sorghum shoot biomass while the zero N fertilizer treatment had the lowest shoot biomass (Table 4.6). Sorghum plants that received of 50 kg N/ha also produced more root biomass, while the lowest root biomass was found on plants that received the application rate of 75 kg N/ha (Table 4.6).

4.2.5 Effect of cropping system on sorghum biomass yield

Planting density had no significant effect on the shoot and root biomass yield of sorghum during the flowering stage (Table 4.6).

4.2.6 Effect of planting density on biomass yield

Cropping system had no significant effect on the shoot and root biomass yield of sorghum at the flowering stage (Table 4.6).

Treatments	Sorghum shoot biomass	Sorghum root biomass
	(g/plant)	(g/plant)
Fertilizer rate (kg N/ha)		
0	32.92±5.25c	8.56±1.38bc
50	63.96±5.83a	15.09±2.03a
75	42.65±4.15bc	6.90±0.55c
100	48.83±4.56b	10.55±1.60abc
125	46.14±7.15bc	12.44±3.03ab
Cropping system		
Monocrop sorghum	45.95±4.06	11.03 ± 1.57
Intercrop sorghum	47.85±3.37	10.38 ± 0.84
Planting density (plants/ha)		
66 667	41.91±3.61	8.71±0.99
53 333	47.36±4.24	$11.14{\pm}1.48$
40 000	$51.43 \pm .56$	12.27 ± 1.96
F statistic		
Fertilizer rate	4.812^{***}	3.577***
Cropping system	0.172 ns	0.183 ns
Planting density	1.437 ns	1.913 ns
Fertilizer rate*cropping system	3.801***	2.825**
Fertilizer rate*planting density	0.768 ns	1.841 ns
Cropping system*planting density	2.810 ns	1.909 ns
Fertilizer rate*cropping system*	0.851 ns	1.554 ns
planting density		

Table 4.6: Below and above ground sorghum biomass yield measured at the flowering stage

Values followed by dissimilar letters in the same column within a treatment are significant at P \leq 0.05 according to Fischer LSD. *: P \leq 0.05; **: P \leq 0.01; ***: P \leq 0.001. NS= not significant. Values in the columns represent the means and their standard errors.



Figure 4.12: Interaction of fertilizer application rate and cropping system on sorghum shoot biomass at the flowering stage. Values followed by dissimilar letters in the same column within a treatment are significant at P \leq 0.05 according to Fischer LSD. Error bars represents standard error.

Intercropped sorghum had the highest shoot biomass for plants that received 0, 75 and 100 kg N/ha compared to monocropped plants (Figure 4.12). For plants that received 125 kg N/ha, monocropped plants exhibited the highest shoot dry matter compared with intercropped ones (Figure 4.12).



Figure 4.13: Interaction of fertilizer rate and cropping system on sorghum root biomass. Values followed by dissimilar letters in the same column within a treatment are significant at P \leq 0.05 according to Fischer LSD. Error bars represents standard error.

Regarding the interaction of fertilizer application rate and cropping system on root biomass, there were no significant differences between the root biomass of monocrops and intercrops for the plants that received 0 and 75 kg N/ha (Figure 4.13). At the application of 50 and 125 kg N/ha the root dry matter of monocropped sorghum was significantly higher relative to that of intercropped sorghum. However the reverse was true at 100 kg N/ha with roots of intercropped plants being significantly higher in weight than those of monocrops (Figure 4.13).

4.2.7 Sorghum grain yield and yield components

Fertilizer rate, cropping system, planting density and their interactions had no significant effect on 1000 seed weight, total yield, panicle length and panicle weight of sorghum (Table 4.7).

Treatments	1000 seed	Sorghum yield	Panicle	Panicle
	weight (g)	(kg ha ⁻¹)	length (cm)	weight (g)
Fertilizer rate (kg N/ha)				
0	26.12±0.34	4078.50±501.26	28.21±0.45	72.83 ± 5.02
50	24.37±0.69	3725.19 ± 578.80	26.69 ± 0.75	66.90 ± 7.77
75	26.81±0.46	4927.43 ± 560.42	27.90 ± 0.65	73.76±5.15
100	25.72 ± 0.67	3977.96±521.72	26.90 ± 0.85	65.07 ± 6.56
125	26.61 ± 0.40	4469.91±663.10	28.99 ± 0.41	73.17±4.18
Cropping system				
Monocrop sorghum	25.53 ± 0.37	4555.66±347.28	27.85 ± 0.46	69.36±4.06
Intercrop sorghum	26.20 ± 0.39	3915.94±370.61	27.38 ± 0.44	70.52 ± 3.74
Planting density (plants/ha)				
66 667	25.65 ± 0.48	4270.11±416.38	27.03 ± 0.57	65.63 ± 5.24
53 333	25.80 ± 0.52	4383.45±451.13	28.14 ± 0.57	70.47 ± 4.54
40 000	26.14 ± 0.40	4053.83±451.13	27.72 ± 0.50	74.03 ± 4.42
F statistic				
Fertilizer rate	2.07 ns	0.69 ns	1.50 ns	0.40 ns
Cropping system	1.42 ns	1.59 ns	0.17 ns	0.19 ns
Planting density	0.40 ns	0.14 ns	0.90 ns	0.84 ns
Fertilizer rate*cropping system	0.15 ns	1.05 ns	0.18 ns	0.37 ns
Fertilizer rate*planting density	0.60 ns	0.49 ns	0.43 ns	0.29 ns
Cropping system*planting density	0.82 ns	0.14 ns	0.32 ns	0.05 ns
Fertilizer rate*cropping system* planting density	0.68 ns	0.31 ns	0.30 ns	0.34 ns

Table 4.7: Sorghum yield and yield components

Ns= not significant. Values in the columns represent the means and their standard errors.

There was a positive correlation between sorghum yield and panicle length, between panicle weight and 1000 seed weight and between sorghum yield and 1000 seed weight in sorghum (Table 4.8).

Table 4.8: Pearson correlation coefficient	for sorghum yield and yield components
--	--

	1000 seed weight (g)	Sorghum yield (kg ha ⁻¹)	Panicle length (cm)	Panicle weight (g)
1000 seed weight (g)	1			
Sorghum yield (kg/ha)	0.221 ^{ns}	1		
Panicle length (cm)	0.878^{***}	0.127^{ns}	1	
Panicle weight (g)	0.568^{***}	0.805^{***}	0.844^{***}	1

Values for correlation coefficient are significant at P \leq 0.05 according to Pearson correlation. *: P \leq 0.05; **: P \leq 0.01; ***: P \leq 0.001. NS= not significant.

4.2.8 Cowpea grain yield and yield components

Fertilizer rate, cropping system, planting density and their interactions had no significant effect

on pod length, pod weight, number of seeds per pod 100 seed weight and total yield in cowpea

(Table 4.9).

Treatments	Pod length (cm)	Pod weight (g)	Number of seeds/pod	100 Seed weight (g)	Cowpea yield (kg/ha)
Fertilizer rate (kg N/ha)					
0	21.09 ± 0.20	2.96 ± 0.07	17.34 ± 0.28	11.50 ± 0.18	1774.35 ± 197.41
50	20.92 ± 0.22	2.79 ± 0.07	16.79±0.33	11.38 ± 0.09	1601.27±234.97
75	20.71±0.21	2.78 ± 0.05	16.69 ± 0.32	11.31±0.14	1727.88±197.41
100	20.77±0.20	2.70 ± 0.07	16.23±0.31	11.20 ± 0.17	1886.76±197.41
125	20.87 ± 0.18	2.82 ± 0.08	16.77±0.32	11.27 ± 0.22	1761.82 ± 205.48
Cropping system					
Monocrop cowpea	21.03±0.22	2.81 ± 0.05	16.96±0.17	11.44 ± 0.09	1778.70±124.86
Intercrop cowpea	20.72±0.13	2.80 ± 0.04	16.56 ± 0.22	11.21 ± 0.11	1721.33±136.77
Planting density (plants/ha)					
66 667	20.69±0.16	2.74 ± 0.05	16.48 ± 0.26	11.36±0.14	1788.01±152.92
53 333	20.92±0.15	2.83 ± 0.05	16.77±0.23	11.40 ± 0.11	1925.04±160.38
40 000	20.99±0.19	2.85 ± 0.06	17.03±0.25	11.22 ± 0.14	1537.00±167.51
F statistic					
Fertilizer rate	0.54 ns	1.70 ns	1.56 ns	0.39 ns	0.23 ns
Cropping system	3.10 ns	0.00 ns	1.94 ns	1.90 ns	0.10 ns
Planting density	1.07 ns	1.06 ns	1.30 ns	0.44 ns	1.43 ns
Fertilizer rate*cropping	1.87 ns	1.40 ns	1.28 ns	0.30 ns	1.57 ns
system		0.70	0.50	0.1.4	0.4.4
Fertilizer rate*planting	1.45 ns	0.50 ns	0.53 ns	0.16 ns	0.46 ns
density					
Cropping system*planting	0.50 ns	0.12 ns	0.38 ns	0.05 ns	0.13 ns
density					
Fertilizer rate*cropping	1.32 ns	0.38 ns	0.96 ns	0.57 ns	1.10 ns
system [*] planting density					

Table 4.9: Cowpea yield and yield components

NS= not significant. Values in the columns represent the means and their standard errors.

There was a strong positive correlation between pod length and pod weight and between pod weight and number of seeds per pod of cowpea (Table 4.10).

	Pod length (cm)	Pod weight (g)	No of seeds/pod	100 seed weight (g)	Cowpea yield (kg/ha)
Pod length (cm)	1				
Pod weight (g)	0.677^{***}	1			
No of seeds/pod	0.496^{***}	0.699^{***}	1		
100 seed weight (g)	0.268^{**}	0.488^{***}	0.267^{**}	1	
Cowpea yield (kg/ha)	0.145 ^{ns}	0.321*	0.392^{**}	0.278^{*}	1

Table 4.10: Pearson correlation coefficient for cowpea yield and yield components

Values for correlation coefficient are significant at P \leq 0.05 according to Pearson correlation. *: P \leq 0.05; **: P \leq 0.01; ***: P \leq 0.001. NS= not significant

4.3 Nitrogen use efficiency and nutrients uptake

4.3.1.1 Nitrogen use efficiency in sorghum

Application of LAN significantly ($p \le 0.01$) affected NUE during the flowering stage (Table 4.11). The highest NUE was found on plants that received 50 kg N/ha while further increase in N fertilizer application rate decreased NUE. Cropping system and planting density had no significant effect on sorghum NUE during the flowering stage. Fertilizer rate and planting density did not have significant effect on NUE of sorghum at harvest. However, cropping system significantly ($p \le 0.05$) affected NUE at harvest, with monocropped plants having higher NUE relative to intercropped plants (Table 4.11).

Treatments	Shoot NUE at flowering	Grain NUE at harvest
	(kg/kg)	(kg/kg)
Fertilizer rate (kg N/ha)		
50	30.65±6.20a	-4.32 ± 7.28
75	5.61±6.20b	7.58 ± 7.05
100	7.83±6.20b	0.79 ± 6.56
125	4.68±6.20b	-2.30 ± 8.34
Cropping system		
Monocrop sorghum	17.38 ± 4.39	9.04±4.98a
Intercrop sorghum	7.01±4.39	-8.16±5.38b
Planting density (plants/ha)		
66 667	5.30±5.37	-6.33±5.95
53 333	15.98 ± 5.37	11.33±6.55
40 000	15.30 ± 5.37	-3.68 ± 6.55
F statistic		
Fertilizer rate	3.98 **	0.52 ns
Cropping system	2.80 ns	5.51 *
Planting density	1.24 ns	2.22 ns
Fertilizer rate*cropping system	0.63 ns	0.18 ns
Fertilizer rate*planting density	0.33 ns	1.06 ns
Cropping system*planting density	0.73 ns	3.35 *
Fertilizer rate*cropping system*	0.21 ns	0.80 ns
planting density		

Table 4.11: Nitrogen use efficiency (NUE) at flowering and at harvest in sorghum

Values followed by dissimilar letters in the same column within a treatment are significant at P \leq 0.05 according to Fischer LSD. *: P \leq 0.05; **: P \leq 0.01; ***: P \leq 0.001. ns=not significant. Values in the columns represent the means and their standard errors.



Figure 4.14: The interaction of cropping system and planting density on NUE of sorghum at harvest. Values followed by dissimilar letters in the same column within a treatment are significant at P \leq 0.05 according to Fischer LSD. Error bars represents standard error.

At harvest, monocropped sorghum plants from the high and middle planting densities showed significantly higher NUE compared to intercropped plants (Figure 4.14). At 40 000 plants/ha, there were no significant differences in NUE between monocropped and intercropped sorghum plants (Figure 4.14).

4.3.2 Nitrogen use efficiency in cowpea

Fertilizer rate, cropping system, planting density and their interactions did not have any significant effect on NUE of cowpea at flowering stage and at harvest (Table 4.12).

Table 4.12: Nitrogen use efficiency (NUE) in cowpea at flowering and at harvest

Treatments	Shoot NUE at flowering	Grain NUE at harvest
	(kg/kg)	(kg/kg)
Fertilizer rate (kg N/ha)		2 2
50	-3.90 ± 2.05	-2.45 ± 3.50
75	-6.31±2.05	-0.65 ± 2.94
100	-2.01 ± 2.05	1.12 ± 2.94
125	-0.59 ± 2.05	-0.28 ± 3.06
Cropping system		
Monocrop cowpea	-4.69 ± 1.45	$1.01{\pm}2.08$
Intercrop cowpea	-1.72 ± 1.45	-2.14 ± 2.33
Planting density (plants/ha)		
66 667	-1.78 ± 1.78	0.26 ± 2.55
53 333	$-4.10{\pm}1.78$	2.39 ± 2.70
40 000	-3.74 ± 1.78	-4.63 ± 2.85
F statistic		
Fertilizer rate	1.47 ns	0.21 ns
Cropping system	2.12 ns	1.02 ns
Planting density	0.50 ns	1.70 ns
Fertilizer rate*cropping system	1.61 ns	1.36 ns
Fertilizer rate*planting density	0.14 ns	0.35 ns
Cropping system*planting density	1.96 ns	0.01 ns
Fertilizer rate*cropping system* planting density	0.30 ns	0.81 ns

Values followed by dissimilar letters in the same column within a treatment are significant at P \leq 0.05 according to Fischer LSD. *: P \leq 0.05; **: P \leq 0.01; ***: P \leq 0.001. ns=not significant. Values in the columns represent the means and their standard errors.

4.3.3 Effect of nitrogen fertilizer application rate on cowpea nutrient uptake

Application of LAN to cowpea decreased the uptake of macronutrients (Ca, Mg, K, N and P) (Table 4.13). The control plants had the highest Mg, Ca, K, Na and Fe uptake compared to those that received LAN. Variation of nitrogen application rate did not have any significant effect on the uptake of Zn and Cu (Table 4.13).

4.3.4 Effect of cropping system on cowpea nutrient uptake

Cropping system did not have a significant effect on the uptake of Mg, K, P, Na, Fe, Cu, Zn and N in cowpea (Table 4.13). Ca uptake in cowpea was significantly affected by cropping system, with monocropped cowpea plants showing higher Ca content relative to intercropped ones (Table 4.13).

4.3.5 Effect of planting density on cowpea nutrient uptake

The uptake of Ca, Mg, P and N in cowpea, significantly increased with a decrease in planting density. Planting density did not have any significant effect on the uptake of K, Fe, Na, Zn and Cu in cowpea (Table 4.13).
Treatments	Ν	Р	K	Ca	Mg	Na	Fe	Cu	Zn
			•	— mg/plant -					
Fertilizer rate (kg N/ha)									
0	1102.9±59.8a	157.9±9.5a	932.2±55.2a	418.8±27.5a	137.1±8.7a	33.2±2.6a	31.2±3.1a	0.34 ± 0.02	1.75 ± 0.17
50	932.5±61.7b	131.3±9.7ab	768.3±56.4b	285.4±28.1b	89.8±8.8b	27.0±2.6abc	17.0±3.1b	0.26 ± 0.02	1.57 ± 0.17
75	827.4±61.0b	97.2±9.4c	653.2±54.5b	197.4±27.2c	83.2±8.5b	20.2±2.7c	14.2±3.0b	0.27 ± 0.02	1.51±0.16
100	973.0±60.5ab	118.8±9.3bc	683.4±54.1b	262.7±27.0bc	96.4±8.5b	24.0±2.5bc	27.1±3.0b	0.30 ± 0.02	1.59 ± 0.16
125	981.3±67.4ab	121.4±10.5b	750.4±60.8b	279.0±30.0b	95.3±9.4b	28.2±2.9ab	29.1±3.3b	0.28 ± 0.02	1.88 ± 0.18
Cropping system									
Monocrop cowpea	942.6±39.8	121.9±6.3	767.4±36.5	319.1±18.1a	103.8 ± 6.0	25.6±1.7	23.5 ± 2.0	0.30 ± 0.01	1.70 ± 0.11
Intercrop cowpea	984.2±38.8	128.7±6.0	747.6±37.8	258.2±17.3b	96.9 ± 5.4	27.5±1.7	24.1±1.9	0.28 ± 0.01	1.62 ± 0.11
Plant density (plants/ha)									
66 667	867.3±48.4b	104.6±7.4b	730.1±43.2	262.1±21.4b	89.2±6.7b	24.3±2.1	22.7±2.4	0.27 ± 0.02	1.56±0.13
53 333	967.8±48.4ab	130.4±7.6a	747.3±43.8	273.2±21.9ab	93.7±6.9b	24.9±2.1	24.1±2.4	0.29 ± 0.02	1.55±0.13
40 000	1055.1±47.6a	141.0±7.5a	795.1±43.6	330.7±21.8a	118.1±6.8a	30.5±2.1	24.7 ± 2.4	0.31±0.02	1.88±0.13
F statistic									
Fertilizer rate	2.69^{*}	5.44***	3.90^{***}	8.67^{***}	6.01***	3.51***	5.87^{***}	1.92 ns	0.76 ns
Cropping system	0.56 ns	0.62 ns	0.16 ns	5.93**	0.75 ns	0.59 ns	0.04 ns	0.36 ns	0.29 ns
Plant density	3.84 *	6.31 ***	0.60 ns	2.89^{*}	5.23***	2.80 ns	0.19 ns	1.29 ns	2.05 ns
Fertilizer rate*cropping system	2.44 *	1.61 ns	2.17 ns	4.89***	5.82 ***	1.77 ns	4.34***	5.93***	2.37^{*}
Fertilizer rate*plant density	1.01 ns	1.26 ns	0.63 ns	1.38 ns	0.89 ns	1.27 ns	0.91 ns	1.08 ns	0.56 ns
Cropping system*plant density	0.87 ns	0.63 ns	0.12 ns	0.28 ns	0.67 ns	0.67 ns	0.36 ns	0.34 ns	0.73 ns
Fertilizer rate*cropping	0.73 ns	1.36 ns	1.69 ns	2.39**	2.57***	3.47***	2.25 *	1.95 ns	2.19^{*}
system* plant density	-								

Table 4.13: Nutrient uptake in cowpea shoot at the flowering stage

Values followed by dissimilar letters in the same column within a treatment are significant at P \leq 0.05 according to Fischer LSD. *: P \leq 0.05; **: P \leq 0.01; ***: P \leq 0.001. ns=not significant. Values in the columns represent the means and their standard errors.



Figure 4.15: Interactive effect of N fertilizer application rate and cropping system on the uptake of: A) N, B) Ca, C) Mg, D) Fe, E) Cu and F) Zn in cowpea shoot. Values followed by

dissimilar letters in the same column within a treatment are significant at P \leq 0.05 according to Fischer LSD. Error bars represents standard error.

At 0 and 125 kg N/ha application rate, the monocropped cowpea had the greatest N, Mg and Fe uptake compared to intercropped plants (Figure 4.15A, C and D). There were no significant differences in the uptake of these elements between sole and intercropped cowpea for plants that received 50 kg N/ha. Interestingly, intercropped plants had higher uptake of N, Mg and Fe over sole plants that received 75 and 100 kg N/ha (Figure 4.15A, C and D). Similarly, sole plants that received 0 and 125 kg N/ha exhibited significantly higher uptake of Ca, Cu and Zn (Figure 4.15 B, E and F). Furthermore, at 75 kg N/ha, intercropped plants showed a significantly higher uptake of Ca, Cu and Zn relative to monocropped ones (Figure 4.15 B, E and F).



Figure 4.16: The interactive effect of N fertilizer application rate, cropping system and planting density on the uptake of: A) Ca, B) Mg and C) Na in cowpea shoot. Values followed by dissimilar letters in the same column within a treatment are significant at P \leq 0.05 according to Fischer LSD. Error bars represents standard error.

Figure 4.16 shows the interaction of fertilizer application, cropping system and planting density on the uptake of Ca, Mg and Na by cowpea. For monocropped cowpea planted at the highest and lowest densities, application of fertilizer reduced the uptake of Ca and Mg (Figure 4.16 A and B). Similarly for intercropped sorghum sown at 53 333 plants/ha, fertilizer application reduced the uptake of Ca, Mg and Na (Figure 4.16 A – C).

For monocropped cowpea, it is evident that LAN application reduced the uptake of Mg especially at highest and lowest planting densities (Figure 4.16B). At the middle density, plants that received 125 kg N/ha exhibited the highest Mg uptake followed by control plants. With respect to intercropped cowpea however, the trend was different. For plants that were sown at the highest and lowest densities, those that received 100 kg N/ha showed the highest Mg uptake (Figure 4.16B). Regarding the middle density, the trend for Mg was similar to monocropped plants with control plants displaying the highest uptake of Mg (Figure 4.16B).

For monocropped plants sown at highest density, Na uptake was higher on plants that received 125 kg of N/ha (Figure 4.16C). However for plants planted at a lower density, Na uptake was higher on the control plants. With respect to the intercropped, for plants that were sown at a lower density, cowpea plants that received LAN had higher Na uptake relative to control plants (Figure 4.16C). However on plants that were sown at the middle density, control plants had the highest Na uptake compared with plants that received LAN (Figure 4.16C).



Figure 4.17: The interactive effect of N fertilizer application rate, cropping system and planting density on the uptake of: A) Fe and B) Zn in cowpea shoot. Values followed by dissimilar letters in the same column within a treatment are significant at P \leq 0.05 according to Fischer LSD. Error bars represents standard error.

With respect to monocropped plants, control cowpea plants planted at 66 667 and 40 000 plants/ha had the greatest Fe uptake that is, fertilizer application reduced Fe uptake (Figure 4.17A). At the middle planting density, plants that received the highest fertilizer dose showed the highest Fe uptake followed by control plants (Figure 4.17A). Regarding intercropping, for

the highest and lowest planting densities, plants that received 100 kg N/ha exhibited the highest Fe uptake. For the middle density, application of fertilizer reduced the uptake of Fe (Figure 4.17A).

Although fertilizer application had no effect on Zn uptake, (Table 4.13), there was a significant fertilizer x cropping system x planting density interaction (Figure 4.17B). At 66 667 and 40 000 plants/ha, fertilizer application reduced the uptake of Zn in monocropped cowpea. Furthermore, for the 66 667 planting density, there were significant differences in Zn uptake between control plants and those that received 125 kg N/ha (Figure 4.17B). Interestingly at 40 000 plants/ha, control plants also exhibited the highest Zn uptake. With respect to intercropped cowpea, at 66 667 plants/ha, there were no significant differences in the uptake of Zn among plants that received 50 – 100 kg N/ha (Figure 4.17B). For the 53 333 plants/ha, cowpea plants received 100 kg N/ha showed the highest Zn uptake followed by the control plants. For the lowest density, plants that received the highest fertilizer exhibited the highest Zn uptake (Figure 4.17B).

4.3.6 Effect of nitrogen fertilizer application rate on sorghum nutrient uptake

In sorghum, the uptake of Ca, Mg, K, P, Cu, Zn and N was greatest on plants that received 50 kg N/ha than on other N application rates (Table 4.14). Furthermore plants that received 100 kg N/ha also showed the highest Cu intake. Nitrogen fertilizer application rate had no significant effect on Fe, Na and Mn uptake in sorghum (Table 4.14).

4.3.7 Effect of cropping system and planting density on sorghum nutrient uptake

Cropping system and plant density had no significant effect on the uptake of Ca, Mg, K, P, Fe, Na, Mn, Cu, Zn and N in sorghum (Table 4.14).

Treatments	Ν	Р	K	Ca	Mg	Na	Fe	Mn	Cu	Zn
			•	— mg/plant -						
Fertilizer rate (kg N/ha)										
0	471.2±113.0c	120.9±27.4b	1005.8±173.7b	129.8±27.6b	72.8±14.6b	$10.4{\pm}1.8$	6.0±1.4	12.6±1.8	0.2±0.1b	2.2±0.5b
50	1032.7±97.9a	261.5±23.7a	1780.0±150.4a	238.4±23.9a	124.8±12.6a	10.6±1.6	10.9 ± 1.2	11.9±1.6	0.4±0.1a	4.3±0.4a
75	683.0±101.9bc	127.1±24.7b	1034.1±156.4b	127.3±24.8b	79.5±13.1b	10.9 ± 1.7	7.1±1.2	13.7±1.7	0.3±0.1ab	3.1±0.5ab
100	814.4±97.9ab	153.3±23.7b	1243.2±150.4b	156.2±23.9b	99.0±12.6ab	$12.4{\pm}1.6$	$8.0{\pm}1.2$	10.7±1.6	0.4±0.1a	3.5±0.4ab
125	760.6±105.7bc	155.6±25.6b	1202.5±162.5b	134.3±25.8b	83.3±13.6b	12.5±1.7	7.9±1.3	$10.4{\pm}1.7$	0.7±0.1ab	2.7±0.5b
Cropping system										
Monocrop sorghum	733.1±64.9	153.2±15.7	1207.1±99.7	$140.4{\pm}15.8$	85.0 ± 8.4	11.8 ± 1.1	7.88 ± 0.8	11.3±1.1	0.3 ± 0.03	2.99±0.3
Intercrop sorghum	771.7±65.9	174.2±16.0	1299.2±101.3	173.9±16.1	98.8 ± 8.5	13.2±1.1	8.07 ± 0.8	$12.4{\pm}1.1$	0.4 ± 0.03	3.36±0.3
Plant density (plants/ha)										
66 667	641.1±81.3	145.7±19.7	1193.6±124.9	144.6±19.8	87.1±10.5	12.2±1.3	7.18 ± 1.0	11.3±1.3	0.3 ± 0.04	2.9 ± 0.4
53 333	757.9±77.7	159.6±18.8	1228.6±119.4	157.5±18.9	93.3±10.0	12.4±1.3	8.00 ± 0.9	10.9±1.3	0.4 ± 0.04	3.4±0.3
40 000	858.2±81.3	185.7±19.7	1337.1±124.9	169.3±19.8	95.3±10.5	12.9±1.3	8.75 ± 1.0	13.4±1.3	0.4 ± 0.04	3.2±0.4
F statistic										
Fertilizer rate	3.78 **	5.47***	4.07^{**}	3.68**	2.52^{*}	2.22 ns	2.15 ns	0.66 ns	2.64^{*}	3.14^{*}
Cropping system	0.17 ns	0.88 ns	0.42 ns	2.21 ns	1.34 ns	0.82 ns	0.03 ns	0.56 ns	0.25 ns	0.81 ns
Plant density	1.79 ns	1.06 ns	0.36 ns	0.39 ns	0.16 ns	0.08 ns	0.61 ns	1.06 ns	0.06 ns	0.60 ns
Fertilizer rate*cropping	3.14 *	4.26**	4.81^{**}	2.98^{*}	5.42***	3.12*	1.71 ns	2.45*	3.69**	3.19*
system			0.50	0.00	0.00	0.51		o .	0.57	0.50
Fertilizer rate*plant density	0.70 ns	0.78 ns	0.63 ns	0.90 ns	0.88 ns	0.61ns	0.79 ns	0.45 ns	0.65 ns	0.68
Cropping system*plant density	2.11 ns	4.09*	5.93 **	4.25**	4.07*	4.47**	3.50*	3.10*	2.03 ns	4.32**
Fertilizer rate*cropping system* plant density	1.02 ns	0.96 ns	0.92 ns	1.00 ns	0.70 ns	0.78 ns	0.55 ns	1.84 ns	1.07 ns	0.57

Table 4.14: Nutrient uptake in sorghum shoot at the flowering stage

Values followed by dissimilar letters in the same column within a treatment are significant at P \leq 0.05 according to Fischer LSD. *: P \leq 0.05; **: P \leq 0.01; ***: P \leq 0.001. ns=not significant. Values in the columns represent the means and their standard errors.



Figure 4.18: The interaction of fertilizer application rate and cropping system on the uptake of: A) N, B) P, C) K, D) Ca, E) Mg and F) Na in sorghum. Values followed by dissimilar letters in the same column within a treatment are significant at P \leq 0.05 according to Fischer LSD. Error bars represents standard error.

It is noteworthy that the application of fertilizer affected the uptake of N, P, K Mg, Na Cu and Zn in a very similar pattern (Figures 4.18A - F). For example, at 0, 75 and 100 kg N/ha, intercropped sorghum plants exhibited higher uptake nutrients compared to monocropped ones. At 50 kg N/ha, for all the nutrients except Ca, there were no significant differences in their uptake between sole and intercropped sorghum plants (Figures 4.18A - F). Furthermore, at 125 kg N/ha, monocropped plants showed higher uptake of N, P, K, Ca, Mg and Na (Figures 4.18A - F).



Figure 4.19: Interactive effect of N fertilizer application rate and cropping system on the uptake of: A) Mn, B) Cu and C) Zn in sorghum shoot. Values followed by dissimilar letters in the same column within a treatment are significant at P \leq 0.05 according to Fischer LSD. Error bars represents standard error.

Interestingly, the interaction of fertilizer application and cropping system for micronutrients was similar to those of macronutrients, especially for Cu and Zn (Figure 4.19 B and C). For Mn, from 0 to 75 kg N/ha, intercropped sorghum exhibited a higher uptake relative to sole plants, and above 75 kg N/ha, the reverse was true (Figure 4.19A).



Figure 4.20: Interactive effect of planting density and cropping system on the uptake of: A) P, B) K, C) Ca and D) Mg in sorghum shoot. Values followed by dissimilar letters in the same column within a treatment are significant at P \leq 0.05 according to Fischer LSD. Error bars represents standard error.

The interaction of planting density and cropping system influenced the uptake of P, K, Ca and Mg in sorghum (Figure 4.20). At the highest density, intercropped sorghum exhibited higher

uptake of P, K, Ca and Mg compared to sole plants. Interestingly, this trend was reversed at the next density of 53 333 plants/ha with monocropped plants showing significantly higher macronutrients uptake (Figure 4.20 A – D). At 40 000 plants/ha, there were no significant differences in the uptake of P, K and Mg (Figure 4.20 A, B, D) between monocropped and intercropped sorghum plants. The uptake of Ca by sorghum at this planting density was an exception, with intercropped plants showing higher values compared to sole plants (Figure 4.20 C).



Figure 4.21: Interactive effect of planting density and cropping system on the uptake of: A) Na, B) Fe, C) Mn and D) Zn in sorghum shoot. Values followed by dissimilar letters in the same

column within a treatment are significant at P \leq 0.05 according to Fischer LSD. Error bars represents standard error.

The interactive effect of planting density and cropping system on the uptake of Na was similar to that of micronutrients (Figure 4.21 A - D). At the highest density, sorghum intercrops exhibited higher Na, Fe, Mg and Zn uptake relative to sole sorghum plants. Similar to the uptake of macronutrients (Figure 4.20), this trend was reversed for the 53 333 plants/ha density, that is sole sorghum plants showed higher uptake values. At the lowest planting density, (40 000 plants/ha), there were no significant differences in the uptake of Fe and Na between monocropped and intercropped sorghum plants. For Mn and Zn uptake, intercropped plants had higher values (Figure 4.21 C and D).

4.4 Efficiency of intercropping system

4.4.1 Land equivalent ratio (LER)

LER calculated from grain yield was found to be 1.83.

Chapter 5

5 Discussion

5.1 Effect of nitrogen application and planting density on the growth of intercropped sorghum and cowpea

5.1.1 Photosynthetic parameters of cowpea

Photosynthesis is a key process in the establishment of the plant assimilates (Zakariyya and Prawoto, 2015). The effects of planting density, N application, cropping systems and their interactions on photosynthetic parameters (photosynthesis, stomatal conductance transpiration, internal carbon dioxide and chlorophyll) has not been fully appraised under Botswana conditions despite their influence on plant growth and productivity. In this study, cowpea with no fertilizer application (control plants) had higher photosynthetic rates irrespective of cropping system and planting density. The higher photosynthetic rates of control cowpea plants may have stimulated an increase in cowpea shoot biomass because these plants also had a higher shoot biomass than those that received fertilizer (Table 4.1 and 4.4). Therefore in this study nitrogen application depressed cowpea growth. However, Otieno *et al.* (2007) reported that nitrogen application improved the growth of grain legumes (lablab, common bean, green grams and lima bean).

Plant spacing in the field is very important in facilitating aeration and light penetration in to plant canopy for optimizing rate of photosynthesis (Ouji *et al.*, 2016). In this experiment, planting density had a significant ($p \le 0.01$) effect on the rate of photosynthesis in cowpea, with the moderate planting density of 53 333 plants/ha having the highest photosynthetic rates (Table 4.1). This is possibly because solar radiation absorbance is low at thin densities and coefficient of their photosynthetic output is very low, moreover, sufficient sunlight is not absorbed in thick densities which has high leaf area index, but photosynthetic output is very low due to mutual shadowing leaves, therefore maximum sunlight absorbance throughout growth season is very important in canopy (Naseri *et al.*, 2012). Zhang *et al.* (2016) found that in tobacco the moderate plant spacing was conducive for photosynthesis while the higher and lower plant spacing, were unfavourable for the rate of photosynthesis. Similarly Franic *et al.* (2015), found that photosynthetic performance of maize significantly declined with higher maize planting density. On the contrary Wilson *et al.* (2012), found that planting density did not affect the rate of photosynthesis in pigeonpea.

The rate of photosynthesis was significantly the same for both cowpea monocrop and intercrop (Table 4.1). This might be because cowpea is shade tolerant and compatible as an intercrop with cereal crops (Mekonnen *et al.*, 2016), therefore sorghum intercropping did not prevent cowpea from light necessary for photosynthesis. The higher rates of stomatal conductance in cowpea plants that did not receive any fertilizer increased their internal CO₂ leading to higher assimilation of photosynthates. The higher internal carbon dioxide found on plants that received 0 and 100 kg N/ha may be responsible for their increased rate of photosynthesis. (Table 4.1). The main purpose of photosynthesis is to fix carbon dioxide in the leaf to make carbon, therefore higher internal carbon dioxide may lead to higher photosynthetic rates when other factors are not limiting. Thus plants are able to derive their own carbon source directly from inorganic carbon dioxide in the air, by chemically fixing it into an organic form through the process of photosynthesis (Bueckert, 2013).

Intercropped cowpea had significantly ($p \le 0.001$) higher rates of transpiration compared to monocropped cowpea (Table 4.1). Similarly, Walker and Ogindo (2003) found that intercropping maize with bean resulted in higher transpiration rates compared to their sole crops with a transpiration loss of 5% and 6% more than the sole maize and bean respectively. Plants that received 75 and 125 kg N/ha showed higher transpirational water loss (Table 4.1). Therefore, excess nitrogen may be the one that caused cowpea plants that received 125 kg N/ha to have higher transpiration rates and burning of older leaves. This is because, excess nitrogen is believed to increase transpirational water loss, lowering the solute potential, hence the water potential of the soil solution, resulting in increased plant water loss, leading to foliar burn (Liu *et al.*, 2012).

Fertilizer application at the rate of 50 and 75 kg N/ha increased chlorophyll content in cowpea (Table 4.1). At 75 kg N/ha intercropped plants exhibited higher chlorophyll content compared to monocropped plants (Figure 4.1C). After 75 kg N/ha, application of fertilizer reduced the chlorophyll content of cowpea. Kulsum *et al.* (2007) found that chlorophyll content linearly increase with increase in nitrogen fertilizer rate in blackgram. Zhang *et al.* (2014) also found that N fertilization resulted in higher leaf chlorophyll content of lettuce.

The reason why monocropped cowpea had significantly ($p \le 0.001$) higher WUE than intercropped cowpea irrespective of N fertilizer rate (Figure 4.1D), could be that in an intercrop system there can be increased competition of available moisture leading to high water demand and as a result available water is not efficiently used for plant growth and productivity. Similarly Makoi *et al.* (2010) found that sorghum and cowpea plants under monoculture had much higher WUE relative to those in intercropping. Water-use efficiency increased with increase in planting density (Table 4.1). In this study during the planting season, temperatures were extremely high, (with an average of 29.2 0 C) therefore in densely populated cowpea plants soil evaporation was reduced by the cover and most of the water loss is by transpiration and this increased WUE. Similarly Sawargaonkar *et al.* (2013) found that increasing plant spacing (equivalent to lowering planting density) increased WUE is sorghum. Shaheen *et al.* (2010) found that mulching increased WUE in sorghum because it provided soil cover. In another study, Wilson *et al.* (2012) found that WUE was not significantly affected by planting density in pigeonpea. Makoi *et al.* (2010) found that WUE was high on sorghum and cowpea plants that were planted at a lower density of 83 333 plant/ha.

5.1.2 Photosynthetic parameters in sorghum

Intercropped sorghum at 50 and 75 kg N/ha had higher stomatal conductance (Figure 4.7A) which consequently led to higher internal CO₂ concentration and significantly higher transpirational pull and therefore water loss (Figure 4.7B and 4.7C respectively). A higher transpirational pull is associated with more nutrients being uptaken by plant roots leading to high biomass (Figure 4.12). This implies that intercropped sorghum efficiently assimilates food as it was also shown by the higher shoot biomass of the intercropped sorghum when it was not applied with N fertilizer. Ghosh et al. (2006) has found that intercropping sorghum with soybean increased photosynthetic rate of the intercropped sorghum by 1.2 %. Photosynthesis assimilation in sorghum was positively correlated with both stomatal conductance and transpiration (Figure 4.8 A, B and C). This suggests that there is a relationship between stomatal conductance, transpiration and photosynthesis in sorghum. This is true because, stomata are the pores on a leaf surface through which plants regulate the uptake of CO₂ for photosynthesis against the loss of water through transpiration (Haworth et al., 2011). Therefore the relationship between stomatal conductance and photosynthesis is due the fact that stomata controls the exchange of CO₂ and water which are vital for the process of photosynthesis. According to Haworth et al. (2011), a plant with high stomatal conductance incurs higher rates of water loss and associated risks of desiccation. However, when moisture is not limiting a higher water loss is associated with higher nutrient uptake.

5.1.3 Days to flowering in sorghum and cowpea

Flowering in cowpea was significantly ($p \le 0.05$) affected by fertilizer application (Table 4.3). The application rate of 75 kg N/ha speeded up flowering in cowpea. This might be due to the beneficial effect of N application on promoting reproductive growth. According to Satodiya *et al.* (2015) more availability of nutrients enhance early flowering. Control cowpea plants had delayed flowering. This might be because more rapid leaf area development and delayed flowering are options for increasing nitrogen storage and hence, nitrogen fixation activity and crop yield (Sinclair and Vadez, 2012). Cropping system and planting density did not have any significant effect on number of days to 50% flowering in cowpea. Similarly Satodiya *et al.* (2015) found that days to 50% flowering in cowpea did not differ significantly with planting density. With regard to sorghum the treatments did not have any significant effect on days to 50% flowering (Table 4.3). Buah *et al.* (2012) has found that nitrogen application induced early flowering in sorghum.

5.2 Effect of nitrogen application, cropping system and planting density on yield and yield components

5.2.1 Cowpea nodulation and biomass yield

The number of nodules were high on cowpea plants that received 0 and 50 kg N/ha while higher levels of N reduced the number of cowpea nodules irrespective of cropping system (Figure 4.9). High N in soil inhibits nodulation in legumes because it supresses root-hair curling and inhibiting rhizobial infection and when applied at later stages after nodule formation may inhibit nodule development (Streeter and Wong, 1988; Singh and Usha, 2003; Zahran, 1999; Wahab *et al.*, 1996). These plants also had higher photosynthesis, stomatal conductance and WUE. Among the plants that received 0 and 50 kg N/ha, monocropped plants had the highest nodulation (nodule number and nodule biomass) compared with intercropped plants (Figure 4.9). In general, cropping system did not have any significant effect on the number of root nodules. Oroka (2010) also found that cropping system did not affect the number of nodules in cowpea while Sibhatu *et al.* (2015) have found that intercropping reduces the number of nodules in cowpea.

Lower application rates of N fertilizer (0 and 50 kg N/ha) had an effect on increasing nodule biomass while higher application rates of N fertilizer resulted in lower nodule biomass. Streeter and Wong (1988) pointed out that high soil NO_3^- may allow the plant to conserve its energy, since in overall terms more energy is required to fix N₂ than to utilize NO₃⁻. Therefore lower nodule biomass under high N application in cowpea is due to the plant reducing nitrogen fixation process to save energy that is used during N₂ fixation and hence using the N that is readily available from the chemical N fertilizer. Xiao et al. (2004) found that N application seemed to reduce both the number and mass of nodule in fababean. Cowpea when planted as a monocrop had significantly ($p \le 0.001$) higher nodule biomass but when intercropped with sorghum it had a lower nodule biomass (Table 4.4). Similarly Egbe and Egbo (2011) found that intercropping cowpea varieties with maize reduced cowpea nodule biomass compared to when planted as sole cowpea. Makoi et al. (2009) also found that intercropping of cowpea and sorghum significantly reduced the nodule biomass of cowpea compared with monocropped cowpea. This reduction in cowpea nodule biomass when cowpea was intercropped with sorghum could be due to interspecific competition of resources such as water. The number of nodules positively correlated with nodule biomass hence an increase in the number of nodules may lead to an increase in the nodule weight even though about 68% of increase in nodule number can be associated with increase in nodule weight (Table 4.5).

The for higher shoot biomass on control cowpea plants irrespective of planting density and cropping system is due to the fact that these plants were found to have the highest rate of photosynthesis. Similarly Belane and Dakora (2011) found that cowpea genotypes with high leaf photosynthetic rates showed greater stomatal conductance, high transpiration rates, increased water-use efficiency, and greater dry matter yield, while those with low photosynthetic rates exhibited low stomatal conductance, low transpiration rates, low water-use efficiency, and low dry matter yield. Planting density significantly affected cowpea shoot

biomass (Table 4.4). At higher density of 66 667 plants/ha cowpea shoot biomass was significantly lower but at lower densities (of 53 333 and 40 000 plants/ha), cowpea shoot biomass was higher. The lower shoot biomass at higher density is probably due to competition for available resources. Similarly Makoi *et al.* (2009) found that increasing cowpea density reduced the dry matter of shoots, roots, pods and nodules, and therefore whole plant biomass. Although there was no significant difference between shoot biomass of monocrop cowpea and intercropped cowpea, Abusuwar and Bakshawain (2012) found that cowpea grown as a intercrop consistently produced the lowest dry matter yield compared to monocrop. This means that cowpea efficiently uses available resources when it is grown alone with no N fertilizer application than when it is grown alone with higher application rate of nitrogen. Cowpea shoot biomass positively correlated with root biomass therefore the increase and decrease in cowpea root biomass can be explained by the increase and/or decrease in shoot biomass (Table 4.5).

5.2.2 Sorghum biomass yield

Sorghum plants that did not receive LAN fertilizer had a lower shoot biomass compared with to those that did (Table 4.6). Similarly Turgut *et al.* (2005) found that the lowest forage and dry matter yield of sorghum was associated with no nitrogen treatment. Almodares *et al.* (2009) also found that biomass of sorghum and maize were increased significantly by increasing nitrogen fertilizer level. In this study, intercropped sorghum yielded more shoot biomass than monocropped sorghum under all nitrogen applications except for when application rate of 125 kg/ha was applied (Figure 4.12). This means that when nitrogen levels are low in the soil, intercropped sorghum can efficiently make use of available resources and/or it was because intercropped sorghum was indirectly benefiting from nitrogen fixed by cowpea. Dakora and Keya (1997) reported that N transfer does occur in intercropping system. Xiao *et al.* (2004) found that there was N transfer from fababean to wheat through root interaction with distance

and root contact being the main factors affecting N transfer. Sorghum plants that received 50 kg N/ha also had significantly higher root and shoot biomass than other N treatments (Table 4.6). This implies that these plants developed more root biomass as a way of survival mechanism, therefore they were able to explore more soil to acquire more nutrients and leading to increased nutrients acquisition by the plant and hence more shoot biomass. According to Ågren and Franklin (2003), when nutrient availability increases, plants allocate relatively less to their roots, which is consistent with a resource optimization hypothesis as increasing nutrient availability means that less effort is required to acquire this source.

5.2.3 Yield and yield components of sorghum and cowpea

A positive correlation between sorghum yield and panicle weight and between panicle length and panicle weight may imply that an increase in panicle weight may be increased by panicle length and consequently an increase in grain yield (Table 4.8). With respect to cowpea, a positive correlation between number of seeds per pod and pod weight, implied that the weight of the pod may have been increased by the number of the seeds per pod (Table 4.10). Although N fertilizers are known to increase crop productivity, in this study N application was ineffective in increasing significant crop grain yield mostly because of the extremely low rainfall and high temperatures that occurred during the growing season. According to Malik *et al.* (2013) under low rainfall conditions, the use of chemical fertilizers to satisfy nutrient requirements of plants is often ineffective, because of inadequate moisture for mass flow and diffusion. Many researchers have found that fertilizer rate, cropping system, planting density and their interactions had significant effect on yield and yield components of sorghum and cowpea although it is not so in this study. Turgut *et al.* (2005) found that sweet sorghum seed yield was highly responsive to nitrogen fertilizer application with the highest seed yield being obtained in plots fertilized with 150 kg N/ha. Reports of sorghum whether as an monocrop or intercrop are varied. For example, Refay *et al.* (2013) found a significant increment of sorghum grain yield per plant when grain sorghum was intercropped with cowpea. Oseni and Aliyu (2010) found a significantly higher grain and stover yields of both sorghum and cowpea in sole compared to the intercrop. Latha and Singh (2003) found that sorghum grain yield was higher when grown as sole crop than when it was intercropped. Fernandez *et al.* (2012) found that neither row spacing nor plant populations had a significant difference in 1000 seed weight of sorghum under rainfed conditions in a two year study.

5.3 Effects of nitrogen application and planting density on the nutrient uptake of sorghum and cowpea intercrop

5.3.1 Nitrogen use efficiency in sorghum and cowpea

Nitrogen fertilizer application significantly affected the agronomic NUE of sorghum during the flowering stage. The highest NUE was found on sorghum plants that received the lowest fertilizer rate of 50 kg N/ha while further increase in N fertilizer application rate decreased NUE of sorghum under rainfed conditions (Table 4.11). Similarly Sawargaonkar *et al.* (2013) found that N application increased NUE and in that study applying N above 90 kg/ha reduced NUE of sorghum in the semi-arid conditions. Rahman *et al.* (2014) also found that NUE declined with higher levels of fertilizer N applied. In this study, the efficiency of applied N fertilizer was low in an intercrop system as seen by the highest NUE on the monocrop sorghum irrespective of planting density (Figure 4.14). This means that intercropping sorghum with cowpea may have reduced the amount of mineral N fertilizer required by sorghum through indirect benefit from nitrogen fixation. Similarly Sarr *et al.* (2008) found that millet monocrop had the greatest NUE compared with millet intercropped with cowpea.

Nitrogen application rate, cropping system, planting density and their interactions did not have any significant effect on NUE of cowpea at flowering stage and at harvest (Table 4.12) possibly because cowpea is able to meet its nitrogen demand through the process of nitrogen fixation. In general the efficiency of applied LAN was very low in cowpea compared to that of sorghum. This is mainly because legumes are less reliant on inorganic N fertilizer than many other nonlegume crops such as cereals and pasture grasses because of their N_2 fixing ability (Chen, 2006). Thus they can potentially fix about 80% of their own nitrogen need and in addition can contribute to the yield of subsequent crops but all these potential benefits can be harnessed only under certain conditions (Rao, 2014).

5.3.2 Sorghum and cowpea shoot nutrient uptake

5.3.2.1 Cowpea nutrient uptake

The human diet depends directly on the mineral composition of plants and, therefore an increase in nutrient content and availability in agricultural products will have a positive impact on human nutrition (Lopez-Arredondo *et al.*, 2013). Fertilizer application reduced the uptake of N, P, K, Ca, Mg and Fe in cowpea because control plants exhibited significantly higher contents of these nutrients. According to Weisany *et al.* (2013) these mineral nutrients are amongst those that are known to be essential for the legume-Rhizobium symbiosis. Belane *et al.* (2014) found that cowpea genotypes that showed superior symbiotic performance consistently exhibited greater accumulation of minerals. In general, nutrient uptake was higher on control plants and this might have been the reason why these plants had higher growth, photosynthetic WUE and biomass yield than plants that received LAN. The higher N uptake in the control plants is for the reason that NO_3^- inhibits N_2 fixation (Streeter and Wong, 1988). Although cowpea fixes N_2 during the seedling stage when the cotyledon reserves has been exhausted it may suffer N deficiency therefore giving a low dose of N fertilizer may be a good

strategy before biological N fixation kicks in (Abayomi *et al.*, 2008). The uptake of Ca, Mg, P and N was significantly higher on plants planted at a lower density of 40 000 plants/ha. This is probably because at a higher planting density there is increased competition for nutrients. According to Makoi *et al.* (2010) high planting density leads to intense plant-to-plant competition which can decrease the uptake of various nutrients.

5.3.2.2 Sorghum nutrient uptake

The application of limestone ammonium nitrate improved the uptake of N, Cu and Zn in sorghum shoot compared to the control plants. Xue *et al.* (2014) found that N fertilizer application increased Cu and Zn uptake in maize shoot in all stages of growth thus showing that optimized N management is an applicable strategy to improve micronutrient nutrition for maximum yield. According to Hafeez *et al.* (2013), Zn deficiency can be ameliorated in plants with the application of nitrogen fertilizers thus increase in N can increase Zn. In sorghum, the uptake of N, P, K, Ca, Mg, Cu and Zn was higher in plants that received 50 kg N/ha than for other N application rates. Increased nutrient uptake on sorghum plants that received 50 kg N/ha may have been caused by higher NUE and root and shoot biomass yield of these plants (Table 4.2, 4.6, 4.14).

In this study cropping system and planting density had no significant effect on the uptake of N, P, K, Ca, Mg, Na, Fe, Mn, Na, Cu and Zn in sorghum. This implies that sorghum did not significantly compete with cowpea for nutrients probably because they have different rooting system. Increased efficiency on the use of resources in intercropping may occur because the component crops use the resources either at different times, acquire resources from different parts of the soil or in different forms (Echarte *et al.*, 2011). Intercropping of sorghum with cowpea improved its nutrient uptake significantly as the interaction of fertilizer application and

cropping system has shown that for plants that received 0, 75 and 100 kg N/ha, intercropping increased the uptake of N, P, K, Ca, Mg, Mn, Cu and Zn in sorghum. Although the mechanism that triggered high uptake of N, P, K, Ca, Mg, Mn, Cu and Zn on intercropped sorghum plants that received 0 kg N/ha is unknown in this study, other authors suggest that legume may be of benefit to cereal cropping system due to contribution of N from N₂ fixation and improving bioavailability of sparingly soluble P (Carsky et al., 2000). According to Brooker et al. (2015) mechanisms that enhance soil mineral availability have been identified from intercropping systems, but these processes have not been thoroughly examined. Latha and Singh (2003) found that intercropping did not significantly affect nutrient uptake and yield of sorghum as nutrient uptake was higher in sole cropping than in intercropping. The lower/space planting density have shown to have no significant effect on the uptake of P, K, Mg, Fe and Na irrespective of cropping system, suggesting that there was no or less competition of nutrients at low density. However at a higher density of 66 667 plants/ha the uptake of the same nutrients were reduced by monocropping mainly because similar species compete with each other since their nutrient uptake peak is at the same time relative to intercropping. In intercropping, there is an efficient utilization of resources because of different crop species which have different requirements of light, water and nutrients (Martin et al., 2006).

5.4 Efficiency of intercropping system

5.4.1 Land equivalent ratio (LER)

LER of 1.83 shows that intercropping sorghum with cowpea was efficient as it had a yield advantage of 83%. Several researchers have found that intercropping system is efficient and have the potential to increase the long-term sustainability of food production under low inputs in many parts of the world (Brooker *et al.*, 2015; Naim *et al.*, 2013; Ndakidemi and Dakora, 2007; Zougmore *et al.*, 2000).

Conclusions

In general N application under rainfed conditions had little effect on the growth and yield of both intercropped and monocropped cowpea. While it increased the growth and biomass yield of both intercropped and monocrop sorghum, with N fertilizer rate of 50 kg/ha being most efficient in sorghum. Planting density of 53 333 plants/ha increased the growth and biomass yield of sorghum and cowpea as monocrop and as intercrop. Intercropping enhanced sorghum growth as it was seen by higher photosynthetic rates of intercropped sorghum relative to monocropped sorghum. Application of 0 and 50 kg N/ha in cowpea and sorghum respectively increased nutrient uptake of both monocropped and intercropped sorghum and cowpea under different planting densities. In general competition for available nutrients by sorghum-cowpea intercrop was not significant and thus sorghum and cowpea can be suitable cropping system to be adopted by smallholder farmers in sub-Saharan Africa. The increase in plant growth, biomass yield, NUE, WUE and nutrient uptake due to N application, cropping system and planting density did not translate to increment in grain yield of sorghum and cowpea mainly because the 2015/2016 growing season was an abnormal one characterized with extremely low rainfall and higher temperatures. Intercropping sorghum with cowpea was efficient than monocropping in resource utilization as shown by LER value that is greater than one.

Recommendations

Since this study was only one year trial another trials are needed to verify the results. Similar studies should be conducted on different agro-ecological regions, for atleast two seasons. More leguminous plants with high nitrogen fixing capability should be included to fix more nitrogen and reduce fertilizer cost by smallholder farmers. Modelling should be included to help policy makers in planning to assist farmers on improving crop production to increase food security in a sustainable manner. Further studies are needed to; quantify N transferred from cowpea to sorghum in an intercrop system.

References

- Abayomi, Y.A., Ajibade, T.V., Samuel, O.F., & Sa'adudeen, B. F. (2008). Growth and yield responses of cowpea (*Vigna unguiculata* (L) walp) genotypes to nitrogen fertilizer (NPK) application in the Southern Guinea Savanna zone of Nigeria. *Asian Journal of Plant Sciences*, 7(2), 170–176.
- Abunyewa, A. A., Ferguson, R. B., Wortmann, C. S., & Mason, S. C. (2017). Grain sorghum nitrogen use as affected by planting practice and nitrogen rate. *Journal of Soil Science and Plant Nutrition*, 17(1), 155–166.
- Abusuwar, A. O., & Bakshawain, A. A. (2012). Effect of chemical fertilizers on yield and nutritive value of intercropped Sudan grass (*Sorghum sudanense*) and cowpea (*Vigna unguiculata* L. Walp) forages grown in an adverse environment of western Saudi Arabi. *African Journal of Microbiology Research*, 6(14), 3485–3491.
- Adeleke, M. A., & Haruna, I. M. (2012). Residual nitrogen contributions from grain legumes to the growth and development of succeeding maize crop. *ISRN Agronomy*. 2012. 10–15.
- Adjei-Nsiah, S., Kuyper, T., Leeuwis, C., Abekoe, M., Cobbinah, J., Sakyi-Dawson, O., & Giller, K. (2008). Farmers' agronomic and social evaluation of productivity, yield and N₂ -fixation in different cowpea varieties and their subsequent residual N effects on a succeeding maize crop. *Nutrient Cycling in Agroecosystems*, 80(3), 199–209.
- African union special summit of the heads of state and government. (2006). Abuja declaration on fertilizer summit for an African green revolution. Abuja.
- Ågren, G. I., & Franklin, O. (2003). Root: shoot ratios, optimization and nitrogen productivity. *Annals of Botany*, 92(6), 795–800.

- Ajeigbe, H., Mohammed, S., Adeosun, J., & Ihedioha, D. (2010). Farmers' guide to increased productivity of improved legume cereal cropping systems in the savannas of Nigeria Farmers' guide to increased productivity of improved legume cereal cropping systems.
 IITA. Ibadan, Nigeria, 104.
- Al-Mujahidy, S. J., Hassan, M., Rahman, M., & Mamum-Or-Rashid, A. N. (2013). Isolation and characterization of Rhizobium spp. and determination of their potency for growth factor production. *International Research Journal of Biotechnology*, 4(7), 117–123.
- Alhaji, I. H. (2008). Yield performance of some cowpea varieties under sole and intercropping with maize at Bauchi, Nigeria. An International Multidisciplinary Journal, Ethiopia, 2(3), 278–291.
- Alla, W. A. H., Shalaby, E. M., Dawood, R. A., & Zohry, A. A. (2014). Effect of cowpea (Vigna sinensis L) with maize (Zea mays L.) intercropping on yield and its components. International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering, 8(11), 1227–1233.
- Almodares, A., Jafarinia, M., & Hadi, M. R. (2009). The effects of nitrogen fertilizer on chemical compositions in corn and sweet sorghum. *American-Eurasian J. Agric. & Environ. Sci.*, 6(4), 441–446.
- Amedie, B., Hiremath, S. M., Chittapur, B. M., Halikatti, S., & Chimmad, V. (2004). Intercropping of grain legumes in sorghum. *Karnataka Journal of Agriculture Science*, 17(1), 22–27.

Bacon, P.E. (ed). (1995). Nitrogen fertilization in the environment. Marcel Dekker Inc. New York.

Bado, B.V, Batiano, A., & Cescas, M. (2006). Assessment of cowpea and groundnut contributionsto soil fertility and succeeding sorghum yields in the Guinean savannah zone of Burkina Faso

(West Africa). *Biology and Fertility of Soil*, 43(2), 171–176.

- Bationo, A., Traore, Z., Kimetu, J., Bagayoko, M., Kihara, J., Bado, V., ... Koala, S. (2003). *Cropping systems in the Sudano-sahelian zone: Implications on soil fertility management. Syngenta Foundation Symposium on Sustainable Agriculture in the Sahel. Lessons and Opportunities for Action.*
- Bayu, W. Rethman, N.F.G., & Hammes, P. S. (2005). Growth and yield compensation in sorghum *(Sorghum bicolor L. Moench)* as a function of planting density and nitrogen fertilizer in semiarid areas of northeastern Ethiopia. *South African Journal of Plant and Soil*, 22(2), 76–83.
- Belane, A. K., & Dakora, F. D. (2011). Photosynthesis, symbiotic N and C accumulation in leaves of 30 nodulated cowpea genotypes grown in the field at Wa in the Guinea savanna of Ghana. *Field Crops Research*, 124(3), 279–287.
- Belane, A. K., Pule-Meulenberg, F., Makhubedu, T. I., & Dakora, F. D. (2014). Nitrogen fixation and symbiosis-induced accumulation of mineral nutrients by cowpea (*Vigna unguiculata* L. Walp). *Crop and Pasture Science*, 65(3), 250–258.
- Bojović, B., & Marković, A. (2009). Correlation between nitrogen and chlorophyll content in wheat (*Triticum aestivum* L.). *Kragujevac Journal of Science*, *31*(2009), 69–74.
- Brooker, R. W., Bennett, A. E., Cong, W. F., Daniell, T. J., George, T. S., Hallett, P. D., ... White,
 P. J. (2015). Improving intercropping: a synthesis of research in agronomy, plant physiology and research review improving intercropping: a synthesis of research in agronomy, plant physiology and ecology. *New Phytologist*, 206(1), 107–117.
- Buah, S. S. J., & Mwinkaara, S. (2009). Response of sorghum to nitrogen fertilizer and plant density in the Guinea Savanna Zone. *Journal of Agonomy*, 8(4), 124–130.

- Buah, S. S. J., Kombiok, J. M., & Abatania, L. N. (2012). Grain sorghum response to NPK fertilizer in the Guinea Savanna of Ghana. *Journal of Crop Improvement*, 26(1), 101–115.
- Bueckert, R. A. (2013). Photosynthetic carbon fixation and crops. *PS&C Prairie Soils & Crops Journal*, 6, 64–77.
- Camara, O., & Heinemann, E. (2006). Overview of the fertilizer situation in africa: In *background paper for Africa fertilizer summit* (p. 21). Abuja.
- Carsky, R. J., Vanlauwe, B., & Lyasse, O. (2000). Cowpea rotation as a resource management technology for cereal-based systems in the savannas of West Africa. In *Challenges and opportunities for enhancing sustainable cowpea production: proceedings of the World Cowpea Conference III held at IITA* (pp. 252–266).
- Chantereneau, J., & Nicou, R. (1994). The tropical agriculturalist; sorghum. The Macmillan Press
- Chen, J. (2006). The combined use of chemical and organic fertilizers and/or biofertilizer for crop growth and soil fertility. In *International workshop on sustained management of the soilrhizosphere system for efficient crop production and fertilizer use* (Vol. 16, p. 20). Land Development Department Bangkok Thailand.
- Chimonyo, V. G., Modi, A., & Mabhaudhi, T. (2016a). Assessment of sorghum–cowpea intercrop system under waterlimited conditions using a decision support tool. *Water SA*, *42*(2), 317–327.
- Chimonyo, V. G. P., Modi, A. T., & Mabhaudhi, T. (2016b). Water use and productivity of a sorghum–cowpea–bottle gourd intercrop system. *Agricultural Water Management*, 165(2016), 82–96.
- Cocking, E. C. (2005). Intracellular colonization of cereals and other crop plants by nitrogen-fixing 86

bacteria for reduced inputs of synthetic nitrogen fertilizers. *In Vitro Cell. Dev. Biol.*—*Plant*, 41(4), 369–373.

- Crews, T. E., & Peoples, M. B. (2004). Legume versus fertilizer sources of nitrogen: Ecological tradeoffs and human needs. *Agriculture, Ecosystems and Environment, 102*(3), 279–297.
- Dakora, F. D., & Keya, S. O. (1997). Contribution of legume nitrogen fixation to sustainable agriculture in Sub Saharan Africa. *Soil Biology and Biochemistry Bio*, 29(5/6), 809–817.
- Dixon, R., & Kahn, D. (2004). Genetic regulation of biological nitrogen fixation. *Nature Reviews Microbiology*, 2(8), 621–631.
- Drake, B., Gonzalez-Meler, M., & Long, S. (1997). More efficient plants: a consequence of rising atmospheric CO₂. *Annu. Rev. Plant. Physiol. Plant. Mol. Bio*, 48, 609–639.
- Echarte, L., Maggiora, A. Della, Cerrudo, D., Gonzalez, V. H., Abbate, P., Cerrudo, A., ... Calviño, P. (2011). Yield response to plant density of maize and sunflower intercropped with soybean. *Field Crops Research*, 121(3), 423–429.
- Egbe, O. (2010). Effects of plant density of intercropped soybean with tall sorghum on competitive ability of soybean and economic yield at Otobi, Benue State, Nigeria. *Journal of Cereals and Oilseeds*, *1*(1), 1–10.
- Egbe, M. O., & Egbo, C. (2011). Nodulation, nitrogen fixation and harvest index of extra- shortand short-duration cowpea varieties intercropped with maize at Otobi , Benue state, Nigeria. *Journal of Animal and Plant Science*, *10*(3), 1315–1324.
- Estefan, G., Sommer, R., & Ryan, J. (2013). Methods of Soil , Plant , and Water Analysis: A manual for the West Asia and North Africa region (3rd edition). Beirut: International Center for Agricultural Research in the Dry Areas (ICARDA).

- Ewansiha, S. U., Kamara, A. Y., Chiezey, U. F., & Onyibe, J. E. (2014). Agronomic responses of diverse cowpea cultivars to planting date and cropping system. Tropical Agriculture (Trinidad), 91(2), 116-130.
- Fernandez, C. J., Fromme, D. D., & Grichar, W. J. (2012). Grain sorghum response to row spacing and plant populations in the Texas Coastal Bend region. International Journal of Agronomy, 2012, 6.
- Franic, M., Mazur, M., Volenik, M., Brkic, J., Brkic, A., & Simic, D. (2015). Effect of plant density on agronomic traits and photosynthetic performance in the maize IBM population. Poljoprivreda, 21(2), 36-40.
- Gerik, T. J., Rosenthal, W. D., Vanderlip, R. L., & Wade, L.J. (2004). Simulating seed number in grain sorghum from increases in plant dry weight. Agronomy Journal, 96(5), 1222–1230.
- Ghosh, N. (2004). Reducing dependence on chemical fertilizers and its financial implications for farmers in India. *Ecological Economics*, 49(2), 149–162.
- Ghosh, P. K., Bandyopadhyay, K. K., Wanjari, R. H., Manna, M. C., Misra, A. K., Mohanty, M., & Subba Rao, A. (2007). Legume effect for enhancing productivity and nutrient useefficiency in major cropping system- an Indian perspective: A Review. Journal of Sustainable Agriculture, 30(1), 59-86.
- Ghosh, P. K., Manna, M. C., Bandyopadhyay, K. K., Ajay, Tripathi, A. K., Wanjari, R. H., ... Subba Rao, A. (2006). Interspecific interaction and nutrient use in soybean/sorghum intercropping system. Agronomy Journal, 98(4), 1097–1108.
- Giller, K. E. (2001). Nitrogen fixation in tropical cropping systems. Cabi.
- Giller, K. E., & Cadisch, G. (1995). Future benefits from biological nitrogen fixation: An 88

ecological approach to agriculture. Plant and Soil, 174(1-2), 255-277.

- Hafeez, B., Khanif, Y. M., & Saleem, M. (2013). Role of zinc in plant nutrition- A review. *American Journal of Experimental Agriculture*, *3*(2), 374–391.
- Haworth, M., Elliott-Kingston, C., & McElwain, J. C. (2011). Stomatal control as a driver of plant evolution. *Journal of Experimental Botany*, 62(8), 2419–2423.
- Hilhorst, T., & Muchena, F.(Eds). (2000). Nutrients on the move soil fertility dynamics in African farming systems. International Institute for Environment and Development. London.
- Hopkins W.G., & Huner N.P.A. (2009). Introduction to plant physiology. 4th edition. John Wiley and Sons Inc. Hoboken.
- Ibrahim, H., Singh, L., & Babaji, G. (2014). Evaluating yield performance of cowpea varieties under sole and intercropping with sorghum at Bauchi, Nigeria. *Ethiopian Journal of Environmental Studies and Management*, 7, 709–719.
- International Fund for Agricultural Development (IFAD) & United Nations Environment Programme (UNEP). (2013). Smallholders, food security, and the environment. International Fund for Agricultural Development. Rome.
- Karanja, S. M., Kibe, A. M., Karogo, P. N., & Mwangi, M. (2014). Effects of intercrop population density and row orientation on growth and yields of sorghum - cowpea cropping systems in semi arid Rongai, Kenya. *Journal of Agricultural Science*, 6(5), 34–43.
- Karikari, S., Chaba, O., & Molosiwa, B. (1999). Effects of intercropping bambara groundnut on pearl millet, sorghum and maize in Botswana. *African Crop Science Journal*, 7(2), 143–152.
- Khan, M. A., Ali, K., Hussain, Z., & Afridi, A. (2012). Impact of maize-legume intercropping on weeds and maize crops. *Pakistan Journal of Weed Science Research*, 18(1), 127–136.

- Kombiok, J.M., Buah S.S.J., & Sogbedji, J.M. (2012). Enhancing soil fertility for cereal crop production through biological practices and the integration of organic and in-organic fertilizers in northern savanna zone of Ghana.
- Kulsum, M., Baque, M., & Karim, M. (2007). Effects of different nitrogen levels on the leaf chlorophyll content nutrient concentration and nutrient uptake pattern of blackgram. *Pakistan Journal of Biological Sciences*, 10(2), 250–254.
- Kusumi, K., Hirotsuka, S., Kumamaru, T., & Iba, K. (2012). Increased leaf photosynthesis caused by changes elevated in stomatal in Posidonia oceanica cadmium induces DNA conductance in a rice mutant deficient in SLAC1, a guard cell methylation and patterning anion channel protein. *Journal of Experimental Botany*, 63(2), 2–11.
- Lara, M. V., & Andreo, C. S. (2011). C₄ plants adaptation to high levels of CO₂ and to drought environments. In *Abiotic Stress in Plants Mechanisms and Adaptations* (pp. 415–428).
 InTech.
- Latha, K. R., & Singh, R. D. (2003). Effects of cropping system and fertilizer levels on the nutrient uptake and yield by sorghum in rainfed vertisols. *Indian Journal of Research*, *37*(3), 209–213.

Lea, P.J., & Morot-Gaudry, J.F. (eds). (2001). Plant nitrogen. Springer. Berlin.

Ltd. London.

- Leakey, A. D. B., Ainsworth, E. A., Bernacchi, C. J., Rogers, A., Long, S. P., & Ort, D. R. (2009). Elevated CO₂ effects on plant carbon, nitrogen, and water relations: Six important lessons from FACE. *Journal of Experimental Botany*, 60(10), 2859–2876.
- Lithourgidis, A. S., Dordas, C. A., Damalas, C. A., & Vlachostergios, D. N. (2011). Annual intercrops: An alternative pathway for sustainable agriculture. *Australian Journal of Crop*

Science, *5*(4), 396–410.

- Liu, G., Li, Y., & Alva, A. K. (2012). Water potential vs pressure in relation to water movement and transpiration in plants. *International Journal of Agronomy and Plant Production*, 3(10), 369–373.
- Lopez-Arredondo, D., Leyva-González, M. A., Alatorre-Cobos, F., & Herrera-Estrella, L. (2013).
 Biotechnology of nutrient uptake and assimilation in plants. *International Journal of Developmental Biology*, 57, 595–610.
- Machado, S. (2009). Does intercropping have a role in modern agriculture? *Journal of Soil and Water Conservation*, 64(2), 55–57.
- Makoi, J. H. J. R., Chimphango, S. B. M., & Dakora, F. D. (2009). Effect of legume plant density and mixed culture on symbiotic N₂ fixation in five cowpea (*Vigna unguiculata* L. Walp.) genotypes in South Africa. *Symbiosis*, 48(1), 57–67.
- Makoi, J. H. J. R., Chimphango, S. B. M., & Dakora, F. D. (2010). Photosynthesis, water-use efficiency and δ13C of five cowpea genotypes grown in mixed culture and at different densities with. *Photosynthetica*, 48(1), 143–155.
- Malik, M. A., Khan, K. S., Marschner, P., & Fayyaz-ul-Hassan. (2013). Microbial biomass, nutrient availability and nutrient uptake by wheat in two soils with organic amendments. *Journal of Soil Science and Plant Nutrition*, 13(4), 955–966.
- Marandu, A. E. T., Semu, E., Mrema, J. P., & Nyaki, A. S. (2010). Quantification of atmospheric
 N₂ fixed by cowpea, pigeonpea and greengram grown on ferralsols in Muheza District,
 Tanzania. *Tanzania Journal of Agricultural Sciences*, 10(1), 29–37.
- Martin J.H., Walden, R.P., & Stamp D.L. (2006). Principles of field crop production. 4th edition. Pearson Education Inc. New Jersey.
- Matusso, J.M.M., Mugwe, J.N., & Mucheru-Muna, M. (2014). Potential role of cereal-legume intercropping systems in integrated soil fertility management in smallholder farming systems of Sub-Saharan Africa. *Research Journal of Agriculture and Environmental Management*, 3(3), 162–174.
- Mekonnen, G., Sharma, J., Negatu, L., & Tana, T. (2016). Growth and yield response of cowpea (*Vigna unguiculata* L. Walp.) to integrated use of planting pattern and herbicide mixtures in Wollo, northern Ethiopia. *Advances in Crop Science and Technology*, 4(6), 10.

Millar, C.E. (2007). Soil Fertility. Biotech books. New Delhi.

- Moriri, S., Owoeye, L.G., & Mariga, I.K. (2010). Influence of component crop densities and planting patterns on maize production in dry land maize/cowpea intercropping systems. *African Journal of Agricultural Research*, 5(11), 1200–1207.
- Muller-Samann, K.M., & Kotschi, J. (1994). Sustaining growth: soil fertility management in tropical smallholdings. Weikersheim. Margraf Verlag.
- Musa, E. M., Elsheikh, E. A. E., Mohamed Ahmed, I. A., & Babiker, E. E. (2012). Intercropping Sorghum (*Sorghum bicolor* L.) and Cowpea (*Vigna unguiculata* L.): Effect of bradyrhizobium inoculation and fertilization on minerals composition of sorghum seeds. *ISRN Agronomy*, 2012, 1–9.
- Naab, J. B., Chimphango, S. M. B., & Dakora, F. D. (2009). N₂ fixation in cowpea plants grown in farmers's fields in the upper West region of Ghana, measured using 15N natural abundance. *Symbiosis*, *48*(1–3), 37–46.

- Naim, A. M. El, Kilali, B. A., Hassan, A. E., & Ahmed, M. F. (2013). Agronomic evaluation of sorghum and cowpea intercropped at different spatial arrangements. *Journal of Renewable Agriculture*, 1(2), 11–16.
- Naseri, R., Soleymanifard, A., Khoshkhabar, H., Mirzaei, A., & Nazaralizadeh, K. (2012). Effect of plant density on grain yield, yield components and associated traits of three durum wheat cultivars in western Iran. *International Journal of Agriculture and Crop Sciences*, *4*(2), 79-85.
- Ndakidemi, P. A., & Dakora, F. D. (2007). Yield components of nodulated cowpea (*Vigna unguiculata*) and maize (*Zea mays*) plants grown with exogenous phosphorus in different cropping systems. *Australian Journal of Experimental Agriculture*, 47(5), 583–589.
- Noordwijk, M. van, Cadisch, G., & Ong, C. K. (Eds.). (2004). Below-ground interactions in tropical agroecosystems: concepts and models with multiple plant components. Wallingford: CABI.
- Nthabiseng, T. R., Irvine, K. M., & Moshibudi, P. M. (2015). Response of a maize or dry bean intercrop to maize density and dry bean arrangement under rainfed conditions. *International Journal of Agronomy and Agricultural Research (IJAAR) ISSN:*, *6*(6), 18–29.
- Ojiem, J. O., Vanlauwe, B., De Ridder, N., & Giller, K. E. (2007). Niche-based assessment of contributions of legumes to the nitrogen economy of western Kenya smallholder farms. *Plant and Soil*, 292, 119–135.
- Omae, H., Saidou, A. K., & Tobita, S. (2014). Improving millet-cowpea productivity and soil fertility with crop rotation, row arrangement and cowpea density in the Sahel, west Africa. *American-Eurasian J. Agric. & Environ. Sci, 14*(2), 110–115.

Oroka, F. O. (2010). Responses of rice and cowpea intercropping to nitrogen fertilizer and plant

population (1): nodulation, nitrogen fixation and agronomic efficiency. *World Journal of Agricultural Sciences*, 6(2), 154–159.

- Oseni, T. O., & Aliyu, I. G. (2010). Effect of row arrangements on sorghum-cowpea intercrops in the semi arid savannah of Nigeria. *International Journal of Agriculture and Biology*, 12(1), 137–140.
- Otieno, P. E., Muthomi, J. W., Chemining'wa, G. N., & Nderitu, J. H. (2007). Effect of rhizobia inoculation, farmyard manure and nitrogen fertilizer on growth, nodulation and yield of selected food grain legumes. In *African Crop Science Conference Proceedings* (Vol. 8, pp. 305–312).
- Ouji, A., El-Bok, S., Youssef, N. O. B. N., Rouaissi, M., Mouelhi, M., Younes, M., & Kharrat, M. (2016). Impact of row spacing and seeding rate on yield components of lentil (*Lens culinaris* L.). *Journal of New Sciences*, 25(2), 1138–1144.
- Outlaw, W. H., & Vlieghere-He, X. (2001). Transpiration rate. an important factor controlling the sucrose content of the guard cell apoplast of broad bean. *Plant Physiology*, *126*(4), 1716– 1724.
- Paul, E.A. (ed). (2007). Soil microbiology, ecology and biochemistry. 3rd edition. Academic Press. Burlington.
- Plaster, E.J. (2009). Soil science and management. 5th edition. Delmar. New York.
- Prsa, I., Stampar, F., Vodnik, D., & Veberic, R. (2007). Influence of nitrogen on leaf chlorophyll content and photosynthesis of "Golden Delicious" apple. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science*, 57(3), 283–289.

- Pule-Meulenberg, F., Belane, A. K., Krasova-Wade, T., & Dakora, F. D. (2010). Symbiotic functioning and bradyrhizobial biodiversity of cowpea (*Vigna unguiculata* L. Walp.) in Africa. *BMC Microbiology*, 10(89), 12.
- Rahman, M. M., Islam, M. A., Azirun, M. S., & Boyce, A. N. (2014). Tropical legume crop rotation and nitrogen fertilizer effects on agronomic and nitrogen efficiency of rice. *The Scientific World Journal*, (2014), 11.
- Rao, D. L. N. (2014). Recent advances in biological nitrogen fixation in agricultural systems. Proceedings of the Indian National Science Academy, 80(2), 359–378.
- Rashid, A., Khan, R., & Farooq, M. A. (2004). Effect of legume intercropping on sorghum production. *Pakistan Journal of Agricultural Science*, *41*(3–4), 109–113.
- Reeuwijk, L.(ed). (2002). Procedures for soil analysis. International soil reference and information centre (6th edition). Food and Agriculture Organization of the United Nations.
- Refay, Y. A., Alderfasi, A. A., Selim, M. M., & Awad, K. (2013). Evaluation of variety, cropping pattern and plant density on growth and yield production of grain sorghum -cowpea under limited water supply condition growth, yield and yield component characters of sorghum. *Journal of Agriculture and Veterinary Science*, 2(3), 24–29.
- Rockstrom, J. (2000). Water resources management in smallholder farms in Eastern and Southern Africa: An overview. *Physics and Chemistry of the Earth, Part B: Hydrology, Oceans and Atmosphere*, 25(3), 275–283.
- Sadava, D., Hillis, D., Heller, C.H., & Berenbaum, M.R. (2011). Life; the science of biology. 9th edition. Sinauer Associates, Inc. Gordonsville.
- Sarr, P. S., Khouma, M., Sene, M., Guisse, A., Badiane, N., & Yamakawa, T. (2008). Effect of 95

pearl millet – cowpea cropping systems on nitrogen recovery, nitrogen use efficiency and biological fixation using the N tracer technique. *Soil Science and Plant Nutrition*, *54*(1), 142–147.

- Satodiya, B. N., Patel, H. C., & Soni, N. V. (2015). Effect of planting density and integrated nutrient management on flowering, growth and yield of vegetable cowpea (*Vigna unguiculata* (L) Walp). *The Asian Journal of Horticulture*, *10*(2), 232–236.
- Sawargaonkar, G. L., Patil, M. D., Wani, S. P., Pavani, E., Reddy, B. V. S. R., & Marimuthu, S. (2013). Nitrogen response and water use efficiency of sweet sorghum cultivars. *Field Crops Research*, 149, 245–251.
- Shaheen, A., Ali, S., Stewart, B. A., Naeem, M. A., & Jilani, G. (2010). Mulching and synergistic use of organic and chemical fertilizers enhances the yield, nutrient uptake and water use efficiency of sorghum. *African Journal of Agricultural Research*, 5(16), 2178–2183.
- Shata, S. M., Mahmoud, A., & Siam, S. (2007). Improving calcareous soil productivity by integrated effect of intercropping and fertilizer. *Research Journal of Agriculture and Biological Sciences*, 3(6), 733–739.
- Sanginga, N., & Woomer P.L. (eds). (2009). Integrated soil fertility management in Africa; principles, practices and developmental process. Tropical soil biology and fertility institute of the international center for tropical agriculture. Nairobi.
- Sheaffer, C.C., & Moncada, K.M. (2009). Introduction to agronomy: Food, crops and environment. Delmar Cengage learning. New York.
- Sher, A., Barbanti, L., Ansar, M., Manaf, A., & Kaleem, S. (2011). Late harvest associated with P and S fertilization enhances yield and quality of forage sorghum (*Sorghum bicolor* (L.)

Moench), grown as a rainfed crop in Pakistan. *African Journal of Agricultural Reseearch*, 6(29), 6232–6239.

- Sibhatu, B. (2016). Evaluation of cowpea plant density and nitrogen fertilizer for productivity of sorghum/cowpea intercrops at Abergelle. *Journal of Natural Sciences Research*, 6(3), 112– 121.
- Sibhatu, B., Belete, K., & Tessma, T. (2015). Effect of cowpea density and nitrogen fertilizer on a sorghum-cowpea intercropping system in Kobo, northern Ethiopia. *International Journal of Agriculture and Forestry*, 5(6), 305–317.
- Sinclair, T. R., & Vadez, V. (2012). The future of grain legumes in cropping systems. *Crop and Pasture Science*, *63*(6), 501–512.
- Singh, B., & Usha, K. (2003). Nodulation and symbiotic nitrogen fixation of cowpea genotypes as affected by fertilizer nitrogen. *Journal of Plant Nutrition*, *26*(2), 463–473.
- Souza, D. M. D, Morais, A. P. O., Matsushige, I., & Rosa, L. A. (2016). Development of alternative methods for determining soil organic matter. *Revista Brasileira de Ciencia Do Solo*, 40, 1–17.
- Sprent, J. I., Odee, D. W., & Dakora, F. D. (2010). African legumes: A vital but under-utilized resource. *Journal of Experimental Botany*, *61*(5), 1257–1265.

Statistic Botswana. (2015). 2013 Annual Agricultural Survey Report. Gaborone: Author.

- Streeter, J., & Wong, P. P. (1988). Inhibition of legume nodule formation and N₂ fixation by nitrate. *Critical Reviews in Plant Sciences*, 7(1), 1–23.
- Tajul, M. I., Alam, M. M., Hossain, S. M. M., Naher, K., Rafii, M. Y., & Latif, M. A. (2013).

Influence of plant population and nitrogen-fertilizer at various levels on growth and growth efficiency of maize. *The Scientific World Journal*, 2013, 9.

- Tsubo, M., Walker, S., & Ogindo, H. O. (2005). A simulation model of cereal-legume intercropping systems for semi-arid regions: I. Model development. *Field Crops Research*, 93(1), 10–22.
- Turgut, I., Bilgili, U., Duman, A., & Acikgoz, E. (2005). Production of sweet sorghum (*Sorghum bicolor* L . Moench) increases with increased plant densities and nitrogen fertilizer levels.
 Acta Agriculturae Scandinavica Section B Soil And Plant Science, 55(3), 236–240.
- Wahab, A. M., Zahran, H. H., & Abd-Alla, M. H. (1996). Root-hair infection and nodulation of four grain legumes as affected by the form and the application time of nitrogen fertilizer.
 Folia Microbiologica, 41(4), 303–308.
- Walker, S., & Ogindo, H. O. (2003). The water budget of rainfed maize and bean intercrop. *Physics and Chemistry of the Earth*, 28(2003), 919–926.
- Weisany, W., Raei, Y., & Allahverdipoor, K. H. (2013). Role of some of mineral nutrients in biological nitrogen fixation. *Bull. Env. Pharmacol. Life Sci*, 2(4), 77–84.
- Wilson, C., Hui, D., Nwaneri, E., Wang, J., Deng, Q., Duseja, D. &Tegegne, F. (2012). Effects of planting dates, densities, and varieties on ecophysiology of pigeonpea in the southeastern United States. *Agricultural Sciences*, 3(2), 147–152.
- World Bank Group data. http://wdi.worldbank.org/table/3.2#
- Wortmann, C. S., Mamo, M., & Dobermann, A. (2007). Nitrogen response of grain sorghum in rotation with soybean. *Agronomy Journal*, 99(3), 808–813.

- Xiao, Y., Li, L., & Zhang, F. (2004). Effect of root contact on interspecific competition and N transfer between wheat and fababean using direct and indirect 15 N techniques. *Plant and Soil*, 262, 45–54.
- Xue, Y., Yue, S., Zhang, W., Liu, D., Cui, Z., Chen, X., ... Zou, C. (2014). Zinc, iron, manganese and copper uptake requirement in response to nitrogen supply and the increased grain yield of summer maize. *PLoS ONE*, 9(4), 1–12.
- Zahran, H. H. (1999). Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. *Microbiology and Molecular Biology Reviews*, 63(4), 968–989.
- Zakariyya, F., & Prawoto, A. A. (2015). Stomatal conductance and chlorophyll characteristics and their relationship with yield of some cocoa clones under tectona grandis, leucaena sp., and cassia surattensis. *Pelita Perkebunan*, 31(2), 99–108.
- Zhang, J., Bei, Z., Zhang, Y., & Cao, L. (2014). Growth characteristics, water and nitrogen use efficiencies of spinach in different water and nitrogen levels. *Sains Malaysiana*, 43(11), 1665–1671.
- Zhang, Z., Liu, Q., He, K., Feng, X., Cheng, Y., Deng, Y., ... Li, L. (2016). Effects of nitrogen application and plant spacing on photosynthesis. *Agricultural Science and Technology*, 17(3), 645–649.
- Zougmore, R., Kambou, F. N., Ouattara, K., & Guillobez, S. (2000). Sorghum-cowpea intercropping: an effective technique against runoff and soil erosion in the sahel (Saria, Burkina Faso). *Arid Soil Research and Rehabilitation*, *14*(4), 329–342.