BIOLOGICAL NITROGEN FIXATION IN LEGUME-LEGUME AND LEGUME-CEREAL INTERCROPS: EFFECTS ON YIELDS OF SUBSEQUENT MAIZE CROP IN CENTRAL MALAWI

KESTON OLIVER WILLARD NJIRA

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN SOIL AND WATER MANAGEMENT OF SOKOINE UNIVERSITY OF AGRICULTURE, MOROGORO, TANZANIA

2016

EXTENDED ABSTRACT

Declining soil fertility continues to be one of the most important challenges in Malawi's crop production systems, with nitrogen considered as the most limiting nutrient element. However, food legume crops such as pigeon pea (PP) and cowpea (CP) are popular amongst smallholder farmers as they contribute to food security and protein nutrition, source of income to farmers and contribute to soil fertility improvement through biological nitrogen fixation (BNF). They are grown in various cropping systems such as sole cropping, legume-cereal and legume-legume or "doubled-up" intercrops. However, information on BNF, crop productivity, vesicular arbuscular mycorrhizal (VAM) fungal colonisation and contributions of the pigeon pea-cowpea intercrop to subsequent maize (MZ) yields and grain quality is scanty. Therefore, a study to address the aforementioned information gaps was conducted in the 2013/14 and 2014/15 cropping seasons on the Chromic Luvisols of Lilongwe and Dowa districts of Central Malawi. Specific study sites were at the Lilongwe University of Agriculture and Natural Resources (LUANAR), Department of Crop and Soil Sciences Research Farm (14^{o} 11′ S, 33^{o} 46′ E) and at Nachisaka Extension Planning Area (EPA) (13^{o} 37′ S, 33^{o} 56′ E) in Lilongwe and Dowa districts, respectively. The study was aimed at optimizing pigeon pea and cowpea intercropping practices for increased yields of subsequent maize in rotation. Specifically, it included evaluation of the extent of nodulation and BNF by the PP and CP and their productivity in sole cropping, legume-legume and legume-cereal intercropping systems. Additionally, the VAM fungal colonisation of the PP and CP-based systems and rotational maize, and its contribution to the BNF and crop growth was also assessed. Furthermore, N mineralization patterns in the legume-based system plots with residues retained and the rotational maize plant N uptake were assessed. Finally, maize grain and

total dry matter (TDM) yields, harvest index percentages (HI %), grain crude protein contents and nitrogen use efficiencies (NUE) in the subsequent season were evaluated. In the first cropping season (2013/14), the experiment was arranged in the randomized complete block design (RCBD) whereby pigeon pea, cowpea and maize were grown as sole crops, legume-cereal and legume-legume intercrops. The split plot design was used in the second cropping season to grow maize in an integrated soil fertility management (ISFM) approach in which the legume-based systems with residues retained in their plots were the main plots and the 0, 45, 90 and 120 kg ha^{-1} N fertilizer applications formed the sub-plots. Results showed significant effects of the cropping systems on the performance of the PP, CP and MZ crops. Nodulation was significantly increased ($P < 0.05$) under sole cropping. Sole cropped PP nodule dry weights were significantly higher ($P < 0.05$) by 25% and 48% than those of PP in intercrops with CP and MZ, respectively, in the Lilongwe site. Similarly, the nodule dry weights were also significantly higher ($P < 0.05$) by 25% and 46% compared with those in PP in intercrops with CP and MZ, respectively, in the Dowa site. Significant differences in PP nodule numbers were noted for Dowa, with only slight differences in Lilongwe site. Furthermore, sole cropped CP produced significantly higher ($P < 0.05$) nodule dry weights by 38% and 36% than that in CP in an intercrop with PP or MZ, respectively, in the Lilongwe site. Similarly, intercropping systems decreased the percentage of nitrogen derived from the air (%N_{dfa}) and the total amount of N_2 fixed by each of the two legume species. The highest amount of biologically fixed N or N_2 fixed (92.9 kg ha^{-1}), which was significantly higher ($P < 0.05$) than that by the PP under both the PP-CP and PP-MZ intercrops by 31% and 36%, respectively, was noted in the Dowa site. However, a comparison of the overall cropping system BNF contribution per unit area showed the combined amount of biologically fixed N (82.9 kg ha^{-1}) from the two component legume crops in the PP-CP “doubled-up” was comparable to that by the sole cropped PP, at the Dowa site. Furthermore, the PP-CP doubled up BNF at Dowa was significantly higher ($P < 0.05$) than the amounts of N_2 fixed by the sole cropped CP (62.5 kg N ha^{-1}), pigeon pea in the PP-MZ intercrop (59.9 kg N ha^{-1}) or CP in the CP-MZ intercrop (13.1 kg N ha^{-1}). However, a different trend was noted at the Lilongwe site. Although the biologically fixed N (85.7 kg ha^{-1}) by the sole cropped pigeon pea was similarly the highest, the combined amount of N_2 fixed (57.4 kg N ha^{-1}) by the PP and CP in the pigeon pea-cowpea "doubled-up" was significantly lower than that by the sole pigeon pea, by 33%. From this study it was concluded that both legume-legume and legume-cereal intercropping reduces nodulation and BNF per plant but the overall amount of nitrogen fixed per unit area by the PP-CP "doubled up" can be comparable to that by the sole cropped PP depending on environmental conditions. Similar to the BNF, grain and TDM yields per plant and HI%, were also decreased by the intercropping systems. However, the productivity by all the intercropping combinations (PP+MZ, PP+CP and MZ+CP) was higher than under sole cropping as they all resulted in LERs of greater than one and positive monetary advantage index (MAI) values. The PP+MZ intercrop showed to be the most beneficial in terms of both yields and monetary gains as it produced highest LERs and MAI values at both sites of Lilongwe and Dowa. Furthermore, the partial LERs, relative N and P yields showed maize to be the most resilient when intercropped with either PP or CP whereas cowpea was the most suppressed when intercropped with either PP or MZ. Additionally, the VAM fungal colonisation was not affected by the PP and CP-based cropping systems such as sole cropping, legume-cereal and legume-legume intercrops. However, a weak positive relationship was noted between VAM fungal colonisation and yields, P uptake or BNF. Furthermore, all the legume-based cropping systems led to significant increases of the VAM fungal colonisation of the subsequent maize roots by ranges of 39 to 50% and 15 to 36% in the Lilongwe and Dowa sites, respectively, which showed potential of the PP and CP based systems in influencing the P uptake enhancing VAM associations. Furthermore,

interactive effect of the legume residues and inorganic fertilizer led to higher maize grain yields by a range of 30% under treatment that was previously CP+MZ intercrop (1689 kg ha^{-1}) to 59% under treatment that was previously sole cropped CP (2864 kg ha^{-1}) at 0 kg N ha^{-1} fertilizer application than the treatment that was previously sole cropped MZ (1178 kg ha^{-1}), in the Lilongwe site. Similarly, at the highest rate of N application, 120 kg N ha^{-1}, treatments that were previously legume-based produced higher grain yields than the treatment that was previously sole cropped MZ (3277 kg ha^{-1}) by a range of 28% under treatment that was previously CP+MZ intercrop (4525 kg ha^{-1}) to 42% under treatment that was previously sole cropped CP (5665 kg N ha^{-1}), at the Lilongwe site. A similar trend was observed at the Dowa site. Furthermore, from this study it was shown that mixing high quality pigeon pea and cowpea with the low quality maize residues increased mineralization rates, N uptake, and nitrogen use efficiency by the maize grown after the legumes in rotation, with implications on yields. In addition, increasing inorganic N application increased maize grain crude protein content in both study sites, which indicates increased grain quality. Therefore, it was concluded that for smallholder farmers on the Chromic Luvisols of Lilongwe and Dowa districts, central Malawi, an ISFM approach involving PP and CP, either as sole crops, legume-legume or legume-cereal intercrops can substantially increase rotational maize yields, both quantitatively and qualitatively with the implication on reducing the investment costs of inorganic fertilizers.

DECLARATION

I, Keston Oliver Willard Njira, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

....

Keston O. W. Njira Date

(PhD Candidate)

The above declaration is confirmed

....

Prof. E. Semu Date

(Supervisor)

....

Prof. J. P. Mrema Date

(Supervisor)

....

Dr. P. C. Nalivata Date

(Supervisor)

COPYRIGHT

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my academic supervisors, Prof. E. Semu and Prof. J. P. Mrema from the Department of Soil and Geological Sciences at Sokoine University of Agriculture (SUA) and Dr P. C. Nalivata from the Lilongwe University of Agriculture and Natural Resources (LUANAR) for their tireless efforts in checking my work and giving me constructive advices from proposal development, research period and to the writing of this dissertation.

I thank the Alliance for Green Revolution in Africa (AGRA) for fully funding my doctoral studies. I believe this will go a long way in bringing satisfaction to my ambitions but also in contributing to the development of my country. I also thank the Department of Soil and Geological Sciences at SUA for providing a conducive environment for my studies. Dr A. Kaaya, the AGRA Soil Health Project Coordinator at SUA is appreciated for his availability in situations when need for his intervention was coming up.

I thank my employer, the LUANAR for giving me a study leave. I appreciate the Head, Department of Crop and Soil Sciences (DCSS) at LUANAR and the Dowa district farmer, Mr Yohane Ngomacheza for providing land for my field trials. I also thank Mr B. Msukwa, the laboratory technician in the DCSS at LUANAR, for his assistance during plant and soil analyses. I appreciate, Mr Chiwaya, a Nachisaka (Dowa) Extension Planning Area (EPA) Agricultural Extension Development Officer (AEDO) for his assistance in coordinating me with the farmer. I am not forgetting my former Head of Department at LUANAR, Dr V. H. Kabambe for his kindness and encouragement when I was applying for the scholarship.

I thank my parents for the love and care, and for giving me good upbringing that has led me to reach this far. I also thank my sisters, Delibe (late) and Rhoda, and brothers, Edwin (late) and Alick for the social, moral and financial support, and the care they provided to me since a tender age as the youngest member of the family. I also thank my wife, Chikondi Bukani Njira for her love, support and prayers she provided to me during the course of my studies.

Above all, I thank God for keeping my life in good health, and blessing me with the ambition and ability to reach this far.

DEDICATION

This dissertation is dedicated to my daughters, Chitsanzo, Trinity and Divine. I love you all. Your presence adds motivation for my hard work. I also dedicate it to my father, Oliver, and mother, Lefiness, for raising me up with discipline and good Christian upbringing that have always helped me to be gentle and focused.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS, ACRONYMS AND SYMBOLS

ADB	African Development Bank
ADP	Adenosine Diphosphate
AGRA	Alliance for Green Revolution in Africa
ANOVA	Analysis of Variance
ASA	American Society of Agronomy
ATP	Adenosine Triphosphate
B	Boron
BNF	Biolological Nitrogen Fixation
Ca	Calcium
CEC	Cation Exchange Capacity
CP	Cowpea
Cu	Copper
EPA	Extension Planning Area
FAO	Food and Agriculture Organisation of the United Nations
Fd	Ferrodoxin
Fe	Iron
H_2O_2	Hydrogen peroxide
ha	Hectare
HI	Harvest Index
HSD	Honest Significant Difference
ICRISAT	International Crops Research Institute for Semi-Arid Tropics
INM	Integrated Nutrient Management
ISFM	Integrated Soil Fertility Management
K	Potassium

kg	Kilogram
KOH	Potassium hydroxide
LER	Land Equivalent Ratio
LSD	Least Significant Difference
LUANAR	Lilongwe University of Agriculture and Natural Resources
m	Metre
MAI	Ministry of Agriculture and Irrigation
Mg	Magnesium
mg	Milligram
Mn	Manganese
Mo	Molybdenum
MoAFS	Ministry of Agriculture and Food Security
MZ	Maize
N	Nitrogen
N_2	Dinitrogen
NAD	Nicotinamide Adenine Dinucleotide
NADH	Reduced Nicotinamide Adenine Dinucleotide
NB	*Nota Bene*
N_{dfa}	Nitrogen Derived from the Air
NH_3	Ammonia
NiR	Nitrite Reductase
NO_3^-	Nitrate
NR	Nitrate Reductase
NUE	Nitrogen Use Efficiency
P	Phosphorus
PFP_N	Partial Factor Productivity of Applied Nitrogen

PP	Pigeon Pea
PRLC	Percent Root Length Colonised
RCBD	Randomized Complete Block Design
RNY	Relative Nitrogen Yield
RPY	Relative Phosphorus Yield
RYT	Relative Yield Total
SAI	Sustainable Agriculture Intensification
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
SSSA	Soil Science Society of America
TDM	Total Dry Matter
UNESCO	United Nations Educational, Scientific and Cultural Organization
VAM	Vesicular Arbuscular Mycorrhiza
Zn	Zinc

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Soil Fertility Challenges and their Interventions in Sub-Saharan Africa

Soil degradation is one of the main challenges that affect agricultural production in many countries of tropical and subtropical regions, with an estimated 65% of arable land already damaged in Sub-Saharan Africa, leading to low productivity (FAO, 2001; The Montpeller Panel, 2014). Lal (1997) noted that soil degradation is essentially the decline in soil quality or reduction in its productivity and environmental regulatory capacity. Soil degradation is characterized by nutrient depletion, soil erosion, compaction, accumulation of harmful components through salinisation, acidification and other processes, and soil organic matter reduction (The Montpeller Panel, 2014; Lal, 2015; Singh and Ryan, 2015; Zigore *et al.,* 2015). Earlier studies show that countries most affected include Kenya, Malawi, Burundi, Ethiopia, Lesotho and Rwanda (Henao and Baante, 1999). Furthermore, on average, the sub-Saharan Africa lose 22 kg N, 2.5 kg P and 15 kg K per hectare annually (Stoorvogel and Smaling, 1990). The loss of nutrients in sub-Saharan Africa soils is due factors such as erosion, reduced fallows and under-replenishment of nutrients (FAO, 2001).

The degradation and low soil productivity are exacerbated by a number of factors including low inherent soil fertility that is characterized by low CECs and generally low in N and P and poor soil management practices (Bationo *et al.,* 2012). Additionally, increasing human population pressures result in reduced fallows and lead to nutrient mining (Drechel *et al.*, 2001; Ndakidemi *et al.*, 2006). Furthermore, Tully *et al.* (2015) emphasized that the main cause of soil degradation leading to poor soil health for Sub-Saharan African countries is the expansion and intensification of agriculture to feed the growing human populations. The consequence of degrading soils and poor soil health is the high prevalence of food and nutrition insecurity (Singh and Ryan, 2015), and inefficiencies in the returns to inputs such as fertilizers, seed and labour and management in smallholder farms in Sub-Saharan Africa (Sanchez, 2002; Mekuria *et al.,* 2002; Bationo *et al.,* 2004).

Many interventions and possible solutions have been proposed to tackle the challenge of low soil fertility and crop productivity, including use of inorganic fertilizers, organic resources such as farm-yard manures and composts, agroforestry, green manures and integration of legumes in cropping systems. Adoption of most of these practices faces various challenges, including biophysical or technological, socio-economic and policy issues (Ajayi *et al.,* 2007; Sanginga and Woomer, 2009). The use of inorganic fertilizers faces the challenge of low financial power by most of the smallholder farmers to purchase these fertilizers (Alliance for Green Revolution in Africa, AGRA, 2014). Challenges associated with the use of organic resources such as composts, crop residues, livestock and farm-yard manure include low quality (Palm *et al*., 1997), bulkiness, low availability of livestock to produce manure and competing priorities on where and how to use crop residues, whether for managing soil or feeding livestock (Ajayi *et al.,* 2007; Giller *et al.*, 2009; Rusinamodzi *et al*., 2015).

However, the increasing population pressures and environmental deterioration has led to emphasis on the quest for sustainable agriculture intensification (SAI) that recognizes the need for enhanced agricultural productivity while ensuring the protection of the ecosystems and improvement in their resilience (Vanlauwe *et al.,* 2014; Lampkin *et al.*, 2015). Furthermore, Vanlauwe *et al.* (2015) recommends integrated soil fertility management (ISFM) as a good approach to achieving the SAI whereby agronomic efficiency, use of the right germplasm and adaptation to the local conditions are considered while combining inorganic and organic plant nutrient resources for soil fertility improvement.

For the case of Malawi, challenges of human population pressures are high, for instance, the fact that on average, a farming household has less than a hectare of land (Ricker-Gilbert *et al*., 2014) and nutrient depletion from the soils is prominent in derailing agricultural production. Smaling *et al.* (1997) reported estimates of around 40 kg N ha^{-1}, 6.6 kg P ha^{-1} and 33.2 kg K ha^{-1} annual losses from Malawi soils while Munthali *et al.* (2007) reported that N is the major challenge for plant growth, with P, S, B and Zn also becoming challenges as well, depending on location. The use of inorganic fertilizers is often beyond the reach of most smallholder farmers because of their high prices (African Development Bank (ADB), 2011). The government of Malawi has advocated favourable policies for reducing soil fertility challenges, for instance, through the implementation of the farm input subsidies over the years, which has shown small but statistically significant improvements of maize yields (Ricker-Gilbert *et al*., 2014). However, in recent years, the policy of fertilizer subsidies has faced the challenge of rising fertilizer costs on the international markets (Dorward and Chirwa, 2011) and this threatens its sustainability.

Therefore, an ISFM approach can be ideal as it helps to reduce the burden of high costs of inorganic fertilizers while combined with organic resources. The integration of food grain legumes in various cropping systems is popular among Malawi's smallholder farmers as it offers multiple benefits including food and nutrition security, increased income and soil fertility improvement through biological nitrogen fixation (BNF) (Kerr *et al*., 2007; Phiri *et al.*, 2012).

1.2 The BNF and Legume-*Rhizobium* Symbiosis

Biological nitrogen fixation is a biochemical process by which the inert dinitrogen (N_2) gas of the atmosphere is converted to reactive N, ammonia (NH_3) in the presence of a biological catalyst, nitrogenase (Brady and Weil, 2008; Sylvia *et al.,* 2005). Nitrogenase is commonly produced by certain prokaryotic species referred to as diazotrophs (Giller, 2001; Santi *et al*., 2013). The common diazotrophs include several species of symbiotic *Rhizobium*, actinomycetes and cyanobacteria, and free living prokaryotes such as *Azospirillum, Herbaspirillum* and *Azotobacter* (Sylvia *et al*., 2005; Brady and Weil, 2008; Santi *et al.,* 2013). It should be noted that the term *Rhizobium* is retained historically but it represents a number of microbial groups, with current information indicating more than 10 genera that include *Allorhizobium, Bradyrhizobium, Mesorhizobium, Rhizobium, Sinorhizobium (Ensifer), Azorhizobium, Burkholderia, Phyllobacterium, Microvirga, Ochrobacterium, Methylobacterium and Shinella* (Weir, 2016).

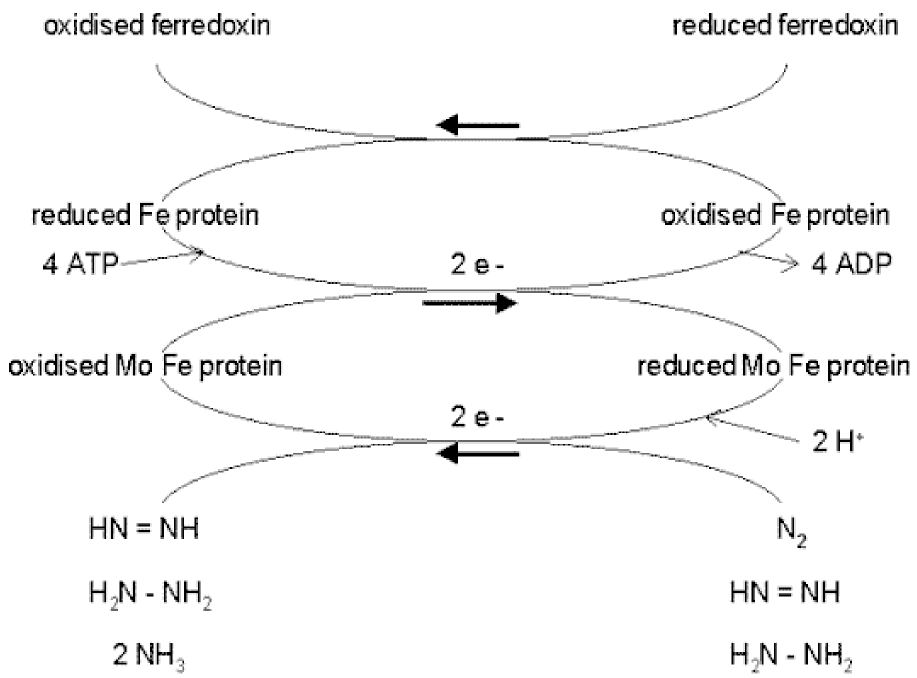
The most efficient nitrogen fixing processes take place in symbiotic or mutualistic relationship with plants. The common mutualistic relationship is the association between rhizobia and roots of legume plants where N_2 is converted to NH_3 in the plant nodules. The NH_3 produced in this interaction benefits the plant as it is taken up and assimilated into amino acids and proteins while the *Rhizobium* microsymbiont benefits from the protected environment and the supply of photosynthates (Sylvia *et al.,* 2005). Significant amounts of N (see Section 1.5) are fixed in these relationships between *Rhizobium* and roots of legumes (Giller, 2001; Havlin *et al.,* 2005; Sylvia *et al.,* 2005; Brady and Weil, 2008).

1.3 The Biochemistry of the Biological Nitrogen Fixation Process

This section elucidates the biochemical process of nitrogen fixation. Reaction (i) is a summary of a series of reactions that take place during biological nitrogen fixation according to Giller (2001):

$$N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16P_i \quad \text{(i)}$$

The ATP generated from respiration (oxidative degradation of sugars and related molecules) in the plant is used as the source of energy for the cleavage and reduction of N_2 into ammonia (NH_3) (Brady and Weil, 2008). The reaction depends on the catalytic activity of nitrogenase. Usually, nitrogenase in rhizobia exists as a complex containing two protein components (Giller, 2001; Sylvia *et al.*, 2005; Nelson and Cox, 2008). Component one contains the active site where N_2 is actually reduced and is known as the molybdenum iron (MoFe) protein or dinitrogenase, whereas component two provides electrons to component one for N_2 reduction and is known as the Fe protein or dinitrogenase reductase (Giller, 2001; Sylvia *et al.*, 2005; Cheng, 2008). Figure 1.1 illustrates the biochemical process of nitrogen fixation as catalysed nitrogenase components.



Source: Deacon, 1997

Figure 1.1: The biochemical process of nitrogen fixation as catalysed by nitrogenase components.

Nitrogenase activity integrates with the normal metabolism with that of a plant by diverting reducing equivalents, electrons, to the ferrodoxin or flavodoxin that donates electrons to the Fe protein (Taiz and Zeiger, 2006). Firstly, the Fe protein is reduced by electrons donated by ferrodoxin (electron donors) (Giller, 2001). The reduced Fe protein binds two molecules of magnesium adenosine triphosphate (MgATP) and reduces MoFe protein (transfering electrons) (Giller, 2001; Cheng, 2008). For each electron transfer from Fe protein to FeMo protein, there is an accompanying hydrolysis of two bound MgATP molecules to give two MgADP molecules. The involvement of ATP molecules in the process provides the energy for breaking the triple bond of N_2 and allows the hydrogen atoms to combine with the N atoms. The MoFe protein donates two electrons that are accompanied by two hydrogen atoms per cycle to N_2 to produce NH=NH.

In two further cycles of this process (each requiring electrons donated by ferrodoxin), HN=NH is reduced to $H_2N\text{-}NH_2$ and this in turn is reduced to $2NH_3$ (Giller, 2001). It should be noted that for every N_2 fixation reaction eight electrons are transferred, out of these six are needed to reduce N_2 to two molecules of NH_3 and the remaining two to produce one molecule of H_2 (Nelson and Cox, 2008). Each electron transfer is accompanied by hydrolysis of two molecules of ATP, hence 16ATP molecules hydrolyzed to 16ADP and $16P_i$ molecules in the process (Giller, 2001; Cheng, 2008).

1.4 Factors that Affect the Rate of Biological Nitrogen Fixation In Grain Legumes

The rate of nitrogen fixation is affected by a number of factors, including temperature, moisture, soil reaction, available nitrogen, presence of effective rhizobial stains, availability of various essential plant nutrients, cropping systems, tillage practices and the influence of vesicular arbuscular mycorrhizal (VAM) (Giller, 2001; Sylvia *et al.,* 2005; Havlin *et al.,* 2005; Brady and Weil, 2008; Makoi and Ndakidemi, 2009; Mohammadi *et al.,* 2012).

Temperature affects nitrogen fixation through its effect on the survival and metabolic activities of rhizobia as high temperatures denature proteins including enzymes (Coyne, 1999) and very low temperatures lead to loss of microbial membrane function and reduced affinity for substrates (Nedwell, 1999). Extreme temperatures also affect nodulation by reducing signal exchange between the symbiotic partners, thus, preventing root hair infection, bacteroid differentiation within the nodules, and the activity of nitrogenase (Mohammadi *et al.,* 2012; Lebrazi and Benbrahim, 2014). Somasegaran and Hoben (1994) reported 25 to 30^{o}C as the optimum temperature for most rhizobial strains but recent studies show a lot of variations, with many studies reporting between 20 and 35^{o}C as rhizobial favourable temperature range (Mohammadi *et al.,* 2012; Gundale *et al*., 2012; Abd-Alla *et al.*, 2014). However, various strains are adapted differently in different environments. For instance, Pinto *et al.* (1998) reported varied capacities of rhizobia in tolerating heat at temperatures in the range of 35 to 39^{o}C. In other studies, over 90% of cowpea rhizobia isolated from the Sahelian Savanna of Niger were reported to grow at 40^{o}C (Abd-Alla *et al.*, 2014). The variations in microbial tolerance to different ranges of temperatures are attributed to genetic differences whereby strains that tolerate very high temperatures and very low temperatures possess heat shock genes and cold shock genes, respectively (Nedwell, 1999; Cavicchioli *et al*., 2001; Rodrigues *et al*., 2006). These genes enable the tolerant microbial strains to produce heat stable and cold stable proteins in hot and cold environments, respectively, and thereby enabling them to carry out their metabolic processes normally (Coyne, 1999).

Plant nutrients may affect nitrogen fixation both negatively and positively. Mohammadi *et al.* (2012) reported a negative exponential relationship between N fertilizer rate and N2-fixation. Excess nitrate in the soil can reduce nitrogenase activity (Havlin *et al.*, 2005). This is because nitrogenase is a depressible enzyme in that its production is inhibited by the presence of the product (Sylvia *et al.,* 2005). However, Tong-Min and Israel (1991) reported reduced nitrogen fixation in soybean due to P deficiency that affected the activity of nitrogenase within the root nodules. On the other hand, Azziz *et al.* (2016) reported increase in BNF in soybean with addition of P fertilizer on the low P Fluvic Luvisols of Ghana. Nitrogen fixation increased by 60% in red kidney beans due to the addition of sulphur (S) in S deficient soils of Michigan (Janssen and Vitosh, 1974).

The presence or absence of effective rhizobia also affects BNF. Some indigenous rhizobial strains are more competitive than introduced strains while they are only infective but produce less effective nodules, and this acts as a barrier to the more effective strains (Mulongoy, 1992). In a study that elucidated a high degree of genetic diversity among strains of rhizobia, only a small fraction was shown to be symbiotically effective on their host plants (Bala and Giller, 2001). This was attributed to differences in compatibility between the host and the rhizobia. Furthermore, the presence of effective rhizobial strains is to some extent influenced by environmental factors, with extreme values of soil pH, moisture levels and salinity affecting their survival negatively (Vriezen *et al*., 2007; Abd-Alla *et al*., 2014). Dry and saline environments reduce the survival of microorganisms by leading to cytoplasm shrinkage, damage to membranes and ribosome structure, and decreased growth (Abd-Alla *et al*., 2014). A number of studies have shown reduction in nitrogenase activity, nodule numbers and BNF due to salt stress and dry conditions (Abd-Alla *et al*., 1998; Shereen *et al*., 1998). It should be noted that dry conditions due to dry spells or drought are becoming more frequent in some areas due to climate variability. However, some reports suggest that climate change may have some positive effects on BNF. With well adapted drought resistant microsymbionts, the carbon dioxide (CO_2) accumulation in the atmosphere may lead to increased photosynthetic rate that has a positive influence on the nitrogen fixing symbiosis (Rao, 2014). On the one hand, reported optimum pH range for rhizobia is 6.0 to 7.0 (Somasegaran and Hoben, 1994). On the other hand, extreme soil reaction levels affect nitrogenase activity though some rhizobial strains have shown good adaptation at pH of 4.5 and others at pH of 11.5 (Abd-Alla *et al*., 2014).

Furthermore, a number of studies show that legume varieties, tillage practices, cropping systems and vesicular arbuscular mycorrhizal (VAM) fungi influence nodulation and BNF. Different varieties have different genetic potential as to how much nitrogen they can biologically fix. For instance, high biomass producing and long duration pigeon pea varieties are known to be more efficient in biological nitrogen fixation than the short duration varieties (Rao and Dart, 1987). On the other hand, research shows that reduced tillage and residue retention have a positive influence on nodulation and BNF. In a study done in Kenya, Kihara *et al.* (2011) reported increased nodule numbers, nodule dry weights and percent of nitrogen derived from the air (%N_{dfa}) in soybean under reduced tillage with residue retention as compared to results in conventional tillage without residue retention. Montanez (2002) noted that reduced tillage lead to periodic decrease in N mineralization and increase in its immobilization, which in return reduces amount of available N and this eventually boosts biological nitrogen fixation. Reviews on the effects of cropping systems and VAM fungi on BNF are included in sections 1.6 and 1.10, respectively.

1.5 Significance of Legumes in Alleviating Soil Fertility Problems in Sub-Saharan Africa

Legume crops have the advantage in obtaining nitrogen (N) through biological nitrogen fixation (Giller, 2001). Biological N_2 fixation involves the reduction of the inert dinitrogen gas (N_2) into ammonia (NH_3) in the presence of the enzyme nitrogenase (Brady and Weil, 2008). The process of BNF contributes to the increase of nitrogen in the soil and improves soil fertility. Ojiem *et al.* (2007) and Nyemba and Dakora (2010) reported a BNF range of 33 to 124 kg N ha^{-1} by groundnuts, whereas Egbe *et al.* (2007) and Njira *et al.* (2012) reported a BNF range of 20 to 124 kg N ha^{-1} by pigeon pea from studies done in sub-Saharan Africa. Benefits of legume N_2 fixation include soil fertility improvement (mainly through legume plant residues left after harvesting), savings on fertilizer costs and extra cash income from sale of crop surpluses and improved protein nutrition (Mpepereki, 2003; Sanginga and Woomer, 2009).

Adoption of legume N_2 fixation technologies in crop production is important because in the first place, decline in soil fertility is a big problem in parts of Sub-Saharan Africa. It is considered as a widespread limitation in growth and yield improvement in many maize based-cropping and farming systems throughout East and Southern Africa (Buresh *et al.*, 1997; Mekuria *et al.*, 2004).

Soil fertility can be improved through the use of mineral fertilizers. However, their use by resource-poor farmers is limited by the prohibitive high prices which are often beyond the reach by these farmers, resulting in the chronic food insecurity in Africa (Druilhe and Barreiro-Hurle, 2012). Palm *et al.* (1997) suggested that solutions to smallholder farmers' soil fertility problems may be obtained through strategic combination of organic resources, particularly from N_2 fixing legumes, with small amounts of mineral fertilizers, that is adoption of integrated soil fertility management (ISFM).

1.6 Legume-cereal and Legume-Legume Cropping Systems

In Malawi, legumes are commonly grown as sole crops and intercrops with cereals such as maize (International Crops Research Institute for Semi-Arid Tropics, ICRISAT/Ministry of Agriculture and Irrigation, MAI, 2000). However, other farmers also grow legumes in intercrops with other legumes, for instance pigeon pea with groundnuts and this is commonly referred to as "doubled-up" legume technology (ICRISAT/MAI, 2000). Farmers in Kasungu district, central Malawi, ranked highly the doubled-up legume technology of pigeon pea and groundnuts, citing food security and soil fertility benefits as reasons (Phiri *et al*., 2012). Research shows that growth and total N_2 fixed by the legume in an intercrop depends on complementarities between the crops for optimizing resources such as nutrients from the soil, moisture and light (Giller, 2001). For instance the slow growth of pigeon pea offers little competition to other crops and does not affect yields of some cereals such as maize (Giller, 2001). However, a number of contrasting results have been obtained on BNF and productivity of various intercropping systems. Ghosh *et al.* (2007) reported pigeon pea/groundnuts as the most beneficial intercrop in terms of resource use while Ghosh *et al.* (2006) reported competition for soil N in a pigeon pea/soybean intercrop, which was attributed to the growing habits of the two crops. Furthermore, in a study done in Malawi, Mhango (2011) reported 44% lower N balance in a pigeon pea-groundnut intercrop whereas Njira *et al.* (2012) reported suppression of pigeon pea BNF in an intercrop with soybean. While there are a number of studies in other cropping systems, very few studies have been done to evaluate BNF in legume/legume intercrops, specifically the pigeon pea-cowpea intercrop.

1.7 Cowpea and Pigeon Pea Cropping Systems as Used in Malawi

Cowpea is one of the most important food legumes in the semi-arid tropics. It is known to resist heat stress and to tolerate droughts (Hall, 2004). It is a multifunctional crop that is important for human food, animal feed, income from sales and soil fertility improvement through biological nitrogen fixation in its symbiotic association with *Bradyrhizobium* spp (Timko and Singh, 2008). In Malawi, cowpea is grown both as a sole crop or an intercrop with other crops especially maize (Ministry of Agriculture and Food Security, MoAFS, 2012). Literature shows that not much research has been done on estimating its BNF quantities when it is grown in different cropping systems.

Pigeon pea is a multipurpose drought tolerant legume crop that provides resource poor farmers with many benefits including protein rich grain, fuel, fodder, fencing material, improved soil fertility and control of erosion (Siambi *et al.,* 1992). In Southern Malawi, where it is mostly grown, it is commonly intercropped with maize (Kamanga, 2002), but in recent years pigeon pea is also grown in other parts of the country. It can also be intercropped with other crops including legumes such as groundnuts and soybean (ICRISAT/MAI, 2000; Kanyama-Phiri *et al.,* 2000; Mhango *et al.,* 2012; Njira *et al.*, 2012).

1.8 The fate of N_2 Fixed by the Legume-*Rhizobium* Association

Biological reduction of atmospheric N_2 to ammonia (NH_3) (N_2 fixation) provides about 65% of the biosphere available N (Mokhele *et al.,* 2012). Most of the ammonia is contributed by legume-rhizobia symbioses, which is initiated by the infection of legume hosts, resulting in the formation of root nodules where the N_2 fixation process takes place (Lodwig *et al.,* 2003). It should be noted that nitrogen from other sources such as fertilizers and organic N also dissociate into NH_4^+ and NO_3^-, which eventually, are also taken up by plants. The most usual fate of biologically fixed N (NH_3) is assimilation by the plant because it is contained in the plant nodules (Serraj, 2004). Plants make use of N for biosynthesis, when it is in a more reduced state (McCashin, 2000), for instance NH_4^+. Hence, after plant uptake, two enzymes are involved in converting NO_3^- to NH_4^+. These are nitrate reductase (NR) that catalyzes the conversion of NO_3^- to nitrite (NO_2^-) commonly in the cytosol of the leaves, and nitrite reductase (NiR) that catalyzes the conversion of NO_2^- to NH_4^+, mostly after translocation of NO_2^- into the plastids/chloroplasts. It should be noted that nitrate reduction also takes place in the roots which commonly depends on electron carriers such as nicotinamide adenine dinucleotide (NADH) and ferrodoxin (Fd) in the process as electron donors (Taiz and Zeiger, 2006). The two equations (ii) and (iii) below summarize the reduction processes of NO_3^- to NH_4^+ (McCashin, 2000; Taiz and Zeiger, 2006).

$$NO_3^- + NADH + H^+ + 2e^- \xrightarrow{NR} NO_2^- + NAD^+ + H_2O \text{ .... (ii)}$$

$$NO_2^- + 6Fd\,(Fe^{2+}) + 8H^+ + 6e^- \xrightarrow{NiR} NH_4^+ + 6Fd\,(Fe^{3+}) + 2H_2O \text{...............(iii)}$$

The two enzymes are inducible, meaning that the presence of NO_3^- will induce the synthesis of NR whereas the presence of NO_2^- will induce the synthesis of NiR (McCashin, 2000). Once formed, NH_4^+ can be incorporated into carbon compounds by reacting with the amino acid glutamate (glutamic acid) to form glutamine (GLN). This reaction is catalyzed by an enzyme called glutamine synthetase (GS) (equation iv).

$$\text{Glutamate} + NH_4^+ + \text{ATP} \xrightarrow{\text{GS}} \text{Glutamine} + \text{ADP} + P_i \text{ .... (iv)}$$

In another step, one molecule of glutamine from the first step is then used by glutamine-2-oxoglutarate-amino-transferase (GOGAT or glutamate synthase) to aminate one molecule of 2-oxoglutaric acid, a product of the Kreb's cycle. The net effect of the process is the generation of two molecules of glutamate (equation v) (Giller, 2001; Taiz and Zeiger, 2006). One molecule continues with the cycle whereas the other is used in the formation of other amino acids and proteins (Giller, 2001).

$$\text{Glutamine} + 2\text{–oxoglutarate} + \text{NADH} + H^+ \xrightarrow{\text{GOGAT}} 2\text{ glutamate} + \text{NAD}^+ \text{ ....(v)}$$

The other fates of fixed N predominate after a plant finishes its life cycle and dies and these follow various processes such as decomposition, mineralization (aminization and ammonification), and nitrification in the soil (Brady and Weil, 2008). Ammonification converts organic N in residues into NH_3 that becomes susceptible to volatilization, or protonation to NH_4^+ that in turn is also easily nitrified to NO_3^- in aerobic conditions (Brady and Weil, 2008; Havlin *et al.*, 2005). Nitrate also has a number of fates, either to be taken up by growing plants or lost through leaching or denitrification (Sylvia *et al.*, 2005; Paul, 2007).

1.9 Mineralization Patterns of Crop Residues

Egbe *et al.* (2007) and Svubure *et al.* (2010) reported superior performance of subsequent maize yields following incorporation of crop residues of food grain legumes, namely pigeon pea, groundnuts, soybean, bambara groundnut and common bean as compared to maize following incorporation of maize crop residues or fallow systems. Residue quality as determined by N, lignin and polyphenol contents is one of the factors that affect the rate of nutrient release and availability for plant uptake (Sanginga and Woomer, 2009). Palm *et al.* (2001) assigned organic resources into basic categories depending on N, lignin and polyphenol contents and made the following recommendations:

(a) High quality organic resources having N > 2.5%, lignin < 15% and polyphenols < 4% should be incorporated directly.

(b) Organic resources having N > 2.5%, lignin > 15% and polyphenols > 4% should be mixed with organic fertilizer or high quality residues.

(c) Organic resources having N < 2.5% and lignin < 15% should be mixed with fertilizer or added to compost.

(d) Lowest quality organic resources having N <2.5% and lignin > 15% should be applied to the soil surface as mulch for erosion and water control.

Research involving mixtures of same quantities of pigeon pea roots plus 2.5 t ha^{-1} maize stover and pigeon pea roots with 5 t ha^{-1} maize stover led to 15 and 35% immobilization of N respectively in Malawi (Makumba and Akinifesi, 2008). In another study Gentile *et al.* (2009) in Kenya, reported an early season N release of 22 kg N ha^{-1} from tithonia leafy biomass with 120 kg N ha^{-1} inorganic N application and an immobilization of 34 kg N ha^{-1} from maize stover with 120 kg N ha^{-1} inorganic N application. However, quantitative information on mineralization patterns of crop residues from legume/legume and legume/cereal intercrops is scanty.

1.10 Vesicular Arbuscular Mycorrhizae Contributions to Plant Nutrition as Influenced by Legume-Based Cropping Systems

Mycorrhiza is a mutualistic association between roots of plant and certain species of fungi. The most common mycorrhizal fungal group reported in over 80% of plant species is referred to as vesicular arbuscular mycorrhizae (VAM) or arbuscular mycorrhiza (AM) (Brundrett, 2004). Mycorrhiza is an adaptive symbiosis that allows the fungi to get energy from the plant's photosynthates while providing a number of benefits to the plant, including enhancement of uptake of P and other nutrients (N, K, Zn) by increasing the root surface area, and producing enzymes and organic acids that solubilise the mineral nutrients (Bolan, 1991; Sylvia *et al*., 2005; Brady and Weil, 2008). Marschner and Dell (1994) reported that VAM can account for 80%, 25% and 10% of a plant's P, N and K uptake, respectively. Furthermore VAM fungal colonisation is also reported to protect some plants from effects of heavy metals by reducing their uptake (Borie *et al*., 2002; Singh, 2012). It also increases water uptake, and protect plants from some pathogens and weeds (Makoi and Ndakidemi, 2009).

Furthermore, some studies show that VAM fungal colonisation enhances BNF through synergistic or additive interactions with rhizobial species. The tripartite symbiotic relationship involving the plant, rhizobia and mycorrhizal fungi has been reported to be beneficial to the BNF process as it enhances soil P uptake, since increasing P levels are associated with increased BNF (Liu *et al*., 2000; Makoi and Ndakidemi, 2009; Lupayi *et al*., 2011; Fageria, 2012). Abd-Alla *et al*. (2014b), in a study in a slightly alkaline to alkaline (pH 7.5 – 8.7) soil in Egypt, reported a synergistic interaction between VAM fungi and *Rhizobium leguminosarum* bv. v*iciae* that led to increase in number and mass of nodules, nitrogenase activity, leghaemoglobin content of nodules, amount of N fixed and dry matter yields of faba bean (*Vicia faba*). Furthermore, the VAM fungi and *Rhizobium leguminosarum* bv. v*iciae* synergistic interaction boosted the VAM fungal colonisation from 40% to 87% over the control as the alkalinity level increased (Abd-Alla *et al*., 2014b). It should be noted that depending on environmental conditions, microbial strains and legume species under study the tripartite symbiosis or interactions of VAM fungi, rhizobia and plant lead to contrasting results. Co-inoculation of common bean (*Phaseolus vulgaris*) with VAM fungi and *Rhizobium* led to lower nodule counts and dry weights compared to inoculation with *Rhizobium* alone (Ballesteros-Almanza *et al*., 2010). In this study the negative effects by the tripartite symbiosis were attributed to competition for photosynthates under the drought tress conditions in which the experiment was conducted (Ballesteros-Almanza *et al*., 2010).

Mycorrhizae proliferation is affected by a number of factors including cropping and rotational systems, and nutrient levels such as of P in the soil. Bagayoko *et al.* (2000) in Niger observed native vesicular arbuscular mycorrhia colonization of 27% on pearl millet roots in a monoculture system and 45% colonization when millet followed cowpea in rotation, with direct positive correlation to grain yield. Lekberg *et al.* (2008) in Zimbabwe reported 45% and 40% maize mycorrhizal colonization after lablab and pigeon pea, respectively, which were slightly higher than mycorrhizal colonization after maize. Furthermore, positive correlations were reported between shoot dry weight of maize and mycorrhizal colonization for maize grown after lablab, pigeon pea and maize (Lekberg *et al*., 2008). Residual effects of mycorrhizal inoculation in Nigeria resulted in 41% higher maize yield than maize yield obtained after non-mycorrhizal maize (Dania *et al.*, 2013). However, information on mycorrhizal colonization in a pigeon pea/cowpea intercrop by both native and inoculated arbuscular mycorrhizal fungi and their effects on yields of subsequent maize crop is scanty. Hence, the need to further address the research gaps.

1.11 Justification

Nitrogen is the major limiting nutrient element in Malawi soils with respect to growth and productivity of maize (Snapp, 1998; Makumba, 2003; Tamene *et al.*, 2015). This poses a serious challenge to Malawi's smallholder farmers as the majority of them apply ≤ 50% of the recommended rate (92 kg N ha^{-1}) of N fertilizer, due to high fertilizer costs (African Development Bank, ADB, 2011). Legume cropping systems and crop residue management are some of the alternative ways of improving soil fertility through the supply of biologically fixed nitrogen (Sanginga and Woomer, 2009).

In Malawi, commonly grown legumes include cowpea (*Vigna unguiculata*), groundnuts (*Arachis hypogaea*), pigeon pea (*Cajanus cajan*), common bean (*Phaseolus vulgaris*) and soybean (*Glycine max*) (Kamanga, 2002). They are grown in different cropping systems including sole cropping, legume-cereal intercropping, and legumes intercropped with other legumes (legume/legume intercrops) in what is commonly known as "doubled-up" legume technology (ICRISAT/MAI, 2000; Mhango, 2011). Ngwira *et al.* (2012) reported 35% higher maize grain yield following pigeon pea/groundnuts intercrop than in a continuous maize monocropping system in Malawi. Some research has been done on sole cropped legumes, legume/cereal intercrops and legume/legume intercrops but not much has been done on evaluating the amounts of biologically fixed N in legume/legume intercrops. Therefore, there is a need to evaluate the amount of biologically fixed N in legume/legume intercrops in comparison with the amount of N_2 fixed in sole legume crops and in legume/cereal intercrops. Very little has been done in evaluating BNF in different cropping systems involving cowpea, which is widely grown in Malawi. Cowpea is also reported to participate in symbiotic relationship with mycorrhizal fungi that improve uptake of P and other nutrients (Bagayoko *et al.*, 2000). However, there is no documentation on the effect of intercrops of pigeon pea and cowpea or maize on vesicular arbuscular mycorrhizae (VAM). Therefore, comparative quantification of BNF, assessment of mycorrhizae colonization and determination of the crop residue effects from the sole cropping, legume/legume and legume/cereal intercrop systems on maize yields is necessary for appropriate ISFM recommendations in Malawi.

1.12 Objectives

1.12.1 Overall objective

To optimize pigeon pea and cowpea intercropping practices for increased yields of subsequent maize in rotation.

1.12.2 Specific objectives

i. To determine the effects of sole cropping and intercropping systems on biological nitrogen fixation by pigeon pea and cowpea and their productivity.
ii. To determine N-release patterns of residues from sole cropping, legume/legume and legume/cereal intercrop systems of pigeon pea, cowpea and maize for synchronization with N uptake by subsequent maize crop.
iii. To determine the effects of sole cropping, legume/legume and legume/cereal intercrop systems of pigeon pea, cowpea and maize on vesicular arbuscular mycorrhizal fungi colonization and their implications on BNF.
iv. To determine the effects of crop residues from the different cropping systems together with supplemental N on the yields of a subsequent maize crop.

1.13 Organization of the Dissertation

This dissertation is organized into six chapters. Chapter One covers the extended introduction containing the background information that include the review of literature on the status of soil fertility in sub-Saharan Africa in general and Malawi in particular. It also covers a review on factors that affect the legume-*Rhizobium* symbiosis and BNF, legume-based cropping systems and their significance in soil fertility improvement, mycorrhizae in maize and legume-based systems and their implications on BNF, and crop residue mineralization patterns and their effects on sub-sequent maize yields. Finally, it states the objectives of the study and outlines the organization of the dissertation.

Chapter Two covers effects of cropping systems on BNF; Chapter Three covers the assessment of legume-based systems productivity while Chapter Four covers VAM fungal colonization in the legume-based systems and on rotational maize following the legume based systems. Chapter Five covers the mineralization patterns of different crop residues, and the effects of integrated legume-based residual N and inorganic N on maize yields. Finally, Chapter Six is a summary of Conclusions from all the previous chapters and Recommendations based on results reported in this dissertation.

REFERNCES

Abd-Alla, M. H., El-Enamy, A. E., Nafady, N. A., Khalaf, D. M. and Morsy, F. M. (2014b). Synergistic interaction of *Rhizobium leguminosarum* bv. *Viciae* and arbuscular mycorrhizal fungi as a plant growth promoting biofertilizers for bean (*Vicia faba* L.) in alkaline soil. *Microbiological Research* 169: 49 – 58.

Abd-Alla, M. H., Issa, A. A. and Ohyama, T. (2014). Impact of harsh environmental conditions on nodule formation and nitrogen fixation of legumes. In Tech. [cnd.intech open.com/pdfs-wm/45747.pdf] site visited on 05/05/2016.

Abd-Alla, M. H., Voung, T. D. and Harper J. E. (1998). Genotypic differences in dinitrogen fixation response to NaCl stress in intact and grafted soybean. *Crop Science* 38: 72 – 77.

Adu-Gyamfi, J. J., Myaka, F. A., Sakala, W. D., Odguard, R., Vesterage, J. M. and Hogh-Jensen, H. (2007). Biological nitrogen fixation and nitrogen and phosphorus budget in farmer-managed intercrops of maize-pigeon pea in semi-arid Southern and Eastern Africa. *Plant and Soil* 295: 127 – 136.

African Development Bank (2011). *Interim Country Strategy Paper for Malawi*. African Development Bank, 20pp.

Ajayi, O. C., Akinifesi, F. K., Sileshi, G. and Chakeredza, S. 2007. Adoption of soil fertility replenishment technologies in southern Africa: Lessons learnt and the way forward. *Natural Resources Forum* 31: 306 – 317.

Alliance for Green Revolution in Africa (2014). *Africa Agriculture Status Report: Climate Change and Smallholder Agriculture in sub-Saharan Africa.* Alliance for Green Revolution in Africa, Nairobi, Kenya. 216pp.

Azziz, A. L. A., Ahiabor, B. D. K., Opoku, A. and Abaidoo, R. C. (2016). Contribution of *Rhizobium* inoculants and phosphorus fertilizer on biological nitrogen fixation, growth and grain yield of three soybean varieties on a Fluvic Luvisol. *American Journal of Experimental Agriculture* 10(2): 1 – 11.

Bakayoko, M., Buerkert, A., Lung, G., Bationo, A. and Romheld, V. (2000). Cereal/legume rotation effects on cereal growth in Sudano-Sahelian West Africa: Soil mineral nitrogen, mycorrhiza and nematodes. *Plant and Soil* 218: 103 – 116.

Bala, A. and Giller, K. E. (2001). Symbiotic specificity of tropical tree rhizobia for host legumes. *New Phytologist* 149: 495 – 507.

Ballesteros-Almanza, L., Altamirano-Hernandez, J., Peña-Cabriales, J. J. Santoyo, G., Sanchez-Yañez, J. M., Valencia-Cantero, E., Macias-Rodriguez, L., Lopez-Bucio, J., Cardenas-Navarro, R. and Farias-Rodriguez, R. (2010). Effect of co-inoculation with mycorrhiza and rhizobia on the nodule trehalose content of different bean genotypes. *The Open Microbiology Journal* 4: 83 – 92.

Bationo, A., Hartemink, A., Lungu, O., Naimi, M., Okoth, P., Smailing, E., Thiombiano, L. and Waswa, B. (2012). Knowing Africa soils to improve fertilizer recommendation. [www. alfredhartemink.nl/2012%20-%20Bationa,%20Hartemink%20et%2] site visited on 14/11/2015.

Bationo, A., Kimetu, J., Ikerra, S., Kimani, S., Mugendi, D., Odendo, M., Silver, M., Swift, M. J., and Sanginga, N. (2004). The African Network for Soil Biology and Fertility: New challenges and opportunities. In: *Managing Nutrient Cycles to Sustain Soil Fertility in Sub-Saharan Africa.* (Edited by Bationo, A.), Academy Science Publishers, Nairobi, Kenya. pp. 1 – 24.

Bolan, N. S. (1991). A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. *Plant and Soil* 134(2): 189 – 207.

Borie, F., Redel, Y., Rubio, R., Rouanet, J. L., Barea, J. M. (2002). Interactions between crop residue application and mycorrhizal development and some soil-root interface properties and mineral acquisition by plants in an acidic soil. *Biology and Fertility of Soils* 36: 151 – 160.

Brady, N. C. and Weil, R. R. (2008). *The Nature and Properties of Soils*. (Revised 14th Ed.), Pearson Prentice Hall, New Jersey. 975pp.

Brundrett, M. C. (2004). Diversity and classification of mycorrhizal associations. *Biological Reviews* 79: 473 – 495.

Buresh, R. J., Sanchez, P. A. and Calhoun, F. (Eds) (1997). *Replenishing Soil Fertility in Africa*. SSSA Special Publication No.51. Soil Science Society of America. 251pp.

Cavicchioli, R., Saunders, N. and Thomas, T. (2001). Cold shock response in microorganisms. In: Extremphiles Basic Concepts. Encyclopia of life support systems. [www.oxford.e olss.net] site visited on 17/05/2016.

Cheng, Q. (2008). Perspectives in biological nitrogen fixation research. *Journal of Integrated Plant Biology* 50(7): 784 – 796.

Coyne, M. S. (1999). *Soil Microbiology: An Exploratory Approach*. (2nd Ed.), Delmar Publishers, Bonn. 462pp.

Dania, S. O., Fagbola, O. and Dania, M. I. (2013). Response of maize-pigeon pea intercrop to arbuscular mycorrhizal fungi in nutrient depleted soil. *Annual Review and Research in Biology* 3(3): 232 – 245.

Deacon, J. (1997). The microbial world: the nitrogen cycle and nitrogen fixation. Institute of Cell and Molecular Biology, University of Edinburgh. [http://archive.bio.ed.ac.uk/jdeacon/ microbes/nitrogen.htm] site visited on 02/06/2016.

Dorward, A. and Chirwa, E. (2011). The Malawi agriculture input subsidy programme: 2005/06 to 2008/09. *International Journal of Agriculture Sustainability* 9(1): 232 – 247.

Drechsel, P., Gyiele, L., Kunze, D. and Cofie, O. (2001). Population density, soil nutrient depletion and economic growth in sub-Saharan Africa. *Ecological Economics* 38: 251 – 258.

Druilhe, Z. and Barreiro-Hurle, J. (2012). *Fertilizer Subsidies in Sub-Saharan Africa. Agricultural Development and Economics.* Working Paper No. 04. Food and Agriculture Organization, Rome. 64pp.

Egbe, O. M., Idoga, S. and Idoko, J. A. (2007). Preliminary investigation of residual effects of pigeon pea geonotypes intercropped with maize in Southern Guinea Savanna of Nigeria. *Journal of Sustainable Development in Agriculture and Environment* 3: 58 – 75.

Fageria, N. K. (2012). *The Role of Plant Roots in Crop Production*. CRC Press, Boca Raton. 468pp.

Food and Agricultural Organization of the United Nations (FAO) (2001). *Soil Fertility Management in Support of Food Security in sub-Saharan Africa.* FAO, Rome. 55pp.

Gentile, R., Vanlauwe, B., Van Kessel, C. and Six, J. (2009). Managing N availability and losses by combining fertilizer-N with quality residues in Kenya. *Agriculture, Ecosystems and Environment* 131: 308 – 314.

Ghosh, P. K., Bandyopadhyay, K. K., Wanjari, R. H., Manna, M. C., Misra, A. K., Mohanty, M. and Rao, A. S. (2007). Legume effect for enhancing productivity and nutrient use-efficiency in major cropping system. An Indian perspective: A review. *Journal of Sustainable Agriculture* 30(1): 59 – 86.

Ghosh, P. K., Mohanty, M., Bandayopadhy, K. K., Painuli, D. K. and Misra, A. K. (2006). Growth, competition, yields advantage and economics in soybean/pigeon pea intercropping system in semi-arid tropics of India II. Effect of nutrient management. *Field Crops Research* 96: 90 – 97.

Ghosh, P. K., Tripathi, A. K., Bandyopadhayay, K. K. and Manna, M. C. (2009). Assessment of nutrient competition and nutrient requirement in soybean sorghum intercropping system. *European Journal of Agronomy* 31: 43 – 50.

Giller, K. E. (2001). *Nitrogen Fixation in Tropical Cropping Systems.* (2^{nd} Ed.), Commonwealth for Agriculture Bureau International, Wallingford. 423pp.

Giller, K. E., Cadish, G., Ebaliostis, C., Adams, E., Sakala, W. D. and Mafongoya, P. L. (1997). Building nitrogen capital in Africa. In: *Replenishing Soil Fertility in Africa.* (Edited by Buresh, J. R., Sanchez, P. A. and Calhoun, F.), SSSA Special Publication No. 51. USA. pp. 47-61.

Giller, K. E., Witter, E., Corbeels, M. and Tittonell, P. (2009). Conservation agriculture and smallholder farming in Africa: the heretics view. *Field Crops Research* 114: 23 – 24.

Giovannetti, M. and Mosse, B. (1980). An evaluation of techniques for vesicular arbscular mycorrhizal infection in roots. *New Phytologist* 84: 489 – 500.

Gundale, M. J., Nilson, M., Bansal, S. and Jaderland, A. (2001). The interactive effect of temperature and light on biological nitrogen fixation. *New Phytologist* 194: 453 – 463.

Hall, A. E. (2004). Breeding for adaptation to drought and heat in cowpea. *European Journal of Agronomy* 21(4): 447 – 454.

Havlin, J. L., Beaton, J. D., Tisdale, S. L. and Nelson, W. L. (2005). *Soil Fertility and Fertilizers: An Introduction to Nutrient Management*. (7^{th} Ed.), Pearson Prentice Hall, New Jersey. 515pp.

Henao. J. and Baanante, C. (1999). Nutrient depletion in the agricultural soils of Africa. A 2020 vision for food, agriculture and environment. 2020 Brief. 2pp.

International Crops Research Institute for Semi-Arid Tropics/Ministry of Agriculture and Irrigation (2000). *Cost-effective Soil Fertility Options for Smallholder Farmers in Malawi.* ICRISAT, Bulawayo and MAI, Lilongwe. 24pp.

Janssen, K. A. and Vitosh, M. L. (1974). Effect of lime, sulfur and molybdenum on nitrogen fixation and yield of dark red kidney beans. *Agronomy Journal* 66: 736 – 740.

Kamanga, B. C. G. (2002). *Farmer Experimentation to Access the Potential of Legumes in Maize-Based Cropping Systems in Malawi.* Risk Management Working Paper No. 02. CIMMYT, Mexico.16pp.

Kanyama-Phiri, G. Y., Snapp, S., Kamanga, B. and Wellard, K. (2000). *Towards Integrated Soil Fertility Management in Malawi: Incorporating Participatory Approaches in Agricultural Research.* Working Paper Managing Africa Soils No.11. Russel Press, London. 28pp.

Kerr, R. B., Snapp, S., Chirwa, M., Shumba, L. and Msachi, R. (2007). Participatory research on legume diversification with Malawian smallholder farmers for improved human nutrition and soil fertility. *Experimental Agriculture* 43(04): 437 – 453.

Kihara, J., Martius, C., Bationo, A. and Vlerk, P. L. G. (2011). Effects of tillage and crop residue application on soybean biological nitrogen fixation in a topical Ferrasol. *Environments* 1: 22 – 37.

Kumwenda, J. D.T., Waddington, S., Snapp, S. S., Jones, R. B. and Blackie, M. J. (1997). Soil fertility management in smallholder maize-based cropping systems of Eastern and Southern Africa: In: *The Emerging Maize Revolution.* (Edited by Eicher, C). Michigan State University, Michigan. pp. 153 – 172.

Lal, R. (1997). Degradation and resilience of soils. *Philosophical Transactions of the Royal Society* 352: 997 – 1010.

Lal, R. (2015). Restoring soil quality to mitigate soil degradation. *Sustainability* 7(5): 5875 – 5895.

Lampkin, N. H., Pearce, B. D., Leake, A. R., Creissen, H., Gerrard, C. L., Girling, R., Lloyd, S., Padel, S., Smith, J., Smith, L. G., Vieweger, A. and Wolfe, M. S. (2015). *The Role of Agroecology in Sustainable Intensification. Report for the Land Use Policy Group*. Organic Research Centre, UK. 151pp.

Lebrazi, S. and Benbrahim, K. F. (2014). Environmental stress conditions affecting N_2 fixing Rhizobium-legume symbiosis and adaptation mechanisms. *African Journal of Microbiology Research* 8(53): 4053 – 4061.

Lekberg, V., Koide, R. T. and Twomlow, S. J. (2008). Effects of agricultural management practices on arbuscular mycorrhizal fungal abundance in low input cropping systems of Southern Africa: A case study from Zimbabwe. *Biology and Fertility of Soils* 44: 917 – 923.

Liu, A., Hamel, C., Hamitton, R. I., Ma, B. L. and Smith, D. L. (2000). Acquistion of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levls. *Mycorrhiza* 9: 331 – 336.

Lodwig, E. M., Hosie, A. H. F., Bourdes, A., Findlay, K., Allaway, D., Karunakaran, R., Downie, J. A. and Poole, P. S. (2003). Amino-acid cycling drives nitrogen fixation in the Legume-*Rhizobium* symbiosis. *Nature* 422: 722 – 726.

Lupwayi, N. Z., Kennedy, A. C. and Chirwa, R. M. (2011). Grain legume impacts on soil biological processes in Sub-Saharan Africa. *African Journal of Plant Science* 5(1): 1 – 7.

Makoi, H. J. R. and Ndakidemi, P. A. (2009). The agronomic potential of vesicular-arbuscular mycorrhiza (VAM) in cereals-legume mixtures in Africa. *African Journal of Microbiology Research* 3(11): 667 – 675.

Makumba, W. (2003). Nitrogen use efficiency and carbon sequestration in legume tree-based agroforestry system: A case study of Malawi. Thesis for the Award of PhD Degree at Wageningen University, The Netherlands, 208pp.

Makumba, W. and Akinifesi, F. K. (2008). Mineralization and N-use efficiency of tree legume prunings from fertilizer tree systems and low quality crop residues in Malawi. *African Journal of Biotechnology* 7(18): 3266 – 3274.

Marschner, H. and Dell, B. (1994). Nutrient uptake in mycorrhizal symbiosis. *Plant and Soil* 159(1): 89 – 102.

McCashin, B. G. (2000). *Induction of Nitrate Reductase in Plant Shoots*. Department of Biological Sciences, University of Alberta. 30pp.

Mekuria, M., Waddington, S. and Siziba, S. (2004). Breaking the "fertilizer poverty" and food insecurity traps in smallholder maize based systems in southern Africa. [http://www. ageconsearch.umn.edu/bitstream/9515/1/cp 04me01.pdf] site visited on 20/08/2012.

Mekuria, M. and Waddington, S. R. (2002). Initiatives to encourage farmer adoption of soil fertility technologies for maize-based cropping systems in southern Africa. In: *Natural Resources Management in African Agriculture: Understanding and Improving Current Practices.* (Edited by. Barrett, C. B., Place, F. and Aboud, A. A). Commonwealth for Agriculture Bureau International, Wallingford, UK. pp. 219 – 233.

Mhango W.G., Snapp S. S. and Kanyama-Phiri, G. Y. (2012). Opportunities and constraints to legume diversification for sustainable maize production on smallholder farms in Malawi. *Renewable Agriculture and Food Systems* 28: 234 – 244.

Mhango, W. G. (2011). Nitrogen budgets in legume based cropping systems in Northern Malawi. Dissertation for the Award of PhD Degree at Michigan State University, USA, 257pp.

Ministry of Agriculture and Food Security (2012). *Guide to Agricultural Production and Natural Resource Management in Malawi*. Agricultural Communications Branch, Lilongwe, Malawi. 366pp.

Mloza-Banda, H. R. (1994). *Principles and Practices of Crop Management: Field Study Guide.* Crop Science Department. Bunda College of Agriculture, Malawi. 137pp.

Mohammadi, K., Shohrabi, Y., Gholamreza, H., Khalesro, S. and Majidi, M. (2012). Effective factors on biological nitrogen fixation. *African Journal of Agricultural Research* 7(12): 1782 – 1788.

Mokhele, B., Zhan, X., Yang, G. and Zhang, X. (2012). Nitrogen assimilation in crops and its affecting factors. *Canadian Journal of Plant Science* 92: 399 – 405.

Montanez, A. (2002). Overview and case studies on biological nitrogen fixation: Perspectives and limitations. *Biological Management of Soil Ecosystems for Sustainable Agriculture* 182: 204 – 224.

Mpepereki, S. and Pompi, S. (2003). Promoting BNF technologies among smallholder farmers: A success story from Zimbabwe. In: *Grain Legumes and Green Manures for Soil Fertility in Southern Africa: Taking Stock of Progress.* (Edited by Waddington, S. R.), CIMMYT, Zimbabwe. pp. 1 – 33.

Mulongoy, K. (1992). Technical paper 2: Biological nitrogen fixation. The AFNETA Alley Farming Training Manual Volume. Alley Farming Network for Tropical Africa. [http://www. fao.org/wairdocs/ILRI/x5546E/x5546e05.htm] site visited on 04/10/2015.

Munthali, M. W. (2007). Integrated soil fertility management technologies: A counteract to existing milestone in obtaining achievable economical crop yields in cultivated lands of poor smallholder farmers in Malawi. In: *Advances in Integrated Soil Fertility Management in sub-Saharan Africa: Challenges and Opportunities.* (Edited by Bationo, A., Waswa, B., Kihara, J. and Kimetu, J.), Springer Netherlands. pp. 531 – 536.

Ndakidemi, P. A., Dakora, F. D., Nkonya, C., Ringo, D. and Mansoor, D. (2006). Yield and economic benefits of common bean (*Phaseolous vulgaris*) and Soybean (*Glycine max*) inoculation in northern Tanzania. *Australian of Journal of Experimental Agriculture* 46(4): 571 – 577.

Nedwell, D. B. (1999). Effect of low temperature on microbial growth: lowered affinity for substrates limits growth at low temperature. *FEMS Microbiology Ecology* 30(2): 101 – 111.

Nelson, D. L. and Cox, M. M. (2008). *Lehninger Principles of Biochemistry*. (5th Edition), WH. Freeman and Company, USA. 1158pp.

Ngwira, A. R., Kabambe, V. H., Kambauwa, G. Mhango, W. G. and Mwale, C. D. (2012). Scaling out best fit legume technologies for soil fertility enhancement among smallholder farmers in Malawi. *African Journal of Agricultural Research* 7(6): 918 – 228.

Njira, K. O. W., Nalivata, P. C., Kanyama-Phiri, G. Y. and Lowole, M. W. (2012). Biological nitrogen fixation in sole and doubled-up legume cropping systems in the sandy soils of Kasungu, Central Malawi. *Journal of Soil Science and Environmental Management* 3(9): 224 – 230.

Nyemba, R. C. and Dakora, F. D. (2010). Evaluating nitrogen fixation by food legumes in farmers' fields in three agroecological zones of Zambia using ^{15}N natural abundance. *Biology and Fertility of Soils* 46: 461 – 470.

Ojiem, J. O., Vanlauwe, B., Ridder, N. and 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.

Palm, C. A. and Sanchez, P. A. (1991). Nitrogen release from the leaves of some tropical legumes as affected by their lignin and polyphenolic contents. *Soil Biology and Biochemistry* 28: 83 – 88.

Palm, C. A., Gachengo, C. N., Delve, R. J., Cadish, G. and Giller, K. E. (2001). Organic inputs for soil fertility management: Some rules and tools. *Agriculture, Ecosystems and Environment* 83: 27 – 42.

Palm, C. A., Myers, R. J. K. and Nandwa, S. M. (1997). Combined use of organic and inorganic nutrient sources for soil fertility maintenance and replenishment. In: *Replenishing Soil Fertility in Africa.* (Edited by Buresh, R. J., Sanchez, P. A. and Calhoun, F.) SSSA Special Publication No 51. Soil Science Society of America, Madison, Wisconsin. pp. 151 – 192.

Paul, E. A. (Ed.) (2007). *Soil Microbiology, Ecology and Biochemistry.* (3rd Edition), Elsevier, Amsterdam. 514pp.

Peoples, M. B., Faizah, A. W., Rerkasem, B. and Herritedge, D. F. (1989). *Methods for Evaluating Nitrogen Fixation by Nodulating Legumes in the Field*. Australian Centre for International Agriculture Research, Canberra. 89pp.

Phiri A. D. K., Kanyama-Phiri, G.Y. and Snapps, S. (1999). Maize and sesbania production in relay cropping at three landscape positions in Malawi. *Agroforestry Systems* 47: 153 – 162.

Phiri, A. T., Mhango, W. G., Njoloma, J. P., Kanyama-Phiri, G. Y. and Lowole, M. W. (2012). Farmers evaluation of integrated soil fertility management methods in Northern Kasungu, Central Malawi. *Journal of Agricultural Extension and Rural Development* 4(11): 340 – 346.

Pinto, P. P., Raposeiras, R., Macedo, A. M., Seldin, L., Paira, E. and Sa, N. M. H. (1998). Effects of high temperature on survival, symbiotic performance and genomic modifications of bean nodulating *Rhizobium* strains. *Revista de Microbiologia* 29(4): 295 – 300.

Rao, D. L. N. (2014). Recent advances in nitrogen fixation in agricultural systems. *Proceedings of the Indian Natural Science Academy* 80(2): 359 – 378.

Rao, J. V. D. K. K. and Dart P. J. (1987). Nodulation, nitrogen fixation and nitrogen uptake in pigeonpea (*Cajanus cajan* (L.) Millsp) of different maturity groups. *Plant and Soil* 99(2): 255 – 266.

Ricker-Gilbert, J. Jumbe, C. and Chamberlin, J. (2014). How does population density influence agricultural intensification? Evidence from Malawi. *Food Policy* 48: 114 – 128.

Rodrigues, C. S., Laranjo, M. and Oliveira, S. (2006). Effect of heat and pH stress in the growth of chickpea mesorhizobia. *Current Microbiology* 53(1): 1 – 7.

Rusinamodzi, L., Van Wijk, M. T., Corbeels, M., Rufino, M. C. and Giller, K. (2015). Maize crop residue uses and trade-offs on smallholder crop-livestock farms in Zimbabwe: Economic implications of intensification. *Agriculture, Ecosystems and Environment* 214: 31 – 45.

Sanchez, P. E. (2002). Soil Fertility and Hunger in Africa. *Science* 295: 2019 – 2020.

Sanginga, N. and Woomer, P. L. (eds) (2009). *Integrated Soil Fertility Management in Africa: Principles, Practices and Developmental Process.* Tropical Soil Biology Institute of the International Centre for Tropical Agriculture, Nairobi. 263pp.

Santi, C., Bogusz, D. and Franche, C. (2013). Biological nitrogen fixation in non-legume plants. *Annals of Botany* 111(5): 743 – 763.

Serraj, R. (2004). *Symbiotic Nitrogen Fixation:Prospects of Enhanced Application in Tropical Agriculture.* Oxford and IBH Publishing Company, New Delhi. 367pp.

Shereen, A., Ansari, R., Naqvi, S. S. M. and Soomro, A. Q. (1998). Effect of salinity on *Rhizobium* species, nodulation and growth of soybean (*Glycine max* L.). *Pakistan Journal Botany* 30: 75 – 81.

Singh, B. and Ryan, J. (2015). Managing fertilizers to enhance soil health. IFA. [www.fertilizer.org/library] site visited on 16/11/2015.

Singh, P. K. (2012). Role of glomalin related soil protein produced by arbuscular mycorrhizal fungi: A review. *Agricultural Science Research Journal* 2(3): 119 – 125.

Smaling, E. M. A., Nandwa, S. M. and Jansen, B. H. (1997). Soil fertility in Africa is at stake. In: Replenishing Soil Fertility in Africa. (Edited by Buresh, J. R., Sanchez, P. A. and Calhoun, F.). SSSA Special Publication, USA. pp. 47 – 61.

Snapp, S. S. (1998). Soil nutrient status of smallholder farms in Malawi. *Communications in Soil Science and Plant Analysis* 29(17-18): 2571 – 2588.

Somasegaran, P. and Hoben, H. J. (1994). *Handbook for rhizobia: Methods in Rhizobium-legume technology*. Springer-Verlag. New York. 450pp.

Sorrantonio, M. (1991). *Methodologies for Screening Soil-improving Legumes*. Rodale Institute. Kutztown, USA. 118pp.

Stoorvogel, J. J. and Smaling, E. M. A. (1990). *Assessment of Soil Nutrient Depletion in Sub-Saharan Africa: 1983-2000.* Report No. 28. Winand Staring Centre, Wageningen, The Netherlands. 585pp.

Svubure, O., Mpepereki, S. and Makonese, F. (2010). Sustainability in maize-based cropping systems in rural areas of Zimbabwe: an assessment of the residual soil fertility effects of grain legumes on maize (*Zea mays* [L.]) under field conditions. *International Journal of Engineering, Science and Technology* 2(7): 141 – 148.

Sylvia, D. M., Furhmann, J. J., Hartel, P. G. and Zuberer D. A. (2005). *Principles and Application of Soil Microbiology*. (2nd Ed), Pearson Prentice Hall, New Jersey. 640pp.

Taiz, L. and Zeiger, E. (2006). *Plant Physiology*. (4th Ed.), Sinauer Associates, Inc., Publishers, Sanderland. 764pp.

Tamene, L., Mponela, P., Ndengu, G. and Kihara, J. (2015). Assessment of maize yield gap and major determinant factors between smallholder farmers in the Dedza district of Malawi. [http: /link.springer.comarticle/10.1007/s10705-015-9692-7] site visited on 04/122015.

The Montpeller Panel (2014). *No Ordinary Matter: Conserving, Restoring and Enhancing Africa's Soils*. Agriculture for Impact, 37pp.

Timko, M. P. and Singh, B. B. (2008). Cowpea, a multifunctional legume. In: *Genomics of Tropical Crop Plants.* (Edited by Moore, P. H. and Ming, R.), Springer, New York. pp. 227 – 258.

Tong-Min, S. and Israel, D. W. (1991). Energy status and functioning of phosphorus-deficient soybean nodules. *Plant Physiol*ogy 97(3): 928 – 935.

Tully, K., Sullivan, C., Weil, R. and Sanchez, P. (2015). The state of soil degradation in sub-Saharan Africa: Baselines, trajectories and solutions. Sustainability 7: 6523 – 6552.

Vanlauwe, B., Coyne, D., Gockowski, J., Hauser, S., Huising, J., Masso, C., Nzigueheba, G., Schut, M. and Van Asten, P. (2014). Sustainable intensification and African smallholder farmer. *Current Opinion in Environmental Sustainability* 8: 15 – 22.

Vanlauwe, B., Descheemaeker, K., Giller, K. E., Huising, J., Merckx, R., Nziguheba, G., Wendt, J. and Zingore, S. (2015). Integrated soil fertility Management in sub-Saharan Africa: Unravelling local adaptation. *Soil* 1: 491 – 508.

Vriezen, J. A., De Bruijn, F. J. and Nusslein, K. (2007). Responses of rhizobia to desiccation in relation to osmotic stress, oxygen, and temperature. *Applied and Environmental Microbiology* 73(11): 3451 – 3459.

Weir, B. S. (2016). The current taxonomy of rhizobia. New Zealand Rhizobia website. [http://www.rhizobia.co.nz/taxonomy/rhizobia] site visited on 13/05/2016.

Zingore, S., Mutegi, J., Agesa, B., Tamene, L. and Kihara, J. (2015). Soil degradation in Sub-Saharan Africa and crop production options. *Better Crops* 99(1): 24 – 26.

CHAPTER TWO

2.0 BIOLOGICAL NITROGEN FIXATION BY PIGEON PEA AND COWPEA GROWN IN "DOUBLED-UP" AND OTHER CROPPING SYSTEMS ON THE LUVISOLS OF CENTRAL MALAWI

Abstract

Legumes form a very important component in Malawi's cropping systems especially due to their roles in food security, income generation and soil fertility improvement through biological nitrogen fixation (BNF). They are commonly grown in various cropping systems including sole cropping, cereal-legume intercrops and legume-legume ("doubled-up") intercrops. However, information on BNF by the pigeon pea and cowpea under doubled-up system is scanty. Therefore, a comparative study was conducted at two sites of Lilongwe and Dowa in the 2013/14 growing season, to quantify the amounts of biologically fixed nitrogen in the three legume cropping systems. The experiments were laid out in a randomized complete block design and biological nitrogen fixation was estimated using the modified nitrogen difference method. Results showed that there were significant differences ($P < 0.05$) in nodule numbers, nodule dry weights, $\%N_{dfa}$ and quantity of N_2 fixed per unit area due to cropping systems effects at both sites. Sole cropped pigeon pea produced the highest N_2 fixed (92.9 kg N ha^{-1}) which was significantly higher by 86%, 30%, 36% than the amounts fixed in the cowpea-maize intercrop, sole cowpea and pigeon pea-maize intercrop, respectively, in the Dowa site. On the other hand, the total sum of the amounts of N_2 fixed (82.9 kg N ha^{-1}) by the component crops in the pigeon pea-cowpea "doubled-up" was comparable to that by sole cropped pigeon pea in the Dowa site. However, for Lilongwe site the doubled-up cropping system total amount of biologically fixed nitrogen (57.4 kg N ha^{-1}) was significantly lower than that by the sole cropped pigeon pea (85.7 kg N ha^{-1}) by 33%. The 57.4 kg N ha^{-1} was not significantly different from the biologically fixed nitrogen by the sole cropped cowpea and pigeon pea-maize intercrop but was significantly higher ($P < 0.05$) than that by cowpea-maize intercrop (10.2 kg N ha^{-1}) by 82%. From this study it can be noted that all three legume cropping systems can lead to substantial amounts of biologically fixed nitrogen, but their implementation should consider both combinations and environmental factors for specific sites.

Key words: Biological nitrogen fixation, cropping systems, legume-legume intercrop, soil fertility.

2.1 Introduction

The quest for high agricultural productivity in many parts of the world is hampered by many factors including climate variability, growing human populations that put more pressure on land resources and reduced productivity of the soils (AGRA, 2014; Reynolds *et al*., 2015). In Sub-Saharan Africa, the challenge of declining soil productivity is enormous, which is also exacerbated by low economic status of most smallholder farmers who cannot afford enough quantities of inorganic fertilizers to effectively replenish nutrients in their farms (Druille and Barreto-Hurle, 2012; AGRA, 2014). In Malawi, declining soil fertility, with reference to nitrogen as the major limiting nutrient for crop growth, has been the biggest challenge in sustainably achieving optimum yields of maize, the main staple crop of the country (Kumwenda *et al*., 1997; Makumba, 2003). Although various technologies of soil management have been developed by researchers and some practised by smallholder farmers, they face numerous challenges in many parts of Sub-Saharan Africa, including Malawi, in terms of adoption by smallholder farmers due to various reasons. These include transportation costs and low level of ownership of livestock that could produce manure (Ajayi *et al.*, 2007) and low nutrient content of many organic soil amendments (Palm *et al.*, 1997).

However, legumes that are known to participate in symbiotic N_2 fixation are commonly grown in many parts of Sub-Saharan Africa. In Malawi, commonly grown legumes include common beans (*Phaseolus vulgaris*), cowpea (*Vigna ungiculata*), soybean (*Glycine max*), groundnut (*Arachis hypogaea*) and pigeon pea (*Cajanus cajan*) (Mhango *et al.*, 2012; Ngwira *et al.*, 2012). Snapp *et al.* (2014) reported that 35 to 50% of maize plots surveyed in Malawi integrated some legumes. Legumes offer many advantages to farmers, including increased income, protein source and soil fertility improvement through biological nitrogen fixation (BNF). Biological nitrogen fixation is achieved through the involvement of legumes in a mutualistic relationship with bacteria, mainly rhizobia. Rhizobia is a generalized name but is a group that includes various genera such as *Allorrhizobium*, *Bradyrhizobium*, *Rhizobium*, *Sinorhizobium* and *Mesorhizobium* (Giller, 2001; Syvia *et al.*, 2005; Berrada and Fikri-Benbrahim, 2014). Various amounts of biologically fixed N have been reported from studies done in Sub-Saharan Africa, ranging from 13 to 167 kg ha^{-1} for pigeon pea (Giller, 2001), 33 to 124 kg ha^{-1} for groundnuts (Nyemba and Dakora, 2010; Mhango, 2011) and 47 to 105 kg ha^{-1} for cowpea (Giller, 2001).

The amounts of biologically fixed nitrogen and their subsequent contribution to soil fertility improvement vary according to a number of factors, including available plant nutrients such as macro- and micronutrients, soil reaction, cropping systems used and other environmental factors (Giller, 2001; Sylvia *et al.*, 2005; Brady and Weil, 2008; Mohammadi *et al.,* 2012). In Malawi, farmers grow legumes in various cropping systems, including sole cropping, cereal-legume intercrops and legume-legume intercropping which is popularly known as "doubled-up" legume technology (ICRISAT/MAI, 2000; Mhango *et al.*, 2012).

A doubled-up legume technology is where a legume is intercropped with another legume and this is commonly done by involving a tall legume such as pigeon pea and other relatively short legumes such as groundnuts, soybean or cowpea (ICRISAT/MAI, 2000). Farmers ranked doubled-up legumes as being highly beneficial, citing reasons such as increased food security, labour saving and soil fertility improvement (Phiri *et al*., 2012). However, most information on the quantities of biologically fixed N is based on sole crops. Mhango (2011) and Njira *et al.* (2012) reported on biological nitrogen fixation of doubled-ups of pigeon pea-groundnuts (42-82.8 kg ha^{-1}) and pigeon pea-soybean (53.6 kg ha^{-1}) whereas Phiri *et al.* (2014) reported soil fertility improvement due to pigeon pea-groundnut intercrop.

However, not much has been done to evaluate quantities of biologically fixed N under doubled-ups involving most of the recent legume varieties and information on pigeon pea-cowpea doubled-up BNF is scanty. Therefore, the objective of this study was to assess nodulation and quantify the amount of nitrogen that is biologically fixed per unit area by each of pigeon pea and cowpea in a doubled-up cropping system, their combined amount of N and in comparison with N_2 fixed when grown as sole crops or in cereal-legume intercrops. In this dissertation, the terms N_2 fixed and biologically fixed nitrogen (N) are used interchangeably.

2.2 Materials and Methods

2.2.1 Site identification and characterization of soils of the study sites

Two sites, Lilongwe and Dowa, both in the Central region of Malawi were identified for the study. The two sites were considered to increase the scope of identifying consistence and repeatability of treatment effects as recommended by Nielsen (2010). In Lilongwe district, the experiment was conducted at the Lilongwe University of Agriculture and Natural Resources (LUANAR) research farm (14^{o} 11′ S, 33^{o} 46′ E) within the Mkwinda Extension Planning Area (EPA), specifically at Bunda, whereas in Dowa district the experiment was conducted at Nachisaka EPA (13^{o} 37′ S, 33^{o} 56′ E). Rain gauges were installed at both sites for rainfall monitoring. Soils of the two sites have been classified as Chromic Luvisols (Typic Hapludalfs) (Chilimba *et al*., 2011; Mutegi *et al.,* 2015).

Soil samples were collected using an auger from depths of 0 - 20 cm and 20 - 40 cm, based on the simple random sampling plan, before planting and after harvesting according to Anderson and Ingram (1993). The sampling during pre-planting involved sampling from six points in Lilongwe and nine points in Dowa and for each of these sites soils were pooled into depth-wise composite samples. The nine points in the Dowa site were considered because the slope was slightly higher than that of the Lilongwe site which was generally flat. These were air dried and analysed for various parameters namely: soil texture by the hydrometer method (Bouyoucos, 1962), pH in water by the potentiometric method (Thomas, 1996), total nitrogen (N%) by micro-Kjedahl method (Bremner and Mulvaney 1982), soil extractable phosphorus (P) by Mehlich-3 method (Mehlich, 1984) and boron using the hot water soluble method as outlined by Anderson and Ingram (1989). Organic carbon (C) was determined using the wet oxidation method by dichromate (Nelson and Sommers, 1982), exchangeable bases, iron (Fe), manganese (Mn) and zinc (Zn) by Mehlich-3 method (Mehlich, 1984). Post-harvest sampling involved sampling soils from each plot for analysis of mineral N that was used in the modified nitrogen difference method for determination of biologically fixed N according to Peoples *et al.* (1989), described in detail in Section 2.2.4.

2.2.2 Experimental design and plot layout

The experiment was laid out in the randomized complete block design (RCBD) with three replicates at both sites. Treatments included sole cropped cowpea, sole cropped pigeon pea, pigeon pea-cowpea intercrop, cowpea-maize intercrop, pigeon pea-maize intercrop and sole cropped maize. Varieties planted were the *Alectra vogelii*-resistant cowpea known as *Mkanakaufiti* (IT99K-494-6), the medium duration pigeon pea known as *Mwayiwathu alimi* (ICEAP 00557), and the *Mkangala* (DKC 8053) variety of maize. The treatments were replicated three times. The plot of sole cropped maize (without fertilizer) was included to serve the purpose of reference crop in the determination of BNF by the modified nitrogen difference method (Peoples *et al*., 1989). All the maize in the intercrops was not fertilized to avoid confounding effects on BNF through N transfer that might occur. Furthermore, the nitrogen difference method (Sections 2.2.1 and 2.2.4) is more reliable in low N than in higher soil N conditions (Danso *et al.,* 1992).

The size of each treatment plot was 15 m by 7 m and included 20 ridges of 7 m long at the spacing of 75 cm. The big plot size was used purposively in preparation for the following season split plot design that was superimposed on these trials (described in Chapter Four and Five). Three pigeon pea seeds were planted per planting station at 90 cm between planting stations within the row/ridge at the ridge spacing of 75 cm in both sole and intercrop according to MoAFS (2012). In-row intercropping was done by planting either cowpea or maize between pigeon pea planting stations within the ridge/row according to MoAFS (2012). In sole cowpea, two seeds were planted per planting station at a spacing of 20 cm between planting stations along the ridge and at a distance of 75 cm between ridges, whereas the intercropped cowpea was planted at the same distance of 20 cm, which made three planting stations per every space between two pigeon pea planting stations. Similar to pigeon pea, three maize seeds were planted per planting station at

90 cm between planting stations within the row/ridge at a ridge spacing of 75 cm in both sole and intercrop. This made the planting pattern of intercropped maize and pigeon pea involving the planting station of pigeon pea being systematically in the middle of the space between maize planting stations.

2.2.3 Nodulation assessment, plant sampling and analysis

Nodulation assessment was conducted both in cowpea and pigeon pea at 50% flowering of each crop. These activities were conducted at different times as these crops grow and reach specific growth stages at different periods. Nodulation assessment included careful uprooting of plants, counting number of nodules, recording fresh and dry weights of nodules and determining the effectiveness of the nodules by slicing them and checking their internal colours. Effective nodules are identified by pink, red or brown colours while other colours such as green, white and yellow mean non-effectiveness (Peoples *et al.*, 1989; Sylvia *et al*., 2005). Ten plants were randomly sampled from each plot of cowpea and nodules numbers from each of these plants were recorded. The procedure of sampling for pigeon pea plants was similar to that of sampling cowpea. However, eight plants were sampled for pigeon pea since its plant population was lower than that of cowpea. Furthermore, 10 nodules were randomly sampled, each nodule cut into halves to check the internal colour for determination of effectiveness. Number of effective nodules was expressed as the percentage of effectiveness. Fresh and dry weights were also taken from the total number of nodules per plant. Plant samples were collected from the fields at 50% flowering from each of the legumes and at tasseling stage for maize for analysis of % N.

2.2.4 Determination of biological nitrogen fixation and percentage of nitrogen derived from the atmosphere (%N_{dfa})

The total N percentage determined in the plant samples was multiplied by total dry matter yields of each crop. Biological nitrogen fixation was determined using the modified Nitrogen-Difference method (Peoples *et al*., 1989). In the N-difference method N_2 fixed is estimated from the difference between total plant nitrogen (N yield) of an N_2-fixing legume and a reference crop (non-N_2-fixing). The modified technique is intended to improve accuracy of measurements when a non-nodulating legume is not available and the two plant species being used are not well matched. This is achieved by determining the difference of post-harvest soil N in the legume and control plots (Evans and Taylor, 1987). Hence, in this study maize root system is not well matched with those of either pigeon pea or cowpea. Therefore, the formula used was as in the following equation (vi):

$$\mathrm{Q} = [\mathrm{N\ yield\ (legume)} - \mathrm{N\ yield\ (control)}] + [\mathrm{N\ soil\ (legume)} - \mathrm{N\ soil\ (control)}] \quad \text{……..(vi)}$$

where:

Q (kg ha^{-1})	=	Quantity of the biologically fixed nitrogen
N yield [legume] (kg ha^{-1})	=	Nitrogen yield of a legume
N yield [control] (kg ha^{-1})	=	Nitrogen yield of a non-fixing plant
N soil (kg ha^{-1})	=	Post-harvest soil nitrogen in legume or control plot

The percentage of nitrogen derived from the atmosphere (%N_{dfa}) was determined as follows:

$$\%\mathrm{N_{dfa}} = \frac{\mathrm{N_2\ fixed}}{\mathrm{N\ yield}} \times 100 \quad \text{…..…………………..…..(vii)}$$

where:

N_2 fixed is the biologically fixed nitrogen

N yield is the total N uptake

2.2.5 Determination of total dry matter yields

The total dry matter yields (total biomass yields) were determined on a per hectare basis for all the crops, which are cowpea, pigeon pea and maize. A net plot of 13.5m by 4.3m was demarcated and all the plants for a specific crop were cut at ground level for the total above ground biomass. These were weighed for fresh weights and samples taken to the laboratory for oven drying and determination of dry matter yields according to Mloza-Banda (1994).

2.2.6 Statistical and data analysis

All the obtained data were subjected to analysis of variance (ANOVA) using Genstat 15th edition statistical package based on the statistical model presented in equation (viii). Consideration of plant population of cowpea was made, as it was planted with different populations in the sole and intercropped systems. This was achieved by determination of parameters that needed cowpea plant population on per plant basis and where possible covariate analyses were applied according to Gomez and Gomez (1984). Means were separated using the least significant difference test (LSD) at 5% level of significance.

$$Y_{ij} = \mu + \beta_j + \tau_i + \varepsilon_{ij}$$

Where:

Y_{ij} = measured variable from the ith treatment group in the jth block,

μ = overall mean

τ_i = the effect of the ith treatment

B_j = the effect of the jth block

ε_{ij} = random error from the ith group in the jth block

2.3 Results

2.3.1 Rainfall amounts at the two study sites in the 2013/14 cropping season

Figure 2.1 shows the amount of rainfall at the two study sites. Results show the highest monthly rainfall in the months of January and February for Lilongwe and Dowa, respectively. Lilongwe site received higher total amount of rainfall (1205 mm) than the Dowa site (758 mm). However, the total amounts of rainfall at both sites are within the required rainfall amount ranges for the crops that were planted in this study, based on MoAFS (2012). Rainfall distribution was favourable at both sites especially for cowpea and maize as it was moderately high in the month of February when these two crops were podding and tasseling, respectively.

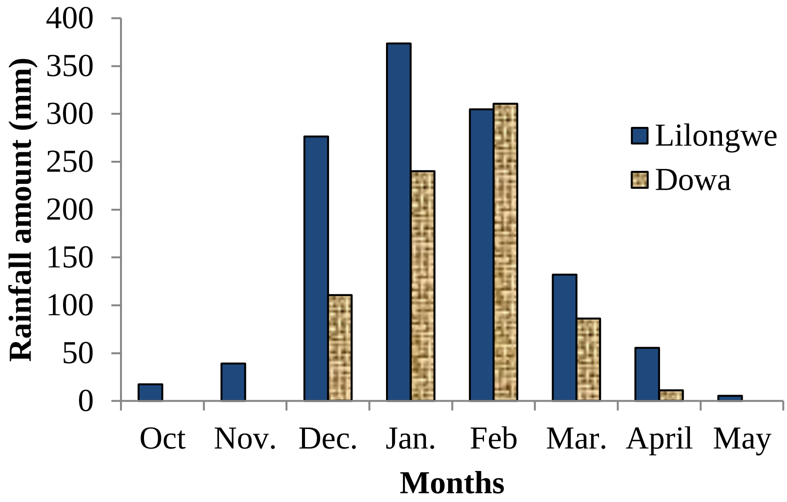


Figure 2.1: Rainfall amounts for Lilongwe and Dowa sites in the 2013/14 cropping season

2.3.2 Soil properties of the Lilongwe and Dowa study sites

Results of soil properties for the two study sites are shown in Table 2.1 whereas criteria for rating and critical values or ranges are presented in Appendix 1. The topsoil mean values indicated moderately acid for Lilongwe and slightly acid for Dowa site whereas the subsoils of both sites were slightly acidic with the same mean value (Table 2.1). Dowa soil had high organic matter for both top and subsoil whereas Lilongwe showed low organic matter for both depth ranges (Table 2.1). Total N was low at both sites compared with its critical value (Appendix 1) but available P (Mehlich-3 P) was moderately high in the topsoil and medium in the subsoil for Dowa as compared to its critical value (Appendix 1). On the other hand, Lilongwe site soils showed high available P in both depth ranges. Exchangeable Mg was medium for both sites and depths. Both sites showed medium and high exchangeable Ca in the top and subsoil, respectively. Furthermore, exchangeable K was above the critical values at both sites. The Zn values were much higher for the Dowa soil by 79% and 77% than the Lilongwe soil in the topsoil and subsoils, respectively, but values in both sites were higher than its critical value (Appendix 1). Soil textural classes for the topsoil of both sites were sandy clay loam whereas the subsoil showed clay loam for Dowa and loam for Lilongwe.

Table 2.1: Soil properties of the Lilongwe and Dowa study sites in Malawi

Soil property	Lilongwe 0 – 20 cm	Rating	Dowa 0 – 20 cm	Rating	Lilongwe 20 – 40 cm	Rating	Dowa 20 – 40 cm	Rating
pH_{water} 1:2.5	6.00	Moderately acid	6.20	Slightly acid	6.2	Slightly acid	6.2	Slightly acid
Organic C (%)	1.10	Low	2.8	Medium	0.9	Low	2.5	Medium
SOM (%)	1.80	Low	4.7	High	1.6	Low	4.4	High
Total N (%)	0.05	Very low	0.14	Medium	0.05	Very low	0.10	Low
Mehlich-3 P (mg/kg)	57	High	41	High	28	Adequate	21	Low
Mg ($cmol_+$/kg)	1.24	High	0.99	High	1.18	High	1.12	High
Ca ($cmol_+$/kg)	4.24	High	4.78	High	5.2	High	5.3	High
K ($cmol_+$/kg)	0.35	High	0.23	Adequate	0.22	Adequate	0.23	Adequate
Fe (mg/kg)	19.11	Adequate	19.2	Adequate	15.6	Adequate	19.0	Adequate
Zn (mg/kg)	2.56	Adequate	12.2	High	4.0	Adequate	17.4	High
Mn (mg/kg)	10.3	High	17.1	High	11.49	High	15.4	High
B (mg/kg)	0.58	Adequate	0.15	Adequate	0.08	Low	0.16	Adequate
Bulk density (g/cm^3)	1.53	-	1.45	-	1.54	-	1.52	-
Particle size distribution								
Sand	46.4		46.7		47.6		45	-
Silt	22.7		23.3		29.1		24.4	-
Clay	30.9		30.0		23.3		30.6	-
Textural class	Sand clay loam		Sandy clay loam		Loam		Clay loam	-

NB: hyphen (-) = no information
Critical values/ranges and references obtained from are in Appendix 1
Ratings are according to references as presented in Appendix 1

2.3.3 Nodulation of pigeon pea as affected by cropping system at the two study site

Table 2.2 shows that there were significant differences ($p < 0.05$) in pigeon pea nodule dry weights as influenced by cropping system at both sites. Nodule dry weights were significantly higher ($p < 0.05$) in sole cropped pigeon pea by 25% and 48% than that of pigeon pea in the pigeon pea-cowpea and pigeon pea-maize intercrops, respectively, at the Lilongwe site. Similarly, at the Dowa site, sole pigeon pea showed significantly higher ($p < 0.05$) nodule dry weights by 28% and 46% than that of pigeon pea in pigeon pea-cowpea and pigeon pea-maize intercrops, respectively. Furthermore, at the Dowa site, nodule numbers were significantly higher ($p < 0.05$) in the sole cropped pigeon pea than those of pigeon pea in the pigeon pea-maize intercrops by 31% and slightly higher than those in the pigeon pea-cowpea intercrop. However, at the Lilongwe site, nodule numbers were only slightly higher in the sole cropped pigeon pea than that of pigeon pea in the pigeon pea-cowpea and pigeon pea-maize intercrops. Furthermore, no significant differences were observed in nodule effectiveness at both study sites.

2.3.4 Nodulation of cowpea as affected by cropping system at the two study sites

The effect of cropping system on the extent of nodulation was as presented in Table 2.3. Results show that sole cropped cowpea produced significantly ($P < 0.05$) higher nodule numbers than those of cowpea in the cowpea-maize intercrop by 38%, at the Dowa site. However, sole cropped cowpea nodule numbers were only slightly higher in the Lilongwe site. On the other hand, significant differences were observed in cowpea nodule dry weights in both Lilongwe and Dowa sites. Sole cowpea produced significantly higher ($P < 0.05$) nodule dry weights by 38% and 36% than those of cowpea in the cowpea-pigeon pea and cowpea-maize intercrops, respectively. No significant differences were observed in cowpea nodule effectiveness in both sites of the study.

Table 2.2: Pigeon pea nodule numbers, dry weights and effectiveness as affected by cropping system at the two study sites

Parameter	Cropping system	Site: Lilongwe	Site: Dowa
Nodule numbers/plant	PP	12	13
	PP+CP	10	12
	PP+MZ	9	9
	LSD (0.05)	2.6	3.8
	F pr.	0.085	0.045
	CV %	11.2	15.0
Nodule dry weight (mg/plant)	PP	335.6	353.6
	PP+CP	251.5	254.3
	PP+MZ	174.7	191.9
	LSD (0.05)	47.1	125.5
	F pr.	0.002	0.045
	CV %	8.2	20.8
Nodule effectiveness (%)	PP	66.7	66.7
	PP+CP	53.3	63.3
	PP+MZ	56.7	63.3
	LSD (0.05)	15.1	15.1
	F pr.	0.145	0.79
	CV %	11.3	10.3

NB: (i)Separation of means was based on LSD as 5% significant level; CV = coefficient of variation; F pr. = F probability

(ii)PP = pigeon pea; CP = cowpea and MZ = maize; PP+CP = PP intercropped with CP; PP+MZ = PP intercropped with MZ

Table 2.3: Cowpea nodule numbers, dry weights and effectiveness as affected by cropping system at the two study sites

Parameter	Cropping system	Site	
		Lilongwe	Dowa
Nodule numbers/plant	CP	11	16
	CP+PP	8	12
	CP+MZ	8	10
	LSD (0.05)	3.99	5.16
	F pr.	0.256	0.043
	CV %	19.9	18.3
Nodule dry weight (mg/plant)	CP	519.1	711.4
	CP+PP	323.7	492.8
	CP+MZ	330.7	419.9
	LSD (0.05)	73.1	205.8
	F pr.	0.003	0.037
	CV %	8.2	16.8
Nodule effectiveness (%)	CP	83.3	86.7
	CP+PP	76.7	86.7
	CP+MZ	80.0	80.0
	LSD (0.05)	18.5	17.7
	F pr.	0.64	0.54
	CV %	10.2	9.3

NB: (i)Separation of means was based on the LSD at 5% significant level; CV = coefficient of variation; F pr. = F probability

2.3.5 Biological nitrogen fixation by pigeon pea and cowpea as influenced by cropping system at the Lilongwe and Dowa sites

2.3.5.1 Quantities of N_2 fixed and $\%N_{dfa}$ by pigeon pea at Lilongwe site

The amounts of biologically fixed N by pigeon pea and cowpea on a per plant basis and the % Ndfa, as influenced by cropping system are as presented in Table 2.4. The per plant basis analysis was intended to see the performance of the crop as it grows in different cropping systems whereas per hectare basis (Figure 2.2) was done specifically to show quantities for each crop per unit area, and consideration of plant population was done by covariate analysis on the cowpea data as it was sown in different plant populations in the different cropping systems. Results show that there were significant differences ($P < 0.05$) in biologically fixed N by the legumes as influenced by cropping system at both study sites (Fig. 2.2). In the Lilongwe site, sole cropped pigeon pea showed significantly higher ($P < 0.05$) N_2 fixed per plant (Table 2.4) than that by pigeon pea under both intercrops of pigeon pea-cowpea and pigeon pea-maize by 42% and 33%, respectively.

On the other hand, N_2 fixed per hectare by pigeon pea (Figure 2.2) was also significantly higher ($P < 0.05$) in the sole pigeon pea than that by pigeon pea in the pigeon pea-cowpea or pigeon pea-maize intercrops by 47% and 36%, respectively. However, no significant differences were noted in the N_2 fixed by pigeon pea in the pigeon pea-cowpea and pigeon pea-maize intercrops. The $\%N_{dfa}$, in the Lilongwe site (Table 2.4) was significantly higher ($P < 0.05$) in the sole pigeon pea by 17% and 12%, respectively, than that by pigeon pea in the pigeon pea-cowpea and pigeon pea-maize intercrops. Comparison of results on pigeon pea $\%N_{dfa}$ in the pigeon pea-cowpea and pigeon pea-maize intercrops showed no significant differences.

2.3.5.2 Quantities of N_2 fixed and $\%N_{dfa}$ by pigeon pea at Dowa site

Similar to the Lilongwe site, at the Dowa site, there were significant differences ($P < 0.05$) in the N_2 fixed for both per plant (Table 2.4) and per hectare basis (Figure 2.2). Sole cropped pigeon pea N_2 fixed per plant was significantly higher ($P < 0.05$) than that by pigeon pea in the pigeon pea-cowpea and pigeon pea-maize intercrops by 27% and 28%, respectively. On the other hand, N_2 fixed per hectare was also significantly higher ($P < 0.05$) in the sole cropped pigeon pea than those by pigeon pea in the pigeon pea-cowpea and pigeon pea-maize intercrops by 31% and 36%, respectively. No significant differences were observed in N_2 fixed by pigeon pea in the pigeon pea-cowpea and pigeon pea-maize intercrops. Furthermore, sole cropped pigeon pea showed significantly higher ($P < 0.05$) $\%N_{dfa}$ than that by pigeon pea in the pigeon pea-cowpea and pigeon pea-maize intercrop, by 10% and 12%, respectively. No significant differences were observed in $\%N_{dfa}$ by pigeon pea in the pigeon pea-cowpea and pigeon pea-maize intercrops.

2.3.5.3 Quantities of N2 fixed and %Ndfa by cowpea at Lilongwe site

Results of biological nitrogen fixation by cowpea indicate that there were significant differences ($P < 0.05$) in N_2 fixed and $\%N_{dfa}$ as influenced by cropping system. The N_2 content per plant in sole cropped cowpea (Table 2.4) was significantly higher ($P < 0.05$) than that by cowpea in the intercrops with pigeon pea and maize, by 70% over each of the intercropped cowpea. Furthermore, the $\%N_{dfa}$ by the sole cowpea was significantly higher ($P < 0.05$) than that by cowpea in an intercrop with pigeon pea and with maize by 58% and 61%, respectively. There were no significant differences in N_2 fixed per plant by cowpea in the cowpea-pigeon pea or cowpea-maize intercrops.

Table 2.4: Pigeon pea and cowpea biological nitrogen fixation and Ndfa as affected by cropping system at the two study sites

Crop	Cropping system	Lilongwe site		Dowa site	
		N_2 fixed/ plant (g)	N_{dfa} (%)	N_2 fixed/ plant (g)	N_{dfa} (%)
PP	PP	1.90	75.7	2.61	76.0
	PP+CP	1.11	62.6	1.91	68.7
	PP+MZ	1.27	66.6	1.89	66.7
	LSD (0.05)	0.59	7.34	0.59	6.49
	F pr.	0.042	0.018	0.044	0.036
	CV (%)	15.1	4.7	12.1	4.1
CP	CP	0.48	69.4	0.50	68.8
	CP+PP	0.14	28.7	0.27	37.3
	CP+MZ	0.14	27.2	0.17	29.5
	LSD	0.22	19.9	0.13	14.1
	F pr.	0.021	0.007	0.005	0.003
	CV (%)	38.7	21	17.9	13.8

NB: Separation of means was based on the LSD at 5% significant level

2.3.5.4 Quantities of N2 fixed and %Ndfa by cowpea at Dowa site

Results of N_2 fixed at the Dowa site show that there were significant differences ($P < 0.05$) as influenced by cropping system (Table 2.4). Sole cropped cowpea produced significantly higher ($P < 0.05$) plant N content by 48% and 66% than that of cowpea in an intercrop with pigeon pea or maize. Furthermore, the $\%N_{dfa}$ by sole cowpea was also significantly higher ($P < 0.05$) than that by cowpea in the cowpea-pigeon pea and cowpea-maize intercrops, by 46% and 57%, respectively (Table 2.4).

2.3.6 Total biologically fixed N for the different overall cropping systems

The cropping systems included sole crops, legume-legume and cereal-legume combinations. The legume-legume plot was the same size as others while it contained two plant species that were biologically contributing nitrogen to the system. Therefore, this section shows comparisons where the amount of nitrogen fixed by a doubled-up cropping system is the summation of the N_2 fixed from the component crops, that is pigeon pea and cowpea added together. Results show significant differences ($P <0.05$) as influenced by cropping system (Figure 2.3-A and B).

Sole cropped pigeon pea had the highest amount of N_2 fixed at both study sites. At the Dowa site, sole cropped pigeon pea produced 30%, 36% and 86% higher amounts of biologically fixed N than those by the sole cropped cowpea, pigeon pea-maize and cowpea-maize intercrops, respectively. Similarly, the sum of N_2 fixed by pigeon pea and cowpea in the “doubled-up” system was comparable to that of the sole cropped pigeon pea. The total sum of the amounts of N_2 fixed by pigeon pea-cowpea intercrop at the Dowa site was 21, 28 and 84% more than the biologically fixed N contributed by the sole cropped cowpea, pigeon pea-maize and cowpea-maize intercrops, respectively.

Similarly, at the Lilongwe site, sole cropped pigeon pea produced the highest amount of N_2 fixed. However, it was not significantly higher than that by sole cropped cowpea but was significantly higher than the total sum of the amounts of N_2 fixed by the pigeon pea-cowpea "doubled-up", by 33%. On the other hand the pigeon pea-cowpea "double-up" N_2 fixed was not significantly different from those in the sole cropped cowpea or pigeon-pea maize intercrop but was significantly higher ($P < 0.05$) than that of the cowpea-maize intercrop, by 82%.

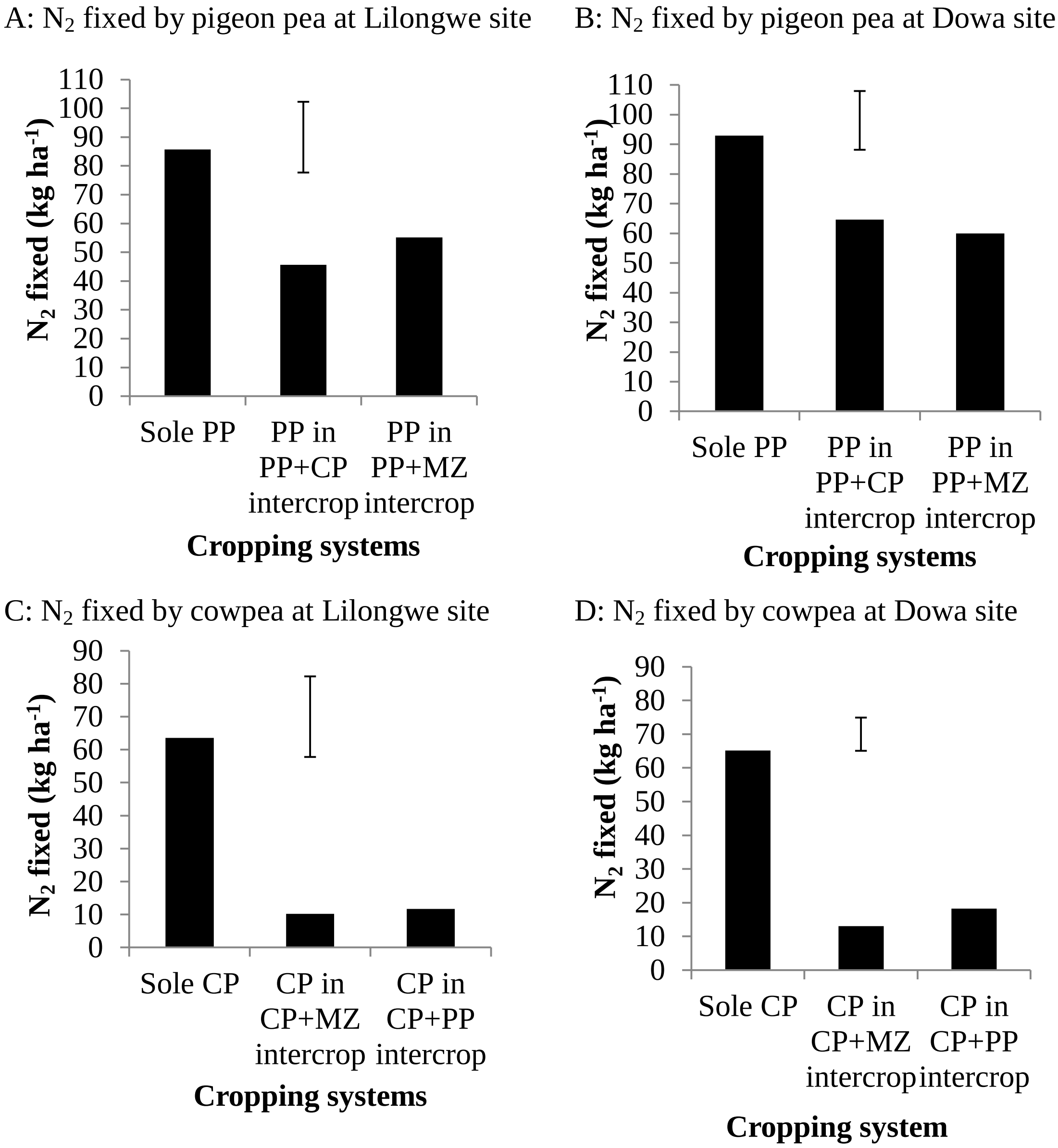
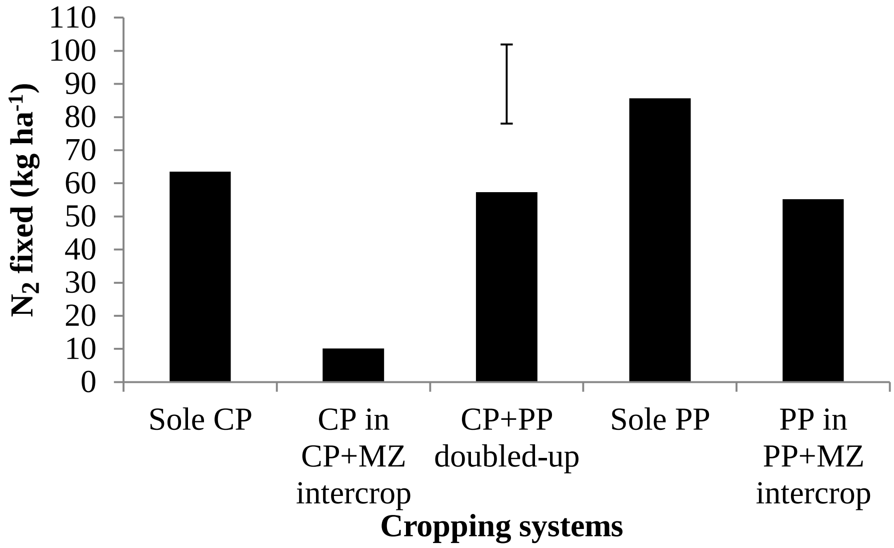


Figure 2.2: Quantities of N2 biologically fixed by pigeon pea and cowpea grown in different cropping systems.

NB: (i) PP = pigeon pea; CP = cowpea and Mz = maize; PP+CP = PP intercropped with CP; PP+MZ = PP intercropped with MZ; Covariate analysis was done on the cowpea N_2 fixed

(ii) The vertical bar within each graph represents the LSD

A: N_2 fixed at Lilongwe site under different cropping system



B: N_2 fixed at Dowa site under different cropping systems

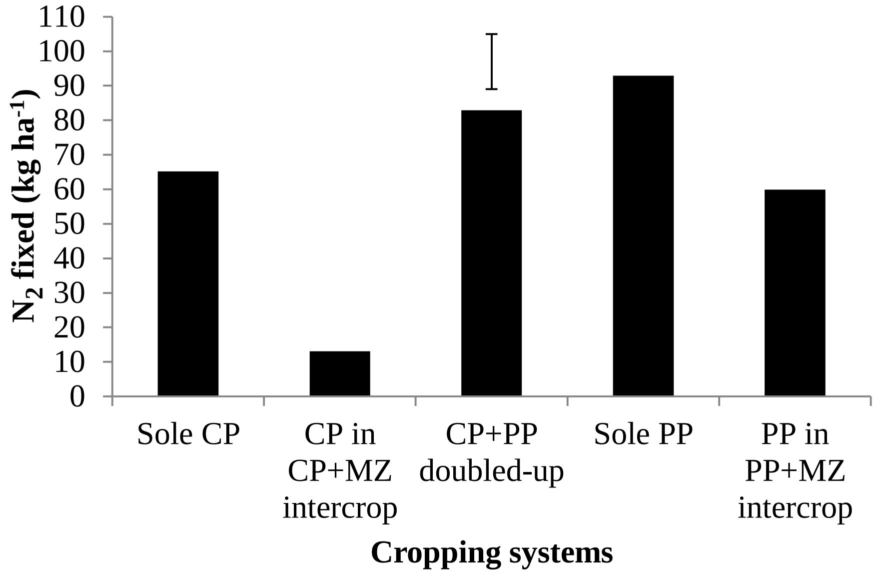


Figure 2.3: Total amounts of biologically fixed nitrogen (kg N ha-1) contributed by the legume components

NB: (i) The amount of biologically fixed nitrogen in the CP+PP "doubled-up" is the sum of N_2 fixed by each of the PP and CP components.

(ii) The vertical bar within each graph represents the LSD

2.4 Discussion

Growth, nodulation and biological nitrogen fixation of legume plants are influenced by many factors including soil temperature, soil reaction, essential macro- and micro-nutrients, presence of effective microbial symbionts and cropping systems (Giller, 2001; Mohammadi *et al*., 2012). In this study, cropping system effects have been consistent in most of the parameters determined and statistical differences in nodulation and N_2 fixed of both cowpea and pigeon pea have to some extent been influenced by the conditions of the sites where these crops were grown. The significantly higher pigeon pea nodule numbers, nodule weights, N_2 fixed and $\%N_{dfa}$ in sole cropping than in the intercrops as observed in this study could be attributed to a number of factors such as inter-specific competition between the two plant species for nutrients, light or moisture. Pigeon pea that was intercropped with maize or cowpea faced competition for growth resources including nutrients. Ghosh *et al*. (2006) reported reduction in relative nitrogen yields in pigeon pea when intercropped with soybean, which was attributed to competition for N, whereas Katayama *et al*. (1995) in a similar study in a shallow Alfisol in India reported reduction in $\%N_{dfa}$ when pigeon pea was intercropped with various crops including cowpea. Pigeon pea reduction in growth and N_2 fixation as influenced by intercropping with various crops such as maize, soybean, groundnuts or sorghum has been reported in a number of similar studies (Egbe, 2007; Mhango, 2011; Njira *et al*., 2012: Egbe *et al*., 2015).

Long and medium duration pigeon pea varieties are known for their slow growth that gives the crop a good quality for intercropping as it allows the companion crop to grow and reach maturity before pigeon pea completes its life cycle. This characteristic makes it susceptible to inter-specific competition depending on planting pattern and the type of companion crop. In this study the companion crops, cowpea and maize, all have shorter and faster growth habits than pigeon pea, which can offer stiff competition to pigeon pea in the early growth stages. Mangla *et al*. (2011) noted that slow growers are out-competed when faced with inter-specific competition for nutrients such as N.

Similarly, cowpea indicated significant reduction in number of nodules, N_2 fixed and $\%N_{dfa}$ in intercrops with pigeon pea or maize which can be attributed to competition for growth resources. Similar observations have been reported in a number of other studies. Van Kassel and Roskoski (1998) reported stiff competition in a cowpea-maize intercrop where the intercropped maize took twice as much N as the cowpea as compared to similar amounts that were taken up in sole crops, whereas Makoi *et al*. (2008) reported reduction in cowpea plant growth and %N in an intercrop with sorghum. However, Katayama *et al.* (1995) observed no significant differences in $\%N_{dfa}$ by cowpea in sole cropping and in an intercrop with pigeon pea which was attributed to the indeterminate, spreading and climbing nature of the cowpea variety used in that experiment which gave it an advantage in exploiting resources.

The present study has shown that the overall contribution of N per unit area by a cropping system with consideration of both component crops in the pigeon pea-cowpea intercrop (summing up their biologically fixed N) depended on both the system and site effects. The Dowa site indicated comparable results in terms of N_2 fixed (kg N ha^{-1}) in the sole pigeon pea and pigeon pea-cowpea doubled-up, but in the Lilongwe site the doubled-up system showed significant reduction in the overall N_2 fixed. This shows some dynamics in how cropping systems may impact the performance of the crops in different environments. As to the sites under this study, phosphorus, which is needed much in BNF, was above critical values in the 0 - 20 cm depth at both sites. However, N and soil organic matter levels for Lilongwe soils were low whereas Dowa site showed medium N level and high organic matter levels based on ratings by Landon (1991).

High amounts of N in the soil are reported to suppress BNF through reduction of nitrogenase activity, for instance when nitrates are perpetually in high amounts (Havlin *et al*., 2005). However, small additional sources or starter amounts of N have been reported to enhance BNF in legumes where soils are low in N (Mulongoy, 1995; Adu-Gyamfi *et al*., 1997; Ahmed *et al.*, 2014). On the other hand, in a number of studies, increase in soil organic matter has been associated with increased BNF. Increased nodulation and BNF due to increase in soil organic matter has been reported in soybean (Lawson *et al*., 1995; Santos *et al*., 2011; Coskan and Dogan, 2011; Hayat *et al.*, 2012). Similarly, in this study, N level and the higher organic matter levels for Dowa could have contributed to the increase in nodulation and BNF of cowpea and pigeon pea. Mangla *et al.* (2011) reported reduction in the effects of inter-specific competition when N was increased to an intercropping system of two different plant species. On the other hand, soil organic matter has many functions in the soil including acting as a soil nutrient supply and reserve for metabolically active microbial community, increasing water holding capacity and enhancing chelation and bioavailability of micronutrients (Sylvia *et al*., 2005; Brady and Weil, 2008; Fageria, 2012). All these factors are very important in the N_2 fixation process as they enhance the healthy growth of both the legume plant and the microbial symbionts, and this may have occurred in the present study. It should be noted that a high coefficient of variation (38.7) was noted on the amounts of N_2 fixed for the Lilongwe site and this was attributed to observed differences in nodulation across replicates.

2.5 Conclusions

From this study it can be concluded that both cropping systems and site of the study had an influence on nodulation, $\%N_{dfa}$ and the total amount of N_2 fixed by the two legume species in the different cropping systems. At the Dowa site, sole cropped pigeon pea produced the highest amount of biologically fixed N (92.9 kg ha^{-1}), which was significantly higher than that by the pigeon pea in both the pigeon pea-cowpea and pigeon pea-maize intercrops by 31% and 36%, respectively. On the other hand, comparison of the overall cropping system contribution per unit area, the total sum of the amounts of biologically fixed N (82.9 kg ha^{-1}) from two component crops in the pigeon pea-cowpea "doubled-up" was comparable to that by the sole cropped pigeon pea but was significantly higher than the amounts of N_2 fixed by sole cowpea (62.5 kg N ha^{-1}), pigeon pea in the pigeon pea-maize intercrop (59.9 kg N ha^{-1}) or cowpea in the cowpea-maize intercrop (13.1 kg N ha^{-1}). However, the trend was different at the Lilongwe site. Although the biologically fixed N (85.7 kg ha^{-1}) by the sole cropped pigeon pea was similarly the highest, the total sum of the amounts of N_2 fixed (57.4 kg N ha^{-1}) by the component crops in the pigeon pea-cowpea "doubled-up" was significantly lower than that by the sole pigeon pea, by 33%. In this study it was noted that competition for resources due to type of cropping system and differences in environmental factors can be important factors influencing the performance of legume components in legume-legume, legume-cereal and sole cropped legume systems. Therefore, implementation of cropping systems that integrate legumes should follow a thorough evaluation of site-specific soil and other environmental conditions.

References

Adu-Gyamfi, J. J. and Ito, O. (1997). *Nitrogen management in sorghum/pigeonpea intercrop.* In: *Extending nitrogen fixation research to farmers' fields: Proceedings of an International Workshop on Managing Legume Nitrogen Fixation in the Cropping Systems of Asia*, 20-24 August 1996, ICRISAT Asia Center, Patancheru, Andhra Pradesh, India. pp. 3 – 15.

Ahmed, A. E., Mukhtar, N. O., Babiker, H. M. and Adam A. I. (2014). Effect of nitrogen fertilization and *Bradyrhizobium* inoculation on the growth, symbiotic properties and yield of pigeon peas. *Journal of Natural Resources and Environmental Studies* 3: 27 – 31.

Ajayi, O. C., Akinifesi, F. K., Sileshi, G. and Chakeredza, S. (2007). Adoption of soil fertility replenishment technologies in southern Africa: Lessons learnt and the way forward. *Natural Resources Forum* 31: 306 – 317.

Alliance for Green Revolution in Africa (2014). *Africa Agriculture Status Report:* Climate Change and Smallholder Agriculture in Sub-Saharan Africa, Nairobi, Kenya. 216pp.

Anderson J. M. and Ingram, J. S. I. (1989). *Tropical Soil Biology and Fertility: A Handbook of Methods of Analysis.* Commonwealth for Agriculture Bureau International, Wallingford. 117pp.

Anderson, J. M. and Ingraham, J. S. I. (1993). *Tropical Soil Biology and Fertility: A Handbook of Methods.* Commonwealth for Agriculture Bureau International, Wallingford. 221pp.

Berrada, H. and Fikri-Benbrahim, K. (2014). Taxonomy of rhizobia: current perspectives. *British Microbiology Research Journal* 4(6): 616 – 639.

Bouyoucos, G. J. (1962). Hydrometer method improved for making particle size analyses of soils. *Agronomy Journal* 54(5): 464 – 465.

Brady, N. C. and Weil, R. R. (2008). *The Nature and Properties of Soils*. (Revised 14th Ed.), Pearson Prentice Hall, New Jersey, 975pp.

Bremner, J. M. and Mulvaney, C. S. (1982). Nitrogen total. In: *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*. (Edited by Page, A. L.), ASA and SSSA. Madison, Wisconsin. pp. 595 – 624.

Chilimba, A. D. C., Mughogho, S. K. and Wendt, J. (1999). Mechlich 3 or modified Olsen for soil testing in Malawi. *Communications in Soil and Plant Analysis* 30(8): 1231 – 1250.

Chilimba, A. D. C., Young, S. D., Black, C. R., Rogerson, K. B., Ander, E.L., Watts, M. J., Lammel, J and Broadly, M. R. (2011). Maize grain and soil surveys reveal suboptimal dietary selenium intake is widespread in Malawi. *Scientific Reports* 1: 72.

Coskan, A. and Dogan, K. (2011). Symbiotic nitrogen fixation in soybean, soybean physiology and biochemistry. [http://www.intechopen.com/books/soybean-physiology-andiochemis try/ symb iotic-nitrogen-fixation-insoybean] site visited on 02/10/2015.

Danso, S. K. A. and Sanginga, N. (1992). Biological nitrogen fixation in trees in agroecosystems. In: *Biological Nitrogen Fixation for Sustainable Agriculture*. (Edited by Ladha, J. K., George, T. and BohBohlool, B. B.), Kluwer Academic Publishers. Dordrecht. pp. 177 – 196.

Druille, Z. and Barreto-Hurle, J. (2012). *Fertilizer subsidies in sub-Saharan Africa.* Agricultural Development and Economics Working Paper No. 4. Food and Agriculture Organization, Rome. 64pp.

Egbe, M. O., Ibrahim, N. B., Aor, F. T. (2015). Effect of low rates of nitrogen and phophorus fertilizers on growth and yield of intercropped pigeonpea with sorghum in Makurdi, Nigeria. *Journal of Biology, Agriculture and Healthcare* 5(8): 156 – 162.

Egbe, O. M. (2007). Assessment of biological nitrogen fixation potential of pigeon pea genotypes intercropped with sorghum for soil fertility improvement in Southern Guinea Savanna of Nigeria. *Journal of Agriculture, Food, Environment and Extension* 6(1): 33 – 45.

Evans, J. and Taylor, A. C. (1987). Nitrogen fixation and accretion by grain legumes. *Journal of Australian Institute of Agricultural Research* 53: 309 – 322.

Fageria, N. K. (2012). Role of soil organic matter in managing sustainability of cropping systems. *Communications in Soil Science and Plant Analysis* 43(16): 2063 – 2113.

Ghosh, P. K., Mohanty, M., Bandayopadhy, K. K., Painuli, D. K. and Misra, A. K. (2006). Growth, competition, yields advantage and economics in soybean/pigeon pea Intercropping system in semi-arid tropics of India II. Effect of nutrient management. *Field Crops Research* 96: 90 – 97.

Giller, K. E. (2001). *Nitrogen Fixation in Tropical Cropping Systems.* Commonwealth for Agriculture Bureau International, Wallingford, UK. 423pp.

Gomez, K. A. and Gomez, A. A. 1984. *Statistical Procedures for Agricultural Research*. (2nd Ed), John Wiley and Sons New York. 680pp.

Havlin, J. L., Beaton, J. D., Tisdale, S. L. and Nelson, W. L. (2005). *Soil Fertility and Fertilizers: An Introduction to Nutrient Management*. (7th Ed.), Pearson Prentice Hall, New Jersey, 515pp.

Hayat, R., Iftikhar-ul-Hassan, M., Akram, S., Sheirdill, R. A. and Ahmad, M. (2012). Evaluation of compost application for improving legumes yield and N_2-fixation. *African Journal of Biotechnology* 11(41): 9758 – 9764.

International Crops Research Institute for Semi-Arid Tropics/Ministry of Agriculture and Irrigation (2000). *Cost-effective Soil Fertility Options for Smallholder Farmers in Malawi.* ICRISAT, Bulawayo, Zimbabwe. 24pp.

Katayama K., Ito, O., Matsunanga, R., Adu-Gyamfi, J. J., Rao, T. P., Anders, M. M., and Lee, K. K. (1995). Nitrogen balance and root behaviour in four pigeon pea intercropping systems. *Fertilizer Research* 42: 315 – 319.

Kumwenda, J. D. T., Waddington, S., Snapp, S. S., Jones, R. B. and Blackie, M. J. (1997). Soil fertility management in smallholder maize-based cropping systems of Eastern and Southern Africa*:* In: *The Emerging Maize Revolution.* (Edited by Eicher, C). Michigan State University, USA. pp. 153 – 172.

Landon, J. R. (Ed.) (1991) *Booker Tropical Soil Manual. A Handbook for Soil Survey and Agricultural Land Evaluation in the Tropics and Subtropics.* John Wiley and Sons, Inc., New York. 474pp.

Lawson, I. Y. D., Muramatsu, K. and Nioh, I. (1995). Effect of organic matter on growth, nodulation and nitrogen fixation of soybean growth under acid and saline conditions. *Soil Science and Plant Nutrition* 41(4): 724 – 728.

Makoi, J. H. J. R., Chimphango, S. B. M., and Dakora, F. D. (2008). Symbiotic N nutrition and yield of cowpea genotypes under different plant densities and cropping systems. In: Biological Nitrogen Fixation: Towards poverty alleviation through sustainable agriculture. (Edited by Dakora, F. D., Chimphango, S. M. B., Valentine, A. J., Elmerich, C. and Newton, W. E.). *Proceedings of the 15th International Congress and 12th International Conference of the African Association of Biological Nitrogen Fixation.* Springer. pp. 117 – 118.

Makumba, W. (2003). Nitrogen use efficiency and carbon sequestration in legume tree-based agroforestry system: A case study of Malawi. Thesis for Award of PhD Degree at Wageningen University, The Netherlands, 208pp.

Mangla, S Sheley, R. L., James, J. J. and Radosevich, S. R. (2011). Intra and interspecific competition among invasive and native species during early stages of plant growth. *Plant Ecology* 212: 531 – 542.

Mehlich, A. (1984). Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. *Communications in Soil Science and Plant Analysis* 15(12): 1409 – 1416.

Mhango, W. G. (2011). Nitrogen budgets in legume based cropping systems in Northern Malawi. Dissertation for the Award of PhD Degree at Michigan State University, USA, 257pp.

Mhango, W. G., Snapp, S. S. and G.Y. Kanyama-Phiri. (2012). Opportunities and constraints to legume diversification for sustainable maize production on smallholder farms in Malawi. *Renewable Agriculture and Food Systems* 28: 234 – 244.

Ministry of Agriculture and Food Security (2012). *Guide to Agricultural Production and Natural Resource Management in Malawi*. Agricultural Communications Branch, Lilongwe. Malawi, 366pp.

Mloza-Banda, H. R. (1994). *Principles and Practices of Crop Management: Field Study Guide.* Crop Science Department, Bunda College of Agriculture, Malawi. 137pp.

Mohammadi, K., Shohrabi, Y., Gholamreza, H., Khalesro, S. and Majidi, M. (2012). Effective factors on biological nitrogen fixation. *African Journal of Agricultural Research* 7(12): 1782 – 1788.

Mulongoy, K., (1992). Technical paper 2: Biological nitrogen fixation. The AFNETA Alley Farming Training Manual Volume. Alley Farming Network for Tropical Africa.[http://www.fao.org/wairdocs/ILRI/x5546E/x5546e05.htm] site visited on 04/10/2015.

Mutegi, J., Kabambe, V., Zingore, S., Harawa, R. and Wairegi, L. (2015). *The Fertilizer Reccomendation Issues in Malawi: Gaps, Challenges, Opportunities and Guidelines.* Soil Health Consortium, Malawi. 54pp.

Nelson, D. W. and Sommers, L. E. (1996). Total carbon and organic matter. In: *Methods of Soil Analysis. Part 3. Chemical Methods.* (Edited by Sparks, D. L.), SSSA Book Series No. 51. Soil Science Society of America, Madison, Wisconsin, USA. pp. 961 – 1010.

Ngwira, A. R., Kabambe, V. H., Kambauwa, G., Mhango, W. G. and Mwale, C. D. (2012). Scaling out best fit legume technologies for soil fertility enhancement among smallholder farmers in Malawi. *African Journal of Agricultural Research* 7(6): 918 – 228.

Nielsen, R. B. (2010). A practical guide to on-farm research. Purdue University Department of Agronomy. 10pp. [http://www.kingcorn.org/news/timeless/OnFarmResearch.pdf] site visited on 08/05/2013.

Njira, K. O. W., Nalivata, P. C., Kanyama-Phiri, G. Y. and Lowole, M. W. (2012). Biological nitrogen fixation in sole and doubled-up legume cropping systems in the sandy soils of Kasungu, Central Malawi. *Journal of Soil Science and Environmental Management* 3(9): 224 – 230.

Nyemba, R. C. and Dakora, F. D. (2010). Evaluating nitrogen fixation by food legumes in farmers' fields in three agroecological zones of Zambia using ^{15}N natural abundance. *Biology and Fertility of Soils* 46: 461 – 470.

Palm, C.A., Myers, R. J. K. and Nandwa, S. M. (1997). Combined use of organic and inorganic nutrient sources for soil fertility maintenance and replenishment. In: *Replenishing Soil Fertility In Africa*(Edited by Buresh, R. J., Sanchez, P. A. and Calhoun, F.) SSSA Special Publication Number 51. Soil Science Society of America, Madison, Wisconsin. pp. 151 – 192.

Peoples, M. B., Faizah, A. W., Rerkasem, B., and Herritedge, D. F. (1989). *Methods for Evaluating Nitrogen Fixation by Nodulating Legumes in the Field*. Australian Centre for International Agriculture Research, Canberra. 89pp.

Phiri, A. T., Mhango, W. G., Njoloma, J. P., Kanyama-Phiri, G. Y. and Lowole, M. W. (2012). Farmers evaluation of integrated soil fertility management methods in Northern Kasungu, Central Malawi. *Journal of Agricultural Extension and Rural Development* 4(11): 340 – 346 .

Phiri, A. T., Msaky, J. J., Mrema, J., Kanyama-Phiri, G. Y. and Msanya, B. M. (2014). Effect of pigeon pea-groundnut intercropping system on selected soil properties. *International Journal of Plant and Soil Science* 3(4): 397 – 407.

Reynolds, T. W., Waddington, S. R., Anderson, C. L., Chew, A., True, Z. and Cullen, A. (2015). Environmental impacts and constraints associated with the production of major food crops in sub-Saharan Africa and South Asia. *Food Security* 7: 795 – 822.

Santos, J. A., Nunes, L. A. P. L., Melo, W. J., Figueiredo, M. B. V., Singh, R. P., Bezerra, A. A. C. and Araujo, S. F. (2011). Growth, nodulation and nitrogen fixation of cowpea in soils amended with composted tannery sludge. *Brazilian Journal of Soil Science* 35: 1865 – 1871.

Snapp, S. S., Jayne, T. S., Mhango, W., Benson, T. And Ricker-Gilbert, J. (2014). Maize yield response to nitrogen in Malawi's smallholder production systems. *Paper Prepared For The National Symposium Eight Years of FISP- Impact And What Next? Bingu International Conference Centre*, Lilongwe 14 – 15 July 2014. pp. 1 – 22.

Sylvia, D. M., Furhmann, J. J., Hartel, P. G. and Zuberer D. A. (2005). *Principles and Application of Soil Mmicrobiology*. (2nd Ed.), Pearson Prentice Hall, New Jersey. 640pp.

Thomas, G. W. (1996). Soil pH and soil acidity. In: *Methods of Soil Analysis. Part 3. Chemical Methods.* (Edited by Sparks, D. L.), SSSA Book Series No. 5. Soil Science Society of America, Madison, Wisconsin. pp. 475 – 490.

Van Kassel, C. and Roskoski, J. P. (1988). Row spacing effects on N_2-fixation, N-yield and soil N uptake of intercropped cowpea and maize. *Plant and Soil* 111: 11 – 23.

Wendt, J. W. (1996). *Chemical Analysis Manual of Soil and Plant Samples*. Rockefeller Foundation and Department of Agricultural Research and Technical Services, Lilongwe, Malawi. 96pp.

CHAPTER THREE

3.0 PRODUCTIVITY OF PIGEON PEA, COWPEA AND MAIZE UNDER SOLE CROPPING, LEGUME-LEGUME AND LEGUME-CEREAL INTERCROPS ON THE LUVISOLS OF CENTRAL MALAWI

Abstract

A study was conducted in the Lilongwe and Dowa districts of Central Malawi to assess the performance and productivity of pigeon pea (PP), cowpea (CP) and maize (MZ) when grown as sole crops, legume-legume and legume-cereal intercrops. The experiment was laid out in a randomized complete block design (RCBD). Analysis of variance for yields was carried out on a per plant basis and the productivity was assessed by using various competition indices including the land equivalent ratio (LER), relative dry matter yield (RDY), relative nutrient yields and the monetary advantage index (MAI). Results showed significantly higher ($P < 0.05$) grain yield per plant for the sole cropped pigeon pea and cowpea than their intercrops, but only slight differences were observed for maize, at both study sites. However, for overall productivity, results showed higher productivity in terms of yields (LER>1) and monetary gains (positive MAI values) in all the intercropping systems than sole cropping, at both study sites. Furthermore, the PP+MZ intercrop was the most productive system in terms of yields and monetary gains as it produced the highest LER and MAI values. The following LERs were obtained: PP+MZ (1.57), PP+CP (1.18) and MZ+CP (1.22) for the Lilongwe site and PP+MZ (1.86), PP+CP (1.63) and MZ+CP (1.26) for the Dowa site. The CP consistently produced lower partial LERs in both study sites than the other component crops when intercropped with either MZ or PP. Furthermore, CP growth was limited by both N and P in both study sites as compared to MZ and PP, whose growth was mostly limited by N. This implied that CP was less competitive and adaptive for the prevailing conditions. Although the study showed higher

productivity with intercropping, it is recommended that implementation of intercropping systems with various crop combinations should consider crop competitive interactions. Furthermore, research needs to be done on site specific fertilizer recommendations for various intercropping systems.

Keywords: Legume-legume intercrop, land equivalent ratio, legume-cereal intercrop, cropping system productivity, monetary advantage index, Malawi

3.1 Introduction

Legume crops form an important part of crop production in Malawi and other Sub-Saharan African countries. They offer a number of benefits to smallholder farmers including soil fertility improvement through biological nitrogen fixation (BNF) and addition of soil organic matter (Drinkwater *et al.*, 1998). Legumes contribute to food security and human nutrition, and are a good source of protein to resource poor farmers, especially in areas where availability of meat is low (Giller, 2001; Kerr *et al.*, 2007). In Malawi, legumes that are commonly grown include common beans (*Phaseolus vulgaris*), groundnuts (*Arachis hypogaea*), cowpea (*Vigna unguiculata*), pigeon pea (*Cajanus cajan*), soybean (*Glycine max*) and Bambara groundnuts (*Vigna subterranea*) among others (Kamanga 2002; Mhango *et al.*, 2012). Legumes are commonly grown as sole crops/monocrops and legume-cereal intercrops, and they are also intercropped with other crops such as cassava and sweet potatoes. Some farmers intercrop legumes with other legumes in what is commonly known as doubled-up legume technology (ICRISAT/MAI, 2000; Mhango, 2011). Examples of doubled-up legume cropping systems are where pigeon pea is intercropped with groundnuts or pigeon pea intercropped with soybean (ICRISAT/MAI, 2000).

Doubled-up legumes are important to farmers who have scarce labour or land, as they allow them to grow more legumes with the same investment and labour. Farmers in Malawi consistently ranked doubled-up legumes highly among organic soil fertility options (Kanyama-Phiri *et al*., 2000; Phiri *et al.*, 2012). Legumes are also rotated with maize in which a legume crop is followed by maize in order for the maize to benefit from the organic N left in the legume residues. Furthermore, legumes are popular among Malawian smallholder farmers that have small pieces of land due to population pressure. Population pressure is a common challange in Malawi, with an average of less than a hectare per household (Ricker-Gilbert *et al*., 2014).

Although popular, intercropping practices have challenges such as difficulties in mechanization, application of herbicides, interactions that lead to suppression of component crops yields, and nutrient management. Very often cropping patterns and densities bring challenges in yield evaluation, and therefore, competition indices such as land equivalent ratios (LERs) and monetary advantage indices (MAIs) that measures productivity are usually used in evaluating their performances. For the case of studies done in the Malawi environment, positive results have been reported on LERs in maize-pigeon pea and pigeon pea-groundnuts intercrops (Mhango, 2011; Phiri *et al*., 2013). However, no comprehensive studies have been done on most of recent varieties. On the other hand, information on productivity of the pigeon pea-cowpea doubled-up is scanty. Therefore, the objective of this study was to evaluate and compare the productivity of the sole cropped system, legume-cereal intercrop and doubled-up legumes involving pigeon pea, cowpea and maize.

3.2 Materials And Methods

3.2.1 Description of the study sites

The study was conducted in Central Malawi, at two sites of Lilongwe and Dowa districts in the 2013/14 cropping season. The study sites detailed information on coordinates, rainfall and soil properties is presented in Chapter Two, Sections 2.2.1, 2.2.1 and 2.3.1, and 2.2.1 and 2.3.2, respectively.

3.2.2 Treatment description

The treatments included sole crops, legume-legume and maize-legume intercrops as follows: sole cropped pigeon pea, sole cropped cowpea, sole cropped maize, pigeon pea-cowpea intercrop, pigeon pea-maize intercrop, and maize-cowpea intercrop arranged in a randomized complete block design (RCBD). Detailed information on plot sizes, planting patterns, spacing within and between the rows, and crop varieties used is presented in Chapter Two Section 2.2.2.

3.2.3 Harvesting and determination of yields and harvest indices

Harvesting was done from a net plot of 13 m X 5 m which was obtained by leaving a one metre as guard space from each side of the 15 m X 7 m gross plot. This was done as a standard procedure (Mloza-Banda, 1994) to avoid outside interference. The different crop species in these experiments reached maturity at different periods of the season and, therefore, were also harvested at different periods. The first to be harvested was cowpea, followed by maize and lastly pigeon pea.

For cowpea, harvesting involved cutting of stover at ground level to get all the above ground biomass including all the pods which were at this period un-shattered. Then, the above ground biomass was weighed and recorded as total above ground fresh weight or total biomass. The pods were unshelled and all the net plot grain was weighed and recorded as net plot fresh weight. Then a sample of 100 seeds was taken up by following a quartering procedure (Kanyama-Phiri, 2010, Personal Communication). The 100 seed samples from each net plot were oven dried at 70°C for 48 hours. The moisture content of the seed was determined by using the formula in equation (ix).

$$\text{Seed moisture content (\%)} = \frac{W1 - W2}{W1} \times 100 \quad \text{..............(ix)}$$

Where:

W1 = weight of seed before oven drying

W2 = weight of seed after oven drying

The grain yield per hectare was determined at a predetermined moisture percentage of 10 by using the fresh net plot grain weight, moisture percentage from the 100 seed sample, and plot size according to Mloza-Banda (1994). For the determination of total dry matter yields, a one kg sample of fresh stover was taken to the laboratory for oven drying and determination of dry matter. Then, the total dry matter yields were obtained from the addition of the stover dry matter plus the grain yield. Similar procedures were followed in obtaining grain and total dry matter yields for maize and pigeon pea. However, the predetermined moisture percentages used were 12.5 for maize and 10 for pigeon pea. Determination of yield per plant was done by dividing the yield by the crop stand count that was taken during harvesting time. The yield per plant determination was necessary because cowpea was planted with a lower population in the intercrop than it was done in the sole crop. The harvest index percentages (HI%) were calculated according to Mloza-Banda (1994) as shown in equation (x).

$$\text{Harvest index percentage} = \frac{\text{grain yield}}{\text{total dry matter yield}} \times 100 \quad \ldots\ldots\ldots(\text{x})$$

3.2.4 Assessment of productivity of the cropping systems using land equivalent ratios, monetary advantage index, relative yield total and relative nutrient yields indices

3.2.4.1 Land Equivalent Ratios (LERs)

Intercrop productivity was measured using land equivalent ratios (LERs). The LER is defined as the ratio of the land size needed under sole cropping to land size needed under intercropping at the same level of management to give an equal amount of grain yield or economic yield (Willey and Osiru, 1972). It measures the effects of both beneficial and negative interactions between the intercropped species. It is calculated by dividing intercrop yields with yields of pure stands of each component crop and the resulting rations are added up. In interpretation, an LER of 1.0 means that the amount of land required for a component crop in an intercrop is the same as that required when the component crop is grown in pure stand. On the other hand, an LER of greater than 1.0, shows advantage with intercropping while an LER number of less than 1.0 shows a disadvantage with intercropping. The division result for each component crop before adding up to get an LER is referred to as a partial LER. The partial LER can indicate which component crop is dominating or is suppressed in exploiting resources (Dhima *et al*., 2007). The LER and partial LER are calculated as illustrated algebraically in equation (xi) by Dhima *et al.* (2007).

$$\mathrm{LER} = \mathrm{LER_A} + \mathrm{LER_B} \qquad \text{………………………(xi)}$$

Where:

$$\mathrm{LER_A} = \frac{\mathrm{Y_{AB}}}{\mathrm{Y_A}}$$

$$\mathrm{LER_B} = \frac{\mathrm{Y_{BA}}}{\mathrm{Y_B}}$$

Where:

A and B are crop species under sole or intercropping

Y_{AB} is grain yield of crop A in an intercrop with crop B

Y_{BA} is grain yield of crop B in an intercrop with crop A

Y_A and Y_B are grain yields of sole crop A and sole crop B, respectively

3.2.4.2 Monetary Advantage Index (MAI)

The MAI provides information on the economic advantage or disadvantage of the cropping system whereby positive values show economic advantage (Dhima *et al*., 2007). Furthermore, Yilmaz *et al.* (2008) report that the higher the MAI value the more profitable the cropping systems. The MAI was calculated as in equation (xii).

$$\mathrm{MAI} = \frac{\text{(Value of combined intercrops)}}{\mathrm{LER}} \times \text{(LER-1)} \qquad \text{………(xii)}$$

The crop prices for the study area were obtained from key informants such as lead farmers, extension officers, and visitations to markets where farmers from the study sites usually sell their produce. This information was also compared with the minimum farm gate prices advertised by the Ministry of Agriculture, Irrigation and Water Development (MoAIWD) of the Government of Malawi (MoAIWD, 2014) and a registered commodity traders company “Auction Holdings Commodity Exchange (AHCX)”, advertising in the

Malawi's popular newspapers, "The Nation" and "The Daily Times" (AHCX, 2014). It should be noted that all prices obtained were in Malawi kwacha currency but the data in results section was presented as indices which are unitless.

3.2.4.3 Relative yield total, relative dry matter yield and relative nutrient yields

Relative yield total (RYT) is the degree to which various components in an ecosystem share common resources (Tofinga, 1993). The RYT is similar to the LER but when using the RYT what is considered is the total dry matter yield or the whole biomass instead of only grain or economic yield as is done with the LER. As illustrated by Ghosh *et al.* (2009), the formulae for relative yield totals for the PP+CP, PP+MZ and MZ+CP are as shown in equations (xiii) to (xv).

$$RYT_{pp+cp} = \frac{IDM_{pp}}{SDM_{pp}} + \frac{IDM_{cp}}{SDM_{cp}} \quad \text{..................(xiii)}$$

$$RYT_{pp+mz} = \frac{IDM_{PP}}{SDM_{pp}} + \frac{IDM_{mz}}{SDM_{mz}} \quad \text{..................(xiv)}$$

$$RYT_{mz+cp} = \frac{IDM_{mz}}{SDM_{mz}} + \frac{IDM_{cp}}{SDM_{cp}} \quad \text{..................(xv)}$$

Where:

IDM and SDM are intercropped species and sole cropped species dry matter, respectively, whereas pp, cp, and mz stand for pigeon pea, cowpea and maize, respectively, in reference to the formula.

NB: The RYT values as index values are unitless.

One of the main factors affecting plant growth is the nutrients from the soil. Therefore, the plant N uptake and P uptake as components of some major nutrients affecting plant growth were determined and used in computing nutrient uptake indices, which are relative

N yield (RNY) and relative P yield (RPY). These were compared with relative dry matter yield (RDY) according to Ghosh *et al*. (2009). This helped to determine how these nutrients limited the growth of the component crops in the different cropping systems but also to determine which component crops were more resilient or adaptive than others. These indices were determined as shown in equations (xvi) to (xviii) according to Ghosh *et al*. (2009).

$$\mathrm{RDY_a} = \frac{\text{intercrop dry matter yield of species a}}{\text{sole crop dry matter yield of species a}} \quad \text{……………(xvi)}$$

$$\mathrm{RNY_a} = \frac{\text{N uptake by intercrop species a}}{\text{N uptake by sole crop species a}} \quad \text{……………..(xvii)}$$

$$\mathrm{RPY_a} = \frac{\text{P uptake of intercrop species a}}{\text{P uptake of sole crop species a}} \quad \text{……..………..(xviii)}$$

Results of the computations from equations (xv) to (xviii) were interpreted according to Ghosh *et al.* (2009) as follows: RDY $\geq$ RNY implies N is less exploited by the intercropped species than they do on other resources, meaning that N is limiting the performance of the intercropped species. On the other hand RDY $\leq$ RNY means N is exploited more by the intercropped species than they are exploiting on other resources. Therefore this indicates that N is not limiting the growth and performance of the intercropped systems. The same interpretation applies to RPY. However RYT is interpreted as follows: RYT > 1 but < 2 shows partial competition for resources (Tofinga, 1993).

3.2.5 Data Analysis

Data on yields and harvest indices were subjected to analysis of variance (ANOVA) using Genstat 15th edition based on the model as presented in Section 2.2.6. Means were separated using the least significant difference (LSD) at P = 0.05. Furthermore, the productivity for the different cropping systems was mostly assessed and evaluated by using various indices such as the LER, partial LER, MAI, RYT, RDY, RNY and RPY. It should be noted that the indices are unitless.

3.3 Results

3.3.1 Pigeon pea, cowpea and maize yields per plant and harvest indices percentages (HI%) for the Lilongwe site

The total dry matter and grain yields per plant as influenced by cropping systems results are presented in Table 3.1. Sole cropped PP dry matter yield was significantly higher ($P < 0.05$) than that of PP in each of the PP+CP and PP+MZ intercrops by 23%. Similarly, sole cropped PP grain yield was significantly higher ($P < 0.05$) than that of PP in PP+MZ by 36% but was only slightly higher than that of PP in the PP+CP intercrop. However, there were no significant differences in PP harvest index percentages (HI%).

Similarly, for cowpea, the sole cropped CP produced significantly higher ($P < 0.05$) grain yields than that of CP in PP+CP and MZ+CP intercrops by 19% and 15%, respectively. However, no significant differences were observed for the cowpea HI%. Furthermore, for maize, no significant differences were observed in the yields and HI% as influenced by cropping systems, though MZ yields in the MZ+CP intercrop showed slightly lower values.

Table 3.1: Yields and yield components of pigeon pea, cowpea and maize as affected by cropping systems at Lilongwe and Dowa sites

Crop	Cropping systems	Lilongwe site			Dowa site		
		Total dry matter/plant (g)	Grain yield/plant (g)	HI (%)	Total dry matter/plant (g)	Grain yield/plant (g)	HI (%)
Pigeon pea	PP	101.8	16.5	16.1	115.5	12.4	10.7
	PP+CP	77.0	11.9	15.5	95.2	11.2	11.8
	PP+MZ	77.5	10.5	13.5	93.8	12.5	13.3
	$LSD_{(0.05)}$	19.2	4.15	6.71	18.8	3.25	4.96
	F. pr	0.033	0.048	0.477	0.018	0.914	0.340
	CV (%)	20.3	39.2	19.8	12.5	33.1	51.3
Cowpea	CP	22.6	6.2	27.4	25.3	6.3	27.2
	CP+PP	19.2	5.0	26.0	23.4	4.6	19.7
	CP+MZ	21.2	5.3	25.5	21.0	4.7	22.4
	$LSD_{(0.05)}$	7.01	0.512	8.56	3.21	1.31	5.25
	F. pr	0.459	0.007	0.561	0.177	0.035	0.039
	CV (%)	14.7	4.1	14.5	6.3	11.1	10.0
Maize	MZ	61.3	20.5	33.8	111.0	47.8	43.2
	MZ+PP	69.6	19.0	27.4	99.0	40.7	41.5
	MZ+CP	48.4	13.9	27.3	94.4	39.6	42.6
	$LSD_{(0.05)}$	44.2	14.5	11.3	42.4	10.2	15.19
	F. pr	0.477	0.485	0.300	0.580	0.165	0.952
	CV (%)	32.6	36.1	17.0	18.4	10.6	15.8

NB: Separation of means was based on LSD at 5% significant level; F. pr = F probability; CV = coefficient of variation

3.3.2 Pigeon pea, cowpea and maize yields per plant and harvest indices percentages (HI%) for the Dowa site

For the Dowa site, sole cropped PP total dry matter yield was significantly higher ($P < 0.05$) than that of PP in PP+CP and PP+MZ intercrops by 18% and 19%, respectively (Table 3.1). However, there were no significant differences in grain yields and HI%. For cowpea, sole cropped CP produced significantly higher grain yield than that of CP in PP+CP and MZ+CP intercrops by 27% and 25%, respectively. Similarly, HI% of sole cropped CP was significantly higher ($P < 0.05$) than that of CP in PP+CP by 28% but was only slightly higher than that of CP in the MZ+CP intercrop. As for the Lilongwe site, at the Dowa site, MZ yields were consistently lower for MZ in the MZ+CP intercrop than in the other cropping systems. However, no significant differences were observed.

3.3.3 Intercrop productivity as measured by competition and monetary advantage indices for Lilongwe site

3.3.3.1 Land Equivalent Ratios (LERs) and Partial LERs for Lilongwe site

Results in Table 3.2 show the highest grain yield productivity using LERs where pigeon pea was intercropped with maize at the Lilongwe site. The benefits of intercropping over sole cropping were as follows: 57% by PP+MZ, 22% by MZ+CP and 18% by PP+CP intercropping systems. To check which component crop was more competitive than the other in exploiting resources, the partial LERs (Table 3.2) show that at the Lilogwe site, PP was more competitive when intercropped with CP. On the other hand, maize showed higher partial LERs when intercropped with either PP or CP, which means it was more competitive than the partner component crops. Furthermore, in both cases of intercropping CP with either MZ or PP, CP produced lower partial LERs than the partner component crops that means it was less competitive.

3.3.3.2 Monetary Advantage Index (MAI) for Lilongwe site

Results show positive numbers (Table 3.2) that means there was an economic advantage from all the intercropping systems. Furthermore, amongst the three intercropping systems, Table 3.2 shows that the PP+MZ intercrop was the most beneficial as it indicated the highest MAI value.

3.3.4 Intercrop productivity as measured by competition and monetary advantage indices for Dowa site

3.3.4.1 Land Equivalent Ratios (LERs) and Partial LERs for Dowa site

Similar to the Lilongwe site, Table 3.2 shows the highest grain yield productivity using LERs where pigeon pea was intercropped with maize, also for Dowa site. The benefits of intercropping over sole cropping were as follows: 86% by PP+MZ, 63% by PP+CP and 26% by MZ+CP intercropping systems. The partial LERs (Table 3.2) show that PP was more competitive when intercropped with CP. However, maize showed higher partial LERs when intercropped with either PP or CP, meaning that it was more competitive than the partner component crops. Furthermore, in both cases, intercropping CP with either MZ or PP produced lower partial LERs than the partner component crops, implying less competiveness by the CP.

Table 3.2: Land equivalent ratios (LER) and monetary advantage index (MAI)

Cropping system	Partial values of LERs						LER		MAI	
	Lilongwe			Dowa			Lilongwe	Dowa	Lilongwe	Dowa
	PP	CP	MZ	PP	CP	MZ	LER	LER	MAI	MAI
PP+CP intercrop	0.68	0.5	NA	1.06	0.57	NA	1.18	1.63	25804	36163
PP+MZ intercrop	0.65	NA	0.92	1.01	NA	0.85	1.57	1.86	53592	90756
MZ+CP intercrop	NA	0.48	0.74	NA	0.43	0.83	1.22	1.26	17509	34752

NB: (i) In the above table data are presented as mean values of indices and therefore are unit-less.

(ii) NA = Not applicable

(iii) LER values have the following implications: LER > 1 intercropping is advantageous compared to sole cropping; LER < 1 intercropping is disadvantageous; LER = 1 the same piece of land is needed for the same level of productivity

(iv) MAI values have the following implications: positive values show economic advantage while negative values show economic losses to the farmer.

3.3.4.2 Monetary Advantage Index (MAI) for Dowa site

Similar to the Lilongwe site, Table 3.2 shows all positive numbers for MAI, which means there was an economic advantage by all the intercropping systems also for the Dowa site. Furthermore, PP+MZ intercrop were observed to be more beneficial to farmers as it indicated the highest MAI value.

3.3.5 Competition for nutrient uptake in the various cropping systems for the Lilongwe site

Results in Table 3.3 show that both intercropping combinations that involved PP (PP+CP and PP+MZ) indicated partial competition for resources (RYT > 1 but < 2). On the other hand, Figure 3.1 shows that N was limiting the growth of both component crops (RDY ≥ RNY) but P was not a limiting factor (RDY≤ RPY) in the growth of PP in both the PP+CP and PP+MZ intercrops.

Table 3.3: Relative yield totals for the different cropping systems for the Lilongwe and Dowa sites

Crop	Cropping systems	Relative Yield Total (RYT)	
		Lilongwe	Dowa
Pigeon pea	PP+CP	1.26	1.38
	PP+MZ	1.93	1.54
Cowpea	CP+PP	1.26	1.38
	CP+MZ	1.30	1.13
Maize	MZ+PP	1.93	1.54
	MZ+CP	1.30	1.13

NB: (i) Mean values of RYT are presented in the table above.

(iii) RYT has the following implications: RYT < 1 but > 2 means there was partial competition for resources.

For cowpea, Figure 3.2 shows that both N and P limited the growth of cowpea in both PP+CP and MZ+CP intercrops (RDY ≥ RNY and RDY ≥ RPY), in the Lilongwe site. However, for maize, Figure1 shows that N was limiting the growth of maize in both intercrops of MZ+PP and MZ+CP (RDY ≥ RNY) but P was not a limiting factor (RDY≤ RPY).

3.3.6 Competition for nutrient uptake in the various cropping systems for the Dowa site

Similar to the Lilongwe site, for the Dowa site, Table 3.3 indicated partial competition for resources (RYT > 1 but < 2) in both intercropping combinations that included PP that is the PP + CP and PP + MZ intercrops. Furthermore, Figure 3.2 shows that nitrogen was limiting the growth of both component crops (RDY ≥ RNY) but P was not a limiting factor (RDY≤ RPY) in the growth of PP in the PP+CP intercrop in the Dowa site. However, P showed to be a limiting factor to PP growth in the PP+MZ intercrop (RDY ≥ RPY).

Similarly**,** as the case of the Lilongwe site, Figure 3.2 shows that both N and P limited the growth of cowpea in both intercropping systems of PP+CP and MZ+CP (RDY ≥ RNY and RDY ≥ RPY) in the Dowa site. On the other hand, for MZ, in the Dowa site, N was limiting where maize was intercropped with CP i.e. MZ+CP but not in the MZ+PP intercrop. Furthermore, in the Dowa site P was also limiting maize growth where it was intercropped with CP.

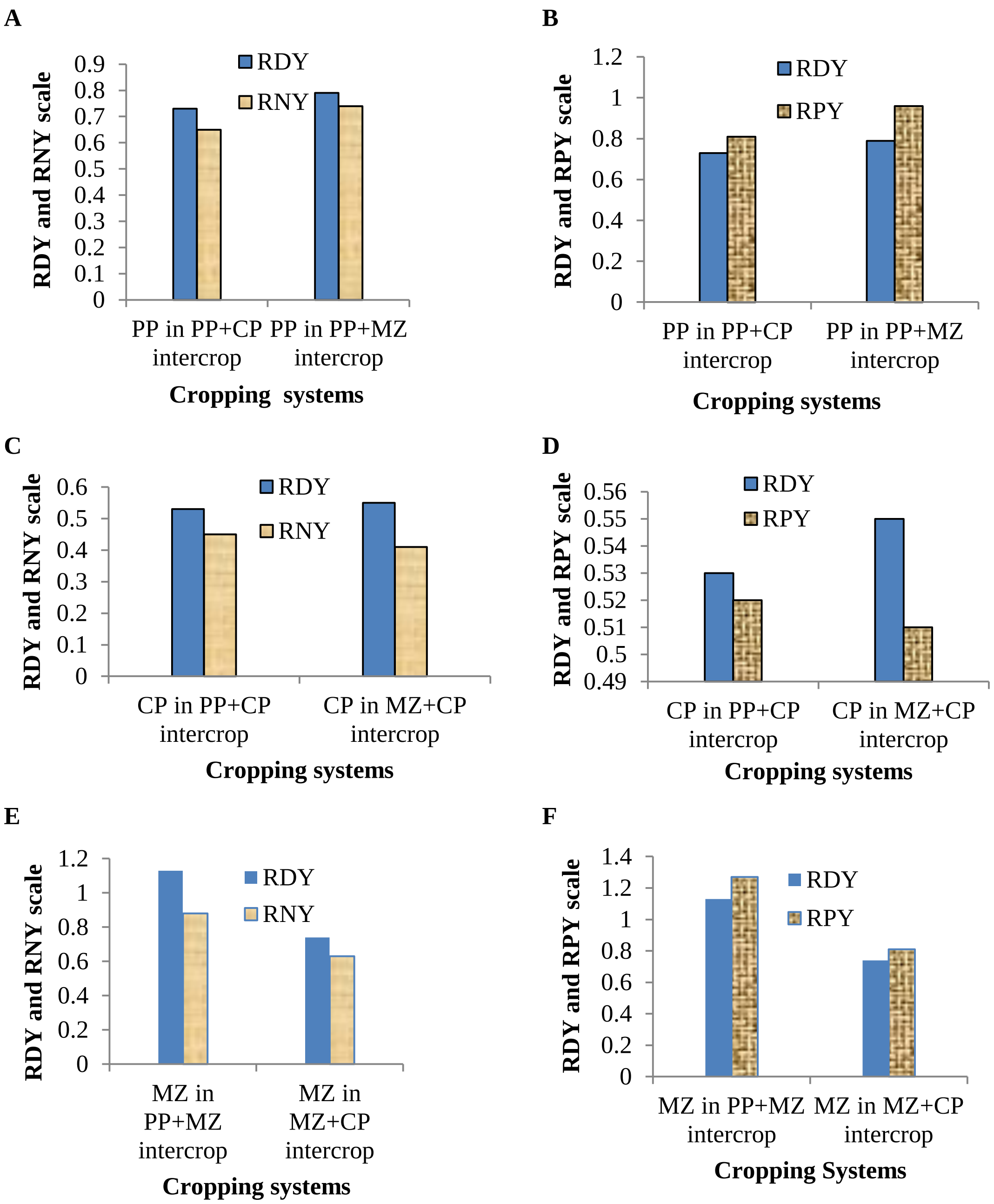


Figure 3.1: Competition for N and P uptake as illustrated by relative dry matter yield (RDY), relative N yield (RNY) and relative P yield (RPY) for the Lilongwe site

NB: i) A: Pigeon pea in competition for N uptake; B: Pigeon pea in competition for P uptake; C: Cowpea in competition for N uptake; D: Cowpea in competition for P uptake; E: Maize in competition for N uptake; F: Maize in competition for N uptake

ii) RDY ≥ RNY implies N is limiting growth; RDY ≥ RPY implies P is limiting growth

(iii) The Y axis shows values of index mean values of RDY and RNY or RDY and RPY

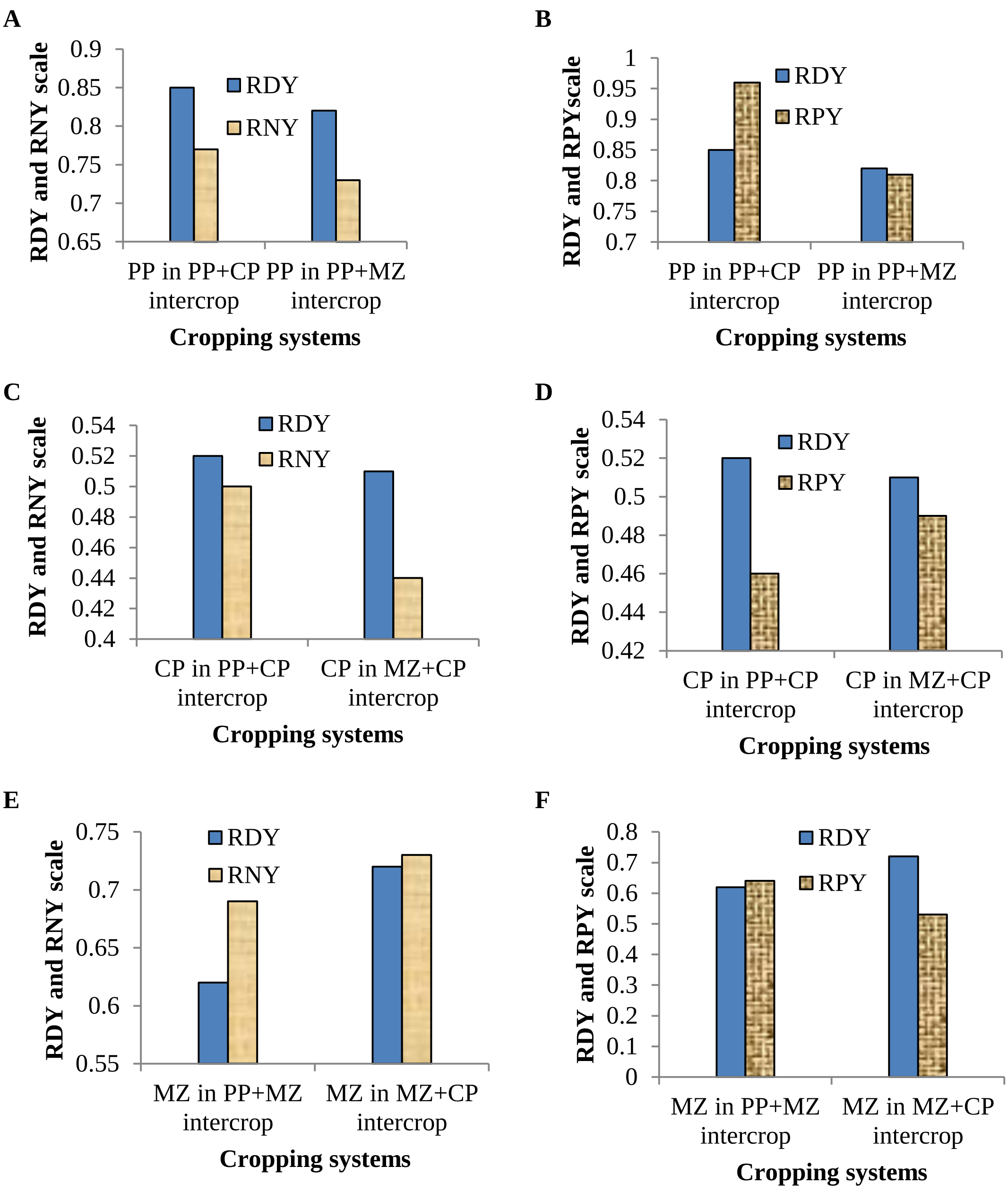


Figure 3.2: Competition for N and P uptake as illustrated by relative dry matter yield (RDY), relative N yield (RNY) and relative P yield (RPY) for the Dowa site

NB: i) A: Pigeon pea in competition for N uptake; B: Pigeon pea in competition for P uptake; C: Cowpea in competition for N uptake; D: Cowpea in competition for P uptake; E: Maize in competition for N uptake; F: Maize in competition for N uptake

ii) RDY ≥ RNY implies N is limiting growth; RDY ≥ RPY implies P is limiting growth

(iii) The Y axis shows values of index mean values of RDY and RNY or RDY and RPY

3.4 Discussion

Many intercropping systems are characterized by interactions between the component crops that may affect the performance of one or both of the component crops. In this study, performance of the cropping systems in terms crop yields, productivity, nutrient uptake and utilization and monetary advantage were evaluated. Total dry matter and grain yields have been consistently higher in the sole crops than in the intercrops in both pigeon pea and cowpea for both study sites, with slight differences. This shows the effect of competition for resources such as nutrient from the soil and sunlight. In similar studies, Legwaila *et al.* (2012) and Abd El-Lateef *et al*. (2015) reported lower grain and dry matter yields per plant in cowpea and maize intercrops than their sole crops. However, in this present study, considering the LERs, the overall productivity was higher for all the three intercrops (PP+CP, PP+MZ, MZ+CP) than that of sole crops. This is also consistent with a number of similar studies where higher LERs have been reported. Mathews *et al.* (2001) and Mhango (2011) also reported higher LERs in a pigeon pea-maize intercrop though considering individual crop yields, sole cropping produced higher yields than those by the same crops in an intercrop. Similarly, Yilmaz *et al.* (2008) reported higher LERs in a maize-cowpea intercrops planted in different patterns. Higher LERs are mostly attributed to higher land or resources utilization efficiency, which can also be the case in this study.

On the other hand, individual component crops showed different performances in their resource utilization. Partial LERs indicated that PP was more competitive than CP, whereas MZ was more competitive than both CP and PP. On the other hand CP indicated that it was less competitive than both the MZ and PP. This is consistent with results by Saudy and El-Bagoury (2014) which showed in a similar study that maize was more dominant and cowpea was the dominated crop. This can be attributed to the MZ greater height that shades the cowpea but also the fast growth habit that enables it to out-compete the pigeon pea which is commonly characterized by the early slow growth. Furthermore, Ghosh *et al*. (2009) noted cereals to be suppressive components in intercropping systems.

Similarly, the present study showed that there are some differences in the individual component crops on how they take up and utilize nutrients when grown in different cropping systems. For instance, cowpea growth was limited by both N and P in both study sites. That is its RDY was higher than both of its RNY and RPY, which was different from the PP and MZ situation that were mostly limited by N. This can be attributed to a number of factors including functions of these nutrients in different crops. For instance, N is needed in large amounts by all plants for promotion of rapid growth and other functions. On the other hand, apart from common functions of P, it is also needed for biological nitrogen fixation and therefore it is needed in high amount in legumes, including cowpea and pigeon pea. However, the difference between CP and PP is that PP can have reduced effects of the competition since it continues to grow after harvesting other crops. Pigeon pea is also deep rooted and it exploits nutrients from even the lower layers of the soil. Furthermore, PP root exudates are also reported to have the ability to solubilise Al-P and Fe-P forms (Subbarao et *al.,* 1997; Ishikawa *et al*., 2002). Furthermore, Edje (2014) reported high amounts of P (56.6 mg/kg) and Fe (196.2 mg/kg) in pigeon pea litter, which were attributed to the effect of root exudates.

Cropping systems productivity not only measures performance of the crop in terms of yields but the other concern of farmers is in the monetary value of their intervention. The present study showed that the PP+MZ intercrop showed the highest monetary advantage.

This can be attributed to a number of factors including the prices offered to pigeon pea on the market was the highest during the market season of this study. Similarly, maize was not much affected by the competition for growth resources, which implies its yield was relatively high. On the other hand, cowpea was the most suppressed crop in the cropping systems and, therefore, its yield was much more reduced in such that it could not fetch more money.

3.5 Conclusions

Intercropping systems continue to be popular among smallholder farmers in Malawi because of the benefits they bring, such as food security and soil fertility benefits. Therefore, it is important to continue evaluating some of the crop combinations practised by farmers to ensure productivity. From this study, it can be concluded that all the intercropping systems compared were more productive than sole cropping since they all resulted in LERs of greater than one. Furthermore, they were also more productive in terms of monetary gains as they all produced positive MAI values. Though all the intercropping systems (PP+MZ, PP+CP and MZ+CP) have many advantages, from this study, the PP+MZ intercrop is the most beneficial in terms of both yields and monetary gains as it produced highest LERs and MAI values in both sites of Lilongwe and Dowa. The study has also elucidated that maize is the most resilient crop when grown either in PP+MZ or MZ+CP intercrops whereas cowpea is the most suppressed when intercropped with either PP or MZ. Therefore, it can be recommended that prudent considerations should be taken into account when planning intercropping systems as far as crop competition for nutrients is concerned. Furthermore, studies are needed on the intercropping systems to derive site-specific fertilizer recommendations.

References

Abd El-Lateef, E. M., Abd El-Salam, M. S., El-Habbasha, S. F. and Ahmed, M. A. (2015). Effect of maize-cowpea intercropping on light interception, yield and land use efficiency. *International Journal of ChemTech Research* 8(6): 556 – 564.

Auction Holdings Commodity Exchange (AHCX) (2014). *Sales Statistics For Commodities In The 2013/14 Marketing Season.* The daily times, August 11, 2014. 18pp.

Chilimba, A. D. C., Young, S. D., Black, C. R., Rogerson, K. B., Ander, E. L., Watts, M. J., Lammel, J and Broadly, M. R. (2011). Maize grain and soil surveys reveal suboptimal dietary selenium intake is widespread in Malawi. *Scientific Reports* 1:72.

Dhima, K. V. A., Ruthourgidis, A. A., Vasilakoglou, I. B. and Dordas, C. A. (2007). Competition indices of common vetch and cereal intercrops in two seeding ratio. *Field Crop Research* 100: 249 – 246.

Drinkwater, L. E., Wagoner, P. and Sarrantonio, M. (1998). Legume based cropping systems have reduced carbon and nitrogen losses. *Nature* 396: 262-264.

Edje, O. T. (2014). Effects of intercropping maize (*Zea mays* L) with pigeon pea (*Cajanus cajan* L.) on seed yield, litter and fuel wood in Mildveld Swaziland. *African Journal of Agricultural Sciences and Technologies* 1(2): 1 – 8.

Ghosh, P. K., Tripathi, A. K., Bandyopadhayay, K.K. and Manna, M. C. (2009). Assessment of nutrient competition and nutrient requirement in soybean sorghum intercropping system. *European Journal of Agronomy* 31: 43 – 50.

Giller, K. E. (2001). *Nitrogen Fixation in Tropical Cropping Systems.* Commonwealth for Agriculture Bureau International, Wallingford. UK. 423pp.

International Crops Research Institute for Semi-Arid Tropics/Ministry of Agriculture and Irrigation (2000). *Cost-effective Soil Fertility Options For Smallholder Farmers In Malawi.* ICRISAT, Bulawayo, Zimbabwe. 24pp.

Ishikawa, S., Adu-Gyamfi, J. J., Nakamura, T., Yoshihara, T., Watanabe, T. and Wagatsuma, T. (2002). Genotypic variability in phosphorus solubilizing activity of root exudates by pigeon pea grown in low-nutrient environments. *Plant and Soil* 245: 71 – 81.

Kamanga, B. C. G. (2002). *Farmer Experimentation to Access the Potentiontial of legumes in Maize-Based Cropping Systems in Malawi*. Risk Management Working Paper No. 02. CIMMYT, Mexico. 16pp.

Kanyama-Phiri, G. Y., Snapp, S., Kamanga, B. and Wellard, K. (2000). *Towards integrated soil fertility management in Malawi: Incorporating participatory approaches in agricultural research.* Working Paper Managing Africa Soils No.11. International Institute for Environment and Development, London, UK. 28pp.

Kerr, R. B., Snapp, S., Chirwa, M., Shumba, L. and Msachi, R. (2007). Participatory research on legume diversification with Malawian smallholder farmers for improved human nutrition and soil fertility. *Eperimental Agriculture* 43: 437 – 453.

Landon, J. R. (Ed.) (1991) *Booker Tropical Soil Manual. A handbook for Soil Survey and Agricultural Land Evaluation in the Tropics and Subtropics.* John Wiley and Sons, Inc., New York. 474pp.

Legwaila, G. M., Marokane, T. K. and Mojeremane, W. (2012). Effect of intercropping on the performance of maize and cowpea in Botswana. *International Journal of Agriculture and Forestry* 2(6): 307 – 310.

Mathews, C. Jones, R. B. and Saxena, K. B. (2001). Maize and pigeon pea intercropping in Mpumalanga, South Africa. International Chickpea and Pigeon pea Newsletter 8. [http://oar.icrisat.org/id/eprint/4895] site visited on 24/03/2016.

Mhango, W. G., Snapp, S. S. and Phiri, G. Y. K. (2012). Opportunities and constraints to legume production on smallholder farms in Malawi. *Renewable Agriculture and Food Systems*28: 234 – 244.

Ministry of Agriculture and Food Security, MoAFS. (2012). *Guide to Agricultural Production and Natural Resource Management in Malawi*. Agricultural Communications Branch. Lilongwe, Malawi. 366pp.

Ministry of Agriculture, Irrigation and Water Development (2014). *Minimum Farm Gate Prices for Strategic Crops for The 2014/15 Agricultural Season*. The Nation, Tuesday, May 11, 2014, 19pp.

Mloza-Banda, H. R. (1994). *Principles and Practices of Crop Management: Field Study Guide.* Crop Science Department, Bunda College of Agriculture, Lilongwe. 137pp.

Mutegi, J., Kabambe, V., Zingore, S., Harawa, R. and Wairegi, L. (2015) *The Fertilizer Recommendation Issues in Malawi: Gaps, Challenges, Opportunities and Guidelines.* Soil Health Consortium, Malawi. 54pp.

Njira, K. O. W., Nalivata, P. C., Kanyama-Phiri, G. Y. and Lowole, M. W. (2012). Biological nitrogen fixation in sole and doubled-up legume cropping systems in the sandy soils of Kasungu, Central Malawi. *Journal of Soil Science and Environmental Management* 3(9): 224 – 230.

Phiri, A. T., Njoloma, J. P., Kanyam-Phiri, G. Y., Snapp, S., Lowole, M. W. (2010). Maize yield response to the combined application of Tunduru rock phosphate and Pigeon pea residues in Kasungu, Central Malawi. *Africa Journal of Agricultural Research* 5(11): 1235 – 1242.

Phiri, A. T., Mhango, W. G., Njoloma, J. P., Kanyama-Phiri, G. Y. and Lowole, M. W. (2012). Farmers evaluation of integrated soil fertility management methods in Northern Kasungu, Central Malawi. *Journal of Agricultural Extension and Rural Development* 4(11): 340 – 346.

Phiri, A. T., Msaky, J. J., Mrema, J. P., Kanyama-Phiri, G. Y. and Harawa, R. (2013). Assessment of nutrient and biomass yields of medium and short duration pigeon pea in the pigeon pea-groundnut intercropping systems in Malawi. *Journal of Sustainable Society* 2(1): 36 – 48.

Ricker-Gilbert, J. Jumbe, C. and Chamberlin, J. (2014). How does population density influence agricultural intensification? Evidence from Malawi. *Food Policy* 48: 114 – 128.

Saudy, H. S. and El-Bagoury, K. F. (2014). Quixotic coupling between irrigation system and maize-cowpea intercropping for weed suppression and water preservation. *African Crop Science Journal* 22(2): 97 – 108.

Subbarao, G. V., Ae, N. and Otani, T. (1997). Genotypic variation in iron- and aluminium-phosphate solubilizing activity of pigeon pea exudates under P-deficient conditions. *Soil Science and Plant Nutrition* 43(2): 295 – 305.

Tofinga, M. P. (1993). Design *and Analysis of Mixed Cropping Experiments for Indigenous Pacific Island Agroforestry*. Forest Service General Technical Report No.140. United States Department of Agriculture, USA. 5pp.

Willey, R. W. and Osiru, D.S.O. (1972). Studies on mixtures of maize and beans (*Phaseolous vulgaris*, L.) with particular reference to plant population. *Journal of Agricultural Science* 79: 517 – 529.

Yilmaz, S., Atak, M. and Erayman, M. (2008). Identification of advantages of maize-legume intercropping over solitary cropping through competition indices in Eastern Mediterranean region. *Turkish Journal of Agriculture and Forestry* 32: 111 – 119.

CHAPTER FOUR

4.0 ASSESSMENT OF VAM FUNGAL COLONISATION IN PIGEON PEA, COWPEA AND MAIZE DIVERSIFIED CROPPING SYSTEMS AND MAIZE SHORT ROTATION ON LUVISOLS OF CENTRAL MALAWI

Abstract

Mycorrhizal associations contribute to the sustainability of crop production systems through their roles in nutrient cycling and other benefits in the soil-plant ecosystems. A two year study was conducted on the Luvisols of the Lilongwe and Dowa districts of Central Malawi to assess the vesicular-arbuscular mycorrhizal (VAM) colonisation levels in pigeon pea, cowpea and maize grown in diversified legume systems (sole cropping, legume-cereal and legume-legume intercropping), and in maize grown in short rotation as affected by the previous cropping system and N fertilizer application. Experiments were arranged in the randomized complete block design (RCBD) and split plot design in the first and second years, respectively. Results showed no significant differences ($p < 0.05$) in VAM fungal colonisation of roots in each crop as influenced by cropping systems in the legume-based cropping systems. However, year two results showed that all treatments that included legumes whether grown as a sole crop, in legume-cereal or in legume-legume cropping systems in the previous year, had significantly higher ($p < 0.05$) VAM fungal colonisation of the rotational maize crop roots by a range 39% to 50% and 19% to 47% than those in maize supplied and not supplied with N fertilizer, respectively, in a maize-maize short rotation, in the Lilongwe site. Similarly, in the Dowa site, results showed significantly higher ($p < 0.05$) VAM fungal colonisation in maize that followed legume-based systems in rotation ranging from 15% to 36% and 28% to 40% than those in unfertilized and N fertilized maize, respectively, in a maize-maize short rotation. There were no significant differences ($p < 0.05$) in VAM fungal colonisation in the rotational maize due to the fertilizer N application. Furthermore, there were positive correlations between VAM fungal colonisation and the plant P content, dry matter yield, nodule numbers and BNF, but only showed significance for P content in maize ($r = 0.659$, $P < 0.02$), with mostly non-significant results, that can be attributed to the relatively lower VAM fungal colonisation percentages. From this study it can be noted that inclusion of diversified legume systems of pigeon pea and cowpea in maize based systems can improve the proliferation of VAM fungi in its subsequent crop. Further studies may also help to assess the diversity of VAM fungal species and identify more adaptive ones for inoculation studies.

Key words: Legume cropping systems, rotation, VAM fungal colonisation, vesicular-arbuscular mycorrhiza

4.1 Introduction

The sustainable intensification of crop production calls for various approaches including integrated soil fertility management (ISFM) (Giller *et al*., 2015; Lampkin *et al*., 2015). It advocates agricultural productivity while ensuring the maintenance and resilience of the ecosystems (Vanlauwe *et al*., 2014; Heissenhuber *et al*., 2015). On the other hand, soil fertility is one of the main challenges that can continue to derail sustainable agriculture production in many developing countries. In sub-Saharan Africa, most smallholder farmers have limited capabilities to acquire inorganic fertilizers (Alliance for Green Revolution in Africa, AGRA, 2014). Inclusion of legumes that improves soil fertility through biological nitrogen fixation (BNF) and phosphorus acquisition through mycorrhizal associations is of paramount importance.

Mycorrhiza is a mutualistic association between roots of plants and some fungal species. Major groups of mycorrhizae include ectomycorrhizae, endomycorrhizae, ericoid mycorrhizae and orchid mycorrhizae (Brundrett, 2004; Sylvia *et al*., 2005; van der Heijden *et al*., 2015). Endomycorrhizae involves root cortex penetrating fungi under a Phlyum of *Glomeromycota,* associating with over 80% of plant species (Brundrett, 2004; Wang and Qui, 2006). The most reported endomycorrhiza group is commonly referred to as vesicular-arbuscular mycorrhiza (VAM) or arbuscular mycorrhiza (AM) because of their morphological features, the arbuscules and vesicles, that are used for transportation and storage of materials, respectively (Brundrett, 2004; Brady and Weil, 2008). The mycorrhizal association is developed as a mutualistic adaptation benefiting both symbionts. The fungal species benefits carbohydrates and habitat from the plant while providing a number of benefits to the plant. They enhance P uptake and other nutrients by increasing plant root surface area, and producing organic acids and phosphatase enzymes that solubilise P (Bolan, 1991; Momayezi *et al.*, 2015; Gastol *et al.*, 2015). Marschner and Dell (1994) have reported up to 80%, 25%, 10%, 25% and 60% uptake of plant P, N, K, Zn and Cu, respectively, by external hyphae of VAM. Furthermore, some studies have shown synergistic effects of VAM to *Rhizobium*-legume symbiosis that result in BNF increase (Ahiabor *et al.*, 2007; Lupwayi *et al*., 2011). This is achieved through the VAM's enhancement of plant P uptake, which is required in high amounts for BNF (Brady and Weil, 2008). The VAM associations are also reported to be involved in N transfer from legume to cereal in intercropping systems (Makoi and Ndakidemi, 2009). Other benefits to the plant offered by the mycorrhiza include enhancement of the plant's water uptake that increases drought tolerance (Benhiba *et al.*, 2015; Ortiz *et al*., 2015), increasing the plant's resistance against some soil borne pathogens (Declerk *et al*., 2002; Hage-Ahmed *et al*., 2013) and against weed species such as striga (Othira *et al.*, 2012). Furthermore, mycorrhizal fungi produce glomalin, a glycoprotein that binds soil particles and improves the soil structure (Singh, 2012). It also binds heavy metals and improves plant's tolerance to their toxic effects (Singh, 2012).

The proliferation of VAM fungi in an ecosystem is affected by a number of factors. Many studies show that VAM development usually favours low levels of P (Liu *et al.*, 2000) with a few exceptions where additional P increased colonisation levels (Gamage *et al*. 2004), slightly low pH (Kanno *et al*., 2006), warm temperatures and light availability (Fageria, 2012). However, influence of farming practices and cropping systems have shown different outcomes. Conservation practices such as conservation agriculture (CA) have been associated with increased VAM fungal diversity in some studies (Sale *et al.*, 2015) and rotations with some legumes have shown increased VAM fungal colonisation in the crop that follow (Lekberg *et al*., 2008). On the other hand intercrops have led to increase while others to decrease in either VAM fungal colonization or diversity (Jefwa, 2004; Hage-Ahmed *et al*., 2013).

In Malawi, common crops include maize (*Zea mays*), common beans (*Phaseolus vulgaris*), cowpea (*Vigina unguiculata*), pigeon pea (*Cajanus cajan*) and groundnuts (*Arachis hypogaea*) and some agroforestry species. These crops are usually grown in monocrops/sole crops and in intercrops. Jefwa (2004) reported the presence of VAM species of the genera *Glomus, Gigaspora, Acaulospora, Scutellaspora* and *Archaeospora* in a study involving sole cropping and intercropping of maize with agroforestry species of *Gliricidia sepium*, *Sesbania sesban* and *Sesbania macratha* on the soils of Southern Malawi. However, despite the importance of VAM in the cropping systems, information on the status of VAM in the field crops such as pigeon pea and cowpea grown in sole crops or intercropped with maize or as legume/legume intercrops on the Malawi soils is scanty. Therefore, the aim of this study was to assess VAM fungal colonisation status in the cowpea, pigeon pea and maize grown as sole crops, legume-cereal and legume-legume intercrops, and on maize grown after the legume-based systems as a short rotation. Furthermore, it was also aimed at assessment of correlations of VAM fungal colonisation in the intercrops and P uptake, BNF and other yield components.

4.2 Materials and Methods

4.2.1 Site description

The study was conducted in two cropping seasons (2013/14 and 2014/15) in two sites of Central Malawi, in the districts of Lilongwe and Dowa. The experiment was conducted at the Lilongwe University of Agriculture and Natural Resources, Bunda Campus Research farm (14^{o} 11′ S, 33^{o} 46′ E) in the Lilongwe district, whereas in the Dowa district, the experiment was conducted at the Nachisaka Extension Planning Area (EPA) (13^{o} 37′ S, 33^{o} 56′ E). The details for rainfall distribution are provided in Sections 2.2.1 and 2.3.1 whereas details for soil characterization are provided in Sections 2.2.1 and 2.3.2.

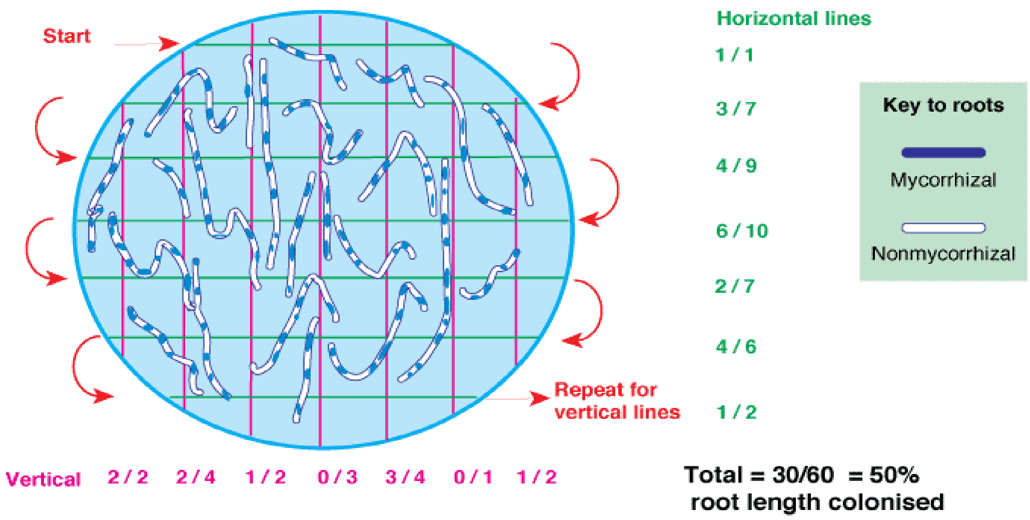
4.2.2 Treatment description

The assessment of VAM fungal colonisation was superimposed on a major experiment where many other variables were tested. The first year involved planting cowpea, pigeon pea and maize as sole crops, legume/legume and legume/cereal in-row intercrops. No fertilizers were applied to all the first season treatments except an additional sole cropped maize treatment that was purposively included as a control for the second season and this received 92 kg N ha^{-1}. Further details of first season treatment description are provided in Section 2.2.2.

The second year (cropping season two) involved testing the residual effects of the different cropping systems on short rotational maize yields by planting maize across all treatment plots. Therefore VAM fungal colonisation was also assessed on the short rotational maize. Each type of crop residue was incorporated in a plot where that specific crop was grown. During VAM colonisation assessment, plots of the previous season were purposively split into two sub-plots hence the split-plot design was achieved with previous cropping system as main factor and N fertilizer levels of 0 kg N ha^{-1} and 23 kg N ha^{-1} as sub plots.

4.2.3 Assessment of VAM fungal colonisation

The process of assessing VAM fungal colonisation in plant roots involved plant sampling to obtain the roots, clearing of roots of various pigments and staining them to make hyphae and VAM key features, *i.e*. arbuscules and vesicules, visible on a compound light microscope, and quantifying of VAM fungal colonisation of roots on a dissecting microscope. Ten plants were sampled per plot of each treatment, uprooted, the roots cut from the stem and gently cleaned and placed in clean plastic bottles, transported while kept in an ice box, and ethanol added to the bottles and kept in a refrigerator at 6 ^{o}C. The clearing and staining of roots were done using a procedure as described by Vierheiling *et al.* (1998) and Cao *et al.* (2013) in which 10% KOH is used for clearing root pigments at 90 ^{o}C for 90 min, blanching with alkaline 10% H_2O_2, acidifying with 0.2M HCl, staining with 5% blue ink in 5% acetic acid (vinegar) and lastly de-staining with vinegar. Verification of VAM presence was done on a compound microscope (10x40 magnification) by considering features as described by Brundrett (2013). Quantification of percent root length colonisation was done using the gridline intersect method as described by Giovanetti and Mosse (1980). Figure 4.1 shows how the counts of root colonisation were done as illustrated by Brundrett (2008).



Source: Brundrett, 2008

Figure 4.1: The illustration of how percent root length colonised is determined using the gridline intersect method.

NB: The diagram shows a grid lined petri dish containing root threads

4.2.4 Statistical analysis

Computation of percentages was done using Microsoft Excel computer package. The Genstat statistical package, 15th edition, was used for determinations of correlations and analysis of variance based on a statistical model as described in Section 2.2.6, and separation of means using the least significant difference (LSD) at 5% level of significance for year one data. Year two data contained multiple comparisons. Therefore, analysis was based on the statistical model described in equation (xix), and the Tukey's honest significant difference (HSD) test at 5% level of significance was used for the separation of means.

$$Y_{ijk} = \mu + \beta_i + \alpha_j + \rho + \tau_k + (\alpha\,\tau)_{jk} + \varepsilon_{ijk} \qquad \text{(xix)}$$

Where:

Y_{ijk} = response variable;

μ = the overall mean;

β_i = ith block effect;

α_j = jth main factor (previous cropping system with residues retained) effect;

ρ_{ij} = ith and jth whole plot random error;

τ_k = kth sub factor (different rates of N fertilizer) effect;

$(\alpha \tau)_{jk}$ = interaction of jth main factor effect and kth sub factor effect;

ε_{ijk} = the random error term for the sub plot.

4.3 Results

4.3.1 The VAM fungal colonisation in pigeon pea, cowpea and maize as influenced by different cropping systems at the Lilongwe and Dowa sites

Table 4.1 shows results of VAM fungal colonisation in pigeon pea, cowpea and maize as affected by different cropping systems. Results show that there were no significant differences ($p < 0.05$) due to cropping system in percent root colonisation by the VAM fungi in all the three crops. However, looking across all the crops, maize had the lowest values of percent root length colonised by VAM fungi in both sites. On the other hand, though not statistically analysed, all values of percent root colonisation by VAM fungi were relatively lower at the Lilongwe site than those of the Dowa site.

4.3.2 The VAM fungal colonisation of maize roots as influenced by the previous cropping systems and N application at the Lilongwe site

Results show that there were signification differences ($p < 0.05$) in percent of VAM fungal colonisation in rotational maize roots as affected by the previous seasons' cropping systems (Figure 4.2). All treatments that involved legumes (legume-based) in the previous season, that is pigeon pea and cowpea, grown as a sole crop or as legume-cereal or a legume-legume intercrop showed significantly higher ($p < 0.05$) percent colonisation of maize roots by VAM fungi ranging from 39% (in previous PP+MZ) to 50% (in previous sole PP) than VAM fungal colonisation in maize not supplied with N fertilizer that followed sole maize. Similarly, the previous season legume-based systems led to significantly higher VAM fungal colonisation, by the range of 19% (in previous PP+MZ) to 47% (in previous PP+CP) in maize supplied with 23 kg N ha^{-1} that was preceded by sole maize of the previous season.

Table 4.1: Effect of cropping system on VAM fungal colonisation on pigeon pea, cowpea and maize at the two study sites

Crop	Cropping system	Lilongwe % VAM col	Dowa % VAM col
PP	Sole PP	25.0	41.1
	PP+CP intercrop	26.9	36.9
	PP+MZ intercrop	26.1	37.4
	LSD	11.6	10.1
	F pr.	0.91	0.51
	CV %	19.8	11.6
CP	Sole CP	33.2	46.7
	CP+PP intercrop	28.2	40.1
	CP+MZ intercrop	25.5	41.2
	LSD	11.0	20.5
	F pr.	0.261	0.660
	CV %	16.8	21.2
MZ	Sole MZ	15.2	24.6
	MZ+PP intercrop	17.3	22.6
	MZ+CP intercrop	14.5	20.8
	Sole MZ+92N	19.4	23.4
	LSD	15.9	4.4
	F pr.	0.872	0.300
	CV %	47.8	9.6

NB: (i)Means were separated based on LSD at 5% significant level

(ii)CP = cowpea; MZ = maize; PP = pigeon pea; 92N = 92 kg N ha^{-1}

The supplemental 23 kg N ha^{-1} to the rotational maize seemed to have a slight positive influence on VAM fungal colonisation levels though no significant differences were obtained. It showed non-statistically different results with the pigeon pea plus maize intercrop effects of VAM fungal colonisation on rotational maize. On the other hand, the 92 kg N ha^{-1} applied to one control plot in the previous season did not show significant effect on VAM fungal colonisation of rotational maize. No significant interaction was observed between previous cropping systems and the 23 kg N ha^{-1} applied to the rotational maize.

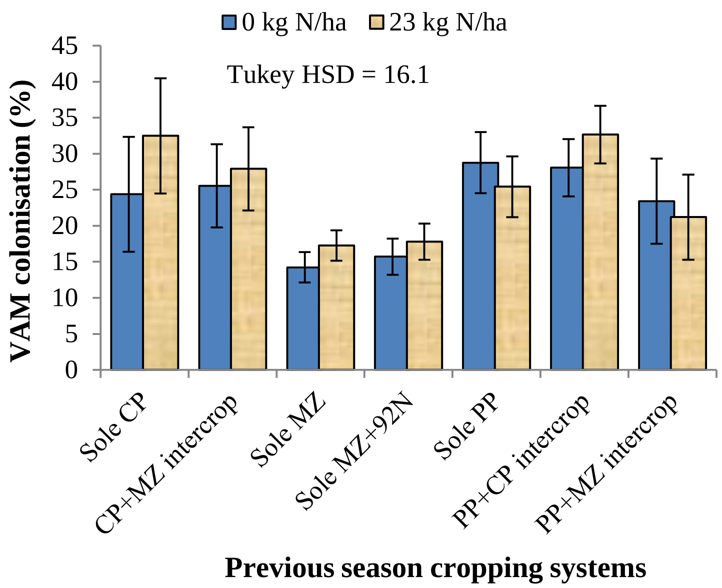


Figure 4.2: VAM colonisation as influenced by previous legume-based cropping systems and N fertilizer application for Lilongwe site

NB: (i)Means were separated using Tukey's HSD at 5% significant level

(ii)CP = cowpea; MZ = maize; PP = pigeon pea

Error bars stand for standard errors of the mean

4.3.3 The VAM fungal colonisation of maize roots as influenced by the previous cropping systems and N application at the Dowa site

Figure 4.3 shows the results for the Dowa site VAM fungal percent colonisation. Similar to the Lilongwe site, results show that there were signification differences ($p < 0.05$) in percent root colonisation as affected by the previous season cropping systems.

All treatments that involved legume-based cropping systems in the previous season showed significantly higher ($p < 0.05$) percent colonisation of maize roots by VAM fungi, ranging from 15% (in previous PP+CP to 36% (in previous PP+MZ) than VAM fungal colonisation in the maize at zero N fertilizer application that followed previous season sole maize. Similarly, where maize was supplied with 23 kg N per ha^{-1}, the previous season legume-based systems led to significantly higher VAM fungal colonisation by the range 28% (in previous sole CP) to 40% (in previous sole PP) in maize roots but the application of N fertilizer did not significantly affect VAM fungal colonisation levels though they showed some slight increases. In both cases there were no significant interaction between the previous cropping system and 23 kg N ha^{-1} fertilizer application.

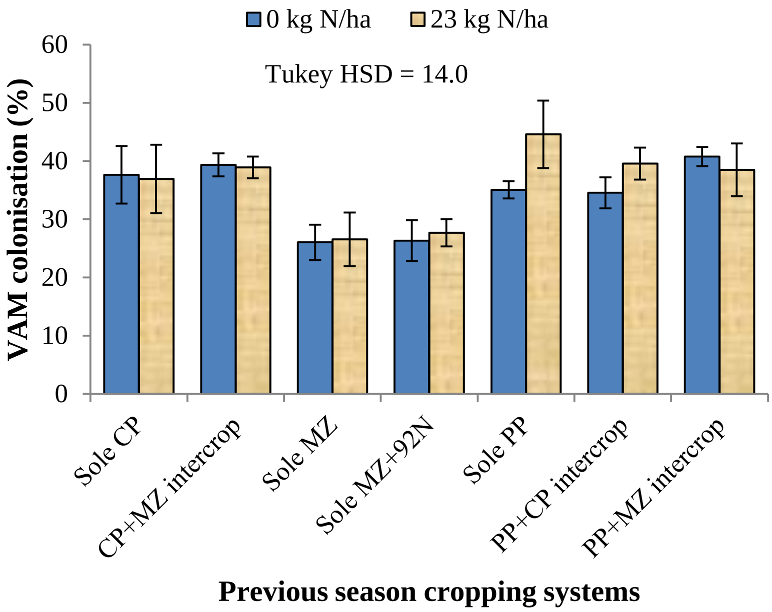


Figure 4.3: VAM colonisation of maize roots as influenced by previous legume-based cropping systems and N fertilizer application for Dowa site

NB: (i)Means were separated using Tukey's HSD at 5% significant level

(ii)CP = cowpea; MZ = maize; PP = pigeon pea

Error bars stand for standard errors of the mean

4.3.4 Pearson correlations between colonisation percentage and plant tissue P concentration, P uptake and total dry matter yields

The Pearson correlation coefficients were determined to assess the association between the VAM fungal colonisation percentage of roots of crops in year one, and the plant tissue P concentration, P uptake, total dry matter yields, BNF/plant, nodule number and nodule dry weights (Data on the above-mentioned parameters are available in Chapter two, sections 2.3.4 – 2.3.5. Results (Table 4.2) show positive correlation coefficients for all parameters but mostly with non-significant P-values with the exception of P content in maize which showed a significant correlation ($r = 0.659$, $p < 0.02$) and a few others with p-values approaching significant levels (from slightly above 0.05 to 0.1).

Table 4.2: Pearson correlations between colonisation percentages and plant tissue P concentration, P uptake, total dry matter yields, BNF/plant, nodule number and nodule dry weights

Crop	Colonisation (%) and parameters	Lilongwe r	Lilongwe P-value	Dowa r	Dowa P-value
PP	% P	0.395	0.293	0.185	0.634
	P uptake	0.349	0.365	0.488	0.182
	TDM	0.170	0.661	0.045	0.909
	BNF	0.191	0.623	0.188	0.627
	Nod. number	0.067	0.861	0.577	0.154
	Nod. dry wt	0.368	0.330	0.470	0.202
CP	% P	0.252	0.513	0.379	0.290
	P-uptake	0.645	0.061	0.557	0.120
	TDM	0.067	0.864	0.660	0.053
	BNF	0.630	0.069	0.479	0.193
	Nod. number	0.155	0.689	0.657	0.055
	Nod. dry wt	0.438	0.238	0.650	0.058
MZ	% P	0.659	0.020*	0.558	0.283
	P-uptake	0.455	0.138	0.210	0.352
	TDM	0.378	0.226	0.101	0.521

NB: (i) Colonisation (%) is the independent variable

(ii)* = Significant at P = 0.05; corr. r = correlation coefficient

(iii) Number of observations (n) under PP and CP statistical analysis = 9; degree of freedom (d.f.) = 8; n for MZ statistical analysis = 12 and d.f. = 11

(iv) Detailed tables on correlations involving the various parameters are in Appendix 2

More detailed information of the correlation matrices is available in Appendices 2A-2F. No correlations were determined for the year two, rotational maize study because of the possibility of many confounding factors since the rotational maize were subjected to a number of factors such as previous season effects, addition of inorganic fertilizers and later in their growth cycle different rates of N fertilizers, whose effects are not wholly covered in this paper.

4.4 Discussion

Elucidation of levels of VAM fungal colonisation in predominant cropping systems in Malawi such as sole cropping, cereal-legume and legume-legume intercrops, can be of importance in the development of sustainable agricultural systems. Results in this study showed that the degree of colonisation of each crop that is pigeon pea, cowpea and maize by VAM, was not affected by the cropping system. However, maize, compared with the other crops, showed relatively low VAM colonisation levels. These observations show both similarities and differences to some studies on VAM colonisation in intercropping systems (Harinikumar *et al.*, 1990). The non-significant differences due to cropping systems can be attributed to the type of crop species involved in the present intercropping study and probably the selectivity of un-explored mycorrhizal fungal species in the soils at the study sites. In a study involving sole maize and maize intercropped with agro-forestry species of *Sesbania* and *Gliricidia* in Southern Malawi on the frequency of occurrence of VAM fungal species, Jefwa (2004) reported that out of the 12 VAM fungal species identified, five species occurred mostly in the maize monocrop whereas the remaining seven were not affected by the cropping systems.

On the other hand, Hage-Ahmed *et al.* (2013) reported three scenarios which showed significant increases and decreases and non-significant differences from an intercropping study involving tomato, where VAM fungal colonisation of tomato was increased in an intercrop with leek, while no significant differences were observed when intercropped with cucumber and basil, but decreased when intercropped with fennel. These observations were attributed to differences in the establishment of symbioses as affected by different root sizes that in turn affect their influence in the soil ecosystem, but also to the effect of VAM species on plant competitions. In the current study, all the three crops are mycorrhizal as reported in many studies (Arihara and Karasawa, 2000; Ahiabor and Hirata, 2003; Dania *et al.*, 2013), therefore, are very unlikely to cause suppressive effects on the intercropping partners. However, since no additional evaluation of VAM fungal species was undertaken in this study, therefore, comprehensive evaluation of the intensities of VAM fungal colonisation was not possible.

However, in the second season there were significantly higher colonisation levels of maize roots by the VAM fungi as influenced by the previous season legume-based cropping systems of sole pigeon pea, sole cowpea and their legume-legume and maize-legume intercrops than in the maize following maize rotational system. This observation is consistent with a number of similar studies. In a study conducted in Zimbabwe, Lekberg *et al.* (2008) reported slightly higher VAM fungal colonisation in maize grown after lablab and pigeon pea than VAM fungal colonisation in maize grown after maize rotational system. On the other hand, Bagayoko *et al.* (2000), in a study done in Niger, reported 45% native VAM fungal colonisation of pearl millet roots when pearl millet followed cowpea in rotation whereas 27% VAM fungal colonisation was observed on pearl millet roots when it followed another pearl millet in rotation. Arihara and Karasawa (2000) reported increased mycorrhizal colonisation and yield components in rotational maize after mycorrhizal crops, which were soybean, sunflower, maize and potato as compared to maize after non-mycorrhizal crops, rape and sugar beet. On the other hand, Lekberg *et al.* (2008) and Dania *et al.* (2013) also reported higher positive correlations of maize yield components with VAM fungal colonisation when maize was grown after lablab or pigeon pea. Furthermore, Njeru *et al.* (2014) reported increased indigenous VAM fungal colonisation of rotational maize roots after cover crop legumes such as hairy vetch (*Vicia villosa*), common pea (*Pisum sativa*) and broad bean (*Vicia faba*) which was attributed to the ability of some crop species to sustain VAM fungal natural communities more than others. Furthermore, Borie *et al.* (2002) in a study comparing residues of a legume (lupine) and non-legume (wheat) effects on VAM fungal proliferation reported higher mycorrhizal fungi colonisation in treatments applied with lupine which was attributed to the higher nutritional content in the legume residues which boosted mycorrhizal development. Therefore, the VAM fungal sustainability and residue quality effects of the legumes can be main factors that increased VAM fungal colonisation in the rotational maize of the present study.

The Pearson correlation coefficients were computed to assess the associations between the VAM fungal colonisation of pigeon pea, cowpea and maize roots, and various parameters including plant P content and BNF, for the first season only (see Section 4.3.4). Results showed positive associations between VAM fungal colonisation of roots of plants under study and their plant tissue P, total P uptake, total dry matter yield, BNF, nodule numbers and nodule dry weight but mostly showed non-significant P-values, except for phosphorus content in maize which had a significant correlation. The weak association and non-significant correlation coefficients predominant in this study could be a contrast to the advantages of VAM fungal colonisation as reported in many studies (Bolan, 1991, Marschner and Dell, 1994; Dania *et al*., 2013). However, VAM studies lead to a number of uncommon observations in terms of associations with plant P contents and even effects due to available soil P which was relatively high in this study (Grant *et al*., 2005; Thorne *et al*., 2013). Furthermore, both levels of soil P and levels of VAM colonisation could be the reasons for observations in this study. Though, some authors have considered 40% as high colonisation, but based on meta-analysis data of 91 laboratory and field based studies Treseder (2012) reports that response ratio of plant biomass and plant P concentration increase as percent root length colonised (PRLC) increases and the benefit of PRLC become distinctly prominent if PRLC reaches 60% or more. Therefore, the VAM root length colonisation levels that never reached 60% and rarely exceeded 40% in this study could be the main factor contributing to the weak positive associations observed between VAM fungal colonisation and parameters such as plant P content, BNF and dry matter yields.

4.5 Conclusions

From this study, it can be concluded that VAM fungal colonisation was not affected by the legume-based cropping systems such as sole cropping, cereal-legume and legume-legume intercrops involving pigeon pea, cowpea and maize. On the other hand, all the legume-based cropping systems showed significant positive effect on VAM fungal colonisation of the subsequent maize grown in short rotation. Furthermore, there were positive correlations between plant roots' VAM fungal colonisation and the plant P content, nodule numbers, BNF and total dry matter yields. Therefore, integrating diversified legume-based cropping systems can be a good approach in promoting VAM fungal proliferation that contributes to increasing plant P uptake, which also has positive effects on BNF and crop yields. Furthermore, the increased P acquisition and BNF are among the key components of soil health improvement for sustainable agriculture production, in most soils of sub-Saharan Africa. Additionally, more research needs to be done to address the interactions between cropping systems and existing VAM fungal species, their abundance and diversity on Malawi soils. Isolation of more adapted species for inoculant production can be another good step forward in alleviating soil health problems.

References

Abiahor, B. D. and Hirata, H. (2003). Associative influence of soluble phosphates, rock phosphates and arbuscular mycorrhizal fungus on plant growth and phosphorus uptake of three tropical legumes. *West African Journal of Applied Ecology*. 4: 75-90

Ahiabor, B. D., Fosu, M., Tibo, I. and Sumaila, I. (2007). Comparative nitrogen fixation, native arbuscular mycorrhiza formation and biomass production potentials of some grain legume species grown in the field in the Guinea Savanna Zone of Ghana. *West African Journal of Applied Ecology* 11 (1): 72 - 80.

Alliance for Green Revolution in Africa (2014). Africa Agriculture Status Report: Cclimate change and smallholder agriculture in sub-Saharan Africa. Nairobi, Kenya. 216 pp.

Arihara, J. and Karasawa, T. (2000). Effect of previous crops on arbuscular mycorrhizal formation and growth of succeeding maize. *Soil Science and Plant Nutrition*, 46(1): 43-51.

Bagayoko, M., Buerkert, A., Lung, G., Bationo, A. and Romheld, V. (2000). Cereal/legume rotation effects on cereal growth in Sudano-Sahelian West Africa: Soil mineral nitrogen, mycorrhiza and nematodes. *Plant and Soil* 218: 103-116.

Benhiba, L., Fouad, M. O., Essahibi, A., Ghoulam, C. and Qaddoury, A. (2015). Arbuscular mycorrhizal symbiosis enhanced growth and antioxidant metabolism in date palm subjected to long-term drought. *Trees 29*(6), 1725-1733.

Bolan, N. S. (1991). A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. *Plant and Soil* 134(2):189-207. DOI: 10.1007/BF00012037.

Borie, F., Redel, Y., Rubio, R., Rouanet, J. L., Barea, J. M. (2002). Interactions between crop residue application and mycorrhizal development and some soil-root interface properties and mineral acquisition by plants in an acidic soil. *Biology and Fertility of Soils* 36: 151-160.

Brady, N. C. and Weil, R. R. (2008). *The Nature and Properties of Soils*. Revised 14th ed. Pearson Prentice Hall. New Jersey, USA. 975 pp.

Brundrett, M. C. (2004). Diversity and classification of mycorrhizal associations. *Biological Reviews* 79: 473-495.

Brundrett, M. C. (2013). Methods for quantifying AMF in roots. [*www. researchgate.net/file.PostFileLoader.html?id...assetKey*] site visited on 09/11/2014.

Brundrett, M. C. 2008. Mycorrhizal Associations: The Web Resource. [http://mycorrhizas.info] site visited on 06/02/2013.

Cao, M., Wu, Q. and Zou, Y. (2013). An improved ink-acetic acid technique for staining arbuscular mycorrhizas of citrus. *International Journal of Agriculture and Biology* 15: 386 – 388.

Chilimba, A.D.C., Young, S. D., Black, C. R., Rogerson, K. B., Ander, E.L., Watts, M. J., Lammel, J and Broadly, M. R. (2011). Maize grain and soil surveys reveal suboptimal dietary selenium intake is widespread in Malawi. *Scientific Reports* 1:72 [*www.ncbi.nlm.nih.gov › NCBI › Literature › PubMed Central (PMC)*] site visited on 01/09/2012.

Dania, S. O., Fagbola, O. and Dania, M. I. (2013). Response of maize-pigeon pea intercrop to arbuscular mycorrhizal fungi in nutrient depleted soil. *Annual Review and Research in Biology* 3(3): 232-245.

Declerck, S., Risede, J. M. Rufyikiri, G. and Delvaux, B. (2002). Effects of arbuscular mycorrhizal fungi on severity of root rot of bananas caused by *Cylindrocladium spathiphylli*. *Plant Pathology* 51: 109–115.

Fageria, N. K. (2012). *The role of plant roots in crop production*. CRC Press. Boca Raton. USA 468pp.

Gamage, H. K., Singhakumara, B. M. P. and Ashton, M. S (2004). Effects of Light and Fertilization on Arbuscular Mycorrhizal Colonization and Growth of Tropical Rain-Forest Syzygium Tree Seedlings. *Journal of Tropical Ecology* 20(5): 525-534.

Gastol, M. and Domagala-Swiatkiewicz, I. (2015). Mycorrhizal inoculation of apple in replant soils – enhanced tree growth and mineral nutrient status. *Acta Scientiarum Polonorum Hortorum Cultus* 14(4): 17 – 37.

Giller K. E., Anderson, J. A., Corbeels, M., Kirkgaard, J., Mortensen, D., Erenstein, O., Vanlauwe, B. (2015). Beyond conservation agriculture. *Frontiers in Plant Science* 6 (870): 1-14.

Giovannetti, M. and Mosse, B. (1980). An evaluation of techniques for vesicular arbscular mycorrhizal infection in roots. *New Phytologist* 84: 489-500.

Gomez, K. A. and Gomez, A. A. 1984. *Statistical procedures for agricultural research*. 2nd ed. New York: John Wiley and Sons. 680pp.

Grant, C., Bittman, S., Montreal, M., Plenchette, C. and Morel, C. (2005). Soil and fertilizer phosphorus: effects on plant P supply and mycorrhizal development. *Canadian Journal of Plant Science* 85(1): 3-10.

Hage-Ahmed, K., Krammer, J. and Steinkellner, S. (2013). The intercropping partner affects arbuscular mycorrhizal fungi and Fusarium oxysporum f. sp. lycopersici interactions in tomato. *Mycorrhiza* 23: 543-550.

Harinikumar, K. M., Bagyaraj, D. J. and Mallesha, B. C. (1990). Effect of intercropping and organic soil amendments on native VA mycorrhizal fungi in an oxisol. *Arid Land Research and Management* 4(3): 193-197.

Heissenhuber, A., Bloss, E. and Kramer, C. (2015). Sustainable intensification of food production. *Trakia Journal of Sciences* 3: 214 – 221.

Jefwa, J. M. (2004). The taxonomy and ecology of arbuscular mycorrhizal fungi (AMF) in agroforestry systems in Malawi. [*repository.up.ac.za/bits tream/hand le/2263/27135/00front.pdf?...1*] site visited on 06/11/2015.

Kanno, T., Saito, M., Macedo, M. C. M., Nakamura, T. And Miranda, C. H. B. (2006). Importance of indigenous arbuscular mycorrhiza for growth and phosphorus uptake in tropical forage grasses growing on an acid, infertile soil from the Brazilian savannas. *Tropical Grasslands* 40: 94-101.

Lampkin, N. H., Pearce, B. D., Leake, A. R., Creissen, H., Gerrard, C. L., Girling, R., Lloyd, S., Padel, S., Smith, J., Smith, L. G., Vieweger, A., and Wolfe, M. S. (2015). The *role of agroecology in sustainable intensification*. *Report for the Land Use Policy Group*. Organic Research Centre, Elm Farm and Game & Wildlife Conservation Trust. 151pp.

Lekberg, V., Koide, R. T. And Twomlow, S. J. (2008). Effects of agricultural management practices on arbuscular mycorrhizal fungal abundance in low input cropping systems of Southern Africa: A case study from Zimbabwe. *Biology and Fertility of Soils* 44: 917-923.

Liu, A., Hamel, C., Hamitton, R. I., Ma, B. L., Smith, D. L. (2000). Acquistion of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levls. *Mycorrhiza* 9:331-336. DOI: 10.1007/s00720050277.

Lupwayi, N. Z., Kennedy, A. C. and Chirwa, R. M. (2011). Grain legume impacts on soil biological processes in Sub-Saharan Africa. *African Journal of Plant Science* 5(1): 1-7.

Makoi HJR and Ndakidemi PA. 2009. The agronomic potential of vesicular-arbuscular mycorrhiza (VAM) in cereals-legume mixtures in Africa. *African Journal of Microbiological Research* 3(11): 667-675.

Marschner, H. and Dell, B. (1994). Nutrient uptake in mycorrhizal symbiosis. *Plant and Soil* 159(1):89-102.

Ministry of Agriculture and Food Security (2012). *Guide to Agricultural Production and Natural Resource Management in Malawi*. Agricultural Communications Branch. Lilongwe. Malawi, 366 pp.

Momayezi, M., Pereira, R. C. and Self, R. (2015). Biochar and mycorrhizal fungi influence on nutrient uptake by two pasture species in New Zealand. *International Journal of Biosciences* 6(6): 52-60.

Mutegi, J., Kabambe, V., Zingore, S., Harawa, R. and Wairegi, L. (2015) The *Fertilizer Reccomendation Issues in Malawi: Gaps, Challenges, Opportunities and Guidelines.* Soil Health Consortium of Malawi. 54 pp.

Njeru, E. M., Avio, L., SBrana, C., Turrini, A., Bocci, G., Barberi, P. and Giovannetti, M. (2014). First evidence for major cover crop effects on arbuscular mycorrhizal fungi and organic maize growth. *Agronomy for Sustainable Development* 34: 841-848.

Ortiz, N., Armada, E., Duque, E., Roldán, A. and Azcón, R. (2015). Contribution of arbuscular mycorrhizal fungi and/or bacteria to enhancing plant drought tolerance under natural soil conditions: Effectiveness of autochthonous or allochthonous strains. *Journal of Plant Physiology* 174: 87 – 96.

Othira, J. O., Omolo, J. O., Wachira, F. N. and Onek, L. A. (2012). Effectiveness of arbuscular mycorrhizal fungi in protection of maize (*Zea mays* L.) against witchweed (*Striga hermonthica* Del Benth) infestation. *African Journal of Biotechnology and Sustainable Development* 4(3): 37-44.

Sale, V., Aguilera, P., Laczko, E., Mader, P., Berner, A.,Zihlmann, U., Van der Heiden, M. G. A. and Oehl, F. (2015). Impact of conservation tillage and organic farming on the diversity of arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry* 84: 38-52

Singh, P. K. (2012). Role of glomalin related soil protein produced by arbuscular mycorrhizal fungi: A review. *Agricultural Science Research Journal* 2(3): 119-125.

Sylvia, D. M., Furhmann, J. J., Hartel, P. G. and Zuberer D. A. (2005). *Principles and application of soil microbiology.* 2^{nd} (ed.). Pearson Prentice Hall. New Jersey. 640 pp.

Thorne, M., Rhodes, L. and Cardina, J. (2013). Effectivity of arbuscular mycorrhizal fungi collected from reclaimed mine soil and tall grass praire. *Open Journal of Ecology* 3(2): 223-233.

Treseder, K. K. (2012). Percent mycorrhizal root length is a reliable indicator of benefits to host plants: Results from a meta-analysis [http://www. searchgate.net/publication/267291286_Percent_mycorrhizal_root_length_is_a_reliable_indicator_of_benefits_to_host_plants_Results_from_a_meta-analysis] site visited on 09/11/2015.

Van der Heijden, M. G. A., Martin, F. M., Selosse, M. and Sanders, I. R. (2015). Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytologist* 205: 1406-1423.

Vanlauwe, B., Coyne, D., Gockowski, J., Hauser, S., Huising, J., Masso, C., Nziguheba, G., Schut, M., Van Asten, P. (2014). Sustainable intensification and African smallholder farmer. *Current Opinion in Environmental Sustainability* 8: 15 - 22.

Vierheilig, H., Schweiger, P., and Brundrett, M. (2005). An overview of methods for the detection and observation of arbuscular mycorrhizal fungi in roots *Physiologia Plantarum* 125: 393–404.

Wang, B. and Qui, Y. L. (2006). Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* 16(5) 299-363.

CHAPTER FIVE

5.0 EFFECTS OF PIGEON PEA AND COWPEA RESIDUES APPLICATION AND MINERAL N USE ON N DYNAMICS AND YIELDS OF MAIZE ON THE CHROMIC LUVISOLS OF CENTRAL MALAWI

Abstract

An integrated nutrient management study was conducted on the Chromic Luvisols of Lilongwe and Dowa districts of Central Malawi. The objective of the study was to evaluate the pigeon pea (PP) and cowpea (CP) legume-legume and legume-cereal intercropping system residual effects when integrated with inorganic N fertilizer on N mineralization patterns, rotational maize (MZ) N uptake, grain and other yield components including grain protein content (quality), and nitrogen use efficiency (NUE). The study was arranged in a split plot design with the previous legume-based systems (residues retained) as main plots and the 0, 45, 90 and 120 kg N ha^{-1} inorganic N rates contributing the subplots. Field sampled soils during the growing season consistently showed significantly higher ($P < 0.05$) NO_3-N in the previous Sole CP, Sole PP, PP+CP, CP+MZ and PP+MZ intercrops by a mean of 29% in the planting week to 17% in week 11, and 19% in planting week to 14% in week 11 after planting than in the previous Sole MZ, in the Lilongwe and Dowa sites, respectively. Nitrogen uptake by the maize was significantly higher ($P < 0.05$) ranging from 38% in the previous PP+MZ intercrop to 68% in the previous Sole CP at 0 kg N ha^{-1} and 28% in the previous PP+MZ intercrop to 48% in the previous Sole CP at 120 kg N ha^{-1} than in the previous Sole MZ at the same rates of fertilizer application, in the Lilongwe site. Similarly, grain yields were significantly higher ($P < 0.05$) by a range of 30 to 59% at 0 kg N ha^{-1}, and 28 to 42% at

120 kg N ha^{-1} in the previous CP+MZ intercrop and Sole CP, respectively, in the Lilongwe site. Similar trends were observed for the Dowa site. Maize grain protein significantly ($P < 0.05$) increased with increase in inorganic N application which was attributed to timing of the top dressing N fertilizer. The previous legume based systems significantly increased NUE over the previous Sole MZ. From this study it was noted that N uptake, yields and quality and NUE of maize can substantially be increased with an integration of pigeon pea and cowpea residues in their legume-legume, legume-cereal or sole crops with an accompanying reduction in costs of inorganic fertilizers.

Keywords: Pigeon pea, cowpea, maize, N mineralization, N uptake, nitrogen use efficiency

5.1 Introduction

Declining soil fertility continues to be one of the main challenges for sub-Saharan Africa crop production systems (Zingore *et al*., 2015). Continuous soil erosion and nutrient depletion are some of the most pressing challenges in managing soils in most parts of the region (Tamene and Le, 2015). Earlier studies listed Burundi, Malawi, Kenya, Ethiopia, Lesotho and Rwanda as countries with very high nutrient depletion rates (Stoorvogel and Smaling, 1990). The rising costs of inorganic fertilizers and growing human populations are exacerbating the challenges in managing soil fertility and increasing crop production (AGRA, 2014).

For the case of Malawi, where agricultural production is dominated by smallholder maize production, affordability of inorganic fertilizers is one of the crucial challenges (African Development Bank, 2011), with nitrogen being the most limiting element in maize production (Makumba, 2003; Tamene *et al*., 2015). Over the years, the government has shown a political will to improve the situation by implementing farm input subsidy policy which reasonably improved yields in some years but with unproven sustainability (Dorward and Chirwa, 2011; Ricker-Gilbert *et al*., 2014). Similar to many other countries, research and implementation of various soil management interventions, including use of various types of manures, agroforestry technologies and inclusions of legumes in the cropping systems, are promoted. Inclusion of food legumes seems to be the most popular due to a number of factors including food diversity and security, increased income and the soil fertility improvements (Kerr *et al.*, 2007; Kamanga *et al*., 2010; Kamanga *et al*., 2014).

Legumes such as common beans (*Phaseolus vulgaris*), groundnuts (*Arachis hypogaea*), pigeon pea (*Cajanus cajan*), soybean (*Glycine max*) and cowpea (*Vigna unguiculata*) are grown by many farmers in Malawi, both as food and cash crops (Kamanga *et al*., 2010). They are grown in various cropping systems including sole or monocropping, cereal-legume and legume-legume intercrops, also commonly referred to as “doubled up” legume technology (ICRISAT/MAI, 2000).

Soil fertility benefits from legume interventions evaluated through their effects on maize in short rotations have been reported from studies done in Malawi and elsewhere (Sakala *et al.,* 2003; Mhango, 2011). However, very few studies have reported soil fertility benefits from legumes that are grown as legume-legume intercrops. Soil fertility benefits on maize yields from doubled-ups of pigeon pea-groundnuts or pigeon pea-soybean have been reported to be comparable to that of sole crops (Mhango, 2011; Njira *et al*., 2013; Phiri *et al*., 2014). However, information on effects of the combinations of various new varieties of these crops, specifically from a pigeon pea-cowpea doubled up on maize yields and yield components such as quality is scanty. Furthermore, information on the mineralization patterns of various combinations of either legume-legume or a legume-cereal residue is scanty. Therefore the objective of this study was to evaluate and compare the effects of the pigeon pea-cowpea doubled up in comparison with their sole and legume-cereal intercrops on N mineralization patterns and their implications on N uptake, nitrogen use efficiency, yields and grain protein content of maize in short rotation when integrated with inorganic fertilizers.

5.2 Materials and Methods

5.2.1 Site description and characterization

The study was done as a continuation of the 2013/14 legume-based study. Details of the site description are as in Section 2.2.1. However, soil analysis was also carried out for the 2014/15 growing season. Soil samples were collected from each of the plots in the previous legume based treatments in the depth ranges of 0 - 15 cm and 15 - 30 cm. Soils were analysed for total N, available P, soil organic carbon (SOC) and pH using the micro-Kjedahl, Mehlich 3 extraction procedures, wet oxidation and potentiometric method, respectively, as outlined by Anderson and Ingram (1989). Rainfall was recorded from each of the sites during the cropping season.

5.2.2 Treatments and residue quality assessment

The experiments were superimposed on the 2013/14 legume-based treatments. The legume based treatments included sole pigeon pea, sole cowpea, pigeon pea-cowpea intercrop, pigeon pea-maize intercrop, cowpea-maize intercrop, sole maize (unfertilized), and sole maize fertilized at 92 kg N ha^{-1} (Sole MZ+92N), the recommended N rate (MoAFS, 2012) in 15 m x 7 m plots. The crop residues were incorporated in the soil soon after harvesting.

The legume-based cropping systems' treatments were arranged in randomized complete blocks design (RCBD). In the 2014/15 study year, each plot was split into four sub-plots that received different levels of inorganic N as follows: 0, 45, 90 or 120 kg N ha^{-1} (in the 7m x 3.75m subplots). Therefore, the split plot arrangement in RCBD was obtained. The sources of N applied were the basal fertilizer, 23:21:0+4S and the top dressing N fertilizer, urea. One maize seed was planted per each planting station at a distance of 25 cm between planting stations along the ridge/row and ridges were spaced at 75cm. The maize variety used was the DKC 8053. All conventional crop management practices were followed. Nitrogen concentration and C/N ratios were determined in the crop residues before incorporation, to assess their quality according to Palm *et al.* (2001), Havlin *et al*. (2005) and Brady and Weil (2008).

5.2.3 Soil mineral N assessment and plant analysis

Soil samples were collected from the 0 – 20 cm depth by augering a minimum of three randomly selected points from each sub plot. Composite samples were made from soils of each subplot. Then, the composite samples were kept in ice boxes and taken to the laboratory and kept in a refrigerator to avoid further mineralization. The soil mineral N, both nitrate (NO_3^-) and ammonium (NH_4^+), were then extracted using 2M KCl, and then analyzed colorimetrically according to Anderson and Ingram (1989) procedure. These were done as a series of activities from a week of planting (wk 0) to 11 th week after planting (wk 11).

Maize plant samples were collected at tasseling, five plants from each subplot for determination of N concentration based on the procedure as described by Anderson and Ingram (1989). Furthermore, grain samples for N determination were also collected at harvesting.

5.2.4 Harvesting and determination of yields, harvest indices and grain protein content

Harvesting was done after the maize had reached physiological maturity and dried. A net plot of 5.5 m x 1.5 m was demarcated from each subplot leaving guard rows to avoid the outside interference. The maize stalks were cut at ground level with cobs not removed. These were weighed as the total above ground biomass. Then the cobs were detached and the grains were threshed. The net plot grain fresh weight was recorded and a sample of 100 seeds sampled using a quartering procedure (Kanyama-Phiri, personal communication, 2010), and taken to the laboratory. The detailed procedures for determination of grain and total dry matter yields, and harvest indices are presented in Section 3.2.3. Maize grain crude protein content was determined by multiplying grain N concentration by a Jones factor of 6.25 according to FAO (2003).

5.2.5 Determination of N uptake and nitrogen use efficiency

Nitrogen uptake by the maize plant was determined by multiplying the total above ground dry matter (TDM) with plant N concentration. On the other hand, nitrogen use efficiency was determined for applied nitrogen using the partial factor productivity of applied N (PFP_N) which takes into account the use efficiency of both indigenous and applied N resources as illustrated in the following equation (xx) after Dobermann (2005):

$$PFP_N = \frac{Y_N}{F_N} \quad \text{...(xx)}$$

Where

Y_N = kg of yield produced

F_N = kg N applied

5.2.6 Data analysis

Data was subjected to analysis of variance (ANOVA) using Genstat 15th edition statistical package based on a statistical model presented in equation (xix) (Section 4.2.4). Separation of means was achieved with the Tukey's honest significant difference (HSD) test at 5% significant level where the cropping systems and fertilizer N rate factors were analysed. Additionally, where one way ANOVA was used to analyse a specific factor, means were separated by the least significant differences (LSD) at 5% significant level.

5.3 Results

5.3.1 Monthly rainfall at the Lilongwe and Dowa sites in the 2014/15 cropping season

Monthly rainfall distribution for the Lilongwe and Dowa sites in the 2014/15 cropping season is as presented in Fig. 5.1. Lilongwe site received rainfall from November to May with the highest amount of 243 mm in the month of January and an annual total amount of 639 mm. On the other hand the Dowa site received rainfall from the month of December to April with the highest amount of 189 mm in the month of February and an annual total of 577 mm.

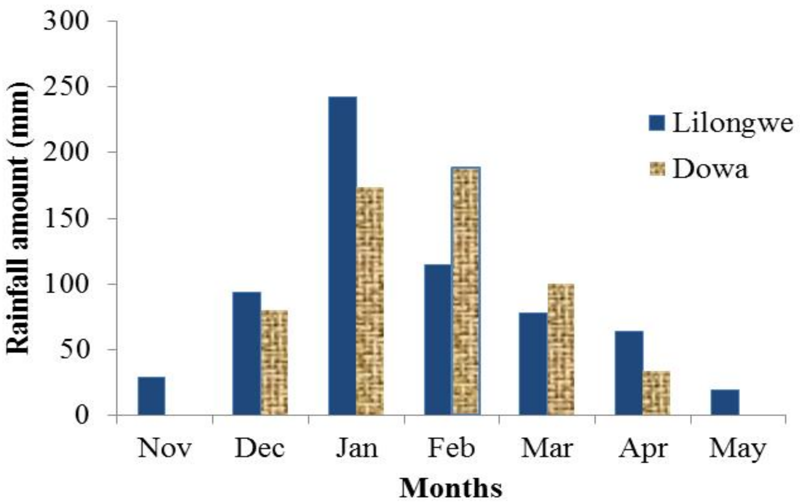


Figure 5.1: Monthly rainfall amounts (mm) during the 2014/15 growing season for Lilongwe and Dowa sites

5.3.2 Selected post-harvest soil properties from the previous legume based cropping systems for the Lilongwe and Dowa sites

Postharvest soil analysis for the Lilongwe site, showed medium to slightly acid soil reaction with pH ranging from 5.7 in the previous sole maize plus 92 kg N ha^{-1} plot (Sole MZ+92N) to 6.1 in a number of previous legume-based treatments in the subsoil (Table 5.1). The overall means pH for both topsoil and subsoil was 6.0. Furthermore, the overall mean total soil N was 0.06 and 0.056 for the topsoil and subsoil, respectively. Soil organic carbon (C) was also low with overall means of 1.2 and 1.1 for the topsoil and subsoil, respectively. On the other hand, the available (Mehlich-3) P was high with overall means of 42.4 and 39.2 mg kg^{-1} for the topsoil and subsoil, respectively (Table 5.1).

For the Dowa site, Table 5.1 shows a slightly acid soil reaction with overall mean pH of 6.2 for both topsoil and subsoil. Total soil N was low to medium, with overall mean values of 0.14 and 0.10 in the topsoil and subsoil, respectively. Soil organic C was medium with overall mean values of 2.75 and 1.95 in the topsoil and subsoil, respectively. On the other hand, available (Mehlich-3) P was low to medium with overall mean values of 29.7 and 26.9mg kg^{-1} for the topsoil and subsoil, respectively.

5.3.3 Stover quality from previous cropping systems for the Lilongwe and Dowa sites

The stover quality was assessed by determining the N concentration and C/N ratios (Table 5.2). Critical values of determining quality were based the work of Palm *et al*. (2001), Havlin *et al.* (2005) and Brady and Weil (2008) on the implication of C/N ratios. Results showed different characteristics of the stover. For the Lilongwe site, Sole cowpea (CP) stover was of the highest quality, with N concentration of 2.7% and C/N ratio of 16.4 while maize stover from the maize-cowpea intercrop showed the lowest quality with N concentration of 0.7% and C/N ratio of 59.4. For the Dowa site, high quality characteristics were depicted by Sole CP and Sole PP, both with N concentration of 2.8 with C/N ratios of 15.9 and 16.4, respectively. Maize stover in the intercrops with cowpea and pigeon pea showed the lowest quality characteristics, with N concentrations of 0.8 and 0.7% and C/N ratios of 63.5 and 58.1, respectively.

Table 5. 1: Selected post-harvest soil properties from the previous legume based cropping systems at the Lilongwe and Dowa sites

Site	Previous cropping system	pH		Total N (%)		Available Mehlich-3 P (mg/kg)		SOC	
		0 - 15 cm	15 - 30 cm	0 - 15 cm	15 - 30 cm	0 - 15 cm	15 - 30 cm	0 - 15 cm	15 - 30 cm
Lilongwe	Sole MZ	6.1	6.1	0.057	0.054	40.9	40.2	1.14	1.07
	Sole MZ+92N	5.7	5.9	0.063	0.059	44.3	38.4	1.25	1.20
	Sole CP	6.1	6.0	0.062	0.058	41.8	39.3	1.24	1.16
	CP+MZ intercrop	6.0	6.1	0.065	0.054	45.5	38.6	1.31	1.08
	Sole PP	6.1	6.1	0.059	0.054	45.0	37.4	1.17	1.09
	PP+CP intercrop	5.9	6.0	0.057	0.055	41.2	39.2	1.14	1.12
	PP+MZ intercrop	6.1	6.0	0.058	0.059	37.9	41.2	1.17	1.19
	Grand mean	6.0	6.0	0.060	0.056	42.4	39.2	1.20	1.13
Dowa	Sole MZ	6.2	6.1	0.12	0.09	28.3	27.5	2.45	1.80
	Sole MZ+92N	6.1	6.3	0.14	0.10	27.5	27.3	2.86	2.00
	Sole CP	6.3	6.3	0.15	0.10	32.1	27.5	2.96	2.09
	CP+MZ intercrop	6.2	6.4	0.14	0.09	34.2	29.3	2.88	1.90
	Sole PP	6.3	6.2	0.13	0.10	32.1	24.7	2.65	2.04
	PP+CP intercrop	6.1	6.0	0.14	0.10	28.3	25.4	2.85	1.95
	PP+MZ intercrop	6.2	6.1	0.13	0.09	25.4	26.8	2.63	1.87
	Grand mean	6.2	6.2	0.14	0.10	29.7	26.9	2.75	1.95

NB: SOC = soil organic carbon; MZ = maize; CP = cowpea; PP = pigeon pea; 92N = 92 kg N ha^{-1}.

Ratings and critical values/ranges for various soil parameters are available in Table 2.1 (Section 2.3.2) and Appendix 1, respectively.

Table 5. 2: Quality of crop residues of the previous cropping systems for the Lilongwe and Dowa sites

Site	Crop in the previous cropping system	Stover N concentration (%)	C/N ratio
Lilongwe	Sole MZ	0.8	55.7
	Sole MZ+92N	1.0	46.4
	Sole CP	2.7	16.4
	Sole PP	2.3	19.5
	CP in CP+MZ intercrop	2.1	21.7
	CP in PP+CP intercrop	2.3	20.2
	PP in PP+CP intercrop	2.1	21.7
	PP in PP+MZ intercrop	2.2	20.8
	MZ in PP+MZ intercrop	0.8	61.3
	MZ in CP+MZ intercrop	0.7	59.4
Dowa	Sole MZ	0.8	55.9
	Sole MZ+92N	1.0	44.8
	Sole CP	2.8	15.9
	Sole PP	2.8	16.4
	CP in CP+MZ intercrop	2.5	18.4
	CP in PP+CP intercrop	2.7	16.7
	PP in PP+CP intercrop	2.5	18.1
	PP in PP+MZ intercrop	2.5	18.3
	MZ in PP+MZ intercrop	0.7	58.1
	MZ in CP+MZ intercrop	0.8	63.5

NB: N (%) < 2.5 implies low quality; C/N > 25 implies low quality

5.3.3 Field soil mineral N patterns in the 2014/15 cropping season as influenced by the previous season cropping systems

5.3.3.1 Field soil mineral N patterns at the Lilongwe site

Fig.5.2 shows mineral N (NO_3^-, NH_4^+) levels sampled from planting week to week 11 after planting. Results are presented only for the legume-based subplots that did not receive N fertilizer application to elucidate the clear effect of the previous cropping systems. The general trend showed decrease in mineral N levels from planting week to the later weeks.

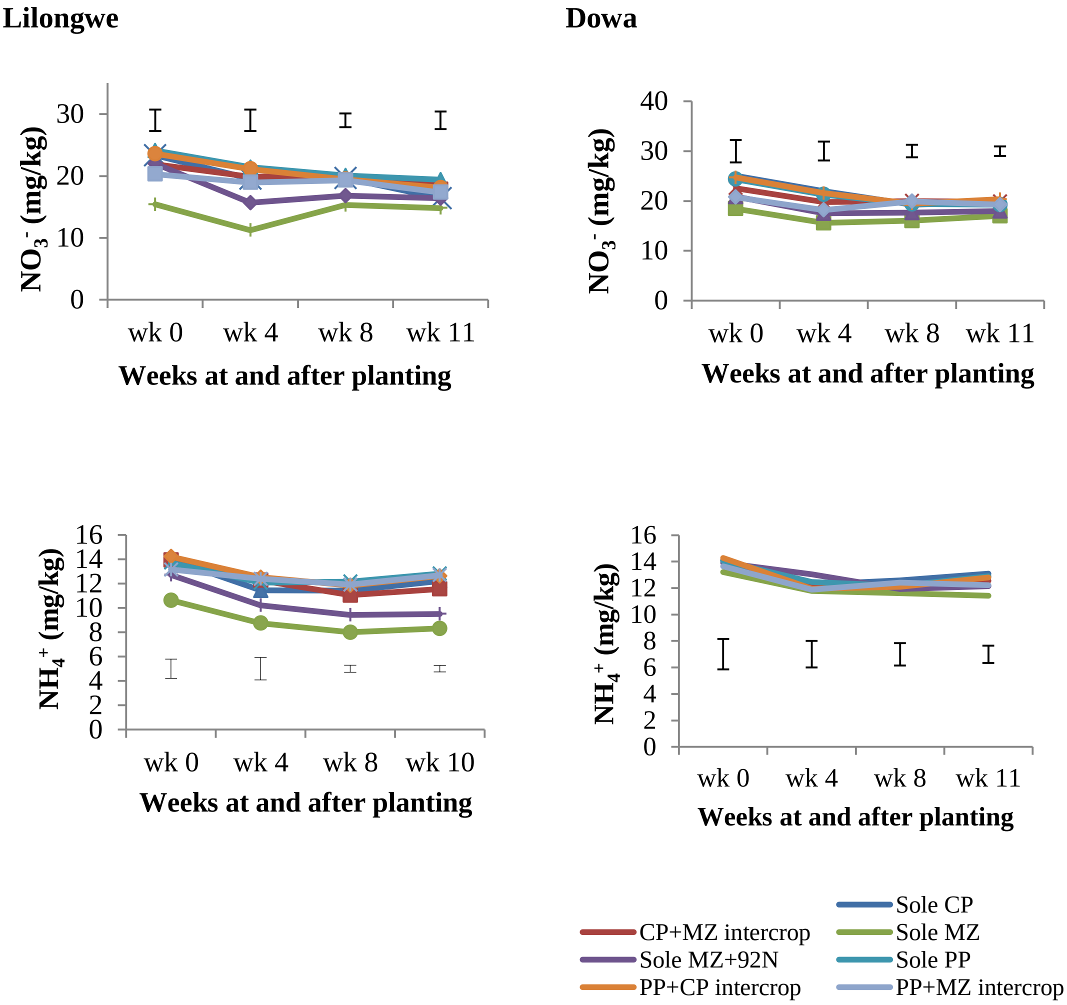


Figure 5.2: Soil mineral N in the maize plots in the 2014/15 cropping season as influenced by the previous season cropping systems at the Lilongwe and Dowa sites

NB: (i) Above analysis show mineral N patterns in the sub plots that did not receive fertilizer N application in the 2014/15 cropping system.

(ii) Vertical bars within the charts represent LSD values for separation of means for each specific week.

There were significant differences ($P < 0.05$) due to the previous cropping systems. Nitrate levels were relatively higher than the amounts of NH_4-N levels. Nitrate N levels for previous legume-based systems were significantly higher ($P < 0.05$) than that in the previous Sole MZ by a mean of 29% in wk 0 to 17% in wk 11 for the Lilongwe site. Furthermore, the plot that was previously Sole MZ+92N showed equally higher NO_3-N levels during the planting week but drastically dropped in the subsequent weeks. There were no significant differences in NO_3-N among plots previously planted to legumes as sole, legume-legume or legume-cereal intercrop. The trend for NH_4-N was somewhat similar to that of NO_3-N.

5.3.3.2 Field soil mineral N patterns at the Dowa site

Similar to the Lilongwe site, in the Dowa site, the trend of mineral N in the soils of the study plots showed a decrease from the planting to week 11 after planting. There were significant differences as influenced by the previous cropping systems. The previous legume based plots showed higher ($P < 0.05$) NO_3-N than that in the previous Sole MZ plots by 19% in wk 0 to and 14% in wk 11. The trend was similar for NH_4-N. However, no significant differences were observed in NH_4-N as influenced by the previous cropping systems.

5.3.4 Nitrogen uptake by subsequent maize plants as influenced by the previous season cropping systems and fertilizer N rates at the Lilongwe and Dowa sites

5.3.4.1 Nitrogen uptake by maize plants at the Lilongwe site

Fig. 5.3 shows N uptake by the maize plants. There were significant differences ($P < 0.05$) as influenced by both cropping system and N fertilizer application. Plots that were previously legume-based showed significantly higher ($P < 0.05$) N uptake than The Sole MZ and Sole MZ+92N. Increasing N application rate led to significant increases in N uptake by the maize plants. However, from plots that were previously legume-based, N uptake amongst the rates of 45, 90 and 120 kg N ha^{-1} were not significantly different but all these were significantly higher ($P < 0.05$) than the treatment with 0 kg N ha^{-1}.

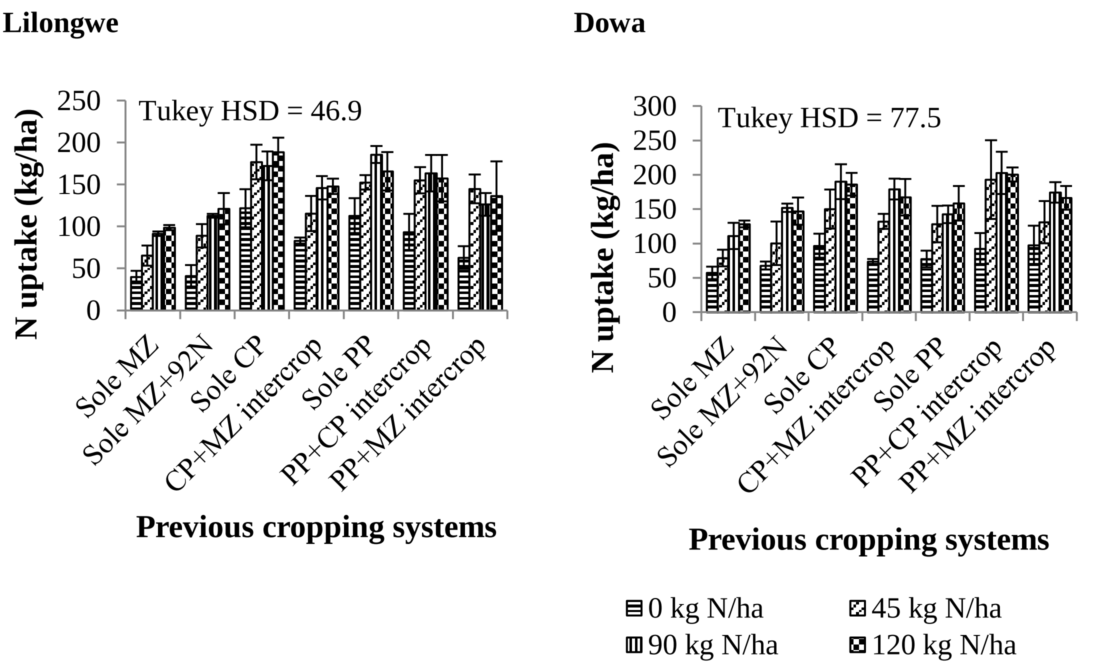


Figure 5.3: N uptake in maize crop as influenced by the previous cropping systems and N fertilizer rate at the Lilongwe and Dowa sites

NB: Means were separated using Tukey HSD test at 5% significant level and its value is shown on the chart; Error bars represent standard errors of the means

The legume-based systems led to higher N uptake by a range of 38% in the PP+MZ intercrop to 68% in the Sole CP at 0 kg N ha^{-1} as compared to that in the Sole MZ. On the other hand, at the highest rate of 120 kg N ha^{-1} the legume based systems led to increases in N uptake ranging from 28% in PP+MZ intercrop to 48% in the Sole CP over the Sole MZ. Plots that previously contained Sole CP and Sole PP show slightly higher N uptakes than the rest of the treatment plots.

5.3.4.2 Nitrogen uptake by maize plants at the Dowa site

Similar to the Lilongwe site, in the Dowa site, plant N uptake by subsequent maize was significantly influenced by both the previous cropping systems and N fertilizer application. Increasing fertilizer N application led to significant increases in N uptake (Fig. 5.3). Nitrogen uptake at 45, 90 and 120 kg N ha^{-1} were all significantly higher ($P < 0.05$) than that at 0 kg N ha^{-1} but were not significantly higher among themselves. The legume based systems led to higher N uptake by a range of 23% in the CP+MZ intercrop to 41% in the PP+MZ at 0 kg N ha^{-1} than that in the Sole MZ. On the other hand, at the highest rate of N (120 kg N ha^{-1}) the legume based systems led to increases in N uptake by a range of 23% in PP+MZ intercrop to 36% in the PP+CP intercrop over the Sole MZ.

5.3.5 Grain and total dry matter yields and harvest indices of subsequent maize as influenced by the previous season cropping systems and fertilizer N rates at the Lilongwe and Dowa sites

5.3.5.1 Maize grain and total dry matter yields and harvest indices at the Lilongwe site

Maize grain and total dry matter or above ground biomass were significantly ($P < 0.05$) influenced by both cropping system and N application (Fig. 5.4). Increasing rate of N application led to increase in yields. Treatments that were previously legume based showed significantly higher ($P < 0.05$) maize grain yields at all the four rates of N fertilizers than that by the treatments under the previous Sole MZ. Amongst the previous legume-based treatments, the 0 kg N ha^{-1} showed significantly lower yields than those by the rest of the higher N rates of 45, 90 and 120 kg N ha^{-1}, which, among themselves, were not significantly different. The previous legume based systems produced significantly higher ($P < 0.05$) grain yields than the previous Sole MZ by a range of 30% under previous CP+MZ intercrop to 59% under previous Sole CP at 0 kg N ha^{-1} fertilizer application. Similarly, at the highest rate of N application, 120 kg N ha^{-1}, the previous legume-based treatments were significantly higher (P <0.05) than the previous Sole MZ by a range of 28% under previous CP+MZ intercrop to 42% under previous Sole CP. The TDM yields followed a similar trend. Furthermore, HI% by the treatments on the previous Sole MZ was significantly lower than the rest of the cropping systems (Fig. 5.5). Additionally, significant differences (P < 0.05) in HI% were also observed as influenced by the rate of N applied for the Lilongwe site.

5.3.5.2 Grain and total dry matter yields and harvest indices of the subsequent maize at the Dowa site

Maize grain yields for the Dowa site were significantly different (P < 0.05) as influenced by both cropping system and fertilizer N rate applied (Fig. 5.4). Treatments that were previously legume-based showed significantly higher (P < 0.01) grain yields than those that were previously Sole MZ at all rates of N fertilizer applied. At the 0 kg N ha^{-1}, they were significantly higher (P < 0.05) by a range of 24% under treatment that was previously CP+MZ intercrop to 48% under the treatment that was previously Sole PP whereas at the 120 kg N ha^{-1} they were significantly higher (P < 0.05) by a range of 35% under previous PP+MZ intercrop to 45% under treatment that was previously PP+CP. On the other hand, the previously Sole MZ+92N treatment yields were not significantly different from the legume based plots' yields at 120 kg N ha^{-1}. Furthermore, the treat previously Sole MZ+92N yield at 45 kg N ha^{-1} was not significantly different from the previous Sole MZ grain yield at the same rate of fertilizer. The harvest indices were also significantly affected by both cropping system and the amount of N applied (Fig. 5.5). Treatments that previously contained legumes produced significantly higher (P < 0.05) HI% than the treatments that were previously Sole MZ. However, the HI% by the previously PP+MZ, CP+MZ and MZ+92N were not significantly different from that under treatment that was previously Sole MZ. Increasing amount of N fertilizer led to significant increases in HI%.

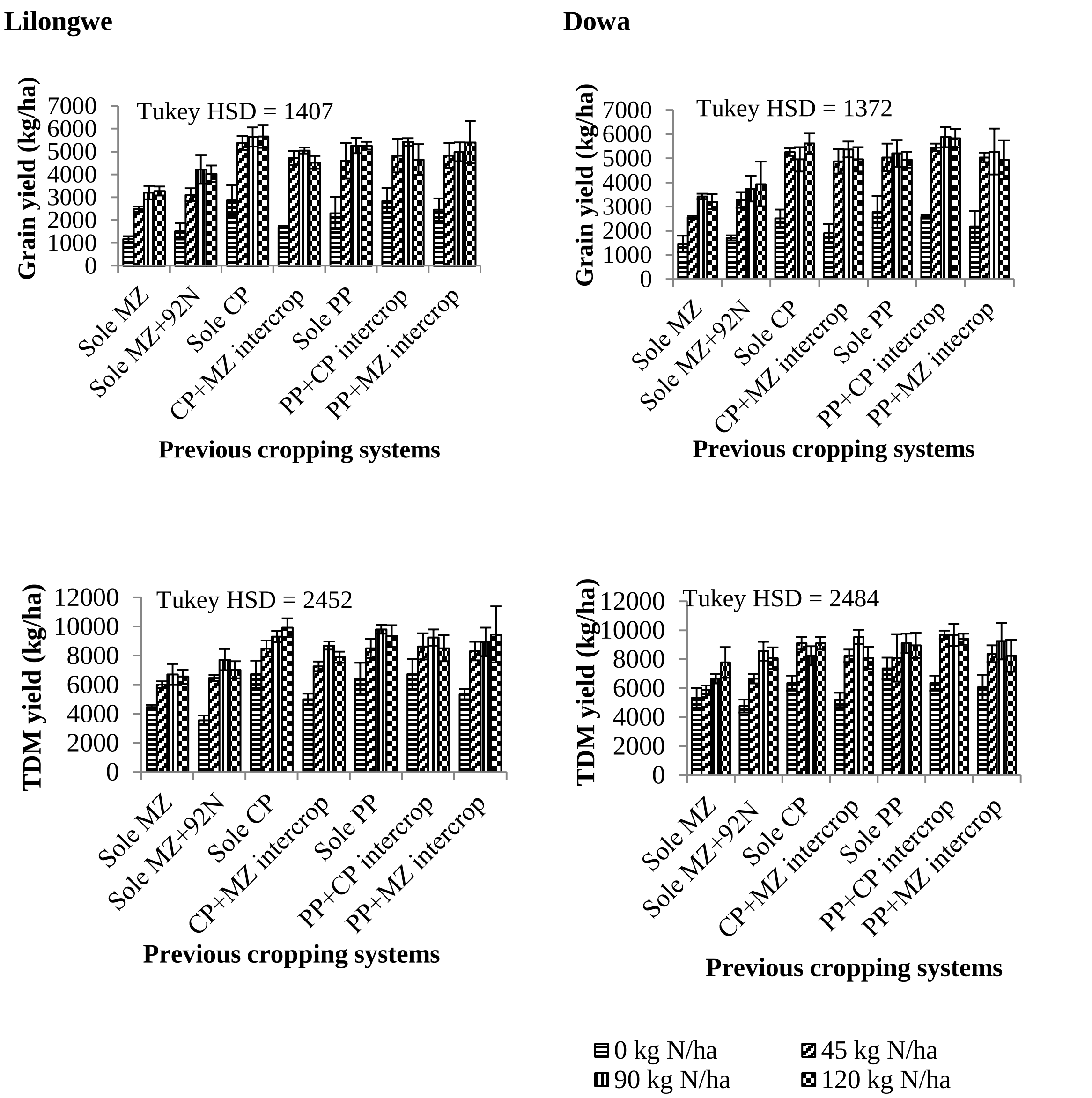


Figure 5.4: Grain and total dry matter (TDM) yields of maize as influenced by the previous cropping systems and N fertilizer rate at the Lilongwe and Dowa study sites

NB: Means were separated using Tukey HSD test at 5% significant level and its value is shown on the bar charts; Error bars represent standard errors of the means

5.3.6 Maize grain protein content as influenced by the previous season cropping systems and fertilizer N rates for the Lilongwe and Dowa sites

5.3.6.1 Maize grain protein content at the Lilongwe site

Maize grain protein content (Fig. 5.5) was significantly different ($P < 0.01$) as influenced by fertilizer N rate applied. Increasing amount of N applied led to increase in maize grain protein content. The trend of protein percentages increased from 0 kg N ha^{-1} to 120 kg N ha^{-1}, with average range values of 9.0 to 11.4% protein content, respectively.

5.3.6.2 Maize grain protein content at the Dowa site

For the Dowa site, the trend in maize grain protein percentage was similar to that of the Lilongwe site. Increasing amount of N led to significant increase in grain protein content (Fig. 5.5) with the lowest and highest amounts at 0 kg N ha^{-1} and 120 kg N ha^{-1}, and average range values of 10.2 to 13.1% protein content, respectively.

5.3.7 Nitrogen use efficiency as influenced by the previous cropping system and fertilizer rate on maize in Lilongwe, Central Malawi

5.3.7.1 Nitrogen use efficiency at the Lilongwe site

The nitrogen use efficiency as demonstrated by the PFP_N showed significant differences ($P < 0.05$) as influenced by cropping systems and amount of fertilizer N applied (Table 5.3). Treatments that were previously legume-based systems were significantly higher in terms of their N-use efficiency in both applied and indigenous N (PFP_N) than those that were previously Sole MZ and Sole MZ+92N. The overall mean values for treatments that were previously Sole MZ and Sole MZ+92N were not significantly different. The highest efficiency for PFP_N was at 45 kg N ha^{-1} in all the treatments and the treatment that was previously Sole CP showed a slightly higher value than the rest.

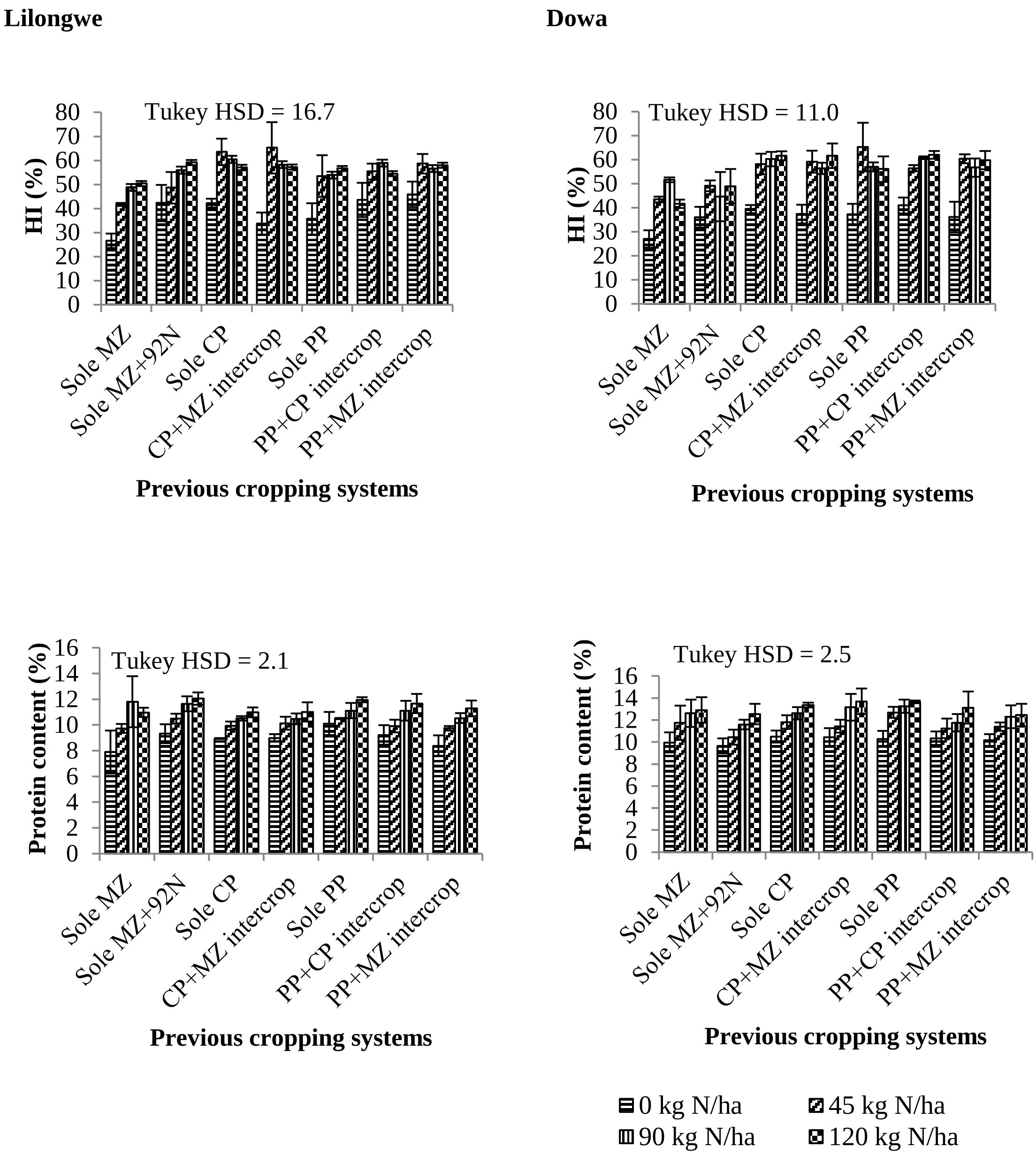


Figure 5. 5: Harvest index percentages (HI%) and grain protein content (%) of maize as influenced by the previous cropping systems and N fertilizer rate at the Lilongwe and Dowa study sites

NB: Means were separated using Tukey HSD test at 5% significant level and its value is shown on the bar charts; Error bars represent standard errors of the means

Table 5.3: Nitrogen use efficiency as influenced by the previous cropping system and fertilizer rate on maize at the Lilongwe and Dowa sites, Central Malawi

Site	Fertilizer rate	Previous cropping systems							
		Sole MZ	Sole MZ+92N	Sole CP	CP+MZ intercrop	Sole PP	PP+CP intercrop	PP+MZ intercrop	Mean (fert. rate)
Lilongwe	0	-	-	-	-	-	-	-	-
	45	55.2	69.1	119.5	104.9	102.3	107.3	107.3	95.1a
	90	35.6	46.9	62.7	56.1	58.4	60.3	55.4	53.6b
	120	27.3	33.6	47.2	37.7	43.8	38.7	45.1	39.1c
	Mean (crop. syst)	39.4b	49.9b	76.5a	66.2a	68.1a	68.8a	69.3a	
	F. pr (CS)	0.001							
	F. pr (FR)	0.001							
	F.pr (CS*FR)	0.048							
	CV (%)	18.4							
Dowa	0	-	-	-	-	-	-	-	-
	45	56.7	72.8	116.9	108.4	112.1	121.3	112.0	100.0a
	90	38.1	41.7	55.1	59.7	57.9	65.3	58.7	53.8b
	120	26.7	32.8	46.8	41.3	41.2	48.6	41.2	39.8c
	Mean (crop. syst)	40.5b	49.1b	72.9a	69.8a	70.4a	78.4a	70.6a	
	F. pr (CS)	0.001							
	F. pr (FR)	0.001							
	F.pr (CS*FR)	0.01							
	CV (%)	14.3							

NB: (i) NUE was not calculated at zero rate of N fertilizer; Means were separated using the Tukey's HSD test at 5% significant level; Means with different letters are significantly different; CS = cropping system; FR = fertilizer rate; symbol * = interaction

(ii) Values in the Table are in kg grain/kg N applied

(iii) NUE > 70 implies very good performance

5.3.7.2 Nitrogen use efficiency at the Dowa site

For the Dowa site, the PFP_N was significantly ($P < 0.05$) influenced by the cropping systems and fertilizer N amount applied (Table 5.3). Similar to the Lilongwe situation, the highest NUE was at 45 kg N ha^{-1} fertilizer application rate. All the former legume-based treatments showed mean PFP_N values that were higher than the common ranges of 40-70 and were all significantly higher ($P < 0.05$) than that under the Sole MZ. The treatment that was previously PP+CP intercrop showed a slightly higher value that the rest.

5.4 Discussion

Integrated nutrient management performance depends on many factors including improved seed, sound crop management, type and amount of inorganic nutrients, cropping systems implemented and the amount and quality of organic resources retained or added to the systems. In this study an integrated nutrient management approach was implemented in which crop residues were retained to the system and different levels of inorganic fertilizers were added. Levels of N concentrations and C/N ratios of the different crop residues used under the present study were similar to those of other past studies (Palm *et al*., 2001; Mtambanenge *et al*., 2007; Abera *et al*., 2013). For both sites sole cropped legume residues showed the highest quality which can be attributed to the nutrient accumulations achieved during the growth of those crops. On the other hand, legume crop residues from intercropped species (Table 5.2) still satisfied the high quality (i.e. high N concentration and low C/N ratios) criteria according to Palm *et al.* (2001) and Brady and Weil (2008).

For the Lilongwe and Dowa sites, soil mineral N (NO_3^{-1} and NH_4^+) patterns showed ranges (Figure 5.2) similar to what have been reported by a number of authors in similar environments (Mhango, 2011; Matusso *et al*., 2014). The significantly higher levels of mineral N in the plots that had legume residues (CP and PP) whether sole cropped or intercropped can be attributed to high quality of the legume residues. The maize residues showed very low N concentrations and very high C/N ratios whereas the legumes, both

CP and PP, showed high quality (Table 5.2). Zeng *et al.* (2010) and Abasi *et al.* (2015) in similar studies with various crop residues including that of maize, groundnuts and soybean reported a positive correlation between N mineralization and initial N contents of the residues. This study confirms that addition of high quality residues to the poor quality ones improves the rate of nutrient mineralization. This can be attributed to the activation of microbial activities in the soil that need reasonable amounts of nitrogen to facilitate the mineralization process and reduce the period of N immobilization.

Similarly, Partey *et al*. (2014) reported increase in mineralization rate of 58% and 55% by mixing maize residues with respectively *Vicia faba* and *Tithonia diversifolia*. Furthermore, significant increases in microbial biomass were also reported with *V. faba* and *T. diversifolia* alone or in combination with maize residues (Partey *et al*., 2014). On the other hand, Sakala *et al.* (2000) reported increased N mineralization with additional amounts of high quality PP residues and N fertilizer to maize residues. It should be noted that the previously Sole MZ+92N plots showed relatively higher amounts of mineral N than that of previously Sole MZ plots. This can be attributed to the slightly higher quality of the residues but also residual N from the previously applied urea, although the highest levels were only more conspicuous at the start of the rains and thereafter it dropped. This implies that the already readily available N from the previous season was leached quickly by the rains. Beauchamp (1987) reported residual benefits of urea on maize in a season immediately after the season in which the urea was applied.

The maize grain yields determined in this study were in similar ranges as maize yields reported by Zingore (2011) for medium to high fertile soils of Malawi and by Tamene *et al.* (2015) for farmers practising good management practices such as integration of NPK fertilizers and animal manure, which are relatively higher than the Malawi farmers' average. The significantly higher maize plant N uptake, grain and TDM yields for plots that were previously Sole CP, Sole PP, and PP+CP intercrop than those that were previously Sole MZ and Sole MZ+92N plots at all fertilizer levels, can be attributed to the combined effect of the high quality of the residues and inorganic N fertilizer, which increased N recovery and yields more than the low quality MZ residues combined with inorganic N fertilizer. Mtambanengwe *et al.* (2007), in a similar study involving *Crotalaria jancea* green manure, *Calliandra calothyrsus* and *Pinus patula* residues as organic amendments in Zimbabwe, observed improvements in maize yields by between 24% and 104% from combined mineral fertilizer and leguminous resources over those from sole fertilizer. On the other hand, Mahama *et al.* (2016), in a similar study, reported greater benefits in maize N uptake due to cowpea and pigeon pea previous cropping systems in the USA than by that in the soybean system. Similar to the Lilongwe trends were also those for the Dowa site with slight differences. For instance, amounts of N uptake in Dowa were above 50 kg N ha^{-1} in all the zero fertilizer plots and in all the previous legume based cropping systems plots compared to Lilongwe levels that were lower in the treatment that was previously Sole MZ. This can be attributed to the initial soil total N and soil organic matter levels which were higher in Dowa than in Lilongwe.

The significantly higher harvest indices due to the previous legume-based cropping systems can be attributed to increased synchrony in the release of N from the high quality residues and the N requirements of the maize crop. This was complemented by the effect of applied N. However, apart from other treatments being significantly higher than the zero N rate, no differences were observed amongst the higher fertilizer rates. Maobe *et al.* (2010) noted the increase in HI with N application from both *Mucucan pruriens* residues and inorganic fertilizer but at only medium rates of N from 30 to 60 kg N ha^{-1} and no further increase in HI at the fertilizer rates above 100 kg N ha^{-1}.

The crude protein percentages of maize grain in the present study are similar to those reported in other studies (Silva *et al*., 2005; Genetic Technologies Limited, 2016). Results in the present study show that increasing the rate of N application led to increase in crude protein content of the maize grain. Blumenthal *et al.* (2008) in a review of importance and effect of N on crop quality, summarises a number of studies where increasing N rate lead to increase in maize grain protein. However, in the present study, grain protein in maize differed significantly due to applied inorganic N fertilizer, but significant differences were not observed due to previous cropping systems at both study sites. This can be attributed to the timing of N application in that the protein content might have been influenced by top dressing N as fertilizer, which was applied closer to the tasseling stage and is more likely to have been channelled to kernel development and filling, as opposed to the N from the cropping system residuals which acted more as a basal fertilizer for vegetative development. In a similar study, increase in crude protein content of maize was reported to be influenced by late N application towards silking (Silva *et al*., 2005).

The previous legume-based systems resulted in significantly higher NUE in terms of PFP_N than by those that were previously Sole MZ or Sole MZ+92N in both study sites, and this can be attributed to interactive effects of the high quality residues and the applied inorganic N. For maize residues with low quality, NUE can be reduced by the longer periods of N immobilization and, in turn, not meeting the synchrony with plant growth stages when the N is greatly needed. This implies that in the maize monoculture systems much of the plant growth would be dependent on the applied N, which is more susceptible to losses through leaching. According to Dobermann (2005), common ranges of NUE are 40 - 70 kg grain kg^{-1} N applied. The PP+CP intercrop and Sole CP NUE values were the highest but the rest of the legume-based systems treatments in this study resulted in far much higher than the stated ranges. According to Dobermann (2005), NUE values above the stated ranges are obtained when there is good crop performance at lower rates of N and in well managed systems. Therefore, the pigeon pea and cowpea-based systems planted either in sole, legume-legume intercrops or legume-cereal intercrops, can all be considered good systems for increased NUE and increased yields for maize in rotation. It should be noted that results of NUE reported in this study differ greatly from the lower values of NUE reported by Snapp *et al.* (2014) based on a survey on farmers' fields in Malawi, where a range of 7 to 14 kg grain kg^{-1} N applied was noted. The low values were attributed to differences in farmers' crop management skills and various challenges including poor soil fertility conditions, labour constraints, droughts and pests and diseases, all of which affect maize growth and yields.

5.5 Conclusions

Integrated soil fertility management (ISFM) aims at achieving sustainable yields while minimizing costs on the part of a farmer. From this study it can be noted that integration of pigeon pea and cowpea either as sole crops, legume-legume or legume-cereal intercrop led to higher N uptake, grain and TDM yields and harvest indices in the rotational maize as compared to the maize-maize monoculture with or without inorganic fertilizer. Interactive effect of the legume residues and inorganic fertilizer led to higher maize grain yields by ranging from 30% under treatment that was previously CP+MZ intercrop (1689 kg ha^{-1}) to 59% under treatment that was previously Sole CP (2864 kg ha^{-1}) at 0 kg N ha^{-1}

fertilizer application than the under treatment that was previously Sole MZ (1178 kg ha^{-1}). Similarly, at the highest rate of N application, 120 kg N ha^{-1}, the previous legume-based treatments produced higher maize grain yields than the treatment that was previously Sole MZ (3277 kg ha^{-1}) by a range of 28% under treatment that was previously CP+MZ intercrop (4525 kg ha^{-1}) to 42% under treatment that was previously Sole CP (5665 kg N ha^{-1}), in the Lilongwe site. For the Dowa site, at the 0 kg N ha^{-1} maize grain yields increase over the treatment that was previously Sole MZ (1454 kg ha^{-1}) ranged from 24% under the previously CP+MZ intercrop (1908 kg ha^{-1}) to 48% under treatment that was previously Sole PP (2792 kg ha^{-1}) whereas at the 120 kg N ha^{-1} increase over the treatment that was previously Sole MZ (3204 kg N ha^{-1}) ranged from 35% under treatment that was previously PP+MZ (4945 kg ha^{-1}) intercrop to 45% under treatment that was previously PP+CP intercrop (5836 kg ha^{-1}). From this study it is noted that mixing high quality pigeon pea or cowpea residues (with high N concentration and narrow C/N ratios) with the low quality maize residues increased N mineralization rates, N uptake, and nitrogen use efficiency by the maize grown after the legumes. Additionally, increasing inorganic N application increased maize grain crude protein content. Therefore, for the smallholder farmers on the Chromic Luvisols of Lilongwe and Dowa districts, Malawi, a rotation of pigeon pea or cowpea in legume-legume, legume-cereal and sole cropping systems can substantially increase maize yields with the implication of lowering the investment costs of inorganic fertilizers. Furthermore, an integrated approach can improve both quantitative yields and quality of the maize grain.

References

Abbasi, M. K., Tahir, M. M., Sabir, N. and Khurshid, M. (2015) Impact of the addition of different plant residues on nitrogen mineralization–immobilization turnover and carbon content of a soil incubated under laboratory conditions. *Solid Earth* 6: 197 – 205.

Abera, G., Wolde-meskel, E. and Bakken, L. R. (2013). Effect of organic residue amendments and soil moisture on N mineralization, maize (*Zea mays*L.) dry biomass and nutrient concentration. *Archives of Agronomy and Soil Science* 59(9): 1263 – 1277.

African Development Bank (2011). Interim country strategy paper for Malawi. ADB. 20pp.

Alliance for Green Revolution in Africa (2014). Africa *Agriculture Status Report: Climate Change and Smallholder Agriculture in Sub-Saharan Africa*. Alliance for Green Revolution in Africa, Nairobi, Kenya. 216pp.

Anderson J. M., Ingram, J. S. I. (1989). *Tropical Soil Biology and Fertility: A Handbook of Methods of Analysis.* Commonwealth for Agriculture Bureau International, Wallingford. 117pp.

Beauchamp, E. G. (1987). Corn response to residual N from urea and manures applied in the previous years. *Canadian Journal of Soil Science* 67: 931 – 942.

Blumenthal, J., Battenspenrger, D., Cassman, K. G., Mason, K. G. and Pavlista**,** A. (2008). Importance of nitrogen on crop quality and health. *In: Nitrogen in the Environment: Sources, Problems and Management.* (Edited by Hatfield, J. L. and Folett, R. F.). Elsevier, Amsterdam. pp. 51 – 70.

Brady, N. C. and Weil, R. R. (2008). *The Nature and Properties of Soils*. Revised 14th ed. Pearson Prentice Hall. New Jersey. 975pp.

Chilimba, A.D.C., Young, S. D., Black, C. R., Rogerson, K. B., Ander, E.L., Watts, M. J., Lammel, J and Broadly, M. R. (2011). Maize grain and soil surveys reveal suboptimal dietary selenium intake is widespread in Malawi. *Scientific Reports* 1:72.

Dobermann, A. R. (2005). Nitrogen use efficiency-the state of the art. Agronomy – Fuculty Publications Paper 316 [http://digitalcommons.unl.edu/ agronomy fac pub/316] site visited on 10/11/2015.

Dorward, A. and Chirwa, E. (2011). The Malawi agriculture input subsidy programme: 2005/06 to 2008/09. *International Journal of Agriculture Sustainability* 9(1): 232 – 247.

Food and Agricultural Organization of the United Nations (2003). *Food Energy – Methods of Analysis and Conversion Factors.* Food and Nutrition Paper No. 77. Food and Agricultural Organization of the United Nations, Rome. 87pp.

Genetic Technologies Limited (2016). Maize for grain. Pioneer Technical Insight. [http://www.pioneer.co.nz/maize-grain/product-information/grain-technical-insights /feeding-maize-grain-to-dairy-cows.html] site visited on 6/04/2016.

Havlin, J. L., Beaton, J. D., Tisdale, S. L. and Nelson, W. L. (2005). *Soil Fertility and Fertilizers: An Introduction to Nutrient Management*. (7th Ed.), Pearson Prentice Hall, New Jersey. 515pp.

Kamanga, B. C. G., Kanyama-Phiri, G. Y. K., Waddington, S. R., Almekinders, C. J. M. and Giller, K. E. (2014). The evaluation and adoption of annual legumes by smallholder maize farmers for soil fertility maintenance and food diversity in Central Malawi. *Food Security* 6(1): 45 – 59.

Kamanga, B. C. G., Whitbread, A., Wall, P., Waddington, S. R., Almekinders, C. and Giller K. E. (2010). Farmer evaluation of phosphorus fertilizer application to annual legumes in Chisepo, Central Malawi.*African Journal of Agricultural Research* 5(8): 668 – 680.

Kerr, R. B., Snapp, S., Chirwa, M., Shumba, L. and Msachi, R. (2007). Participatory research on legume diversification with Malawian smallholder farmers for improved human nutrition and soil fertility. *Experimental Agriculture* 43: 437 – 453.

Mahama, G. Y., Prasad, P. V. V., Roozeboom, K. L., Nippert, J. B. and Rice, C. W. (2016). Response of maize to cover crops, fertilizer nitrogen rates, and economic return. *Agronomy Journal* 108(1): 17 – 31.

Makumba, W. (2003). Nitrogen use efficiency and carbon sequestration in legume tree-based agroforestry system: A case study of Malawi. Thesis for Award of PhD Degree at Wageningen University, The Netherlands, 208pp.

Maobe, S. N., Akundabweni, L. S. M., Mburu, M. W. K., Ndufa, J. K., Mureith, J. G., Gachene, C. K. K., Makini, F. W. and Okello, J. J. (2010). Effects of Mucuna green manure and inorganic urea nitrogen sources and application rates on harvest indices of maize (*Zea mays* L.). *World Journal of Agricultural Sciences* 6(5): 532 – 539.

Matusso, J. M. M., Mungwe, J. N. and Mucheru-Muna, M. (2014). Effects of different maize (*Zea mays* L.) soybean (*Glycine max* (L.) Merrill) intercropping patterns on soil mineral-N, N-uptake and soil properties. *African Journal of Agricultural Research* 9(1): 42 – 55.

Mhango, W. G. (2011). Nitrogen budgets in legume based cropping systems in Northern Malawi. Dissertation for the Award of PhD Degree at Michigan State University, East Lancing, USA, 257pp.

Ministry of Agriculture and Food Security, MoAFS. (2012). *Guide to Agricultural Production and Natural Resource Management in Malawi*. Agricultural Communications Branch, Lilongwe, Malawi. 366pp.

Mloza-Banda, H. R. (1994). *Principles and Practices of Crop Management: Field Study Guide.* Crop Science Department, Bunda College of Agriculture, Lilongwe. 137pp.

Mtambanengwe, F., Mapfumo, P. and Vanlauwe, B. (2007). Comparative short term effects of different quality organic resources on maize productivity under two different environments in Zimbabwe. In: *Advances in Integrated Soil Fertility Management in Sub-Saharan Africa: Challenges and Opportunities* (Edited by Bationo, A., Waswa, B., Kihara, J. and Kimetu, J.), Springer. 1090pp.

Mutegi, J., Kabambe, V., Zingore, S., Harawa, R. and Wairegi, L. (2015) The *Fertilizer Reccomendation Issues in Malawi: Gaps, Challenges, Opportunities and Guidelines.* Soil Health Consortium, Malawi. 54pp.

Njira, K. O. W., Nalivata, P. C., Kanyama-Phiri, G. Y. and Lowole, M. W. (2013). Effects of sole cropped, doubled-up legume residues and inorganic nitrogen fertilizer on maize yields in Kasungu, Central Malawi. *Agricultural Science Research Journal* 3(3): 97 – 106.

Palm, C. A., Gachengo, C. N., Delve, R. J., Cadish, G. and Giller, K. E. (2001). Organic inputs for soil fertility management: Some rules and tools. *Agriculture, Ecosystems and Environment* 83: 27 – 42.

Partey, S. T., Preziosi, R. F. and Robsosn, G. D. (2014). Improving maize residue use in soil fertility restoration by mixing with residues of low C-to-N ratio: effects on C and N mineralization and soil microbial biomass. *Journal of Soil Science and Plant Nutrition* 14(3): 518 – 531.

Phiri, A. T., Msaky, J. J., Mrema, J. P., Kanyama-Phiri, G. Y. and Msanya, B. M. (2014). Effect of pigeon pea-groundnut system on selected soil properties. *International Journal of Plant and Soil Science* 3(4): 397 – 407.

Sakala, W. D., Cadisch, G. and Giller, K. E. (2000). Interactions between residues of maize and pigeonpea and mineral N fertilizers during decomposition and N mineralization. *Soil Biology and Biochemistry* 32: 679 – 688.

Sakala, W. D., Kumwenda, J. T. D. and Saka, A. R. (2003). The Potential of Green Manures to Increase Soil Fertility and Maize Yields in Malawi. *Biological Agriculture and Horticulture* 21(2): 121 – 130.

Silva, P. R. F., Strieder, M. L., Coser, R. P. S., Rambo, L., Sangoi, L., Argenta, G., Forsthofer, E. L., Silva, A. A. (2005). Grain yield and kernel crude protein content increases of maize hybrids with late nitrogen side-dressing. *Scientia Agricola Piracicaba, Brazil* 62(5): 487 – 492.

Snapp, S. S., Jayne, T. S., Mhango, W., Benson, T. And Ricker-Gilbert, J. (2014). Maize yield response to nitrogen in Malawi's smallholder production systems. *Paper Prepared For The National Symposium Eight Years of FISP- Impact And What Next? Bingu International Conference Centre*, Lilongwe 14 – 15 July 2014. pp. 1 – 22.

Stoorvogel, J. J. and Smailing, E. M. A. (1990). *Assessment of soil nutrient depletion in Sub-Saharan Africa: 1983-2000.* Report No. 28. Wageningeni, The Netherlands. 284pp.

Tamene, L. and Le, Q. B. (2015). Estimating soil erosion in sub-Saharan Africa based on landscape similarity mapping and using the revised universal soil loss equation (RUSLE). *Nutrient Cycling in Agroecosystems* 102: 17 – 31.

Tamene, L., Mponela, P., Ndengu, G. and Kihara, J. (2015). Assessment of maize yield gap and major determinant factors between smallholder farmers in the Dedza district of Malawi. [http: /link.springer.comarticle/10.1007/s10705-015-9692-7] site visited on 04/122015.

Zeng, D., Mao, R., Chang, S. X., Li, L. and Yang, D. (2010). Carbon mineralization of tree leaf litter and crop residues from poplar-based agroforestry systems in Northeast China: A laboratory study.*Applied Soil Ecology* 44: 133 – 137.

Zingore, S. (2011). Maize productivity and response to fertilizer use as affected by soil fertility variability, manure application, and cropping system. *Better Crops* 95(1): 4 – 6.

Zingore, S., Mutegi, J., Agesa, B., Tamene, L. and Kihara, J. (2015). Soil degradation in Sub-Saharan Africa and crop production options. *Better Crops* 99(1): 24 – 26.

CHAPTER SIX

6.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1 General Conclusions

Integrated soil fertility management is one of the approaches for achieving sustainable agriculture production. This study was aimed at evaluating the performance of pigeon pea, cowpea and maize in the sole cropping, legume-cereal and legume-legume intercropping systems in terms of nodulation and BNF, crop productivity, influence on VAM fungal colonisation and their contributions to subsequent maize yields under different rates of inorganic N fertilizer.

From this study it was noted that the nodulation (nodule numbers, nodule weights and effectiveness) was influenced by the cropping systems. Sole cropping led to higher nodulation in both legumes and study sites. Sole PP nodule dry weights were higher by 25% and 48% than those of PP in intercrops with CP and MZ, respectively, at the Lilongwe site. Similarly, the nodule dry weights were also higher 25% and 46% compared with that in PP in an intercrop with CP or MZ, respectively, at the Dowa site. Significantly higher nodule numbers were reported at the Dowa site with only slight differences reported for Lilongwe site. Furthermore, Sole CP nodule dry weights were significantly higher by 38% and 36% than those in CP in an intercrop with PP and MZ, respectively, at the Lilongwe site.

Similar to the trend of nodulation, it can be noted from this study that both intercropping systems decreased $\%N_{dfa}$ and the total amount of N_2 fixed by the two legume species. At the Dowa site, sole cropped pigeon pea produced the highest amount of biologically fixed N (92.9 kg ha^{-1}), which was significantly higher than that by the pigeon pea for both the pigeon pea-cowpea and pigeon pea-maize intercrops by 31% and 36%, respectively. However, a comparative evaluation of the overall cropping system contribution per unit area revealed the combined amount of biologically fixed N (82.9 kg ha^{-1}) from the two component legume crops in the pigeon pea-cowpea "doubled-up" which was at par with that of the sole cropped pigeon pea. Furthermore, the PP-CP doubled up BNF in Dowa was significantly higher than the amounts of N_2 fixed by sole cowpea (62.5 kg N ha^{-1}), pigeon pea in the pigeon pea-maize intercrop (59.9 kg N ha^{-1}) or cowpea in the cowpea-maize intercrop (13.1 kg N ha^{-1}). However, observations from the Lilongwe site were different. Although the biologically fixed N (85.7 kg ha^{-1}) by the sole cropped pigeon pea was similarly the highest, the combined amount of N_2 fixed (57.4 kg N ha^{-1}) by the PP and CP in the pigeon pea-cowpea "doubled-up" was significantly lower than that by the sole pigeon pea, by 33%. This showed that competition for resources as affected by cropping systems such as legume-legume and legume-cereal intercrops, or sole cropping is also aggravated by environmental factors. From this study, it can be concluded that both legume-legume and legume-cereal intercropping reduces nodulation, BNF per plant but overall amount of nitrogen fixed per unit area can be comparable to the Sole PP and the PP+CP intercrop, which to some extent may depending on environmental conditions.

Furthermore, from this study it can be noted that the intercropping involving PP, CP or MZ (PP+CP, PP+MZ, CP+MZ) can lead to reduction in grain and TDM yields per plant and HI, compared to their sole crops. However, productivity by all the intercrops under this study was enhanced as they all resulted into LERs of greater than one and positive MAI values. Although all the intercropping systems (PP+MZ, PP+CP and MZ+CP) offer different benefits, the PP+MZ intercrop from this study, was the most beneficial in terms of both yields and monetary gains as it produced highest LERs and MAI values at both sites of Lilongwe and Dowa. Furthermore, the study revealed by the partial LERs, RDY, RNY and RPY determined in this study that some crops were more competitive than others for the specific intercropping combinations. Maize was shown to be the most resilient crop when intercropped with either PP or CP whereas CP was the most suppressed when intercropped with either PP or MZ.

From this study, it can be concluded that VAM fungal colonisation was not affected by the PP and CP based cropping systems such as sole cropping, cereal-legume and legume-legume intercrops. However, a positive relationship was noted between VAM fungal colonisation and yields, P uptake, nodulation and BNF. Furthermore, all the legume-based cropping systems showed significant positive effect on VAM fungal colonisation of the subsequent maize grown in short rotation. There were significant increases ranging from 39 to 50% and 15 to 36% in colonisation of the VAM fungi in the subsequent maize roots in the Lilongwe and Dowa sites, respectively, which was attributed to the ability of some crop species to sustain VAM fungal natural communities than others.

From the ISFM component of this study, it was noted that legume-based systems increased mineral N in the soils during the cropping seasons which, in turn, had an influence on plant N uptake. It could therefore be concluded that integration of pigeon pea and cowpea either as sole crops, legume-legume or legume-cereal intercrop led to higher N uptake, grain and TDM yields and harvest indices in the rotational maize as compared to the maize-maize monoculture with or without inorganic fertilizer. Furthermore, interactive effect of the legume residues and inorganic fertilizer led to higher maize grain yields ranging from 30% under treatment that was previously CP+MZ intercrop (1689 kg ha^{-1}) to 59% under treatment that was previously Sole CP (2864 kg ha^{-1}) at 0 kg N ha^{-1} fertilizer application than the treatment that was previously Sole MZ (1178 kg ha^{-1}).

Similarly, at the highest rate of N application, 120 kg N ha^{-1}, the previous legume-based treatments produced higher grain yields than the treatment that was previously Sole MZ (3277 kg ha^{-1}) by a range of 28% under treatment that was previously CP+MZ intercrop (4525 kg ha^{-1}) to 42% under treatment that was previously Sole CP (5665 kg N ha^{-1}), at the Lilongwe site with a similar trend at the Dowa site. Furthermore, from this study it was shown that mixing high quality pigeon pea and cowpea with the low quality maize residues increases mineralization rates, N uptake, and nitrogen use efficiency by the maize grown after the legumes, with implications on yields. In addition, increasing inorganic N application increased maize grain crude protein content in both study sites and this has an implication on the quality of the grain.

6.1 Recommendations

From the results obtained in the present study the following recommendations are given:

i. Implementation of the legume-legume and legume-cereal intercrops or sole cropping systems involving pigeon pea and cowpea with the aim of improving soil fertility should be encouraged as all the systems lead to significant amounts of biologically fixed N. However, their implementation should be accompanied by thorough evaluation of environmental conditions including soil factors in order to ensure good crop performance and to minimize soil degradation.

ii. There is need for further research on BNF in legume-legume systems involving pigeon pea and cowpea in order to provide more information as to why “doubled-up” legume systems may result into more nitrogen fixed in some sites than others or to get more information on effects of interaction of environment and doubled-up legume systems on BNF.

iii. Evaluation of intercrop productivity should always be considered when implementing various intercropping systems involving pigeon pea and cowpea to ensure efficient utilization of resources.

iv. Further studies should be done on pigeon pea and cowpea legume-legume and legume-cereal intercropping systems involving different planting patterns, densities and different crop varieties to get more information on their competitive ability, general productivity and the BNF.

v. There is a need for more research on assessing VAM fungal species abundance and diversity in different sites on Malawi soils, and their influence on BNF of pigeon pea and cowpea and growth of both legumes and maize. Furthermore, this should include isolation of promising species for VAM inoculant production.

vi. The ISFM approach involving pigeon pea and cowpea in either legume-legume or legume-cereal intercrops should be encouraged as these systems lead to substantial increases in yields of subsequent maize, increased grain quality and nitrogen use efficiency.

vii. Further studies should be undertaken to evaluate the effects of the PP and CP-based ISFM on other maize grain quality attributes such as carbohydrate and oil contents other than protein.

APPENDICES

Appendix 1: Critical values for some selected soil properties

Soil property	Critical values/ range	Reference
pH_{water} 1:2.5	>5.5	Landon (1991)
Organic C (%)	2.35	Chilimba (2007)
SOM (%)	4	Chilimba (2007)
Total N (%)	0.13	Landon (1991)
Mehlich-3 P (mg/kg)	25 - 33	Chilimba (2007)
Mg ($cmol_+$/kg)	0.5	Chilimba (2007)
Ca ($cmol_+$/kg)	2.0	Mehlich (1984)
K ($cmol_+$/kg)	0.2	Wendt (1996); Chilimba *et al.* (1999)
Fe (mg/kg)	2.5 – 4.5	Landon (1991)
Zn (mg/kg)	2.5	Chilimba *et al.* (1999)
Mn (mg/kg)	3	Mehlich (1984)
B (mg/kg)	0.1 – 0.7	Landon (1991)

Appendix 2A: Correlation matrix for VAM fungal colonisation of pigeon pea roots, P uptake BNF and other growth parameters at the Lilongwe site

Parameter	% col	% P	P uptake	TDM	BNF	Nodule number	Nodule dry weight
% col	-						
% P	0.395	-					
P uptake	0.344	0.833*	-				
TDM	0.170	0.467	0.860**	-			
BNF	0.191	0.063	0.523	0.752*	-		
Nodule number	0.069	0.285	0.576	0.618	0.331	-	
Nodule dry weight	0.368	0.4897	0.135	0.121	0.310	0.602	-

NB: *** Signicant at P = 0.001; **Significant at P = 01; *Significant at P = 0.05

TDM = total dry matter; % col = percentage of colonisation

Number of observations (n) = 9; degrees of freedom (d.f) = 8

Appendix 2B: Correlation matrix for VAM fungal colonisation of pigeon pea roots, P uptake BNF and other growth parameters at the Dowa site

Parameter	% col	% P	P uptake	TDM	BNF	Nodule number	Nodule dry weight
% col	-						
% P	0.185	-					
P uptake	0.488	0.714*	-				
TDM	0.045	0.173	0.273	-			
BNF	0.188	0.163	0.640	0.834**	-		
Nodule number	0.517	0.065	0.490	0.195	0.425	-	
Nodule dry weight	0.470	0.191	0.276	0.095	0.268	0.872**	-

NB: *** Signicant at P = 0.001; **Significant at P = 01; *Significant at P = 0.05

TDM = total dry matter; % col = percentage of colonisation

Number of observations (n) = 9; degrees of freedom (d.f) = 8

Appendix 2C: Correlation matrix for VAM fungal colonisation of cowpea roots, P uptake BNF and other growth parameters at the Lilongwe site

Parameter	% col	% P	P uptake	TDM	BNF	Nodule number	Nodule dry weight
% col	-						
% P	0.252	-					
P uptake	0.645	0.546	-				
TDM	0.067	0.271	0.392	-			
BNF	0.630	0.104	0.877*	0.566	-		
Nodule number	0.155	0.690*	0.628***	0.113	0.363	-	
Nodule dry weight	0.438	0.531	0.899	0.233	0.783*	0.849	-

NB: *** Signicant at P = 0.001; **Significant at P = 01; *Significant at P = 0.05

TDM = total dry matter; % col = percentage of colonisation

Number of observations (n) = 9; degrees of freedom (d.f) = 8

Appendix 2D: Correlation matrix for VAM fungal colonisation of cowpea roots, P uptake BNF and other growth parameters at the Dowa site

Parameter	% col	% P	P uptake	TDM	BNF	Nodule number	Nodule dry weight
% col	-						
% P	0.397	-					
P uptake	0.557	0.612	-				
TDM	0.660	0.453	0.496	-			
BNF	0.479	0.005	0.750	0.416	-		
Nodule number	0.657	0.071	0.570	0.483	0.781*	-	
Nodule dry weight	0.650	0.110	0.722	0.489	0.820*	0.965***	-

NB: *** Signicant at P = 0.001; **Significant at P = 01; *Significant at P = 0.05

TDM = total dry matter; % col = percentage of colonisation

Number of observations (n) = 9; degrees of freedom (d.f) = 8

Appendix 2E: Correlation matrix for VAM fungal colonisation maize roots, P uptake BNF and other growth parameters at the Lilongwe site

Parameter	% col	% P	P uptake	TDM
% col	-			
% P	0.659*	-		
P uptake	0.455	0.429	-	
TDM	0.378	0.317	0.988***	-

NB: *** Signicant at P = 0.001; **Significant at P = 01; *Significant at P = 0.05

TDM = total dry matter; % col = percentage of colonisation

Number of observations (n) = 12; degrees of freedom (d.f) = 11

Appendix 2F: Correlation matrix for VAM fungal colonisation maize roots, P uptake BNF and other growth parameters at the Dowa site

Parameter	% col	% P	P uptake	TDM
% col	-			
% P	0.558	-		
P uptake	0.210	0.433	-	
TDM	0.101	0.219	0.930***	-

NB: *** Signicant at P = 0.001; **Significant at P = 01; *Significant at P = 0.05

TDM = total dry matter; % col = percentage of colonisation

Number of observations (n) = 12; degrees of freedom (d.f) = 11