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Biological nitrogen fixation by pigeon pea and cowpea in the "doubled-up" and other cropping systems on the Luvisols of Central Malawi

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Legumes form a very important component in Malawi's cropping systems because of 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 intercrops (also commonly referred to as "doubled-up"). However, information on BNF by the pigeon pea and cowpea under doubled-up system is scanty. Therefore, a study was conducted at two sites of Lilongwe and Dowa in the 2013/14 growing season, to quantify and compare the amounts of biologically fixed nitrogen in the three legume cropping systems. The experiments were laid out in a randomized complete block design and BNF was estimated using the modified nitrogen difference method. Results showed that there were significant differences ($P < 0.05$) in nodule numbers, nodule dry weights, and quantities 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 and 36% than the amounts fixed in the cowpea-maize intercrop (13.1 kg N ha^{-1}), sole cowpea (62.5 kg N ha^{-1}) and pigeon pea-maize intercrop (59.9 kg N ha^{-1}), respectively, at 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 at 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%. 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, cowpea; cropping systems, cereal-legume intercrop; legume-legume intercrop, pigeon pea.

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).

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 for doubled-ups of pigeon pea-groundnuts (42 to 82.8 kg N ha^{-1}) and pigeon pea-soybean (53.6 kg N 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 paper, the terms N_2 fixed and biologically fixed nitrogen (N) are used interchangeably.

MATERIALS AND METHODS

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 research farm within the Mkwinda Extension Planning Area (EPA), whereas in Dowa district the experiment was conducted at Nachisaka EPA. Rain gauges were installed at both sites for rainfall monitoring. Soils of the two sites have been classified as Chromic Luvisols in the World Reference Base System (Typic Hapludalfs in the USDA Soil Taxonomy) (Chilimba et al., 2011; Mutegi et al*.,* 2015).

Soil samples were collected using an auger from depths of 0 - 20 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

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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, extracted using 2 M KCl, followed by colorimetric determination according to Anderson and Ingram (1989) 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 the section for BNF determination.

Experimental design and treatment description

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 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. 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. No rhizobial inoculation was done on both cowpea and pigeon pea. Therefore, nodulation and BNF in this study was dependent on indigenous rhizobia from soil.

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.

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). Therefore, the formula used was as shown in the following equation after Peoples et al. (1989):

$$Q = [\text{N yield (legume)} - \text{N yield (control)}] + [\text{N soil (legume)} - \text{N soil (control)}]$$

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; Control/reference crop = Unfertilized maize.

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

$$\%N_{dfa} = \frac{N_2 \text{ fixed}}{\text{N yield}} \times 100$$

Where: N_2 fixed is the biologically fixed nitrogen; N yield is the total N uptake.

Determination of total dry matter yields

The total dry matter yields (total biomass yields) were needed for the quantification of BNF and were determined for all the crops, which are cowpea, pigeon pea and maize. A net plot of 13.5 m by 4.3 m 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).

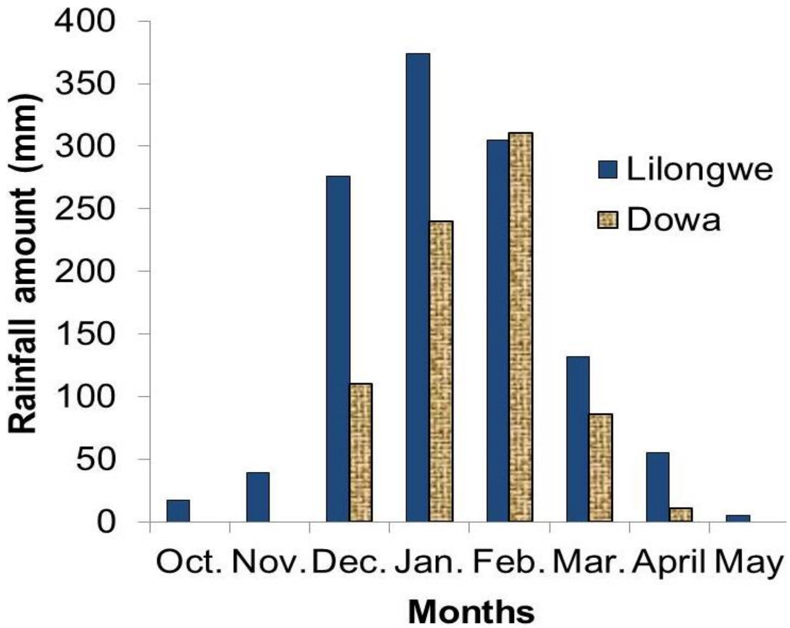

Figure 1. Rainfall amounts for Lilongwe and Dowa sites in the 2013/14 cropping season.

Statistical and data analysis

All the obtained data were subjected to analysis of variance (ANOVA) using Genstat 15^{th} edition statistical package. 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 needed 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.

RESULTS

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

Figure 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.

Soil properties of the Lilongwe and Dowa study sites

Results for the determination of soil properties for the two study sites are shown in Table 1. The topsoil mean values indicated moderately acid for Lilongwe and slightly acid for Dowa site whereas the subsoils of both sites were slightly acid with the same mean value (Table 1). Dowa soil had high organic matter for both top and subsoil whereas Lilongwe showed low organic matter for both depth ranges (Table 1). Further details for various soil properties and their ratings according to Landon (1991) and Chilimba (2007) are presented in Table 1.

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

Table 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

Table 1. Soil properties of the Lilongwe and Dowa study sites in Malawi.

Soil property	Lilongwe		Dowa		Lilongwe		Dowa	
	0 - 20 cm	Rating	0 - 20 cm	Rating	20 - 40 cm	Rating	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	-

Ratings are based on Landon (1991) and Chilimba (2007).

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.

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 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.

Table 2. Pigeon pea 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	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[a]	353.6[a]
	PP+CP	251.5[b]	254.3[ab]
	PP+MZ	174.7[c]	191.9[b]
	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[ns]	15.1[ns]
	F pr.	0.145	0.79
	CV %	11.3	10.3

Means with different letters in the same column are significantly different according to LSD at 5% significant level; ns = non-significant difference; CV = coefficient of variation; F pr. = F probability; PP = pigeon pea; CP = cowpea and MZ = maize; PP+CP = PP intercropped with CP; PP+MZ = PP intercropped with MZ; PP+CP and CP+PP have been used interchangeably in this paper.

No significant differences were observed in cowpea nodule effectiveness in both sites of the study.

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

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 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 2A to B) 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 (Figure 2A to B). In the Lilongwe site, sole cropped pigeon pea showed significantly higher ($P < 0.05$) N_2 fixed per plant (Table 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 2A to B) 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 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.

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 4) and per hectare basis (Figure 2A to B). 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

Table 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[a]
	CP+PP	8	12[ab]
	CP+MZ	8	10[b]
	LSD (0.05)	3.99[ns]	5.16
	F pr.	0.256	0.043
	CV %	19.9	18.3
Nodule dry weight (mg/plant)	CP	519.1[a]	711.4[a]
	CP+PP	323.7[b]	492.8[b]
	CP+MZ	330.7[b]	419.9[b]
	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[ns]	17.7[ns]
	F pr.	0.64	0.54
	CV %	10.2	9.3

Means with different letters in the same column are significantly different according to LSD at 5% significant level; ns = non-significant difference; CV = coefficient of variation; F pr. = F probability; PP = pigeon pea; CP = cowpea and MZ = maize; PP+CP = PP intercropped with CP; PP+MZ = PP intercropped with MZ; PP+CP and CP+PP have been used interchangeably in this paper.

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.

Quantities of N2 fixed and $\%N_{dfa}$ by cowpea at Lilongwe and Dowa sites

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 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.

On the other hand, results of N_2 fixed by cowpea at the Dowa site show that there were significant differences ($P < 0.05$) as influenced by cropping system (Table 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 4). Similar trends were observed on both per plant basis (Table 4) and per hectare basis (Figure 2C to D).

Total biologically fixed N for the different overall cropping systems

The cropping systems included sole crops, legume-

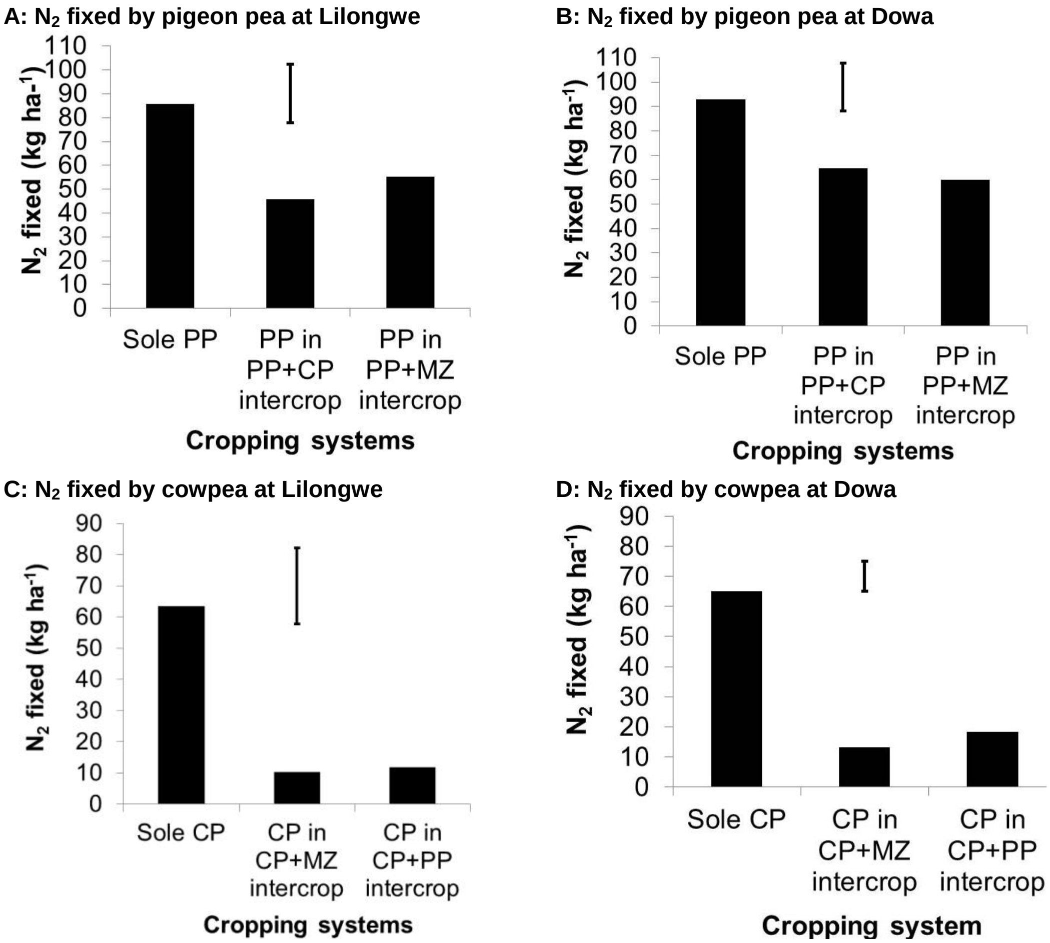

Figure 2. Quantities of N_2 biologically fixed by pigeon pea and cowpea grown in different cropping systems at Lilongwe and Dowa sites. PP = pigeon pea; CP = cowpea and Mz = maize; PP+CP = PP intercropped with CP; PP+MZ = PP intercropped with MZ; PP+CP and CP+PP have been used interchangeably in this paper; covariate analysis was done on the cowpea N_2 fixed; the inserted vertical bar within each graph represents the LSD.

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 3A to 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

Table 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[a]	75.7[a]	2.61[a]	76.0[a]
	PP+CP	1.11[b]	62.6[b]	1.91[b]	68.7[b]
	PP+MZ	1.27[b]	66.6[b]	1.89[b]	66.7[b]
	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[a]	69.4[a]	0.50[a]	68.8[a]
	CP+PP	0.14[b]	28.7[b]	0.27[b]	37.3[b]
	CP+MZ	0.14[b]	27.2[b]	0.17[b]	29.5[b]
	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

Means with different letters in the same column are significantly different according to LSD at 5% significant level; ns = non-significant difference; F pr. = F probability; PP = pigeon pea; CP = cowpea and MZ = maize; PP+CP = PP intercropped with CP; PP+MZ = PP intercropped with MZ; PP+CP and CP+PP have been used interchangeably in this paper.

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%.

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. 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

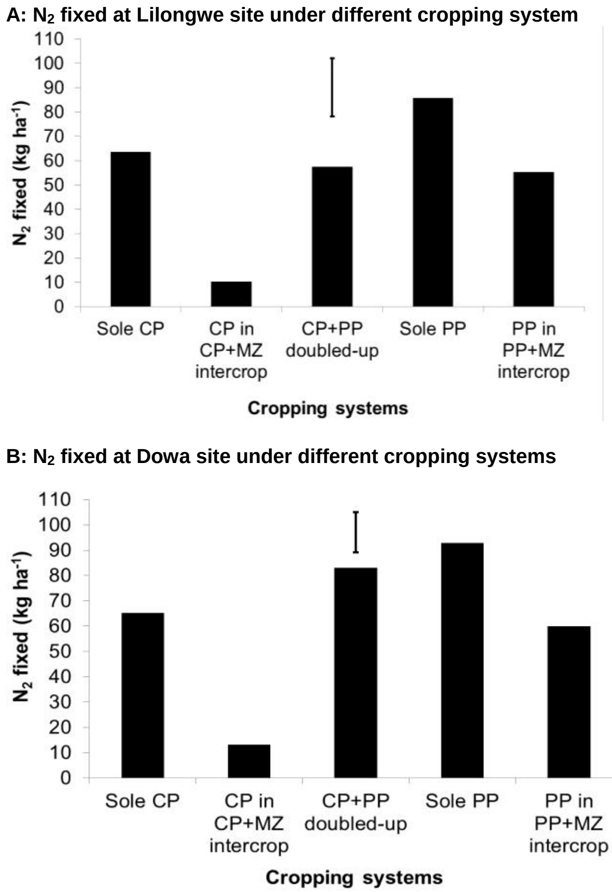

Figure 3. Total amounts of biologically fixed nitrogen (kg N ha^{-1}) contributed by the legume components. The amount of biologically fixed nitrogen in the CP+PP “doubled-up” is the sum of N_2 fixed by each of the PP and CP components. The inserted vertical bar within each graph represents the LSD; PP = pigeon pea; CP = cowpea and Mz = maize; PP+CP = PP intercropped with CP; PP+MZ = PP intercropped with MZ; PP+CP and CP+PP have been used interchangeably in this paper.

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 to some extent the site effects. The Dowa site indicated comparably high amounts 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 in the doubled-up. 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, especially 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). In this study, additional N application was not considered because of the sensitivity of the N-difference method (Danso et al., 1992). 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; 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). 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.

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.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Abbreviations

AFNETA, Alley Farming Network for Tropical Africa; **ICRISAT,** International Crops Research Institute for Semi-Arid Tropics; **MAI,** Ministry of Agriculture and Irrigation; **MoAFS,** Minitry of Agriculture and Food Security; **SSSA,** Soil Science Society of America.

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