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Full Length Research Paper

Biological nitrogen fixation in sole and doubled-up legume cropping systems on the sandy soils of Kasungu, Central Malawi

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Nitrogen is the most limiting element in Malawi soils with respect to crop production. However, grain legumes that are associated with biological nitrogen fixation are commonly grown in the country. Results are reported of a study on evaluation of the effects of sole cropped and doubled-up legume systems (legume-legume intercrop) on biological nitrogen fixation on the Ultisols of Kasungu district, Central Malawi. The modified nitrogen difference method was used to estimate the amount of nitrogen fixed per hectare. The total amount of nitrogen biologically fixed in each cropping system (that is, for intercrops, this means adding together amount of nitrogen fixed by the component crops) showed significantly ($p<0.05$) higher mean nitrogen amount in the pigeon pea/groundnuts doubled-up cropping system (82.8 kg N ha^{-1}) that was 33 and 35% more nitrogen than those of the sole groundnuts (55.8 kg N ha^{-1}) and sole pigeon pea (54.1 kg N ha^{-1}), respectively. However, the pigeon pea/soybean doubled-up (53.6 kg N ha^{-1}) was only significantly higher than that for the sole soybean (35.8 kg N ha^{-1}) but resulted in nitrogen amount similar to that for the sole pigeon pea which implies a large suppression on intercropped pigeon pea biological nitrogen fixation. This could be attributed to competition for light and nutrients presented to the pigeon pea in the pigeon pea/soybean intercrop in early stages of development by the fast growing bushy solitaire soybean.

Key words: Biological nitrogen fixation, doubled-up legume cropping system, nitrogen difference method, sole crop.

INTRODUCTION

Low soil fertility is widely considered as a major factor contributing to low productivity and non-sustainability of existing production systems and long-term food insecurity in Southern and Eastern Africa (Buresh et al., 1997; Mekuria et al., 2004). Mekuria and Waddington (2002) also reported that low soil fertility is a direct contributor to reduced productivity and a major source of inefficiency in the returns to other inputs and management committed to smallholder farms, including nitrogen (N) fertilizer, seed and labour.

Mineral fertilizers improve soil fertility and result in increased yields but are expensive and often beyond the reach of resource poor farmers resulting in the chronic food insecurity in Africa (World Bank, 1996). On the other hand, there are wide spread concerns about the effect of increased use of mineral fertilizer on water resources, soil fauna and soil health (Dorward and Chirwa, 2011). Excessive use of N fertilizers cause problems of acidification and the over-use of N and P fertilizers cause water pollution in the form of eutrophication (Brady and Weil, 2008; Olson, 1987).

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Abbreviations: **BNF,** biological nitrogen fixation; **EPA,** extension planning area; **ICRISAT,** International Crop Research Institute for Semi-Arid Tropics; **MAI,** Ministry of Agriculture and Irrigation; **N,** nitrogen.

Therefore, solutions to smallholder farmers' soil fertility problems may be found in the strategic combination of organic resources, particularly from nitrogen-fixing legumes, with small amounts of mineral fertilizers (Palm et al., 1997). Legumes have unusual advantage in obtaining nitrogen through biological nitrogen fixation (BNF) process by participating in a symbiotic relationship with *Rhizobia* spp. The ability of legumes to fix atmospheric nitrogen in their nodulated roots and plant residues left after harvesting represent a valuable source of organic N (Hayat, 2005). Benefits of legume nitrogen fixation include protein nutrition, soil fertility improvement, savings on fertilizer costs and cash income from sale of crop surpluses (Mpepereki and Pompi, 2002). Legume grain is protein-rich and is an essential part of the diet in many parts of the tropics, particularly where meat is scarce (Giller, 2001). The legume plant assimilates all the nitrogen that it biologically fixes hence, maintaining the balance of global nitrogen cycle keeps nitrogen in a form that will not pollute the environment (Serraj, 2004).

In Malawi, a wide range of legumes is grown traditionally, either as monocrops or in association with cereals especially maize. Groundnut (*Arachis hypogaea*) and bean (*Phaseolus vulgaris*) are the most commonly grown legumes in the country. The Lilongwe and Kasungu plains, the Mzimba and Henga Valley plains, and the Phalombe plain are the leading groundnut producing areas in Malawi. Pigeon pea (*Cajanus cajan*) is the most popular legume for intercropping with maize, particularly in the Southern part of Malawi. It also grows well in all free draining soils and is an important crop mostly to resource-poor farmers throughout the area (Kamanga, 2002). Soybean (*Glycine max*), velvet bean (*Mucuna puriens*), bambara nut (*Vigna subterranea*) are also grown, among others. Legumes can also be grown in association with other legumes in what is known as doubled-up legume technology (legume-legume intercrops) (ICRISAT/MAI, 2000), a cropping system that is also practiced by some farmers in Malawi.

This study was based on the hypothesis that intercropping a legume with another legume will increase amount of nitrogen fixed per unit area (piece of land). The objective of the study was to evaluate the effect of sole legume cropping and legume – legume intercropping on biological nitrogen fixation.

MATERIALS AND METHODS

Site selection and experimental plot layout

The on-farm farmer managed, researcher-designed experiment was conducted in the 2008/2009 cropping season in Kasungu district, Mkanakhothi Extension Planning Area (EPA) in five villages involving 15 farmers. The area receives an average annual rainfall of 680 mm (Phiri et al., 2010). The soils of the area are Ultisols that are dominated by slightly acidic sandy to sandy clay loam soils. Farmers' selection was based on legume production interest. Fields that had no legumes and no application of manure in the previous years were selected. Rainfall data for the area were collected for the cropping season (November to April). Rain gauges were calibrated and installed in two experimental sub-sites (strategic villages of Ndaya and Kaunda). Daily rainfall amounts for the whole period of the study were recorded and total rainfall for each month and the year were calculated.

The experiment was laid out in a randomized complete block design, with 7 treatments on 15 farmers' fields (taking each farmer as a replicate). Each treatment covered a plot 10 × 10 m. Table 1 shows the list of treatments. All the treatments were laid on ridges that were 25 cm high and spaced at 75 cm apart for all the plots. The ridge heights and spacing were done according to the farmer practice in the area. Treatments involving groundnuts included sole cropped groundnuts planted at 15 cm between planting stations along the ridge and 1 seed per planting station and groundnuts in an in-row intercrop with pigeon pea planted at 15 cm between planting stations along the ridge, 1 seed per planting station. Pigeon pea was planted 3 seeds per planting station at distances of 90 cm apart for all treatments. Treatments involving maize were organized as follows: maize not fertilized but intercropped with pigeon pea and a control with sole cropped maize without fertilizer application, were planted in planting stations separated at 90 cm along the ridge and 3 seeds per planting station. Soybean was inoculated with a soybean nodulating *Bradyrhizobium japonicum* and included sole cropped soybean and soybean intercropped with pigeon pea and was sown at 5 cm between planting stations along the ridge, 2 seeds per planting station. In both pigeon pea/soybean and pigeon pea/groundnut intercrops, a distance of 30 cm was reserved between the pigeon pea planting station and its nearest station of groundnuts or soybean in order to provide some space for both component crops in each intercrop. No inorganic fertilizer was applied to any of the crops in all the treatments. The sole cropped maize treatment was used as a reference or control crop on which to base the nitrogen difference method. The pigeon pea plus maize treatment was an additional treatment that was not addressed by the main objective of the paper but it provided extra information apart from the sole cropped and doubled-up legumes.

Soil and plant sampling

The first batches of soils were taken from each farmer's field before planting. Samples were collected randomly from five points on each farmer's field over depth ranges of 0 to 15 cm and 15 to 30 cm. Soils from each depth range were thoroughly mixed and quartering method was used to obtain 500 g composite sample. These samples were stored in plastic bags tied tightly. Second sampling was done at harvesting from each of the treatment plots from all the fields involved and was specially done to obtain postharvest soil N for the modification of the nitrogen difference method. This time samples were taken from only three points in each plot (10 × 10 m), and were mixed in order to obtain composite samples. On every occasion, all samples were collected during dry seasons and were air dried, passed through a 2 mm sieve and then kept in plastic bags ready for laboratory analysis.

Eight maize plants were sampled randomly from each plot at tasseling stage, 16 soybean plants, 8 groundnut plants and 8 pigeon pea plants were sampled randomly from each plot at podding stage. These are the stages when the crops described reach their highest level of dry matter accumulated and contain high levels of N. Determination of number of plants to be sampled led to a highest number for soybean because it had the highest plant population. Samples were oven dried at 70°C for 48 h and ground to powder and kept in plastic bottles ready for laboratory analysis.

Soil and plant analysis

A number of soil analyses were done including texture, determined

Table 1. List of treatments on the effect of cropping system on biological nitrogen fixation.

Number	Treatments
I	Soybean
Ii	Groundnuts
Iii	Pigeon pea
Iv	Pigeon pea + Groundnuts
V	Pigeon pea + Soybean
Vi	Maize + Pigeon pea
Viii	Maize

Varieties used were MH 18 for maize, CG7 for groundnuts, Solitaire for soybean and ICEAP00040 for pigeon pea.

Table 2. Monthly rainfall amount for the study site in the 2008/2009 study period.

Month	Rainfall (mm)
November	15.2
December	97.1
January	139.9
February	186.2
March	189.0
April	89.0
Annual/seasonal total	716

using the Hydrometer method (Anderson and Ingram, 1989), soil pH measured in water (1:2.5) using a pH meter (Wendt, 1996), total organic carbon (% C) by Walkley-Black method adapted for spectrometric determination (Anderson and Ingram, 1989) and later converted to soil organic matter by multiplying the values of organic carbon by 1.72, total nitrogen (% N) by the Kjeldahl method, available P and micronutrients by Mehlich 3 extraction procedures as outlined by Anderson and Ingram (1989). Plant samples were ground and sieved through a 2 mm sieve before being analyzed for total nitrogen and phosphorus. Samples were digested and subjected to colorimetric determination as outlined by Anderson and Ingram (1989).

Determination of biological nitrogen fixation

The modified nitrogen-difference method (technique) as described by Peoples et al. (1989) was used in estimating biologically fixed nitrogen (N_2). In the N-difference technique, the difference between total plant nitrogen of an N_2 fixing legume and a control crop (non-N_2-fixing) is considered to be nitrogen that has been obtained through biological fixation. A modification to this basic principle is considered to improve accuracy of measurements when the legume and a control are not well matched (Evans and Taylor, 1987). In this study, a control crop was sole cropped maize without fertilizer application. The quantity (Q) of biologically fixed N in a modified N-difference technique is calculated as follows:

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

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 [legume] (kg ha^{-1}) = Post-harvest soil nitrogen in a legume plot
N soil [control] (kg ha^{-1}) = Post-harvest soil nitrogen in an un-fertilized maize

The amount of nitrogen was determined per plant and converted to amount per hectare based on total shoot dry matter yield each crop produced per hectare.

Data analysis

Data were subjected to Analysis of Variance (ANOVA) using GenStat edition 14. Means were separated using least significant differences (LSD) at p = 0.05.

RESULTS

Rainfall and soil characterization of the study area

In the 2008/2009 cropping season, the study area received an annual total rainfall of 716 mm with moderately high rainfall in the months of February and March (Table 2). The 2008/2009 total rainfall was within the range of yearly rainfall, 628 to 844 mm received in the area in the past 7 years. Tables 3 and 4 described results of soil characterization of the study sub-sites/villages. The means of total nitrogen in the topsoil ranged from 0.07 to 0.10% with Ndaya indicating a relatively higher value than the rest of the sub-sites. A similar trend appeared in the sub-soil with a range of 0.05 to 0.09%. Both overall means of total soil nitrogen were below the critical value of 0.1%. The ranges of values for available phosphorus (Mehlich-3 P) in the topsoil and subsoil were both below the critical value of 25 ppm with Chisazima and Kaunda indicating relatively higher values of P than the rest of the sub-sites. The mean values of zinc fell above the critical value (2.0 ppm). The ranges of Zn and Fe fell within the range of those reported by Sillanpaa (1982) for Malawi in a global study on micronutrient and nutrient status of soils. All the study sites had moderately acid to acidic soils. On the average, the soils were acidic with the overall mean pH of 5.4 and 5.2 for the topsoil and subsoil respectively. The top soil textures ranged from loamy sandy (coarse-textured) to slight sand clay loam (moderately fine-textured). The subsoil textures ranged from loamy sandy to sandy clay loam (fine-textured). On the average, the top soils were coarse textured whereas the sub oils were comparatively fine textured.

Plant tissue nitrogen concentration and biologically fixed nitrogen by soybean, groundnuts and pigeon pea in different cropping systems

Table 5 shows the concentration of nitrogen in plant

Table 3. Soil texture data of the study sub-sites.

Depth range (cm)	Soil property	Sub-sites					Standard deviation
		Chisazima	Tchezo	Kaunda	Ndaya	Chaguma	
0 -15	Sand	86.0	80.2	80.4	74.4	70.3	6.1
	Silt	2.0	0.7	7.2	8.0	8.0	2.5
	Clay	13.0	19.4	16.0	21.6	25.7	4.9
	Textural class	Loamy sand	Sandy loam	Sandy loam	Sandy clay loam	Sandy clay loam	-
15 -30	Sand	84.6	77.1	70.7	63.8	65.0	8.7
	Silt	6.1	7.1	11.3	15.8	21.4	6.4
	Clay	12.3	19.3	23.7	28.3	24.3	6.1
	Textural class	Loamy sand	Sandy loam	Sandy clay loam	Sandy clay loam	Sandy clay loam	-

Table 4. Initial soil test results for the study sub-sites in the 0 to 15cm and 15 to 30 cm depth ranges.

Site and statistics	Soil properties in different depth ranges											
	Total N (%)		Mehlich-3 P (ppm)		Fe (ppm)		Zn (ppm)		OM (%)		pH $_{(H2O)}$	
	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm
Chisazima	0.07	0.05	23.2	21.4	93.4	101.1	3.56	2.70	1.2	0.9	5.5	5.2
Tchezo	0.08	0.08	10.3	8.5	77.9	83.8	2.25	2.60	1.5	0.9	5.2	5.0
Kaunda	0.08	0.07	20.9	11.6	91.1	89.0	2.64	1.90	1.7	1.2	5.6	5.5
Ndaya	0.10	0.09	11.9	7.3	92.4	83.2	3.03	2.23	2.1	1.1	5.3	5.2
Chaguma	0.09	0.08	4.1	2.2	71.7	90.2	3.31	2.60	1.7	1.0	5.3	5.1
Grand mean	0.08	0.07	14.1	10.2	85.3	89.5	2.96	2.43	1.6	1.0	5.4	5.2
Standard deviation	0.02	0.02	8.2	7.6	15.8	11.5	0.98	0.82	0.5	0.2	0.2	0.2

OM: organic matter.

tissues of groundnuts, soybean and pigeon pea measured at podding and biologically fixed nitrogen. Separation of means for biologically fixed nitrogen was done both per plant and per hectare with covariate analysis to take care of the different plant populations in the sole and intercropped legume systems. There was no significant difference ($p<0.05$) between sole cropped soybean plant tissue nitrogen concen-tration and that of soybean intercropped with pigeon pea. There was no significant difference in nitrogen concentration between sole cropped groundnuts and groundnuts intercropped with pigeon pea. There were significant differences ($p<0.05$) in plant tissue nitrogen among pigeon pea crop-ping systems. Pigeon pea intercropped with soybean indicated significantly ($p<0.05$) lower tissue nitrogen concentration than those in sole cropped pigeon pea, pigeon pea intercropped with groundnuts and pigeon pea intercropped with maize by 11, 9 and 12% respectively.

There were no significant differences ($p<0.05$) in biologically fixed nitrogen between sole cropped soybean and soybean in an intercrop with pigeon

Table 5. Plant tissue nitrogen concentration and biologically fixed nitrogen by sole cropped and doubled-up soybean, groundnuts and pigeon pea cropping systems at podding stage.

Crop	Cropping system and statistics	Plant tissue N %	N biologically fixed per plant (g)	N biologically fixed (kg ha^{-1})
Soybean	Soybean	2.45	0.34	30.6 (35.8)
	Soybean + pigeon pea	2.29	0.36	27.3 (22.8)
	LSD (0.05)	0.25^{NS}	0.14^{NS}	17.2^{NS}
	CV %	12.3	39.5	47.0
Groundnuts	Groundnuts	2.68	1.13	50.4 (55.8)
	Groundnuts + pigeon pea	2.79	0.96	45.9 (28.8)
	LSD (0.05)	0.44^{NS}	0.24^{NS}	31.7^{NS}
	CV %	17	24.5	35.8
Pigeon pea	Pigeon pea	3.32^{a}	3.09^{a}	54.1^{a}
	Pigeon pea + groundnuts	3.25^{a}	3.60^{a}	54.0^{a}
	Pigeon pea + soybean	2.97^{b}	2.24^{b}	30.8^{b}
	Pigeon pea + maize	3.37^{a}	2.47^{ab}	35.9^{ab}
	LSD (0.05)	0.25	0.76	22.3
	CV %	8.5	52.5	52.1

Means with different superscripts within the same column are significantly different ($p < 0.05$); ns, not significant at $p<0.05$; CV, Coefficient of variation; LSD (0.05); Least significant difference at 5%; The numbers in parentheses refer to un-adjusted means and this applies only where covariate analysis was done; Number of replicates (n) = 15.

pea. The same trend was observed for groundnuts in that there were no significant differences ($p<0.05$) in biologically fixed nitrogen between sole cropped groundnuts and groundnuts in an intercrop with pigeon pea. However, there were significant differences ($P<0.05$) in biologically fixed nitrogen among pigeon pea cropping systems. Pigeon pea intercropped with soybean indicated significantly the lowest amount of biologically fixed nitrogen and produced 42 % less nitrogen than the highest amount of biologically fixed nitrogen that was obtained in a sole cropped pigeon pea. Sole cropped pigeon pea biologically fixed nitrogen was not significantly different from those of pigeon pea in an intercrop with groundnuts or maize.

Total biologically fixed nitrogen per unit area resulting from sole and doubled-up soybean, groundnut and pigeon pea cropping systems

Table 6 shows the legume total biologically fixed nitrogen. The legume cropping system total biologically fixed nitrogen is the total amount of nitrogen fixed by a crop in the sole cropping system whereas for an intercrop, it is the total sum of nitrogen fixed by both component crops in that system. Under this study, the sum total amount of nitrogen fixed by the component crops in the pigeon pea/groundnuts intercrop was significantly ($p<0.001$) higher than those fixed by any of the other systems. The pigeon pea/groundnuts intercrop resulted in 33, 35, 35 and 57% more nitrogen than that of sole groundnuts, sole pigeon pea, pigeon pea/soybean intercrop and pigeon pea/maize intercrops respectively, pigeon pea/ soybean intercrop however, resulted in nitrogen significantly higher ($p<0.05$) than that for only sole soybean but produced nitrogen amount similar to that for sole pigeon pea. It also produced significantly ($p<0.05$) lower biologically fixed nitrogen than that produced by the pigeon pea/groundnuts doubled-up.

Table 6. Total biologically fixed nitrogen per unit area resulting from sole and doubled-up soybean, groundnut and pigeon pea cropping systems.

Cropping system and statistics	Biologically fixed nitrogen (kg ha^{-1})
Groundnuts	55.8^b
Soybean	35.8^c
Pigeon pea	54.1^b
Pigeon pea + groundnuts	82.8^a
Pigeon pea + soybean	53.6^b
Pigeon pea + maize	35.9^c
LSD (0.05)	15.6
CV %	21.4

Means with different superscripts within the same column are significantly different ($p < 0.05$); CV, Coefficient of variation; LSD (0.05); least significant difference at 5%.

DISCUSSION

The amount of total nitrogen in the initial soil samples from the study site showed to be below the critical value. Though a disadvantage to plant growth, this result could be of importance to this study as it satisfies one of the conditions that increases the reliability of the nitrogen difference method (Danso, 1995). Havlin et al. (2005) reported that excess nitrate in the soil can reduce nitrogenase activity and hence, reduce nitrogen fixation. The estimates showed that sole pigeon pea tissue nitrogen concentration and biologically fixed nitrogen were not significantly different from those in intercrops with groundnuts and maize, but were significantly higher than those in an intercrop with soybean. This can be attributed to competition for nutrients such as nitrogen and phosphorus that was offered to the pigeon pea in the early stages of development by the fast bushy growing indeterminate solitaire soybean. Field observations revealed fast growth by the soybean whereas pigeon pea showed very slow growth. In a similar study, Ghosh et al. (2006) reported lower relative dry matter yield and relative nitrogen yield in pigeon pea that was intercropped with soybean than the sole cropped pigeon pea. In this study, soybean proved to be a strong competitor with pigeon pea for soil nitrogen during the first half of the growing season. Tobita et al. (1996) and Ghosh et al. (2006) observed that relative dry matter yield and nitrogen yield of pigeon pea increased after harvesting in sorghum/pigeon pea and soybean/ pigeon pea intercrops respectively. Similar to the trend of soybean, Rao and Willey (1980) noted that the indeterminate nature of cowpea reduced the yield of pigeon pea. Contrary to the trend in pigeon pea, soybean did not show significant differences in tissue nitrogen concentration and biologically fixed nitrogen. This can be attributed to its fast growth such that it was able to exploit the soil before the slow growing pigeon pea full establishment.

The non-significant differences in plant tissue nitrogen concentration and biologically fixed nitrogen between sole cropped groundnuts and groundnuts in an intercrop with pigeon pea can be attributed to the growing habits and planting pattern of the component crops. Although, a short crop, groundnuts possibly did not face completion for light from the pigeon pea because of enough spaces that were provided during planting (30 cm reserved between pigeon pea planting station and the nearest groundnut planting station). In a similar study, Katayama et al. (1995) reported lower nitrogen yield for groundnuts intercropped with pigeon pea but there were also no significant differences in the proportion of nitrogen derived from the atmosphere in sole cropped groundnuts and groundnuts in an intercrop with pigeon pea.

The total amount of nitrogen biologically fixed per unit area resulting from the cropping systems (including the sum of biologically fixed nitrogen by component crops) revealed significantly higher amount by the pigeon pea/groundnut intercrop than all other cropping systems and this is attributed to reduced competition to both component crops as compared to the pigeon pea/ soybean intercrop.

Conclusions

According to the results of this study, it can be concluded that the amount of biologically fixed nitrogen was affected differently by the sole and doubled-up legume cropping systems. Pigeon pea/groundnut doubled-up proved to be the most beneficial in terms of nitrogen fixation as it resulted into more nitrogen fixed per unit area (82.8 kg N ha^{-1}) than sole crops of pigeon pea (54.1 kg N ha^{-1}) and groundnuts (55.8 kg N ha^{-1}). Pigeon pea/soybean doubled-up lowered amount of nitrogen fixed by the pigeon pea. Growing habits and planting pattern of component crops seemed to be the most important things to be considered when growing legumes in a doubled-up cropping system.

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