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Research Article

N_2 Fixation of Grain Legumes Leading to Beneficial Effect on the Succeeding Maize Crop

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Abstract

In large parts of sub-Saharan Africa, smallholder yields have remained low and declining, and food security is very low. The decline in yield is due to the loss of soil organic matter (SOM), as farmers generally collect all plant matter as animal feed or cooking fuel. Other factors include unavailability of arable land, inherently low soil fertility, insect pests and diseases, and climate change. In addition to wind and water erosion, much of the land degradation is caused by overgrazing, deforestation and intensive cropping. According to researchers, fertilizer use in Sub-Saharan Africa is low (NPK at 8.8 kg). This situation is similar in South Africa. For millennia, humans have utilized legumes as a source of food, animal fodder, traditional medicine, shelter, fuel etc. Legumes such as Groundnut (*Arachis hypogaea* L.) and Bambara groundnut (*Vigna subterranea* (L.) Verdc.), have that special ability to meet more than half of their N requirements through the biological nitrogen fixing-process.

This is achieved through the symbiotic relationship with nitrogen-bacteria. Legumes species cowpea (*Vigna unguiculata* L.), black-gram (*Vigna mungo* L.) and mung beans/green gram (*Vigna radiata* L.), are known to contribute significant amounts of fixed nitrogen to the cropping systems thereby benefiting subsequent non-legume crops or crops grown in rotation with them. They therefore have the potential to promote and sustain agricultural productivity in the low-input farming systems of Sub-Saharan Africa.

Legumes are commonly grown by smallholder farmers in production systems such as sole cropping, Intercropping and rotational cropping. Crop rotation with legumes contributes nitrogen to the soil through mineralization of the legume residues. As a result, crop rotation with legumes is reported to reduce the rate of N applied to succeeding maize (*Zea mays* L.) crops. But more importantly, organic matter (SOM) build-up from crop rotation is crucial for sustainable production as organic matter (SOM) is the life of soil, improving soil structure and texture.

The aim of this study was to evaluate the effect of legume rotation in enhancing productivity and profitability of smallholder cropping systems and alleviate household food and nutritional insecurity.

Keywords: *Vigna unguiculata; Vigna mungo*; Maize; $NdfN_2$

Introduction

Soil fertility and hunger in Africa are the key elements that required the development of this study. The benefit of including legume crops in cropping systems is mostly associated with their ability to biologically fix atmospheric $NdfN_2$ [30]. These were the results of nitrogen/phosphorus nutrition of maize as influenced by rotating legumes and maize fertilization at different levels. Crop rotation and its impact on WUE and nitrogen use component of biological nitrogen fixation $NdfN_2$ as an efficient source of nitrogen for sustainable agricultural production [11].

Figure a

Cereal/legumes crop rotation have the yield advantage and interspecific interactions on nutrients [23]. Effects of organic legume residues and inorganic fertilizer nitrogen appplication on maize crop in Southern Africa [7], are agricultural production increase means and soil nutrient mining in Africa with implications for resource conservation and replacement development [39]. Sustaining productivity of maize-legumes cropping system through integrated nutrient management practices on the sandy loam soils of South Africa exhibited biological nitrogen fixation $NdfN_2$, as an efficient source of nitrogen for sustainable cultural production [19].

Figure b

Figure c

The yield advantage to subsequent maize crops supplementary by legumes depends on the species and amounts of fixed $NdfN_2$ [20]. The benefits of crop rotation can be attributed to 'nitrogen or BNF effect' or/and. ^{15}N Natural abundance method is based on the differences in the natural abundance of the stable N-isotopes (^{14}N and ^{15}N) [38].

This is evident from the table below showing the soil nutrient status on smallholder farms.

Parameters	pH (KCl)	P	K	Ca	Zn	SOC %
		mg kg^{-1}				
Soil nutrient levels	3.9-7.1	2- 63	12-38	48- 1778	0.3- 16.4	0.03-0.71
Optimum for maize	>5.2	>20.0	>40.0	>200.0	>2.0	>0.75
% farms < optimum	54	76	22	46	77	100

Table 1

Table shows the nutrient levels of soils used for maize production on smallholder farms in Mpumalanga, South Africa (N = 85) (C. Mathews and FD. Dakora, unpubl. data).

Objective of the Study

Specific objectives

To determine shoot δ ^{15}N of bambara-groundnut, groundnut, cowpea, blackgram and mung-bean in order to estimate and compare N derived from fixation (Keletso Mohale; 4.77, Alphonsus K. Belane; 15.64; Felix Dapare Dakora; 40.56) [23]. To determine the residual N benefit by the grain legumes to the succeeding maize crop at varying N-levels [19]. To evaluate the yield components and other parameters of the maize crop, grown without N fertilization when succeeding bambara-groundnut, groundnut, cowpea, blackgram and mung-bean [16].

Materials and Methods

Experimental site was conducted at the Lowveld Research Station at Nelspruit in Mpumalanga Province, South Africa. Experimental design of planting for the first year (2011/2012 cropping season), a field experiment was laid out in a randomized complete block design and replicated four times. Each net plot measured 6.3m x 14.3m and 4 rows per plot with zero N: P: K was added to the crops in Year - 1.

Treatment for the first year field experiment see table 2.

For the second year (2012/2013 cropping season), the treatments were: Major factor; previous seasons crops and minor factor are N levels as follows: N1 - No Nitrogen application, N2 - LAN (28) @ 20kg/ha^{-1}, N3 - LAN (28) @ 40kg/ha^{-1} and N4 - LAN (28) @ 60kg/ha^{-1}. Plant spacing was Row x Row = 90 cm and Plant x Plant = 30 cm.

Legume	Plant spacing	Square Metre area	Plants/m²	Plant Population
1. Groundnuts cv. JL 24	70 x 10cm	$0.07m^2$	14.2857	142 857
2. Cowpeas cv. PAN 311	70 x 20cm	$0.14m^2$	7.1429	71 429
3. Mung-bean cv. VC	50 x 10cm	$0.05m^2$	20	200 000
4. Bambara cv. MB 51(Brianbeck)	70 x 20cm	$0.14m^2$	7.1429	71 429
5. Blackgram cv. (local)	50 x 10cm	$0.05m^2$	20	200 000
6. Maize cv. ZM 521	90 x 30cm	$0.27m^2$	3.7037	37 037

Table 2

Data collection

At flowering, ten plant samples of groundnut, bambara-groundnut, cowpea, blackgram and mung-bean were collected per plot along with the shoots of several non - N_2 - fixing plants. The samples were put into a pre-labelled sample bags. The plants were separated into different plant parts (shoots, roots and nodules). The samples were oven-dried at 60 °C for 72 hours and weighed to determine shoot biomass. The shoots of the test legumes and reference plants were ground (0.50 mm) for $^{15}N/^{14}N$ isotopic analysis. At physiological maturity, harvesting for grain yield was carried out.

Statistical analysis

All data collected were tested for normal distribution, before being subjected to analysis of variance (ANOVA) using Statistica-10.1 (StatSoft Inc., Tulsa, OK, USA). Where the means were significant, the Duncan's multiple range test was used to separate the means at $p \leq 0.05$.

Crops selected

Figure d: Groundnut (*Arachis hypogaea* L.).

Figure e: Mung beans/Green gram (*Vigna radiata* L.).

Figure f: Cowpea (*Vigna unguiculata* L.).

Figure g: Black gram (*Vigna mungo* L.).

Figure h: Bambara groundnut (*Vigna subterranea* (L.) Verdc.).

Figure i: Maize (*Zea mays* L.).

Why these plants were selected??

Maize is the major staple food in Southern Africa and South Africa is the major producer in Africa (average production 10 - 12m tons annually) [2]. Belongs to the grass family Poaceae. Legumes are notable in that most of them have symbiotic nitrogen-fixing bacteria in structures called root nodules [29]. For that reason, they play a key role in crop rotation. They are highly nutritious and important source for plant protein [32]. Groundnut is third most important legume grown in South Africa and a major crop grown by the smallholder and emerging farmers in Mpumalanga [1]. Bambara is a minor but popular crop grown by smallholder farmers in the Mpumalanga lowveld and midlevel regions [7]. Cowpea is a major dual-purpose crop grown for its nutritious seeds and foliage mainly by the smallholder farmers. While mung-bean and blackgram are not grown widely in South Africa. They were included because of their local and international demand. Also previous studies in Mpumalanga have shown their yield potential under local environments.

Results and Discussion

Measurement of the amount of atmospheric nitrogen fixed by N-fixing legumes can be done using various methods. For this work, however, the natural abundance technique as described by Shearer and Kohl (1986) [38] will be used. %N concentration in plant material in plots without N fertilizer application under bambara groundnut, blackgram cowpeas, groundnut and mung bean, was 42.86% N/ha^{-1}, 17.86%N/ha^{-1}, 40.00%N/ha^{-1}, 25.00%N/ha^{-1}, 30.00%N/ha^{-1} respectively [36]. Legumes variation differences where bambara groundnut + Cowpeas = 2.86%N/ha^{-1}, bambara groundnut + mung bean = 12.86%N/ha^{-1}, bambara groundnut + groundnut = 17.86%N/ha^{-1}, bambara groundnut + blackgram = 25.00%N/ha^{-1}. However, contribution of residual effect of blackgram was not significant [36]. Performance of legumes varied greatly between species. Of the legumes, Bambara groundnut produced the highest average of 31.14%N/ha^{-1}, blackgram 6.26%N/ha^{-1}, groundnut 12.48%N/ha^{-1}, cowpeas 24.92%N/ha^{-1}, mung bean 18.70%N/ha^{-1}.

The amount of N_2 fixed is primarily controlled by four principal factors: (1) the effectiveness of the rhizobia ± host plant symbiosis, (2) the strength of the sink, i.e. the ability of the host plant to accumulate N, (3) the amount of available soil N and (4) environmental constraints to N_2 fixation [8]. Much of the N fixed by grain legumes is removed at harvest, the remainder becomes available to subsequent crops following mineralization, may be incorporated into the soil organic matter (SOM) [5], or as with fertilizer N, may be lost from the cropping system. This publication reviews some of the agronomic management practices that affect N_2 fixation. To specified grain legumes, also asking whether grain legumes can provide an overall net N benefit to the soil when grown in rotation with other crops [24].

This review will limit itself to biological N_2 fixation by grain legumes and concentrate on N_2 fixation in grain legume ± cereal rotations [4]. The most commonly used methods for the estimation of N_2 fixation are: (1) N balance, based on the difference in total N between a grain legume and a non-N_2-fixing reference crop, and (2) ^{15}N-isotope dilution, either with enriched ^{15}N-fertilizers [12] or through changes at the natural ^{15}N abundance level [38].

First year: Legumes planting

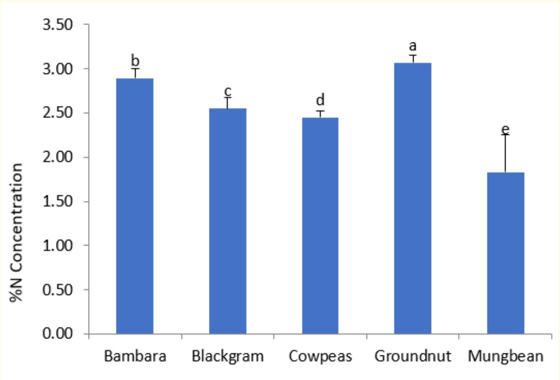

Figure 1: % N concentration in plant material.

Most estimates of N_2 fixation are based solely on above ground plant biomass. But as most of this N is removed in the grain, the importance of belowground deposition of fixed N in maintaining the soil-N balance cannot be ignored [38]. If soil N is sufficient to meet the N demand of the crop, even the most effective rhizobia ± host plant symbiosis will fix little N_2. Data interpretation (See table 3), While mean averages of Bambara groundnut = 31.144%N/concentration/Ha^{-1}, cowpeas = 24.9152%N/concentration/Ha^{-1}, mung bean = 18.6864%N/concentration/Ha^{-1}, groundnut = 12.4576% N/concentration/Ha^{-1} and blackgram = 6.2288%N/concentration/Ha^{-1}.

Therefore, if the control shows a different N uptake pattern than the legume. A subsequent difference in the atom%^{15}N excess value of the legume and cereal, may not solely be the result of an increase in N_2 fixation but also due to the difference in the ^{15}N isotopic composition of the available N pool over time (See figure 3) Most soils are enriched in ^{15}N than ^{14}N isotope due to discrimination against this isotope during volatilization, denitrification, and nutrient uptake by plants [38]. It is a contrast where positive ʒ^{15}N (‰) of groundnuts was achieved, while the most legumes acquired their ʒ^{15}N (‰) from soil N uptake as indicated by figure 3 above. See table 4 give results outcome of figure 3. While averages means of Bambara groundnut = -82 857.2ʒ^{15}N/Ha^{-1}, cowpeas = -66 285.76ʒ^{15}N/Ha^{-1}, mungbean = -49 714.32ʒ^{15}N/Ha^{-1}, blackgram = -33 142.88ʒ^{15}N/Ha^{-1} and groundnut = -16 571.44ʒ^{15}N/Ha^{-1}.

Genotypes/Legume crops	%N concentration/ sample	Square Metre area	Plants/m^2	%N (kg) concentration/ m^2	%N concentration/ Ha^{-1}
Bambara groundnut	30g/sample^{-1} %N	0.14m^2	14.2857	0.42	42.86
Blackgram	25g/sample^{-1} %N	0.05m^2	7.1429	0.17	17.86
Cowpeas	20g/sample^{-1} %N	0.14m^2	20.00	0.40	40.00
Groundnut	35g/sample^{-1} %N	0.07m^2	7.1429	0.25	25.00
Mungbean	15g/sample^{-1} %N	0.05m^2	20	0.30	30.00

Table 3: % N concentration in plant material.

Figure 2: δ15N mill (‰) in plant material.

Measurement of ^{13}C/^{12}C isotopic ratio is a method that is based on mass spectrometric analysis of ^{15}N/^{14}N isotope ratio in finely ground plant tissues. Of which determined the concentration of C in (%C) and ^{13}C/^{12}C isotopic ratios of groundnut, Bambara groundnut, cowpea, blackgram, mungbeans and reference plant species were similarly analysed, as described for ^{15}N. Pee Dee Belemnite limestone was included as a standard notation. The isotopic ration of C was reported in the standard notation relative to the Pee Dee Belemnite standard as Farquhar, Ehleringer and Hubick (1989). Findings in table 5 are outcome of % Carbon concentration in plant material. While averages means of cowpeas = 58.4%C/concentration/ha^{-1}, mungbean = 46.72%C/concentration/ha^{-1}, Bambara groundnut = 35.04%C/concentration/ha^{-1}, groundnut = 23.36%C/concentration/ha^{-1}and blackgram = 11.68%C/concentration/ha^{-1}.

Genotypes/Legume crops	ʒ^{15}N mill(‰)/sample	Square Meter area	Plants/m^2	ʒ^{15}N/m^2	ʒ^{15}N/ Ha^{-1}
Bambara groundnut	- 1.00	0.14m^2	14.2857	-14.29	-142 857
Blackgram	- 0.80	0.05m^2	7.1429	-5.71	-57 143
Cowpeas	- 0.60	0.14m^2	20.00	-12.00	-120 000
Groundnut	0.20	0.07m^2	7.1429	1.43	14 286
Mungbean	- 0.40	0.05m^2	20	-8.00	-80 000

Table 4: δ^{15} N mill (‰) in plant material.

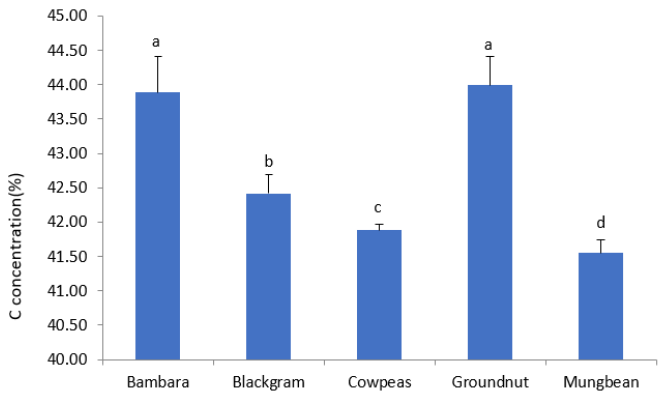

Figure 3: % Carbon concentration in plant material.

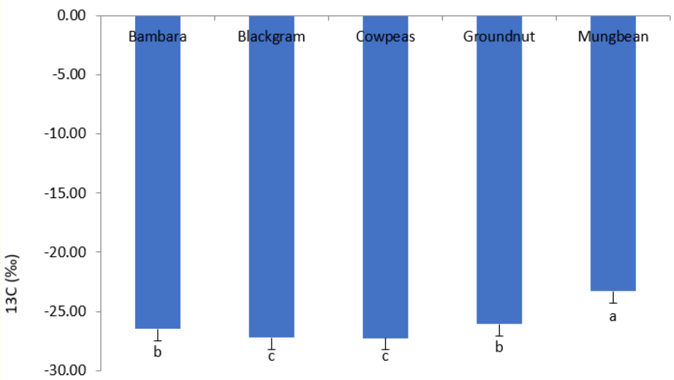

Figure 4: δ^{13}C mill (‰) in plant material.

Genotypes/Legume crops	%C concentration/sample	Square Metre area	Plants/ m²	%C kg/concentration/m²	%C/concentration/ Ha-1
Bambara groundnut	44.00g (%C/plant-1)	0.14m²	14.2857	0.629	63
Blackgram	42.50g (%C/plant-1)	0.05m²	7.1429	0.303	30
Cowpeas	42.00g (%C/plant-1)	0.14m²	20.00	0.840	84
Groundnut	44.50g (%C/plant-1)	0.07m²	7.1429	0.317	32
Mungbean	41.50g (%C/plant-1)	0.05m²	20	0. 830	83

Table 5: % Carbon concentration in plant material.

Where δ^{13}C is the mean ^{13}C natural abundance of the sample in parts per mill (‰), R sample and R standard the $^{13}C/^{12}C$ abundance ratios represents the δ^{13}C mill (‰) in plant material of sample of groundnut, Bambara-groundnut, cowpea, blackgram and mungbean and standard notation respectively. The $^{13}C/^{12}C$ standard used in this study was the isotopic ratio of Belemnite Pee Dee limestone formation of Craig (1957), a universally accepted standard. The below table 6 illustrate discussion data interpretation of figure 5. While averages means of cowpeas = 3 500 004 ʒ^{13}N mill (‰)/ha^{-1}, mungbean = 2 800 003.2 ʒ^{13}N mill (‰)/ha^{-1}, Bambara = 2 100 002.4 ʒ^{13}N mill (‰)/ha^{-1}, blackgram = 1 400 001.6 ʒ^{13}N mill (‰)/ ha^{-1}and groundnut = 7 000 000.8 ʒ^{13}N mill (‰)/ha^{-1}.

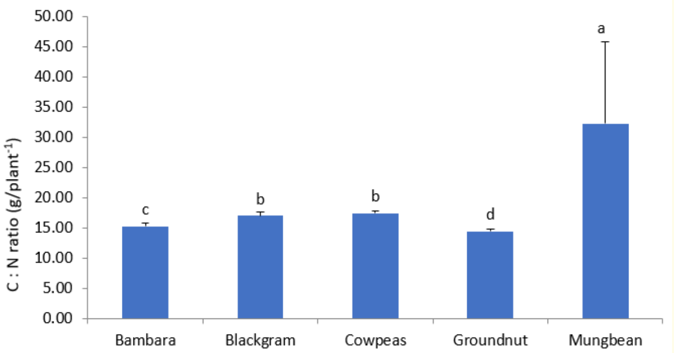

Figure 5: C: N Ratio in plant material.

Genotypes/Legume crops	ʒ^{13}N mill(‰)/sample	Square Metre area	Plants/m²	ʒ^{13}N mill(‰)/m²	ʒ^{13}N mill(‰)/Ha-1
Bambara groundnut	-25.00 parts per mill (‰)	0.14m²	14.2857	-357.14	-3 571 425
Blackgram	-30.00 parts per mill (‰)	0.05m²	7.1429	-214.29	-2 142 870
Cowpeas	-30.00 parts per mill (‰)	0.14m²	20.00	-600.00	-6 000 000
Groundnut	-25.00 parts per mill (‰)	0.07m²	7.1429	-178.57	-1 785 725
Mungbean	-20.00 parts per mill (‰)	0.05m²	20	-400.00	-4 000 000

Table 6: δ^{13}C mill(‰) in plant material.

In the semi-arid prairies the removal or retention of aboveground residue from unfertilized bambara-groundnut, groundnut, cowpeas, blackgram and mungbean, did not alter total soil C and total soil N [18]. Table 7 interpret the above-mentioned figure findings as follows: While the mean variation C:N/(kg)/$ha^{-1}$of mungbean = 29.92C:N (kg)/ha^{-1}, blackgram = 23.933C:N (kg)/ha^{-1}, groundnut = 17.952C:N (kg)/ha^{-1}, cowpeas = 11.968C:N (kg)/ha^{-1} and bambara groundnut = 5.984C:N (kg)/ha^{-1}.

Frequently, by the time a crop has reached pod fill, the available N in the soil has been depleted, and the additional N that is accumulated by the grain legume is derived from N_2 fixation [18] (See figure 7).

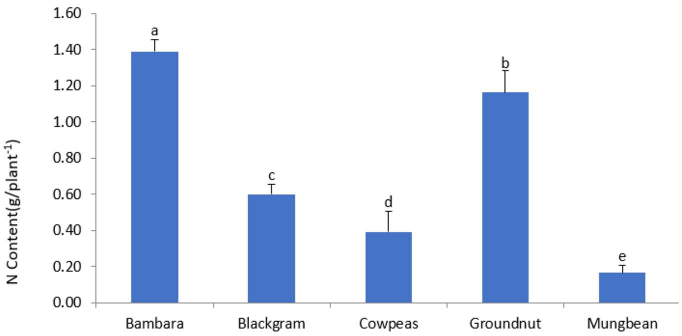

Figure 6: N content in soil.

Genotypes/Legume crops	C : N ratio(g/plant)	Square Metre area	Plants/m^2	C : N/m^2	C : N (kg)/Ha^{-1}
Bambara groundnut	0.15 g/$plant^{-1}$	0.14m^2	14.2857	0.002	10.7
Blackgram	0.18 g/$plant^{-1}$	0.05m^2	7.1429	0.001	36.0
Cowpeas	0.18 g/$plant^{-1}$	0.14m^2	20.00	0.004	12.9
Groundnut	0.14 g/$plant^{-1}$	0.07m^2	7.1429	0.001	20.0
Mungbean	0.35 g/$plant^{-1}$	0.05m^2	20.00	0.007	70.0

Table 7: C: N Ratio in plant material.

In contrast, those legumes caused less nitrogen depletion, and mineral nitrogen contents at the end of maize present thereafter. The above figure explanation of data findings (See table 8). While averages means of groundnut = 91.8 N content (kg)/ha^{-1}, blackgram = 73.44 N content (kg)/ha^{-1}, Bambara = 55.08 N content (kg)/ha^{-1}, mungbean = 36.72 N content (kg)/$ha^{-1}$and cowpeas = 18.36 N content (kg)/ha^{-1}.

The value of grain legumes in cropping systems is predicated on the ability of the grain legume to fix the majority of its N. Unlike %NdfN_2, the total amount of N_2 fixed is yield dependent (See figure 3.7). The ^{15}N natural abundance is then expressed in a relative delta

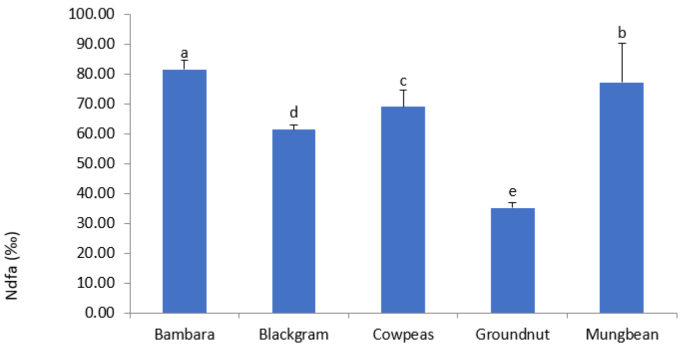

Figure 7: NdfN_2 (‰) in plant material.

Genotypes/Legume crops	N content (g/plant-¹)	Square Metre area	Plants/m^2	N content (kg)/m^2	N content (kg)/Ha^{-1}
Bambara groundnut	1.40 g/$plant^{-1}$	0.14m^2	14.2857	0.019	100
Blackgram	0.60 g/$plant^{-1}$	0.05m^2	7.1429	0.004	120
Cowpeas	0.40 g/$plant^{-1}$	0.14m^2	20.00	0.008	28
Groundnut	1.20 g/$plant^{-1}$	0.07m^2	7.1429	0.008	171
Mungbean	0.20 g/$plant^{-1}$	0.05m^2	20	0.004	40

Table 8: N content in soil.

(δ) notation, which is the ‰ deviation of the ^{15}N natural abundance of the sample from atmosphere N_2 (0.366 atom ‰ ^{15}N). The isotopic composition is thereafter measured according to the following relationship [22]: The most commonly used methods for the estimation of N_2 fixation are: N balance, based on the difference in total N between a grain legume and a non - N_2 fixing reference crop; ^{15}N - isotope dilution, either with enriched ^{15}N fertilizers (Fried., *et al.* 1983) or through changes at the natural ^{15}N abundance level [38]. Discussion of figure 8 would be find in table 9 below. The averages mean variation $NdfN_2$ (‰)/ha^{-1}of mungbean = 9 371 434 $NdfN_2$ (‰)/ha^{-1}, cowpeas = 7 497 142 $NdfN_2$ (‰)/ha^{-1}, Bambara groundnut = 5 622 860 $NdfN_2$ (‰)/ha^{-1}, blackgram = 3 748 573 $NdfN_2$ (‰)/ha^{-1}, and groundnut = 1 874 286 $NdfN_2$ (‰)/ha^{-1}.

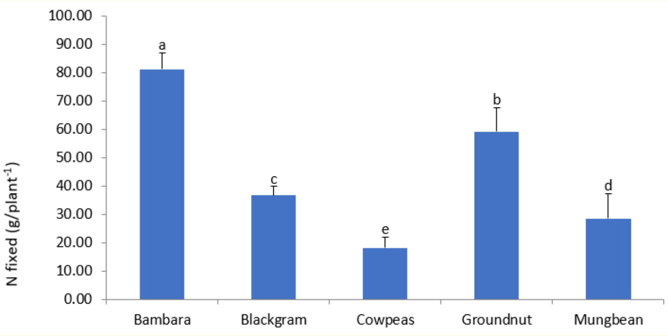

Figure 8: N Fixed in soil.

Genotypes/Legume crops	$NdfN_2$(‰)	Square Metre area	Plants/m²	$NdfN_2$(‰)/m²	$NdfN_2$(‰)/Ha^{-1}
Bambara groundnut	80.00 (‰)	0.14m²	14.2857	1 143	11 428 560
Blackgram	60.00 (‰)	0.05m²	7.1429	429	4 285 740
Cowpeas	70.00 (‰)	0.14m²	20.00	1 400	14 000 000
Groundnut	30.00 (‰)	0.07m²	7.1429	214	2 142 870
Mungbean	75.00 (‰)	0.05m²	20	1 500	15 000 000

Table 9: $NdfN_2$ (‰) in plant material.

Clearly, bambara and groundnut were a better scavenger of available soil N than blackgram, mungbeans and cowpea. N Fixed (kg/ha^{-1}) had a more extensive N fixed (80 g/plant^{-1}) and greater amount of N accumulated (42.86%) per unit concentration than other legumes in the experimental trial (See figure 3.8). As growing conditions in this comparison cannot be standardized, changes over time in the amount of N_2 fixed are not due solely to a crop response to available N but also depend on environmental and management factors that influence crop growth and total N accumulation (Figure 9) [9]. Data discussion of N fixed interaction are showed in, table 10 below. The ranking averages mean variation of Bambara groundnut = 5 866/N fixed kg/ha^{-1}, mungbean = 4 693/N fixed kg/ha^{-1}, groundnut = 3 520/N fixed kg/ha^{-1}, cowpeas = 2 346/N fixed kg/ha^{-1}and blackgram = 1 173/N fixed kg/ha^{-1}.

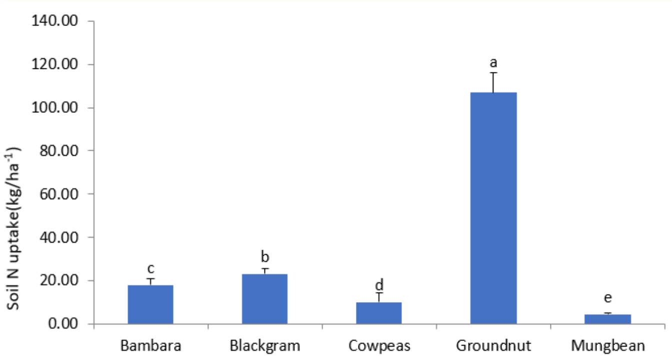

Figure 9: Soil N Uptake.

Genotypes/Legume crops	N fixed (g/plant^{-1})	Square Metre area	Plants/m²	N fixed (kg/m²)	N fixed kg/Ha^{-1}
Bambara groundnut	80g/plant^{-1}	0.14m²	14.2857	1.143	11 430
Blackgram	40g/plant^{-1}	0.05m²	7.1429	0.29	2 900
Cowpeas	20g/plant^{-1}	0.14m²	20.00	0.40	4 000
Groundnut	70g/plant^{-1}	0.07m²	7.1429	0.50	5 000
Mungbean	30g/plant^{-1}	0.05m²	20	0.60	6 000

Table 10: N Fixed in soil.

Therefore, developing grain legumes which are less sensitive to mineral N should not be pursued, unless there is increase in N-uptake (a sink) and an improvement in the overall use efficiency of available N [15]. Furthermore, N uptake by maize increased when grain legumes were part of the rotation as the N supply power of the soil increased when grain legumes were included [38]. As for discussion, the key factor controlling N_2 remains the degree to which N demand can be met by available soil N (See figure 10).

Cultivation of grain legumes will increase total soil N only when the amount of fixed N not removed from the site is greater than the amount of soil N removed in the grain or residue [13]. Data discussion of (Figure 10) is determined in table 11 below. While mean averages of groundnut = 3 029 Soil N uptake/(kg/ha^{-1}), bambara groundnut = 2 423 Soil N uptake/(kg/ha^{-1}), cowpeas = 1817 Soil N uptake/(kg/ha^{-1}), blackgram = 1 211 Soil N uptake/(kg/ha^{-1}) and mungbean = 605 Soil N uptake/(kg/ha^{-1}).

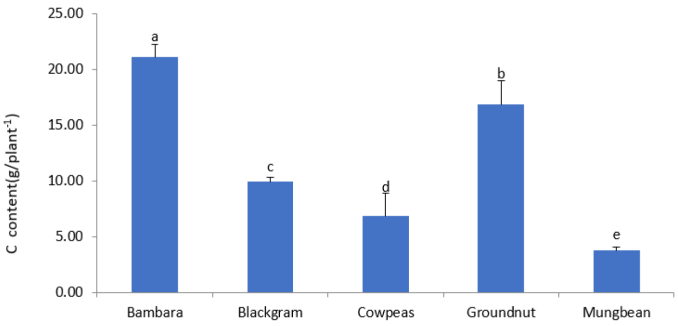

Figure 10: Carbon content.

Genotypes/Legume crops	Soil N uptake (g/Sample^{-1})	Square Metre area	Plants/m^2	Soil N uptake (kg) /m^2	Soil N uptake (kg/Ha^{-1})
Bambara groundnut	15g/plant^{-1}	0.14m^2	14.2857	0.214	2 143
Blackgram	20g/plant^{-1}	0.05m^2	7.1429	0.142	1 429
Cowpeas	10g/plant^{-1}	0.14m^2	20.00	0.200	2 000
Groundnut	120g/plant^{-1}	0.07m^2	7.1429	0.857	8 571
Mungbean	5g/plant^{-1}	0.05m^2	20	0.100	1 000

Table 11: Soil N uptake.

Shoot C content was the shoot C content per plant calculated as the product of %C and shoot dry matter weight. An adequate supply of plant nutrients is essential for the growth of crop plants. About 25 or 30 chemical elements are found in plants while carbon, oxygen, and hydrogen are most abundant. The influence on the carbon and nitrogen content of the soil also indicated that grain legumes cultivated in succession to maize produced significantly higher yields. Studies done in India indicated that crop rotation with leguminous plants did not only increase the yield of the successive maize, but that the total nitrogen and organic carbon content of the soil was increased [5]. Interpretation of data in Figure: 3.10 findings are in table 3.10 below. While the mean averages variation ranking of bambara groundnut = 1 622.8/C content (kg/ha^{-1}), groundnut = 1 298.24/C content (kg/ha^{-1}), cowpeas = 973.68/C content (kg/ha^{-1}), mungbean = 649.12/C content (kg/ha^{-1}) and blackgram = 324.56/C content (kg/ha^{-1}).

Genotypes/Legume crops	C content (g/plant^{-1})	Square Metre area	Plants/m^2	C content (kg) /m^2	C content (kg)/Ha^{-1}
Bambara groundnut	25 C (g/plant^{-1})	0.14m^2	14.2857	0.357	3 571
Blackgram	10 C (g/plant^{-1})	0.05m^2	7.1429	0.071	7 14
Cowpeas	7 C (g/plant^{-1})	0.14m^2	20.00	0.140	1 400
Groundnut	20 C (g/plant^{-1})	0.07m^2	7.1429	0.142	1 429
Mungbean	5 C (g/plant^{-1})	0.05m^2	20	0.100	1 000

Table a

Second year: Crop rotation studies

However, increases in N-deposition in soil do not necessarily reflect an enrichment of the soil N pool, as increases in N input also lead to a potential increase in N losses due to leaching, denitrification, and volatilization. Unfertilized Bambara groundnut, groundnut, cowpea plus black-gram, mung-bean and monocropped maize rotations increased total soil N content after time factor [25]. Although the grain and the aboveground residues was removed, the belowground N contribution was large enough to increase total soil N content and enhance net N mineralization. Additionally, increased fertilizer use leads to an increase in seed yield [5], to increased residue production, and subsequently to an increase in available soil-N.

Between 45 and 75% of the N in the aboveground biomass of grain legumes is removed in the grain at harvest [33]; the harvest index of Bambara groundnut, groundnut, cowpea, black-gram mung-beans and maize (control) ranges from 0.34% (maize) to 0.45% (mung-bean) mean percentages. N harvest index changes with differences in phenology (leafless or semi-leafless), severity of pests, time of seeding, and microclimate variability [21]. Clearly, if grain legumes are to contribute significant amounts of N to the cropping system, %NdfN_2 must be high or the N harvest index low.

Maize grain yield after legumes revealed the fundamentally relationship of maize planted after bambara-groundnut where 60kg/N/ha^{-1} applied was 1 200kg/ha^{-1}. While maize after ground-

Previous crop and N levels	Shoot Biomass (kg/ha)	Grain yield (kg/ha)	Total Above ground Biomass (Kg/ha)	Harvest Index (%)
Maize after Bambara	1071 ± 39b	834 ± 43b	1905 ± 80b	44 ± 0.6ab
Maize after Groundnut	1160 ± 53a	877 ± 43a	2037 ± 95a	43 ± 0.3bc
Maize after Blackgram	959 ± 22d	698 ± 35c	1657 ± 42e	42 ± 1.0c
Maize after Cowpea	1018 ± 28c	819 ± 45b	1838 ± 72c	44 ± 0.8ab
Maize after Mung beans	981 ± 40d	806 ± 35b	1787 ± 74d	45 ± 0.4a
Maize after maize	965 ± 22d	725 ± 59c	1690 ± 79e	42 ± 1.7c
F statistics	55***	28***	67***	6***

Table 12: Influence of selected legumes on the growth and yield of succeeding maize crop, Nelspruit, 2012-2013 cropping season.

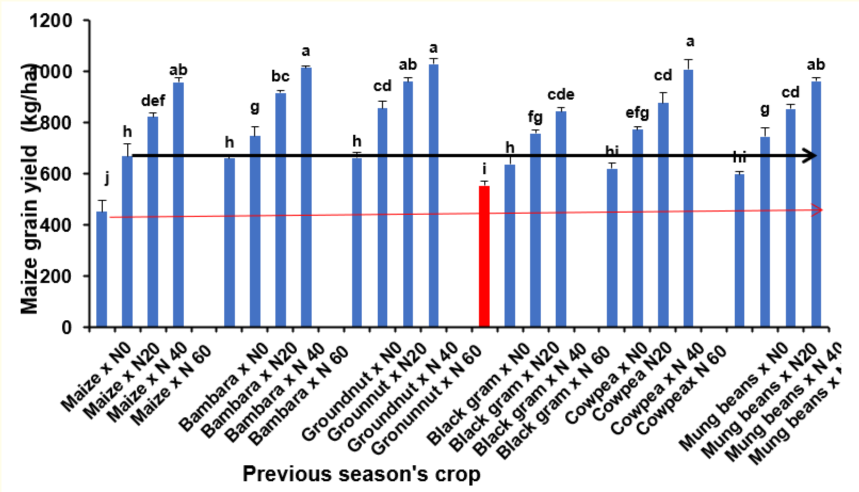

Figure 11: Grain yield (kg/ha^{-1} of maize at different level N application.

nut, grain yield also was 1 200kg/ha-1. The same figure number of maize planted after cowpeas was achieved of 1 200kg/ha-1 where 60kg/N/ha-1 was applied. Grain yield of maize planted after black-gram was 1 000kg/ha-1 where 60kg/N/ha-1 was applied with the same quantity produced by maize after mung-bean where 60kg/N/ha-1 was applied [17]. Maize grain yield after maize yielded 800kg/ha-1 as the lowest grain yield produced where 60kg/N/ha-1 was applied.

Results of maize after Bambara-groundnut grain yield where 40kg/ha-1 N applied gave production yield of 1 000kg/ha-1and where maize planted after groundnut 40kg/ha-1 N applied quantity grain yield was 1 000kg/ha-1 with the same amount as maize planted after cowpeas achieved quantity of 1 000kg/ha-1 [23]. Where 40kg/ha-1 N applied to maize after black-gram 800kg/ha-1 grain yield of maize was harvested. And mung-bean also produced 800kg/ha-1grain yield where maize was planted with 40kg/ha-1 N applied. It is where maize was planted after maize with 40kg/ha-1 N applied, where 600kg/ha-1 grain yield of lowest quantity was achieved.

Where maize was planted with 20kg N/ha-1 after bambara-groundnut, the grain yield of 800kg/ha-1 was in the same quantity with maize planted after groundnut and cowpeas with the same amount of 20kg N/ha-1. Maize planted after black-gram grain yield production quantity was 600kg/ha-1 with the application of 20kg N/ha-1 [31].

The N/20kg/ha-1 applied to maize after mungbean produced grain yield of 600kg/ha-1, while maize planted after maize grain yield production was 400kg/ha-1, as the lowest quantity obtained [26].

If maize was planted after bambaragroundnut and zero N application made, the grain yield quantity obtained was 600kg/ha-1. The same amount of 600kg/ha-1 was produced by maize planted after groundnut and cowpeas with the zero N application. Zero N application of maize planted after black-gram and mung-bean grain yield per ha-1 was 400kg/ha-1, recorded the lowest where Zero N/kg/ha-1 used [31].

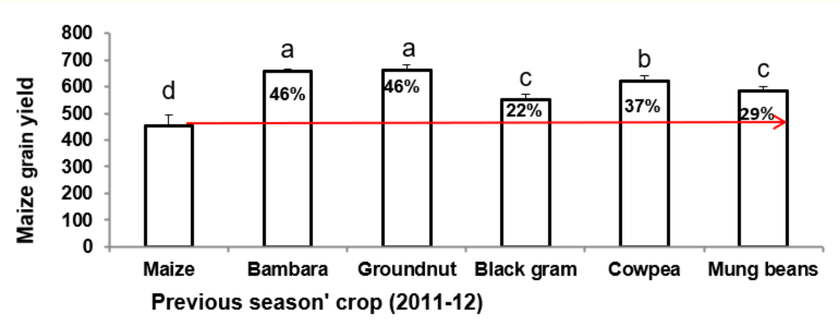

Figure 12: Interaction effect of previous crop and N levels on grain yield of maize planted in rotation during the 2012/2013.

The maize grain yield succeeding legumes benefit of maize after maize N application at different levels (20kg N/ha-1, 40kg N/ha-1 and 60kg/ha-1) contribution was 0 - 19%. Maize after black-gram with all different levels N application as above-mentioned increased from 19% to 22% above the average percentage of maize after maize with all different levels N application. mung-bean succeeded by maize with the application of inorganic nitrogen fertilizer at different levels (20kg N/ha-1, 40kg N/ha-1 and 60kg/ha-1) was 29% increase above maize after black-gram 22% average percentage [35].

Cowpea followed by maize production harvest increased by 8% with the total average percentage of 37% above maize after mung-bean 29% increase. Where bambara-groundnut and groundnut followed by maize, interestingly the different N levels (20kg N/ha-1, 40kg N/ha-1 and 60kg/ha-1) contribution was 46% simultaneously, which revealed same benefit to succeeding rotated maize crop. The outcome results indicate that Ndfa fixed by different legumes genotypes have different benefit in production grain harvest of the following cereal crop. Bambara-groundnut and groundnut genotypes increased grain harvest twofold than maize after maize different levels N application [23].

Conclusion

According to researchers, fertilizer use in Sub-Saharan Africa is low (NPK at 8.8 kg). This situation is similar in South Africa. Legumes such as Groundnut (*Arachis hypogaea* L.) [9] and Bambara groundnut (*Vigna subterranea* (L.) Verdc.) [31], have that special ability to meet more than half of their N requirements through the biological nitrogen fixing-process. Legumes species cowpea (*Vigna unguiculata* L.), blackgram *(Vigna mungo* L.) and mung beans/ green gram (*Vigna radiata* L.) [17], are known to contribute significant amounts of fixed nitrogen to the cropping systems thereby benefiting subsequent non-legume crops or crops grown in rotation with them.

Crop rotation and its impact on WUE and nitrogen use component of biological nitrogen fixation $NdfN_2$ as an efficient source of nitrogen for sustainable agricultural production [37]. Cereal/ legumes crop rotation have the yield advantage and interspecific interactions on nutrients. The yield advantage to subsequent maize crops supplementary by legumes depends on the species and amounts of fixed $NdfN_2$ [40]. Specific objectives was to determine shoot $\delta^{15}N$ of bambara-groundnut, groundnut, cowpea, blackgram and mung-bean in order to estimate and compare N derived from fixation.

The yield increase due to legume rotation at zero nitrogen ranged from 500 to 2000kg/ha^{-1} over maize monoculture (Figure). Mono-cropping led to yield/economic loss (47%). Residual fertilizer benefit was up to 500 kg/ha^{-1} (Figure). This would contribute towards mitigating climate change. Thus, the economic benefit to farmer can be over 47 % excluding the savings made on fertilizer use by including legumes in rotation. Inclusion of legumes in cropping systems as rotational crop is an environmentally-safe option for smallholder farmers in Africa to rejuvenate inherently low fertile soils, enhance household food security as well as raise economic returns while mitigating climate change.

Only field studies that used the ^{15}N-isotope dilution (enriched and natural abundance) or a non-nodulating isoline (maize) and the N difference method to estimate N_2 fixation are included. Similarly, a significant decline in the atom%^{15}N excess value of legumes grown in rotation with a non-nodulating maize crop cannot be interpreted as coming from N transfer and makes conclusions about possible changes in N_2 fixation by rotating grain legumes precarious. Quantifying N transfer between legumes and non-legumes using ^{15}N-isotope dilution methods is dependent on the assumption that changes in the atom$^{\%15}N$ value of the rotating non-legume are caused by transfer of less ^{15}N enriched N from the N_2-fixing legume to the non-legume. The decline in the atom%^{15}N should not reflect a change in competition for N between the legume (Bambara-groundnut, Groundnut, Cowpea, Blackgram, Mung-bean and non-legume (Maize).

Obviously, decline in the atom%^{15}N excess value of the rotated non-N_2-fixing maize was not due to an increase in N_2 fixation, but was likely the result of temporal and spatial differences in the accumulation of available soil ^{15}N by the six plant species over time. Rotating legumes and non-legume increases the opportunity for N-use complimentarily [4]. Determining possible increases in N_2 fixation and N transfer to crops grown after association with legumes remains challenging as the ^{15}N-isotope dilution technique is the only method suited to the study of changes in N_2 fixation and N transfer in rotated systems.

When the ^{15}N-isotope dilution approach is used to quantify difference in N_2 fixation between mono and rotated grain legumes, the assumption is made that the additional dilution of ^{15}N in the rotation of legume compared to the mono-cropped legume is caused by an increase in N_2 fixation. Similarly, Brendel., *et al*. [10] found estimates of N_2 fixation by paired legumes pretense plants grown under different levels of ^{15}N-enrichment 166 above [5] 165 ± 181 were not correlated. It is apparent from these results that the techniques used to obtain field-scale estimates of N_2 fixation by grain legumes will remain semi-quantitative at best.

Using ^{15}N-tracers, George and Singleton [37] noted that field grown Bambara-groundnut, groundnut, cowpea, blackgram and mung-bean was twice as efficient in accumulating mineral N as maize. The ^{15}N-isotope dilution method measures uptake of ^{15}N in an N_2-fixing crop comparing it to that of a reference crop that does not fix N_2. Differences in seasonal N accumulation patterns of legume and reference crop under field conditions, the concurrent decline in atom ‰ ^{15}N of the available soil N pool, and differences in root distribution can lead to erroneous estimates of N_2 fixation.

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Dedication

Untimely passing of Dr Cherian Mathews and his family openly handover his intellectual gift to the Tshwane University of Technology supportive measure of quality scientific studies geared for the benefit of the Biological Nitrogen Fixation colleagues improving their qualifications.

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