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Rotation Benefits From N_2-Fixing Grain Legumes to Cereals: From Increases in Seed Yield and Quality to Greater Household Cash-Income by a Following Maize Crop

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We investigated Bambara groundnut, groundnut, mung bean, cowpea, and black gram for use as biofertilizers in cropping systems. The ^{15}N natural abundance technique was used to measure N_2 fixation in this study. The percent of N derived from fixation by mung bean (*Vigna radiata* L. Wilczek), Bambara groundnut (*Vigna subterranea* L. Verdc.), cowpea (*Vigna unguiculata* L. Walp.), black gram (*Vigna mungo* L.), and groundnut (*Arachis hypogaea* L) was 98, 83, 79, 66, and 45% respectively. Nitrogen contribution from these legumes was 83, 67, 39, 36, and 32 kg.ha^{-1} respectively for Bambara groundnut, groundnut, mung bean, black gram, and cowpea. Maize grain yield without N fertilizer was 2,449, 2,291, 2,204, 2,046, and 1,671 kg.ha^{-1}, respectively, for maize following groundnut, Bambara groundnut, cowpea, mung bean, black gram, and maize. Grain yield increase of maize after legumes without N fertilizer was 47, 46, 37, 32, and 22%, respectively, for groundnut, Bambara groundnut, cowpea, mung bean, and black gram. Supplying 0 to 60 kg N ha^{-1} to maize plants increased shoot DM from 3,264 to 4,279 kg.ha^{-1}, grain yield from 2,184 to 3,586 kg.ha^{-1}, and whole-plant DM from 5,448 to 7,865 kg.ha^{-1}, which represented a 31, 64, and 44% increase with N fertilizer supply from 0 to 60 kg N ha^{-1}. Symbiotic N benefit of preceding legumes to maize without N fertilizer was 20–40 kg N. ha^{-1} in fertilizer equivalents. The preceding legumes increased maize grain concentrations of P, Ca, S, Fe, Mn, and Zn in zero-N plots relative to maize after maize. There was a 225, 222, 154, 149, and 108% increase in marginal returns of maize after groundnut, Bambara groundnut, cowpea, mung bean, and black gram, respectively, without N fertilizer.

Keywords: grain legumes, N_2 fixation, biofertilizers, crop rotation, grain nutritional quality, mineral nutrients, household cash income, marginal returns

INTRODUCTION

Maize is a major staple food crop in Africa, especially in Southern Africa, and is the most important component of smallholder cropping systems in the continent. In Africa, maize is either grown as a monoculture or intercropped with cowpea, groundnut, or Bambara groundnut without any specific planting pattern or rotational system (Mathews and Beck, 1994). Though a staple, maize production in Africa is often constrained by drought and low rainfall, as observed during the 2018 drought in Southern Africa. But maize production can also be low on farmers' fields due to other abiotic factors.

Although commercial farmers in countries like South Africa can obtain maize yields of 4,210–6,470 kg ha^{-1}, grain yield is <2,000 kg ha^{-1} on smallholder fields due to inherently low soil fertility, severe soil degradation, intensive cereal monoculture, low inputs, and inappropriate land use (Von Loeper et al., 2016; DAFF, 2018). While the use of mineral fertilizers could improve maize yields in Africa, they are inaccessible to resource-poor farmers due to their high cost. The low or lack of fertilizer use is currently the major factor limiting increased crop yields (Sinclair and Vadez, 2012) as, on average, only about 8.8 kg NPK fertilizer is applied per hectare by smallholder farmers in Africa (Henao and Baanante, 2006). The inclusion of N_2-fixing legumes in traditional cropping systems can improve soil N fertility and increase crop yields for enhanced food/nutritional security (Walley et al., 2007; Lithourgidis et al., 2011; Ngwira et al., 2012).

The main food legumes cultivated in Africa include cowpea, groundnut, Bambara groundnut, pigeon pea, common bean, and in recent times, soybean. Whether intercropped or cultivated as a monoculture, these legumes are often rotated with cereal crops. While crop rotation is an age-old practice that is not novel, the science behind it is still not properly understood. The work presented here is only a small part of a wider study to understand the changes in soil fertility, soil health, and soil microbial populations using metagenomics. Although all these factors influence yields of cereal crops rotated with legumes, the soil nutrient enrichment, increase in the population of beneficial microbes, and reduction in pathogenic microbes have not been quantified.

About 32 years ago, Dakora et al. (1987) showed that monocultured groundnut and cowpea, respectively, derived 79 and 89% of their N nutrition from symbiotic N_2 fixation, contributed 101 and 210 kg N ha^{-1}, and increased grain yield by 89 and 95% in zero-N plots when maize was rotated with groundnut and cowpea as preceding crops in Northern Ghana. The net N returns to soil in leguminous residues were 68 kg ha^{-1} for groundnut and 150 kg ha^{-1} for cowpea, while the N benefit of each legume to maize in the rotation was equivalent to 60 kg ha^{-1} of N fertilizer based on grain and dry matter yields (Dakora et al., 1987). Clearly, those findings have shown that crop rotation has the potential to improve soil health and increase plant productivity.

In Africa, the N_2-fixing ability and diversity of native soil rhizobia nodulating cowpea, groundnut, Bambara groundnut, common bean, soybean, and Kersting's bean have been established (Chibeba et al., 2017; Puozaa et al., 2017, 2019; Zinga et al., 2017; Chidebe et al., 2018; Gyogluu et al., 2018; Mohammed et al., 2018, 2019). However, their N contribution in cropping systems is still not properly understood. We also do not know the rotation effects of these legumes on the growth, grain yield, and quality of following cereal crops. The aim of this study was to evaluate N contribution by groundnut (*Arachis hypogaea* L.), Bambara groundnut (*Vigna subterranea* L. Verdc.), cowpea (*Vigna unguiculata* L.), black gram (*Vigna mungo* L.), and mung bean (*Vigna radiata* L. Wilczek), and to assess their rotation effect on grain yield, quality, and economics of a following maize crop.

MATERIALS AND METHODS

Description of Experimental Site and Type of Trials

Field experiments were conducted during 2011//2012 and 2012/2013 cropping seasons at Nelspruit (25°26′25″ S, 30°58′57″ E and 640 m above sea level), in the Mpumalanga Province of South Africa. The field trial in 2011/2012 evaluated five grain legumes for plant growth and N_2 contribution, while the field experiment in 2012/2013 measured the rotation effect of each legume on the grain yield and quality of a following maize crop. The rainfall received was 288 mm during the 2011/2012 planting season and 465 mm in the 2012/2013 cropping season. Classified as Avalon series, the soil at the study site is a deep and well-drained sandy-loam (8% clay) with pH 5.95, soil organic carbon (SOC) 0.18%, total nitrogen (N) 7.6 $mg.kg^{-1}$, plant-available phosphorus (P) 19.91 $mg.kg^{-1}$, and potassium (K) 38.86 $mg.kg^{-1}$ (SCWG, 1991).

Field Plot Techniques

The field experiment in 2011/2012 was laid using a randomized complete block design with four replications. A maize plot was included in each replication for assessing the residual effect of these legumes to a following maize crop planted in the 2012/2013 cropping season. Each plot measured 14.4 × 6.3 m in 2011/2012 and the legume species were planted to achieve the plant population shown in **Table 1**. Before planting, 10 soil samples were randomly cored at a depth of 0–30 cm across the experimental site, pooled, sieved, and analyzed for total N, extractable P, K, calcium (Ca), magnesium (Mg) copper, (Cu), zinc (Zn), manganese (Mn), and iron (Fe).

Measurement of Soil Properties

Soil pH was determined in 1 M KCl solution (Black et al., 1965) using a pH meter. The percent soil organic carbon (SOC) was determined as described by Walkley and Black (1934). Extractable P, K, Ca, Zn, and Mg in the soil were determined using the Ambic-1 method developed by Van der Merwe et al. (1984). Extractable P and K were measured on a continuous flow analyzer, and Ca, Mg, and Zn on an atomic-absorption spectrophotometer using an air-acetylene flame. Total N was determined by Kjeldahl digestion (AOAC, 1990).

TABLE 1 | Source and B-values of grain legumes used for estimating %Ndfa.

Grain legume	B-value (‰)	References	Plants.ha^{-1}
Groundnut	−2.700	Nyemba and Dakora, 2010	142,000
Bambara groundnut	−1.400	Nyemba and Dakora, 2010	71,000
Cowpea	−1.759	Belane and Dakora, 2010	200,000
Black gram	−2.200	Unkovich et al., 2008	71,000
Mung bean	−1.140	Unkovich et al., 2008	100,000
Maize	-		37,037

TABLE 2 | Shoot $\delta^{15}N$ (‰) values of reference plants used for estimating %Ndfa in legumes.

Sample no.	Common name	Botanical name	Sample size (n)	$\delta^{15}N$ (‰)
1	Sickle thorn	*Asparagus falcatus*	5	+0.88
2	Starbur	*Acanthospermum hispidum*	2	+1.20
3	Khaki weed	*Alternanthera pungens*	5	+1.50
4	Pig weed	*Amaranthus spinosus*	6	+1.40
5	Wandering jew	*Commelina benghalensis*	8	+1.17
6	Milk weed	*Euphorbia hirta*	10	+0.48
7	Shoe black plant	*Hibuscus rosa-sinesis*	3	+2.64
8	Morning glory	*Ipomoea purpurea*	5	+0.28
9	Calabash	*Lagenaria siceraria*	3	+2.04
10	Brazil pusley	*Richardia brasiliensis*	3	+6.30
11	Castor	*Ricinus communis*	2	+0.27
12	Sesame	*Sesamum indicum*	3	+2.18
13	Devils thorn	*Tribulus terrestris*	3	+7.79
14	Coat buttons	*Tridax procumbens*	1	+1.24
15	Cocklebur	*Xathium strumarium*	2	+0.76
		Combined mean		+2.01

The number of plants sampled (n) per species were pooled, oven-dried, ground, and analyzed for $^{15}N/^{14}N$ ratio.

Plant Sampling and Processing

At flowering to early pod-filling stage, 10 plants were dug out from each plot, and separated into shoots, roots, and nodules. The shoot samples were oven-dried at 70°C for 48 h, weighed, and ground to a fine powder (0.85 mm) for analysis of ^{15}N and ^{13}C. The number of nodules per plant was recorded before oven-drying (70°C) to determine dry weight. Non-legume plants growing inside the plots were concurrently sampled and processed as was done for the legumes. At physiological maturity, 10 plants from the inner plots were harvested for yield determination.

$^{15}N/^{14}N$ Isotopic Analysis of Cowpea Shoots

To determine the $^{15}N/^{14}N$ ratios of plant samples, about 2.0–2.5 mg of plant material was weighed in tin capsules, loaded onto the mass spectrometer, and analyzed using a Carlo Erba NA1500 elemental analyzer (Fisons Instruments SpA, Strada, Rivoltana, Italy) coupled to a Finan MAT252 mass spectrometer (Finnigan, MAT CombH, Bremen, Germany) via a Conflo II open-split device. An internal standard (*Nasturtium* spp.) was included in every five runs to correct for machine error during isotopic fractionation. The isotopic analysis was done for both legumes and reference plants. The combined average $\delta^{15}N$ signature of the non-legume reference plants (+2.01‰) was used to determine the %Ndfa of the test legumes (**Table 2**). The isotopic composition of ^{15}N was measured as (Junk and Svec, 1958; Mariotti, 1983):

$$\delta^{15}\mathrm{N}\,(‰) = \frac{\left({}^{15}\mathrm{N}/{}^{14}\mathrm{N}\right)_{\mathrm{sample}} - \left({}^{15}\mathrm{N}/{}^{14}\mathrm{N}\right)_{\mathrm{atm}}}{\left({}^{15}\mathrm{N}/{}^{14}\mathrm{N}\right)_{\mathrm{atm}}} \times 1000$$

where $^{15}N/^{14}N_{sample}$ was the abundance ratio of ^{15}N and ^{14}N in the plant sample and $^{15}N/^{14}N_{atm}$ was the abundance ratio of ^{15}N and ^{14}N in the atmosphere.

Percent N Derived From Atmospheric N_2 Fixation and N-Fixed

The percent N derived from N_2 fixation (%Ndfa) by the selected legume species was determined as (Shearer and Kohl, 1986; Unkovich et al., 2008):

$$\%\mathrm{Ndfa} = \frac{\delta^{15}\mathrm{N}_{\mathrm{ref}} - \delta^{15}\mathrm{N}_{\mathrm{leg}}}{\delta^{15}\mathrm{N}_{\mathrm{ref}} - \mathrm{B}_{\mathrm{value}}} \times 100$$

where $\delta^{15}N_{ref}$ is the combined mean ^{15}N natural abundance of the non-legume plant species sampled from the experimental plots and used as reference plants (**Table 2**), $\delta^{15}N_{leg}$ is the ^{15}N natural abundance of the legumes tested, and the B value is the ^{15}N natural abundance of the test legumes (Bambara groundnut, groundnut, cowpea, mung bean, and black gram) solely dependent on N_2 fixation for their N nutrition. The B values of the test legumes used in this study are shown in **Table 1**. The B value incorporates the isotopic fractionation associated with N_2 fixation and replaces the value of atmospheric N_2 (Shearer and Kohl, 1986). The amount of N-fixed was calculated as (Maskey et al., 2001):

$$\mathrm{N-fixed} = \%\mathrm{Ndfa} \times \mathrm{legume\ biomass\ N}$$

The N content of plants was estimated as the product of %N and shoot biomass as (Pausch et al., 1996):

$$\mathrm{Shoot\ N} = \mathrm{dry\ matter\ of\ shoot} \times \%\mathrm{N\ of\ shoot}$$

The soil N uptake was calculated as the difference between total N in the shoots and N-fixed.

$^{13}C/^{12}C$ Isotopic Analysis

The $^{13}C/^{12}C$ isotopic ratios in shoots of the test legumes were similarly analyzed as described for $^{15}N/^{14}N$ and reported in the standard notation relative to Pee Dee Belemnite standard as (Farquhar et al., 1989):

$$\delta^{13}\mathrm{C} = \left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}} - 1\right) \times 1000$$

where $\delta^{13}C$ is the ^{13}C natural abundance of the plant shoot sample expressed in parts per million (‰), and R_{sample} and $R_{standard}$ the $^{13}C/^{12}C$ abundance ratios of the shoot sample (Bambara groundnut, groundnut, cowpea, mung bean, and black gram) and standard, respectively. The $^{13}C/^{12}C_{standard}$ used was the isotopic ratio of Belemnite Pee Dee limestone formation (Craig, 1957), a universally accepted standard. Shoot C content per plant was calculated as the product of %C and shoot dry matter weight.

Legume/Maize Rotation

After harvesting the grain, the biomass of each legume was incorporated back into its plots and planted with maize (cv ZM521) as a following crop in the next cropping season. Maize planted after maize was included as control. A spacing of 90 × 30 cm (plant population = 37 037 plants.ha^{-1}) was used. The plots (14.4 × 6.3 m) of preceding legume and maize crops planted in 2011/2012 were each divided into four sub-plots measuring 6.3 × 3.6 m with four rows per plot, and four N levels (N0, N20, N40, and N60 kg.ha^{-1}) super-imposed as sub-treatments using a factorial design with four replications. The five preceding grain legumes and maize planted in the year-1 trial represented the main treatment, and N levels (N0, N20, N40, and N60), the sub-treatments. The trial was planted on 05/10/2012; N was applied (N0, N20, N40, and N60 kg.ha^{-1}) as limestone ammonium nitrate (LAN 28% N) at planting and 9 weeks after planting prior to tasseling. At physiological maturity, shoot dry matter (DM) yield, grain yield, and harvest index were determined from 10 plants per plot. The harvest index was calculated as grain DM divided by the whole-plant oven-dried (60°C) weight times 100 as (Donald and Hamblin, 1976).

$$\text{Harvest index (HI)} = (\text{grain DM}/\text{whole-plant DM}) \times 100\%.$$

Determination of Mineral Nutrients in Maize Grain

The mineral nutrients in maize grain were analyzed at the Soil, Water, and Plant Laboratory, Western Cape Department of Agriculture, Elsenburg, South Africa. Briefly, to measure Fe, Zn, Cu, Mn, and B in cowpea leaves and grain, 1.0 g of ground plant sample was ashed in a porcelain crucible at 500°C overnight, followed by dissolving the ash in 5 ml of 6 M HCl (analytical grade) and placing it in an oven at 50°C for 30 min, after which 35 ml of de-ionized water was added. The mixture was filtered through Whatman No. 1 filter paper. Trace element concentration in plant extracts was determined from four replicate samples using inductively coupled plasma mass spectrometry (IRIS/AP HR DUO Themo Electron Corporation, Franklin, Massachusetts, USA) (Ataro et al., 2008).

Economic Analysis of Maize Grain Yield From Crop Rotation

Economic analyses involving monetary value, total variable costs, gross margin, percentage marginal returns, and cost/benefit ratio were done on maize grain yield produced by the subsequent crop treated to four N levels (i.e., N0, N20, N40, and N60). The economic parameters were estimated as (Gomez and Gomez, 1984):

$$\text{Totalvariable costs (TVC)} = \text{seed cost} + \text{land preparation cost} + \text{agrochemicals cost} + \text{labor cost} + \text{N fertilizer cost}$$
$$\text{Monetary value (MV)} = \text{grain yield} \times \text{market price of grain.ton}^{-1}$$
$$\text{Gross margin (GM)} = \text{monetary value} - \text{total variable costs (TVC)}$$
$$\text{Percent marginal returns (\%MR)} = (\text{gross margin}/\text{monetary value}) \times 100$$
$$\text{Cost/benefit ratio (C/B ratio)} = \text{monetary value}/\text{total variable costs.}$$

The maize market price (in South African Rands) of R2000 per ton (DAFF, 2018) and N fertilizer (LAN) cost of R4500 per ton (GRAIN, 2013) were used to estimate the total variable cost. The gross margin per hectare was based on the estimate of total variable costs per hectare for seed, land preparation, agrochemicals, labor, and N fertilizer, which amounted to R2650, R3238, R3575, and R3913 (in South African Rand currency), for the N0, N20, N40, and N60 treatments, respectively (i.e., the total variable cost was TVC_{N0} = R2650; TVC_{N20} = R3238; TVC_{N40} = R3575; TVC_{N60} = R3913).

Correlation Analysis

Correlation analysis was performed to assess if there was any relationship between fixed-N in the shoots of the preceding legume species and plant growth and/or grain yield of the following maize crop.

Statistical Analysis

The data were tested for normal distribution before being subjected to a 1-way or 2-way analysis of variance (ANOVA) using Statistica version 10.1 (Statsoft Inc., 2011). Where there were significant differences, the Dancan's multiple range test was used to separate the means at $p \leq 0.05$. Pearson's correlation was performed to determine the relationships between yield and symbiotic indices.

RESULTS

Soil Characteristics

The soil used for planting the five legumes in 2011/2012 had pH (KCl) 5.95 and contained 7.65 mg.kg^{-1} N, 19.91 mg.kg^{-1} P, 38.86 mg.kg^{-1} K, 143.50 mg.kg^{-1} Ca, 37.66 mg.kg^{-1} Mg, 3.19 mg.kg^{-1} Zn, 0.18% soil organic matter, and 8.0% clay.

$\delta^{15}N$ of Reference Plants

The $\delta^{15}N$ of non-legume plant species used as reference plants are shown in **Table 2**. Their values ranged from +0.27 to 7.79‰, and it is the combined mean value (+2.01‰) of the 15 plant species analyzed that was used to estimate the percent N derived from fixation by test legumes.

Plant Growth, N_2 Fixation, and N Contribution

There were significant differences in the shoot N concentration of the legume species studied, and these ranged from 2.19%

TABLE 3 | Plant growth, root nodulation, symbiotic performance, and soil N uptake of five grain legumes planted in the field at Nelspruit, South Africa, in the 2011/2012 cropping season.

Legume species	Nodule no. per plant	Nodule DM g.plant^{-1}	Shoot DM g.plant^{-1}	δ^{15}N ‰	N conc'n %	N content g.plant^{-1}	Ndfa %	N-fixed kg.ha^{-1}	Soil N uptake kg.ha^{-1}	δ^{13}C ‰	C/N ratio g.g^{-1}
Bambara	10.0 ± 1.7a	0.15 ± 0.01a	46 ± 2.1a	−0.83 ± 0.11b	2.99 ± 0.1a	1.39 ± 0.1a	83 ± 3.4a	83 ± 7.8a	16 ± 1.22b	−26.44 ± 0.04a	14.74 ± 0.21c
Black gram	22.0 ± 2.0a	0.25 ± 0.05a	22 ± 0.2c	−0.78 ± 0.08b	2.44 ± 0.7b	0.55 ± 0.0c	66 ± 1.9a	36 ± 1.2b	18 ± 1.2b	−27.31 ± 0.09b	17.63 ± 0.31b
Cowpea	15.0 ± 8.2a	0.15 ± 0.07a	24 ± 2.9c	−0.97 ± 0.10b	2.38 ± 0.0bc	0.57 ± 0.1c	79 ± 0.2.7a	32 ± 4.5b	8 ± 1.1c	−27.28 ± 0.10b	17.81 ± 0.08b
Groundnut	40.0 ± 11.6a	0.12 ± 0.04a	34 ± 2.8b	−0.10 ± 0.02a	3.12 ± 0.1a	1.05 ± 0.1b	45 ± 0.4b	67 ± 4.4a	83 ± 1.5a	−26.08 ± 0.14a	14.12 ± 0.42c
Mung bean	10.0 ± 0.9a	0.06 ± 0.01a	10 ± 1.0d	−0.91 ± 0.01b	2.19 ± 0.0c	0.21 ± 0.0d	93 ± 0.4a	39 ± 3.6b	3 ± 0.1d	−27.29 ± 0.21b	19.25 ± 0.11a
F-statistics	3.05 ns	2.80 ns	43.94***	20.23***	36.66***	59.97***	75.08***	21.58***	159.23***	19.7***	73.04***

*Values (Mean ± SE) followed by dissimilar letters in a column are significantly different at *p ≤ 0.05, **p ≤ 0.01, or ***p ≤ 0.001, ns = not significant. The B-values used for estimating %Ndfa were −2.70‰ for groundnut, −2.70‰ for Bambara groundnut, and −1.40‰ for cowpea (Nyemba and Dakora, 2010) as well as −2.20‰ for black gram, and −1.14‰ for mung bean (Unkovich et al., 2008).*

in mung bean to 3.12% in groundnut (**Table 3**). Groundnut and Bambara groundnut showed significantly ($p \leq 0.05$) greater shoot N concentrations than the other legumes. But the shoot N concentration of black gram and cowpea were similar. Nitrogen content was significantly greater in Bambara groundnut than the other legumes (**Table 3**).

The results showed that shoot δ^{15}N was significantly greater ($p \leq 0.05$) in groundnut, followed by Bambara groundnut, black gram, cowpea, and mung bean (**Table 3**). The δ^{15}N in the shoots of the five test legumes ranged from −0.97‰ in mung bean to −0.10‰ in groundnut. As a result, percent N derived from atmospheric fixation (%Ndfa) was also in the range of 45% in groundnut to 93% for mung bean, which reflected the shoot δ^{15}N values of the legumes studied (**Table 3**). The amount of N-fixed was, however, significantly ($p \leq 0.05$) greater in groundnut and Bambara groundnut than the other legumes due to larger shoot biomass (**Table 3**). Legume N contribution by shoots ranged from 32 kg. ha^{-1} in cowpea to 83 kg. ha^{-1} in Bambara groundnut. Nitrogen uptake from the soil was significantly greater in groundnut (83 kg. ha^{-1}) and <20 kg. ha^{-1} in the other legumes which derived more N from fixation (**Table 3**).

Water-Use Efficiency and C/N Ratio

A one-way ANOVA of shoot δ^{13}C values showed significant variation between the test legumes (**Table 3**). The δ^{13}C discrimination was markedly greater (less negative) in groundnut (−26.1‰) and Bambara groundnut (−26.4‰) compared to the other legumes, which recorded −27.3‰. The shoot C/N ratios of the test legume species also revealed substantial differences, which ranged from 14.1 g.g^{-1} in groundnut to 19.3 g.g^{-1} for mung bean (**Table 3**). These differences in shoot C/N could imply potential variation in the decomposition of plant biomass when incorporated into soil.

CROP ROTATION STUDIES

Maize Plant Growth and Grain Yield

Shoot biomass of maize after groundnut was much greater, followed by maize after Bambara groundnut as the preceding crop (**Table 4**). In contrast, the shoot dry matter of maize after black gram was the lowest and similar to maize after maize. In the rotation, whole-plant maize DM (shoot + grain DM) was similar in trend to maize shoot DM, with whole-plant maize DM after groundnut being much higher than the others, followed by Bambara groundnut and cowpea (**Table 4**).

Grain yield also differed significantly between and among the rotation systems. Substantial differences were found in the grain yield of maize following food legumes and maize. This was evidenced by the greater grain yield of maize after legumes (except for black gram) than maize after maize (**Table 4**). More specifically, the grain yield of maize was 3,244 kg.ha^{-1} after groundnut, 3,086 kg.ha^{-1} after Bambara groundnut, 3,032 kg.ha^{-1} after cowpea, 2,909 kg.ha^{-1} after mung bean, 2,582 kg.ha^{-1} after black gram, and 2,682 kg.ha^{-1} after maize as preceding crops.

The supply of mineral N to maize plants in the rotation experiment resulted in significantly increased plant accumulation of shoot biomass and whole-plant dry matter (**Table 4**). Increasing N supply to maize grown after legumes markedly increased shoot biomass, total plant DM, and grain yield relative to the zero-N control (**Table 4**). In all instances, grain yield was much greater at N60 (3,586 kg.ha^{-1}), followed by N40 (3,197 kg.ha^{-1}) and N20 (2,722 kg.ha^{-1}), and lowest in control plants receiving N0 (2,184 kg.ha^{-1}). Shoot biomass and whole-plant DM followed the same pattern, and were similarly much higher at N60, followed by N40 and N20, and lowest in the control N0 plots (**Table 4**). In fact, supplying N0 up to N60 to maize plants increased shoot DM from 3,264 to 4,279 kg.ha^{-1}, yield grain from 2,184 to 3,586 kg.ha^{-1}, and whole-plant DM from 5,448 to 7,865 kg.ha^{-1}, which respectively represented a 31, 64, and 44% increase with N fertilizer supply at 0 compared with 60 kg N ha^{-1}.

Symbiotic N benefit in the rotation was assessed by measuring and comparing maize plant growth and yield from the N0 plots of each preceding legume. As shown in **Figure 1**, shoot biomass, grain yield, and whole-plant DM, respectively, recorded 2,905, 1,671, and 4,576 kg. ha^{-1} for maize after maize; 3,440, 2,440, and 5,879 kg. ha^{-1} for maize after Bambara groundnut; 3,558, 2,449, and 6,008 kg. ha^{-1} for maize after groundnut; 3,229, 2,046, and 5,275 kg. ha^{-1} for maize after black gram; 3,328, 2,294, and 5,622 kg. ha^{-1} for maize after cowpea; and 3,214, 2,204, and 5,328 kg. ha^{-1} for maize after mung bean. The increase

TABLE 4 | Two-way ANOVA of the effect of a preceding legume crop on growth, grain yield, and harvest index of maize planted after legumes in the field and super-imposed with four N levels at Nelspruit in the Mpumalanga Province during the 2012/2013 cropping season.

Preceding crop and N level	Shoot dry matter yield	Grain yield	Whole-plant dry matter	Harvest index
	kg ha^{-1}			%
Preceding crop (PC)				
Bambara groundnut	3,998 ± 139b	3,086 ± 158b	7,084 ± 292b	44 ± 0.63ab
Groundnut	4,245 ± 175a	3,244 ± 158a	7,489 ± 324a	43 ± 0.33bc
Black gram	3,497 ± 83d	2,582 ± 129d	6,079 ± 198d	43 ± 0.67bc
Cowpea	3,710 ± 85c	3,032 ± 166bc	6,741 ± 241c	44 ± 0.77ab
Mung bean	3,661 ± 128c	2,909 ± 154c	6,570 ± 277c	45 ± 0.42a
Maize	3,365 ± 117d	2,682 ± 218d	6,047 ± 331d	42 ± 1.66c
N level				
N0	3,264 ± 64d	2,184 ± 71d	5,448 ± 126d	41 ± 0.89b
N20	3,563 ± 67c	2,722 ± 76c	6,285 ± 132c	43 ± 0.48b
N40	3,878 ± 90b	3,197 ± 64b	7,075 ± 144b	45 ± 0.36a
N60	4,279 ± 106a	3,586 ± 61a	7,865 ± 150a	45 ± 0.42a
F-statistics				
PC	45.3***	29.8***	75.1***	5.2**
N-Level	122.0***	260.0***	381***	31.7***
PC × N level	2.1*	1.9*	2.4*	5.4***

Whole-plant dry matter = shoot biomass + grain dry matter.
*Values (Mean ± S.E.M) followed by dissimilar letters in a column are significantly different at *$p \leq 0.05$, **$p \leq 0.01$, or ***$p \leq 0.001$.*

in rotation benefit of maize after legume crops in the N0 plots ranged from 8 to 22% for shoot biomass, and 22–47% for grain yield (**Figures 1**, **2**).

The fixed-N benefit of the five preceding grain legumes to the following maize crop was estimated using maize grain yield, shoot biomass, and shoot + grain dry matter yield from zero-N plots (**Figures 1**, **2**). Comparing the grain yield from zero-N plots of maize planted after legumes with grain yield of maize after maize receiving N fertilizer showed that the symbiotic N benefit of preceding legumes to the following maize crop was about 20 kg.ha^{-1} in fertilizer-N equivalents for each of the test legumes (**Figure 2**). A similar comparison using whole-plant dry matter yield also showed that the fixed-N benefit was about 20 kg N.ha^{-1} for the five test legumes, except groundnut, which was 40 kg.ha^{-1} (**Figure 2**). Black gram as a preceding legume had little effect on shoot biomass when compared to maize after maize.

Plant harvest index (calculated as grain DM/whole-plant biomass × 100) was much higher for maize planted after mung bean as the preceding crop, followed by cowpea and Bambara groundnut, and lowest for maize after maize, maize after black gram, and maize after groundnut (**Table 4**). With N supply, however, the plant harvest index was significantly increased, and was greater at N60 and N40, followed by N20, and then N0 (**Table 4**).

Nutritional Quality of Maize Grain

Planting maize after legumes significantly increased maize grain quality relative to maize after maize. As shown in **Figure 3**, the grain of maize planted after groundnut recorded higher percentage increases in the concentrations of S, Mn, Fe, and Ca over maize after maize. The percentage increase in the grain levels of P, Zn, Mn, Fe, Cu, and Ca were also significant for Bambara groundnut as the preceding crop. Similar % increases in the concentrations of P, Ca, Zn, Mn, and Fe were found in maize grain after black gram as a preceding crop, just as maize after mung bean recorded a marked increase in the levels of P, Ca, Zn, S, Mn, Fe, and Cu in maize grain. Here, Fe and Cu were the only mineral nutrients whose concentrations were increased in the grain of maize grown after cowpea relative to maize after maize (**Figure 3**).

Economic Analysis of Maize Grain Yield From Rotation

A two-Way ANOVA of selected economic indicators revealed marked differences in profit margins and financial returns on maize grain yield from the positive effect of preceding legume crops. The significantly high maize grain yield after groundnut cultivation led to much greater monetary returns measured in South African currency (Rand) when compared to the other preceding legumes (**Table 5**). The grain yield was next highest in plots with Bambara groundnut and cowpea as preceding crops, and this also led to higher cash income from grain sale (**Table 5**). The gross margin, marginal returns, and the cost/benefit ratio of maize grain sale were similar in trend to the monetary value for each preceding crop (**Table 5**).

In this study, all the four economic parameters (i.e., monetary value, gross margin, marginal returns, and cost/benefit analysis) were expectedly and consistently higher with increasing N fertilizer application from N0 to N60 (**Table 5**). The monetary value of maize grain was thus generally greater with exogenous N supply due to the higher grain yields at increased N levels (**Table 5**). In contrast, the gross margin (GM) and percentage

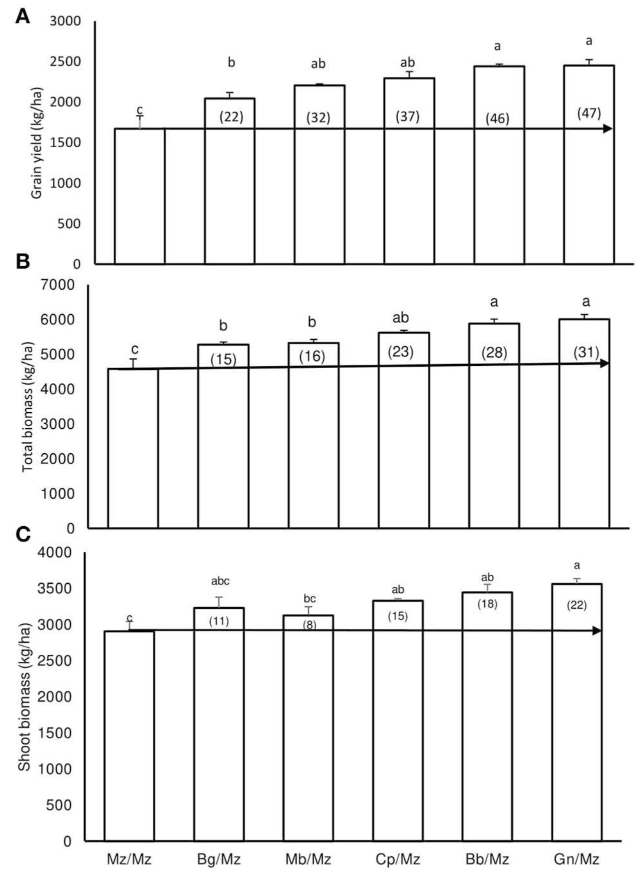

FIGURE 1 | A comparison of **(A)** grain yield, **(B)** whole-plant DM, and **(C)** shoot biomass of maize planted after maize with maize after legumes from zero-N plots at Nelspruit, Mpumalanga Province, in the 2012/2013 cropping season. Values in brackets represent % increases in yield of maize after legume relative to maize after maize as the preceding crop in zero-N plots. The horizontal line delineates increases in yield from zero-N plots caused by a preceding legume crop relative to a maize after maize monoculture. (Mz/Mz, maize after maize; Bg/Mz, maize after black gram; Mb/Mz, maize after mung bean; Cp/Mz, maize after cowpea; Bb/Mz, maize after Bambara bean; and Gn/Mz, maize after groundnut).

marginal returns (%MR) on the sale of maize grain was lower at the higher levels of N application due to the high cost of N fertilizers and the greater labor cost of N application when compared to N0 treatment (**Figure 4**). In essence, the monetary gain as cash income from N contribution by legumes in the cropping system was much higher after cost/benefit analysis than a cereal/cereal system. The increase in financial benefit with regards to percent marginal returns from these legumes over maize after maize at zero-N application was 225, 222, 154, 149, and 108% for groundnut, Bambara groundnut, cowpea, mung bean, and black gram, respectively (**Figure 5**).

Correlation Analysis

There was a strong and significant correlation between the amount of N-fixed by the preceding legumes and grain yield of the following crop, as well as N-fixed by preceding legumes and plant growth (shoot DM) of the following maize crop (**Figure 6**). Thus, symbiotic N in legume residues significantly promoted plant growth and grain yield of the subsequent maize crop in rotation.

DISCUSSION

Legume N Contribution and Water-Use Efficiency

Smallholder farmers account for over 70% of the crops produced in Africa and their grain yield is generally low due to both biotic and abiotic factors. Inherently high climate variability (Lal, 2004), low soil fertility (Dakora and Keya, 1997), high incidence of diseases, insect pests, and parasitic weeds are a major challenge to food production in Africa (Giles, 2007; Chianu et al., 2012). Nutrient depletion in soil is also a major problem affecting crop production by smallholder and resource-poor farmers in developing countries, where most of the grain legumes are produced. Although fertilizer use could increase crop yields in Africa, only a low 8.8 kg NPK fertilizer is applied per hectare per year due to the high cost and inaccessibility to resource-poor farmers (Henao and Baanante, 2006). Therefore, strategies are needed to develop sustainably green and affordable technologies for use by smallholder and resource-poor farmers in Africa in order to enhance soil productivity and ensure food/nutritional security.

In this study, the symbiotic performance of the five grain legumes studied (namely, Bambara groundnut, black gram, cowpea, groundnut, and mung bean) were evaluated in a field trial at Nelspruit in the Mpumalanga Lowveld of South Africa during the 2011/2012 cropping season as a first step in identifying effective technologies for small-scale farmers. The non-N_2-fixing reference plant species used to estimate soil N uptake by the test legumes showed greater $\delta^{15}N$ values (**Table 2**) than the grain legumes, which indicated that the %Ndfa of the legumes studied were reliably estimated using the ^{15}N natural abundance technique (Unkovich et al., 2008).

An assessment of legume performance in the field showed significant differences in plant growth of all the test species, with Bambara groundnut and groundnut exhibiting markedly greater shoot biomass production than the other legumes due to species differences and/or greater shoot N concentration and content. These results are consistent with those of Nyambati et al. (2011), who found a greater increased shoot biomass in Jack bean than hyacinth bean due to high N accumulation in the former.

Shoot $\delta^{15}N$ is a measure of symbiotic functioning in nodulated legumes, with low $\delta^{15}N$ values indicating high N_2 fixation, and greater $\delta^{15}N$ depicting low N_2 fixation. In this study, groundnut showed significantly higher $\delta^{15}N$ values, which resulted in less N derived from atmospheric N_2 fixation relative to the other legumes (**Table 3**). In fact, the %Ndfa was >66% in all the grain legumes, except groundnut which was 45%. As to be expected, this low %Ndfa of groundnut could not meet all of its N requirements. As a result, there was an increased soil N uptake by groundnut, up to 83 kg. ha^{-1} compared with 3–18 kg.ha^{-1} for the other legumes that obtained over 65% of their N nutrition from symbiosis. But the 45% N derived from fixation by groundnut

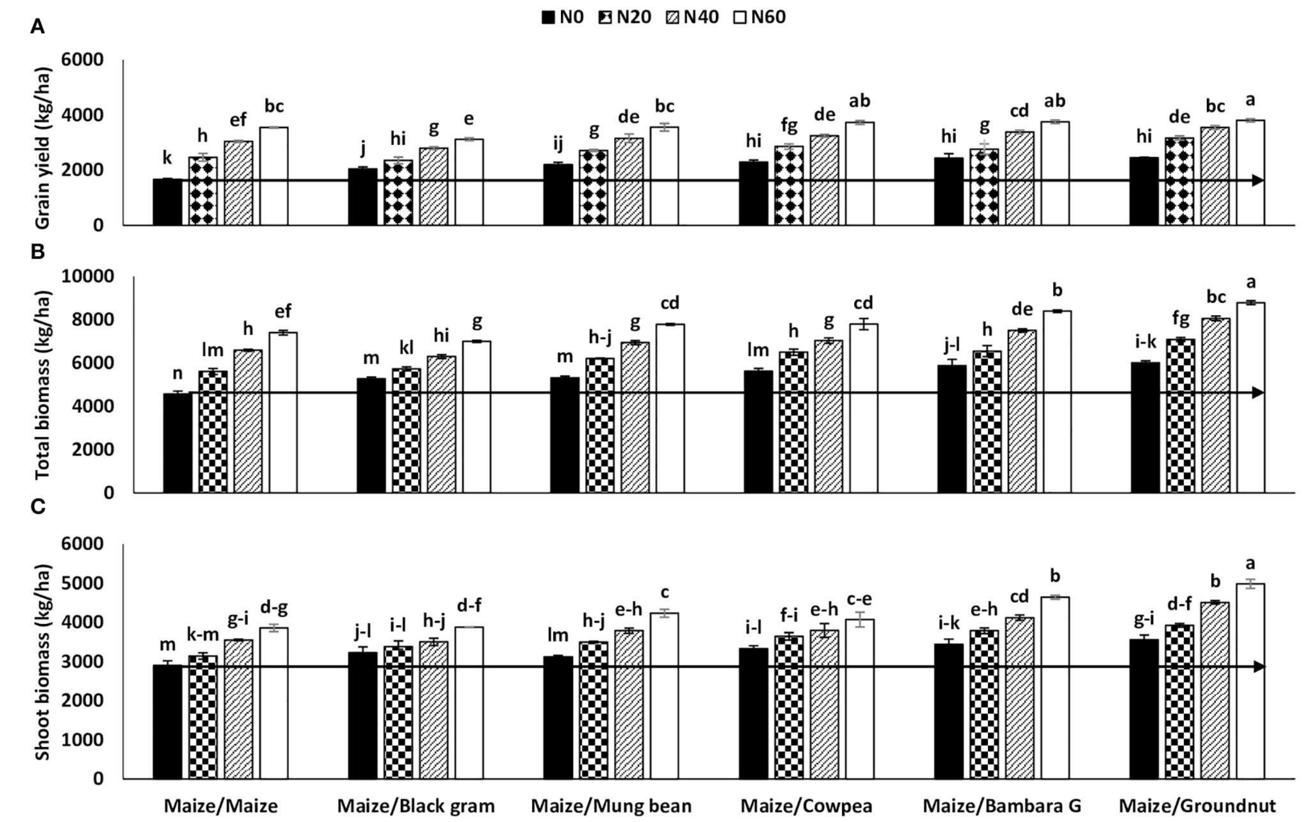

FIGURE 2 | Interactive effect of preceding crop and N fertilizer level on **(A)** grain yield, **(B)** total biomass, and **(C)** shoot biomass of maize planted in rotation at Nelspruit, Mpumalanga Province during the 2012/2013 cropping season. The horizontal line delineates increases in yield caused by N20, N40, and N60 relative to N0.

was nevertheless within the range reported for this legume in Ghana (32–57%), Zambia (27–70%), and South Africa (23–67%) (Nyemba and Dakora, 2010; Mokgehle et al., 2014; Oteng-frimpong and Dakora, 2018). Based on this study, the inclusion of food legumes in cropping systems of smallholder farmers can be a cost-effective and environmentally safe way of enhancing the N nutrition of both the legume and succeeding crops. The net result is sustainably increased yields with reduced soil N uptake, thus eliminating fertilizer N use, which can increase N_2O emission, and hence, global warming.

With climate change, there is a need to select crop species that are drought-tolerant for use in environments with low soil moisture, typically found under rain-fed, dry land conditions in Africa. In C3 plant species, which include members of the Leguminosae, long-term water-use efficiency (or drought tolerance) is commonly measured from analysis of tissue composition of ^{13}C and ^{12}C, the natural isotopes of carbon (Farquhar et al., 1989). In this study, shoot $\delta^{13}C$, which represents a measure of water-use efficiency, ranged from −27.31‰ in mung bean to −26.08‰ in groundnut, a clear indication of significant variations in the water relations of the five legumes tested. The $\delta^{13}C$ values obtained here were similar to those reported elsewhere for cowpea (Makoi et al., 2010) and Bambara groundnut (Mohale et al., 2014). Furthermore, Bambara groundnut and groundnut, which showed much greater $\delta^{13}C$ values (−26.44‰ and −26.08‰, respectively), contributed the highest N to the cropping system, while legume species with the least $\delta^{13}C$ (low water-use efficiency), made much smaller N contributions (**Table 3**). These findings suggest a functional relationship between symbiotic N nutrition and water-use efficiency in nodulated legumes. The significantly greater $\delta^{13}C$ values of Bambara groundnut and groundnut (i.e., better water-use efficiency) was strongly linked to the markedly high shoot N concentration and N content, as well as the increased amount of N-fixed by these legumes, which together supported greater plant growth and biomass accumulation (**Table 3**). These findings could prove useful when selecting legume species for enhanced N_2 fixation and tolerance to drought for use in a changing climate.

Maize Growth, Grain Yield, and Quality in a Legume/Cereal Rotation

Crop rotation is an ancient practice used for sustaining soil productivity, and involves planned successive cultivation of different crops in a specific order on the same field (Karlen et al., 1994). Legumes generally meet their N requirements from symbiotic N_2 fixation, while improving the N nutrition of associated non-legume crops either through N transfer

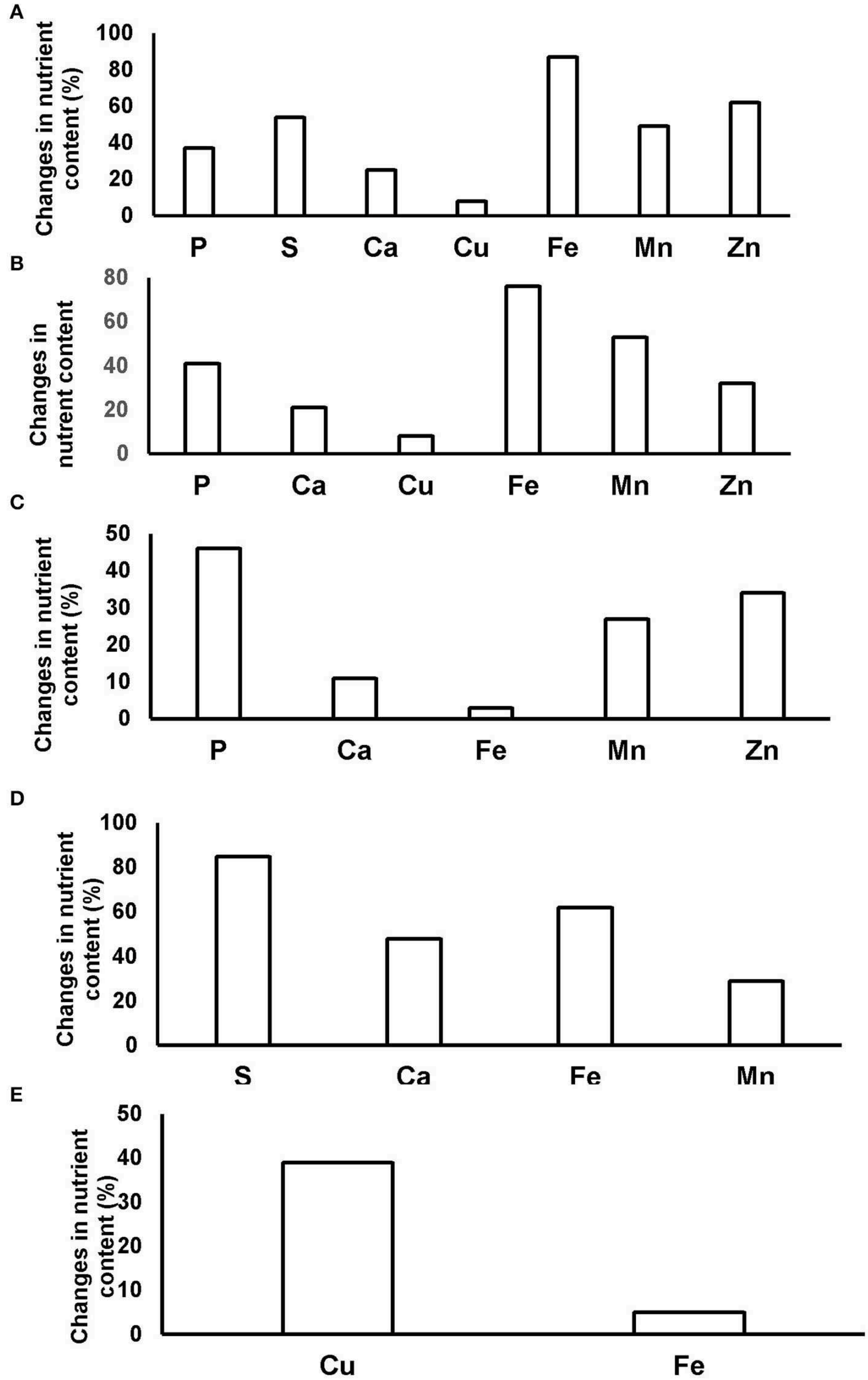

FIGURE 3 | Percentage increases in mineral nutrient composition of maize grain from zero-N plots of **(A)** mung bean, **(B)** Bambara groundnut, **(C)** black gram, **(D)** groundnut, and **(E)** cowpea over maize grain from zero-N plots of maize planted after maize. % mineral A = [(mineral A content of grain from zero-N plots of maize after legume–mineral A content of grain from maize planted after maize from zero-N plots)/(mineral A content of grain from maize grown after maize from zero-N plots)] × 100. Minerals not shown in **Figure 3** did not change significantly relative to maize after maize from zero-N plots.

(Eaglesham et al., 1981), enhancing the N-sparing effect of soil N by the legume, and/or increasing the release of symbiotic N by residues to following crops (Angus et al., 2006; Lithourgidis et al., 2011; Espinoza et al., 2012). Several studies have found substantial N contribution by legumes to subsequent crops, which led to increased plant growth

TABLE 5 | Two-Way ANOVA of the effect of the preceding crop on economic parameters of maize planted in rotation with four N-levels at Nelspruit in the Mpumalanga Province during the 2012/2013 cropping season.

Preceding crop and N-level	Monetary value	Gross margin	Marginal returns	Cost/Benefit ratio
	Currency (South African rand)		%	
Preceding crop (PC)				
Bambara	6,172 ± 317b	2,828 ± 188b	45 ± 1.03ab	1.84 ± 0.03b
Groundnut	6,489 ± 316a	3,145 ± 181a	48 ± 0.70a	1.93 ± 0.03a
Black gram	5,163 ± 257d	1,819 ± 132d	35 ± 1.18c	1.54 ± 0.03d
Cowpea	6,063 ± 231bc	2,719 ± 202bc	44 ± 1.01b	1.80 ± 0.03bc
Mung bean	5,818 ± 308c	2,474 ± 172c	42 ± 0.80b	1.73 ± 0.02c
Maize	5,364 ± 435d	2,020 ± 302d	35 ± 3.66c	1.58 ± 0.07d
N-Level				
N0	4,368 ± 143d	1,718 ± 143d	38 ± 2.57b	1.65 ± 0.05b
N20	5,444 ± 152c	2,206 ± 152c	40 ± 1.76b	1.68 ± 0.05b
N40	6,395 ± 129b	2,820 ± 129b	44 ± 1.15a	1.79 ± 0.04a
N60	7,172 ± 123a	3,259 ± 123a	45 ± 0.99a	1.83 ± 0.03a
F-statistics				
PC	259.7***	29.4***	19.8***	28.9***
N-Level	1.9*	81.1***	10.5***	13.8***
PC × N-level	1.9*	1.9*	2.9*	2.4*

*Means (±S.E.M) followed by dissimilar letters in a column are significantly different at *p ≤ 0.05, **p ≤ 0.01, and ***p ≤ 0.001.*

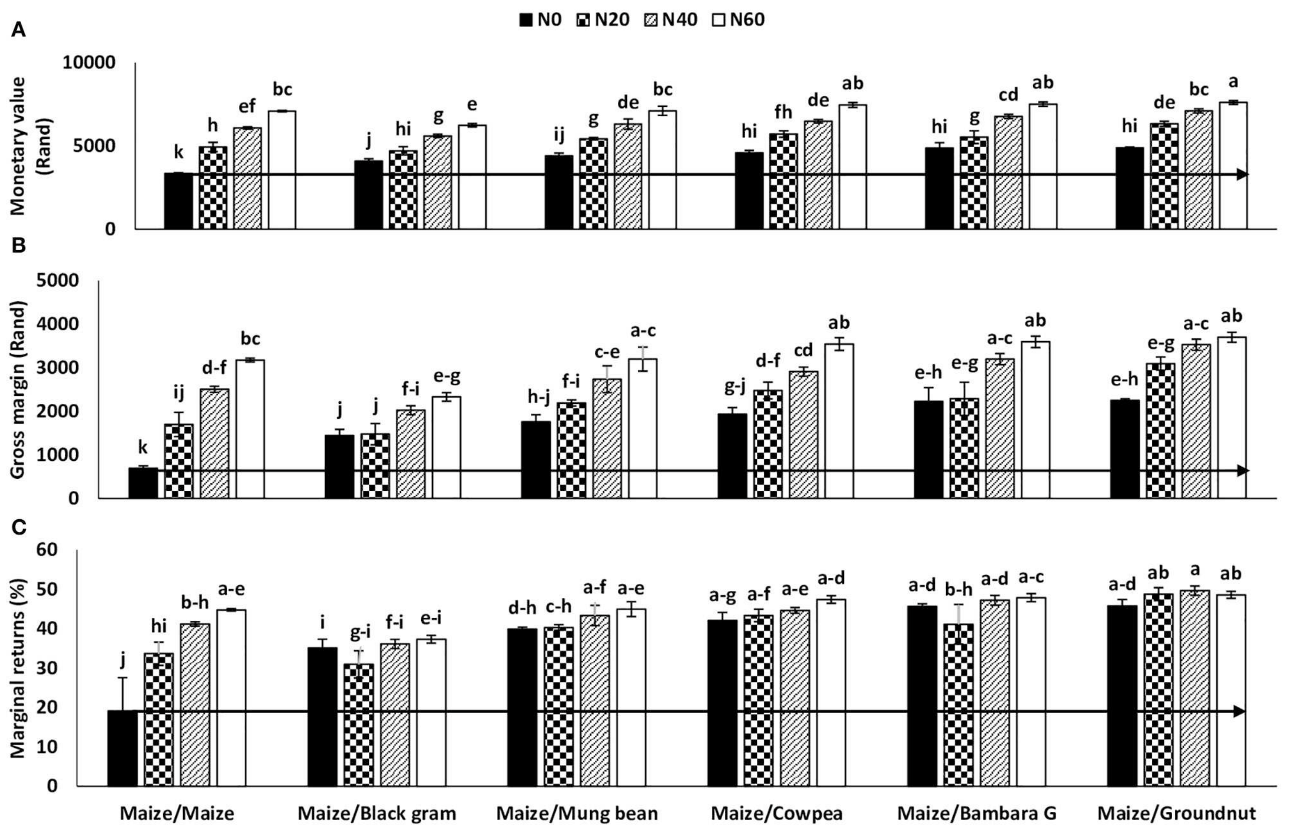

FIGURE 4 | Interaction effect of preceding crop and N fertilizer rate on the **(A)** monetary value, **(B)** gross margin, and **(C)** marginal returns of maize planted in rotation at Nelspruit, Mpumalanga Province during the 2012/2013 cropping season. The horizontal line delineates increases in yield caused by N20, N40, and N60 relative to N0.

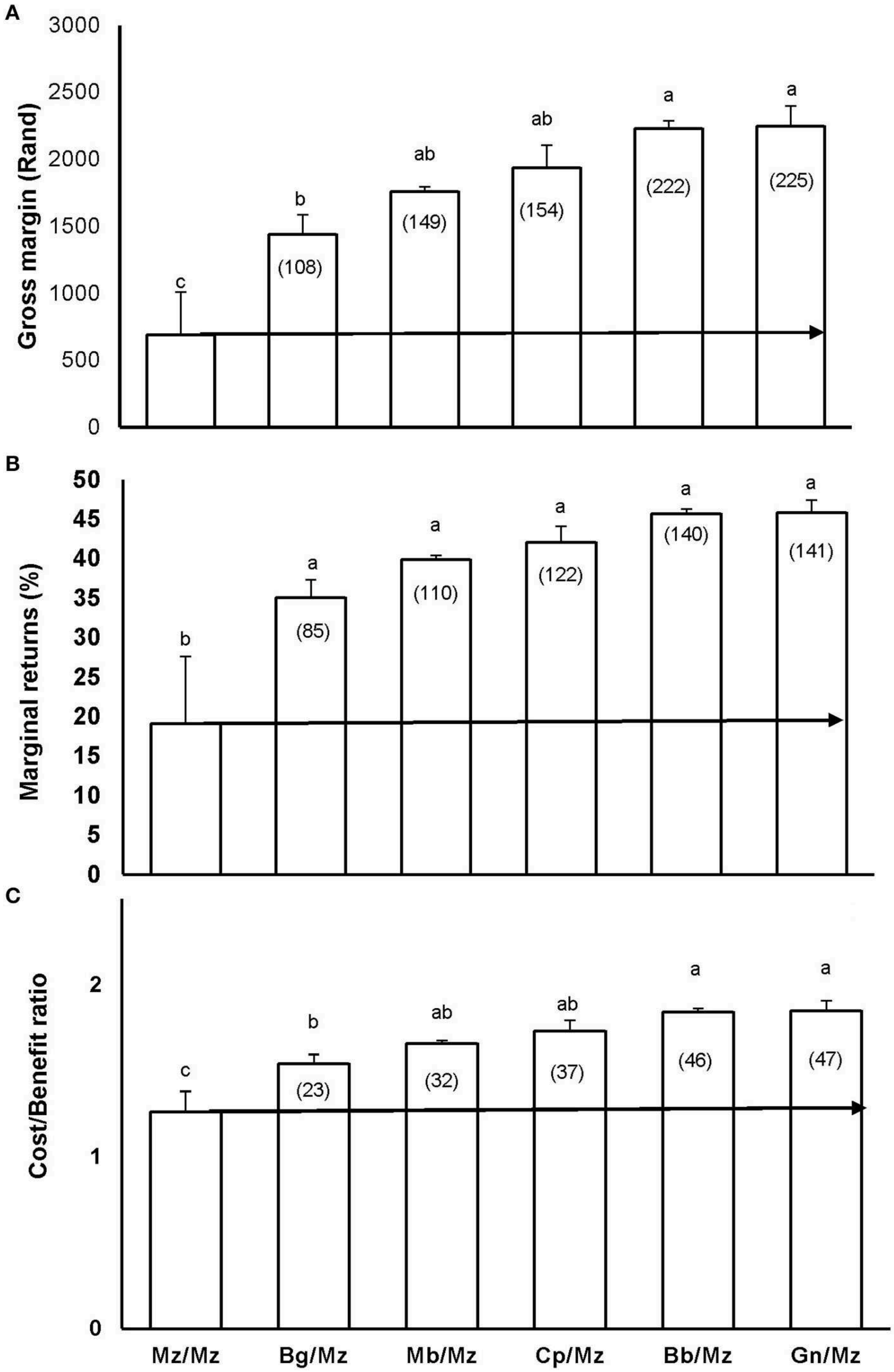

FIGURE 5 | A comparison of **(A)** gross margin, **(B)** marginal returns, and **(C)** cost/benefit ratio of maize after legumes with maize after maize at zero-N application at Nelspruit, Mpumalanga Province, in the 2012/2013 cropping season. Values in brackets represent % increases in yield of maize after legume over maize after maize in zero-N plots of the preceding crop. The horizontal line delineates increases in parameter caused by N20, N40, and N60 relative to N0. (Mz/Mz, maize after maize; Bg/Mz, maize after black gram; Mb/Mz, maize after mung bean; Cp/Mz, maize after cowpea; Bb/Mz, maize after Bambara bean; and Gn/Mz, maize after groundnut).

and grain yield of the following cereal crops (Dakora et al., 1987; Angus et al., 2014; Kirkegaard and Ryan, 2014; Abdel-Galil et al., 2016). The inclusion of symbiotic legumes in cropping systems can therefore help to reduce increased use of N fertilizers in global agriculture, and thus decrease N_2O gas emissions and global warming, thereby mitigating climate change (Dudeja and Duhan, 2005; Sengupta et al., 2015; Stewart, 2015).

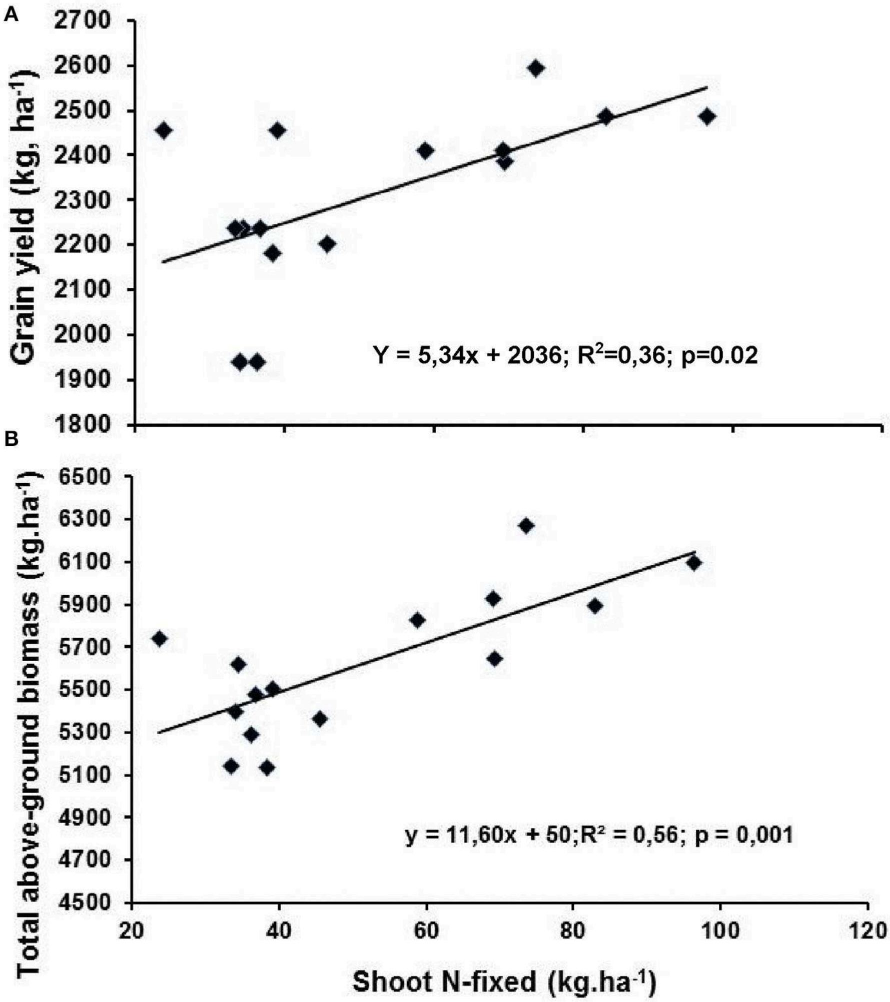

FIGURE 6 | Relationship between **(A)** average amount of N-fixed by all preceding legumes vs. maize grain yield from zero-N plots of the five grain legumes, and **(B)** average amount of N-fixed by all preceding legume and maize shoot DM from zero-N plots of the five grain legumes at Nelspruit, Mpumalanga Province, in the 2012/2013 season.

A comparison of the effect of symbiotic N contribution by the five food legumes to the following maize crop in this study revealed a significant increase in plant growth, shoot biomass, and grain yield of maize planted after legumes relative to maize after maize. The increase in grain yield of maize after legumes was 769, 777, 623, 533, and 375 $kg.ha^{-1}$, respectively, for Bambara groundnut, groundnut, cowpea, mung bean, and black gram from zero-N plots. The positive effect of legumes was also evident from the shoot biomass of maize planted after legumes. The increase in shoot dry matter yield was 535, 653, 423, 324, and 219 $kg.ha^{-1}$, respectively, for Bambara groundnut, groundnut, cowpea, mung bean, and black gram when the zero-N treatments were compared for all five legumes. These increases in maize biomass and grain yield could be attributed to the quantum of symbiotic N in legume residues. This was evidenced by the significant correlations found between the mean amount of N-fixed by all preceding legume vs. plant growth (shoot biomass) or grain yield of the following maize crop (**Figure 6**). Although the soil samples from the maize rotation were mixed up and therefore not analyzed in this study, reports from the same environment revealed soil N was 3.2–4.2% after pigeon pea and 1.0% after sole maize in 2009 (Mathew and Dakora, unpubl. data). These increases could, however, also be attributed to the inclusion of legumes in the cropping system which improved soil fertility and chemical characteristics (McCallum et al., 2004), and probably altered the populations of specific microbes in the rhizosphere to the benefit of the following crops (Osborne et al., 2000; Kirkegaard et al., 2008). As a result, cereal yields after legumes are often 40–80% greater than cereal after cereal without N fertilizer, and these increases can be as high as 450–1,000 kg of additional grain yield per hectare (Seymour et al., 2012). In fact, Dakora et al. (1987) found 89 and 95% increases in grain yield from the zero-N plots of maize after cowpea and groundnut as preceding crops when compared to a maize after maize monoculture. In this study, the increase in grain yield ranged from 375 to 777 kg.ha^{-1}, a finding consistent with the results of maize grain yield after groundnut and cowpea in Ghana (Dakora et al., 1987), or after mung bean, black gram, and soybean in Pakistan (Malik et al., 2006; Naveed et al., 2017).

Furthermore, this study revealed marked differences between and among the test legumes in their ability to promote yield increases of maize planted after legumes. As preceding crops, Bambara groundnut and groundnut, for example, caused greater increase in maize yield than the other legumes (Malik et al., 2006; Naveed et al., 2017). In contrast, there was a small increase in grain yield where maize was planted after black gram, a response similar to maize after maize. These differences in the effect of preceding legumes on maize plant growth and grain yield were likely due to the C/N ratios of the test legume species (**Table 3**). For example, even though Bambara groundnut and groundnut showed much greater shoot biomass, the two species also recorded significantly higher shoot N concentration, N content, and amounts of N-fixed (**Table 3**). As a result, Bambara groundnut and groundnut revealed the lowest C/N ratios among the five legumes tested (**Table 3**). It has, however, been known for a long time that plant residues with low C/N ratios tend to decompose faster under warm tropical conditions (Marschner, 1995; Nicolardot et al., 2001), leading to increased release of N and other mineral nutrients for uptake by subsequent crops. In this study, the low C/N ratio in shoots of Bambara groundnut and groundnut probably led to their increased decomposition and greater release of N and other nutrient elements for uptake by the maize crop following Bambara groundnut and groundnut. This would explain the better growth and increased grain yield of maize after Bambara groundnut and groundnut as preceding crops (**Table 4**). This argument is re-enforced by the results of mineral analysis of maize grain, which showed that maize after Bambara groundnut accumulated more mineral nutrients in grain (P, Ca, Fe, Mn, Zn, and Cu), in the same way that maize after groundnut showed greater mineral concentration (Ca, S, Fe, and Mn) in its grain (**Figure 3**).

The increase in maize grain yield caused by exogenous N supply relative to the zero-N treatment in this study was 355, 711, and 1,101 kg.ha^{-1} for 20, 40, and 60 kg N.ha^{-1}. Nitrogen fertilization thus resulted in 11, 22, and 34% increase in grain yield from supplying 20, 40, and 60 kg N ha^{-1}, respectively, when compared to the zero-N plots, which contained only symbiotic and endogenous soil N. The consistent increase in grain yield with increasing supplemental N suggests that N from the preceding legumes' residues was alone inadequate at meeting the N demand of the following maize crop to produce economic yields (**Supplementary Table 1**). The same could be said of the shoot + grain dry matter yield (**Supplementary Table 1**). The increase in plant growth with exogenous N supply was similar in pattern to grain yield, which confirmed the relationship between dry matter accumulation and grain production (Zhaosu, 1993; Zhang et al., 2008). Based on the grain yield, shoot biomass, and whole-plant dry matter, the symbiotic N benefit of legumes to the succeeding maize crop in rotation was estimated to be between 20 and 40 kg N.ha^{-1} fertilizer equivalents (**Supplementary Table 1** and **Figure 2**). These results are consistent with the findings of previous studies, which also found 20–60 kg N.ha^{-1} fertilizer equivalent when maize was planted as a following crop after grain legumes (Dakora et al., 1987; Myaka et al., 2006).

Economic Benefit of Legume Inclusion in Cropping Systems

For resource-poor farmers in Africa, 375–777 kg extra grain yield per hectare from the inclusion of grain legumes in the cropping system represents a substantial increase in household food security from BNF technology, which should be tapped for farmers' use, especially with the current high cost of N fertilizers. The monetary value of including legumes in the cropping system was also estimated, and found to be substantial, especially when measured against the commonly practiced cereal-after-cereal rotation of smallholder farmers in Africa. The increase in marginal returns recorded in this study was 222, 225, 154, 149, and 108% for Bambara groundnut, groundnut, cowpea, mung bean, and black gram, respectively, over a monoculture of maize after maize without N fertilizer (**Figure 5**). The monetary value of maize after legumes was significantly greater because of the higher maize grain yield produced in the zero-N plots of maize after legumes when compared to the zero-N treatment of maize after maize (**Table 5**). Although the maize grain yield was significantly higher with increased N supply, which resulted in a greater cash income for farmers, the percentage marginal returns from grain yield were markedly lower when the variable costs of fertilizer and labor were included (**Table 5** and **Figure 4**).

The legume/cereal rotation in this study also significantly improved the nutritional quality of maize planted after legumes. Whether produced as food for human consumption or feed for livestock, the grain from maize grown after legumes showed significant percentage increases in the concentrations of dietarily-important mineral nutrients relative to maize after maize (**Figure 3**). A number of studies have similarly found an increase in the levels of protein and nutritionally-important mineral nutrients in the grain of cereal crops planted after legumes (Hauggaard-Nielsen et al., 2006; Lithourgidis and Dordas, 2010). Taken together, this study has demonstrated that the inclusion of nodulated grain legumes as biofertilizers in cropping systems in Africa can significantly increase the grain yield of a following cereal crop, enhance the nutritional quality of maize grain, and overcome food insecurity of small-scale farmers, in addition to raising their household cash-income levels in a sustainable and environmentally-friendly manner.

CONCLUSION

Taken together, this study has demonstrated that the inclusion of nodulated grain legumes as biofertilizers in cropping systems in Africa can significantly increase the grain yield of a following cereal crop, enhance the nutritional quality of maize grain, and overcome food insecurity of small-scale farmers, in addition to raising the levels of their household cash-income in a sustainable and environmentally-friendly manner. The results of this study have provided impetus for conducting similar studies on traditionally neglected and under-researched African food legumes such as the Kersting's groundnut, African yam bean, and mucuna (velvet bean).

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

DL conducted the field experiments, collected and prepared plant samples for isotopes and nutrient analyses, and took part in drafting the manuscript. CM assisted in plant sampling, data analysis, and took part in drafting the manuscript. FD was the supervisor of DL, conceptualized the work, edited the final manuscript, and provided funding for the work. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2020.00094/full#supplementary-material

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