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Nitrogen Fixation and Nutritional Yield of Cowpea-Amaranth Intercrop

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Abstract: Nutrient-poor soils coupled with micronutrient deficiency among many rural resource-poor communities remain a challenge in sub-Saharan Africa. Nutrient-poor soils can be managed through various soil amendment or fertilisation strategies. Micronutrients can be supplied through plants. The study was aimed at determining the symbiotic nitrogen fixation of cowpea as well as the contribution of inter-cropping under varying levels of nitrogen, phosphorus, and potassium (NPK) fertilisation. In addition, the amount of micronutrients supplied by cowpea and amaranth were determined. The experiment was laid out in a 2 × 4 factorial treatment structure in a completely randomised design, with inter-cropping (cowpea and amaranth) and fertiliser (control, 25%, 50%, and 100% of the recommended NPK levels) as treatment factors with four replications. Symbiotic N₂ fixation of cowpea decreased from 341–448 kgN.ha^{−1} to 77–91 kgN.ha^{−1} for the first year and 557–227 kgN.ha^{−1} to 92–164 kgN.ha^{−1} for the second year with fertilisation. The iron and zinc nutritional yield increased (61–210 g.ha^{−1} for first year and 304–867 g.ha^{−1}, for second year), proportional to fertiliser application to both crops. The research shows the benefits of leguminous crops in soil nutrient fertility and inorganic fertilisation with inter-cropping in managing micronutrient deficiency to meet the nutritional needs of rural communities.

Keywords: cowpea; amaranth; inter-cropping; N₂ fixation; hidden hunger; nutritional yield

1. Introduction

Nutrient-poor soils and micronutrient deficiency (also known as “hidden hunger”) are two interrelated challenges, affecting rural resource-poor farming communities in sub-Saharan Africa [1,2]. Hidden hunger is defined as a scenario where intake and absorption of minerals (in particular, essential micronutrients such as zinc and iron) are below amounts needed to maintain good health and development [3]. Poor soil fertility can be addressed through exogenous sources of fertilisation such as the application of organic/inorganic fertilisers and sometimes the use of leguminous crops through their symbiotic N₂ fixation, which provide bio-fertilisers in cropping systems [4]. Low soil fertility affects crop production in rural resource-poor farming communities, reducing nutritional food

security, leading to malnutrition [2,5]. Lack of nitrogen (N), phosphorus (P) and potassium (K) (NPK) in sub-Saharan African soils tends to reduce micronutrient accumulation in plant tissues [6]. There is, in fact, a synergistic relationship between macro-mineral elements such as NPK in soils and trace element accumulation in plant tissues [7], hence the relatedness of the two factors. For example, N and P play an important role in the development of roots, which leads to acquisition, and subsequent translocation of nutrients to edible plant parts constituting economic yield [8]. It is, therefore, important to address these factors in combination due to an escalating rate of population growth which is estimated at 3.09% per annum in Sub-Saharan Africa [9] and hence the need for additional micronutrients in human diets. When soils are low in macro-mineral nutrient elements, it can translate to reduced trace element accumulation in plant tissues, thus escalating “hidden hunger”.

These measures, such as medicine based supplements, food enrichments or nutrients powder inclusion to meals are, however, inadequate to meet the increasing population’s nutritional food needs, largely because of unaffordability [10]. In the quest to address nutritional food insecurity, food-based approaches involving vegetable production in smallholder farming systems are now employed as alternative solutions as they have been proven sustainable [11]. In addition, crop production approaches have also been confirmed effective in addressing household nutritional security [12].

Crop production approaches involve the growth of nitrogen-fixing leguminous crops, such as cowpea (*Vigna unguiculata* L. Walp), which is able to fix up to 337 kg N. ha⁻¹ [13]. In addition, cowpea can supply essential micronutrients [14], resulting in improved nutritional yield. When growing cowpea under subsistence farming system, some rural communities add, for example, inorganic fertiliser in addition to inter-cropping in order to optimise limited agricultural productive land [15]. Inter-cropping by rural resource-poor farmers dates back to ancient civilisation and is today still being practiced [16]. Some common inter-cropping systems entail a combination of a legume and non-legume crop [17]; for example, cowpea and amaranth (*Amaranthus cruentus* L.), both of which are utilised as indigenous vegetables. These indigenous vegetable crops are nutrient-dense particularly in crucial micro-elements such as iron and zinc [18]. Through inter-cropping, the physical, chemical and biological properties of the soil are improved, leading to increased crop growth and yield [19]. In order to measure the yield benefit of inter-cropping, several ratios are commonly used such as land equivalent ratio (LER). [20]. In addition, it has been shown that through inter-cropping (in consideration of benefit from two companion crops), there is more bioavailability of micro-nutrients in terms of concentration, when compared to sole cropping [21]. Micro-nutrient concentration becomes valuable to rural resource-poor communities especially in relation to available land and hence, the concept of nutritional yield (NY). Nutritional yield is defined as a unit of measure, which is a function of edible biomass (for example, leaves) and mineral nutritional composition in plant tissues [22].

There is a paucity of information on the link between symbiotic nitrogen fixation and fertilisation of mostly neglected indigenous vegetables such as cowpea and amaranth grown under inter-cropping system. Furthermore, the information on the effect of different fertilisation levels on nutritional yield of these crops is also limited. This study, therefore, hypothesised that (i) nitrogen fixation in cowpea will be affected by different fertiliser levels, and (ii) cowpea-amaranth intercrop will contribute to an improved NY compared to sole cropping of each crop. The objectives of the study were to assess the effect of fertiliser application and inter-cropping on the nodulation, nitrogen accumulation and symbiotic nitrogen fixation of cowpea. In addition, the effect of fertiliser application and inter-cropping on iron and zinc concentration, as well as nutritional yield of cowpea and amaranth were also assessed.

2. Materials and Methods

2.1. Site Description and Environmental Conditions

The experiment was conducted at the Agricultural Research Council (ARC), Vegetables and Ornamental Plant campus in Roodeplaat, Pretoria, South Africa (25°35′ S, 28°21′ E, 1165 masl) during 2014/15 and 2015/16 summer seasons, from November to January as described by Mndzebele et al. [23].

The description of the soils in which the experimental sites were carried out, as well as the rainfall and temperature conditions during the trials were as detailed by Mndzebele et al. [23].

2.2. Experimental Treatments, Layout and Plot Management

The experimental treatments, layout and the management of the experiment were as described by Mndzebele et al. [23]. These entailed the amount of fertiliser applied, seedling preparation, planting, inter- and intra-row spacing as well as irrigation amounts applied.

Fertiliser (NPK) was applied based on the recommended cowpea requirement taking into consideration the lower fertiliser requirements of the legume. The experiment was laid out in a 2×4 factorial treatment structure in a completely randomised design (CRD) with four replications.

Irrigation scheduling was based on crop water requirement (ET_c) of each crop, either in a sole cropping or in inter-cropping. Crop coefficient (K_c) values for amaranth and cowpea were obtained from Beletse et al. [24].

2.3. Data Collection

2.3.1. Determination of Symbiotic N_2 Fixation in Cowpea

To determine symbiotic N_2 fixation, nodulation (number and mass of nodules formed), relative ureide-N (%RU-N), percentage N derived from N_2 fixation (%Ndfa), N-fixed and soil N uptake of cowpea were determined. The relative ureide-N (%RU-N), percentage N derived from N_2 fixation (%Ndfa), N-fixed and soil N uptake were computed as a factor of above-ground biomass shown in Mndzebele et al. [23]. The choice of the method was informed by the simplicity in the analyses and less costly equipment. Sampling was done for the ureides in the xylem sap, in which ureide N and nitrate N concentrations were obtained to calculate %RU-N, %Ndfa, N-fixed and soil N uptake in cowpea. The %Ndfa was determined using the calibration equation based on root-bleeding sap by Herridge and Peoples [25]. To determine the relative ureide-N abundance, equation 1 was used as shown in Table 1. The amount of N-fixed was calculated as Shearer and Kohl [26], as shown in Table 1. The total soil N uptake was calculated as indicated in Table 1.

Table 1. Equations utilised to compute selected factors.

Equations	Description	Number
Relative ureide abundance (%)	$[\text{ureide-N}/\text{ureide-N} + \text{nitrate-N}] \times 100$	1
N-fixed	$\%N_{\text{dfa}} \times \text{legume biomass-N}$; where, legume biomass N was the N content of AGB.	2
The total soil N uptake	It was calculated by computing the difference between plant total N and N-fixed.	3
$NY_{\text{cowpea (Fe and Zn)}} = [\text{MC} \times \text{AGEB} \times 10]$	$NY_{\text{cowpea (Fe and Zn)}}$ is the cowpea above-ground edible biomass nutritional yield (NY) in g ha^{-1} for iron (Fe) and zinc (Zn); MC is mass concentrations of Fe and Zn ($\text{mg } 100 \text{ g}^{-1}$); AGEB is the above-ground edible biomass (t ha^{-1}).	4
$NY_{\text{amaranth (Fe and Zn)}} = [\text{MC} \times \text{AGEB} \times 10]$	$NY_{\text{amaranth (Fe and Zn)}}$ is the amaranth above-ground edible biomass nutritional yield (NY) g ha^{-1} for iron (Fe) and zinc (Zn); MC is mass concentrations of Fe and Zn ($\text{mg } 100 \text{ g}^{-1}$); AGEB is the above-ground edible biomass (t ha^{-1}).	5
$NY_{\text{Total}} = NY_{\text{cowpea (Fe and Zn)}} + NY_{\text{amaranth (Fe and Zn)}}$	NY_{Total} (g ha^{-1}) is total nutritional yield for iron (Fe) and zinc (Zn); $NY_{\text{cowpea (Fe and Zn)}}$ is the cowpea above-ground edible biomass nutritional yield (NY. g ha^{-1}) for iron (Fe) and zinc (Zn); $NY_{\text{amaranth (Fe and Zn)}}$ is the amaranth above-ground edible biomass nutritional yield (NY) g ha^{-1} for iron (Fe) and zinc (Zn).	6

2.3.2. N-Accumulation, Ureide-and Tissue Nitrate-N of Cowpea at Physiological Maturity

Shoots washed with distilled water to remove debris, were dried and analysed for mineral element concentration. The dried samples were digested using perchloric + nitric acid methods. A mass of 0.5 g of dry sample was digested in a 100 mL volumetric flask containing 7 mL HNO₃ (conc. nitric acid) and 3 mL HClO₄ (perchloric acid) at 180 °C [27]. The digested samples were analysed using the combustion method to determine the concentrations of the nitrogen element. The N content, indicating N-accumulation of cowpea was calculated as a product of N% and sample mass [28]. The sample mass to calculate N-content was obtained from Mndzebele et al. [23]. The ureide-N and nitrate-N were analysed using the Rimini-Schryver reaction as described by Young and Conway [29], in Unkovich et al. [30]. Nitrates in cowpea were quantified using the salicylic acid method of Cataldo et al. [31], as outlined by Unkovich et al. [30].

2.3.3. Measurement of Mineral Element Concentrations in Plant Tissue

Dried leaf samples were analysed for mineral element concentration at the Agricultural Research Council–Soil, Climate, and Water (ARC-SCW) laboratory, Pretoria, South Africa. The dried samples were digested using perchloric + nitric acid methodology. A mass of 0.5 g of dry sample was digested in a 100 mL volumetric flask with 7 mL HNO₃ (concentrated nitric acid) and 3 mL HClO₄ (perchloric acid) at 180 °C [27]. The digested samples were analysed using an Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) to determine the concentrations of the micronutrient elements (specifically Fe and Zn).

2.3.4. Nutritional Yield

Nutritional yield (NY) was calculated for cowpea and amaranth using equations 4 to 6 (Table 1). The computations included the concentrations of Fe and Zn and the above ground edible biomass (AGEB), obtained from Mndzebele et al. [23], for each crop. The total NY factoring both amaranth and cowpea were also determined as shown in equations 4 to 6 in Table 1.

2.3.5. Statistical Analysis

Data were subjected to a two-way analysis of variance with fertiliser levels and cropping system as the main factors. Data analysis was performed using GenStat statistical software (Version 19, VSN International, Hemel Hempstead, UK). Statistically significant treatment means were separated using Fisher least significant difference test at $p \leq 0.05$.

3. Results

3.1. N-Accumulation, Ureide, and Tissue Nitrate-Nitrogen of Cowpea at Physiological Maturity

There was a significant interaction between cropping systems and fertiliser levels with regard to N%, shoot N-content, ureide and tissue NO₃ concentration in cowpea tissues (Table 2). The highest N% and shoot N-content were recorded at 25% NPK fertilisation in both-cropping systems, with the lowest obtained at 100% NPK (Table 2). Nitrogen accumulation (N% and N-content) increased up to 50% NPK fertiliser level in the first season, in both cropping systems. The lowest N-accumulation was observed in the control as well as 100% NPK fertilisation treatments (Table 2). The highest ureide concentration was shown in the interaction of the control fertiliser level with inter-cropping. Ureide concentration showed an inverse proportion in response to fertilisation from control (0%) treatment, up to 100% NPK fertiliser level (Table 2) in both seasons. There were significant ($p < 0.05$) interactions between cropping system and fertiliser level on tissue nitrate concentration in both seasons, with a characteristic increase as the fertiliser level increased up to 50% NPK and a sharp decline at 100% NPK although the concentrations were not significantly different from those of the control (Table 2).

Table 2. Percentage N, shoot N-content, ureides and tissue NO₃-concentration of cowpea fertilised with four different fertiliser (NPK) levels in 2014/15 (S1) and 2015/16 (S2) seasons.

Cropping System [2014/15(S1)]	Fertiliser Level	N	Shoot N-Content	Ureide	Tissue NO ₃ -Concentration	
		%	g.plant ⁻¹	mM	%	
Sole	Fertiliser Levels					
	CONTROL	5.3 ± 0.1a ¹	2.6 ± 0.18b ²	0.05 ± 0.025b ¹	1.5 ± 0.17cd ³	
	25%NPK	5.4 ± 0.2a ¹	4.0 ± 0.30a ¹	0.02 ± 0.002d ²	2.9 ± 0.09b ²	
	50%NPK	4.7 ± 0.2a ¹	4.3 ± 0.28a ¹	0.02 ± 0.003d ²	3.9 ± 0.34a ¹	
	100%NPK	4.7 ± 0.3a ¹	3.6 ± 0.29a ¹	0.01 ± 0.003d ²	1.2 ± 0.04d ³	
	Intercrop	CONTROL	3.4 ± 0.3b ²	1.6 ± 0.24bc ²	0.06 ± 0.013a ¹	2.7 ± 0.25b ¹
		25%NPK	4.9 ± 0.3a ¹	3.5 ± 0.11a ¹	0.05 ± 0.011b ²	1.6 ± 0.05cd ³
		50%NPK	4.8 ± 0.2a ¹	1.8 ± 0.24bc ²	0.03 ± 0.005c ³	2.5 ± 0.18b ¹²
		100%NPK	3.9 ± 0.2b ²	1.0 ± 0.02c ³	0.03 ± 0.013c ³	1.8 ± 0.12c ²
	LSD (<i>p</i> -value)	Cropping System	0.3 (<0.001)	0.39 (<0.001)	0.002 (<0.001)	0.28 (0.155)
	LSD (<i>p</i> -value)	Fertiliser Levels	0.5 (0.007)	0.55 (<0.001)	0.003 (<0.001)	0.40 (<0.001)
	LSD (<i>p</i> -value)	Cropping System X Fertiliser Levels	0.7 (0.005)	0.77 (0.001)	0.005 (<0.001)	0.56 (<0.001)
Cropping System [2015/16(S2)]	Fertiliser Levels					
	CONTROL	3.5 ± 0.1b ²	2.5 ± 0.02b ²	0.052 ± 0.024b ¹	1.8 ± 0.12c ³	
	25%NPK	5.6 ± 0.4a ¹	4.4 ± 0.30a ¹	0.058 ± 0.058b ¹	3.5 ± 0.54bc ²	
	50%NPK	3.6 ± 0.1b ²	2.9 ± 0.10b ²	0.045 ± 0.012bc ¹	5.1 ± 0.36b ¹	
	100%NPK	3.5 ± 0.1b ²	1.8 ± 0.08cd ³	0.046 ± 0.004bc ¹	1.3 ± 0.14c ³	
	Intercrop	CONTROL	2.1 ± 0.3c ²	1.4 ± 0.20d ³	0.084 ± 0.052a ³	3.9 ± 0.31bc ²
		25%NPK	3.5 ± 0.2b ¹	2.3 ± 0.12bc ¹²	0.054 ± 0.009b ³	5.7 ± 1.38b ¹²
		50%NPK	3.6 ± 0.3b ¹	2.8 ± 0.38b ¹	0.034 ± 0.036cd ²	8.7 ± 0.23a ¹
		100%NPK	3.9 ± 0.2b ¹	2.0 ± 0.09cd ²³	0.025 ± 0.029d ¹	1.9 ± 0.15c ²
	LSD (<i>p</i> -value)	Cropping System	0.4 (<0.001)	0.29 (<0.001)	0.007 (0.774)	1.24 (0.002)
	LSD (<i>p</i> -value)	Fertiliser Levels	0.6 (<0.001)	0.40 (<0.001)	0.010 (<0.001)	1.76 (<0.001)
	LSD (<i>p</i> -value)	Cropping System X Fertiliser Levels	0.8 (0.002)	0.57 (<0.001)	0.014 (<0.001)	2.48 (0.387)

Mean ± SE (*n* = 12) values in each column followed by different letters indicate significant differences between treatments (cropping system and fertiliser levels). Numerical values that have been superscripted compare means of each cropping system at different fertiliser levels (*p* ≤ 0.05).

3.2. Symbiotic N_2 Fixation in Cowpea

There was a significant ($p \leq 0.05$) increase in nodulation (nodule biomass and number of nodules) of cowpea at physiological maturity in sole and inter-cropping when fertilisation was increased from control (0% NPK) up to 50% NPK, with an abrupt decline at 100% NPK (Table 3). The lowest nodulation was observed at the 100% NPK fertiliser level in both seasons, in sole and inter-cropping although not significantly different from those obtained at 25% NPK (Table 3). Inter-cropping in combination with 50% NPK fertilisation gave the highest nodule mass, in both seasons. In addition, the highest number of nodules was shown in sole-cropping in combination with 25% NPK fertilisation in both seasons. Cropping system comparison showed more nodule mass and the number of nodules in sole cropping relative to intercropping. The relative ureide-N (%RU-N) and the percentage N derived from N_2 fixation (%Ndfa) in cowpea was inversely proportional to the amount of fertiliser applied, with a significant decrease from control (0%) to 100% NPK in both cropping systems (sole and inter-cropping) in both seasons (Table 3).

N-fixed significantly decreased with the added amount of fertiliser application from the control (0% NPK) up to the 100% NPK in sole cropping system in the first season. However, there was an exception with the other treatment where there was a gradual increase from the control (0% NPK) until 25% NPK, which was the highest, while 100% NPK gave the lowest. There was a general characteristic increase in soil N-uptake with increase in fertilisation up to 50% NPK in both cropping systems and in both seasons. On the other hand, inter-cropping recorded the highest soil N-uptake in combination with 50% NPK in the second season (Table 3).

3.3. Nutrient Concentration and Nutritional Yield in Cowpea and Amaranth

Overall, there was a significant characteristic increase in iron (Fe) and zinc (Zn) concentration of cowpea and amaranth leaves as the fertiliser level increased from the control (0%) to 100% NPK treatments in sole and inter-cropping and in both seasons (Table 4). Similarly, nutritional yield (Fe-NY, Zn-NY and Fe+Zn-NY) (Table 5) followed the same trend to that of the nutritional concentration except for the intercropping treatments, which showed an increase from 0% NPK to 25% NPK followed by a decline beyond these fertiliser levels for both crops in both seasons.

Table 3. Nodule mass, number of nodules, %RU-N, %Ndfa, N-fixed and soil N uptake of cowpea fertilised with four different fertiliser (NPK) levels in 2014/15 and 2015/16 seasons.

Cropping System [2014/15(S1)]	Fertiliser Levels	Nodule Mass	Nodule Number	RU-N	%Ndfa	N-Fixed	Soil N-Uptake
		g.plant ⁻¹	per.plant ⁻¹	%	%	kgN.ha ⁻¹	kg.ha ⁻¹
Sole	CONTROL	6.0 ± 0.2d ³	54 ± 4bc ¹²	75 ± 2a ¹	91 ± 3a ¹	341 ± 23b ¹	34 ± 2b ²
	25%NPK	9.0 ± 0.3c ²	99 ± 5a ¹	32 ± 1c ²	24 ± 2c ²	138 ± 18de ²	438 ± 32a ¹
	50%NPK	11.3 ± 0.6ab ¹	59 ± 4b ¹²	28 ± 2d ³	17 ± 1d ³	104 ± 5def ²³	509 ± 37a ¹
	100%NPK	6.0 ± 0.4d ³	27 ± 1c ²	26 ± 3d ³	15 ± 1d ⁴	77 ± 2f ³	438 ± 34a ¹
Intercrop	CONTROL	6.0 ± 0.4d ²	34 ± 3bc ²	73 ± 5a ¹	88 ± 4a ¹	202 ± 2c ²	28 ± 3b ³
	25%NPK	10.3 ± 0.5bc ¹	30 ± 2bc ²	73 ± 3a ¹	88 ± 2a ¹	448 ± 17a ¹	61 ± 3b ²
	50%NPK	12.7 ± 0.5a ¹	43 ± 2bc ¹	54 ± 3b ²	57 ± 5b ²	149 ± 2d ³	113 ± 11b ¹
	100%NPK	5.0 ± 0.4d ²	35 ± 2bc ¹²	53 ± 5b ²	61 ± 1b ²	91 ± 9ef ⁴	58 ± 4b ²
LSD (<i>p</i> -value)	C. System	1.1 (0.426)	14 (0.003)	2 (<0.001)	3 (<0.001)	23 (<0.001)	45 (<0.001)
LSD (<i>p</i> -value)	F. Levels	1.5 (<0.001)	20 (0.023)	3 (<0.001)	4 (<0.001)	33 (<0.001)	64 (<0.001)
LSD (<i>p</i> -value)	C. System X F. Levels	2.2 (0.330)	28 (0.008)	4 (<0.001)	6 (<0.001)	47 (<0.001)	90 (<0.001)
Cropping System [2015/16(S2)]		Fertiliser Levels					
Sole	CONTROL	3.7 ± 0.3de ³	42 ± 4bc ¹²	74 ± 1a ¹	88 ± 2a ¹	319 ± 3bcd ²	43 ± 2cd ²
	25%NPK	6.0 ± 0.4bd ²	76 ± 4a ¹	74 ± 1a ¹	89 ± 2a ¹	557 ± 2a ¹	69 ± 5cd ²
	50%NPK	8.3 ± 0.7abc ¹	45 ± 2b ¹²	68 ± 2ab ¹	80 ± 2ab ¹	330 ± 5b ²	82 ± 3b ¹
	100%NPK	3.0 ± 0.6e ³	21 ± 1c ²	56 ± 3cd ²	62 ± 2cd ²	164 ± 4bc ²	101 ± 2b ¹
Intercrop	CONTROL	4.0 ± 0.5de ²	23 ± 2bc ²	76 ± 3a ¹	92 ± 4a ¹	185 ± 17cd ¹	16 ± 2d ³
	25%NPK	8.3 ± 0.6b ¹	26 ± 2bc ²	62 ± 2bc ²	70 ± 3bc ²	227 ± 6bcd ¹	97 ± 6bc ²³
	50%NPK	10.7 ± 0.5a ¹	33 ± 2bc ¹	50 ± 1d ³	51 ± 2d ³	207 ± 3d ¹	199 ± 3b ¹
	100%NPK	3.0 ± 0.5e ²	27 ± 2bc ¹²	37 ± 1e ⁴	32 ± 2e ⁴	92 ± 6d ¹	195 ± 12a ²
LSD (<i>p</i> -value)	C. System	1.1 (0.033)	11 (0.003)	5 (<0.001)	8 (<0.001)	48 (<0.001)	34 (0.015)
LSD (<i>p</i> -value)	F. Levels	1.6 (<0.001)	16 (0.023)	7 (<0.001)	11 (<0.001)	69 (<0.001)	48 (<0.001)
LSD (<i>p</i> -value)	C. System X F. Levels	2.3 (0.279)	22 (0.008)	10 (0.006)	16 (0.006)	97 (0.059)	68 (0.007)

Mean ± SE (*n* = 12) values in each column followed by different letters indicate significant differences between treatments (cropping system and fertiliser levels). Numerical values that have been superscripted compare means of each cropping system at different fertiliser levels (*p* ≤ 0.05). C. System and F. levels represents cropping system and fertiliser level respectively.

Table 4. Moisture content, iron mass concentration (Fe) and zinc mass concentration (Zn) of cowpea and amaranth under sole and inter-cropping systems at four fertiliser (NPK) levels in 2014/15 and 2015/16 summer seasons.

Cropping System [2014/15(S1)]		Cowpea			Amaranth		
		Moisture Content	Fe	Zn	Moisture Content	Fe	Zn
	Fertiliser Levels		mg.100g ⁻¹	mg.100g ⁻¹		mg.100g ⁻¹	mg.100g ⁻¹
Sole	CONTROL	0.869 ± 0.006d ³	25.7 ± 1.5d ⁴	2.2 ± 0.1e ³	0.908 ± 0.006b ¹	41.9 ± 1.7c ²	3.1 ± 0.03a ¹
	25%NPK	0.908 ± 0.006b ²	60.4 ± 1.0c ³	3.4 ± 0.1cd ²	0.916 ± 0.005ab ¹	44.3 ± 4.2bc ¹²	3.1 ± 0.16a ¹
	50%NPK	0.919 ± 0.006ab ¹²	75.1 ± 5.3b ²	3.6 ± 0.1bcd ²	0.918 ± 0.005ab ¹	45.7 ± 1.5bc ¹²	3.5 ± 0.33a ¹
	100%NPK	0.927 ± 0.004a ¹	92.3 ± 4.1a ¹	4.4 ± 0.1a ¹	0.931 ± 0.013ab ¹	54.8 ± 4.0ab ¹	3.7 ± 0.34a ¹
Intercrop	CONTROL	0.843 ± 0.008e ³	23.7 ± 1.4d ³	3.3 ± 0.1d ²	0.912 ± 0.008ab ¹	41.9 ± 1.7c ²	3.1 ± 0.08a ¹
	25%NPK	0.847 ± 0.008e ³	24.1 ± 1.4d ³	3.3 ± 0.1d ²	0.917 ± 0.010ab ¹	48.0 ± 2.1bc ²	3.2 ± 0.16a ¹
	50%NPK	0.890 ± 0.008c ²	52.3 ± 1.6c ²	3.8 ± 0.1bc ¹	0.930 ± 0.005ab ¹	54.8 ± 4.0ab ¹²	3.5 ± 0.33a ¹
	100%NPK	0.917 ± 0.005ab ¹	68.9 ± 3.5b ¹	4.0 ± 0.1ab ¹	0.939 ± 0.011a ¹	63.6 ± 7.7a ¹	3.7 ± 0.34a ¹
LSD (<i>p</i> -value)	Cropping System	0.02 (<0.001)	4.90 (<0.001)	0.270 (0.056)	0.02 (0.319)	6.18 (0.060)	0.1 (0.922)
LSD (<i>p</i> -value)	Fertiliser Levels	0.01 (<0.001)	6.11 (<0.001)	0.282 (<0.001)	0.02 (0.040)	8.14 (0.001)	0.5 (0.022)
LSD (<i>p</i> -value)	Cropping System X Fertiliser Levels	0.01 (0.001)	8.07 (<0.001)	0.385 (<0.001)	0.02 (0.896)	10.96 (0.594)	0.6(1.000)
Cropping System [2015/16(S2)]		Fertiliser Levels					
Sole	CONTROL	0.816 ± 0.005b ¹	66.5 ± 3.0c ³	3.1 ± 0.1c ²	0.903 ± 0.009ab ¹	64.0 ± 2.6e ⁴	4.0 ± 0.4bcd ²
	25%NPK	0.806 ± 0.006b ¹	82.0 ± 5.7b ²³	3.1 ± 0.2c ²	0.908 ± 0.005ab ¹	77.8 ± 4.4d ³	4.1 ± 0.2bcd ²
	50%NPK	0.826 ± 0.009ab ¹	91.7 ± 3.3b ²	3.4 ± 0.1bc ¹²	0.911 ± 0.006ab ¹	90.9 ± 2.3c ²	4.8 ± 0.2ab ¹²
	100%NPK	0.811 ± 0.007b ¹	141.5 ± 10.4a ¹	3.7 ± 0.2ab ¹	0.913 ± 0.006ab ¹	110.5 ± 1.6b ¹	5.6 ± 0.5a ¹
Intercrop	CONTROL	0.804 ± 0.003bc ²	67.5 ± 1.8c ³	3.2 ± 0.3c ²	0.896 ± 0.008b ²	79.5 ± 2.7d ³	3.1 ± 0.2d ³
	25%NPK	0.841 ± 0.007a ¹	82.8 ± 5.8b ²	3.2 ± 0.1bc ²	0.911 ± 0.003ab ¹²	85.1 ± 2.3cd ³	3.5 ± 0.3cd ²³
	50%NPK	0.784 ± 0.009c ³	84.3 ± 4.6b ²	3.5 ± 0.2abc ¹²	0.913 ± 0.009ab ¹²	106.2 ± 2.6b ²	3.8 ± 0.3bcd ²
	100%NPK	0.752 ± 0.016d ⁴	95.3 ± 2.5b ¹	3.9 ± 0.1a ¹	0.917 ± 0.005a ¹	129.5 ± 3.7a ¹	4.4 ± 0.3bc ¹
LSD (<i>p</i> -value)	Cropping System	0.02 (0.918)	9.78 (<0.001)	0.20 (0.001)	0.02 (0.918)	9.33 (<0.001)	1.6 (0.001)
LSD (<i>p</i> -value)	Fertiliser Levels	0.01 (0.100)	10.11 (<0.001)	0.32 (0.002)	0.01 (0.100)	5.46 (<0.001)	0.4 (0.002)
LSD (<i>p</i> -value)	Cropping System X Fertiliser Levels	0.02 (0.792)	13.86 (0.258)	0.41 (0.871)	0.02 (0.792)	9.47 (0.258)	1.5 (0.871)

Mean ± SE (*n* = 12) values in each column followed by different letters indicate significant differences between treatments (cropping system and fertiliser levels). Numerical values that have been superscripted compare means of each cropping system at different fertiliser levels (*p* ≤ 0.05).

Table 5. Iron nutritional yield (Fe-NY), Zinc nutritional yield (Zn-NY) and combined zinc and iron nutritional yield (Fe+Zn-NY) of cowpea and amaranth under sole and inter-cropping systems at four fertiliser (NPK) levels in 2014/15 and 2015/16 summer seasons.

Crops (2014/15)	Fertiliser Level	Fe-NY	Zn- NY	Zn+Fe-NY
g.ha ⁻¹				
Amaranth	CONTROL	48 ± 2efg ²	3.5 ± 0.5h ³	52 ± 2ef ²
	25%NPK	76 ± 4ef ²	5.3 ± 0.9gh ²³	81 ± 4ef ²
	50%NPK	97 ± 3e ²	7.7 ± 0.8g ²	105 ± 3e ²
	100%NPK	197 ± 1cd ¹	13.3 ± 1.3ef ¹	210 ± 1d ¹
Cowpea	CONTROL	19 ± 1g ²	12.4 ± 0.3f ³	31 ± 1f ²
	25%NPK	31 ± 3fg ¹	21.4 ± 1.6c ¹	53 ± 3ef ¹
	50%NPK	33 ± 1fg ¹	16.6 ± 1.7de ²	50 ± 1f ¹
	100%NPK	41 ± 2fg ¹	20.0 ± 8cd ¹²	61 ± 2ef ¹
Amaranth and Cowpea	CONTROL	172 ± 14d ³	21.0 ± 1.8c ³	193 ± 13d ³
	25%NPK	234 ± 34c ³	28.2 ± 1.7b ²	262 ± 35c ³
	50%NPK	514 ± 22b ²	36.5 ± 2.4a ¹	550 ± 21b ²
	100%NPK	605 ± 20a ¹	35.5 ± 2.1a ¹	641 ± 21a ¹
LSD (<i>p</i> -value)	Crops	28 (<0.001)	1.9 (<0.001)	29 (<0.001)
LSD (<i>p</i> -value)	Fertiliser Level	32 (<0.001)	2.2 (0.003)	33 (<0.001)
LSD (<i>p</i> -value)	Cropping System X Fertiliser Level	55 (<0.001)	3.8 (0.588)	58 (<0.001)
Crops (2015/16)				
Amaranth	CONTROL	67 ± 6h ³	4.2 ± g ³	71 ± 6h ³
	25%NPK	89 ± 4h ³	4.7 ± g ³	94 ± 4h ³
	50%NPK	162 ± 7gh ²	8.5 ± fg ²	170 ± 7gh ²
	100%NPK	290 ± 3g ¹	14.5 ± f ¹	304 ± 3g ¹
Cowpea	CONTROL	857 ± 12def ³	39.5 ± 2.3cd ²	896 ± 12def ³
	25%NPK	1617 ± 9a ¹	61.4 ± 3.2a ¹	1678 ± 9a ¹
	50%NPK	1298 ± 16b ²	47.5 ± 2.5b ²	1346 ± 16b ²
	100%-NPK	1084 ± 13c ²³	28.3 ± 2.6e ³	1112 ± 13c ²³
Amaranth and Cowpea	CONTROL	770 ± 16f ²	35.9 ± 2.8cd ¹²	805 ± 17f ²
	25%NPK	1025 ± 28cd ¹	40.7 ± 2.2c ¹	1066 ± 28cd ¹
	50%NPK	962 ± 27cde ¹	39.5 ± 2.9cd ¹	1001 ± 28cde ¹
	100%NPK	835 ± 12ef ²	32.8 ± 2.5de ¹²	867 ± 12ef ²
LSD (<i>p</i> -value)	Crops	78 (<0.001)	4.1 (<0.001)	78 (<0.001)
LSD (<i>p</i> -value)	Fertiliser Levels	90 (<0.001)	4.7 (<0.001)	90 (<0.001)
LSD (<i>p</i> -value)	Crops × Fertiliser Levels	155 (<0.001)	8.2 (<0.001)	156 (<0.001)

Mean ± SE (*n* = 18) values in each column followed by different letters indicate significant differences between treatments (crops and fertiliser levels). Numerical values that have been superscripted compare means of each cropping system at different fertiliser levels (*p* ≤ 0.05).

4. Discussion

This study hypothesised that (i) nitrogen fixation in cowpea will be affected by different fertiliser levels, and (ii) cowpea-amaranth intercrop will contribute to improved NY compared to sole cropping of each crop. The objectives of the study were therefore to assess the effect of fertiliser application and inter-cropping on the nodulation, nitrogen accumulation as well as symbiotic nitrogen fixation of cowpea. In addition, iron and zinc concentration, nutritional yields of cowpea and amaranth were also assessed.

Nitrogen accumulation indicators include among others, the ureide-N and nitrate-N. The ureide-N was markedly higher in control treatments and decreased with increasing NPK fertiliser application treatments (Table 2). On the other hand, nitrate-N was significantly enhanced by an increased application of fertiliser treatment with the highest at 25% NPK and 50% NPK and lowest in cowpea grown with 100% NPK fertiliser (Table 2). The overall results of this study indicated that both the ureide- and nitrate-N decreased by the application of 100% NPK. This demonstrates that the application of the highest NPK suppressed ureide- N and nitrate-N concentrations in cowpea, regardless of the cropping system, sole crop or intercropped with amaranth. Studies have shown that the application of higher levels of NPK in legumes tends to suppress the ureide- N and nitrate-N concentrations [32]. All these N-accumulation processes lead to biomass accrual by cowpea, and plant growth. In general, the co-application of these nutrient elements at optimum concentrations increased the uptake and accumulation of N in crops [33]. Overall, the application of NPK at optimum concentrations results in synergistic interaction and promote not only the growth but the uptake and accumulation of N in plant tissue [7], as found in this study.

Symbiotic performance of cowpea indicated by nodulation as well as N_2 fixation showed some degree of sensitivity to both fertilisation [34] and inter-cropping [35]. The higher nodulation (number of nodules and nodule mass) at physiological maturity in cowpea in treatments with fertiliser levels below the recommended rate (Table 3) could be attributed to the application of the 25% of NPK, which probably served as starter N, P, and K, and was therefore crucial in stimulating nodule formation for symbiotic functioning [36]. By contrast, the study showed the least nodulation in cowpea, occurring at 100% NPK (Table 3), which somewhat confirms the assertion that higher mineral NPK levels tend to inhibit nodule formation in legumes [37]. Other studies have also indicated the sensitivity of nodulation to fertiliser application [37]. Generally, there is greater nodulation in the sole cropping [38] when compared to inter-cropping which shows lower amounts [39]. The reduced nodulation observed in the inter-cropping system (in consideration of individual crops), could have been caused by cowpea competition with amaranth, especially for below-ground resources. Inter-cropping results in competition between crops due to proximity with the companion crop [40], thus, resulting in low nodulation as obtained in this study. Higher nodulation in fertiliser treatments below the 100% NPK are perhaps an indication that the soil only required some level of NPK for the cowpea crop to kick start symbiotic performance (Table 3), in which root nodules function effectively. Indications through other studies have shown that increased fertiliser amounts inhibit nodulation [41]. This study satisfies the hypothesis that nitrogen fixation in cowpea will be higher at low fertiliser amounts as shown by the results.

The results also indicated the dependence on atmospheric N_2 (%Ndfa) and N-fixed in cowpea at physiological maturity showed an inverse relation to additional fertilisation in both seasons (Table 3). According to Anglade et al. [42], legumes tend to increase their %Ndfa, whilst decreasing N assimilation. The %Ndfa, and N-fixed in response to fertiliser application level is likely caused by a competition for carbon use through sink-source assimilation partitioning. The carbon costs associated with %Ndfa and N-fixed are substantial and therefore the process occurs and increases at the expense of the biomass accumulation given that N_2 fixation is an energetically expensive process [43]. The reduced dependence on symbiotic N_2 fixation by intercropped cowpea for its N nutrition could have been caused by competition for soil N by the companion crop amaranth. This study corroborates with an earlier study [43] which showed lower %Ndfa by the intercropped legume due to competition

from the non-legume crop. In the current study, the control and/or 25% NPK treatments, though not significantly different from each other, exhibited greater %Nd_{fa}, whereas the application of 100% NPK reduced %Nd_{fa}. With legumes, normally the %Nd_{fa} is usually enhanced when grown under low soil-N compared to that planted with the supply of high N [44]. Also, with the application of higher NPK, the legume reduced its symbiotic performance competitive ability. The reduced N accumulation in the inter-cropping system could have been because the non-legume crop is more competitive for soil N, especially inorganic N and the competition forces the legume to rely only on N₂ fixation, which does not supply as much N [19]. The addition of higher N fertilisers can result in a decrease in the richness, diversity, and composition of soil bacterial communities [45]. That being the case, the decreased microbial community, some of which are responsible for solubilisation of soil N, could result in their inefficacy and therefore reduced N uptake. Understanding cowpea N-accumulation dynamics under varying levels of fertilisation is, therefore, crucial in optimising crop production particularly for the resource-constrained farmers. The cowpea crop tends to depend on atmospheric N₂ under limited N conditions and thus induces N-accumulation in the soil. The findings of this study corroborate with others, such as Singh and Usha [46].

Our results showed that micronutrient (Fe and Zn) concentrations increased with additional fertiliser application in both cowpea and amaranth across seasons (Table 4). On a crop-by-crop basis, cowpea and amaranth bio-accumulate trace mineral nutrients more in sole cropping when compared to inter-cropping system. Fertiliser application, as well as cropping system, play a key role in micronutrient accumulation of legumes and non-legumes [47]. Cowpea had more micronutrients (Fe and Zn) at 25% NPK fertiliser application level compared to amaranth that increased at the recommended 100% NPK rate. The behaviour of cowpea agrees with the assumption that legumes have the ability of more nutrient bioaccumulation when compared to non-legumes [48], such as observed in this study. Higher mineral element concentrations in sole cropping is in line with the theory that non-legumes and legumes show capabilities of acquiring Fe and Zn in an environment with minimal competition from companion plants such as in sole stands relative to intercrops. Similar to other studies such as Dakora and Belane [49], our study found that there is more Fe concentration than Zn in crops, for example, cowpea and amaranth. The reasons for more iron than zinc on earth generally, is due to phytates bioavailability [50].

Micronutrient considerations become valuable to the vulnerable in society, which include women and children under the age of five [51,52], if they are able to quantify amounts of vegetables to be planted in available land to meet sufficient nutrition, using a concept known as nutritional yield (NY). Nutritional yield is affected, amongst other factors, by fertiliser application as well as inter-cropping. In this study, the NY-values for both seasons and for both crops increased in response to fertiliser application (Table 5). Generally, cowpea had more Fe-NY and Zn-NY, relative to amaranth (Table 5). The NY findings of the combination of the crops in an intercrop showed more Fe-NY and Zn-NY. This shows the advantage of inter-cropping to NY. This study provided insights into the benefits related to NY that can be derived from inter-cropping. The farming community particularly the resource-constrained group could be encouraged to consider more than one crop in limited land area to optimise nutrition in plant tissues, primarily trace elements associated with hidden hunger. The value of the determination of NY with inter-cropping of indigenous vegetables (cowpea and amaranth) on various NPK fertility rates in the soil is to provide more benefits to optimise on limited productive land and eventually minimise trace element deficiency in plant tissues. The variable NY's from cowpea and amaranth especially when combined in an inter-cropping are key in expanding options for the vulnerable in society.

5. Conclusions

Cowpea, through symbiotic nitrogen fixation and N-accumulation, was able to contribute N to the soil. However, the application of fertiliser at 100% NPK reduced the amounts. Symbiotic nitrogen fixation in cowpea was reduced at 100% NPK fertiliser levels. However, it was higher at

lower fertiliser levels such the control (0% NPK) and 25% NPK. Land utilisation efficiency showed benefits in an intercrop, which resulted in increased accumulation of Fe and Zn in plant tissues of both crops, hence increasing nutritional yield. Inter-cropping of cowpea with amaranth contributed to improved NY compared to sole cropping. The study alludes to the importance of minimum resource utilisation to provide more zinc and iron nutrition for the vulnerable in rural communities. The study has implications for improvement of soil fertility in nutrient-poor soils. Through intercropping of cowpea and amaranth, malnutrition in rural resource-poor households can be reduced. Practically, it is recommended, therefore, that smallholder farmers intercrop while reducing fertiliser application by up to 50% of the recommended dosages. Future recommendations informed by the study is crop rotation to test the residual benefit of cowpea to other vegetables. In addition, nitrogen benefit studies will be important in the future to equate the amount of N-fixed by cowpea to the actual amounts of nitrogen fertiliser to be saved.

Author Contributions: B.M., T.M., A.T.M. and M.F., conceptualised the research project while B.M., executed the experiments and data collection. B.M. and B.N. analysed the data and wrote the original draft manuscript. B.N., M.F., M.N., S.A.K., S.A., T.M., and A.T.M. reviewed and edited the manuscript. T.M., A.T.M. and B.N. did critical review, redrafting and supervised the student's research project. All authors have read and agreed to the published version of the manuscript.

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