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Research article

Improving soil fertility through dual inoculation with arbuscular mycorrhizal fungi and *Rhizobium* on a eutric cambisol cultivated with forage legumes in a semi-arid region

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ABSTRACT

The Sub-Saharan region of southern Africa is characterized by high temperatures, low rainfall, and poor land-use management practices such as continuous cropping without replenishment of soil nutrients. The combination of these factors has resulted in nutrient depletion and land degradation. The current study aimed at investigating the effect of arbuscular mycorrhizal fungi (AMF) and *Rhizobium* bacteria inoculation on soil chemical properties in field-grown forage legumes, namely, *Mucuna pruriens* (mucuna), *Lablab purpureus* (lablab) and *Vigna unguiculata* (cowpea), in the semi-arid region of the Eastern Cape Province (South Africa). Forage legumes were inoculated with the AMF species *Paraglomus occulum* and the *Rhizobia* bacteria species *Bradyrhizobium* strain and grown for 120 days. Soil samples were collected in the following sequence: prior to planting, before flowering and after harvesting the forage legumes in each of the two seasons (2017/2018 and 2018/2019) and soil chemical properties were determined using standard procedures. The results showed that the addition of dual inoculation over time greatly improved soil chemical properties when compared to the control treatment. This was advocated by the significant ($P \leq 0.05$) increase in soil pH, soil organic carbon, soil organic matter, total nitrogen, phosphorus, calcium, potassium, magnesium, sodium, sulfur and iron in soils. The concentration of cation exchange capacity was significantly ($P \leq 0.05$) higher in cowpea treated with *Rhizobium* as compared to other treatment combinations. The control treatment of mucuna forage greatly improved the concentrations of manganese, boron, copper, molybdenum, and zinc over other treatment combinations only before the flowering stage. However, the concentrations of micronutrients were significantly higher on the treatment combination of lablab and single inoculation of AMF after harvesting. Generally, dual inoculation with AMF and *Rhizobia* enhanced soil properties when compared to a single inoculation or untreated control.

1. Introduction

Communal farmers in the Eastern Cape Province of South Africa face several challenges including low soil fertility, degraded soils

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and loss of biodiversity due to poor management practices and climate change [1–3]. Majority of the province's available land is used to grow cereals that is consumed by humans and to feed certain types of livestock including pigs and poultry [4]. On the other end, the land available to produce crops that can used as feed for ruminants remains scanty. These challenges have immensely affected the productive capacity of forage grasses and legumes produced in the province. Production levels have also been affected by the soils in the Eastern Cape Province that are deficient in nitrogen (N), have a low available phosphorus (P), and little micronutrient content [2,5]. Furthermore, these soils have low organic matter, as such weeds compete with crops by limiting moisture and nutrients, which negatively affect crop yield [5].

To avert this, many farmers in the Eastern Cape Province as well as, in the Sub-Saharan semi-arid regions of Africa have used synthetic fertilizers to correct soil nutrient deficiencies (particularly N and P) [6,7]. However, excessive use of these fertilizers has been associated with groundwater pollution, land degradation and leads to low soil pH as well as accelerated soil acidity [8–10]. Consequently, this can impede the achievement of the Sustainable Development Goals (SDGs 15) which focuses on Life on Land. The principal aim of SDG15 is to save soils by reducing soil nutrient loss and pollution load towards redress of different concerns related to pollution, food security, and health [11]. Therefore, it is necessary to find cheap, eco-friendly alternatives and safe natural sources of phytonutrients to balance and restore soil health.

Such alternatives and sources which are meant to improve soil fertility and soil health status include the use of beneficial bacteria and forage legumes which have been used for biological nitrogen fixation (BFN) [12]. Dual inoculation of *Rhizobia* and arbuscular mycorrhizal fungi (AMF) results in a tripartite mutualistic symbiosis [13,14] and plays a vital role in correcting the problem of low soil fertility, while increasing nutrient uptake, and thereby reducing the high input costs of chemical fertilizers [15–17]. The AMF are obligate biotrophs that form endosymbiotic relations with the roots of vascular plants [18].

Munir et al. [8] reported that AMF inoculation could enable the root systems of the host plant to grow more intensely and deeper in the soil, allowing water to be taken more efficiently. As a result, AMF promotes the uptake of mineral nutrients in the host plant, reduces the infestation of diseases, improves tolerance to heavy metals, salinity and drought stresses, as well as increasing the photosynthetic activity [19–22]. Moreover, the inoculation of *Rhizobium* bacteria forms a symbiotic relationship with the host plant to improve soil N content through biological fixation [23]. Thus, while the synergistic interactions enhance N fixation and nutrient uptake in the plant host, the biofertilization could assist as AMF is responsible for P uptake, thus accelerating biological fixation [24]. This is imperative, especially in the Sub-Saharan region of Africa, where N and P deficits are key determinants limiting plant growth and mineral nutrition [6]. According to Halder et al. [25], AMF not only improves N and P uptake but also enhances the root surface and mobilization of limited nutrients such as zinc (Zn), iron (Fe), and copper (Cu). These results in plant-microbe interactions improve soil fertility, soil pH, and nutrient availability in soil profiles [6]. Therefore, the present study assessed the effect of AMF and *Rhizobium* inoculation on soil chemical properties in field-grown forage legumes in the Eastern Cape Province of South Africa. It is hypothesized that the application of AMF and *Rhizobium* inoculation will improve soil chemical characteristics.

Table 1
The initial soil physicochemical properties of the study field in the 2017/2018 and 2018/2019 growing seasons. Sampling was done at the depth of 0–30 cm.

Physical properties	2017/2018	2018/2019
Sand (%)	64.20	64.20
Clay(%)	19.80	19.80
Silt (%)	16.00	16.00
Soil texture class	Sandy loam	Sandy loam
Chemical properties		
Soil pH	5.60	5.90
Cation exchange capacity (cmol/kg)	6.20	5.50
Soil organic carbon (g.kg^{-1})	6.40	6.90
Soil organic matter	9.60	10.20
Total nitrogen (%)	0.06	0.06
Available phosphorus (g.kg^{-1})	0.32	0.41
Potassium (g.kg^{-1})	6.02	5.67
Calcium (g.kg^{-1})	5.35	5.12
Magnesium (g.kg^{-1})	1.48	1.57
Sodium (g.kg^{-1})	0.58	0.64
Sulfur (g.kg^{-1})	16.92	15.02
Iron (g.kg^{-1})	21.22	20.34
Manganese (mg.kg^{-1})	398.00	356.00
Zinc (mg.kg^{-1})	26.00	24.00
Copper (mg.kg^{-1})	52.00	46.00
Boron (mg.kg^{-1})	44.00	40.00
Molybdenum (mg.kg^{-1})	58.00	50.00

2. Materials and methods

2.1. Description of the study site

The current field study was conducted during summer growing seasons (i.e., 2017/18 and 2018/2019) at the University of Fort Hare Research Farm in the Eastern Cape Province (32°46'15.8" S and 26°50'52.3" E). The farm lies at an altitude of about 535 m above sea level (m.a.s.l.) and has an average annual rainfall of about 575 mm, that is received mostly between November and March. The minimum, average and maximum temperatures are 11.10 °C, 17.80 °C and 24.60 °C, respectively (see Table 1 in Mpongwana et al. [26] for more details on temperature and rainfall of the study site during the two growing seasons). The soils at the farm are of alluvial origin and are classified as Eutric Cambisols using the International Union of Soil Sciences (IUSS) Working Group World Reference Base (WRB) [27]. The soil's texture is 64.2 % sand, 16.0 % silt, and 19.8 % clay (Table 1). The soil texture was determined using Bouyoucos hydrometer method of soil particle size analysis described by Okalebo et al. [28]. The farm consist of grass species such as *Themeda triandra* and *Cymbopogon plurinodis,* as well as woody plants like *Accacia karroo* and shrubs encroaching on some parts of the grazing lands [29].

2.2. Experimental design, treatments and soil sampling

A 3 × 2 × 2 factorial experiment was carried out in a randomized complete block design (RCBD) with 12 treatments and four replications. The factors comprised of three legume species, namely, mucuna (*Mucuna pruriens*), cowpea (*Vigna unguiculata* L. Walp.) and lablab (*Lablab purpureus* L.); two arbuscular mycorrhizal fungi (with and without AMF inoculation); and two *Rhizobium* inoculation (with and without *Rhizobium* inoculation). Prior to planting, the forage legume seeds were treated with Mycoroot™ products that contained a combination of arbuscular mycorrhizal isolates, which included *Claroideoglomus etunicatum, Funneliformis mosseae*, *Gigaspora gigantean*, *Paraglomus occulum* and *Rhizophagus clarus* [26]. The production laboratory at Rhodes University (South Africa) conducted routine quality controls [30], and one product had 10 spores per gramme. The rate for Mycoroot™ products inoculation on seeds was based on the optimum recommended rate of 45 kg P ha^{-1} [31]. Prior to seeding, a *Rhizobium* inoculum product (*Bradyrhizobium japonicum* strain) was applied to the seeds. The suggested single superphosphate rate of 40 kg P ha^{-1}, containing 10.50 % phosphate (0:46:0 %; N: P_2O_5:K_2O) was applied to the plots [26]. The seeds of cowpea, lablab and mucuna were planted using a 50 kg ha^{-1} seeding rate (resulting in 37 037 plants per ha), at a depth of 4–6 cm, with 0.9 × 0.3 m inter- and intra-row spacing.

The current study used the same open field trials as proposed by Mpongwana et al. [26], which found that dual inoculation of AMF and *Rhizobia* bacteria improved plant growth, nodulation, and biomass production in all three legumes. As a result, further details on plant development circumstances and results are presented in our prior work [26]. The samples were collected randomly from the individual treated soils of the field trials before and after the planting of the forage legumes. Samples were taken from 48 plots, each measuring at 4 × 4 m, separated by 1 m spacing between them and 2 m spacing between the blocks (i.e.,59 × 22 m field size) [26]. Five sub-samples were randomly collected from each plot at a depth of 0–15 cm using an auger and mixed thoroughly to obtain a composite homogenous mixture of 250 g. The samples were put in polyethylene plastic bags and sent to the laboratory for analysis. About 20 g of the soil samples collected were dried in open air, ground, and sieved (<2 mm) for the determination of chemical properties.

2.3. Soil chemical determination

Soil properties that were analyzed include pH, soil organic carbon (SOC) and soil organic matter (SOM), cation exchange capacity (CEC), soil total N, soil P availability, Magnesium (Mg), Calcium (Ca), sodium (Na), K, Fe, Zn, Cu, manganese (Mn), Boron (B), Sulfur (S), and Molybdenum (Mo). Briefly, soil pH was determined in water (1:2.5) using the glass electrode pH meter, whereas SOC and SOM were determined using the Walkey-Black method [32]. The determination of total N was done using a high-temperature combustion method (LECO TruSpec CN analyzer) [33], while the available P was extracted with Bray 1 solution and determined using a T60 Ultra-Visible spectrophotometer (SL 171 model, Hyderabad, India) [34]. The exchangeable cations (Mg, Ca, Na, and K) together with CEC were determined by the NH_4OAc extraction method [35], while B was determined by the $CaCl_2$ extraction method. Copper, Fe, Mn, and Zn were extracted using ethylene-diamine-tetra-acetic acid (EDTA) and determined by atomic absorption spectroscopy (AAS) as suggested by Beyers and Coetzer [36]. Table 1 below shows the soil physicochemical properties of the study site prior to the start of a field trial.

2.4. Statistical analyses

Data on soil chemical properties were analyzed using a three-way analysis of variance (ANOVA) through SAS software. Separation of means were done using Least Significant Difference (LSD) at significant level of 5 %. Similar to the study by Mpongwana et al. [26], the statistical model shown below was used:

$$Y_{ijk} = \mu + B_h + C_i + D_j + E_k + (CD)_{ij} + (CE)_{jk} + (DE)_{jk} + (CDE)_{ijk} + eijk$$

"Where: Y_{ijk} = is the dependent variable and μ = overall mean; B_h = h^{th} block effect (h = 1, 2, 3, 4); C_i = i^{th} effect of legume species (i = 1, 2, 3); D_j = j^{th} effect of AMF (j = 1, 2); E_k = k^{th} effect of Rhizobium inoculum (i = 1, 2); CD = ij^{th} interaction between legumes species and AMF; CE = ikth interaction between legumes species and Rhizobium inoculum; DE = jkth interaction between AMF and Rhizobium inoculum; CDE_{ijk} = ijkth interaction of legumes species, AMF and Rhizobium inoculum; eijk = residual error" [26].

3. Results

3.1. The soil chemical properties before flowering as influenced by legume variety, AMF and Rhizobium

The significant interactions ($P \leq 0.05$) between forage legume varieties and inoculation with AMF and *Rhizobia* on soil chemical properties before flowering stage in two seasons are shown in this subsection. The results showed that the soil pH significantly increased over time owing to AMF inoculation on different legume varieties. The initial soil pH was slightly acidic before planting but greatly increased ($P \leq 0.001$) to alkaline conditions during the first season due to the inoculation with AMF and *Rhizobium* bacteria as shown in Table 2. However, the soil pH was neutral and lower in the second season than in the first season. The most noticeable effects concerning the dual inoculation for soil pH in both seasons were observed under mucuna + AMF + *Rhizobium* (MAR) treatment. Findings revealed that lablab control (LC) performed poorly. Likewise, there was a significant 3-way interaction of the factors (legume, AMF and *Rhizobium*) on CEC prior to flowering in the first season (Table 2). The results showed that the addition of a single inoculation of *Rhizobium* bacteria significantly ($P \leq 0.05$) improved CEC during the first season before flowering as compared to other treatment combinations. The improvement was mostly noticeable under cowpea + *Rhizobium* (CR) and lablab + *Rhizobium* (LR) which were not significant ($P > 0.05$) from other treatment combinations before flowering in the first season. However, when dual inoculation was applied in the treatment of lablab + AMF + *Rhizobium* (LAR), there was a significant reduction on CEC. Also, there was no significant interaction ($P > 0.05$) among legume, AMF and *Rhizobium* on CEC before flowering in the second season.

The significant 3-way interaction ($P \leq 0.001$) among legume variety, AMF, and *Rhizobium* on SOC before flowering during the first season is also shown in Table 2. The results of the study indicated that the MAR treatment had the highest SOC during the first season, followed by cowpea + AMF + *Rhizobium* (CAR). The cowpea control (CC) and LC did not differ significantly ($P > 0.05$) and had a low SOC. There were a significant 3-way interactions ($P \leq 0.01$) observed on SOC between legume variety, AMF and *Rhizobium* in the second season. The significant influence on SOC was observed under treatment combinations of CAR and MAR. However, LC treatment had substantial negative effect on SOC before flowering during the second season. The results indicated a significant 3-way interaction ($P \leq 0.001$) effect of legume variety, AMF, and *Rhizobium* on SOM before flowering in the first season (Table 2). Findings further reveal that the application of MAR increased SOM in comparison to CAR and LAR treatments. The optimum SOM during the second season was recorded under MAR treatment combination, whereas the minimum was observed under LAR treatment.

In Table 3, the legume variety, AMF, and *Rhizobium* significantly ($P \leq 0.05$) shows a 3-way interaction for total N in the soil before flowering in the first season. The remarkable significant effect ($P \leq 0.05$) of total N was observed on CAR treatment. However, LAR treatment had the lowest total N when compared to other treatments. In the second season, the legume variety, AMF and *Rhizobium* showed a significant ($P \leq 0.05$) 3-way interaction for total N before flowering. The maximum total N concentration was recorded in

Table 2
Effects of legume variety, arbuscular mycorrhizal fungi (AMF) and *Rhizobium* inoculation on soil pH, CEC, SOC and SOM contents before flowering.

Treatments	Soil pH (water)		CEC (cmol/kg)		SOC (g.kg^{-1})		SOM (g.kg^{-1})	
	2017/2018	2018/2019	2017/2018	2018/2019	2017/2018	2018/2019	2017/2018	2018/2019
Cowpea control	6.00^{i}	5.00^{g}	6.60^{ab}	5.2	8.10^{j}	10.00^{g}	13.90^{j}	17.20^{f}
Cowpea + *Rhizobium*	6.50^{g}	5.40^{f}	6.80^{a}	5.3	9.50^{h}	11.70^{f}	16.30^{h}	20.20^{e}
Cowpea + AMF	7.60^{d}	6.30^{c}	6.40^{b}	5.0	10.00^{f}	11.40^{f}	17.30^{f}	21.40^{d}
Cowpea + AMF + *Rhizobium*	8.10^{b}	6.80^{b}	6.70^{a}	5.3	11.70^{b}	14.90^{a}	20.10^{b}	25.00^{a}
Lablab control	5.90^{j}	4.90^{h}	6.50^{b}	5.1	7.90^{j}	9.60^{h}	13.70^{j}	16.40^{g}
Lablab + *Rhizobium*	6.40^{g}	5.40^{f}	6.80^{a}	5.3	9.40^{h}	11.60^{f}	16.10^{h}	19.90^{e}
Lablab + AMF	7.30^{e}	6.10^{d}	6.60^{ab}	5.2	10.30^{e}	12.80^{d}	17.70^{e}	22.00^{d}
Lablab + AMF + *Rhizobium*	8.00^{c}	6.70^{b}	6.40^{b}	5.0	11.50^{c}	14.20^{b}	19.70^{c}	24.00^{b}
Mucuna control	6.10^{h}	5.10^{g}	6.50^{b}	5.1	8.30^{i}	10.30^{g}	14.30^{i}	17.70^{f}
Mucuna + *Rhizobium*	6.80^{f}	5.70^{e}	6.70^{a}	5.3	9.70^{g}	12.10^{e}	16.70^{g}	20.70^{e}
Mucuna + AMF	7.90^{c}	6.60^{b}	6.70^{a}	5.2	10.90^{d}	13.50^{c}	18.80^{d}	23.30^{c}
Mucuna + AMF + *Rhizobium*	8.40^{a}	7.00^{a}	6.70^{a}	5.2	12.00^{a}	14.90^{a}	20.70^{a}	25.70^{a}
SEM	0.04	0.04	0.10	0.08	0.06	0.11	0.10	0.19
F-test Probabilities								
Legume	***	***	ns	ns	***	***	***	***
Rhizobium	***	***	*	ns	***	***	***	***
AMF	***	***	ns	ns	***	***	***	***
Legume + *Rhizobium*	ns	ns	ns	ns	**	ns	**	ns
Legume + AMF	**	***	ns	ns	***	**	***	**
Rhizobium + AMF	ns	ns	ns	ns	ns	ns	ns	ns
Legume + *Rhizobium* + AMF	***	***	*	ns	***	**	***	**

[abc] Means with dissimilar superscripts in the similar column vary significantly. *, **, and *** indicate significance level at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively; [ns] = non-significant, SEM = Standard error of means. CEC = cation exchange capacity, SOC = soil organic carbon, SOM = soil organic matter.

Table 3
Effects of legume variety, arbuscular mycorrhizal fungi (AMF) and *Rhizobium* inoculation on soil N, P and S (g.kg^{-1}) before flowering.

Treatments	Total N		Available P		S	
	2017/2018	2018/2019	2017/2018	2018/2019	2017/2018	2018/2019
Cowpea control	0.03^{f}	0.04^{f}	0.31^{g}	1.02^{d}	10.27^{h}	11.70^{h}
Cowpea + *Rhizobium*	0.08^{b}	0.11^{a}	0.70^{d}	0.95^{d}	11.19^{f}	14.03^{d}
Cowpea + AMF	0.07^{d}	0.08^{d}	0.92^{c}	1.05^{d}	10.42^{gh}	13.06^{f}
Cowpea + AMF + *Rhizobium*	0.09^{a}	0.09^{b}	1.18^{a}	1.24^{c}	14.62^{b}	13.91^{e}
Lablab control	0.02^{g}	0.06^{e}	0.26^{g}	0.84^{e}	9.75^{i}	12.20^{g}
Lablab + *Rhizobium*	0.07^{c}	0.09^{b}	0.62^{e}	0.35^{h}	10.82^{g}	13.57^{e}
Lablab + AMF	0.05^{e}	0.08^{c}	0.78^{d}	0.52^{f}	11.89^{e}	14.92^{d}
Lablab + AMF + *Rhizobium*	0.08^{b}	0.02^{g}	1.05^{b}	0.41^{g}	13.88^{c}	13.39^{e}
Mucuna control	0.04^{f}	0.05^{f}	0.39^{f}	1.59^{a}	11.26^{f}	17.39^{b}
Mucuna + *Rhizobium*	0.06^{e}	0.09^{b}	0.76^{d}	1.41^{b}	13.33^{d}	16.71^{c}
Mucuna + AMF	0.06^{d}	0.07^{d}	0.92^{c}	1.55^{a}	11.10^{fg}	18.35^{b}
Mucuna + AMF + *Rhizobium*	0.08^{b}	0.07^{e}	1.15^{a}	1.23^{c}	16.81^{a}	20.07^{a}
SEM	0.003	0.004	0.029	0.042	0.136	0.183
F-test Probabilities						
Legume	***	***	***	***	***	***
Rhizobium	***	***	***	***	***	***
AMF	***	***	***	***	***	***
Legume + *Rhizobium*	***	***	ns	**	***	***
Legume + AMF	ns	ns	ns	*	***	***
Rhizobium + AMF	***	***	***	***	***	***
Legume + *Rhizobium* + AMF	*	**	ns	ns	***	***

[abc] Means with dissimilar superscripts in the similar column vary significantly. *, **, and *** indicate significance level at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively; [ns] = non-significant. SEM = Standard error of means. Total N = Nitrogen, Available P = Phosphorus, S = Sulfur.

sole inoculation of CR bacteria, while the minimum total N was observed under LAR treatment. The CAR and CAR treatments had the highest significant effect ($P \leq 0.001$) on available P content when compared to other treatment combinations before flowering during the first season. However, the CC and LC treatments had significantly ($P \leq 0.05$) the lowest available P. In the second season, mucuna control (MC) treatment had a higher available P content as compared to MAR, whereas the lowest concentration was recorded under sole LR.

The 3-way interactive effects of legume variety, *Rhizobium* bacteria and AMF on S concentration were significantly different ($P \leq 0.001$) before flowering in both seasons. For instance, the S concentration within the soil was higher on dual inoculation treatment combination MAR and lower on LC treatment during the first season. Similar trends of dual inoculation effect were also observed as MAR treatment had higher S content, whereas lowest concentration of S was observed on CAR treatment during the second season.

Table 4 below shows a 2-way interaction on Ca content as affected by legume, AMF, and *Rhizobium* bacteria in the soil before

Table 4
Effects of legume variety, arbuscular mycorrhizal fungi (AMF) and *Rhizobium* inoculation on soil Ca, K, Mg and Na (g.kg^{-1}) before flowering.

Treatments	Ca		K		Mg		Na	
	2017/2018	2018/2019	2017/2018	2018/2019	2017/2018	2018/2019	2017/2018	2018/2019
Cowpea control	4.58^{de}	3.50^{f}	3.33^{ef}	2.44^{c}	2.38^{g}	2.58^{c}	0.36^{f}	0.33^{g}
Cowpea + *Rhizobium*	4.66^{d}	3.69^{d}	4.15^{d}	2.97^{b}	2.74^{d}	2.76^{b}	0.45^{e}	0.40^{f}
Cowpea + AMF	4.68^{d}	3.71^{d}	4.49^{d}	3.36^{a}	3.16^{b}	3.23^{a}	0.25^{h}	0.31^{h}
Cowpea + AMF + *Rhizobium*	5.08^{b}	3.78^{c}	6.59^{a}	3.48^{a}	3.68^{a}	2.78^{b}	0.68^{a}	0.30^{h}
Lablab control	4.46^{e}	3.40^{g}	2.29^{g}	1.73^{f}	2.49^{f}	2.05^{h}	0.54^{c}	0.42^{e}
Lablab + *Rhizobium*	4.31^{f}	3.40^{g}	3.17^{f}	1.18^{g}	1.97^{h}	2.19^{f}	0.33^{f}	0.46^{d}
Lablab + AMF	4.52^{e}	3.57^{e}	3.45^{e}	1.87^{f}	2.58^{e}	2.09^{g}	0.42^{e}	0.23^{i}
Lablab + AMF + *Rhizobium*	4.79^{cd}	3.55^{e}	5.60^{c}	1.71^{f}	2.93^{c}	1.73^{i}	0.45^{e}	0.42^{e}
Mucuna control	4.66^{d}	3.80^{c}	3.51^{e}	1.80^{f}	2.33^{g}	2.27^{e}	0.34^{f}	0.50^{c}
Mucuna + *Rhizobium*	4.81^{c}	3.81^{c}	3.85^{e}	2.09^{e}	2.46^{f}	2.16^{f}	0.49^{d}	0.57^{b}
Mucuna + AMF	4.77^{cd}	4.16^{a}	4.59^{d}	2.51^{c}	2.77^{d}	2.43^{d}	0.61^{b}	0.63^{a}
Mucuna + AMF + *Rhizobium*	5.25^{a}	4.03^{b}	6.17^{b}	2.26^{d}	3.14^{b}	2.41^{d}	0.69^{a}	0.64^{a}
SEM	0.031	0.043	0.081	0.064	0.018	0.017	0.013	0.012
F-test Probabilities								
Legume	***	***	***	***	***	***	***	***
Rhizobium	***	***	***	***	***	***	***	***
AMF	***	***	***	***	***	***	***	***
Legume + *Rhizobium*	***	**	***	**	***	***	***	***
Legume + AMF	ns	*	ns	*	***	***	***	***
Rhizobium + AMF	***	***	***	***	***	***	ns	*
Legume + *Rhizobium* + AMF	ns	ns	ns	ns	***	***	**	***

[abc] Means with dissimilar superscripts in the similar column vary significantly. *, **, and *** indicate significance level at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively; [ns] = non-significant. SEM = Standard error of means. Ca = Calcium, K = Potassium, Mg = Magnesium, Na = Sodium.

flowering in both seasons. The highest Ca concentration was recorded under the treatment combination of MAR, while the minimum concentration was observed on LR during the first season. In the second season, the sole treatment inoculation of mucuna + AMF (MA) had a significantly better Ca content than LC and LR treatments. There was a 2-way interaction of the factors (legume, AMF and *Rhizobium*) on the concentration of K in the soil before flowering in both growing seasons. The CAR treatment had the optimum K content, while LC was the lowest in the first season. In the second season, the K content was significantly higher on CAR and cowpea + AMF (CA) treatments. The lowest Ca content was observed on the sole combination of LR. There was a significant 3-way interaction on the soil Mg between legume variety, AMF, and *Rhizobium* bacteria for both seasons before flowering as shown in Table 4. The dual inoculation significantly improved ($P \leq 0.001$) Mg content as compared to the single and un-inoculated treatments. The CAR treatment had significantly the highest Mg content, whereas the lowest content was recorded on the LR treatment in the first season. In contrast, the single inoculation of CA treatment significantly increased Mg content compared to LAR in the second season. The concentration of Na in the soil during the first season was significantly ($P \leq 0.01$) affected by the 3-way interaction of legume variety, AMF, and *Rhizobium* bacteria. This was observed through an increase in Na concentration on MAR and CAR treatments. Similarly, there was a 3-way interaction of the factors (legume, AMF, and *Rhizobium*) in Na concentration before flowering in the second season. The prominent increase on Na contents ($P \leq 0.001$) was observed under MAR and MA treatments, whereas LAR treatment resulted in a decrease in Na.

Table 5 shows that there was a significant 3-way interaction ($P \leq 0.01$) effect of legume variety, AMF, and *Rhizobium* bacteria on the Mn and Cu contents of the soil before flowering during the first season. The highest Mn content was found in single inoculation and un-inoculated treatments. This was demonstrated by the MC treatment with the highest Mn content, which did not differ significantly ($P > 0.05$) from the lablab + AMF (LA) and CA treatments. The MA treatment had the lowest Mn content, which was not significantly different ($P > 0.05$) from other treatment combinations except MC and LA treatments. Nevertheless, there was no significant difference ($P > 0.05$) among all treatment combinations in Mn content for the second season. Regarding Cu content, results show that there was no remarkable effect of dual inoculation, except for the sole inoculation with AMF, which only influenced a particular legume. The highest Cu content was recorded under MC, which did not differ significantly ($P > 0.05$) with LC and LA treatments. On the other hand, mucuna + *Rhizobium* bacteria (MR) had the lowest Cu content but did not differ ($P > 0.05$) with the other treatment combinations, except for MC and LA. Nonetheless, Cu content had a significant 3-way interaction effect ($P \leq 0.05$) during the second season as influenced by dual inoculation. In addition, during the second season, the maximum dual inoculation effect was observed on LAR and LA, whereas the lowest Cu content was on MR.

3.2. *Correlation matrix of soil chemical properties before flowering*

A correlation matrix amongst the selected 16 soil chemical properties before flowering during the two growing seasons (2017/2018 and 2018/2019) is presented in Table 6. A significantly positive correlation matrix ($P \leq 0.001$) of the soil chemical properties between soil pH and the following parameters: SOC ($R^2 = 0.33$), SOM ($R^2 = 0.12$), CEC ($R^2 = 0.34$), total N ($R^2 = 0.23$), available soil P ($R^2 = 0.18$), K ($R^2 = 0.12$), Ca ($R^2 = 0.21$), Mg ($R^2 = 0.16$), Na ($R^2 = 0.26$), and S ($R^2 = 0.24$) were observed. A significant positive ($P \leq 0.001$) correlation was also observed between soil SOC and SOM ($R^2 = 0.56$), CEC ($R^2 = 0.34$), total N ($R^2 = 0.27$), available soil P ($R^2 = 0.18$), K ($R^2 = 0.19$), Ca ($R^2 = 0.24$), Mg ($R^2 = 0.16$), Na ($R^2 = 0.26$), and S ($R^2 = 0.29$). Similarly, positive ($P \leq 0.001$) trends were

Table 5
Effects of legume variety, arbuscular mycorrhizal fungi (AMF) and *Rhizobium* inoculation on Mn, B, Cu and Mo ($mg.kg^{-1}$) before flowering.

Treatments	Mn		B		Cu		Mo	
	2017/2018	2018/2019	2017/2018	2018/2019	2017/2018	2018/2019	2017/2018	2018/2019
Cowpea control	567.00^{b}	472.00	54.92^{b}	62.00	35.67^{c}	43.50^{cd}	51.58^{bc}	56.00^{b}
Cowpea + *Rhizobium*	547.00^{b}	468.00	53.08^{b}	61.00	38.67^{c}	45.75^{bc}	49.92^{bc}	56.92^{b}
Cowpea + AMF	583.00^{ab}	460.00	51.42^{b}	61.50	39.67^{bc}	42.75^{cd}	51.92^{bc}	56.83^{b}
Cowpea + AMF + *Rhizobium*	541.00^{b}	473.00	55.16^{b}	62.08	38.83^{c}	45.75^{bc}	54.50^{bc}	57.33^{b}
Lablab control	582.00^{b}	509.00	54.83^{b}	75.50	40.33^{abc}	48.08^{b}	57.33^{a}	60.33^{a}
Lablab + *Rhizobium*	567.00^{b}	573.00	52.58^{b}	65.25	36.08^{c}	49.08^{b}	48.92^{c}	60.75^{a}
Lablab + AMF	625.00^{a}	547.00	66.67^{a}	74.92	47.00^{ab}	55.00^{a}	60.50^{a}	63.50^{a}
Lablab + AMF + *Rhizobium*	543.00^{b}	495.00	55.33^{b}	62.08	33.33^{c}	59.00^{a}	49.58^{bc}	62.50^{a}
Mucuna control	653.00^{a}	495.00	67.00^{a}	57.75	48.08^{a}	40.83^{cd}	61.08^{a}	54.08^{bc}
Mucuna + *Rhizobium*	530.00^{b}	480.00	50.92^{b}	57.58	32.92^{c}	39.08^{d}	50.83^{bc}	52.08^{c}
Mucuna + AMF	524.00^{b}	495.00	54.08^{b}	59.75	36.58^{c}	43.25^{cd}	51.75^{bc}	53.58^{c}
MAR	566.00^{b}	482.00	58.08^{b}	59.25	38.17^{c}	45.75^{b}	55.25^{ab}	53.50^{c}
SEM	26.25	22.81	3.02	3.38	2.89	3.53	2.18	2.27
F-test Probabilities								
Legume	ns	ns	ns	ns	ns	ns	ns	ns
Rhizobium	**	**	*	**	**	**	***	*
AMF	ns	ns	ns	ns	ns	ns	ns	*
Legume + *Rhizobium*	ns	ns	ns	ns	*	ns	**	ns
Legume + AMF	ns	ns	*	ns	ns	ns	ns	ns
Rhizobium + AMF	ns	ns	ns	ns	ns	ns	*	*
Legume + *Rhizobium* + AMF	**	ns	**	ns	**	*	*	*

[abc] Means with dissimilar superscripts in the similar column vary significantly. *, **, and *** indicate significance level at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively; [ns] = non-significant. SEM = Standard error of means. Mn = Manganese, B = Boron, Cu = Copper, Mo = Molybdenum.

Table 6
The mean correlation analysis among soil chemical properties before flowering in two growing seasons.

	pH	SOC	SOM	CEC	N	P	K	Ca	Mg	Na	S	Fe	Mn	B	Cu	Mo
									2017/2018							
pH		0.95***	0.95***	0.24**	0.61***	0.87***	0.86***	0.56***	0.69***	0.82***	0.72***	−0.03	−0.12	−0.03	−0.09	−0.04
SOC	–		1.00***	0.24**	0.67***	0.87***	0.87***	0.55***	0.69***	0.82***	0.79***	−0.14	−0.23**	−0.09	−0.15	−0.08
SOM	–	–		0.24**	0.67***	0.87***	0.87***	0.55***	0.69***	0.82***	0.79***	−0.14	−0.23**	−0.09	−0.15	−0.08
CEC	–	–	–		0.68***	0.55***	0.41***	0.79***	0.68***	0.42***	0.43***	−0.16	−0.12	−0.18*	−0.23**	−0.15
N	–	–	–	–		0.87***	0.79***	0.76***	0.88***	0.70***	0.67***	−0.14	−0.23**	−0.17*	−0.22**	−0.15
P	–	–	–	–	–		0.89***	0.78***	0.88***	0.85***	0.77***	−0.15	−0.21**	−0.11	−0.19**	−0.09
K	–	–	–	–	–	–		0.76***	0.86***	0.87***	0.83***	−0.18	−0.21**	−0.11	−0.16**	−0.08
Ca	–	–	–	–	–	–	–		0.87***	0.76***	0.75***	−0.32***	−0.16	−0.12	−0.16	−0.01
Mg	–	–	–	–	–	–	–	–		0.82***	0.69***	−0.22	−0.14	−0.09	−0.09	0.01
Na	–	–	–	–	–	–	–	–	–		0.75***	−0.25**	−0.21**	−0.11	−0.12	0.01
S	–	–	–	–	–	–	–	–	–	–		−0.21	−0.13	−0.01	−0.10	0.03
Fe	–	–	–	–	–	–	–	–	–	–	–		0.48	0.30	0.29	0.14
Mn	–	–	–	–	–	–	–	–	–	–	–	–		0.84	0.84	0.77
B	–	–	–	–	–	–	–	–	–	–	–	–	–		0.87	0.88
Cu	–	–	–	–	–	–	–	–	–	–	–	–	–	–		0.88
Mo	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
2018/2019																
pH		0.82***	0.83***	−0.12*	0.45**	0.74***	0.75***	0.44***	0.54***	0.22	0.12	−0.02	−0.08	−0.01	−0.04	−0.03
SOC	–		1.00***	0.16**	0.53***	0.64***	0.59***	0.34***	0.48***	0.46	0.58	−0.04	−0.12	−0.05	−0.11	−0.03
SOM	–	–	−0.16**		0.53***	0.64***	0.59***	0.34***	0.48***	0.57	0.58	−0.04	−0.12	−0.05	−0.11	−0.03
CEC	–	–	–		0.68***	0.32**	0.34***	0.65***	0.89***	0.34	0.49	−0.18	−0.07	−0.12	−0.13	−0.13
N	–	–	–	–		0.45***	0.56***	0.56***	0.97***	0.56	0.59	−0.24**	−0.14**	−0.10*	−0.13**	−0.13*
P	–	–	–	–	–		0.45***	0.67**	0.56**	0.66	0.56	−0.11	−0.15*	−0.05	−0.13**	−0.05
K	–	–	–	–	–	–		0.54**	0.78**	0.65	0.45	−0.12	−0.13**	−0.07	−0.15**	−0.04
Ca	–	–	–	–	–	–	–		0.82***	0.45	0.79**	−0.42***	−0.12	−0.12	−0.12	−0.05
Mg	–	–	–	–	–	–	–	–		0.88*	0.65**	−0.12	−0.11	−0.17	−0.08	0.07
Na	–	–	–	–	–	–	–	–	–		0.98***	−0.11	−0.15**	−0.11	−0.14	0.03
S	–	–	–	–	–	–	–	–	–	–		−0.21	−0.03	−0.08	−0.16	0.07
Fe	–	–	–	–	–	–	–	–	–	–	–		0.53	0.23	0.56	0.11
Mn	–	–	–	–	–	–	–	–	–	–	–	–		0.75	0.67	0.44
B	–	–	–	–	–	–	–	–	–	–	–	–	–		0.78	0.78
Cu	–	–	–	–	–	–	–	–	–	–	–	–	–	–		0.69
Mo	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	

*, **, and *** indicate significance level at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively. SOC = soil organic carbon, SOM = soil organic matter, CEC = cation exchange capacity, N = Nitrogen, P = Phosphorus, K = Potassium, Ca = Calcium, Mg = Magnesium, Na = Sodium, S = Sulfur, Fe = Iron, Mn = Manganese, B = Boron, Cu = Copper, Mo = Molybdenum.

also observed for SOM, CEC, total N, available soil P, K, Ca, Mg, Na, and S, which positively correlated with each other. However, the SOC and SOM were negatively ($P \leq 0.01$) correlated to Mn. On the other hand, CEC capacity was negatively ($P \leq 0.05$) correlated to B and Cu. Total N showed a significantly ($P \leq 0.01$) negative correlation between Mn, B, and Cu. Further to this, available soil P and K were negatively ($P \leq 0.01$) correlated to Mn and Cu. The negative ($P \leq 0.001$) correlation was observed between Ca and Fe, whereas Na negatively ($P \leq 0.01$) correlated between Fe and Mn during the first season.

In the second season, the soil chemical properties significantly ($P \leq 0.001$) showed multicollinearity among each other. For example, soil pH had a strong positive ($P \leq 0.001$) correlation with SOC ($R^2 = 0.26$), SOM ($R^2 = 0.23$), total N ($R^2 = 0.15$), available soil P ($R^2 = 0.12$), K ($R^2 = 0.07$), Ca ($R^2 = 0.16$), and Mg ($R^2 = 0.12$) but a negative ($P \leq 0.01$) correlation with CEC. On the other hand, the SOC, SOM, total soil N, available soil P, K, Ca, and Mg showed a positive correlation amongst each other but a negative correlation to the CEC. Additionally, total soil N showed a significantly ($P \leq 0.01$) negative correlation with the following soil chemical properties; Fe, Mn, B, Cu, and Mo. Available soil P and K were both negatively ($P \leq 0.01$) correlated to Mn and Cu. Whereas Ca was positively related to Mg and S but indicated a negative ($P \leq 0.001$) correlation to Fe.

3.3. *Soil chemical properties after harvesting as affected by legume variety, AMF and Rhizobium*

The significant interactions ($P \leq 0.001$) among legume variety, AMF, and *Rhizobium* for soil pH after a harvesting period for two seasons are shown in Table 7. The MAR treatment showed a maximum value of soil pH when compared to other treatments during the 2017/2018 season. However, a considerable decrease in soil pH was observed under LC treatment. Similarly, soil pH significantly ($P \leq 0.001$) showed a 3-way interaction of treatments (legumes, AMF, and *Rhizobium* bacteria) for the second season. The high soil pH was observed on the CR treatment, whereas the LC had a significantly low pH. In terms of CEC, there was no significant ($P > 0.05$) interaction between treatments during both seasons, and this indicates that neither dual nor single inoculation had any effect on soil CEC. On the other hand, the SOC and SOM contents were significantly affected ($P \leq 0.001$) in a 2-way interaction due to treatment factors in both growing seasons after harvesting. For instance, during the first season, the content of SOC increased by 14.20 g kg^{-1} on CAR as compared to LC owing to dual inoculation. However, for the second season, the highest ($P \leq 0.001$) SOC was recorded under CA, followed by CR and CAR treatments, respectively. The CAR treatment produced the highest SOM in the first season, whereas the un-inoculated treatment of LC significantly reduced the SOM in the second season (Table 7).

In Table 8, the significant 2-way synergy of the factors (legume, AMF, and *Rhizobium* bacteria) for total N, available P, and S over two seasons is illustrated. The dual inoculation application significantly improved the total soil N. For example, the CAR treatment gave the highest total N (0.09 g kg^{-1}) in comparison to the LC treatment in the first season. Likewise, the treatment of CR significantly ($P \leq 0.01$) enhanced total soil N by 19.60 % as compared to LA treatment during the second season. The minimum total soil N in the LA treatment infers that AMF had a negative impact on total N. The amount of available soil P increased with dual inoculation; for instance, CAR and MAR treatments, which were not significantly different ($P > 0.05$) from each other, had the highest P content. Although the findings demonstrated that the dual inoculation had an impact on P, the LAR treatment negatively affected soil P during the first season. Furthermore, the highest P content was found on CR and CAR treatments, which did not vary significantly ($P > 0.05$)

Table 7
Effects of legume variety, arbuscular mycorrhizal fungi (AMF) and *Rhizobium* inoculation on soil physical properties after harvesting.

Treatments	pH (water)		CEC (cmol/kg)		SOC ($g.kg^{-1}$)		SOM ($g.kg^{-1}$)	
	2017/2018	2018/2019	2017/2018	2018/2019	2017/2018	2018/2019	2017/2018	2018/2019
Cowpea control	6.50^{h}	7.40^{d}	7.50	6.60	11.80^{c}	12.60^{c}	20.30^{c}	21.70^{c}
Cowpea + *Rhizobium*	6.90^{g}	7.80^{a}	7.90	6.70	13.60^{b}	14.50^{b}	23.40^{b}	24.90^{b}
Cowpea + AMF	8.00^{d}	7.50^{c}	7.50	6.40	13.70^{b}	15.10^{a}	23.60^{b}	26.00^{a}
Cowpea + AMF + *Rhizobium*	8.50^{b}	7.60^{b}	8.00	6.80	14.20^{a}	14.70^{b}	24.30^{a}	25.20^{b}
Lablab control	6.30^{i}	5.60^{j}	7.30	6.20	8.40^{i}	8.90^{i}	14.40^{i}	15.30^{h}
Lablab + *Rhizobium*	6.80^{g}	6.10^{h}	7.80	6.60	8.50^{hi}	9.10^{i}	17.40^{g}	18.60^{f}
Lablab + AMF	7.70^{e}	5.80^{i}	7.80	6.60	8.70^{h}	9.30^{h}	14.70^{h}	21.00^{d}
Lablab + AMF + *Rhizobium*	8.40^{c}	5.80^{i}	7.90	6.70	10.10^{g}	11.20^{f}	15.00^{h}	19.30^{f}
Mucuna control	6.50^{h}	6.80^{f}	7.30	6.20	10.60^{f}	10.80^{g}	18.20^{f}	16.00^{g}
Mucuna + *Rhizobium*	7.30^{f}	6.50^{g}	7.80	6.60	10.50^{f}	11.20^{f}	18.10^{f}	19.30^{e}
Mucuna + AMF	8.30^{c}	7.10^{e}	7.80	6.60	11.00^{e}	12.20^{d}	19.00^{e}	15.70^{g}
Mucuna + AMF + *Rhizobium*	8.80^{a}	6.20^{h}	7.90	6.70	11.40^{d}	11.80^{e}	19.70^{d}	20.20^{d}
SEM	0.04	0.04	0.10	0.09	0.12	0.15	0.21	0.26
F-test Probabilities								
Legume	***	***	ns	ns	***	***	***	***
Rhizobium	***	***	***	ns	***	***	***	***
AMF	***	***	***	ns	***	***	***	***
Legume + *Rhizobium*	*	ns	ns	ns	*	ns	*	ns
Legume + AMF	**	*	ns	ns	**	**	**	**
Rhizobium + AMF	ns	ns	ns	ns	***	***	***	***
Legume + *Rhizobium* + AMF	***	***	ns	ns	ns	ns	ns	ns

[abc] Means with dissimilar superscripts in the similar column vary significantly; *, **, and *** indicate significance level at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively; [ns] = non-significant. SEM = Standard error of means. CEC = cation exchange capacity, SOC = soil organic carbon, SOM = soil organic matter.

Table 8
Effects of legume variety, arbuscular mycorrhizal fungi (AMF) and *Rhizobium* inoculation on soil N, P and S ($g.kg^{-1}$) content after harvesting.

Treatments	Total N		Available P		S	
	2017/2018	2018/2019	2017/2018	2018/2019	2017/2018	2018/2019
Cowpea control	0.03^{e}	0.09^{b}	0.18^{g}	1.09^{b}	9.32^{h}	11.57^{f}
Cowpea + *Rhizobium*	0.08^{b}	0.11^{a}	0.64^{e}	1.06^{b}	9.66^{g}	11.98^{e}
Cowpea + AMF	0.07^{c}	0.09^{b}	0.84^{c}	1.25^{a}	9.30^{h}	11.55^{f}
Cowpea + AMF + *Rhizobium*	0.09^{a}	0.09^{b}	1.03^{a}	1.23^{a}	9.97^{f}	11.24^{g}
Lablab control	0.02^{f}	0.06^{d}	0.63^{e}	0.72^{d}	9.04^{i}	11.93^{e}
Lablab + *Rhizobium*	0.07^{bc}	0.05^{e}	0.46^{f}	0.39^{f}	10.03^{f}	12.45^{d}
Lablab + AMF	0.05^{d}	0.02^{f}	0.72^{d}	0.47^{e}	11.31^{e}	14.04^{c}
Lablab + AMF + *Rhizobium*	0.08^{b}	0.07^{d}	0.12^{h}	0.45^{e}	12.80^{c}	12.38^{d}
Mucuna control	0.04^{e}	0.08^{b}	0.19^{g}	0.89^{c}	9.56^{g}	15.88^{b}
Mucuna + *Rhizobium*	0.06^{d}	0.08^{c}	0.63^{e}	0.81^{c}	11.82^{d}	14.67^{c}
Mucuna + AMF	0.06^{cd}	0.07^{c}	0.87^{bc}	1.07^{b}	12.82^{b}	15.90^{b}
Mucuna + AMF + *Rhizobium*	0.08^{b}	0.04^{e}	0.98^{ab}	0.89^{c}	15.34^{a}	19.02^{a}
SEM	0.00	0.00	0.05	0.06	0.15	0.20
F-test Probabilities						
Legume	***	***	***	***	***	***
Rhizobium	***	***	***	***	***	***
AMF	***	***	***	***	***	***
Legume + *Rhizobium*	***	***	ns	ns	***	***
Legume + AMF	ns	ns	*	ns	***	***
Rhizobium + AMF	*	**	***	**	***	***
Legume + *Rhizobium* + AMF	ns	ns	ns	ns	***	***

[abc] Means with dissimilar superscripts in the similar column vary significantly. *, **, and *** indicate significance level at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively; [ns] = non-significant. SEM = Standard error of means. Total N = Nitrogen, available P = Phosphorus, S = Sulfur.

from each other during the second season. In contrast, the LR treatment had significantly ($P \leq 0.01$) the lowest P content than other treatment combinations. The significant 3-way interactions ($P \leq 0.001$) among the legume variety, AMF, and *Rhizobium* bacteria on S content over two seasons are also shown, with MAR treatment positively ($P \leq 0.001$) affecting soil S content in both seasons, while LC treatment negatively affected S content for the first season.

The findings indicated that there was a 3-way interaction effect ($P \leq 0.01$) between legume variety, AMF, and *Rhizobium* on soil Ca during the first season, as highlighted in Table 9. The amount of soil Ca content significantly improved due to the dual inoculation under the treatment of MAR, while the LC had low Ca content. In the second season, the treatment factors significantly ($P \leq 0.01$) showed a 2-way interaction for soil Ca. The LC, LR, and LAR treatments had exceptionally low soil Ca values of 3.10, 3.25, and 3.19 g kg^{-1}, respectively, which were not different ($P > 0.05$) among each other. However, the MAR treatment did not differ significantly ($P > 0.05$) from the MA treatment and recorded the highest soil Ca values of 3.86 and 3.79 g kg^{-1}, respectively. Additionally, there was a

Table 9
Effects of legume variety, arbuscular mycorrhizal fungi (AMF) and *Rhizobium* inoculation on soil Ca, K, Mg and Na ($g.kg^{-1}$) content after harvesting.

Treatments	Ca		K		Mg		Na	
	2017/2018	2018/2019	2017/2018	2018/2019	2017/2018	2018/2019	2017/2018	2018/2019
Cowpea control	4.28^{e}	3.46^{c}	2.89^{f}	1.56^{g}	2.28^{g}	3.24^{b}	0.44^{f}	0.52^{d}
Cowpea + *Rhizobium*	4.52^{c}	3.58^{c}	3.05^{e}	1.67^{f}	3.39^{c}	3.03^{c}	0.56^{e}	0.54^{d}
Cowpea + AMF	4.48^{c}	3.55^{c}	3.72^{d}	1.57^{g}	3.36^{b}	3.00^{c}	0.66^{c}	0.50^{d}
Cowpea + AMF + *Rhizobium*	4.79^{ab}	3.48^{c}	5.69^{a}	1.92^{c}	3.79^{a}	3.39^{a}	0.77^{a}	0.59^{c}
Lablab control	4.26^{e}	3.10^{e}	1.89^{h}	1.43^{h}	2.34^{h}	1.95^{g}	0.33^{g}	0.32^{f}
Lablab + *Rhizobium*	4.10^{f}	3.25^{e}	2.67^{g}	1.46^{h}	2.76^{f}	2.47^{e}	0.44^{f}	0.43^{e}
Lablab + AMF	4.37^{de}	3.39^{d}	2.62^{g}	1.42^{h}	2.86^{e}	2.36^{f}	0.51^{e}	0.42^{e}
Lablab + AMF + *Rhizobium*	4.68^{b}	3.19^{e}	4.72^{c}	1.66^{f}	3.33^{c}	2.44^{f}	0.54^{e}	0.43^{e}
Mucuna control	4.31^{de}	3.70^{b}	2.88^{f}	2.03^{d}	2.84^{e}	2.56^{e}	0.43^{f}	0.64^{c}
Mucuna + *Rhizobium*	4.44^{cd}	3.52^{c}	3.03^{e}	2.58^{c}	3.06^{d}	2.74^{d}	0.60^{d}	0.75^{a}
Mucuna + AMF	4.40^{cd}	3.79^{ab}	3.51^{d}	3.10^{a}	3.32^{c}	2.97^{c}	0.72^{b}	0.70^{b}
Mucuna + AMF + *Rhizobium*	4.87^{a}	3.86^{a}	5.33^{b}	2.91^{b}	3.65^{b}	2.98^{c}	0.78^{a}	0.76^{a}
SEM	0.05	0.04	0.08	0.06	0.02	0.02	0.01	0.01
F-test Probabilities								
Legume	***	***	***	***	***	***	***	***
Rhizobium	***	***	***	***	***	***	***	***
AMF	***	***	***	***	***	***	***	***
Legume + *Rhizobium*	***	**	**	*	***	***	**	***
Legume + AMF	ns	***	***	**	ns	***	***	***
Rhizobium + AMF	***	ns	***	***	ns	***	***	*
Legume + *Rhizobium* + AMF	**	ns	ns	ns	***	***	**	***

[abc] Means with dissimilar superscripts in the similar column vary significantly. *, **, and *** indicate significance level at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively; [ns] = non-significant. SEM: Standard error of means. Ca = Calcium, K = Potassium, Mg = Magnesium, Na = Sodium.

significant ($P \leq 0.01$) 2-way synergy among treatment factors (legume variety, AMF, and *Rhizobium*) on soil K over two seasons. Compared to LC, CAR treatment significantly ($P \leq 0.01$) increased the soil K by 33.20 % for the first season. Whereas during the second season, MA treatment improved soil K by 46.10 %, 47.10 %, and 45.80 % when compared to LC, LR, and LA treatments, respectively.

The results further showed the significant 3-way effect of legume variety, AMF, and *Rhizobium* bacteria on soil Mg and Na over two seasons (Table 9). The dual inoculation significantly enhanced soil Mg content, particularly in cowpea over other legumes. In both seasons, the CAR accumulated significantly ($P \leq 0.01$) more soil Mg than other treatment combinations, whereas LC significantly recorded the least soil Mg content in both seasons. Moreover, the dual inoculation greatly influenced Na content, as MAR and CAR treatments resulted in high Na contents (0.78 and 0.77 g kg^{-1}, respectively) during the first season. However, mucuna outcompeted other legumes in the second season, with the most noticeable increase being observed under MAR treatment, followed by MR and MA treatments (0.76, 0.75, and 0.70 g kg^{-1}, respectively).

Table 10 shows that there were no significant ($P > 0.05$) interactions among treatment factors observed on soil Mn content in both seasons, with neither single nor dual inoculation having any effect on Mn. Moreover, the significant 3-way interaction of treatment factors for B and Cu was shown in the soil over two seasons. The most noticeable effect on B content for the first season was observed under LA, followed by MAR and CAR treatments, while the un-inoculated CC and LC treatments had a low B content. Additionally, no significant differences ($P > 0.05$) were observed between the LA and LAR treatments. Results further highlight that on Cu, the LA treatment significantly improved its content in both seasons, and a similar trend was observed for Mo content in the soil over two seasons.

3.4. Correlation matrix of soil chemical properties after harvesting

The significant correlation analysis of the soil chemical properties after harvesting for the seasons 2017/2018 and 2018/2019 is captured in Table 11. During the first season, soil chemical properties were positively correlated with each other. For example, the soil pH showed a positive ($P \leq 0.001$) correlation with SOC ($R^2 = 0.12$), SOM ($R^2 = 0.09$), CEC ($R^2 = 0.46$), total N ($R^2 = 0.55$), available soil P ($R^2 = 0.88$), K ($R^2 = 0.70$), Ca ($R^2 = 0.25$), Mg ($R^2 = 0.42$), Na ($R^2 = 0.63$), and S ($R^2 = 0.48$). In contrast, B, Cu, and Mo were negatively ($P \leq 0.05$) correlated with other soil chemical properties, except for soil pH, SOC, or SOM. However, Fe and Mn showed no significant ($P > 0.05$) interaction with each other, as well as with other soil properties. The soil pH showed a great positive ($P \leq 0.001$) correlation among soil properties in the subsequent season. Despite this, a negative correlation was observed between B, Cu, and Mo with soil pH, SOC, SOM, CEC, total N, available P, and K. Findings also show that Fe negatively correlated ($P \leq 0.05$) with Ca, Mg, and Na. However, Fe and Mn showed no significant ($P > 0.05$) interaction with soil pH, SOC, SOM, or total soil N.

4. Discussion

The synergistic interaction of legume varieties, AMF, and *Rhizobium* bacteria generally responded better as compared to control or sole inoculation, with significant differences observed on soil pH, SOM, SOC, CEC, and other soil nutrients for both seasons. The soil pH

Table 10
Effects of legume variety, arbuscular mycorrhizal fungi (AMF) and *Rhizobium* inoculation on soil Mn, B, Cu and Mo ($mg.kg^{-1}$) content after harvesting.

Treatments	Mn		B		Cu		Mo	
	2017/2018	2018/2019	2017/2018	2018/2019	2017/2018	2018/2019	2017/2018	2018/2019
Cowpea control	601.00	521.00	58.41^{d}	67.67^{d}	40.50^{e}	47.42^{d}	60.17^{e}	66.00^{e}
Cowpea + *Rhizobium*	624.00	540.00	79.08^{b}	89.42^{c}	61.42^{b}	68.83^{c}	71.92^{c}	77.33^{c}
Cowpea + AMF	663.00	571.00	77.42^{c}	89.58^{c}	61.58^{b}	56.83^{b}	75.17^{b}	72.08^{d}
Cowpea + AMF + *Rhizobium*	624.00	539.00	82.92^{a}	68.00^{c}	64.83^{b}	68.33^{c}	75.58^{b}	71.08^{d}
Lablab control	591.00	509.00	59.42^{d}	95.17^{b}	48.41^{e}	72.92^{bc}	64.50^{d}	83.25^{b}
Lablab + *Rhizobium*	642.00	554.00	78.25^{bc}	90.25^{c}	58.75^{d}	72.08^{bc}	71.17^{c}	84.83^{b}
Lablab + AMF	702.00	606.00	89.67^{a}	103.08^{a}	70.83^{a}	83.17^{a}	81.00^{a}	89.33^{a}
Lablab + AMF + *Rhizobium*	592.00	509.00	78.83^{bc}	99.58^{a}	58.25^{d}	75.75^{b}	76.25^{b}	83.75^{b}
Mucuna control	666.00	575.00	77.08^{c}	89.33^{c}	60.33^{b}	70.00^{bc}	65.50^{d}	82.83^{b}
Mucuna + *Rhizobium*	609.00	528.00	77.42^{c}	90.92^{c}	59.67^{c}	69.75^{c}	70.58^{c}	79.25^{c}
Mucuna + AMF	599.00	517.00	78.92^{bc}	90.75^{c}	59.00^{c}	69.25^{c}	72.58^{c}	79.83^{c}
Mucuna + AMF + *Rhizobium*	646.00	557.00	86.50^{ab}	90.67^{c}	62.08^{b}	72.33^{bc}	76.83^{b}	78.33^{c}
SEM	27.32	23.60	3.07	3.53	3.06	3.62	2.09	2.33
F-test probabilities								
Legume	ns	ns	*	*	ns	ns	ns	ns
Rhizobium	ns	ns	***	***	*	*	***	***
AMF	ns	ns	***	***	***	***	***	***
Legume + *Rhizobium*	ns	ns	*	ns	**	*	ns	ns
Legume + AMF	ns	ns	ns	*	**	**	ns	ns
Rhizobium + AMF	ns	ns	***	***	***	***	***	***
Legume + *Rhizobium* + AMF	ns	ns	***	***	**	*	ns	ns

[abc] Means with dissimilar superscripts in the similar column vary significantly. *, **, and *** indicate significance level at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively; [ns] = non-significant. SEM = Standard error of means. Mn = Manganese, B = Boron, Cu = Copper, Mo = Molybdenum.

Table 11
The mean correlation analysis of selected soil chemical properties after harvesting in the two growing seasons.

	pH	SOC	SOM	CEC	N	P	K	Ca	Mg	Na	S	Fe	Mn	B	Cu	Mo
									2017/2018							
pH		0.76***	0.75***	0.23***	0.43***	0.72***	0.65***	0.32***	0.54***	0.75***	0.56***	−0.11	−0.03	−0.23	−0.06	−0.23
SOC	–		1.00***	0.24***	0.87***	0.95***	0.67***	0.27***	0.43***	0.67***	0.54***	−0.02	−0.02	−0.15	0.22*	0.32
SOM	–	–		0.24***	0.87***	0.94***	0.67***	0.27***	0.36***	0.56***	0.53***	−0.01	−0.02	−0.15	0.22	0.32
CEC	–	–	–		0.43***	0.21***	0.43***	0.63***	0.85***	0.34***	0.34***	−0.05	−0.13	−0.18*	−0.14**	−0.12*
N	–	–	–	–		0.53***	0.64***	0.46***	0.83***	0.65***	0.67***	−0.02	−0.06	−0.21**	−0.21**	−0.14**
P	–	–	–	–	–		0.68***	0.34***	0.44***	0.73***	0.68***	−0.04	−0.04	0.25**	0.33***	0.44***
K	–	–	–	–	–	–		0.89**	0.79***	0.71***	0.63***	−0.10	−0.05	0.23***	0.34***	0.45***
Ca	–	–	–	–	–	–	–		0.94***	0.54***	0.57***	−0.12	−0.03	0.14*	0.32***	0.43***
Mg	–	–	–	–	–	–	–	–		0.92***	0.55***	−0.16	−0.07	0.23**	0.34**	0.47***
Na	–	–	–	–	–	–	–	–	–		0.53***	−0.16	−0.05	0.35**	0.30**	0.48***
S	–	–	–	–	–	–	–	–	–	–		−0.10	−0.02	0.32***	0.30*	0.39***
Fe	–	–	–	–	–	–	–	–	–	–	–		−0.21	0.29***	0.27*	0.07***
Mn	–	–	–	–	–	–	–	–	–	–	–	–		0.78***	0.75*	0.43***
B	–	–	–	–	–	–	–	–	–	–	–	–	–		0.79*	0.84***
Cu	–	–	–	–	–	–	–	–	–	–	–	–	–	–		0.89***
Mo	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
2018/2019																
pH		0.92***	0.92***	0.36***	0.61***	0.83***	0.80***	0.47***	0.63***	0.80***	0.70***	0.02	0.07	−0.49**	−0.03**	−0.45**
SOC	–		1.00***	0.31***	0.71***	0.80***	0.81***	0.43***	0.59***	0.73***	0.77***	−0.05	−0.04	−0.43**	−0.31*	−0.44**
SOM	–	–		0.31***	0.71***	0.80***	0.81***	0.43***	0.59***	0.73***	0.77***	−0.05	−0.04	−0.43**	−0.31*	−0.44**
CEC	–	–	–		0.60***	0.37***	0.56***	0.75***	0.77***	0.51***	0.55***	−0.09	0.67	−0.22**	−0.20**	−0.15*
N	–	–	–	–		0.67***	0.78***	0.59***	0.77***	0.70***	0.70***	−0.04	−0.01	−0.40*	−0.30*	−0.40*
P	–	–	–	–	–		0.72***	0.49***	0.66***	0.84***	0.59***	−0.05	0.18*	0.55***	0.43***	0.53***
K	–	–	–	–	–	–		0.73***	0.84***	0.82***	0.79***	−0.12	−0.01	0.33***	0.26***	0.36***
Ca	–	–	–	–	–	–	–		0.90***	0.66***	0.67***	−0.28**	−0.02	0.22**	0.26**	0.30***
Mg	–	–	–	–	–	–	–	–		0.84***	0.68***	−0.23**	−0.07	0.37***	0.38***	0.43***
Na	–	–	–	–	–	–	–	–	–		0.66***	−0.20**	−0.01	0.40***	0.33***	0.44***
S	–	–	–	–	–	–	–	–	–	–		−0.13	0.04	0.39***	0.33***	0.38***
Fe	–	–	–	–	–	–	–	–	–	–	–		0.41***	0.22**	0.21**	0.04
Mn	–	–	–	–	–	–	–	–	–	–	–	–		0.66***	0.67***	0.48***
B	–	–	–	–	–	–	–	–	–	–	–	–	–		0.76***	0.72***
Cu	–	–	–	–	–	–	–	–	–	–	–	–	–	–		0.80***
Mo	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	

*, **, and *** indicate significance level at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively. SOC = soil organic carbon, SOM = soil organic matter, CEC = cation exchange capacity, N = Nitrogen, P = Phosphorus, K = Potassium, Ca = Calcium, Mg = Magnesium, Na = Sodium, S = Sulfur, Fe = Iron, Mn = Manganese, B = Boron, Cu = Copper, Mo = Molybdenum.

increased from acidic (initial) to alkaline (before flowering and after harvesting), as influenced mostly by the inoculation interaction of AMF and *Rhizobium* bacteria. This increase may be due to the AMF immobilizing plant intra-radical hyphal cell wall components through secreting compounds such as glomalin inside fungal cells that alter the metal concentration in plants [37]. The reduction of soil pH during the second season of the current is supported by a study conducted by Moila et al. [31], which reported that the ability of P solubilizing to reduce soil pH depends on the potential of soil protons released by an AMF strain. As such, AMF acts as a metal sink by reducing local concentrations in soils while increasing the soil pH to create a more suitable environment for the growth of plants [38]. According to Okonji et al. [39], the increase in soil pH could be due to phosphate-induced incorporation of P, which raises soil pH and reduces concentrations of toxic aluminum compounds by creating insoluble variscite-like material [Al $(OH)_2$ H_2 PO_4] precipitates in soil. Although dual inoculation continued to improve soil pH over time as it progressed to the second season, observations were made that the soil pH was more acidic as compared to the first season. Thus, the decline in soil pH may be due to soil acidification caused by soil mineral leaching from high rainfall amount received during the second season. This could be promoted by the combined reaction of water (H_2O) and carbon dioxide (CO_2) to form weak carbonic acid. An earlier study by Chien [40] advances the idea that weak carbonic acid releases H^+ ions and bicarbonate (HCO_3), and these ions replace Ca ions and mix with bicarbonate ions to create calcium bicarbonate. This calcium bicarbonate is then leached from the soil profile and results in increased acidity.

There was a slight increase in CEC before the sowing period as compared to before flowering stage. Despite this, CEC increased significantly after harvesting in both seasons. Similarly, extant literature shows that the interaction of legume variety, AMF, and *Rhizobium* bacteria inoculation results in high CEC content in soils [38,41]. The CEC proliferation could be attributed to an increase in soil pH from the initial to harvesting stage. In addition, the results of the study indicate that the dual inoculation has the hyphal potential to change the acidic soil to an alkaline soil due to negative charges that hold the soil and alter microbial activity or proliferation, consequently resulting in an increase in CEC as supported by Marschner et al. [42] and Orwin et al. [43]. Furthermore, the increased soil CEC due to dual inoculated treatments could be attributed to high levels of SOM, which ultimately enhance soil fertility, as demonstrated in this study by the positive correlation matrix between CEC, soil pH, and SOM.

The AMF and *Rhizobium* bacteria, and their interaction, significantly enhanced SOC and SOM. The results showed that dual inoculation had a positive effect on SOC and SOM throughout the sampling stages as compared to un-inoculated soils. The noticeable effect of dual inoculation was mostly recorded during the second season, as SOC and SOM were higher as compared to the first season. In terms of treatment factors and legume comparison, the MAR treatment had high SOC and SOM for the first season before and after flowering. The CAR and MAR treatments had maximum SOC and SOM before flowering for the second season. The increase might be attributed to the symbiotic relationship between plant hosts, AMF, and *Rhizobium* bacteria that directly inhibit the activity of microbiota that can decompose plant litter and, in the process, increase the abundance of soil microbes that improve these soil properties [44,45]. According to Toljander et al. [46] and Verbruggen et al. [47], exudate components found in AMF include glucose, organic acids, polysaccharides (glycogen, oligosaccharides), and polymer compounds (gellan gum), among which are refractory and add to the SOM content. The richness of SOM could guarantee a good supply of major elements such as N, P, K, Ca, and Fe through the process of mineralization [48]. The findings of the present study align with research done by Shoko et al. [49] and Cheng et al. [44] found that SOM and SOC increased more after harvesting, and this was attributable to the addition of above-ground biomass from mucuna and cowpea as green manure, which provided plant materials to the soil microorganisms to decompose. Therefore, the interaction effect of AMF and *Rhizobium* bacteria could improve the rate of decomposition of organic material, which circuitously influences decomposition by interacting with other soil microorganisms.

Microbial dual inoculation showed a tremendous improvement in soil macronutrients as compared to individual inoculations and/or un-inoculated treatments. However, control treatment and the single inoculation of either AMF or *Rhizobium* bacteria enhanced micronutrient content more in comparison to dual inoculation. Even so, mucuna and cowpea were highly associated with soil macronutrient concentrations as compared to lablab forage. The current results correspond with various studies that reported that the dual inoculation of biofertilizers significantly increased the concentration of soil macronutrients (i.e., total N, P, K, and Ca) [25,50,51]. In contrast, Benaffari et al. [14] showed that the amounts of K, Ca, and Fe were lower in the biofertilizer treatments of the postharvest field soils. These observations were relatively explained by their absorption into the mycorrhizal and *Rhizobium*-treated plants. The increase of these minerals in the current study could be associated with solubilization by AMF through the release of organic acid anions such as citrate, malate, and oxalate, as concluded in research by Meena et al. [15]. According to Benaffari et al. [14], AMF has the potential to enhance the chemical and nutritional quality of the soil using various mechanisms, such as soil structure and aggregates, as well as P solubilization through the release of glomalin. The AMF has strong mycelia, which expand the area of roots available for absorption of nutrients such as P by releasing extracellular phosphatase enzyme [6,38,45]. Konvalinková et al. [23] reported that AMF hyphae have a strong affinity for inorganic P, and because of their small diameter than roots, they can explore inaccessible soil pores and increase inorganic P translocation. On the other hand, the increase in total N could also be attributed to dual inoculation, which reduces N leaching caused by this natural mineral's distinctive properties. The AMF and *Rhizobium* bacteria fix N cations such as ammonium with the assistance of nitrifying bacteria, which can take up NH_4^+ or NO_3^- and improve soil total N content [6]. In this study, the dual inoculation caused an elevation of soil pH and CEC, which led to low N leaching due to soil ion neutralization. Also, *Rhizobium* and related soil N fixation enzymes perform best at nearly neutral soil pH.

Nevertheless, dual or single inoculation with AMF and *Rhizobium* bacteria had no significant effect on soil micronutrient concentration. However, the most notable increase in soil micronutrient concentration was observed under the control treatment of the mucuna legume. In contrast, several studies have reported that either dual or single microbial inoculation enhances the content of soil micronutrients when compared to control [6,23,38]. Also, similar observations were made by Mortimer et al. [52] and Halder et al. [25], who showed that the synergistic effects of the dual application of AMF and *Rhizobium* enhanced soil Mn and Cu concentrations. The lack of response of dual or single inoculation to improve soil micronutrients in the current study might be due to the inactive of microbial inoculation to compete with native microorganisms to survive in the soil environment. These findings are commensurate with studies conducted by Singh [53,54]. Further, this could be governed by the properties of the soil, AMF exudates, or climatic conditions which result in microbial inoculation being inactive. Generally, the significant impact of dual inoculation on soil chemical properties found in the current study has very limited comparative studies. Golubkina et al. [55] deduced that the efficacy of AMF application depends on the plant's genetic peculiarities, AMF species, characteristics of the soil and other environmental factors. Moila et al. [31] concluded that the buildup of the soil nutrients usually takes a very long time to respond to soil-applied treatments. Thus, single- or two-cycle season experiments may not show a positive soil mineral response to applied treatments.

5. Conclusion

The dual inoculation of forage legume species with AMF and *Rhizobium* bacteria enhanced soil properties. The effect of the dual inoculation can have synergistic effect on the uptake of nutrients, soil aggregate formation and stabilization. Thus, biofertilization have the potential to reduce the overuse use of synthetic fertilizers, which remain a major concern for emerging farmers. Although AMF inoculation can have positive responses in soils with low fertility, they are more likely to occur in soils where low available P status has been rectified with the application of superphosphate. The presence of P in the soil activates *Rhizobium* bacteria, which then improves the soil N biological fixation. However, the concentrations of micronutrients were negatively affected by dual inoculation as compared to the control treatment. Moreover, the current study showed that a single inoculum of either bacteria or fungi did not have any effect on soil chemical properties. Finally, it must be acknowledged that a study of this nature needs to be carried out over an extended period to further support and substantiate the positive response of dual inoculation with organic fertilizers on soil properties and should also be tested on different soil types.

Data availability statement

Data will be made available on request.

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CRediT authorship contribution statement

Sanele Mpongwana: Writing - original draft, Writing - review & editing, Methodology, Data curation, Investigation, Visualization, Formal analysis. **Alen Manyevere:** Writing - review & editing, Validation, Supervision, Methodology, Funding Acquisition, Project administration, Resources, Conceptualization. **Johnfisher Mupangwa:** Writing - review & editing, Validation, Supervision, Methodology, Funding acquisition, Project administration, Resources, Conceptualization. **Conference Thando Mpendulo:** Writing - review & editing, Validation, Supervision, Methodology, Funding Acquisition, Project administration, Resources, Conceptualization. **Chuene Victor Mashamaite:** Writing - original draft, Writing - review & editing, Methodology, Visualization, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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