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Research article

Dual inoculation of soybean with *Rhizophagus irregularis* and commercial *Bradyrhizobium japonicum* increases nitrogen fixation and growth in organic and conventional soils

Nicholas Mawira Gitonga[1,2,*], Gilbert Koskey[3], Ezekiel Mugendi Njeru[1], John M. Maingi[1] and Richard Cheruiyot[4]

[1] Department of Biochemistry, Microbiology and Biotechnology, Kenyatta University, Nairobi, Kenya
[2] Department of Biological and Physical Sciences, Karatina University, Karatina, Kenya
[3] Institute of Life Sciences, Sant'Anna School of Advanced Studies, Pisa, Italy
[4] Department of Plant Sciences, Kenyatta University, Nairobi, Kenya

* Correspondence: Email: ngitonga@karu.ac.ke.

Abstract: Soil amendment with beneficial microorganisms is gaining popularity among farmers to alleviate the decline of soil fertility and to increase food production and maintain environmental quality. However, farm management greatly influence soil microbial abundance and function, which overly affects crop growth and development. In this work, greenhouse experiments involving soybeans were conducted to evaluate the effects of bradyrhizobia and arbuscular mycorrhizal fungi (AMF) dual inoculation on nodulation, AMF root colonization, growth and nutrient acquisition under contrasting farming systems. The experimental treatments were AMF and/or bradyrhizobia inoculation and dual inoculation on SC squire soybean variety. The exotic AMF inoculants used were *Funneliformis mosseae* (BEG 12) and *Rhizophagus irregularis* (BEG 44) while bacteria were commercial *Bradyrhizobium japonicum* (USDA110) and native bradyrhizobia isolates. Experiments with soil samples from organic and conventional farms were set out using a completely randomized design with three replicates. The results demonstrated that bradyrhizobia and AMF dual inoculation consistently and significantly enhanced soybean nodule dry weight (NDW), shoot dry weight (SDW) and AMF root colonization compared with individual bradyrhizobia, AMF and non-inoculated control. Moreover, organic soil significantly ($p = 0.001$) increased soybean SDW, NDW and AMF root colonization compared to conventional soil. Remarkably, shoot nutrients content differed in organic and conventional farming where, shoot nitrogen, phosphorus, potassium and organic carbon were higher in organic farming than the latter. Among individual inoculants, *Rhizophagus irregularis* outperformed *Funneliformis mosseae,* while commercial *Bradyrhizobium japonicum* showed higher performance than native bradyrhizobia. Our results demonstrated the importance of organic farming, AMF and bradyrhizobia dual inoculation in enhancing soybean growth and nutrient acquisition. However, field trials should be assessed to determine the good performance of bradyrhizobia and AMF dual inoculation in organic farming before being popularized and adopted by farmers as a sustainable agronomical management strategy to increase soil fertility and food productivity.

Keywords: dual inoculation; soybean; bradyrhizobia; arbuscular mycorrhizal fungi; organic; conventional farming

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1. Introduction

Soybean (*Glycine max* L. Merrill) is an important legume with high nutritional value for human health and socio-economic well-being of the low-income rural populations across the globe [1,2]. Soybean seeds contain about 21% oil, 40% protein and 34% carbohydrate contents [3]. Soybean serves as a source of food and food supplements for human and as livestock feed in most countries [4]. Countries with high population growth, for example in the sub-Saharan Africa, highly rely on the cultivation of this legume in their efforts to address food insecurity and related challenges [5]. In Kenya, soybean is cultivated as a food diet and for income by majorly the rural smallholder farmers [6–8] who face financial and environmental resource constraints. For instance, the current decline in soybean productivity in smallholder setups has been attributed to the climatic challenges [9] including unpredictable drought recurrence [10], soil nutrient deficiency [11] due to excessive leaching and acidity, inappropriate use of fertilizers [12] and poor soil management practices [13].

Various mechanisms have been put in place to address the challenges facing smallholder soybean production especially under conventional farm management systems. The most applied strategy that addresses the deficiency of soil nutrients is the use of chemical inputs [12] and development of promiscuous soybean varieties with ability to form effective symbiotic interaction with indigenous bradyrhizobia communities in the soils [14]. However, the strategy of chemical applications has led to the excessive and inappropriate use of large quantities of inorganic fertilizers, herbicides and pesticides to improve soybean production. These strategies have consequently caused serious environmental health concerns, loss of biodiversity and soil fertility depletion [15,16], thus rendering the conventional farming systems unsustainable and environmentally non-productive [17]. It is estimated that only 30–35% of nitrogen (N) fertilizer and 8–10% of phosphorus (P) fertilizer applied by the farmers in conventional management system is absorbed and utilized by the soybean plants [18,19], of which, the same amount of nutrients could be drawn from the soil if the crop is optimally managed organically [13] and with additional inoculation of beneficial plant growth promoting microorganisms such as N-fixing bradyrhizobia [1,14] and P-solubilizing arbuscular mycorrhizal fungi [20].

Organic farming has emerged as a potential alternative in terms of the growing demand of healthy food supply, long-term sustainability, and concerns regarding environmental pollution [20]. Organic farming systems rely on measures that stimulate resilience and sustainability of the agroecosystem by enhancing incorporation of organic matter and beneficial microorganisms that efficiently avail nutrients to the plants, promote fertility and health of the soil. Furthermore, by reducing the application of inorganic nitrogen fertilizers, organic farming systems tends to stabilize soil pH, reduce overall cost of production and increase economic returns to the smallholder farmers [21,22]. This system could be viable for soybean production and soil health restoration in African tropical agroecological zones with typically highly leached acidic soil.

Soybeans significantly depend on their rhizospheric symbionts for nutrient acquisition, growth and dry matter accumulation. Beneficial rhizospheric interactions play a crucial role in maintaining soil ecological balance and, therefore, to the sustainability of both natural and soil ecosystems. More so, the interactions positively influence plant health, soil quality, and soybean yield [23]. Exclusively, employing nitrogen-fixing bradyrhizobia bacteria and AMF in soybean production are important agricultural practices with the potential to enhance fixation of atmospheric nitrogen, solubilization and mobilization of phosphorus and other essential soil nutrients that enhance soybean growth, nutrition, and yield.

Bradyrhizobia-AMF-soybean symbiotic association is essential in facilitation of nutrients (N, P and Zn) and water [24]. Their tripartite interactions improve soil nutrient mobility and uptake efficiency, soil fertility and plant nutrition [25]. Moreover, studies have hypothesized that optimizing AMF and/or bradyrhizobia through inoculation and dual inoculation could further enhance growth and increase yields of soybean and other legumes. Abd-Alla et al. [26] revealed that symbiotic interaction of AMF and *Bradyrhizobium japonicum* strains exerts positive effects on plant growth by improving P and N availability while a study by Ruiz-Lozano et al. [27] confirmed a positive effect of the interactions between AMF and rhizobia under drought conditions. Although field studies show that organic farming tend to increase nitrogen fixation and soybean growth than conventional farming; the effect of bradyrhizobia and AMF dual inoculation in the two farming systems has not been tested fully in Eastern Kenya. Secondly, little attention has been focused on the possibility of raising the inoculum potential of bradyrhizobia and AMF through organic farming; a strategy that would be fundamental towards the development of sustainable agriculture, increased crop growth, food production and enhanced soil fertility.

The study hypothesized that soybean inoculation *versus* dual inoculation with AMF and/or bradyrhizobia increase AMF root colonization, nodulation, soil fertility, nutrient acquisition and growth in organic than conventionally managed farm soils. In view of all these, greenhouse experiments were carried out between September 2016 and April 2017 to investigate the effect of inoculation and dual inoculation of AMF and/or bradyrhizobia on soybean root colonization, nodulation and nutrient acquisition in leached and acidic soil obtained from organic and conventional farming systems in Kenya. Understanding the effects of inoculation and dual inoculation with AMF and/or bradyrhizobia on soybean production under low pH and leached soils is important as it forms the basis upon which cheap and affordable biofertilizers can be developed and adopted by farmers as alternatives for a sustainable agricultural production. The study achieved its aim with the results demonstrating the importance of organic farming and dual inoculation with AMF and bradyrhizobia in enhancing biological soil fertility, nutrient acquisition and sustainable production of soybean while maintaining environmental quality.

2. Materials and methods

2.1. Experimental site and source of rooting medium

Soil samples were collected in September 2016 from three strictly organic and three conventionally managed farmers' fields in Meru South, Tharaka Nithi County, Kenya and used as the growth and rooting medium. The identified organic farms were designated as GO, JO and HO, located at 0°22′16.6″S 37°38′49.5″E; 0°22′18.8″S 37°38′44.2″E and 0°22′21.0″S 37°38′43.9″E respectively; while the conventional farms were designated as GC, JC and HC, respectively located at 0°22′18.2″S 37°38′50.6″E; 0°22′20.1″S 37°38′42.4″E and 0°22′19.7″S 37°38′45.5″E. All the farms had no previous history of bradyrhizobia inoculation or soybean cultivation. The organic farmers were trained and certified. The three organic farms had three years after conversion and no recent history of herbicide, pesticide and inorganic fertilizer application while conventional farms had a long history of herbicide, pesticide and inorganic fertilizer application.

The upper 20 cm of soil was sampled from 12 different points along the diagonally and across every selected farm prior to the onset of short rains. Samples from each farm were mixed thoroughly to make a homogenous composite sample. One kilogram of the composite sample from each farm was packaged independently in sterile khaki bags, sealed and transported to the laboratory for storage at 4 ℃, analysis and greenhouse assay. The soils were air-dried, ground, and passed through a 2-mm sieve prior to analysis [28]. Soil pH was determined using a pH meter (Hach, HQ411d, UK) in a prepared soil-water suspension ratio of 1: 2.5. The soil organic carbon was determined by Walkley-Black combustion method [29] while nitrogen concentration was determined following the Kjeldahl method (Hanon K9840 Kjeldahl apparatus) as described by Sáez-Plaza et al. [30]. The available phosphorus (P) and potassium (K) were determined according to Mehlich-3 (M-3) procedures [31]. The soils are Humic Nitisols [32], well weathered, leached and typically acidic. The soil (rooting medium) for use in the greenhouse assay was sterilized in an autoclave at 0.11 MPa, 121 ℃ for two hours.

2.2. Plant material and inoculants

The SC squire soybean variety was used as the test plant because of its superior performance in the field experiments compared to other soybean varieties. Furthermore, it is a promiscuous soybean variety that nodulates with diverse bradyrhizobia species, high yielding and produces large seeds with high oil content [33]. The seeds were sourced from the Kenya Agricultural Research Institute (KARI) Njoro, Nakuru. Two exotic AMF inoculants; *Funneliformis mosseae* (BEG 12) and *Rhizophagus irregularis* (BEG 44) and two bacteria inoculants; commercial *Bradyrhizobium japonicum* (USDA 110) and native bradyrhizobia isolate (NRB26) were used. The exotic AMF inoculants were acquired from INRA, Dijon France and propagated in pot cultures using Bermuda grass (*Cynodon dactylon*). The resulting propagule inoculants of AMF (consisted of 50 spores' g^{-1}, mycelium and root fragments) were used as the mycorrhizal spore inoculants [34]. The commercial *Bradyrhizobium japonicum* (USDA 110) inoculant was obtained from MEA Company Nakuru-Kenya, sold under license from the Microbiological Resources Centre (MIRCEN), University of Nairobi while the native bradyrhizobia (NRB26) was isolated from the root nodules of SC squire soybean used in the field trapping experiment. The native bradyrhizobia (NRB26) was used because of its superior performance compared with the other native bradyrhizobia soybean isolates.

2.3. Experimental design

A completely randomized design (CRD) with three replicates was used. The SC squire soybeans were inoculated with native bradyrhizobia (NRB26), commercial *Bradyrhizobium japonicum*

(USDA110), *Funneliformis mosseae* (BEG 12) and *Rhizophagus irregularis* (BEG 44) while dual inoculation consisted of a combination of two inoculants (as shown in Table 1) mixed at an equal ratio of 1: 1 (v/v). The controls were left un-inoculated and consisted of only sterilized soil. Treatments requiring AMF inoculation were supplied with 10 g of AMF inoculant against forty grams (40 g) of sterilized soil mixed in a 50 mL falcon tube during transplanting. Seedlings inoculation with the bradyrhizobia inoculant was achieved by applying 1 mL (10^9 colony forming units) of the respective bradyrhizobia inoculant seven days after planting as described by Somasegaran et al. [35]. The application method for treatments requiring AMF + *Bradyrhizobium* co-inoculation was adopted and modified from previous co-inoculation studies [36,37]. A total of 11 treatments were represented giving a total of 33 falcon tubes constituting the whole experimental layout (Table 1).

Table 1. The composition of 11 different treatments represented in greenhouse experiments.

Treatment	Composition
T1	Native bradyrhizobia (NRB)
T2	*Bradyrhizobium japonicum* (CB)
T3	*Funneliformis mosseae* (FM)
T4	*Rhizophagus irregularis* (RI)
T5	Native bradyrhizobia + *Bradyrhizobium japonicum* (NRB + CB)/ or T1 + T2
T6	*Funneliformis mosseae* + *Rhizophagus irregularis* (FM + RI)/ or T3 + T4
T7	Native bradyrhizobia + *Funneliformis mosseae* (NRB + FM) or T1 + T3
T8	Native bradyrhizobia + *Rhizophagus irregularis* (NRB + RI) or T1 + T4
T9	*Bradyrhizobium japonicum* +*Funneliformis mosseae* (CB + FM) or T2 + T3
T10	*Bradyrhizobium japonicum* + *Rhizophagus irregularis* (CB + RI) or T2 + T4
T11	Absolute control without inoculation (C)

2.3 *Soybean pre-germination, planting and management*

Healthy soybean seeds of good viability (85% germination) were selected and surface-sterilized with 3% sodium hypochlorite solution for 5 minutes, rinsed with 8 changes of sterile distilled water, and soaked in clean sterile distilled water for one hour to allow imbibition [35]. Twelve seeds were transferred aseptically to the Petri dishes containing 2% water agar plates and incubated upside down at 28 °C for 4 days in the dark [38]. Aseptically, two seedlings whose radicles attained a length of 1–2 cm after the incubation period were transferred onto falcon tubes filled with the rooting media (and 10g AMF inoculant for only AMF treatments) and moistened with sterilized distilled water. Watering was done after every 4 days interval using sterile distilled water. Seven days after planting, thinning was done to reduce the plants to one seedling in each falcon tube and inoculation was done using freshly made 1mL of bradyrhizobia broth culture for treatments requiring bradyrhizobia. The greenhouse conditions had 12 hours of natural light and darkness and an average temperature of 25 °C and 50%–80% relative humidity during the growing period.

2.4. *Soybean harvesting*

Forty-five days after planting, the plants were harvested for roots and shoots dry weight, and nutrient (N, P and K) content assessment. The plants were gently removed from the falcon tubes together with the adhering soil clods and placed on a course sieve, washed with a gentle stream of water. The roots were observed for the presence of nodules, nodule internal coloration and nodule number. The shoots were cut at the cotyledonary node separating plant shoots from the roots and stored separately in labeled khaki paper bags. The root nodules from each treatment were counted to determine the nodule number (NN) per plant, detached and dried. Nodule characteristics such as color and dry weight (NDW) were also recorded [39]. The plant shoots and roots were oven dried at 65 °C until a constant dry weight was obtained.

2.5. *Root staining and mycorrhizal colonization*

About 1g of roots from each sample was thoroughly cleaned with tap water and placed in falcon tubes, cleared with 10% KOH in water bath at 80 °C for 15 minutes, neutralized in 2% aqueous HCl acid and stained with 0.05% trypan blue in lactic acid [40]. The percentage AMF root colonization was assessed under a dissecting microscope at × 40 magnifications using the gridline intersect method [41].

2.6. *Shoot nutrient analysis*

The previously dried shoot samples (dried at 65 °C to a constant dry weight) were then ground into fine particles that can pass a 1 mm sieve. The concentration of nitrogen in the shoots was determined following the Kjeldahl method (Hanon K9840 Kjeldahl apparatus) as described by Sáez-Plaza et al. [30]. The shoot nutrient contents for phosphorus (P) and potassium (K) were determined by injecting samples into an inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7500ce, Agilent Technologies, United States). This was carried out following Mehlich-3 procedure [31]. The shoot organic carbon was determined by Walkley-Black combustion method [29]

2.7. *Data analyses*

All data were tested for homogeneity of variance before analyses. Data on all the parameters recorded were statistically subjected to analysis of variance (ANOVA) using SAS 8.1 (SAS Institute Inc., Cary, NC, USA) for multiple comparison analysis. Means separation was carried out using Tukey's Honest Significance difference (HSD) at 5% probability level. The correlations of soybean growth parameters, AMF root colonization, nutrients uptake and shoot organic carbon were analysed using Pearson's correlation (R) coefficients (RStudio in R version 4.0.1).

3. Results

3.1. *Soil characteristics*

The physical and chemical characteristics of the soil varied significantly between organic and conventional farming systems (Table 2). The organic farm soils had higher nutrient and chemical content compared to conventional farm soils. All the soils were characteristically acidic with organic farming recording significantly ($p < 0.0001$) higher pH (5.7) than conventional farming (4.57). Soil from organic farming system had total nitrogen concentration of 2.7 which was significantly ($p = 0.012$) higher than in conventional farming (2.2). Organic farming system led to significant ($p = 0.003$)

variation in organic carbon level. Organic farming significantly (p = 0.001) enhanced the level of available soil phosphorus. Although the level of available soil potassium was higher in organic farming compared to conventional farming, the difference was statistically insignificant (Table 2).

Table 2. Soil characteristics in organic and conventional farms.

	pH	N ($g \cdot kg^{-1}$)	K ($g \cdot kg^{-1}$)	P ($mg \cdot kg^{-1}$)	OC ($g \cdot kg^{-1}$)
Farm Management					
Organic	5.7 ± 0.08a	2.7 ± 0.01a	3.94 ± 0.01a	27.73 ± 0.23a	28.2 ± 0.05a
Conventional	4.57 ± 0.06b	2.2 ± 0.00b	3.74 ± 0.01a	25.50 ± 0.17b	25.4 ± 0.05b
P Values					
Farm Management	<0.0001	0.012	0.996	0.001	0.003

Values within the same column without common letters differ significantly according to Tukey's HSD at $p < 0.05$. P: phosphorus; N: nitrogen; K: potassium; OC: organic carbon.

3.2. Effect of farm management systems and inoculation on AMF root colonization

The percentage of soybean root length colonized by AMF significantly (p = 0.007) varied between the two farm management systems where organic soil recorded higher root colonization than conventional soil (Table 3). The cross-species (bradyrhizobia and mycorrhizal) dual inoculation significantly (p = 0.001) enhanced the percentage AMF root colonization compared to the individual mycorrhizal or bradyrhizobia inoculation.

Although the combination of native bradyrhizobia and AMF resulted in a higher root AMF colonization compared to individual exotic AMF, they were not as effective as the association between the commercial *Bradyrhizobium japonicum* and the exotic AMF in enhancing AMF root colonization (Table 3). Dual inoculation with the mixture of two AMF species resulted in root colonization higher (63.88 ± 0.79%) than that of *Funneliformis mosseae* at 56.67 ± 0.91% exclusively but lower than that of *Rhizophagus irregularis* at 69.82 ± 0.58% alone. Expectedly, the control treatment, native bradyrhizobia, *Bradyrhizobium japonicum* and *Bradyrhizobium japonicum* + native bradyrhizobia did not lead to AMF root colonization on soybean roots (Table 3).

Table 3. Effect of inoculation and dual inoculation of soybean plants with bradyrhizobia and arbuscular mycorrhizal fungi (AMF) on root colonization, nodule number, nodule dry weight, shoot dry weight and root dry weight in the greenhouse.

	AMF (%)	NN	NDW ($g \cdot plant^{-1}$)	SDW ($g \cdot plant^{-1}$)	RDW ($g \cdot plant^{-1}$)
Farm Management					
Organic	44.42 ± 6.01a	10.91 ± 1.67a	0.09 ± 0.01a	1.67 ± 0.05a	0.54 ± 0.02a
Conventional	43.55 ± 5.88b	9.45 ± 1.48b	0.08 ± 0.02a	1.45 ± 0.04b	0.46 ± 0.02b
Inoculation					
CB	0.00 ± 0.00	13.33 ± 1.20cd	0.13 ± 0.01bcd	1.45 ± 0.05fg	0.42 ± 0.02ef
CB + FM	73.89 ± 0.81ab	21.50 ± 1.26ab	0.15 ± 0.03ab	1.88 ± 0.05b	0.61 ± 0.01b
CB + NRB	0.00 ± 0.00	11.00 ± 0.94de	0.11 ± 0.01cd	1.39 ± 0.07gh	0.41 ± 0.03fg
CB + RI	75.83 ± 0.42a	24.67 ± 0.88a	0.16 ± 0.02a	2.03 ± 0.06a	0.69 ± 0.03a

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	AMF (%)	NN	NDW (g·plant^{-1})	SDW (g·plant^{-1})	RDW (g·plant^{-1})
FM	56.67 ± 0.91f	0.00 ± 0.00	0.00 ± 0.00	1.48 ± 0.05efg	0.48 ± 0.02de
FM + RI	63.88 ± 0.79e	0.00 ± 0.00	0.00 ± 0.00	1.54 ± 0.04ef	0.53 ± 0.02cd
NRB	0.00 ± 0.00	9.00 ± 0.86e	0.10 ± 0.01d	1.30 ± 0.05h	0.34 ± 0.01g
NRB + FM	71.13 ± 0.42cd	15.00 ± 0.39cd	0.14 ± 0.01abc	1.72 ± 0.06cd	0.56 ± 0.04bc
NRB + RI	72.65 ± 0.45bc	17.50 ± 1.25bc	0.15 ± 0.02abc	1.80 ± 0.05bc	0.57 ± 0.02bc
RI	69.82 ± 0.58d	0.00 ± 0.00	0.00 ± 0.00	1.59 ± 0.06de	0.54 ± 0.02bcd
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.01 ± 0.04i	0.33 ± 0.02g
P Values					
Farm Management	0.007	0.011	0.061	0.001	0.001
Inoculum	0.001	0.001	0.001	0.001	0.001
Farm Management* Inoculum	0.880	0.889	0.874	0.566	0.754

Values within the same column without common letters differ significantly according to Tukey's HSD at $p < 0.05$. NRB: Native bradyrhizobia; CB: Commercial *Bradyrhizobium japonicum*; FM: *Funneliformis mosseae*; RI: *Rhizophagus irregularis*; AMF: arbuscular mycorrhizal fungi; NN: nodule number; NDW: nodule dry weight; SDW: shoot dry weight; RDW:root dry weight.

3.3. Effect of farm management systems and inoculation on soybean nodulation, root and shoot dry weights

Significant variations due to farm management and inoculations were recorded in the studied parameters of plant nodulation and biomass dry weight. Organic soil significantly increased nodule number ($F_{15,47} = 3.72$, $p = 0.011$), root ($F_{41,112} = 10.3$, $p = 0.001$) and shoot dry weights ($F_{15,47} = 3.00$, $p = 0.001$) plant^{-1} compared to conventionally managed soils. Similarly, cross-species (bradyrhizobia + AMF) dual inoculation significantly enhanced nodule ($F_{15,47} = 7.13$, $p = 0.001$), root ($F_{31,112} = 18.11$, $p = 0.001$), and shoot dry weights ($F_{15,47} = 17.64$, $p = 0.001$) plant^{-1} compared to individual bradyrhizobia, mycorrhizal inoculation or un-inoculated control. Remarkably, dual inoculation with the commercial bradyrhizobia and *Rhizophagus irregularis* consistently outscored all other bradyrhizobia + AMF combinations, their respective individuals and controls in all the studied parameters of plant nodulation and biomass dry weight. Among the single inoculants, soybean inoculation with *Rhizophagus irregularis* significantly enhanced roots and shoots dry weight plant^{-1} compared to *Funneliformis mosseae*. Likewise, inoculation with the commercial *Bradyrhizobium japonicum* resulted to a significantly higher shoot and root dry weights plant^{-1} compared to those inoculated with individual native bradyrhizobia. There was no nodulation recorded for soybean plants treated with only the AMF isolates *Rhizophagus irregularis*, *Funneliformis mosseae*, *Rhizophagus irregularis* + *Funneliformis mosseae* and the control. Notably, there were no significant interactions observed between farm management and inoculation in all the plant parameters (Table 3).

3.4. Effects of farm management system and inoculation on soybean shoot nutrition

Farm management system and inoculation significantly influenced soybean shoot nutrition. A significant variation due to different farm management systems was noted on shoot phosphorus (P)

($F_{10,33}$ = 3556.36, p = 0.001), nitrogen (N) ($F_{10,33}$ = 228.20, p = 0.001), potassium (K) (p = 0.001), and organic carbon (OC) ($F_{10,32}$ = 321.54, p = 0.001) (Table 4). Organic soils significantly increased soybean shoots nutrient content by 14% for P, 22% for N, and 5% for both K and OC compared to the conventional soils. Dual inoculation of soybean plants with bradyrhizobia and AMF increased shoots P, N, K and OC content compared to the respective individual inoculants and un-inoculated controls. Interestingly, dual inoculation of soybeans using commercial *Bradyrhizobium japonicum* and *Rhizophagus irregularis* outperformed all other dual and single inoculations (including the single inoculations with native bradyrhizobia), and the un-inoculated controls in shoot P, N, K and OC content. Among the individual bacterial and fungal inoculants; the commercial *Bradyrhizobium japonicum* and *Rhizophagus irregularis* significantly enhanced shoot P, N, K and OC content compared to the native bradyrhizobia and *Funneliformis mosseae* respectively.

There were significant interactions observed between inoculation and farm management practice on P, N and K shoot nutritional parameters tested (Table 4). For instance, soybean inoculated with *Bradyrhizobium japonicum* + *Rhizophagus irregularis* had the highest shoot P content in soils from organic farms compared with soils from conventional farms. However, dual inoculation with *Bradyrhizobium japonicum* + *Funneliformis mosseae* and *Bradyrhizobium japonicum* + *Rhizophagus irregularis* did not yield any significant difference in P content of soybean shoot grown in both organic and conventional soils (Figure 1A). Likewise, the P content in soybean grown in organic soils and dual inoculated with native bradyrhizobia + *Rhizophagus irregularis* and *Bradyrhizobium japonicum* + *Funneliformis mosseae* did not significantly differ despite receiving different bradyrhizobia and AMF inoculant strains. Similarly, dual inoculation of soybeans with *Bradyrhizobium japonicum* + *Funneliformis mosseae* and *Bradyrhizobium japonicum* + *Rhizophagus irregularis* achieved the highest shoot percentage nitrogen in organic farms compared to conventional farms (Figure 1B).

Table 4. Effect of inoculation and dual inoculation on soybean plant shoot phosphorus (P), nitrogen (N), potassium (K) and organic carbon (OC) in the greenhouse.

	P ($mg·kg^{-1}$)	N ($g·kg^{-1}$)	K ($mg·kg^{-1}$)	OC ($g·kg^{-1}$)
Farm management				
Organic	2885.88 ± 66.71a	25.1 ± 0.07a	12000.15 ± 217.49a	212.1 ± 0.37a
Conventional	2521.48 ± 58.04b	20.6 ± 0.07b	11430.45 ± 223.72b	202.0 ± 0.34a
Inoculation				
CB	2471.83 ± 79.99h	20.4 ± 0.08g	10491.00 ± 184.25h	198.6 ± 0.24d
CB+FM	3139.50 ± 94.58b	28.3 ± 0.11b	13848.33 ± 117.17b	233.6 ± 0.22b
CB+NRB	2371.17 ± 69.41i	19.6 ± 0.07g	10151.00 ± 139.53i	190.5 ± 0.23e
CB+RI	3290.50 ± 94.59a	29.8 ± 0.03a	13994.00 ± 117.11a	242.2 ± 0.22a
FM	2587.17 ± 55.84g	21.9 ± 0.09f	11634.00 ± 117.17g	202.1 ± 0.39d
FM+RI	2663.50 ± 78.93f	22.6 ± 0.07f	11819.00 ± 126.15f	204.7 ± 0.31d
NRB	2304.50 ± 72.22j	18.0 ± 0.04h	9848.33 ± 133.21j	185.1 ± 0.23e
NRB+FM	2903.51 ± 94.58d	25.0 ± 0.11d	12790.32 ± 128.22d	216.7 ± 0.31c
NRB+RI	3087.50 ± 94.59c	26.1 ± 0.11c	13217.00 ± 116.15c	229.8 ± 0.22b
RI	2765.50 ± 96.82e	23.6 ± 0.09e	12007.00 ± 117.17e	205.8 ± 0.24d
Control	2155.83 ± 66.51k	15.5 ± 0.09i	9068.33 ± 107.43k	168.6 ± 0.26f
P values				

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	P (mg·kg^{-1})	N (g·kg^{-1})	K (mg·kg^{-1})	OC (g·kg^{-1})
Farm Management	0.001	0.001	0.001	0.001
Inoculum	0.001	0.001	0.001	0.001
Farm Management* inoculum	0.001	0.019	0.001	0.876

Values within the same column without common letters differ significantly according to Tukey's HSD at $p < 0.05$. NRB: Native bradyrhizobia; CB: Commercial *Bradyrhizobium japonicum*; FM: *Funneliformis mosseae*; RI: *Rhizophagus irregularis*; ppm: parts per million; P: phosphorus; N: nitrogen; K: potassium; OC: organic carbon.

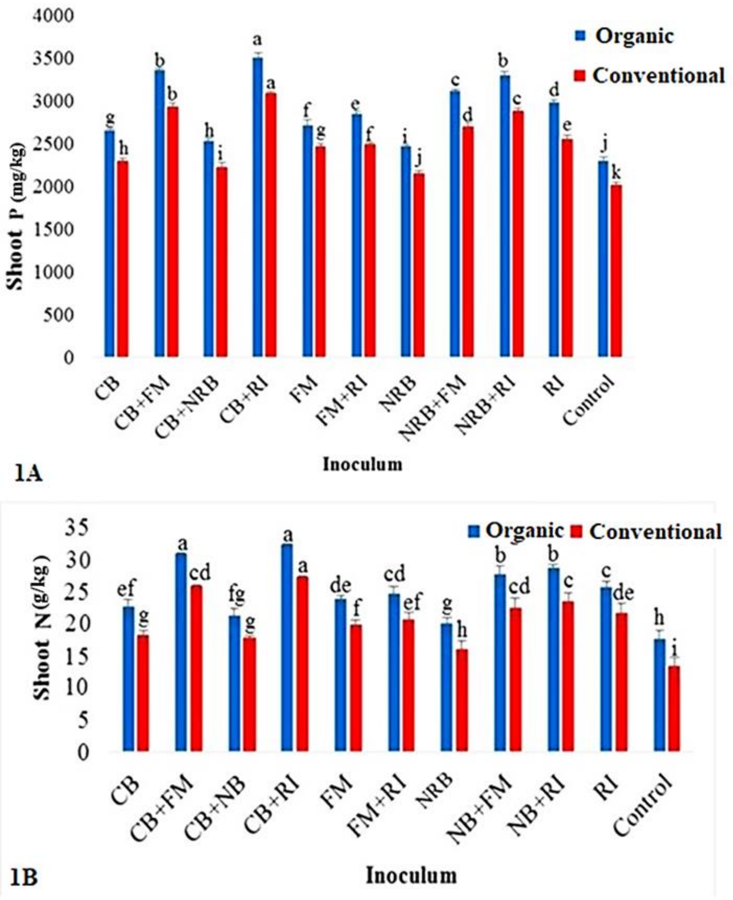

Figure 1. Greenhouse interactive effects of soybean shoot nutrients due to inoculation and dual inoculation in different farm management practices. Bars followed by different letters are significantly different using Tukey's test ($P \leq 0.05$). NRB: Native bradyrhizobia; CB: Commercial *Bradyrhizobium japonicum*; FM: *Funneliformis mosseae*; RI: *Rhizophagus irregularis*.

3.5. *Relationships between AMF colonization, soybean growth and shoot nutrition parameters*

Shoot dry weight had a significant positive correlation with soybean nodule dry weight ($R = 0.582$, $p = 0.001$), OC ($R = 0.947$, $p = 0.001$), shoot P ($R = 0.958$, $p = 0.001$), shoots N concentration ($R = 0.966$, p = 0.001) and shoot K (R = 0.936, p = 0.001) (Table 5). Similarly, AMF root colonization showed strong positive correlation with shoot N, P, K, and shoot OC. Nodule number and dry weight positively correlated with shoot P content, N, and OC, but showed a negative correlation with shoot K. There was also a very strong positive correlation between OC, N, P, K, and shoot dry weight (Table 5).

Table 5. Pearson correlation (R) coefficients between soybean arbuscular mycorrhizal fungi (AMF) root colonization, nodule number (NN), nodule dry weight (NDW), shoot dry weight (SDW), phosphorus (P), Nitrogen (N), potassium (K) and organic carbon (OC) in the greenhouse.

	AMF	NN	NDW	SDW	P	N	K	OC
AMF	1							
NN	+0.266*							
	0.031	1						
NDW	+0.129**	+0.965**	1					
	0.301	0.001						
SDW	+0.754**	+0.686**	+0.582**	1				
	0.001	0.001	0.001					
P	+0.781**	+0.631**	+0.505**	+0.958**	1			
	0.001	0.001	0.001	0.001				
N	+0.764**	+0.624**	+0.499**	+0.966**	+0.987**	1		
	0.001	0.001	0.001	0.001	0.001			
K	+0.904**	−0.606**	−0.478**	+0.936**	+0.942**	+0.936**	1	
	0.001	0.001	0.001	0.001	0.001	0.001		
OC	+0.800**	+0.709**	+0.963**	+0.947**	+0.759**	+0.536**	−0.682**	1
	0.001	0.001	0.001	0.001	0.001	0.001	0.001	

**: Correlation is significant at the 0.01 level (2-tailed); *: Correlation is significant at the 0.05 level (2-tailed).

4. Discussion

4.1. Soil characteristics

The properties of farm soil varied depending on farm management practice. The organic farm soils recorded lower acidity and higher fertility levels compared to conventional farms; which could be due to application of organic fertilizers and amendments that neutralize the low soil acidity and maintain beneficial soil microorganisms that maintain soil nutrient recycling. In organic farming, the application of animals' manure and compost promote the activity and diversity of soil microbes [42] that promote nutrient cycling and enhance soil properties [43]. Organic farm soils, which had a mean pH of 5.7, higher organic carbon content compared to conventional soils with low soil pH of 4.57, consistently supported the tripartite soybean-rhizobia-AMF symbiosis leading to higher averaged values of all the tested parameters. This corresponds with the previous studies that organic farm management allows formation and establishment of a compatible relationship between the host plant and AMF leading to increased AMF root colonization [44]. The increased level of organic nutrients in organic soil favors root and AMF mycelia growth that enhanced AMF root colonization. A study by

Karunasinghe et al. [45] reported that improved nutrients availability facilitates growth of root and AMF mycelia, resulting in a high AMF root colonization.

The conventional farm soils had higher acidity and lower fertility levels, which could be due to extensive application of inorganic fertilizers, heavy leaching and runoff. The low level of soil fertility in conventional farms is attributed to acidity, runoff and leaching, following the use of inorganic chemical fertilizers and other agrochemicals [46]. The relatively higher acidity of the conventional soil used, which is typical of highly weathered East African soils [28], debilitates soil N cycling, P availability and exchange of cations, thus, interfering with the optimal performance of introduced inoculants causing selection pressures. A study carried out by Liu et al. [47] reported up to 90% reduction in arbuscule abundance in tomato roots, which was primarily linked to high acidity. A recent study by Ye et al. [48] also reported soil pH and organic carbon as the most critical factors that regulate the performance and composition of fungal community. The low availability of phosphorus in acidic soil could significantly reduce the process of biological nitrogen fixation [49], and this was reflected in this study with reduced nodulation, shoot dry matter, and phosphorus content in the conventional soils. But interestingly, despite the low soil pH conditions in this study, elite synergistic performance of the commercial *Bradyrhizobium japonicum* and *Rhizophagus irregularis* over the native inoculant combinations in enhancing soybean root colonization, nodulation, growth and shoot nutrition was observed. This is contrary to other previous studies which have shown the superior performance of native isolates over the commercial or exotic inoculants [50].

4.2. Effects of inoculation and dual inoculation on soybean production

Soybean plants require essential elements for their growth and development and through advanced breeding technologies, cultivars such as SC squire that are promiscuous and associate with a diverse range of rhizospheric microorganisms, have been produced to optimize nutrient acquisition through multipartite symbioses [51]. Legumes form tripartite symbiotic associations with rhizobia and AMF that act in synergy to provide various ecosystem services that enhances plant growth, productivity and soil health [52]. The tripartite symbiosis in this study was investigated by dual inoculation of soybean with AMF and bradyrhizobia. The results of this study demonstrated that AMF and bradyrhizobia partners in this tripartite symbiosis did not compete for colonization sites on soybean and therefore they coexisted synergistically in one soybean host plant and enhanced nodulation, nitrogen fixation and nutrients acquisition for the benefit of all the partners. The bradyrhizobia-soybean symbiosis is involved in the fixation of atmospheric nitrogen, while the association with arbuscular mycorrhizal fungi modifies the ability of the plant to take up phosphorus and other nutrients [53].

The results of this study concur with the previous findings that interaction among the participants in tripartite symbiosis was observed to have a significant impact on nitrogen fixation [54]. In dual inoculation of *Vigna unguiculata* (L.) Verdc. with AMF and rhizobia, percentage AMF colonization of roots, the number of nodules and phosphorus absorption was higher than when each was inoculated alone [55]. The rapid and extensive AMF root colonization in the plant leads to early formation of nodules hence a higher nitrogen fixation in plants colonized by both AMF and bradyrhizobia. The interaction between bradyrhizobia and AMF is due to flavonoids secreted by the host plant [56]. A number of flavonoids have the potential of stimulating hyphal growth and branching, which in turn stimulates nodule formation with increase in nitrogen fixation [56].

In low input or organic farm management systems, compatible plant–fungus–rhizobia associations may play a more prominent and critical role in the optimal nutrition of the host plants than in conventional agricultural systems [57]. Therefore, choosing compatible strains of bacteria and fungus when producing inoculants for farmers is imperative to avoid assembling collection of candidates with competitive or antagonistic characteristics. In this study, a combination of commercial *Bradyrhizobium japonicum* with *Rhizophagus irregularis* consistently proved to effectively enhance root colonization, nodulation, shoot and root dry weights, and N, P, K and OC nutrient content of the soybean shoots. This could be associated with their synergistic ability to enhance nutrient availability and absorption compared to the single inoculation of AMF, bradyrhizobia or the un-inoculated control. Previous studies have linked the enhanced nutrient uptake to higher root surface area boosted by the extra-radical mycelium of the fungi especially when *Glomus mosseae* and *Rhizobium* were used [26,58,]. Surprisingly in this study, single inoculation of *Rhizophagus irregularis* had significant performance than the higher levels of AMF diversity in dual inoculations consisting of *Rhizophagus irregularis* and *Funneliformis mosseae* in AMF root colonization and shoot nutrition. Therefore, a clear indication of stronger functional identity than diversity on AMF root colonization and shoot nutrition. This could be due to the competition of the two AMF species for the infection sites on the host plant [59]. *Rhizophagus irregularis* has been commonly used in inoculation of acidic soils due to its adaptability and elite performance in low pH conditions compared to *Funneliformis mosseae* [47].

The superior performance of the commercial *Bradyrhizobium japonicum* inoculant in this study could be attributed to their extensive selection based on their performance under various ecological conditions. Previous study by Tabassum et al. [60] noted that technical advancement in interdisciplinary research and strain improvement are the key aspects for a successful commercialization, utilization and adoption of biological inoculants. Barbosa et al. [61] added that the commercial *Bradyrhizobium japonicum* still holds high symbiotic capabilities with soybeans and in most cases out-competes other strains in colonizing the plant roots. Additionally, other factors such as soil sterilization and laboratory culture maintenance have been shown to alter the optimal strain functionality [62], and could limit the performance of the native inoculants in soybean host plants. It is also noteworthy that this was a greenhouse experiment carried out under controlled conditions and thus there is need to carry out field tests with similar treatments to affirm the current findings.

4.3. *Relationships between AMF colonization, soybean growth and shoot nutrition parameters*

According to the Pearson's correlation coefficients, a strong positive association between nodule dry weight and shoot dry weight supports the biological significance of symbiotic nitrogen fixation in enhancing soybean growth. These findings concur with the previous studies by Ouma et al. [63] that reported that dual inoculation increases nodulation and consequently enhances shoot biomass. The strong positive relationship between AMF root colonization and shoot dry weight depicts the significance of mycorrhizal inoculation in legume production. Mycorrhizal root system promotes the absorption of crucial nutrients such as N, P, K and copper (Cu) by enhancing the absorptive area of root system. Arbuscular mycorrhizal fungi are also able to absorb and transfer both micro and macro nutrients which are necessary for plant growth [64]. Upadhayay et al. [65] reported that AMF network in the soil is able to sustain P transport to the host plant for a long period. This study also revealed that an increase in AMF root colonization, significantly increased shoots organic carbon. The ability of AMF to solubilize different nutrients from the soil and assimilation using their intricate root web [66] could be attributed to the increased organic carbon documented from this study. Previous research by Sofi et al. [67] reported that the same extensive network of AMF fungal filaments contributes to the water uptake and storage for the host plant.

5. Conclusions

This study determined the influence of single inoculation *versus* dual inoculation with AMF and/or bradyrhizobia on root colonization and nodulation in organic and conventional farm soils and the study results strongly support the hypothesis which predicted that; soybean inoculation versus dual inoculation with AMF and/or bradyrhizobia increase AMF root colonization, nodulation, soil fertility, nutrient acquisition and growth in organic than conventionally managed farm soils. The results of the current study demonstrate the importance of organic farming and dual inoculation with AMF and bradyrhizobia in enhancing biological soil fertility, nodulation, AMF root colonization, growth and nutrient acquisition, a key step towards sustainable production of inexpensive healthy foods and maintenance of environmental quality. Therefore, compared to the conventional system, organic farming is sustainable and cost effective for majority of smallholder farmers who are resource-constrained and depend on low input agriculture. Organic farming enhanced the synergistic role of symbiotic microorganisms (AMF and bradyrhizobia) in soybean growth due to increased biological nitrogen fixation and enhanced acquisition of organic carbon, potassium and phosphorus as demonstrated from the soil and shoot analyses. The consistent and outstanding superior performance of *Rhizophagus irregularis* and *Bradyrhizobium japonicum* in both combined and individual state present positive, effective and beneficial synergistic relationship and could be potentially used to optimize organic production as an alternative to chemical fertilizers for agricultural sustainability and reduced cost of crop production. However, further studies should focus on field trials for the exotic strains of AMF used in this greenhouse experiment to establish their competitiveness and beneficial impacts in promoting biological nitrogen fixation, nutrient acquisition and soybean growth in both organic and conventional farms. In addition, further studies on molecular mechanisms involved in the symbiotic associations can be crucial to enhance the present understanding and their application in sustainable organic farming systems.

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Conflict of interest

The authors declare that they have no conflict of interest.

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