



*Agronomy*

ORIGINAL ARTICLE

## Effect of poultry manure and inorganic fertilizer on earthworms and soil fertility: Implication on root nodulation and yield of climbing bean (*Phaseolus vulgaris*)

Christopher Ngosong<sup>1\*</sup>, Ivor karawa Nfor<sup>1</sup>, Clovis Bessong Tanyi<sup>1</sup>, Marie Noela Enyoe Olougou<sup>1</sup>, Lawrence Tatanah Nanganoa<sup>2</sup>, Aaron Suh Tening<sup>1</sup>

<sup>1</sup>Department of Agronomic and Applied Molecular Sciences, Faculty of Agriculture and Veterinary Medicine, University of Buea, P.O.Box 63 Buea, South West Region, Cameroon

<sup>2</sup>Institute of Agricultural Research for Development IRAD, Ekona, PMB 25 Buea, South West Region, Cameroon

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Md Moshir Rahman  
[rahmanag63@bau.edu.bd](mailto:rahmanag63@bau.edu.bd)

\*Corresponding Author  
Christopher Ngosong  
[ngosongk@yahoo.com](mailto:ngosongk@yahoo.com)



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### ABSTRACT

Grain legumes provide dual benefits as a food source and soil fertility or plant nutrition enhancer, but the latter role may be influenced by fertilization regimes. A controlled study was conducted to evaluate the impact of four fertilizer treatments (control–no input, single dose NPK, split dose NPK, and poultry manure) on earthworms and soil fertility, and the implication on the performance of climbing bean (*Phaseolus vulgaris*). The fertilizer amendments significantly affected the soil pH, organic C, N, P, K, Ca, Mg, Na, and ECEC with the highest effect caused by poultry manure. The bean root nodule mass differed significantly across treatments with the highest in control, followed by inorganic fertilizers, and poultry manure. Significant negative correlations occurred between the root nodule mass and soil organic C, N, P, Mg, Ca, and ECEC. Earthworm density differed significantly across treatments with the highest in poultry manure as compared to the control and both single and split dose inorganic fertilizer. Earthworms correlated negatively with soil acidity and positively with soil pH, K, Mg, Ca, and ECEC. The number of bean pods differed significantly across fertilizer treatments with the highest in poultry manure followed by the split dose NPK, which differed from the control and single dose NPK. In addition, the number of bean pods correlated positively with earthworm density and soil organic C, P, Mg, Ca, and ECEC. These findings demonstrate the importance of poultry manure on earthworm population and soil fertility status, and the implication on the performance of climbing beans. Nonetheless, the results highlight potential limitations of poultry manure on root nodulation and nitrogen fixing abilities of climbing bean, which should be considered when designing integrated soil fertility management strategies.

**Keywords:** Fertilization, legumes, manure, nodulation, earthworm, yield

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## 1 Introduction

Feeding the projected 9.8 billion global population by 2050 is a major challenge that requires sustainable increase in crop yields, especially for grain legumes such as climbing bean (*Phaseolus vulgaris*). Grain legumes deliver important benefits because of their dual role as a food source (Broughton et al., 2003; Graham and Vance, 2003) and soil fertility or plant nutrition enhancer (Bildirici and Yilmaz, 2005; Vanlauwe et al., 2010; Bello et al., 2017; Nanganoa et al., 2019). However, crop production in Sub-Saharan Africa (SSA) is constrained by poor and declining soil fertility, with nutrient deficiency often corrected using fertilizers applied at different rates, including higher amounts of nitrogenous inputs (Baki et al., 2015; Sharma et al., 2017; Ngosong et al., 2019). Inorganic fertilizers can cause deleterious effects on the environment that may jeopardise the sustainability of agricultural systems (Shi et al., 2008; Zhou et al., 2013). These environmental effects have necessitated the use of sustainable alternatives that modulate rhizosphere biotic interactions within the nexus of integrated soil fertility management (Sanginga et al., 2003; Mahmood et al., 2017; Nwite and Alu, 2018). For instance, manure amendment is considered a sustainable strategy in arable systems because it adds soil organic matter and essential nutrients (Saha et al., 2007; Hepperly et al., 2009), while improving the rhizosphere microbial community and functions (Albiach et al., 2000; Yadav et al., 2011; Kumar et al., 2015; Sepehrnia et al., 2017). Hence, grain legume cropping systems can provide significant soil fertility benefits by integrating the synergistic effects of manure and soil beneficial microbes or earthworms (Smith et al., 2008; Tagoe et al., 2010; Zhang et al., 2016; Yahyaabadi et al., 2018). Nonetheless, fertilizer regimes may alter the soil biological communities and functions, which might eventually affect nutrient cycling and the productivity of arable soils.

Microorganisms associated with the rhizosphere of leguminous plants exert plant growth promoting abilities via biological nitrogen fixation and phosphorus solubilisation (Verma et al., 2013; Fankem H et al., 2015; Tchakounté et al., 2018). However, the community dynamics and functions of microbes (Ngosong et al., 2010; Tagoe et al., 2010; Zhang et al., 2016) and earthworms (Postma-Blaauw et al., 2006; Sheehan et al., 2006; Smith et al., 2008) can be affected by farm management practices such as crop type, litter and fertilization regimes. Earthworms are soil engineers that improve soil fertility and productivity by altering soil physicochemical properties and biological functions including enhanced enzymatic and nitrogenase activities (Chauhan, 2014). Earthworms represent important microsites for microbes and soils containing earthworms are associated with high microbial diversity and density (e.g. N-fixing bacteria and fungi),

increased organic matter degradation and nutrient mineralisation (Fujii et al., 2012; Bamidele et al., 2014; Aira et al., 2016). Meanwhile, lower earthworm densities associated with farm management practices such as cropping systems and inorganic fertilizers (Norgrove et al., 2011; Spiegel et al., 2018; Yahyaabadi et al., 2018) may affect rhizosphere biotic interactions and reduce the productivity of arable soils. Accordingly, earthworms are used for bio-monitoring and characterisation of soil health and quality (Pères et al., 2011; R. et al., 2014). Therefore, this controlled study was intended to evaluate the effects of poultry manure and inorganic fertilizer on bean root nodulation and the population dynamics of earthworm, in order to infer on the soil fertility status and the performance of climbing bean plants. We hypothesised that poultry manure will enhance soil fertility, earthworm density and bean root nodulation as compared to inorganic fertilizer, which will lead to improvement of the soil fertility status and climbing bean performance.

## 2 Materials and Methods

### 2.1 Experimental site

The study was conducted from April to July 2018 at the Teaching and Research Farm of the Faculty of Agriculture and Veterinary Medicine, University of Buea, Cameroon. The site is located at the foot of the active mount Cameroon (4095 m) volcano in South-western Cameroon (elevation of 1000 m above sea level), and situated between latitudes 4°3' N and 4°12' N and longitudes 9°12' E and 9°20' E. The soils are derived from volcanic rocks and are generally fertile for agricultural production. The site has monomodal rainfall and short dry season with 85–90% relative humidity. Heavy rainfall is from June to October with 2085 mm on the leeward side of the mountain and 9086 mm on windward side. The dry season starts from November to March with 19–30 °C mean monthly air temperature, while the soil temperature at 10 cm depth decreases from 25–15 °C with increasing elevation from 200–2200 m above sea level (Fraser et al., 1998; Proctor et al., 2007).

### 2.2 Experimental setup

The field site has a known history of grain legumes (e.g. soybeans, beans, cowpea, and groundnuts) cultivation, implying existence of indigenous *Rhizobia* communities. A total of 180 m<sup>2</sup> (10 × 18 m) land area was manually cleared using a cutlass. The soil was dug manually to approximately 15 cm depth using a hoe, and homogenised by sieving through 5 mm wire mesh to remove stones and soil debris. Part of the homogenised soil was further sieved with a 2 mm wire mesh and analysed to determine the baseline soil physicochemical properties and the result presented

in Table 1. Black polythene bags (measuring 37 × 45 cm) were filled with 14 kg of the sieved soil and each polybag was perforated with 50 tiny holes to facilitate drainage. The polybags were laid out in the field as completely randomized block design (CRBD) with four treatments (control–no input, single dose NPK, split dose NPK and poultry manure) and each containing 8 replicate polybags per treatment (giving a total of 32 polybags). Treatment polybags were randomly positioned at 1 m apart, while the entire experimental site was surrounded by 2 m buffer zone. Polythene sheets were placed beneath each polybag to prevent bean roots from growing out of polybags into the surrounding field soil.

## 2.3 Soil amendments

The NPK fertilizer (20:10:10 + CaO; ADER® Cameroon) used in this study was purchased from the local market, while poultry manure was collected from the surface of a poultry waste dump heap (i.e. about 6 months old substrate) at the Teaching and Research Farm of the Faculty of Agriculture and Veterinary Medicine, University of Buea. The poultry manure was hand-checked to ensure that no earthworm was present and was thoroughly mixed before use as organic amendment. Granular inorganic fertilizer was applied according to local farming practice, either as a single dose of 10 g NPK at planting, or split dose of 5 g NPK at planting and 5 g at four weeks after planting (i.e. total of 10 g NPK per polybag). The NPK fertilizer was manually applied by ringing at about 5 cm from the stem of plants, while 1 kg poultry manure was applied on the soil surface in each polybag.

## 2.4 Plant cultivation and management

Local landrace climbing bean (*Phaseolus vulgaris*) seeds were purchased from a local agro-shop, four seeds were planted in each polybag at about 3 cm depth (25 April 2018), and later thinned two weeks after germination to two vigorous plants per polybag. Polybags were monitored regularly and the moisture content was supplemented with 1 L tap water every two days, except on rainy days. Each polybag received 5 g molluscicide (MOCID; SAVANA-Horizon Phyto Plus, Cameroon; comprising active ingredient metaldehyde) to control snails and slugs. A mixture of fungicide and insecticide were applied on plants weekly using a knapsack sprayer, based on the local rainfall regime. The contact and polyvalent fungicide (Cotzeb 80WP; SABERO ORGANICS, India; comprising active ingredient mancozebe) was applied at 80 g dissolved in 15 L water. The pyrethroid insecticide (Cigogne 360; SCPA SIVEX international France; comprising active ingredient cypermethrine) was applied at 5 mL dissolved in 15 L water. Plants were staked

at 23 days after planting, using 2.5 m length bamboos to support the climbing vines.

## 2.5 Data collection

### 2.5.1 Root morphology and nodulation

Root morphology and nodulation were evaluated at 65 days after planting. Four randomly selected polybags were split open using a razor blade and the entire content in each bag was placed in a basin filled with tap water. The dissolved content was filtered through 250 mm mesh sieve and roots comprising nodules were gently washed to remove adhering soil particles. Root girth was measured using a graduated tape and expressed in centimetres (cm). Root length was measured starting from the point of branching using a graduated tape (cm) and presented as average length of the main root and one lateral root. The potential for biological nitrogen fixation was assessed through the nodule mass and internal colouration (Muthomi et al., 2009; Ngeno et al., 2012). 100 nodules were randomly handpicked from plant roots, weighed on an electronic balance, and dissected using a blade to assess the internal colouration. Pink and red colours were considered indicative of N-fixation due to the presence of leghemoglobin oxygen carrier that is essential for biological nitrogen fixation.

### 2.5.2 Earthworms and soil properties

Ten weeks after bean seeds were sown (8 July 2018), the remaining four polybags per treatment were sampled to determine the earthworm density and soil physicochemical properties. For earthworm density, the polybags were split open using a razor blade and the remaining soil dispersed on large polythene sheets for visual observation and hand sorting to determine earthworm density. Prior to earthworm sampling, seven sub-soil samples were randomly collected from each polybag at 0–15 cm depth (using 3.5 cm diameter auger), and bulked to form composite samples that were analysed for the soil physicochemical properties. The soil samples were spread on plastic trays and allowed to air-dry at room temperature before sieving through a 2 mm mesh. Soil particle size was determined using the pipette method with sodium hexametaphosphate as dispersing agent (Kalra and Maynard, 1991). Soil pH was determined potentiometrically in both water (H<sub>2</sub>O) and one molar potassium chloride (1 N KCl) solutions after 24 hours in soil suspension (soil/liquid = 1/2.5 w/v) using a digital pH meter (Van Reeuwijk, 1992). Soil exchangeable bases were determined after extraction with 1 N ammonium acetate (NH<sub>4</sub>OAc) solution at pH 7. Calcium (Ca) and magnesium (Mg) were determined by atomic absorption spectrophotometry (AAS), while potassium (K) and Sodium (Na) were analysed by flame photometer (Rowell, 1994;

Benton and Jones, 2001). Exchangeable acidity was determined by KCl extraction method and titrated with 0.01 N NaOH using phenolphthalein indicators (Van Reeuwijk, 1992; Benton and Jones, 2001). Total nitrogen was determined by macrokjeldahl digestion method Bremner and Mulvaney (1982), while available phosphorus (P) was determined by Bray II method (Van Reeuwijk, 1992; Benton and Jones, 2001). Soil organic carbon was determined by Walkley-Black wet digestion method. The Effective Cation Exchange Capacity (ECEC) was determined by the sum of exchangeable cations and acidity, and textural class assigned according to the USDA textural triangle (Van Reeuwijk, 1992).

### 2.5.3 Plant growth and yield components

Plant growth parameters and yield components were recorded at 65 days after planting. Stem girth was measured at plant base above the soil surface using a graduated tape and expressed in centimetres (cm). Total shoot biomass (leaves, stems, branches, and pods) were harvested, oven-dried at 60 °C for 70 hours, weighed on a scale balance and recorded in (g) per plant. The number of bean pods were visually observed and counted per plant. All data sets were presented as mean of the replicate values (Mean ± standard deviation).

## 2.6 Statistical analysis

All data sets were analyzed using the statistical software package STATISTICA 13.2 for Windows. The dependent variables (e.g. soil physicochemical properties, earthworm, nodulation, and plant parameters) were subjected to univariate analysis of variance (ANOVA,  $P < 0.05$ ) to test effect of treatments ( $n=4$ ) as categorical predictors. Significant data means were compared by *posthoc* Tukey's HSD test ( $P < 0.05$ ). Where applicable, Spearman Rank Correlation ( $P < 0.05$ ) was performed to determine the degree of association between dependent variables and the treatments as categorical predictors.

## 3 Results

### 3.1 Soil physicochemical properties

Fertilizer treatments demonstrated significant effects on soil physicochemical properties with the highest influence recorded by poultry manure (Table 2). Soil pH differed ( $P < 0.001$ ) significantly across treatments, with the lowest pH recorded in the inorganic fertilizer treatments as compared to poultry manure and control (Table 2). The post-planting soil pH was highly acidic (4.5–5.0) for both single and split dose NPK fertilizer, moderately acidic (5.6–6.0) for the control, and slightly acidic (6.1–6.5) for the poultry manure. Total

soil nitrogen differed ( $P < 0.001$ ) significantly across treatments with the highest soil N in poultry manure, followed by both single and split dose NPK fertilizer as compared to the control. Soil organic carbon also differed ( $P < 0.001$ ) significantly across treatments with the highest in poultry manure as compared to both single and split dose NPK fertilizer or the control. Significant differences occurred for soil available phosphorus ( $P < 0.001$ ), exchangeable potassium ( $P < 0.001$ ), calcium ( $P < 0.01$ ) and magnesium ( $P < 0.001$ ), with the highest in poultry manure as compared to both single and split dose NPK fertilizer or the control. The sodium content differed ( $P < 0.05$ ) significantly across treatments with the lowest in split dose NPK fertilizer as compared to poultry manure and control. The soil acidity differed ( $P < 0.01$ ) significantly across treatments with the highest in both single and split dose NPK fertilizer as compared to the control and poultry manure. The effective cation exchange capacity (ECEC) differed ( $P < 0.01$ ) significantly across treatments with the highest in poultry manure that differed from inorganic fertilizer and control. Meanwhile, no significant difference occurred for C-N ratio across the different fertilizer treatments.

Table 1. Baseline physicochemical properties of the pre-planting soil

Soil properties	Concentrations
pH (water)	5.72
pH (KCl)	5.37
Organic carbon (%)	2.32
Total nitrogen (%)	0.4
Carbon : Nitrogen	6
Available P (mg kg <sup>-1</sup> )	26
Potassium (cmol kg <sup>-1</sup> )	0.37
Magnesium (cmol kg <sup>-1</sup> )	0.83
Calcium (cmol kg <sup>-1</sup> )	4.77
Sodium (cmol kg <sup>-1</sup> )	23.6
Acidity (cmol kg <sup>-1</sup> )	0.28
ECEC (cmol kg <sup>-1</sup> )	29.85

### 3.2 Root nodulation and earthworms

The rate of root nodulation and earthworm density reflected significant interactions in the rhizosphere of climbing bean plants. The bean root nodule mass represented the N-fixing potential under different fertilization regimes, and ranged from 1–2 g per 100 nodules across treatments that differed ( $P < 0.001$ ) significantly. The highest nodule mass was recorded in the control, followed by inorganic fertilizers, and poultry manure (Fig. 1a). The root nodule colour showed high N-fixation potential that ranged from 80.7–90.7% across treatments, but no significant difference occurred between the different treatments (Table 3). The average earthworm density ranged from 1–26 individuals across treatments (Fig. 1b) that dif-

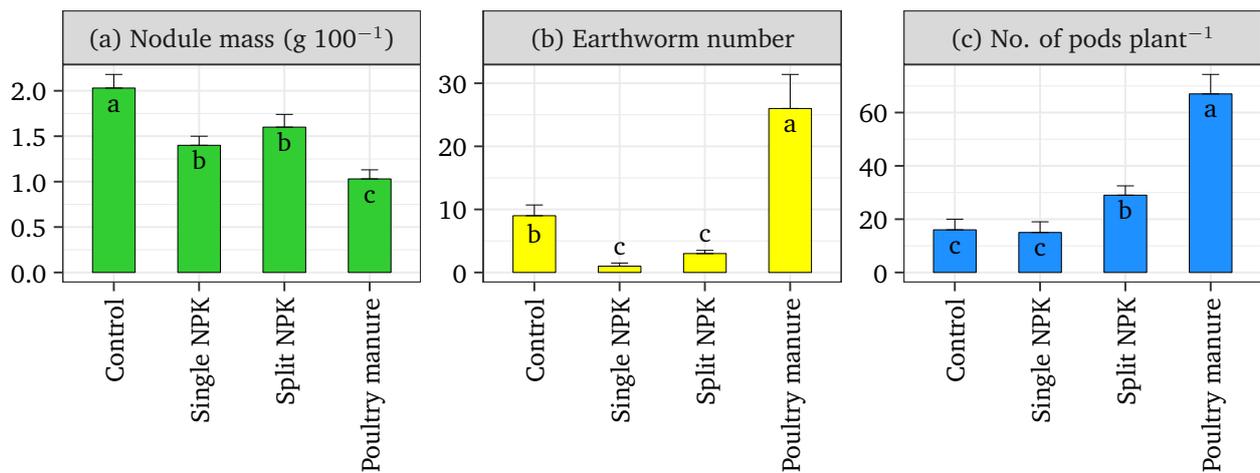


Figure 1. Effect of fertilizer treatments on (a) root nodule mass, (b) number of earthworms, and (c) number of pods. Data with different letters are significantly different ( $P < 0.05$ ).

ferred ( $P < 0.001$ ) significantly, with the highest earthworm density recorded in poultry manure (26), as compared to the control (9), single dose NPK (1) and split dose NPK (2) fertilizer. Significant ( $P < 0.05$ ) correlations occurred between earthworm density and soil physicochemical properties as influenced by the different treatments (Table 4). Earthworm density correlated positively with the number of bean pods and soil physicochemical properties (e.g. pH, potassium, magnesium, calcium, and ECEC), whereas a negative correlation occurred between earthworms and soil acidity (Table 4).

### 3.3 Performance of climbing bean

Fertilizer treatments affected the performance of climbing bean as reflected by the total root biomass that ranged from 14.2–23.2 g across treatments and differed ( $P < 0.001$ ) significantly, with the highest in poultry manure and lowest in control (Table 3). The stem girth ranged from 3.6–5.2 cm per plant and differed ( $P < 0.001$ ) significantly with the lowest in control as compared to inorganic and organic fertilizer treatments, but no such difference occurred for root girth and length (Table 3). Shoot biomass ranged from 95.3–321.9 g and differed ( $P < 0.01$ ) significantly with the highest in poultry manure as compared to the single dose NPK and split dose NPK fertilizer or the control (Table 3). The number of bean pods ranged from 15–67 per plant across treatments and differed ( $P < 0.001$ ) significantly with the highest in poultry manure, followed by the split dose NPK fertilizer that differed from the control and single dose NPK fertilizer (Fig. 1c). Significant correlations occurred between soil and plant parameters (Table 4). Root biomass correlated positively with soil properties (e.g. N, P, Ca, and ECEC). Shoot biomass also correlated positively with soil properties (e.g. pH,

organic C, N, P, Mg, Ca, and ECEC). The number of bean pods correlated positively with soil properties (e.g. organic C, P, Mg, Ca, and ECEC). By contrast, significant negative correlations occurred between the root nodule mass and soil properties (e.g. organic C, N, P, Mg, Ca, and ECEC).

## 4 Discussion

### 4.1 Soil physicochemical properties

The observed variations in soil physicochemical properties are reflective of the fertilizer amendments, with improved soil fertility for both inorganic and organic inputs (Mahmood et al., 2017; Adeyemo et al., 2019). This further highlights the challenges of poor soil fertility in arable systems and emphasize the need for soil fertility improvement strategies. However, greater insights into particular effects on specific soil properties revealed considerable variations, with high potential for negative consequences depending on the type of fertilizer. Accordingly, the observed acidic soil pH of the control treatment corresponds to standard tropical acid soil pH range, but the decrease in pH with inorganic fertilizer highlights its potential deleterious effects, whereas the high soil pH for poultry manure demonstrates its importance as pH buffer for tropical acid soils (Shi et al., 2008; Zhou et al., 2013; Li et al., 2016). Meanwhile, the observed increase in soil nutrients (e.g. N, P, K, C, Mg and Ca) and cation exchange capacity for the poultry manure treatment is consistent with other reports (Dikinya and Mufwanzala, 2010; Adeyemo et al., 2019). This is consistent with the hypothesis of this study, and further confirms the importance of poultry manure as a sustainable soil fertility management practice in agricultural systems. The soil available P content in the control and inorganic treatments could be affected

Table 2. Effect of fertilizer treatments on post-planting soil physicochemical properties

Soil properties	Fertilizer treatments			
	Control	Single NPK	Split NPK	Poultry manure
pH (water)	5.6 ± 0.3b	4.9 ± 0.2c	4.8 ± 0.2c	6.4 ± 0.2a
pH (KCl)	4.9 ± 0.1b	4.4 ± 0.1c	4.4 ± 0.0c	5.9 ± 0.1a
Total nitrogen (%)	0.15 ± 0.03c	0.22 ± 0.02b	0.22 ± 0.05b	0.31 ± 0.02a
Organic carbon (%)	2.4 ± 0.0b	2.5 ± 0.4b	2.4 ± 0.2b	3.9 ± 1.0a
Carbon : Nitrogen	16.8 ± 4.9a	11.3 ± 1.5a	11.0 ± 2.0a	12.5 ± 2.1a
Phosphorus (mg kg <sup>-1</sup> )	18.0 ± 1.8b	28.5 ± 8.7b	35.5 ± 11.0b	449.5 ± 20.7a
Potassium (cmol kg <sup>-1</sup> )	1.25 ± 0.03b	1.09 ± 0.06b	0.98 ± 0.16b	2.19 ± 0.24a
Calcium (cmol kg <sup>-1</sup> )	12.4 ± 1.7b	13.3 ± 4.1b	13.1 ± 2.3b	19.7 ± 2.0a
Magnesium (cmol kg <sup>-1</sup> )	2.7 ± 0.3b	2.7 ± 0.5b	2.9 ± 0.3b	4.5 ± 0.2a
Sodium (cmol kg <sup>-1</sup> )	0.22 ± 0.08a	0.20 ± 0.06a	0.16 ± 0.03b	0.31 ± 0.07a
Acidity (cmol kg <sup>-1</sup> )	0.08 ± 0.02b	0.23 ± 0.05a	0.2 ± 0.05a	0.16 ± 0.04ab
ECEC (cmol kg <sup>-1</sup> )	16.7 ± 2.0b	17.5 ± 4.4b	17.3 ± 2.6b	26.8 ± 2.3a

Values are mean ± standard deviation;

Data within rows with different letters are significantly different (P<0.05)

Table 3. Bean growth, nodulation, and yield parameters as affected by fertilizer treatments

Plant parameters	Fertilizer treatments			
	Control	Single NPK	Split NPK	Poultry manure
Nodule colours (%)	88.6 ± 5.2a	90.7 ± 7.1a	80.7 ± 8.2a	87.9 ± 9.7a
Shoot biomass (g)	95.3 ± 38.8b	147.9 ± 31.6b	145.6 ± 61.9b	321.9 ± 101.3a
Stem girth (cm)	3.6 ± 0.5b	4.5 ± 0.7a	4.5 ± 0.7a	5.2 ± 0.4a
Root biomass (g)	14.2 ± 1.0c	21.5 ± 2.1ab	19.5 ± 2.1b	23.2 ± 1.4a
Root girth (cm)	2.0 ± 0.7a	2.6 ± 0.4a	2.4 ± 0.4a	2.6 ± 0.8a
Root length (cm)	60.1 ± 9.7a	59.1 ± 12.4a	60.8 ± 14.1a	60.8 ± 9.5a

Values are mean ± standard deviation;

Data within rows with different letters are significantly different

Table 4. Correlation (r) of plant parameters and earthworm density with the post-planting soil physicochemical properties

Soil properties	Plant parameters			
	Root biomass	Shoot biomass	Bean pods	Nodule mass
pH (water)	ns	0.58	ns	ns
pH (KCl)	ns	ns	ns	ns
Organic carbon (%)	ns	0.61	ns	-0.61
Total nitrogen (%)	0.71	0.77	0.57	-0.85
Available P (mg kg <sup>-1</sup> )	0.79	0.68	0.72	-0.72
Potassium (cmol kg <sup>-1</sup> )	ns	ns	ns	ns
Magnesium (cmol kg <sup>-1</sup> )	ns	0.73	0.75	-0.74
Calcium (cmol kg <sup>-1</sup> )	0.62	0.68	0.58	-0.72
Acidity (cmol kg <sup>-1</sup> )	ns	ns	ns	ns
ECEC (cmol kg <sup>-1</sup> )	0.62	0.7	0.61	-0.73
Earthworms	ns	ns	0.57	ns

ns = not significant

by high P-sorption capacity of the tropical acid soil in this study with 47.19% clay composition, since high soil clay content correlates with P-sorption (Olatumji et al., 2012; Tening and Foba-Tendo, 2013). The high available P in poultry manure soils is likely due to mineralisation and increased labile soil P pools, coupled with high P content of poultry manure in relation to critical P levels required for mineralisation of soil organic-P to inorganic-P (Szychaj-Fabisiak et al., 2005; Torres-Dorante et al., 2006). Additionally, the high organic carbon and available P in poultry manure soils might be due to inherent rhizosphere biotic interactions of earthworms and beneficial microbes such as phosphate-solubilising bacteria (Zhang et al., 2016).

## 4.2 Root nodulation and earthworms

Earthworms are important in agricultural soils for their contribution to soil structure stabilization, carbon and nitrogen turnover, water flow, microbial dynamics, and effects on biogeochemical cycles (Chauhan, 2014; Bertrand et al., 2015). The lower earthworm density in the control and inorganic fertilizer as compared to poultry manure is consistent with other studies (Norgrove et al., 2011; Spiegel et al., 2018; Yahyaabadi et al., 2018), and strongly support our hypothesis. Although earthworms were not inoculated in this experiment, their occurrence in the different treatments possibly resulted from earthworm cocoons that may have been present in the experimental soil or poultry manure. Earthworm cocoons usually remain dormant in soil for an extended period of time until conditions are favourable, which was probably the case for the poultry manure and control treatments. Environmental factors (e.g. temperature and moisture) and food (e.g. quality and quantity) can influence cocoon production, hatching and growth of earthworms (Ali, 2018). Hence, the low earthworm density in the inorganic fertilizer treatments is likely due to increased soil acidity and toxicity (Spiegel et al., 2018; Yahyaabadi et al., 2018), as compared to favourable soil conditions for cocoons to hatch and develop under poultry manure or control. Accordingly, significant relationships were observed between earthworms and soil properties such as pH and nutrient contents in arable fields (Kanianska et al., 2016). The observed high nodule mass in control without fertilizer addition reflects common knowledge that N-fixation increases under nutrient deficiency as legumes tend to rely on fixed-N, although the N-fixation rate may ultimately depend on the availability of adequate amounts of other essential soil elements (Bildirici and Yilmaz, 2005). Hence, the adequate supply of nitrogen by both inorganic and organic fertilizers likely reduced the overall plant dependence on *Rhizobia* symbiosis for N-fixation, which resulted in the low nodule mass (Tagoe et al., 2010).

Although *Rhizobia* inoculation was not performed in this study, the experimental soil was collected from a field site with history of grain legume cultivation that likely built the communities of symbiotic indigenous *Rhizobia*, which formed symbiosis with the climbing bean plants for N-fixation. Nonetheless, the fertilizer treatments may exhibit idiosyncratic effects on the indigenous *Rhizobia* by enhancing or reducing their potential for N-fixation (Verma et al., 2013; Fankem H et al., 2015). Moreover, the interaction of poultry manure with soil biota improves soil health and fertility leading to greater productivity. Correspondingly, the decrease in root nodule mass under poultry manure as compared to the inorganic fertilizer or control is probably due to constant nutrient supply by poultry manure, which reduced the plant's dependence on N-fixation. This contradicts our hypothesis that poultry manure will enhance root nodulation as compared to inorganic fertilizer. Hence, the improvement of soil fertility and performance of climbing bean under poultry manure is likely due to the high nutrient inputs supplied directly by the poultry manure. Overall, the significant soil fertility benefits observed in this study were likely derived from the synergistic effects of poultry manure and increased biotic interactions including root nodulation/N-fixation and earthworm dynamics (Tagoe et al., 2010; Zhang et al., 2016).

## 4.3 Performance of climbing bean

The higher performance of climbing bean for both inorganic and organic treatments in relation to the unfertilized control treatment is consistent with other reports (Mfilinge et al., 2014; Eboibi et al., 2018; Nwite and Alu, 2018). However, the higher bean performance recorded for the split dose NPK compared to single dose NPK reflects the importance of constant nutrient supply during the entire plant growth period, especially for climbing bean that has a long growth cycle. Similarly, Sharma et al. (2017) reported greater productivity of pole French bean under split inorganic nitrogen fertilizer or vermicompost amendments. In sum the performance of climbing bean was consistent with the soil fertility status and plant nutrient supply (Abdel-Mawgoud et al., 2005). Meanwhile, the observed correlations of some plant parameters with earthworm density is in line with Scheu (2003) who reported significant increase in below and aboveground productivity in the presence of earthworms. Fertilizer inputs affected earthworm density and functions, which likely improved soil fertility and crop performance (Chauhan, 2014; Bertrand et al., 2015). Overall, the observed high climbing bean performance for poultry manure amendment is likely due to improvement in soil fertility resulting from confounding activities of earthworms and root nodulation/N-fixation, which is consistent with

our hypothesis. Hence, the poor climbing bean performance recorded in the control treatment strongly highlight the need for fertilizer inputs to boost soil fertility and productivity in the study area.

## 5 Conclusions

The results of this study demonstrate the importance of poultry manure on earthworms and soil fertility leading to greater crop performance, but highlight potential limitations on root nodulation and nitrogen fixation abilities. This is reflected in the high root nodule mass for the control and inorganic fertilizer treatments as compared to poultry manure. The low earthworm density recorded for both single dose NPK and split dose NPK fertilizer as compared to poultry manure reflects potential soil toxicity resulting from the chemical fertilizer. These findings demonstrate the importance of poultry manure on earthworm dynamics and soil fertility management, and the implication on the high performance of climbing beans.

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## Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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