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Effect of Biochar Type and *Bradyrhizobium japonicum* Seed Inoculation on Soybean Growth, Nodulation and Yield in a Tropical Ferric Acrisol

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Abstract

In tropical environments, most soybean growth studies have utilized rice husk biochar (RHB) in soil, even though it is low in nitrogen, potassium, and phosphorous. This may not give short-term agronomic performance relative to enriched biochar. Moreover, the impact of inoculating soybean seeds with atmospheric nitrogen-fixing bacterium *Bradyrhizobium japonicum* on nodulation and grain yield has produced inconclusive findings in the literature. This research therefore aims to assess the effect of poultry manure (PM), poultry manure biochar (PMB) and RHB alone and in combinations on grain yield, dry shoot and root biomass of soybeans in the semi-deciduous agro-ecological zone. In addition, the effect of *B. japonicum* inoculated and non-inoculated soybean seeds on nodulation and grain yield was also investigated. The treatments followed a split plot design studying inoculation and non-inoculation, soil amendments (eight), and control subplot factors, respectively. The results show that the amendment of a ferric acrisol with 4 $Mg \cdot ha^{-1}$ PM, 10 $Mg \cdot ha^{-1}$ RHB + 2 $Mg \cdot ha^{-1}$ PM, and 5 $Mg \cdot ha^{-1}$ RHB + 4 $Mg \cdot ha^{-1}$ PMB with *B. japonicum* inoculated seed produced significantly greater grain yield ($p = 0.05$). PM treatment had a significant ($p < 0.05$) effect on dry shoot, root, and total shoot biomass weight compared to PMB and RHB. *B. japonicum*-inoculated soybean seeds significantly ($p = 0.014$) increased soybean nodulation. This study suggests that RHB combined with PM or PMB provides a beneficial source of N, P, and K, resulting in improved soybean yield and nodulation in a tropical ferric acrisol.

Keywords

Biochar, Poultry Manure, Soil Fertility, Soil Microorganism, Soybean Production

1. Introduction

Poor resource efficient farming is a major factor contributing to the growing loss of fertile farmlands [1] due to continual soil cultivation without replenishing removed nutrients. Farming is also a highly energy-consuming practice involving the direct use of fuel and electricity for machinery, irrigation and lighting, but also inorganic fertilizer and pesticide consumption [2]. Fossil-based energy resources are dwindling and contribute to global warming, while crucial non-renewable material costs are rising [3]. Green solutions must be found in order to decrease the ecological impact of agriculture, increase soil biochemical quality, and boost soil fertility and productivity. More and more reuse as well as the creation of alternatives for more expensive or less accessible resources are two outcomes of the bioeconomy concept. This concept is essential to sustainable, resource-efficient farming since it reduces the negative environmental effects of agriculture while boosting output and profitability [4].

Implementing bioeconomy initiatives based on better management of plant biomass as a resource for the production of nutrients and energy recovery is a strategic method to manage agricultural waste [5]. This method explores the hidden potential of biomass through the improvement of bio-based value chains [6] and gives low-income farmers the chance to further utilize agricultural waste by turning it into goods with added value like biochar and biofertilizer [7]. This contributes to food security, is a significant step out of poverty in Sub-Saharan Africa (SSA) and mitigates the negative effects of climate change without diminishing the land's nutrient base through waste management technology and soil rehabilitation [8].

The preparation and application of biochar by smallholder farmers is labour intensive hence an effective one-time application for multiple cropping seasons would be desirable. In the best-case scenarios for some regions, extensive use of biochar could save farmers up to 50% of the water they now use to grow crops. The potential use of biochar to reduce nitrogen waste from farming systems also saves energy and costs. There are numerous uses for biochar in agriculture and the environment, making it a potentially affordable carbonaceous resource [9]. The goal of recycling waste is to reduce the use of external inputs, such as fertilizers and pesticides, and instead rely on natural processes to recycle nutrients and maintain soil fertility.

The main basic crops in SSA are cassava (192 million tons per annum), sugar cane (75 million tons per annum), maize (74 million tons per annum), yams (72 million tons per annum), and rice (32 million tons per annum). According to [10], 44 million tons of corn cob, 40 million tons of rice husk, 24 million tons of bagasse, and 17 million tons of cassava stalks (all on dry basis) were produced as harvest residues in 2019. Corn cobs, rice husks, and sugar cane bagasse can all be heated in a low oxygen atmosphere to create biochar [11] [12].

The extent of the biochar-induced agronomic effects depends on the type of biomass feedstock, soil characteristics, local climate and biochar application rate [13] [14] [15] [16]. The local weather could influence the biochar surface chemistry, surface area, pore volumes, morphology and adsorption properties. Biochar surface properties are influenced by biochar types and oxidative conditions, and these changes can gradually alter the physicochemical properties of biochar amended soils, suggesting that these changes are likely to occur during environmental exposure. This implies that these changes have potential effects for altering the physicochemical properties of biochar amended soils [17].

According to [18], the application of biochar in soil results in notable alterations to its physicochemical properties due to a range of natural mechanisms. These mechanisms include fluctuations in temperature, wetting-drying cycles, sunlight irradiation, atmospheric oxygen, root exudates, and microbial activity. Over time, these changes may have a beneficial or negative effect on the performance of biochar in field applications. According to [19], biochar may have a short-live impact on soil characteristics, with the most advantages appearing only one year following its application. Additionally, they pointed out that if biochar is applied more frequently than once, its effects can last longer in the soil.

Application of biochar to soil is a strategy that aims to recover bioenergy from surplus agricultural waste while reintroducing biochar to the soil in the form of minerals and recalcitrant carbon [20] [21]. Biochar may contain a highly stable organic carbon pool with the capacity to reduce climate change [22] [23] [24], retain water and nutrients, and raise the pH of acidic soils, depending on the source material and processing conditions [25] [26] [27] [28] [29].

Rice husks appear to be the most appealing of the main residues available in SSA for biochar production because they do not require additional drying or size reduction [30] [31] [32]. Several studies have reported that rice husk biochar (RHB) has the potential to improve physicochemical soil properties [33] [34]. Adding RHB to silty loam of Typic Hapludults-Ultisols soil improved soil pH, nutrient availability, water retention, and carbon mitigation [35] [36]. Despite these benefits, fresh low-temperature spruce biochar application to soil temporarily reduced initial plant growth due to mineral nitrogen immobilisation [37] requiring a different approach such as charging biochar with nutrients. The majority of biochar research to date have used only pure biochar and have either been carried out in laboratories, greenhouses, or tropical environments [38]. Studies on biochar conducted under field conditions frequently yield contradictory outcomes to those obtained in lab environments [39]. Therefore, trials under field conditions with agronomically relevant fertilizer types and amounts reflect the biochar impact more realistically.

Poultry manure (PM) is a common by-product encountered in animal farming amounting to an annual, estimated 920 million metric tons worldwide and 124 million metric tons in SSA alone [40]. PM is rich in macro and micronutrients but also have numerous contaminants. Its direct application as an organic fertilizer to soil could be impairing human, animal and soil health due to presence of pathogenic strains, a vast array of antibodies and rapid decomposition [41]. Alternative approaches to its safe uses are needed, particularly in developing countries where there is insufficient data and fixed criteria for all the impurities. The rapid decomposition of PM in the tropics means that nutrient retention is a limiting factor to soil productivity [42] [43]. One emerging management tool to maintain higher yields is the addition of RHB [44] while at the same time charging it with valuable plant nutrients such as nitrogen from PM [45] [46]. Conversion of PM to poultry manure biochar (PMB) is another strategy to reduce waste volume, destroy pathogens and antibodies, and produce a nutrient-rich organic amendment for improving soil nutritional status [47] [48] [49]. Though there are several alternative strategies to manage PM, conversion to PMB is considered a safe, reliable, and effective tool due to its stability and the environment-friendly approach of using this organic resource in agriculture[50] [51].

It is critical that protein seed crops such as soybeans receive an adequate supply of nitrogen. Soybean is a crop of global importance, constituting one of the largest sources of vegetable oil and protein feed in the world [52]. Global soybean production was approximately 333 million metric tons in 2019 [53], which is still insufficient to meet the increasing global demand [54]. In SSA, soybean production area has increased exponentially, but yield has remained stagnant at about 1.05 $Mg \cdot ha^{-1}$, compared to the world average of 2.76 $Mg \cdot ha^{-1}$ [55]. The low soybean yield can be attributed to the use of poor-performing varieties, the inadequate application of fertilizers, soil management, and the lack of sustained rhizobial inoculants in soils with no history of soybean production [56] [57] [58].

Soybean yield could be improved with increase in number of nodules due to a greater biological nitrogen fixation for soybean development that later partitioned into seeds, this tends to positively increase seed yield and yield components [59] [60]. In order to jump-start nitrogen-fixation, researchers proposed to inoculate soybean seeds with *Bradyrhizobium* spp. [61] [62] (Gyogluu *et al*., 2016; Ronner *et al*., 2016). *B. japonicum* is a bacterium that enhances the formation of nodules in soybean plants and is responsible for fixing nitrogen at rates up to 300 $kg \cdot ha^{-1}$ [63]. [64] [65] reported a positive increased seed yield as a result of improved nodulation. The practice of biological nitrogen fixation by symbiotic soil bacteria, largely *Bradyrhizobium* spp., is a cheap and natural source of N for soybeans that could significantly reduce the use of inorganic fertilizers and pesticides [66]. However, the use of inorganic nitrogen fertilizers usually reduces nodule formation and nitrogen fixation [67]. In contrast, [68] reported that low yields and other parameters was as a result of drought conditions. A similar finding was reported by [69] that inadequate active nitrogen-fixing nodules may result in significantly reduced plant production and grain yield potential. In some cases, inoculated cultivars did not produce a greater yield, probably due to low organic matter and poor nutrient supply to stimulate inoculated microorganisms [70].

Several reviews of the literature revealed that biochar is mostly enhanced with mineral fertilizer, while others use different feedstocks with clays, mineral rocks, bentonite, chicken manure, sludge, composts, algae, etc. and other nutrient sources [71] [72]. Integration of biochar and inorganic fertilizer has been reported to be more beneficial than the use of either biochar or inorganic fertilizer alone [73] [74]. [75] showed improvement in total carbon (TC), total nitrogen (TN), available P, total exchangeable cations, effective cation exchange capacity (ECEC), and pH, including tissue N, P and K in food crops with the co-application of biochar and NPK. [32] produced P-enriched biochar from chicken litter and coffee husk enriched with phosphoric acid and magnesium oxide combined with triple superphosphate. [76] produced biochars enriched with Mg from poultry litter, pig manure (PMB), and sewage sludge impregnated with a $MgCl_2$ solution to reach approximately 10% of Mg in the biochars. The application of RHB enriched with cattle dung as a soil ameliorant has shown benefits for crops such as maize (*Zea mays*), shallot (*Allium cepa*), and peanut (*Arachis hypogea*), particularly through improving soil properties [77]. Soil biological parameters have also been affected by the addition of biochar. Biochar could especially influence mycorrhizal abundance and/or functioning, allowing improved uptake of nutrients by plants [78]. Moreover, biochar is effective in reducing the need for chemical fertilizer due to bio-fortification and increasing soil microbial population and activity, resulting in more carbon storage in the soil [79] [80].

However, there are no studies on the fertilizer potential of RHB enriched with PM or PMB and the performance of *B. japonicum* inoculant for soybean production in ferric acrisol of the semi-deciduous agro-ecological zone of Ghana. In this paper, we try to make the PM more useful and environmentally friendly combining with RHB when used in agriculture by reducing the ammonia emission and soluble P but not compromising the plant yield.

We hypothesize that the application of RHB or *B. japonicum* alone will not provide as much agronomic value on growth performance, yield and microbial activities compared to RHB enriched with either PM or PMB within one harvest cycle. PM or PMB will counter deficiencies of RHB in N, P and K. Therefore, the overall aim of this paper is to demonstrate the effect of soybean seed inoculation with *B. japonicum* as well as single and combined applications of PM, RHB, and PMB on soybean nodulation, growth and yield in a ferric acrisol in a semi-deciduous agro-ecological zone in SSA.

2. Materials and Methods

2.1. Description of the Study Locations

A field trial was carried out at the Research Farm of the Department of Crop and

Soil Sciences at the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, during the 2017 planting season (May and August). The geographic location of the experimental site in the semi-deciduous agro-ecological zone lies between latitude 06˚63'01.26"N and longitude 01˚55'11.04"W (**Figure 1**), with an elevation of about 356 m above sea level. The experimental site with an unknown history of soybean cultivation and *B. japonicum* inoculation was chosen. [81] investigated the use of soybean inoculant in Argentina and the United States and discovered that the best results were obtained in soil with no history of soybean cultivation, a relatively high level of soil extractable P and S, a high SOM, and in areas with higher rainfall during the early reproductive growth stage in Argentina compared to the United States.

2.2. Weather Conditions

In the semi-deciduous agro-ecological zone, rainfall patterns are bimodal, with an average annual rainfall of about 1500 mm. The major rainy season begins in mid-March and lasts until July, while the minor rainy season falls between September and November. The average monthly temperature ranges between 24 and 28˚C, with an average relative humidity of 88% during the major and 58% during the minor cropping season (Ghana Meteorological Agency, 2012).

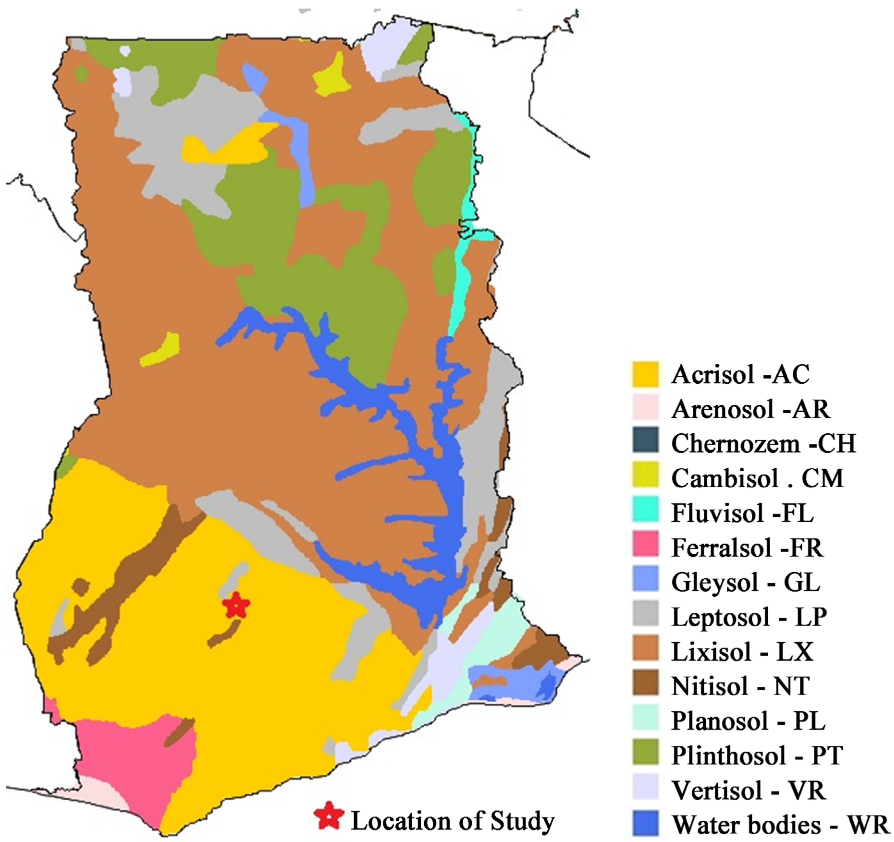

Figure 1. Soil map of Ghana based Harmonised World Soil Map version 1.21. The dominant soil group at the study site is predicted to be Acrisol.

2.3. Soil Description of the Experimental Sites

The soil at the experimental site is a well-drained, sandy loam over reddish-brown and gravelly light clay overlying deeply weathered granite rocks (Supplementary Information Table S1). According to the FAO/UNESCO Soil Map of the World Legend [82], it is classified by [83] as ferric acrisol and is the dominant soil in the study area as represented on soil map of Ghana (Figure 1).

Soil and Climatic Suitability for Soybean Production

Table 1 shows the soil and climatic suitability for soybean production during the 2017 major cropping season in the semi-deciduous agro-ecological zone. The soil pH at the study site was moderately acidic and in good agreement with pH_{H_2O} of 5.10 reported for ferric acrisols (Supplementary Information Table S1). Soil

Table 1. Initial soil physicochemical properties prior to the start of the experiment as well as climatic requirements for soybean production in a ferric acrisol of semi-deciduous agro-ecological zone (adapted from [84].

Soil parameter	Unit	Plain soil	Soil characteristics (Sys *et al.*, 1993)
pH (1:2.5 H_2O)		5.64	S2
Electrical conductivity	($dS \cdot m^{-1}$)	0.6	S1
Organic carbon	(%)	1.81	S2
Total nitrogen	(%)	0.03	NA
Available phosphorus	($mg \cdot kg^{-1}$)	9.4	NA
Calcium	($g \cdot kg^{-1}$)	2.23	NA
Magnesium	($g \cdot kg^{-1}$)	0.11	NA
Potassium	($g \cdot kg^{-1}$)	0.13	NA
Sodium	($g \cdot kg^{-1}$)	0.016	NA
Exchangeable acidity	($cmol(+) \cdot kg^{-1}$)	4.8	NA
ECEC	($cmol(+) \cdot kg^{-1}$)	11.2	NA
BS	(%)	57.1	S1
Texture		SL	S2
Average annual rainfall	mm	1500	NA
Precipitation 1st month	mm	201	S2
Precipitation 2nd month	mm	253	S2
Precipitation 3rd month	mm	138	S1
Precipitation 4th month	mm	91	S1
Average monthly temperature	˚C	24-28	S2
Average relative humidity (major crop season)	%	88	S1

Suitability: S1 (high), S2 (moderate), S3 (marginal), N (unsuitable), NA: Not available.

pH affects aluminium solubility, nutrient availability, biological nitrogen fixation, and soybean cyst nematode population growth [85] [86] [87]. A soil pH of 5.5 - 6.0 results in low aluminium toxicity, good phosphorus availability, enhanced growth of nitrogen-fixing bacteria, and reduced activity of *Heterodera glycines*, a major pest of soybeans. The need for lodging at the study location can therefore be regarded as low.

The electric conductivity (EC) of the plain soil was 0.6 $dS \cdot m^{-1}$ and considered non-saline (**Table 2**). Soil EC readings less than 1 $dS \cdot m^{-1}$ do not impede soybean yield, plant nutrient availability, or microbial processes. Based on the crop tolerance of soybean, the need to flush excessive salts below the root zone is minimal. This result indicates that soil EC can be influenced by the location having high rainfall.

The soil organic carbon (SOC) was moderately suitable for soybean (**Table 2**). The level may further decline if unsustainable farm management is practiced over several cropping seasons. The application of RHB, PM, PMB or their permutations, however, is expected to reverse this trend and maintain healthy levels of SOC for sustained high agricultural productivity.

Soil nitrogen content was low for soybean production at the study site. Low nitrogen is among the major factors limiting the production of soybeans. Soybean nitrogen requirements are met through both nitrogen-fixing bacteria (*Bradyrhizobium* spp.) and residual or mineralized soil nitrogen. Nitrogen application may be beneficial in soils with low residual nitrogen and/or low soil organic matter. On average, the amount of nitrogen required by soybeans is approximately four times that of cereal crops [88].

The available phosphorus concentration in plain soil was found to be 9.4 $mg \cdot kg^{-1}$. This agrees with [89], who reported phosphorous concentrations at the same site of less than 10 $mg \cdot kg^{-1}$. This concentration was rated low, which makes application of phosphorus fertilizer necessary for successful cropping of legumes. Legume plants that depend on biological nitrogen fixation require more phosphorus than plants receiving nitrogen fertilizer since the reduction of atmospheric nitrogen is a very energy-consuming process.

Potassium content was low in the soil. After nitrogen, potassium is the nutrient absorbed in the next largest quantity by soybeans. Potassium plays an important role in the plant's photosynthesis and metabolism. Carbohydrates generated by photosynthesis provide the energy needed by bacteria in nodules to fix atmospheric nitrogen. Fertilization with NPK, organic fertilizer such as PM, and soil amendments such as RHB and PMB, is expected to improve soybean yield.

Sodium content was low, while Mg and relative base saturation ranged from low to medium. Calcium was medium in the studied location.

The textural class is predominantly sandy loam. The soil is moderately suitable for soybean cultivation. The soil has a good depth for root penetration, well-drained, moderate gravel and light clay overlying deeply weathered granite rocks. The soil will benefit from biochar application because it will improve soil aeration, soil structure, reduce bulk density, increase water holding capacity and nutrient uptake.

During the 2017 cropping season, the climatic conditions at the test site was considered highly suitable due to adequate water availability for soybean development during the germination and pod filling that correspond to the first and third months of growing cycle. Similar findings were reported by [90]. The agro-climatic potential soybean yield for the study location is 1630 $kg_{DW} \cdot ha^{-1}$ (Supplementary Information **Figure S1**). The suitability index for the study location was 'moderate', which means that the attainable yield reaches 21-35% of the yield under best conditions (Supplementary Information **Figure S2**).

2.4. Field Preparation

The vegetation at the study site was manually cleared by slashing with a cutlass, then ploughed and left for 7 days for the buried plants to decay before being harrowed with a tractor to a fine tilt. A tape measure, a garden line, and pegs were used to mark the plots. Each block at each experimental site had 18 plots that measured 5 m by 3 m, giving a total of 54 plots with a total land area of 1295 m^2, and 2 m and 1 m spacing between blocks and plots, respectively.

2.5. Experimental Materials

2.5.1. Preparation of Poultry Manure, Rice Husk and Poultry Manure Biochars

RH and PM feedstocks were collected from different rice millers and poultry farms, respectively, and transported for charring to the Soil Research Institute of the Council for Scientific and Industrial Research, Kwadaso, Ghana. RH and PM were air dried for 24 h prior to biochar production. Biochar from RH and PM was produced via pyrolysis in a commercial retort kiln at 600˚C (slow pyrolysis; 3 h holding time) and 300˚C (slow pyrolysis; 1 h holding time), respectively. The biochar yields were 41 wt% and 48 wt% of the dry RH and PM, respectively. RH charred at 600˚C is reported to have a high surface area [91] while [92] reported that the total nitrogen content and cation exchange capacity were best if PM was pyrolyzed at 300˚C. Prior to being applied to the experimental plots, the chemical properties of the organic amendments used as treatments were analyzed (**Table 2**).

2.5.2. Characterization of Biochar and Poultry Manure

Table 3 shows the chemical properties of PM, PMB and RHB. The pH of PMB and RHB was quasi-neutral while PM was found to be slightly alkaline. The total organic carbon content for RHB was >50% with PM and PMB having <22%. The biochar derived from RH had low nitrogen and phosphorus contents and exchangeable bases compared to PMB and PM. Calcium (8.4 ± 2.2 wt%), magnesium (0.62 ± 0.03 wt%) and potassium (2.69 ± 0.17 wt%) contents in PM used as treatment was greater than PMB and RHB as shown in **Table 3**. Based on the chemical properties of RHB, the result shows that it will be more beneficial and

Table 2. Chemical properties of poultry manure as well as poultry manure and rice husk biochars.

Parameter	Unit	PM	PMB	RHB
pH (1:2.5 H_2O)	(/)	7.88	7.16	6.82
Organic carbon	(%)	21.8 ± 3.1	8.3 ± 2.4	52 ± 0.5
Total nitrogen	(%)	2.67 ± 0.05	2.36 ± 0.34	0.59 ± 0.04
Available phosphorus	($mg \cdot kg^{-1}$)	16.20 ± 0.08	12.0 ± 0.11	1.40 ± 0.02
Potassium	($g \cdot kg^{-1}$)	27.0 ± 0.2	12.6 ± 0.1	5.50 ± 0.02
Calcium	($g \cdot kg^{-1}$)	84.0 ± 2.2	33.4 ± 0.4	2.80 ± 0.03
Magnesium	($g \cdot kg^{-1}$)	6.20 ± 0.03	4.70 ± 0.06	1.20 ± 0.01

PM—Poultry manure, PMB—Poultry manure biochar and RHB—Rice husk biochar.

Table 3. Soil treatments at experimental site of the semi-deciduous agro-ecological zone. Values are on $Mg \cdot ha^{-1}$ basis.

Main plot number	Treatment	Main plot *B. japonicum*	Split plot		
			PM	PMB	RHB
1	Control	+	-	-	-
	Control	-	-	-	-
2	10RHB	+	-	-	10
	10RHB	-	-	-	10
3	4PM	+	4	-	-
	4PM	-	4	-	-
4	4PMB	+	-	4	-
	4PMB	-	-	4	-
5	2PM + 10RHB	+	2	-	10
	2PM + 10RHB	-	2	-	10
6	2PMB + 10RHB	+	-	2	10
	2PMB + 10RHB	-	-	2	10
7	4PM + 5RHB	+	4	-	5
	4PM + 5RHB	-	4	-	5
8	2PMB + 5RHB	+	-	2	5
	2PMB + 5RHB	-	-	2	5
9	4PMB + 5RHB	+	-	4	5
	4PMB + 5RHB	-	-	4	5

PM—Poultry manure, PMB—Poultry manure biochar and RHB—Rice husk biochar.

useful when deficient nutrients are enhanced with PM while RHB complement the effect by mitigating Mg losses from the PM.

2.5.3. Soil Treatments

The organic amendments used as treatments investigated in this research are summarized in Table 3. In brief, treatments consisted of 0 $Mg \cdot ha^{-1}$ control, 4 $Mg \cdot ha^{-1}$ PM, 4 $Mg \cdot ha^{-1}$ PMB, 10 $Mg \cdot ha^{-1}$ RHB, 10 $Mg \cdot ha^{-1}$ RHB + 2 $Mg \cdot ha^{-1}$ PMB, 10 $Mg \cdot ha^{-1}$ RHB + 2 $Mg \cdot ha^{-1}$ PM, 5 $Mg \cdot ha^{-1}$ RHB + 4 $Mg \cdot ha^{-1}$ PMB, 5 $Mg \cdot ha^{-1}$ RHB + 4 $Mg \cdot ha^{-1}$ PM, and 5 $Mg \cdot ha^{-1}$ RHB + 2 $Mg \cdot ha^{-1}$ PMB. The biochar application rate largely depends on soil types. However, reviewed literature shows that an application rate of 10 $Mg \cdot ha^{-1}$ is typically used and produces better results for crop production [93] (Liu *et al.*, 2013). We intend to reduce application rates and enrich RHB with dry PM or PMB while still outperforming 10 $Mg \cdot ha^{-1}$ RHB. The treatments were incorporated into the soil on a per-plot basis at a depth of 0.1 m with a soil density of 1600 $kg \cdot m^{-3}$ two weeks prior to planting.

The nutrient content (NC) of treated soil was calculated using Equation (1):

$$NC = \frac{NV \times Area \times D \times BD + TV \times AR}{\left(AR + D \times Area \times BD\right)} \tag{1}$$

where

NV = Nutrient value in the soil [% or $g \cdot kg^{-1}$]

TV = Treatment value [% or $g \cdot kg^{-1}$]

AR = Application rate [$kg \cdot ha^{-1}$]

D = Depth = 0.1 m

A = Area = 10,000 $m \cdot ha^{-1}$

BD = Bulk density of soil = 1600 [$kg \cdot m^{-3}$].

2.6. Experimental Design

The field trial was based on the randomized complete block design arranged in split-plot, replicated three times, with *B. japonicum* inoculum (− and +) as the main plot and RHB with either PM or PMB-levels as sub-plots, nine soil treatments constituting the sub-plot factor. Each replicate contains 18 sub-plots divided evenly between inoculation and non-inoculation. The width between replicates was 2 m, and 1 m for sub-plots. The treatments were applied only once in each row. Individual plot sizes are 3 × 5 m, with a total area of 15 m^2. The total size of the field trial was 1295 m^2.

2.7. Seed Inoculation and Planting

Soygro inoculant with the trade name "SoyCap" was produced by Soygro (Pty) Limited and sold by Nedbank Limited, Rondebosch, South Africa. The inoculant was supplied in a sachet bag. Soybean seeds were either used as received (the control) or wetted with gum arabic and coated with *B. japonicum* inoculant at a dosage of 10 g per kg of seeds. Assuming that one gram of inoculant harbours 10^6 - 10^8 *Bradyrhizobium* cells [94], this equates to 10^4 to 10^6 *Bradyrhizobium* $cell \cdot g^{-1}$ soybean seed. Gum arabic was used to allow the inoculant to stick to the seeds [95]. Seed inoculation was done in a plastic container and air-dried for about 30 min before planting. The "Nangbaar" soybean variety, characterized as an early maturing soybean, obtained from the Fumesua Crop Research Institute of the Council for Scientific and Industrial Research, was used as the test crop. Soybean seeds were sown at a rate of three seeds per hole at 5 - 7 cm depth at 60 cm × 10 cm spacing on May 15 and June 6, 2017 in the semi-deciduous zone, respectively.

2.8. Fertilizer Application

Basal applications of 30 $kg \cdot P \cdot ha^{-1}$ from triple superphosphate and 30 $kg \cdot K \cdot ha^{-1}$ from muriate of potash were applied one week after soybean planting based on the band method [96].

2.9. Soil Sampling and Sample Preparation

Ten core soil samples were randomly collected at a depth of 0 - 15 cm using an auger, following the "W" design for the initial soil sampling. The samples were then mixed thoroughly, and sub-samples were taken for physico-chemical analyses. A composite sample was further air dried, crushed, and sieved through a 2 mm mesh sieve. The prepared samples were put into zip-lock bags and stored in the laboratory awaiting analysis.

2.10. Biochar Characterization

Laboratory analyses of the biochar were carried out prior to its application using standard laboratory protocols as described by [97]: total carbon, available nitrogen, total phosphorus, calcium, and magnesium. Biochar pH was measured at a 1:2.5 ratio of biochar to water using a pH meter (Schott instrument Lab 860). Ash content (AC) was determined by placing a biochar sample in a nickel crucible and heated at 700˚C for 2 h [98]. The AC was calculated as follows (Equation (2)):

$$AC(\%) = \frac{M_{ash}}{M_{biochar}} \times 100 \quad (2)$$

where M_{ash} is the mass of ash (g) and $M_{biochar}$ the dry mass of biochar (g).

Moisture content of the biochar was determined gravimetrically [9].

2.11. Soil Analysis

Soil pH was measured at a 1:2.5 soil-to-distilled water ratio using a pH meter, as described by [99]. A modified Walkley and Black wet oxidation method, as described by [100], was used in the determination of organic carbon. The organic matter was obtained by multiplying the organic carbon value by the van Bemmelen factor (1.72), which converts to percent organic matter as explained by [101]. The Bray-1 method was used to determine available phosphorus with 0.03 *M* NH_4F and 0.025 *M* HCl as the extractant [102]. Total nitrogen was determined by the macro-Kjeldahl digestion and distillation procedure according to [103]. Exchangeable basic cations (Ca, Mg, Na, and K) were extracted using 1.0

M ammonium acetate at pH 7 [18]. Potassium and sodium were estimated using a flame photometer [104]. Calcium and magnesium were determined using the EDTA titration procedure [105]. The exchangeable acidity (hydrogen and aluminium) was estimated in a 1.0 M KCl extract [106]. The hydrometer method, as described by [107], was used to determine the soil texture.

2.12. Measurement of Crop Parameters

2.12.1. Nodulation

At the 50% flowering growth stage, 10 plants from each plot were gently uprooted after the stem was cut about 5 cm above ground level. The roots were carefully dug out to a depth of 30 cm using a spade. The roots and detached nodules collected from the soil were put in ziplock bags and labeled accordingly. To remove adhered soil, the roots were thoroughly washed on a 1 mm sieve mesh under running tap water. The nodules were removed and blotted dry using a paper napkin. The nodules were then counted and oven-dried at 65˚C to a constant weight, after which nodule numbers and oven-dry weights were estimated.

2.12.2. Shoot Dry Biomass Weight

At 50% flowering and harvest, 10 soybean plant stands per plot were harvested, excluding roots, and oven-dried at 60˚C for 2 h to a constant weight using an electronic balance.

2.12.3. Grain Yield

At harvest, soybean plants within the harvestable area (3 m^2) were sampled and weighed, excluding the roots. For grain yield, the harvest was threshed, the seeds collected, air-dried, cleaned, and weighed. To determine seed dry matter, the collected grain was sub-sampled, weighed, and oven dried at 60˚C for 2 h to a constant weight and recorded with an electronic balance per plot [108]. The grain's dried matter was determined by proportion. The following is an estimate of grain yield per hectare (Equation (3)):

$$\text{Grain yield}\left(\text{Mg}\cdot\text{ha}^{-1}\right)=\frac{\text{Grain yield per plot}\times 10000\ \text{m}^2}{\text{Harvest area}\left(\text{m}^2\right)\text{ha}} \tag{3}$$

2.12.4. Harvest Index

After harvest, one hundred and five plants were gathered from each plot and air dried. The total biomass of threshed residue was weighed, sub-sampled, weighed, and oven dried at 60˚C for 2 h. The harvest index (HI) was determined according to Equation (4) [109].

$$\text{HI}=\frac{\text{Economic yield}}{\text{Total biological yield}}\times 100\% \tag{4}$$

where economic yield is the yield per plant [$kg\cdot ha^{-1}$] and biological yield is the summation of dry shoot weight and grain yield [$kg\cdot ha^{-1}$].

2.13. Statistical Analysis

Data obtained for the nodulation, growth, and yield parameters of soybean were statistically analysed using GENSTAT (2012 Edition). Analysis of variance (ANOVA) was carried out to identify significant differences between treatments, after which significant means were separated using the Duncan Multiple Range Test (DMRT) at the 5% level of probability. DMRT was used because it is more useful when larger pairs of means are being compared and is powerful in detecting statistical differences [110].

3. Results and Discussion

3.1. Response of Soybean Growth and Yield to Seed Inoculation, Manure and Biochar Application

Root, shoot and total plant dry mass performances were observed at 50% flowering stage of soybean for *B. japonicum* inoculated and non-inoculated seeds grown in plots amended with single and combined applications of PM, RHB and PMB (Figures 2-4).

Single application of 4 Mg·PM·ha^{-1} significantly increased the growth ($p < 0.05$) of root, shoot and total plant dry mass compared to the control, while 5 Mg·RHB·ha^{-1} + 4 Mg·PMB·ha^{-1} had a significant effect on dry root weight (Table S2). Soybean seeds inoculated with *B. japonicum* also significantly increased

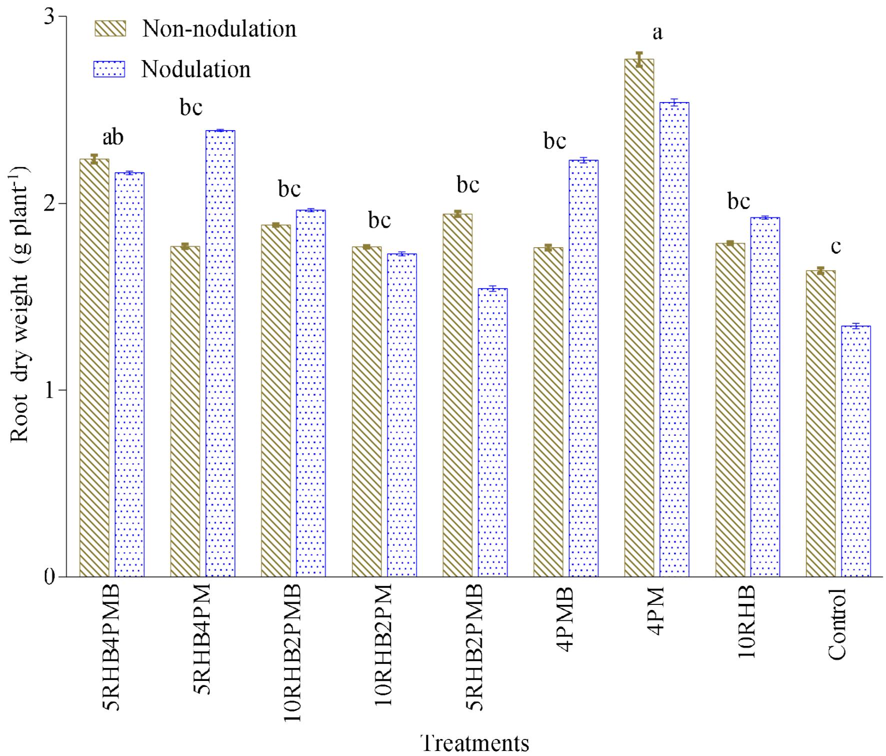

Figure 2. Root dry weight of soybean as influenced by the treatments in semi-deciduous forest agro-ecology. Error bars represent standard deviation of the mean. Bars with the same letters are not significantly different ($p < 0.05$).

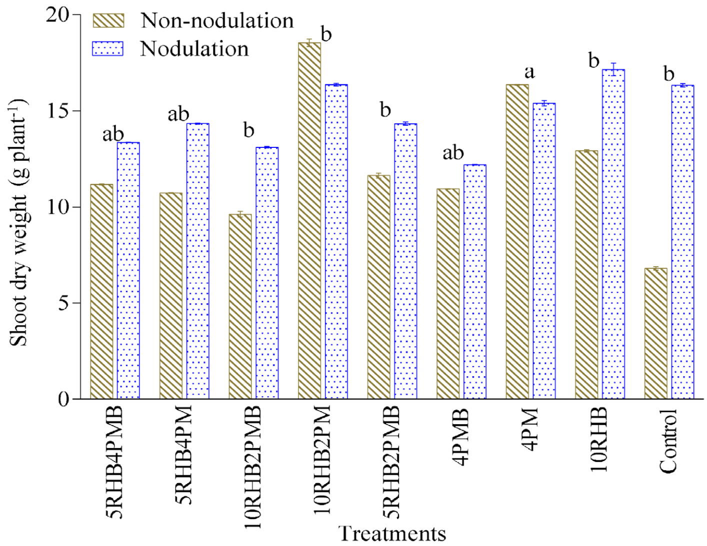

Figure 3. Shoot dry weight of soybean as influenced by the treatments in semi-deciduous forest agro-ecology. Error bars represent standard deviation of the mean. Bars with the same letters are not significantly different ($p < 0.05$).

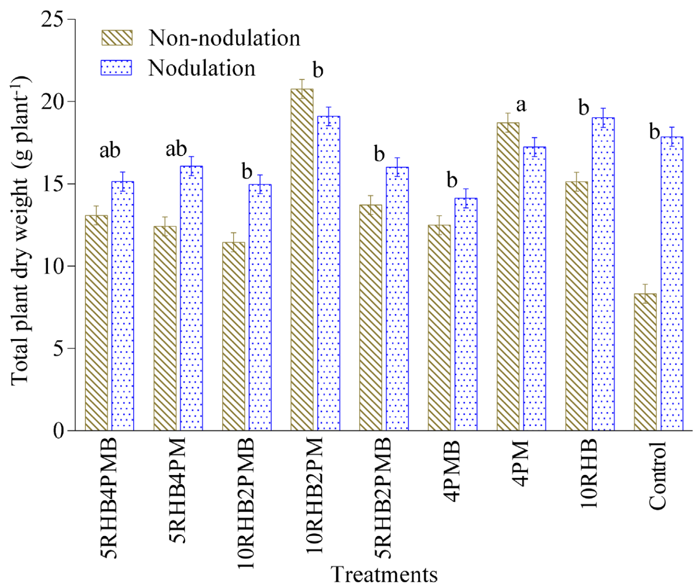

Figure 4. Total plant dry weight of soybean as influenced by the treatments in semi-deciduous forest agro-ecology. Error bars represent standard deviation of the mean. Bars with the same letters are not significantly different ($p < 0.05$).

shoot and total dry mass production. No significant interaction with single and combined application of PM, RHB and PMB were observed.

The response of root dry weight following single and combined applications of PM, RHB and PMB ranged from 1.40 to 2.67 g·plant^{-1} with 4 Mg·ha^{-1} PM having the highest root dry weight, while the lowest root weight was recorded in the control. While all treatments resulted in greater root dry weight, only the application of 4 Mg·PM·ha^{-1} as well as 5 Mg·RHB·ha^{-1} + 4 Mg·PMB·ha^{-1} was statistically significant at the $p = 0.05$ level (Table S2). The observed general increase in root dry weight may be attributed to the improved texture of the sandy loam soil [111].

A similar performance was recorded for dry shoot weight. The plot amended with 4 Mg·ha^{-1} PM produced the heaviest dry shoot weight (17.3 g·plant^{-1}) followed by 5 Mg·ha^{-1} RHB + 4 Mg·ha^{-1} PM (15.9 g·plant^{-1}), 5 Mg·ha^{-1} RHB + 4 Mg·ha^{-1} PMB (14.9 g·plant^{-1}) and 4 Mg·ha^{-1} PMB (12.9 g·plant^{-1}). The lowest dry shoot weights were recorded for the application of 10 Mg·ha^{-1} RHB + 2 Mg·ha^{-1} PMB (11.28 g·plant^{-1}), 5 Mg·ha^{-1} RHB + 2 Mg·ha^{-1} PMB (11.6 g·plant^{-1}) and the control (11.6 g·plant^{-1}). Compared to single application of PM and PMB, the use of RHB + PM as well as RHB + PMB generally resulted in a decrease in shoot dry weight (**Table S2**) and available phosphorus (**Table 4**). This suggests that RHB immobilised phosphorous originating from PM and PMB rendering it temporarily unavailable to the soybean plant. The use of PMB instead of PM also resulted in a general decrease in dry shoot weight and available phosphorous in soil suggesting that it was volatilised and immobilised during pyrolysis.

The total plant dry weight of soybean following amendment with 4 Mg PM ha^{-1} gave the highest yield (19.9 g·plant^{-1}). The total plant dry weight decreased in the following order of treatment: 5RHB + 4PM > 5RHB + 4PMB > 4PMB > 10RHB + 2PM > 10RHB > 5RHB + 2PMB > 10RHB + 2PMB. The lowest total plant dry weight (13.1 g·plant^{-1}) was found in the control (**Table S2**). While all treatments helped to improve the total plant dry weight compared to the control, only the treatment with 4 Mg·PM·ha^{-1} was statistically significant ($p < 0.05$). Addition of PM to the sandy loam arguably improved soil texture for more efficient nutrient uptake while also providing sufficient nutrients for plant growth.

Table S2 shows that *B. japonicum* inoculation generally had a positive influence ($p < 0.05$) on shoot dry weight and total plant dry weight.

Figures S3-S5 showed that root, shoot, and total plant dry weight ($R^2 > 0.2$) of soybean exhibit a weak positive correlation with available N, P, and K, while NPK in soil is found to be strongly correlated to grain yield ($R^2 > 0.7$), as shown

Table 4. Nutrient content of plain and treated ferric acrisol from the semi-deciduous agro-ecological zone.

Soil parameter	Unit	Ctrl	4PM	4PM + 5RHB	2 PM + 10RHB	4PMB	4PMB+ 5RHB	2PMB +5RHB	2 PMB + 10RHB	10RHB
Organic carbon	(%)	1.81	1.86	1.98	2.15	1.83	1.98	1.97	2.13	2.12
Total nitrogen	(%)	0.030	0.037	0.038	0.037	0.036	0.038	0.035	0.036	0.033
Available phosphorus	(mg·kg^{-1})	9.4	49.8	43.5	38.1	39.3	43.5	28.6	32.9	18
Potassium	(g·kg^{-1})	0.125	0.192	0.173	0.192	0.156	0.173	0.157	0.174	0.158
Calcium	(g·kg^{-1})	2.23	2.43	2.31	2.33	2.31	2.31	2.27	2.27	2.23
Magnesium	(g·kg^{-1})	0.107	0.122	0.122	2.15	1.83	1.98	1.97	2.13	2.12

PM—Poultry manure, PMB—Poultry manure biochar and RHB—Rice husk biochar.

in **Figures S6-S8**. As the soil in our experiment was nutrient poor, *B. japonicum* inoculated and non-inoculated seeds grown in plots amended with single and combined applications of PM, RHB, and PMB may have stimulated nodulation, thereby increasing chlorophyll content in leaf tissue and enhancing harvest index, yield, and 100 seed weight. We suggest that the increase in yield was due to an increase in soil pH caused by the more alkaline biochars (**Table 3**), and an increase in NPK rom applications of organic amendment, thereby increasing P availability in this slightly acidic soil (**Table 2**).

3.2. Response of Soybean 100 Seed Weight, Grain Yields and Harvest Index to Seed Inoculation, Manure and Biochar Application

The 100 seed weight, grain and harvest index of inoculated and non-inoculated soybean from plots amended with single and combined applications of PM, RHB and PMB are presented in **Figures 5-7**.

Single and combined applications of PM, RHB and PMB as well as inoculation with *B. japonicum* had no significant ($p > 0.05$) impact on hundred (100) soybean seed weight and harvest index (**Table S3**). Also, there was no significant interaction between single and combined application of PM, RHB and PMB on 100 seed weight and harvest index of inoculated and non-inoculated soybean seeds (**Table S3**).

Single and combined applications of PM, RHB and PMB positively increased grain yields in both inoculated and non-inoculated soybean seed (**Table S3**). Similarly, *B. japonicum* inoculation significantly ($p < 0.05$) increased the yield. However, there was not significant interaction between single and combined

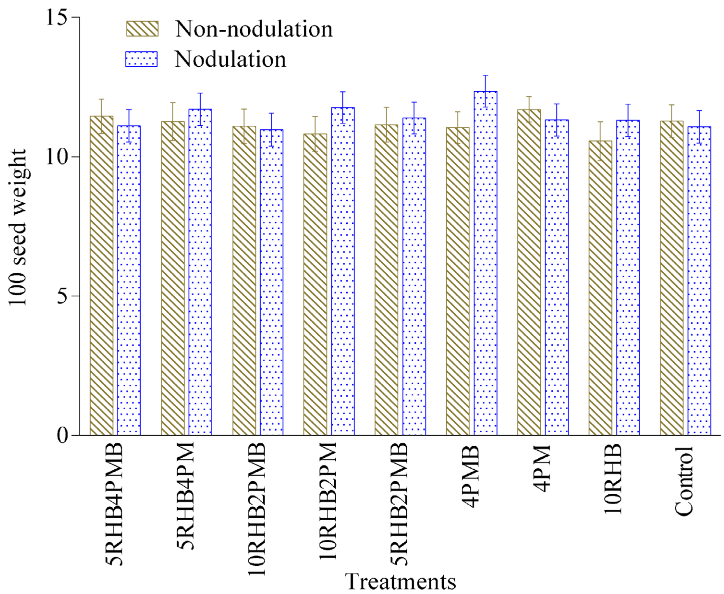

Figure 5. 100 seed weight of soybean as influenced by the treatments in semi-deciduous forest agro-ecology. Error bars represent standard deviation of the mean. The 100 seed weight are not significantly different ($p < 0.05$).

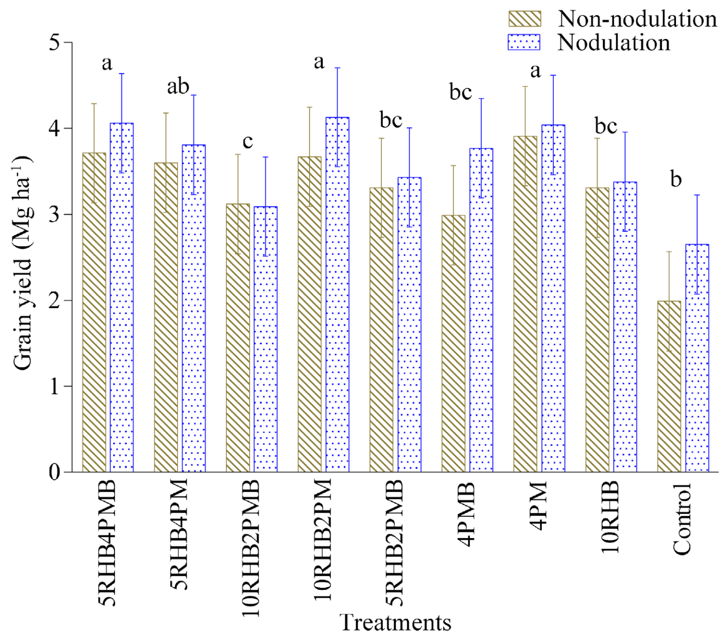

Figure 6. Grain yield of soybean as influenced by the treatments in semi-deciduous forest agro-ecology. Error bars represent standard deviation of the mean. Bars with the same letters are not significantly different ($p < 0.05$).

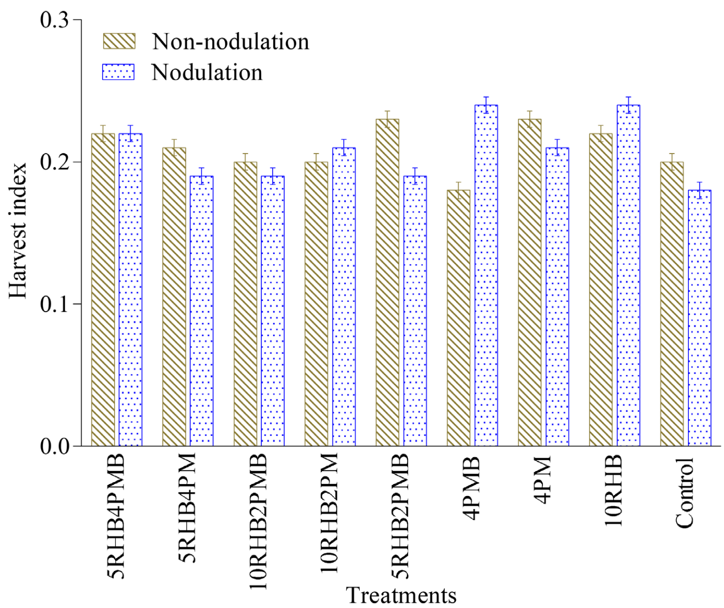

Figure 7. Harvest index of soybean as influenced by the treatments in semi-deciduous forest agro-ecology. Error bars represent standard deviation of the mean. Harvest index is not significantly different ($p < 0.05$).

application of PM, RHB and PMB on the grain yield of inoculated or non-inoculated soybean seeds.

The highest 100 seed weight was recorded under 4 Mg·ha^{-1} PMB (11.7 g) application while 10 RHB and 10 RHB + 2PMB gave the lowest weight. The highest grain yield was recorded for 4 Mg·ha^{-1} PM (3.98 Mg·ha^{-1}) followed by 10RHB +

2PM > 5RHB + 4PMB > 5RHB + 4PM > 4PMB > 5RHB + 2PMB > 10RHB > 10RHB + 2PMB while the lowest 100 seed weight (2.32 $Mg{\cdot}ha^{-1}$) was recorded for control. The highest HI was recorded for 10 $Mg{\cdot}ha^{-1}$ RHB (0.24) as compared to 5 $Mg{\cdot}ha^{-1}$ RHB + 4 $Mg{\cdot}ha^{-1}$ PMB and 5 $Mg{\cdot}ha^{-1}$ RHB + 2 $Mg{\cdot}ha^{-1}$ PMB while the lowest HI (0.19) was recorded for control.

The significant increase in soybean grain yield came as a result of soil treatments in the semi-deciduous agro-ecology due to better soil pH, soil texture, and exchangeable cation along with greater water availability. Inoculation of seeds with *B. japonicum* increased nodulation and grain yield because more atmospheric nitrogen is converted into plant-available ammonium required for protein synthesis. Nitrogen could be a limiting nutrient that affects the seed weight and grain filling because soybean require a lot of nitrogen although it has capacity to fix enough nitrogen with adequate population of biological nitrogen fixing rhizobia bacteria. Addition of 4 Mg PM has greatest grain yield compared to 10 Mg RHB and 4 Mg PMB because pH, P and K increase in soil are greatest. Amendment of 4 Mg PM constituted the highest soil pH, P and K (**Table 3**) which tend to high higher grain yield increase compared to 5RHB + 2PMB with 5RHB + 4PMB and 5RHB + 2PMB with 10RHB + 2PMB.

Soybean plants and symbiotic bacteria require essential nutrients [112] [113]. Out of the three primary nutrients NPK, nitrogen was supplied from both soil and symbiotic legume-bacteria in this field trial. **Table S3** shows the positive impact on grain yield of soybean following application of 4 Mg PM and *B. japonicum* inoculation in the semi-deciduous agro-ecological zone (**Table S3**). The significant increase in 100 seed weight of soybean could be attributed to the nutrient(s) and soil pH derived from biochar and *B. japonicum* inoculation in the in a ferric acrisol of semi-deciduous agro-ecological zone.

The significant increase in grain yield relative to the control agrees with the findings of [114] [115] [116] who reported that combined application of spruce chip biochar with 10 to 30 $Mg{\cdot}ha^{-1}$ rates and inorganic fertilizers increased the yield of soybean due to increased nutrients supplied to plants but not significant. Husk and Major (2011) also reported increased yield of soybean (20%) in biochar amended plot in the first year. Furthermore, [117] reported an increase in growth and yield of soybean with biochar relative to the control (no biochar). Generally, biochar amendments to the soil resulted in improved yield although the results were inconsistent. The possible cause of this inconsistent result in yield could be a result of variation in soil and types of biochar [118] as shown in **Table 3** and **Table 4**.

The significant increase grain yield was observed in the interaction of biochar and location of the experiment. [119], reported variations in the crop yield along geographical locations. [120] also reported that biochar can be more helpful on soils in low-nutrients and acidic relative to fertile soils. Acidic clayey soil with high CEC has high buffering capacity to change following fertilizer application [121].

The HI of soybean ranged from 0.18 to 0.24 as shown in **Figure 7**. The HI did not change significantly in response to single and combined applications of PM, RHB and PMB as well as seed inoculation with *B. japonicum.* A similar HI range was reported by [122] at Bembereke (Sudanian zone), who obtained a soybean HI for the first growing season ranging between 0.18 (obtained with treatments $N_0P_{60}K_{20}Mg_{20}Zn_5$ or $N_{20}P_0K_{20}Mg_{20}Zn_0$) and 0.39 ($N_{40}P_{30}K_{20}Mg_{20}Zn_{10}$). The results show the importance of providing adequate amounts of macro- and micronutrients. The authors concluded that the balance between fertilizer application and plant nutrient demand is essential for ensuring agricultural production because it is effective to prevent nutrient deficiency and excess, especially for soybean.

[123] [124] reported similar result found a positive correlation between the nodulation with biochar and NPK fertilizer applications. They suggested that the synergistic increase in yield was due to a decrease in soil pH caused by biochar and NPK fertilizer applications thereby increasing P availability in this alkaline soil. Application of starter N can increase nodulation, BNF and yield of common bean [125]. Nevertheless, a positive effect of biochar was observed on plant growth in both soybean cultivars, which verified our hypothesis. The positive effect of rice husk-derived biochar on yield was also found in rice [126] and maize [127]. [128] studies reported increases in crop yield due to increased P uptake after the application of biochar. Furthermore, higher yield always resulted from high plant biomass induced by biochar application.

3.3. Response of Soybean Nodule Number and Nodule Dry Weight to Seed Inoculation, Manure and Biochar Application

The response of nodule number and nodule dry weight were examined at 50% flowering of the inoculated and non-inoculated seeds grown in plots amended with single and combined applications of PM, RHB and PMB (**Figure 8** and **Figure 9**).

The results generally show a significant ($p < 0.05$) increase in nodule number and nodule dry weight of inoculated soybean following single and combined application of PM, RHB and PMB. However, no significant effects were observed for the non-inoculated plots.

The results show that 4PM gave the highest nodule number (46 nodules per plant) and the least was recorded for 5RHB + 4PMB (22 nodule per plant) (**Figure 8**).

The effect of biochar and *B. japonicum* inoculation on nodule number of soybean is shown in **Figure 8**. The nodule number was used as an indicator to the amount of biological nitrogen fixed.

Addition of 4 Mg PM achieved the greatest number of nodules and grain yield compared to 10 Mg RHB and 4 Mg PMB because pH, phosphorus and potassium increase in soil were greatest. Phosphorus is more bioavailable in PMB than RHB partially because the pyrolysis temperature used for PMB was lower than RHB. When combined with PM, RHB may temporarily lock up nitrogen

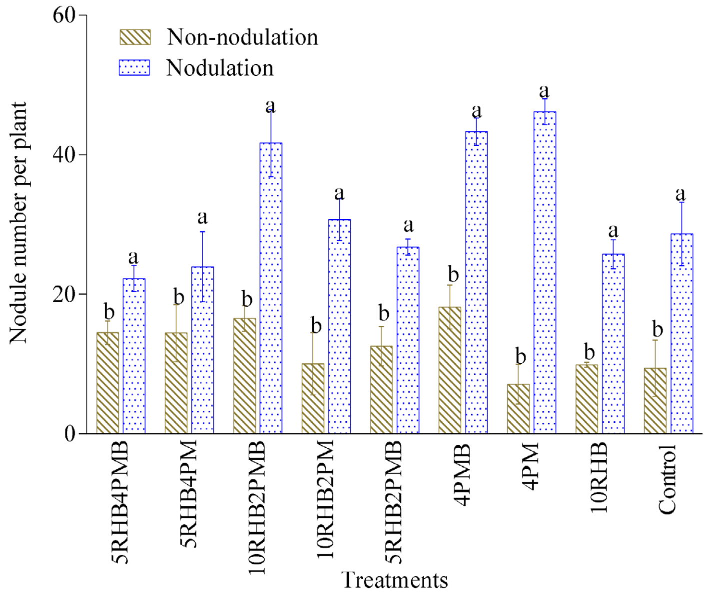

Figure 8. Nodule number per plant of soybean as influenced by the treatments in semi-deciduous forest agro-ecology. Error bars represent standard deviation of the mean. Bars with the same letters are not significantly different (p < 0.05).

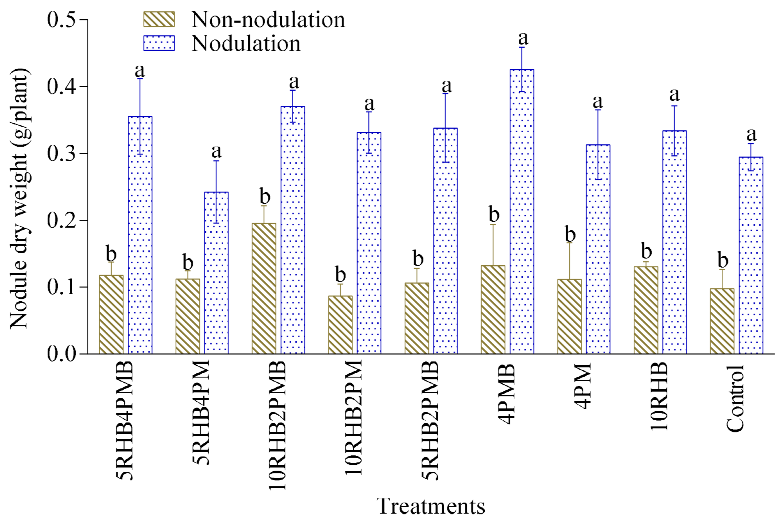

Figure 9. Nodule dry weight per plant of soybean as influenced by the treatments in semi-deciduous forest agro-ecology. Error bars represent standard deviation of the mean. Bars with the same letters are not significantly different ($p < 0.05$).

and phosphorus released from PM, which arguably affected *B. japonicum* growth (compare 4PM with 4PM + 5RHB in terms of root biomass and number of nodules). There was variation in nodule number when RHB and PM was applied but it was not significant (p > 0.05). The variation may be attributed to increased soil pH and organic matter. [129] reported an increase of about 61.5% in nodulation from soybean and cowpea following combined application of rock phosphate and *Bradyrhizobium* spp. inoculum on two benchmark soils in Northern Ghana. The positive increase in nodule number of soybean plants also agrees with the results of [130] and [131] who reported that *B. japonicum* inoculation significantly increased the number of effective nodules of soybean plant grown in predominantly sandy soil classified as Entisols. The authors suggested that their positive findings was a result of higher number of rhizobia introduced into soil that induced symbiotic relationship with the roots of the leguminous that result to fixing nitrogen.

Moreover, application of *B. japonicum* alone significantly increased the nodule number of inoculated soybean by about 96.6% compared to non-inoculated seeds ($p < 0.05$) (**Figure 8**). This indicates that the soil of the experimental site had a low population of compatible rhizobia, which is also supported by the fact that soybeans have not previously been planted in the location. [132] reported that 43 and 79% of the soils in West and East Africa countries soils have less than 10 *Bradyrhizobia* cells·g^{-1} soil. A similar figure was reported by [133] for soils in the northern and western regions of Ghana. [134], on the other hand, reported that native cowpea *Bradyrhizobia* population in Ghanaian soils ranges 0.6×10^1 to 31.0×10^3 cells·g^{-1} soil. In most cases, however, the concentration of indigenous soil *Bradyrhizobia* spp. is still orders of magnitudes lower than the typical concentration on inoculated soybean seeds, ranging from 10^4 to 10^6 cells per gram of soybean seed.

Slow changes in soil pH due to biochar application could also result in slight improvements in the number of nodules on the field. [135] observed an increase in nodule number and nitrogen fixation to nutritive solution adjusted pH, but at pH 7.0 nitrogenase activity dropped drastically, suggesting that optimum pH for *B. japonicum* growth falls between pH 6 and 7. Nodulation failures in acid soil conditions is predominant, particularly in soils of pH < 5.0 [136]. *B. japonicum* is more tolerant at low pH 4.0 - 4.5 than the fast-growing nodule bacteria [137]. However, [138] reported increase in nodulation following combined application of biochar and NPK fertilizer in an alkaline soil. In summary, our results suggest that application of alkaline biochars as well as use of *B. japonicum* inoculated seeds in soils without history of soybean cultivation can be expected to improve the number of nodules.

Results of nodule dry weight showed that 10RHB + 2PMB gave the highest weight (0.19 g·plant^{-1}) and the least was recorded for 10RHB + 2PM (0.08 g·plant^{-1}) (**Figure 9**). **Figure 9** shows that *B. japonicum* inoculation generally increased nodule dry weight of soybean plant in the semi-deciduous agro-ecological zone. A positive increase in nodule dry weight of soybean in response to *B. japonicum* inoculation was also reported by [139]. [140] reported that rhizobia inoculation positively increased nodule dry weight of soybean over control. [141] also found a two to three-fold increase in nodule dry weight and nodule number compared to the control when *B. japonicum* inoculant was applied to soybean. [142] reported that integrated application of *B. japonicum* inoculant and starter nitrogen fertilizer positively enhanced both yield and nodulation of common bean varieties. Similar to the findings of this study, dry weight of nodule and number of nodules of soybean increased under carbonized chicken manure application as reported by [143] [144].

4. Conclusions

Results obtained from the ferric acrisol in the semi-deciduous agro-ecological zone suggest a need to increase soil pH and organic matter for better yield and nodulation of legumes. A single application of RHB, or combined with PM or PMB, can increase the soil organic carbon from 1.81 in the control to 1.97% to 2.15% in the treated soil.

Addition of 4 Mg PM·ha^{-1} as well as 5 Mg RHB·ha^{-1} + 4 Mg PMB·ha^{-1} significantly improved shoot and root dry weight arguably due to improved nutrient access and soil texture, respectively. Soybean production can be increased by applying PM directly or nutrient-rich biochar derived from PM without the help of *B. japonicum* or N. This supports the alternate hypothesis of this study that nutrient-enriched biochar has a higher potential for fertilization than RHB or non-biochar. Application of *B. japonicum*-coated soybean seeds significantly increased nodule number and nodule dry weight in a ferric acrisol of the semi-deciduous agro-ecological zone.

Available phosphorous and potassium found to be correlated with root, shoot and total plant dry weight, while NPK in soil appear to be correlated to grain ($R^2 > 0.7$).

This work demonstrates the possibility of using PM as an alternative to or supplement of synthetic inorganic fertilizer for cultivation of soybeans. PMB produced at 350˚C should be used instead of PM to reduce odour and weight without losing nutrients. PMB can also correct nutrient deficiencies in RHB.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Nomenclatures Section

Nomenclature	Referred to
-	Subtraction
%	Percentage
+	Addition
<	Less than
=	Equal to
>	Greater than
A	Area
C	Carbon
Ca	Calcium
CEC	Cation exchangeable capacity
cm	Centimetre
$cmol(+) \cdot kg^{-1}$	centimoles per kilogram
D	Depth
dS/m	deciSiemens per metre
EC	Electric conductivity
$g \cdot plant^{-1}$	gram per plant
g	gram
HCl	Hydrogen chloride
K	Potassium
KCl	Potassium chloride
$Kg \cdot ha^{-1}$	Kilogram per hectare
Kg	Kilogram
$kg_{DW} \cdot ha^{-1}$	kilogram dry weight per hectare
m^2	Meter square
M_{ash}	Mass of ash (g) and
$M_{biochar}$	Dry mass of biochar (g)
$Mg \cdot ha^{-1}$	Megagram per hectare
$mg \cdot kg^{-1}$	Milligram per kilogram
Mg	Magnesium
mm	Millimetre
NV	Nutrient value in the soil
N	Nitrogen
Na	Sodium
NH_4F	Ammonium fluoride
C	Degree Centigrade
OM	Organic matter
P	Phosphorous
p	Probability
pH	Potential of hydrogen
TV	Treatment value

List of Acronyms

Abbreviation	Meaning
AC	Ash content
AR	Application rate
ANOVA	Analysis of variance
BD	Bulk density of soil
BS	Base saturation
DMRT	Duncan Multiple Range Test
HI	Harvest index
PM	Poultry Manure
PMB	Poultry Manure Biochar
RHB	Rice Husk Biochar
SOC	Soil organic carbon

Supplementary Information

Table S1. Properties of orthic and ferric acrisols according to Harmonised World Soil Map version 1.21.

Soil Unit Symbol (FAO 74)	Af
Soil Unit Name (FAO74)	Ferric Acrisols
Topsoil Texture	Medium
Reference Soil Depth (cm)	100
PHASE1	-
PHASE2	-
Obstacles to Roots (ESDB) (cm)	-
Impermeable Layer (ESDB) (cm)	-
Soil Water Regime (ESDB)	-
Drainage class (0% - 0.5% slope)	Moderately Well
AWC (mm)	150
Gelic Properties	No
Vertic Properties	No
Petric Properties	No
Topsoil (0 - 30 cm)	
Topsoil Sand Fraction (%)	53
Topsoil Silt Fraction (%)	22
Topsoil Clay Fraction (%)	25

Continued

Topsoil USDA Texture Classification	sandy clay loam
Topsoil Reference Bulk Density (kg/dm^3)	1.4
Topsoil Bulk Density (kg/dm^3)	1.4
Topsoil Gravel Content (%)	23
Topsoil Organic Carbon (% weight)	0.98
Topsoil pH (H_2O)	5.1
Topsoil CEC (clay) (cmol/kg)	14
Topsoil CEC (soil) (cmol/kg)	6
Topsoil Base Saturation (%)	49
Topsoil TEB (cmol/kg)	2.7
Topsoil Calcium Carbonate (% weight)	0
Topsoil Gypsum (% weight)	0
Topsoil Sodicity (ESP) (%)	2
Topsoil Salinity (ECe) (dS/m)	0.1
Subsoil (30 - 100 cm)	
Subsoil Sand Fraction (%)	41
Subsoil Silt Fraction (%)	20
Subsoil Clay Fraction (%)	39
Subsoil USDA Texture Classification	clay loam
Subsoil Reference Bulk Density (kg/dm^3)	1.31
Subsoil Bulk Density (kg/dm^3)	1.4
Subsoil Gravel Content (%)	19
Subsoil Organic Carbon (% weight)	0.39
Subsoil pH (H_2O)	5
Subsoil CEC (clay) (cmol/kg)	12
Subsoil CEC (soil) (cmol/kg)	6
Subsoil Base Saturation (%)	33
Subsoil TEB (cmol/kg)	1.3
Subsoil Calcium Carbonate (% weight)	0
Subsoil Gypsum (% weight)	0
Subsoil Sodicity (ESP) (%)	2
Subsoil Salinity (ECe) (dS/m)	0.1

Table S2. Root, shoot and total plant dry weight of soybean in a ferric acrisol of semi-deciduous agro-ecological zone. p values indicate a significant effect at p = 0.05 level.

Treatment	Root dry weight ($g \cdot plant^{-1}$)			Shoot dry weight ($g \cdot plant^{-1}$)			Total plant dry weight ($g \cdot plant^{-1}$)		
	+	−	Mean	+	−	Mean	+	−	Mean
5RHB + 4PMB	2.21	2.16	2.19ab	13.35	11.17	14.88ab	15.14	13.08	17.98ab
5RHB + 4PM	1.77	2.39	2.08bc	14.32	10.71	15.90ab	16.08	12.42	17.98ab
10RHB + 2PMB	1.89	1.96	1.93bc	13.08	9.48	11.28b	14.97	11.45	13.21b
10RHB + 2PM	1.76	1.72	1.74bc	16.29	18.23	12.51b	19.1	20.77	14.25b
5RHB + 2PMB	1.93	1.54	1.74bc	14.27	11.49	11.57b	16.02	13.71	13.21b
4PMB	1.75	2.22	1.99bc	12.19	10.95	12.88ab	14.12	12.49	14.86b
4PM	2.8	2.54	2.67a	15.47	16.33	17.26a	17.24	18.72	19.93a
10RHB	1.78	1.91	1.85bc	16.8	12.97	12.26b	19.01	15.13	14.11b
Control	1.62	1.34	1.40c	16.24	6.98	11.61b	17.86	8.32	13.09b
p-value									
B	0.87			0.03			0.04		
T	0.01			0.04			0.02		
T*B	0.62			0.28			0.32		

B = *B. japonicum*; + = inoculated; − = not inoculated T = Poultry manure (PM), poultry manure biochar (PMB) and rice husk biochar (RHB).

Table S3. Hundred seed weight, grain yields and harvest index of soybean in a ferric acrisol of semi-deciduous agro-ecological zone. p-values indicate a significant effect at p = 0.05.

Treatment	100 seed weight (g)			Grain yield ($Mg \cdot ha^{-1}$)			Harvest index		
	+	−	Mean	+	−	Mean	+	−	Mean
5RHB + 4PMB	11.11	11.45	11.3	4.06	3.72	3.89a	0.22	0.22	0.22
5RHB + 4PM	11.70	11.12	11.4	3.81	3.60	3.70ab	0.19	0.21	0.20
10RHB + 2PMB	10.97	11.01	11.0	3.09	3.12	3.11c	0.19	0.20	0.20
10RHB + 2PM	11.76	10.90	11.3	4.13	3.67	3.90a	0.21	0.20	0.21
5RHB + 2PMB	11.39	11.09	11.2	3.43	3.31	3.37bc	0.19	0.23	0.21
4PMB	12.35	11.03	11.7	3.77	2.99	3.38bc	0.24	0.18	0.22
4PM	11.32	11.69	11.5	4.04	3.91	3.98a	0.21	0.23	0.22
10RHB	11.31	10.67	11.0	3.38	3.31	3.35bc	0.24	0.22	0.24
Control	11.07	11.22	11.2	2.65	1.99	2.32b	0.18	0.20	0.19
p-value									
B	0.29			0.02			0.87		
T	0.28			<0.01			0.24		
T*B	0.07			0.26			0.15		

B = *B. japonicum*; + = inoculated; − = not inoculated.

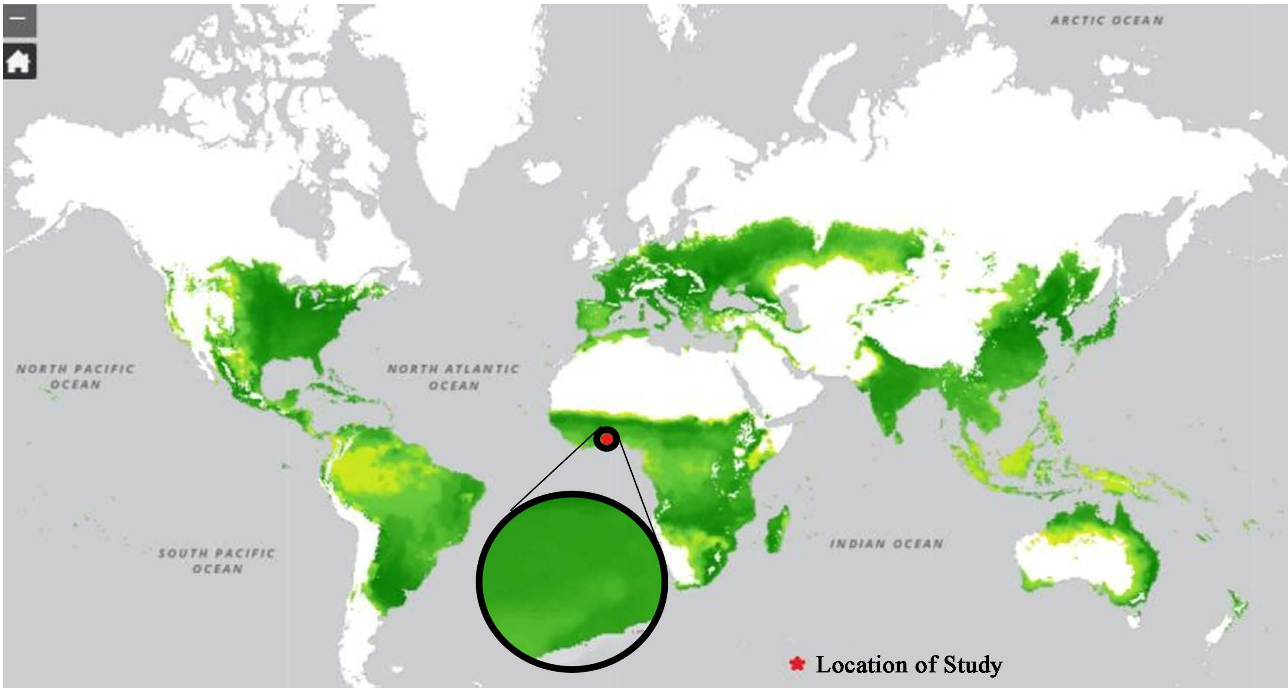

Figure S1. Historical agro-climatic potential yield with regard to temperature, radiation and moisture regimes for soybean between 1981-2010. The displayed potential yield was computed based on an available water content of 200 mm/m under rainfed conditions for subsistence-based farming system (input level "low") (Source: https://gaez-data-portal-hqfao.hub.arcgis.com/).

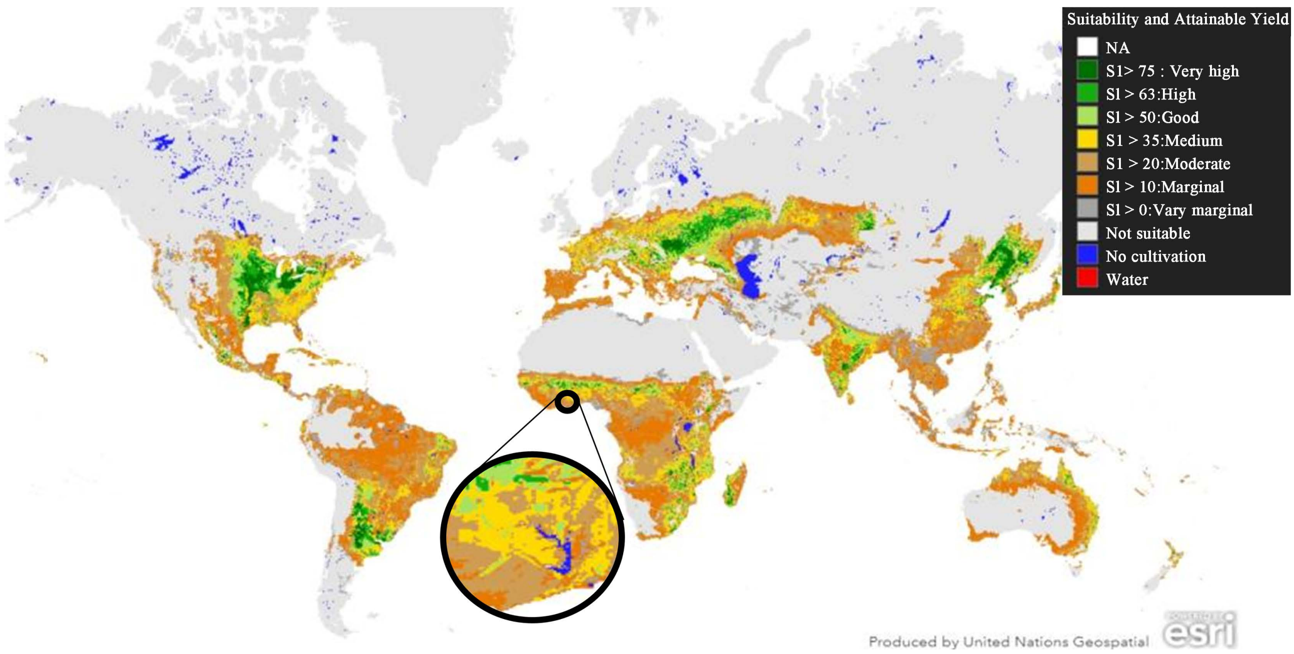

Figure S2. Historical suitability index for soybean between 1981-2010. The suitability index describes the relationship between yield outcomes under optimal growing conditions for a land utilisation type and the yield obtainable under prevailing growing conditions in a particular location. The displayed suitability index was computed based on rainfed conditions for subsistence-based farming system (input level "low") (Source: https://gaez-data-portal-hqfao.hub.arcgis.com/).

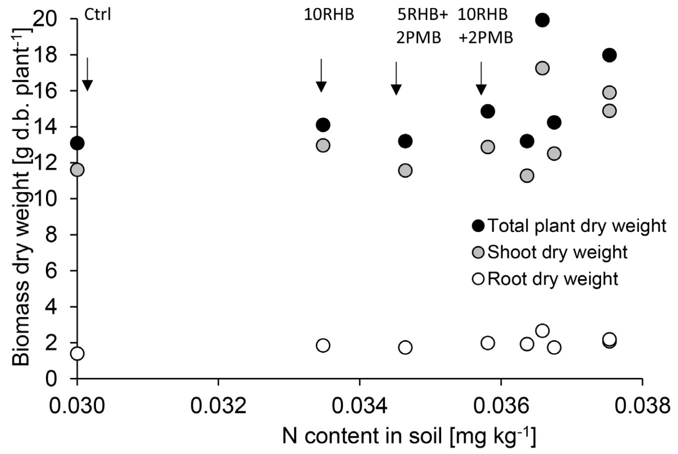

Figure S3. Correlation between nitrogen content in soil and dry weight of roots, shoots and total plants.

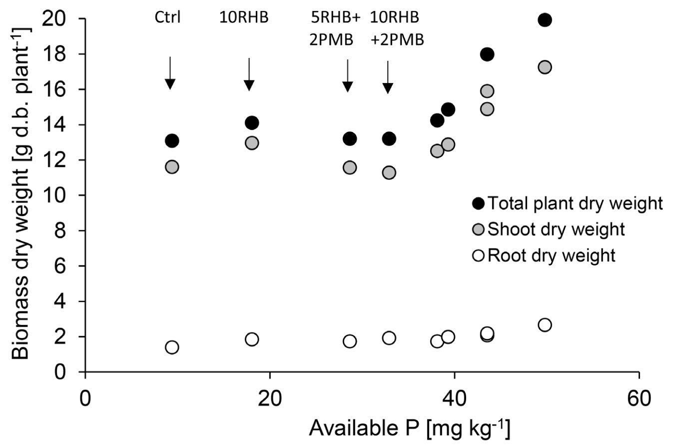

Figure S4. Correlation between available phosphorus content in soil and dry weight of roots, shoots and total plants.

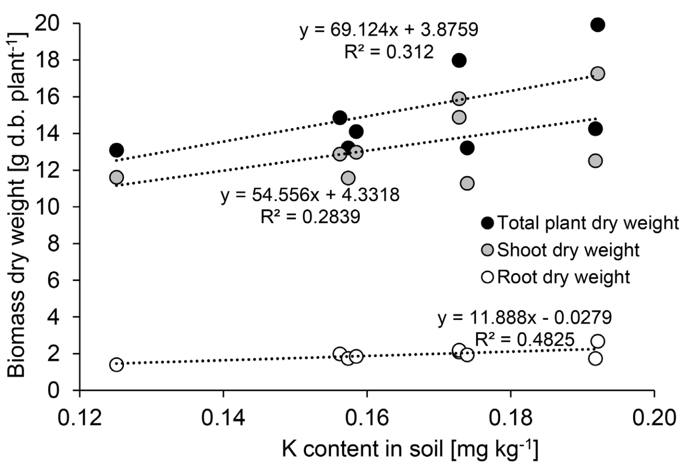

Figure S5. Correlation between potassium content in soil and dry weight of roots, shoots and total plants.

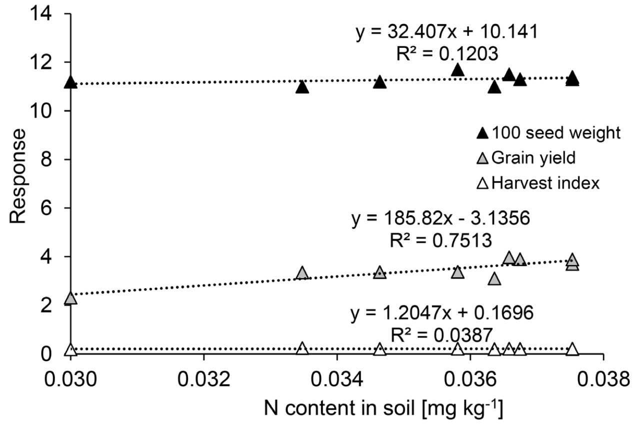

Figure S6. Correlation between nitrogen content in soil and 100 seed weight, grain yield and harvest index of soybean.

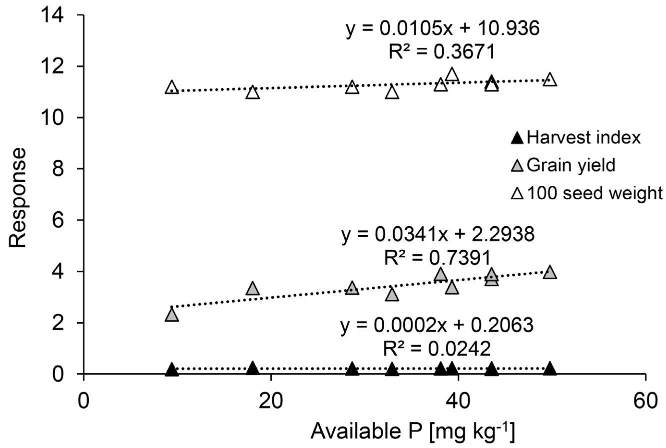

Figure S7. Correlation between available phosphorus content in soil and 100 seed weight, grain yield and harvest index of soybean.

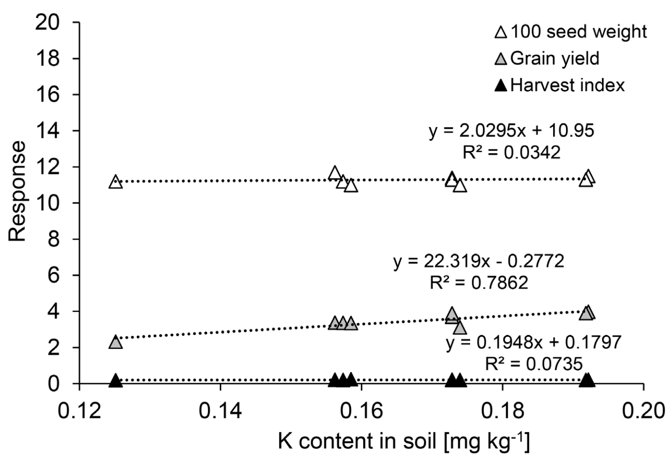

Figure S8. Correlation between potassium content in soil and 100 seed weight, grain yield and harvest index of soybean.