



Soil amendments affect soil chemical properties and soybean yield in the semi-deciduous forest zone of Ghana

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Abstract: Low soil pH can limit soybean growth in the semi-deciduous forest zone of Ghana, where soybean is a potential food security crop. An experiment was set up in 2021 to evaluate the combined effects of soil amendments, phosphorus (P), and rhizobium inoculation on soybean growth and soil chemical properties. To evaluate the interactive effects of soil amendments, phosphorus (P), and rhizobium inoculation on soybean growth and soil chemical properties, an experiment was conducted in 2021.

The treatments were set up in a split-split plot design, with four replications. The main plots were soil amendments (2 tons/ha lime and 5 tons/ha rice husk biochar), the subplots were 0 and 20 kg P ha⁻¹, and the sub-subplots were inoculated with or without rhizobium. The joint application of soil amendment, P, and rhizobium inoculation did not increase the nodule number, shoot biomass, harvest index, haulm weight, 100-seed weight, rainwater use efficiency, and grain yield. However, P alone increased the grain yield of soybean and rainwater use efficiency. Soil amendments, P, and rhizobium inoculation increased the total N, organic C, Ca, and Mg contents in the soil. Soybean nodulation and yield were not significantly affected by the three factors studied. However, it influences the chemical properties of the soil. The application of P alone has the potential to increase soybean growth and yield in the semi-deciduous forest zone of Ghana.

Keywords: rhizobium inoculation, Ghana, soybean nodulation, soil amendment

1. Introduction

Soybean [*Glycine max* (L.) Merrill] is an important yearly leguminous crop in the family Fabaceae [24]. A classification by the United Nations Food and Agriculture Organization groups soybean as an oil seed rather than a pulse [45]. Global soybean production in 2019 was 350 million tons. Sub-Saharan Africa (SSA) accounted for 3.1 million tons of the global production in 2019. One-third of the 3.1 million tons was produced in West Africa [18]. In the past ten years, especially between 2008 and 2018, soybean production in Ghana increased from 74,800 MT to 176,670 MT [32]. Soybean is an important component of the mainly cereal-based systems of farming in the Guinea savannah agroecological zone of Ghana. It is a vital element of food-based oil and poultry industry. Soybean offers livelihood to smallholder farmers via income generated through the sale of the products and offers nutritional security, particularly among underprivileged households [44]. Soybean can be processed into different food recipes that offer high-quality protein to combat malnutrition. It contains all the important amino acids [17], which is why it has been recommended in Ghana for commercial production and utilization as meat alternative [17].

The five northern regions and the northern Volta are responsible for the production of nearly 90% of soybean in Ghana [32]. Recently, soybean production has expanded to the semi-deciduous agroecological zone of Ghana, where the crop could benefit from double cropping due to the bimodal rainfall pattern and could serve as a food security crop [3]. In this part of Ghana, however, soybean production is hindered by low soil pH, which reduces nodulation due to p-fixation [3]. With the application of P fertilizer and rhizobium inoculation, Adjei-Nsiah et al. [1] observed soybean yields as high as 2.4 t ha⁻¹ in the semi-deciduous forest agroecological zone. These yields are equivalent to those obtained in the Guinea savannah agroecological zone under comparable management approaches. Since soybeans thrive in the pH range of slightly acidic to neutral, it is expected that the yield could be even higher if the pH is raised. Acidic soils reduce microbial activity, P availability, and biological nitrogen fixation, all of which have an impact on soybean growth and yield [11]. The addition of rice husk biochar and agricultural lime as soil amendments can increase the pH of acidic soils. Aluminium and manganese toxicity are decreased by agricultural lime, which enhances the plant root system and promotes Ca, Mg, Mo, and P uptake [7]. There is plenty of farm and organic waste, such as rice husks, cocoa pod husks, and corncobs, which can be burnt without oxygen to produce biochar. However, soybean farmers have paid little attention to these feedstocks. By increasing the soil's organic matter content, biochar improves the soil's bulk density, ion exchange capacity, plant nutrient retention, water-holding capacity, and soil structure [19]. Rice husks and cocoa pod husks burnt into biochar at 450 °C had the respective accessible phosphorus

concentration of 531 mg/kg and 3,897 mg/kg [43]. Consequently, biochar can be used as a liming material with the added benefit of being a P source. Although several studies have shown the beneficial effects of P-fertilizer application and rhizobium inoculation on the yield of soybean, there is paucity of information on the combined effects of liming, P-fertilizer application, and rhizobium inoculation in the semi-deciduous forest agroecological zone of Ghana.

Reducing soil acidity to a point where the crop can generate its potential is the first step in enhancing soybean yields in the semi-deciduous forest zone. This is followed by applying P fertilizer to maintain and increase soil fertility and by the introduction of an external rhizobia population through inoculation. Phosphorus, a key nutrient for nitrogen fixation, is often limited in the Ghanaian soil. Nitrogen fixation in grain legumes is improved by seed inoculation with rhizobium strains. Inputs such as biochar, P fertilizer, in addition to Bradyrhizobium inoculants, improve soil microbial activity, biological nitrogen fixation, and the chemical characteristics of acidic soils. This study aimed to evaluate the combined effects of lime (CaCO_3) and rice husk biochar as soil amendments, phosphorus fertilizer, and Bradyrhizobium inoculants on soil chemical properties, soybean nodulation, and yield in the semi-deciduous forest agroecological zone of Ghana. The study objective was based on the hypothesis that biochar and/or lime application reduces acidity, limits P-fixation, and increases soybean yield. By increasing soybean yield, smallholder farmers can obtain extra income and improve their livelihoods. Furthermore, improvements in soil chemical properties could have residual benefits for subsequent crops.

2. Materials and methods

Study site

The research was conducted at the Forest and Horticultural Crops Research Centre (FOHCREC), Kade (6°06'N, 0°49' W), in the Denkyembuor District of the Eastern Region of Ghana. FOHCREC is located in the semi-deciduous forest agroecological zone of Ghana, at an elevation of 135.9 meters above sea level. Rainfall in the region is bimodal, peaking in June and October, with a brief pause in August and a dry spell from November to March. The soil at the experimental site is Forest Ochrosols (Acrisols), which is deep and well-drained [5, 16]. The rainfall distribution at the experimental site is bimodal, averaging slightly above 1,200 mm per year (*Figure S1*). The area records a temperature between 25 and 38 °C [38].

Soils were sampled prior to experiment at 0–20 cm depth via an auger. The soil pH was determined according to a soil:water ratio of 1:2.5 [48]. The total nitrogen content was determined via the Kjeldahl digestion method [9]. Available phosphorus was determined via the Bray 1 method [8]. The amount of organic carbon in the soil

was measured via the wet combustion method [56]. The pH, total N, and organic carbon content of the biochar were measured via the above-mentioned methods.

Experimental layout

The experiment was set up in a split-split plot design arranged in a randomized complete block with four replications. The experimental plot measured 6 m × 3 m with a spacing of 50 cm × 10. The plots were separated by a 1 m alley, while the blocks were separated by a 2 m alley. Soybean seeds were sown at 3 per hill and later thinned to 2 per hill. The TGx 1448-2E soybean variety was used in this experiment. Jenguma has a potential yield of 2.5 t/ha, matures between 110 and 115 days, and is resistant to striga [39]. The treatments evaluated were 5 tons/ha biochar (rice husk biochar), 2 tons/ha lime (CaCO₃), two levels of phosphorus fertilizer (0 and 20 kg P ha⁻¹), Bradyrhizobium inoculation, and a control treatment (no inoculation). Eight weeks before planting, lime was applied using granular calcium carbonate (CaCO₃) (Omya Calciprill 110, Germany).

Application of lime was done 8 weeks prior to planting by drilling at a depth of 10 cm and covering with soil to avoid being blown away by wind. As an additional liming material for the study, biochar obtained from rice husk was used. The rice husk biochar was charred in a furnace at a pyrolysis temperature of 700 °C at the experimental site. The biochar was applied 2 weeks before planting in the drills at a depth of 10 cm. The drills were covered with soil to prevent water or wind erosion. Phosphorus was applied as triple superphosphate at 0 and 20 kg P/ha one week after planting. Phosphorus fertilizer was applied in furrows 10 cm away from the planting line. Nodumax inoculant was used in this experiment. It contained 10¹⁰ cells g⁻¹ of the Bradyrhizobium japonicum strain USDA 110. Nodumax is manufactured by the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Before adding the inoculant at a rate of 5 g per kilogram of seeds, the soybean seeds were soaked in a basin containing gum arabic solution. An even coating was obtained by thoroughly stirring and swirling the slurry. Early morning planting of the inoculated seeds prevented them from being exposed to direct sunlight, which might have lowered the inoculant's quality. The uninoculated seeds were planted first, but on the same day as the inoculated ones, to avoid contamination. To control the weeds, three manual hoe-weeding techniques were employed. One week following emergence, seedlings were treated with an emamectin benzoate solution. To suppress caterpillars and leaf miners, the seedlings were sprayed with 250 mL/15 L of the insecticide emamectin benzoate one week after they had emerged. Utilizing 36 g at a rate of 35 mL/15 L, cypermethrin was used to control grasshoppers. The plants were sprayed with an attack (permethrin (25 g/L) + pirimiphos-methyl (475 g/L)) once every week until they were 6 weeks old. The

field was left fallow for three years prior to maize and soybean cultivation. The experimental field was mechanically prepared with tractor and harrow.

Assessment of soybean growth

The assessment of nodulation and shoot biomass followed a similar pattern as previously reported by [2, 53]. At 50% flowering, ten plants per plot were cut at ground level and uprooted. The root zone was gently washed over a 2-mm mesh sieve under tap water to remove dirt and soil particles. Nodules were counted and then oven-dried at 65 °C for 48 hours along with the shoot to determine their dry weights via a standard electronic scale.

Yield assessment of soybean

Approximately 75% of seeds harden [31] and 95% of plants turn from green to golden yellow when they reach physiological maturity [49]. Plants were harvested at this stage from an area of 16 m² (8 rows of 4 m length) from a 30 m² plot. The pods were then detached from the plants, weighed, and shelled, and the grains were separated from the husks. The grains were oven-dried at 65 °C until constant weight was achieved. The moisture contents of the seeds were determined to be 13% via a Kvister moisture meter. One hundred seeds were counted from each plot after oven drying, and the weights were measured via a standard electronic scale. The total seed weight per plot was divided by the total biomass weight per plot to determine the harvest index of soybean [13]. The agronomic P-use efficiency, defined as the amount of P fertilizer used to produce one kilogram of soybean grain, was determined via the formula of [55]. Rainwater use efficiency was also determined via the formula of [46], which indicates the yield attained per millimetre of rainfall received during the planting season.

Final soil chemical analyses of the experimental plots

Soil samples from each of the 48 plots were taken along the planting line where the treatments were applied. The samples were air-dried and sieved for chemical properties such as total N, available P, pH, and organic carbon. The chemical properties were determined using standard protocols previously described under the section *Study Site*.

Statistical analysis

Analysis of variance (ANOVA) was performed using the GenStat software (12th edition) on data collected during the experiment at 10 weeks after planting

(WAP) for nodule number, one hundred seed weight, grain yield, haulm weight, shoot biomass at the beginning of podding, agronomic P-use efficiency, and rainwater use efficiency. The least significant difference (LSD) test was used to separate the means with a 5% probability. The data were checked for normality via the Shapiro–Wilk test before being subjected to analysis of variance.

3. Results

Initial physicochemical characteristics of the soil and rice husk biochar

The soil of the experimental site had a low level of organic matter, moderate levels of N and exchangeable Ca and Mg, and a strong acidic pH (*Table 1*) [22]. Compared to the soil (0.24%), the total nitrogen content of the rice husk biochar was significantly higher (1.27%). The soil's pH was 5.09, whereas that of the rice husk biochar was 6.85. The soil from the research location has more accessible P (10.59 mg kg⁻¹) than the rice husk biochar (0.09 mg kg⁻¹) (*Table 1*).

Table 1. Initial physical and chemical analysis of the soil and biochar

Soil property	Soil	Rice husk biochar (RHB)
pH (1:2.5 H ₂ O)	5.09	6.85
Total nitrogen (%)	0.24	1.27
Organic carbon (%)	1.32	48.14
Available P (mg kg ⁻¹ soil)	10.59	0.09
Sand (%)	36	
Silt (%)	53	
Clay (%)	11	
Exchangeable cations		
Calcium (Ca ⁺⁺) (cmol ₍₊₎ kg ⁻¹)	3.56	
Potassium (K ⁺) (cmol ₍₊₎ kg ⁻¹)	1.00	
Magnesium (Mg ⁺⁺) (cmol ₍₊₎ kg ⁻¹)	1.32	

Effects of rhizobium inoculation, soil amendments, and phosphorus on soil chemical properties 120 days after treatment

In general, the soil pH initially increased from 5.09 to 5.52 and 5.54 in the plots that received biochar and lime, respectively, seventeen weeks after treatment, although these differences were not significant ($p > 0.865$). However, the effects of inoculation on pH were significant ($p < 0.018$) (*Table 2*). The combined effects of

the soil amendments, P-fertilizer, and rhizobium inoculants significantly increased the total N, soil organic C, exchangeable Ca, and Mg contents in the soil (*Table 2*). The soil-exchangeable K increased only in the plots that received the rhizobium inoculant. Likewise, a significant pH was recorded only for plots that received the rhizobium inoculant.

Effects of rhizobium inoculation, soil amendments, and phosphorus fertilizer on the nodulation, growth, and yield of soybean

The rhizobium inoculant application considerably ($p < 0.001$) increased the number of nodules by 44% when compared to no inoculation (*Table 3*). Although the changes were not statistically significant ($p > 0.05$), the effects of lime and biochar increased the number of nodules by 41% and 62%, respectively, compared to the control. Compared with no P application, the P-fertilizer application alone increased the number of nodules by 44% (*Table 3*). None of the interactions were significant. In comparison to plants that did not receive phosphorus fertilizer, the dry shoot biomass of soybean plants increased by 28% when phosphorus was applied ($p < 0.05$) (*Table 3*).

Table 2. Response of soil chemical and physical properties to soil amendments

Treatment	pH	Total N (%)	OC (%)	Available P (mg kg ⁻¹)	Exchangeable cations (cmol (±) kg ⁻¹)		
					K	Ca	Mg
<i>Rhizobia (R)</i>							
+ RH	5.46	0.17	1.36	8.30	0.37	4.67	2.62
- RH	5.58	0.15	1.29	7.21	0.33	4.91	3.31
<i>Soil amendments (S)</i>							
Biochar	5.52	0.16	1.28	7.11	0.35	4.49	2.57
Lime	5.54	0.16	1.36	7.02	0.37	4.75	3.15
No amendment	5.51	0.17	1.34	9.12	0.34	5.13	3.18
<i>Phosphorus (P)</i>							
P+	5.53	0.16	1.28	6.4	0.36	4.69	2.86
P-	5.52	0.16	1.37	9.11	0.34	4.89	3.07
<i>P values (0.05)</i>							
R	0.018	< 0.001	0.003	0.423	0.012	0.196	< 0.001
S	0.865	0.002	0.019	0.359	0.327	0.019	0.004
P	0.73	0.959	< 0.001	0.05	0.529	0.28	0.184
R × S	0.233	0.008	0.019	0.135	0.386	< 0.001	0.003
R × P	0.097	0.012	0.041	0.42	0.523	0.288	0.191
S × P	0.335	< 0.001	0.064	0.275	0.066	< 0.001	< 0.001
R × S × P	0.843	< 0.001	0.041	0.454	0.22	< 0.001	< 0.001

The interaction between the rhizobium inoculant and P fertilizer had a significant effect on shoot biomass. The application of rhizobium did not significantly ($p > 0.427$) affect the weight of one hundred seeds from the soybean plants, even though the rhizobium-inoculated plots presented a higher mean weight of one hundred seeds (11.77 g) than did the non-inoculated plots (10.84 g) (*Table 3*). None of the treatments had significant effect on 100 seed weight. Although grain yield was 46% higher than that of the plants not receiving inoculants, the impact of rhizobium inoculation on grain yield was not statistically significant ($p > 0.05$) (*Table 3*).

Grain yield increased by about 100% and 14% in the lime- and biochar-amended plots, respectively, although the application of the soil amendments had no apparent impact on yield when compared to the control ($p > 0.444$).

However, phosphorus application significantly ($p < 0.013$) increased the grain yield. The increase in grain yield was 60% relative to that of the plants that did not receive phosphorus (*Table 3*). The harvest index of soybean was not influenced by the treatments. The interactions between the soil amendments and P fertilizer significantly increased the P-use efficiency of the soybean plants (*Table 3*). The application of P fertilizer alone resulted in a significant increase in rainwater use efficiency (*Table 3*). The plots that received P fertilizer had 60% greater rainwater use efficiency than the plots that did not receive P.

Table 3. Response of soybean to soil amendments

Treatment	Nodule number	Shoot biomass (kg ha ⁻¹)	One hundred seed weight (g)	Haulm weight (kg ha ⁻¹)	Harvest index	P-use efficiency (kg grain kg ⁻¹ P)	Rainwater use efficiency (kg ha ⁻¹ mm ⁻¹ rainfall)	Grain yield (kg ha ⁻¹)
<i>Rhizobia (R)</i>								
RH+	7.35	1353.0	11.77	991.0	1.40	2.97	0.15	138.0
RH-	1.03	1139.0	10.84	441.0	1.12	2.0	0.11	94.70
<i>Soil amendments (S)</i>								
Biochar	4.39	1341.0	11.59	742.0	1.30	4.20	0.14	122.70
Lime	5.05	1228.0	11.32	726.0	1.27	1.74	0.13	118.50
No amendment	3.12	1169.0	11.01	681.0	1.20	1.52	0.12	107.80
<i>Phosphorus (P)</i>								
P+	4.94	1398.0	11.95	845.0	1.39	1.39	0.16	143.30
P-	3.44	1095.0	10.66	588.0	1.13	1.13	0.10	89.40

Treatment	Nodule number	Shoot biomass (kg ha ⁻¹)	One hundred seed weight (g)	Haulm weight (kg ha ⁻¹)	Harvest index	P-use efficiency (kg grain kg ⁻¹ P)	Rainwater use efficiency (kg ha ⁻¹ mm ⁻¹ rainfall)	Grain yield (kg ha ⁻¹)
<i>P-values (0.05)</i>								
R	<0.001	0.43	0.61	0.09	0.22	0.69	0.18	0.15
S	0.20	0.74	0.88	0.88	0.84	0.043	0.44	0.45
P	0.15	0.02	0.20	0.018	0.27	0.37	0.013	0.012
R × S	0.42	0.49	0.66	0.64	0.12	0.61	0.14	0.12
R × P	0.35	0.03	0.47	0.73	0.85	0.23	0.26	0.30
S × P	0.59	0.13	0.68	0.67	0.27	0.044	0.85	0.90
R × S × P	0.77	0.14	0.27	0.87	0.98	0.98	0.78	0.52

3.1. Discussions

In addition to having low amounts of organic matter and moderate amounts of N, exchangeable Ca, and Mg, the soil at the experimental site was very acidic [21]. This study's investigation of rice husk biochar and lime as soil amendments is justified by the low pH of 5.09, which may be caused by the intrinsic parent material and/or leaching. Soil pH is essential for nutrient absorption and microbial activity [27]. Low soil pH limits rhizobium growth, root infection, and the overall symbiosis, which in turn affect soybean yield [33]. The organic carbon content of the biochar was high, consistent with the findings by [57], who reported that high heating rates of biochar shortens the time for de-hydration reactions, resulting in high carbon yield. Due to the release of inorganic orthophosphate ions, which combined with the released Ca to form Ca-P compounds and raise the overall P content of the biochar, the accessible P in the rice husk biochar was equally high [26]. Charring biomass can increase the availability of P from plant tissue by cleaving organic phosphorus bonds and forming soluble P ions, according to Shenbagavalli and Mahimairaja [47]. Due to the high intrinsic P content in the feedstock, rice husk biochar has a high total P content. Additionally, the exchangeable calcium and magnesium levels were considerably raised by the soil amendments. The amount of nitrogen from the applied biochar may have contributed to the rise in total nitrogen seen in this study. According to Cross and Sohi [11], biochar mineralization takes place in the soil and causes the release of labile substances like nitrogen. Although plant-based biochar makes up the majority of the total nitrogen pool, it has been demonstrated to include smaller levels of mineral nitrogen [12].

pH is an important soil characteristic and a major determinant of soil fertility. Acidity and alkalinity are associated with the physical and chemical elements of the soil–plant system [29, 35]. According to a prior study by [35], the geochemical

cycle of soil nutrients was impacted by an increase in soil acidity because it accelerated the depletion of essential elements like K, Ca, and Mg and reduced the cation exchange capacity. The phosphorus fertilizer that was administered had a considerable impact on the soil's available P, which was tested 120 days after the treatment had been applied. The increase in the amount of P accessible in the soil was caused by the application of P fertilizer. Compared to several studies that documented higher soybean yield responses to the combined application of inoculant and P fertilizer, the overall grain yield of this study was extremely low. As shown in *Figure S1*, the irregular rainfall pattern during the crop's flowering and podding stages may have contributed to the low yield reported in this study. Low rainfall can result in lower grain yields of up to 74.05% and a 58% decrease in the number of pods, according to other reports [15]. Despite the overall low production, the study's findings demonstrated that soybeans responded to P fertilizer, as grain yield improved by 60% in plots that had P fertilizer. Several authors have reported the response of grain legumes to P fertilization. Numerous processes in legumes, including energy transfer, nodulation, atmospheric nitrogen fixation, flower initiation, fruit development, and seed formation, are known to be significantly impacted by P fertilizer [36, 37]. Adding lime, biochar, or P fertilizer to inoculated seedlings is anticipated to boost soybean grain output. The application of lime or biochar, however, had no effect on the soybean yield. This might be the case since the first year of production did not allow the treatment benefits of soil amendments to be fully realized. Rainfall, or water, is a significant environmental factor that affects plant growth. It is well recognized that a significant barrier to agricultural productivity is moisture stress brought on by unpredictable rainfall patterns [34]. Hatfield and Prueger [20] found that soybean biomass and grain yield declined when there was a water deficit. According to a different study, reducing soybean output during the reproductive cycle due to water stress accelerated leaf senescence and decreased the time needed for seed filling [14]. The main limiting factor for the low yield was moisture stress. Nonetheless, [52] indicated that rhizobia can survive up to a year in Ghanaian soil with the host plant. Giving the residual effect of the lime and biochar, favourable environmental conditions, such as adequate moisture, are likely to increase soybean grain yield in subsequent seasons.

4. Conclusions

The purpose of this study was to test the hypothesis that in Ghana's semi-deciduous forest agroecological zone the combined effects of phosphorus fertilizer application, rhizobium inoculation, and soil amendments enhance soybean nodulation, growth, yield, and soil physical and chemical properties. The results, however, indicate that there is no evidence that the combination of

these three factors increased the soybean grain yield in the research region under the given experimental conditions. Therefore, combining these three factors may not increase soybean yield under similar environmental conditions. However, the combination of lime and rice husk biochar increased the pH and improved the total N, organic carbon, and exchangeable calcium and magnesium contents of the soil. Additionally, the study found that the sole application of phosphorus at a rate of 20 kg ha⁻¹ enhanced soybean grain yield when compared to the control. For soybean grain yield increase in acidic soil, farmers in earlier studies and those with similar environmental conditions are advised to use phosphorus fertilizer at a rate of 20 kg P/ha.

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Supplementary Figure

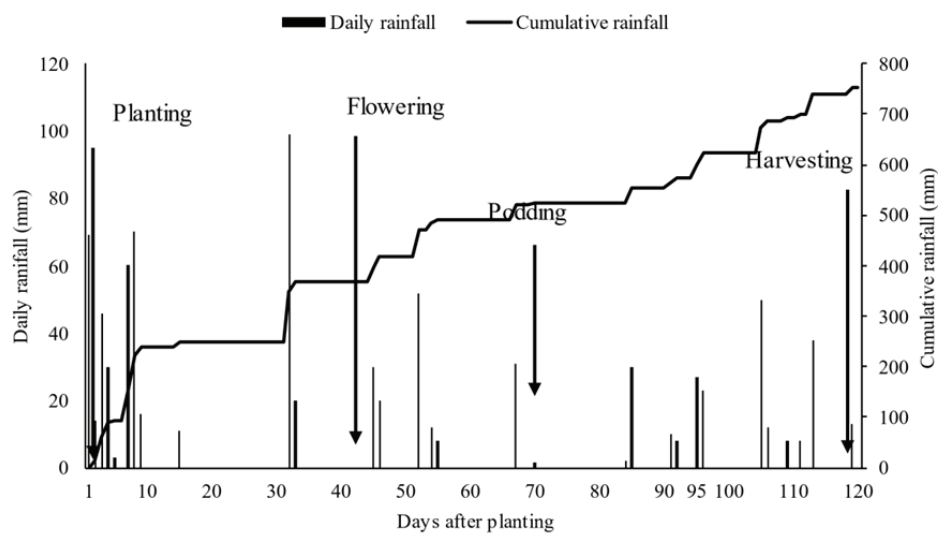


Figure S1. Daily and cumulative rainfall during the growing season in Kade, Ghana