

Article

The Effects of *Gliricidia*-Derived Biochar on Sequential Maize and Bean Farming

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Abstract: The addition of biochar to soils can improve soil fertility and increase agricultural productivity. We carried out a field experiment in which biochar produced from *Gliricidia sepium* (Jacq.) Kunth ex Walp. was added to low-fertility Brazilian planosol and tested to increase the yield of maize (*Zea mays*) and snap beans (*Phaseolus vulgaris* L.) in sequential, organic cultivation. Biochar was applied at a 15 t/ha rate, combined or not with *Azospirillum Brasiliense* inoculation and organic fertilizer (Bokashi). The application of biochar resulted in an increase in soil pH and of the content of macronutrients such as phosphorus and potassium. Contrary to evidence from elsewhere, biochar had a limited effect on increasing maize yield. In the case of beans, when combined with fertilizer, biochar increased the production of beans pods and biomass, but the significant increase was observed only for inoculation. Beans are the principal component of Brazilian diet and increasing productivity of beans is of utmost importance for the poorest in Brazil, and in other tropical countries.

Keywords: biochar; maize and beans; smallholder farming; productivity; Brazil

1. Introduction

Agriculture contributes to food security, job creation and economic growth, yet it can also lead to loss and degradation of natural ecosystems [1,2]. Around the world, smallholder farmers

produce 80% of world food [3]. In Brazil, smallholder agriculture occupies approximately 80 million hectares [4] providing on average 40% of the total Brazilian agricultural production [3]. It also employs approximately 13 million people, corresponding to 79% of Brazil's agricultural workers [5].

Biochar emerged as a potential tool to improve soil conditions and to increase crop productivity, principally for smallholder farmers [6]. Biochar is a solid material that remains following the pyrolysis (decomposition at elevated temperature of organic residues in the absence or with limited access of oxygen; [7]. Biochar can increase crop productivity, diminishing farmers' dependence on external inputs such as lime [8–14].

Biochar can improve soil physical structure and aggregation, resulting in both better retention of moisture in sandy soils [15,16] and improved drainage in clay soils [17,18]. Biochar can increase soil pH [19], soil cation exchange capacity (CEC, [20,21]), sequester pollutants [22,23], and increased carbon storage in soil [24], which may contribute to the mitigation of greenhouse gas emissions [13,25]. Recent literature has also demonstrated that when biochar is mixed with manure, enriched with urine, or co-composted, effect on yield can be even greater [26]. The amendment of biochar to soil can also result in positive microbial effects [27] whereby the biochar may provide a conducive environment for microorganisms. The effect of biochar depends on soil properties, crop type, the rate of biochar applied and biochar properties [11,24,28–30]. In addition, local edapho-climatic conditions are fundamental for benefiting from biochar [24].

Biochar research on sequential cropping systems of maize and beans in Brazil is scarce [31]. Maize is the most cultivated and consumed crop in Brazil [32] with a projected increase in production by 24% in ten years (from 2016/2017) [33]. Beans form the basic diet, being of utmost importance for food safety and nutrition in Brazil [34]. For smallholder farming, even though of low efficiency, both maize and beans are the principal food produced and consumed in Brazil [4]. Between 1996 and 2006, smallholder participation contributed to the increase in the gross value production of maize and beans, from 49 to 52% and 67 to 77%, respectively [5].

Because of the importance of maize and beans for nutrition, especially for the poorest on the planet, it is crucial to search for methods to increase productivity while minimizing the impact on the environment. The scarcity of biochar studies focused on maize and beans in the Brazilian context and the potential benefits for smallholder farming and a global society led to the research presented in this paper. In the context of organic agriculture and smallholder farming, biochar can bring significant benefits [35,36], especially given that in Brazil organic agriculture increased at the rate of 15 to 20% per year between 1995 and 2005 (while other food sectors simultaneously grew only from 4 to 5%; [37]). Worldwide similar trend has been observed [38].

In this study, biochar derived from *Gliricidia sepium* (Jacq.) Kunth ex Walp. was applied to a Brazilian planosol in combination with organic fertilizer and microbial inoculants, and the effect on yield of sequential maize and garden bean cropping was measured. Our hypothesis was that all amendments improve plant productivity but to different extents. The present study is one of the few studies to investigate the amendment of biochar combinations on sequential cropping of vital crops under controlled field conditions.

2. Methods

2.1. Study Area

To evaluate biochar and other soil amendments on maize and snap beans yields, we conducted a field experiment between March 2015 and April 2016 at the Integrated Agroecology Production Experimental Station (also known as “Fazendinha Agroecológica Km 47” in Portuguese), Seropédica municipality, Rio de Janeiro state, latitude 22° 45' S, longitude 43° 41' W at altitude between 30 and 70 m a.s.l. [39] (Figure 1; Supplementary Material). A sequential cultivation of maize (*Zea mays*) variety Caatingueiro in the first cropping cycle and snap beans (*Phaseolus vulgaris*) cv. Alessa in the second cropping cycle was carried out. The climatic conditions in the region, according to Köppen's

classification, are tropical Aw; hot and wet with rain in the summer [40] and drought during tropical winter. Following the FAO (1998) taxonomy [41], the soils in the region were classified as planosols [39,42], and are often degraded with low fertility.

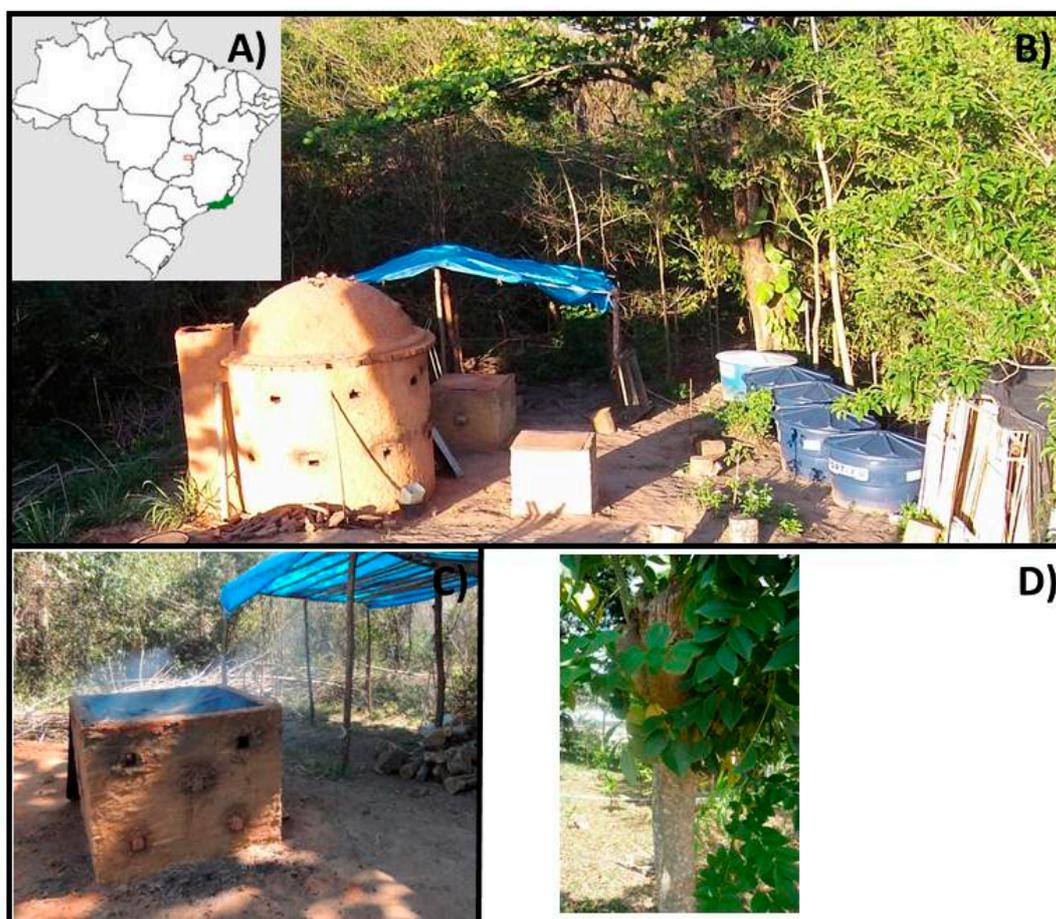


Figure 1. Area where the experiment was carried out (Rio de Janeiro state) (A); biochar production area with different types of ovens located at Embrapa Agrobiologia Experimental Station (B); brick oven used for production of biochar used in this study (C); biomass (*Gliricidia sepium* (Jacq.) Kunth ex Walp.) used for biochar production (D).

2.2. Experimental Design

The experiment followed a factorial scheme using a randomized block design with 24 plots (3.0 × 3.5 m) where three factors were evaluated, each in the presence or absence of biochar, inoculation and fertilization with organic fertilizer [43]. The organic fertilizer consists of compost produced from wheat bran, castor bean cake (*Ricinus communis* L.) and effective microorganisms that consist of beneficial and naturally-occurring mixed cultures of microorganisms, applied as inoculants, to improve soil quality and yield [44]. This resulted in eight treatments tested in triplicate, hereafter referred to as B—biochar; BI—biochar + inoculation; BF—biochar + fertilization; BIF—biochar + inoculation + fertilization; I—inoculation; F—fertilization; IF—inoculation + fertilization; C—control (without any amendment).

Prior to planting, the experimental area was ploughed by harrowing and furrowing. Bokashi was applied at 200 g/m (equivalent to 113 kg/ha of N), a standard dose used in organic agriculture in Brazil [45]. This fertilizer was added only during the cultivation of maize in order to investigate its lasting effects during the second cropping for garden beans. Inoculation was carried out at a rate of 50 g of inoculum for each 10 kg of seeds. A 10% sucrose solution was used as adherent.

Maize seeds were inoculated with *Azospirillum brasilense* (strain sp. 245) and beans seeds co-inoculated with *Rhizobium tropici* (strain CIAT 899) and *A. brasilense* (sp. 245) prior to planting. Inoculum of each bacterium was produced with turf and contained 10^8 colony-forming unit/g. Sixty kg of P_2O_5 /ha was applied to the entire experimental area following [46].

2.3. Biochar

The feedstock used to produce biochar consisted of branches and pruned logs of *Gliricidia sepium* (Jacq.) Kunth ex Walp. of Fabacea family (Figure 1). *Gliricidia* is often used for nitrogen fixation in organic agriculture [47–49]. Below-ground parts of *Gliricidia* are considered most useful as they fix nitrogen, while the above-ground parts may rapidly shadow plants [48–51]. *Gliricidia* spreads rapidly in our experimental area as it does in the region and enters adjacent native forest. In Brazil, *Gliricidia* is often considered as an invasive species.

Biochar was produced in a traditional brick oven with the height of 1 m, and the base of the stove was a square 1×1 m (Figure 1; [52]). The temperature during pyrolysis varied between 350 and 450 °C, and 35 kg of biochar was produced, on average, per 72 h pyrolysis cycle (approximately 30% yield based on amount of biomass used). Biochar was added to the soil once, in the beginning of the experiment when maize was sowed, at a dose of 15 t/ha per experimental plot. At that time, 15 t/ha was considered the most optimal dose given that higher doses were shown environmentally and economically inefficient while lower doses are not always effective [53,54]. Biochar had carbon content of 60% and a nitrogen content of 1%. Total hydrogen content was 2.5% and the biochar had H/C ratio of 0.5. The total cation exchange capacity (CEC), measured in 1 M NH_4NO_3 , was 83 cmol/kg (unwashed sample). In addition, biochar was characterized for dry matter content (99%), pH in water (8.6), total N (1%), Al (0.03 cmol/kg), Ca (44 cmol/kg), K (24 cmol/kg), Mg (12.5 cmol/kg), Na (2.5 cmol/kg) and H^+ (0 cmol/kg). A detailed description of the methods used for our biochar analysis can be found in [12].

2.4. Vegetative Cycles

Two sequential cropping cycles were carried out. Maize (*Caatingueiro*) was planted in March 2015 in rows with line spacing of 1 m. Plants were thinned to a number of six plants per meter. Cobs were harvested 90 days after planting (June 2015) from an area of 2 m² (12 plants). The aerial biomass of maize (shoot biomass) was sampled from an area of 1 m² (six plants).

Following maize harvesting, beans (*Alessa*) were planted in June 2015, on the same plots of maize, using a minimum tillage method with line spacing of 0.5 m. Plants sown in plots previously inoculated with *A. brasilense* were co-inoculated with the same *A. brasilense* strain (sp. 245) and with *Rhizobium tropici* CIAT 899. Rhizobium inoculant is recommended in Brazil for cultivation of common beans [55]. Plants were thinned to eight bean plants per meter. Beans were harvested 60 days after planting in a 1 m² area within each experimental parcel. During bean flowering, five plants were harvested in order to determine the mass and number of nodules. In order to control *Empoasca kremeri* pest, Nim oil spray (*Azadirachta indica* A. Juss) was applied weekly (1.5% solution) during the infestation period. It is common to use extracts from the Nim plant as an insecticide (permitted in Brazil also in organic agriculture) that reduces dependence on synthetic insecticides and diminishes crop production costs [56].

2.5. Soil and Plant Analyses

Soil was sampled with auger at the depth of 10 cm from all experimental plots (composite sample from each of 24 parcels), before and after biochar application, inoculum and/or fertilizer application, and following maize and beans harvest. Soil samples were homogenized and sieved through 2 mm, and analyzed for pH (in water), pf curve (%) at 15,000 and 100 hPa, moisture (%), organic matter (%), total C (g/kg), total N (g/kg), total K (mg/dm³), total P (mg/dm³), total Mg (cmol/dm³) and CEC (potential and effective; cmol/dm³). The potential CEC was measured as the sum of the base cations

Ca^{2+} , Mg^{2+} , Na^{+} and K^{+} in addition to Al^{3+} and H^{+} (cmol/kg). The effective CEC was defined as the sum of base cations, in addition to Al^{3+} (determined using a 1 mol/L KCl solution). PF curves were calculated in Richards pressure chamber. Potassium and phosphorus were analyzed using a Mehlich 1 extractor (0.05 mol/L HCl and 0.0125 mol/L H_2SO_4) while total Mg was measured using a 1 mol/L KCl solution. Nitrogen was determined using the Kjeldahl method. For the measurement of organic matter (OM) content, $\text{Na}_2\text{Cr}_2\text{O}_7 + \text{H}_2\text{SO}_4$ 10 N oxidation was used. C content was determined as $\text{C} = \text{OM}/1.724$ and subsequently the C/N ratio was calculated.

For maize, the following variables were measured: wet and dry above-ground biomass, wet weight of cobs with straw, wet and dry weight of cobs without straw, mass of 100 grains, and cob length and diameter. For beans, the parameters that were determined were: wet and dry weight of pod production and above ground biomass, and number of nodules. Mass of pods has direct relation with beans productivity as it is the main product that will be marketed and is the most important variable in this study. The mass of the pods was collected in an area of 1 m² in each of the plots and subsequently converted to productivity change per hectare. Above ground biomass is also an important indicator as it provides cover material often used in organic agriculture. It was also collected from an area of 1 m² in each of the plots and converted to mass per hectare. The nodules were collected from five bean plants (root), in each of the plots, at the time of full bloom (most active period for nodules). The nodules were counted and weighed. It is also a common variable used for bean-productivity descriptions [57–60]. Results for dry weight are presented in the main paper.

2.6. Statistics

All soil and yield data were converted to normalize residuals (logarithmic transformation) and analysed with repeated Anova and Tukey's average test with a 95% confidence interval using the R Software.

2.7. Biochar Production Costs

In addition to soil and yield analyses, we calculated the time needed to equalize the investment in ovens (payback). The effect of economies of scale was evaluated considering the marginal cost of constructing additional ovens and labour efficiency, according to the economic principles of fixed costs and variable costs [61]. To measure the marginal cost in the construction of ovens, protective equipment (gloves, boots, masks, etc.) and basic tools (chain saw, spade, etc.) that may be used in constructing one or "n" ovens, was considered by calculating the investment over a greater amount of total biochar production. Biochar-production costs were based on real costs of skilled personnel employed throughout the project. To determine labour efficiency, the amount of ovens that one person can feed with biomass per day was considered.

3. Results and Discussion

3.1. Biomass Yields for Maize (*Zea mays*) VAR. *Caatingueiro*

Figure 2 shows the corresponding dry weight for the above ground biomass, dry weight for the maize cob without straw, the grains, the length and the diameter of maize. The discussion given below is focused on Figure 2, while corresponding figures and statistics related to the wet weight data are shown in Figure S1 in the supplementary materials. Tables S1 and S2 provide the statistical analyses carried out on the data set for the ANOVA analysis of the effects of treatment, and the Tukey-test analysis of the effect of treatment on the mass of 100 grains of maize.

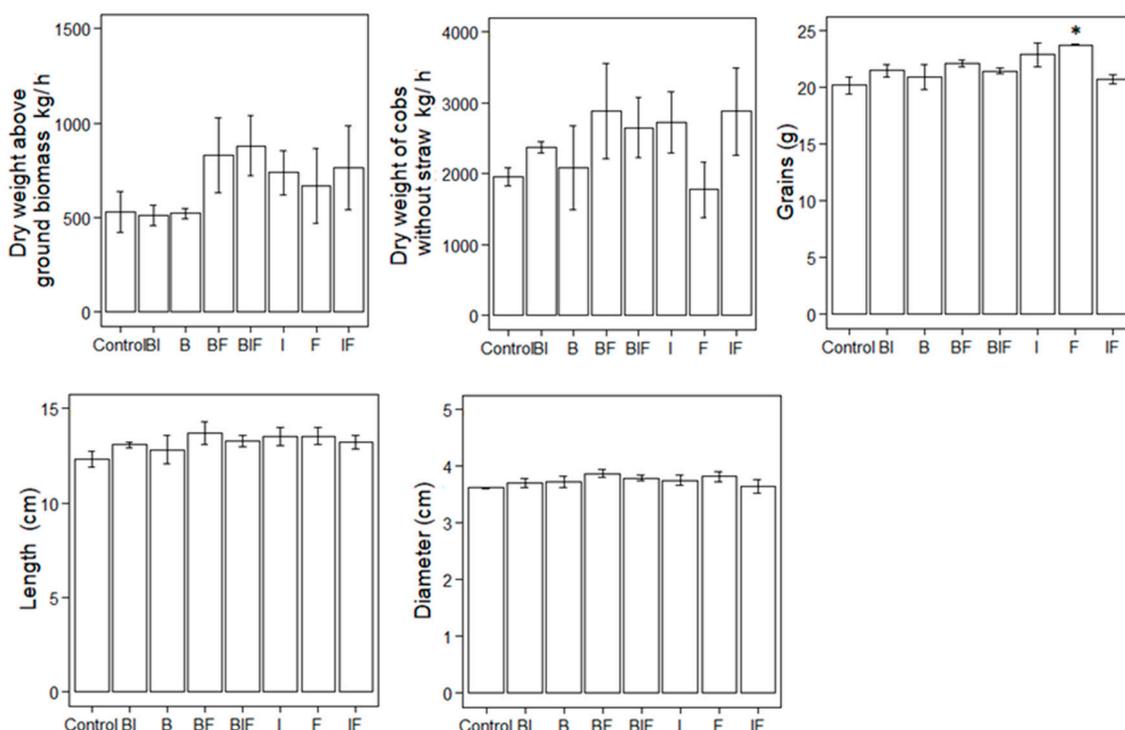


Figure 2. Results of dry weight of above ground biomass, dry weight of cobs without straw, mass of 100 grains, length and diameter of maize, *Caatingueiro*. Treatments are as follows: C—Control, BI—Biochar + Inoculant, B—Biochar, BF—Biochar + Fertilizer, BIF—Biochar + Inoculant + Fertilizer, I—Inoculant, F—Fertilizer, IF—Inoculant + Fertilizer. The data and standard deviation are based on the average of three replicate measurements. Symbol * indicates statistically significant difference (<0.05) between treatment C and F for the grains.

The effect of treatment had a limited effect on the parameters determined in our study. Only the mass of 100 maize grains demonstrated statistically significant treatment effect in case of fertilizer as compared to control ($p < 0.05$). Contrary to literature [9,11,62], we observed that biochar, fertilizer and inoculum, or a combination of these treatments had little effect on maize characteristics, such as dry weight of the above-ground biomass, the maize cob length and the maize-cob diameter. Notably, the dry weight of maize cobs without straw was higher for treatments with combined factors as compared to biochar alone. Consequently, there was a greater above-ground biomass accumulation (dry weight) when biochar and fertilizer were combined, corroborating observations of other authors [11,31,62]. When the fertilizer was present, there was a greater mass of 100 grains than in the inoculated treatments. Yet, in the treatment with fertilizer and inoculant less grain mass was observed than in the treatment with inoculant only. In this combination, the application of fertilizer may have inhibited the action of nitrogen-fixing bacteria. According to [63], when nitrogen is present in the soil heterotrophic bacteria compete with the diazotrophic bacteria for the same compounds, which can cause loss in the diversity of the population of nitrogen-fixing bacteria.

Even though previous studies show a positive effect of amendments (including biochar) on maize productivity in limited-quality soils [9,12], we did not observe this effect in our experiments. On the other hand, similar results were observed in temperate climates where high doses of biochar (50 t/ha) did not lead to significant increase in maize productivity over long term [64]. Other research shows [26,65] that the effect of biochar on crop productivity is a function of a range of factors such as the type of biochar and the amount of biochar added to the soil, where biochar is being applied and how much additional nutrient is added. For instance, it is shown [26] that low-dosage cow-urine-biochar application to root zone in a fertile silt loam soil in Nepal resulted in a more than 300% increase

in pumpkin yield as compared to treatment with urine only. When urine–biochar treatment was compared with a treatment of biochar only, an 85% increase in pumpkin yield was observed [26], which was explained by interaction urine with the biochar whereby urine without biochar might have leached out [66,67]. Similarly, [66] demonstrates that adding co-composted biochar (at 2% dosage) to sandy-poor soil promotes biochar's positive effects on *Chenopodium quinoa* yield (by 305%) by nitrate capture and delivery. Authors also observed that treatments with biochar only decreased yield by 60% when compared to control [66].

In our study, we tested pure biochar as, at the time of designing the study (2013–2014), there was not much evidence on the effect of pure biochar added at smaller quantities to Brazilian soils in the context of organic maize production and the evidence of nutrient-enriched biochar in the context of consecutive cropping in Brazil was scarce. It has also been reported that the biochar effect may be more prominent after a longer time because the CEC of biochar may increase over time [20]. We also acknowledge that other factors may have influenced our experiment such as largely uncontrolled in field conditions biases from the sun and shading patterns or other environmental factors.

3.2. Biomass Yields for Snap Bean (*Phaseolus Vulgaris*) cv. Alessa

Figure 3 shows dry weight of pod production, dry above ground biomass and the number of nodules. Table S3 shows the one-way ANOVA analysis and Table S4 the results of the Tukey-test analysis. The discussion below is focused on the dry weight data and the number of nodules, whilst analysis related to the wet weights can be found in the SI (Figure S2).

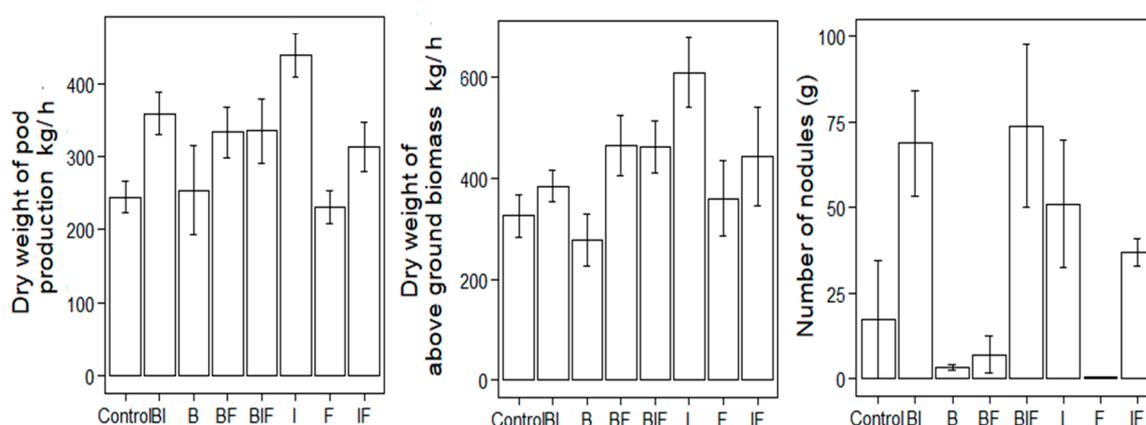


Figure 3. Nodules, dry weight above ground biomass and dry weight of pod production of common beans, cultivar *Alessa*. C—Control, BI -Biochar + Inoculant, B—Biochar, BF—Biochar + Fertilizer, BIF—Biochar + Inoculant + Fertilizer, I—Inoculant, F—Fertilizer, IF—Inoculant + Fertilizer. The data and standard deviation are based on the average of three replicate measurements.

Inoculation with *A. brasilense* and *R. tropici* increased dry weight of pod production to the greatest extent ($p < 0.05$) (nearly doubled; Figure 3). Similar differences were observed for the dry weight of the above ground biomass. The effectiveness of biochar increased when it was added in combination with fertilizer and inoculum, supporting previously published studies [8,12,68]. The number of nodules differed significantly between treatments. Biochar alone, biochar plus fertilizer and fertilizer alone reduced the number of nodules when compared to the control treatment ($p < 0.005$), while other biochar combinations (BI and BIF) had a positive effect. This could also be explained by statistically significant increase in effective CEC of soil in the plots with biochar, inoculant and fertilizer over the duration of experiment (Figure 4).

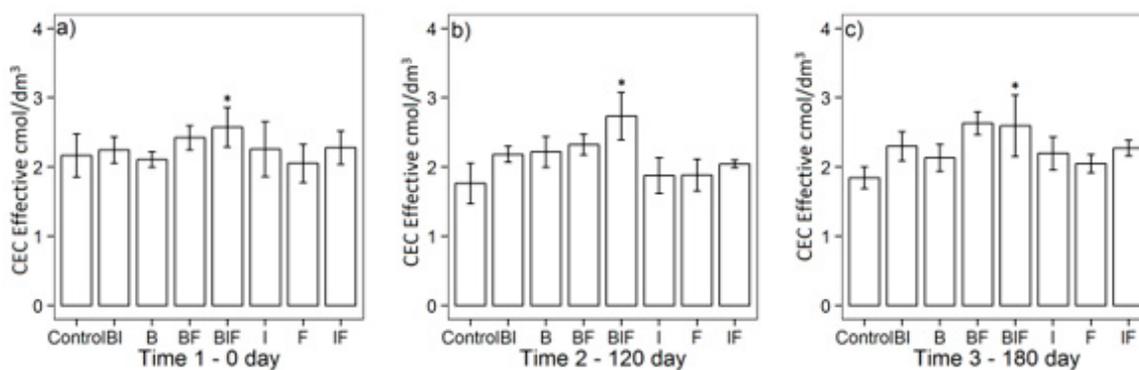


Figure 4. The temporal change in effective cation exchange capacity (CEC) following amendment (day 0), maize harvest (day 120) and bean harvest (day 180). Symbol * indicates statistically significant difference between treatment C and BIF.

Nitrogen is extremely important for bean production [69] and biological fixation is the main source of nitrogen for this plant. It is likely that the inoculant *Rhizobium tropici* stimulated nodules development when biological nitrogen fixation processes occurred [55,70]. The soil C/N ratio was higher in the beans cycle than the maize cycle, supporting this observation ($p < 0.0001$; Figure S6). In addition, *Azospirillum* is known to help plants in conditions of drought [55,71,72]. Thus, the addition of both inoculants promotes plant growth and stimulates the mechanisms of resistance to diseases and environmental stress. The negative impact, as compared with inoculant only, observed following the amendment of fertilizer and biochar may be explained by bacterium immobilization (via binding to biochar) or a toxic effect (of either the biochar or fertilizer on the microbial community) [73,74]. Other studies have indicated that the liming effect of biochar can contribute negatively to N fixation, which could explain the results observed here for the biochar treatments [27]. In addition, some volatile compounds derived from biochar can also contribute to a reduced microbial activity in soil, thus resulting in a high C/N ratio [75]. On the other hand, a positive effect of biochar on biological nitrogen fixation in common beans has been reported [76]. This is an interesting aspect of our research that needs to be further explored.

3.3. Change of Soil Properties over the Two Cropping Seasons

The results of changes in soil chemical attributes following the amendments for time points at 0, 120 days and 180 days are shown in the supporting information (Table S6—composite sample from the entire experimental area and Figures S3–S11 while Tables S7–S8 provide the details of statistical analyses). Throughout the analysis of soil data we assumed a constant bulk density. The soil pH varied both with treatment and with time. The highest pH values were observed in treatments that received biochar (BI, B, BF, BIF). Soil pH increased to 5.9 following the biochar amendment and was statistically higher as compared to the control treatment that had a pH of 4.8 ($p < 0.001$). Of the biochar treatments, treatment with biochar only (B) showed the highest pH value, which increased from 5.1 on day 0 to 6.1 after 120 days. However, after 180 days the pH on the plot with biochar decreased to pH of 5.3. This is not an uncommon phenomenon following the amendment of biochar to soil and may suggest the need for alkaline biochar re-application if pH is a limiting factor for a certain plant growth. Biochar is usually alkaline (pH for biochar used in our study was 8.6) and thus it is expected to increase the pH of an acidic soil [77]. Over time leaching of the alkaline components of the biochar takes place as water percolates the soil [78], therefore the pH can decrease. Contrary to the increase in pH after 120 days in the parcels with biochar amendment, the addition of fertilizer and the inoculant-fertilizer combination resulted in a decrease of soil pH over time.

The highest soil N and P contents were observed during the first maize cropping cycle. The application of biochar had no effect on the contents of N and P, most likely because part of

the N contained in the feedstock is lost in the pyrolysis process [74,79] and because available P depends on the type of biomass feedstock used for the biochar production [80]. The treatments with biochar were characterized by larger K content ($p < 0.0001$), supporting the notion that biochar is rich in K [12]. The content of Mg increased mostly in the case of combined treatments BFI and BF ($p < 0.005$). Soil organic matter content increased significantly with the duration of the experiments but did not differ between the treatments (Figure S7). The soil moisture values were lower after 120 days ($p < 0.0001$), which can be explained by the weather conditions.

There were statistically significant differences between treatments for the effective CEC ($p < 0.05$). The effective CEC for the soil alone was 2.17 cmol/kg on day 0, 1.76 cmol/kg after 120 days and 1.74 cmol/kg after 180 days, while for the biochar treatment was 1.78 cmol/kg on day 0, 2.22 cmol/kg after 120 days and 2.06 cmol/kg after 180 days. The effective CEC was the highest for the BIF treatment. This could be attributed to a combination of high availability of cations in exchangeable form in this treatment, possibly related to the presence of biochar [25]. The potential CEC was the highest after 120 days and decreased after beans harvest after 180 days. The decrease with time may be related to a decrease in soil pH and to biochar properties [25,81]. We also observed no statistically significant differences between treatments and potential CEC.

3.4. Biochar-production Costs

Marginal costs of biochar production decrease with an increasing number of ovens used to produce biochar, demonstrating gains in economies of scale (Figure 5). This gain is associated with the use of labour, since the brick ovens allow one person to manage up to four production units. Due the intensity of labour use, biochar production costs are lower for smallholder agriculture, reducing costs from BRL 1.5/kg to BRL 0.40/kg for small-holder agricultural production (Figure 6) (where BRL 1 equals USD 0.32, on the 20th of September 2017). This cost reduction (economy of scale) in the case of small-holder agriculture did not consider the opportunity costs of labour, and the timber for charcoal is collected inside the farm of smallholders. When in-house labour is used, the reduction in cost can reach 54%. According to census (IBGE, 2006), there were 321 properties with small-holder agriculture in the Seropédica municipality, each of which had an average area of 16 h per property. The estimated area (hectares) that a family of four people can attend and use for the production of biochar over the course of one year is up to 18% of the average area considered as smallholder agriculture in the municipality of Seropédica. It is important to emphasize that in many regions in Brazil, small-holder farmers have difficulty accessing the input markets, due to high costs, low scale for purchase, and poor infrastructure.

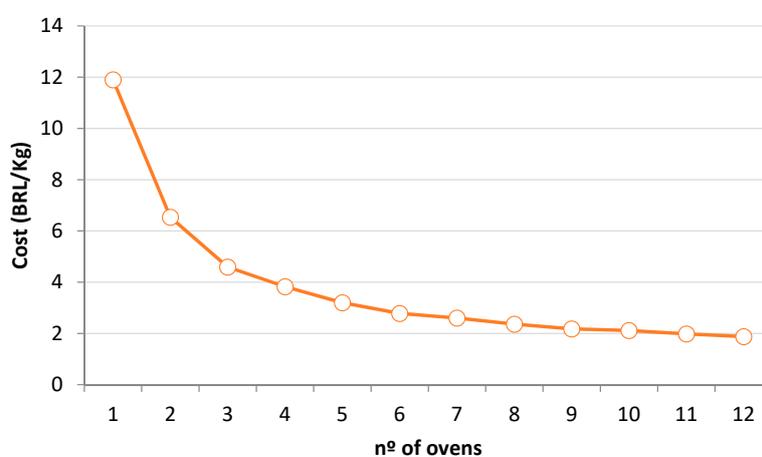


Figure 5. Relation between the number of brick ovens producing biochar simultaneously and costs per kilogram of biochar produced (in Brazilian Real per kilogram where BRL 1 equals approximately USD 0.32) in these ovens.

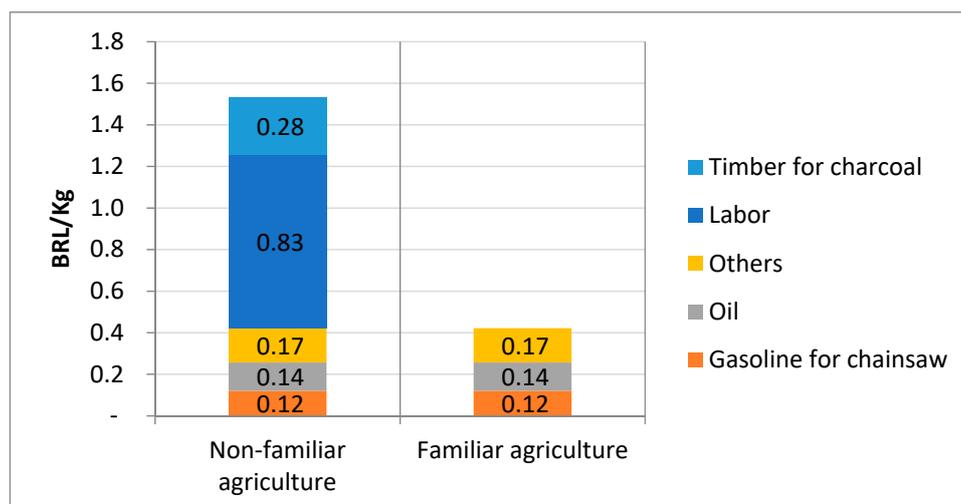


Figure 6. Production cost (in Brazilian Real per kilogram BRL/kg where BRL 1 equals approximately USD 0.32) of biochar in brick oven for large scale (non-family run agriculture) vs small-scale (run by family/small-holder) agricultural practices.

4. Conclusions

Adding biochar to Brazilian soil showed a range of positive effects on soil quality such as diminishing acidity and improving nutrient content. This effect was not, however, directly associated with plant productivity improvement. Notwithstanding several studies reporting that in tropical weathered soil the addition of biochar in combination with fertilizer significantly improved maize production (e.g., [8]) our study shows a limited effect of biochar on maize yields. For beans, amending the soil with inoculant alone produced the best effect likely due to *Rhizobium* and *Azospirillum* contained in the inoculant that facilitate N intake and plant growth, and reduces plant stress to external factors (such as drought that happened during our experiment). In the case of beans, we observed a positive effect of biochar (in combination with inoculant and fertilizer) on pods and biomass production. Beans form a principal component of the basic Brazilian diet, and techniques focused on improving its production have far reaching consequences.

Our results also show that the brick ovens commonly used in rural tropical areas provide the scale gains and lower costs of biochar production; and, using family labour, biochar production costs can decrease by 54%. In this way, biochar could be a viable alternative for small-holder farmers who seek to reduce reliance on external inputs or have limited input market access in regions with poor infrastructure, whilst still maintaining crop yields. On the other hand, the addition of inoculum, even though most promising given field trials, may be difficult to apply by the farmers in practice due to costs and preparation method. Future research could look into the socio-economic aspects of all treatments examined here and extend into study of biochar in longer trials as well as with multiple additions of biochar.

Supplementary Materials: Supplementary materials can be found at www.mdpi.com/2071-1050/10/3/578/s1.

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References

1. Strassburg, B.B.N.; Latawiec, A.E.; Barioni, L.G.; Nobre, C.A.; Da Silva, V.P.; Valentim, J.F.; Vianna, M.; Assad, E.D. When enough should be enough: Improved use of current agricultural lands could meet demands and spare nature in Brazil. *Glob. Environ. Chang.* **2014**, *28*, 84–97. [CrossRef]
2. Strassburg, B.B.N.; Latawiec, A.E.; Creed, A.; Nguyen, N.; Sunnenberg, G.; Miles, L.; Lovett, A.; Joppa, L.; Ashton, R.; Scharlemann, J.P.W.; et al. Biophysical suitability, economic pressure and land-cover change: A global probabilistic approach and insights for REDD+. *Sustain. Sci.* **2014**, *9*, 129–141. [CrossRef]
3. FAO. Family Farmers. Feeding the World, Caring for the Earth. 2014. Available online: <http://www.fao.org/assets/infographics/FAO-Infographic-IYFF14-FamilyFarms-en.pdf> (accessed on 3 March 2017).
4. França, C.G.; Del Grossi, M.E.; Marques, V.P.M.A. *O Censo Agropecuário 2006 e a Agricultura Familiar No Brasil*; Ministério do Desenvolvimento Agrário (MDA): Brasília, Brazil, 2009; p. 96. Available online: <http://www.bb.com.br/docs/pub/siteEsp/agro/dwn/CensoAgropecuario.pdf> (accessed on 3 March 2017).
5. Guanziroli, C.E.; Buainain, A.M.; Di Sabbato, A. Dez Anos de Evolução da Agricultura Familiar no Brasil: (1996 e 2006). *Rev. Econ. Sociol. Rural* **2012**, *50*, 351–370. [CrossRef]
6. Scholz, S.M.; Sembres, T.; Roberts, K.; Whitman, T.; Wilson, K.; Lehmann, J. *Biochar Systems for Smallholders in Developing Countries: Leveraging Current Knowledge and Exploring Future*; A World Bank Studies: Washington, DC, USA, 2014; p. 208.
7. Sohi, S.P.; Krull, E.; Lopez-Capel, E.; Bol, R. A review of biochar and its use and function in soil. *Adv. Agron.* **2010**, *105*, 47–82.
8. Steiner, C.; Teixeira, W.G.; Lehmann, J.; Nehls, T.; de Macedo, J.L.V.; Blum, W.E.H.; Zech, W. Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. *Plant Soil* **2007**, *291*, 275–290. [CrossRef]
9. Major, J.; Rondon, M.; Molina, D.; Riha, S.J.; Lehmann, J. Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol. *Plant Soil* **2010**, *333*, 117–128. [CrossRef]
10. Petter, F.A.; Madari, B.E.; Silva, M.A.S.; Carneiro, M.A.C.; Carvalho, T.M.C.; Junior, B.H.M.; Pacheco, L.P. Fertilidade do solo e produtividade do arroz de terras altas no Cerrado após aplicação de “biochar”. *Pesqui. Agropecu. Bras.* **2012**, *47*, 699–706. [CrossRef]
11. Cornelissen, G.; Martinsen, V.; Shitumbanuma, V.; Alling, V.; Breedveld, G.D.; Rutherford, D.W.; Sparrevik, M.; Hale, S.H.; Obia, A.; Mulder, J. Biochar Effect on Maize Yield and Soil Characteristics in Five Conservation Farming Sites in Zambia. *Agronomy* **2013**, *3*, 256–274. [CrossRef]
12. Martinsen, V.; Mulder, J.; Shitumbanuma, V.; Sparrevik, M.; Borresen, T.; Cornelissen, G. Farmer-led maize biochar trials: Effect on crop yield and soil nutrients under conservation farming. *J. Plant Nutr. Soil Sci.* **2014**, *177*, 681–695. [CrossRef]
13. Lehmann, J.; Joseph, S. Biochar for Environmental Management: An Introduction. In *Biochar for Environmental Management Science and Technology*; Lehmann, J., Joseph, S., Eds.; Earthscan: New York, NY, USA, 2009. Available online: <http://www.biochar-international.org/projects/book> (accessed on 2 February 2017).
14. Verheijen, F.; Jeffery, S.; Bastos, A.C.; van der Velde, M.; Dias, I. *Biochar Application to Soils: A Critical Scientific Review of Effects on Soil Properties, Processes and Functions*; JRC Scientific and International Reports; Office for the Official Publication of the European Communities: Luxembourg, Luxembourg, 2010; p. 166.
15. Basso, A.S.; Miguez, F.E.; Laird, D.A.; Horton, R.; Westgate, M. Assessing potential of biochar for increasing water-holding capacity of sandy soils. *GCB Bioenergy* **2013**, *5*, 132–143. [CrossRef]
16. Obia, A.; Mulder, J.; Martinsen, V.; Cornelisse, G.; Borresen, T. In situ effects of biochar on aggregation, water retention and porosity in light-textured tropical soils. *Soil Tillage Res.* **2016**, *155*, 35–44. [CrossRef]
17. Barnes, R.T.; Gallagher, M.E.; Masiello, C.A.; Liu, Z.; Dugan, B. Biochar-Induced Changes in Soil Hydraulic Conductivity and Dissolved Nutrient Fluxes Constrained by Laboratory Experiments. *PLoS ONE* **2014**, *9*, e108340. [CrossRef] [PubMed]
18. Cernansky, R. Agriculture: Stet-of-the-art soil. *Nature* **2015**, *517*, 258–260. [CrossRef] [PubMed]

19. Deal, C.; Brewer, C.E.; Brown, R.C.; Okure, M.A.E.; Amoding, A. Comparison of kiln-derived and gasifier-derived biochars as soil amendment in the wet tropics. *Biomass Bioenergy* **2012**, *37*, 161–168. [[CrossRef](#)]
20. Cheng, C.-H.; Lehmann, J.; Thies, J.E.; Burton, S.D.; Engelhard, M. Oxidation of black carbon by biotic and abiotic processes. *Org. Geochem.* **2006**, *37*, 1477–1488. [[CrossRef](#)]
21. Novotny, E.H.; Azevedo, E.R.; Bonagamba, T.J.; Cunha, T.J.F.; Madari, B.E.; Benites, V.M.; Hayes, M.H.B. Studies of the compositions of humic acids from Amazonian Dark Earth soils. *Environ. Sci. Technol.* **2007**, *41*, 400–405. [[CrossRef](#)] [[PubMed](#)]
22. Beesley, L.; Moreno-Jiménez, E.; Gomez-Eyles, J.L.; Harris, E.; Robinson, B.; Sizmur, T. A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. *Environ. Pollut.* **2011**, *159*, 3269–3282. [[CrossRef](#)] [[PubMed](#)]
23. Mohan, D.; Sarswat, A.; Ok, Y.S.; Pittman, C.U., Jr. Organic and inorganic contaminants removal from water with biochar, a renewable, low cost and sustainable adsorbent—A critical review. *Bioresour. Technol.* **2014**, *160*, 191–202. [[CrossRef](#)] [[PubMed](#)]
24. Jeffery, S.; Abalos, D.; Prodana, M.; Bastos, A.C.; van Groenigen, J.W.; Hungate, B.A.; Verheijen, F. Biochar boosts tropical but not temperate crop yields. *Environ. Res. Lett.* **2017**, *12*, 053001. [[CrossRef](#)]
25. Novotny, E.H.; Maia, C.M.B.F.; Carvalho, M.T.M.; Madar, B.E. Biochar: Pyrogenic carbon for agricultural use—A critical review. *Revista Brasileira de Ciência do Solo* **2015**, *39*, 321–344. [[CrossRef](#)]
26. Schmidt, H.-P.; Pandit, B.H.; Martinsen, V.; Cornelissen, G.; Conte, P.; Kammann, C.I. Fourfold Increase in Pumpkin Yield in Response to Low-Dosage Root Zone Application of Urine-Enhanced Biochar to a Fertile Tropical Soil. *Agriculture* **2015**, *5*, 723–741. [[CrossRef](#)]
27. Lehmann, J.; Rillig, M.; Thies, J.; Masiello, C.A.; Hockaday, W.C.; Crowley, D. Biochar effects on soil biota: A review. *Soil Biol. Biochem.* **2011**, *43*, 1812–1836. [[CrossRef](#)]
28. Jeffery, S.; Verheijen, F.G.A.; van der Velde, M.; Bastos, A.C. A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. *Agric. Ecosyst. Environ.* **2011**, *144*, 175–187. [[CrossRef](#)]
29. Biederman, L.A.; Harpole, W.S. Biochar and its effects on plant productivity and nutrient cycling: A meta-analysis. *GCB Bioenergy* **2013**, *5*, 202–214. [[CrossRef](#)]
30. Gray, M.; Johnson, M.G.; Dragila, M.I.; Kleber, M. Water uptake in biochars: The roles of porosity and hydrophobicity. *Biomass Bioenergy* **2014**, *61*, 196–205. [[CrossRef](#)]
31. Oliveira, D.M. Biocarvão de Bambu e Fontes de Fósforo Afetando a Disponibilidade de p e o Crescimento de Feijão Caupi e Milho em Latossolo Amarelo Distrófico da Amazônia. Ph.D. Thesis, Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Brazil, 2012.
32. OCDE-FAO. Perspectivas Agrícolas No Brasil: Desafios da Agricultura Brasileira 2015–2024. 2015; p. 54. Available online: <http://www.fao.org.br/download/PA20142015CB.pdf> (accessed on 2 February 2017).
33. MAPA. Projeções do Agronegócio Brasil 2016/2017–2026/2027. Projeções de Longo Prazo. 2017. Available online: <http://www.agricultura.gov.br/assuntos/politica-agricola/todas-publicacoes-de-politica-agricola/projecoes-do-agronegocio/projecoes-do-agronegocio-2017-a-2027-versao-preliminar-25-07-17.pdf/view> (accessed on 5 September 2017).
34. Conab. Evolução dos Custos de Produção de Feijão no Brasil e Sua Rentabilidade—Safrá 2010/2011 a 2015/2016. 2016. Available online: https://www.conab.gov.br/OlalaCMS/uploads/arquivos/17_01_24_14_49_18_compendio_de_estudos_conab_-_volume_5_2017.pdf (accessed on 10 September 2017).
35. Campanhola, C.; Valarini, P.J. A agricultura orgânica e seu potencial para o pequeno agricultor. *Cad. Cienc. Tecnol.* **2001**, *18*, 69–101.
36. Crowder, D.W.; Reganold, J.P. Financial competitiveness of organic agriculture on a global scale. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 7611–7616. [[CrossRef](#)] [[PubMed](#)]
37. Carvalho, J.F. Avaliação de Cultivares de Feijão-Caupi e Feijão Vagem Arbustivo em Sistema Orgânico de Produção. Master's Thesis, Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, Brazil, 2012; pp. 36–62.
38. Latawiec, A.E.; Strassburg, B.B.N.; Rodriguez, A.M.; Matt, E.; Nijbroek, R.; Silos, M. Suriname: Reconciling agricultural development and conservation of unique natural wealth. *Land Use Policy* **2014**, *38*, 627–636. [[CrossRef](#)]

39. Neves, M.C.P.; Guerra, J.G.M.; Carvalho, S.R.; Ribeiro, R.L.D.; Almeida, D.L. Sistema Integrado de Produção Agroecológica ou Fazendinha Agroecológica do km 47. In *Agroecologia: Princípios e Técnica Para Uma Agricultura Orgânica Sustentável*; Embrapa Informação Tecnológica: Brasília, Brazil, 2005; pp. 147–172.
40. Carvalho, D.F.; Faria, R.A.; Sousa, S.A.V.; Borges, H.Q. Espacialização do período de veranico para diferentes níveis de perda de produção na cultura do milho, na bacia do Rio Verde Grande, MG. *Rev. Bras. Eng. Agric. Ambient.* **2000**, *4*, 172–176. [[CrossRef](#)]
41. FAO. *World Reference Base for Soil Resources*; Food and Agriculture Organization of the United Nations: Rome, Italy, 1998.
42. Oliveira, N.G.; De-Polli, H.; Almeida, D.L.; Guerra, J.G.M. Feijão-vagem semeado sobre cobertura viva perene de gramínea e leguminosa e em solo mobilizado, com adubação orgânica. *Pesqui. Agropecu. Bras.* **2006**, *41*, 1361–1367. [[CrossRef](#)]
43. Guedes, R.E.; Guerra, J.; Ribeiro, R.; Coelho, R.; de Paula, P.D.; Moreira, V. *Avaliação de Cultivares de Feijão-De-Vagem de Crescimento Determinado Sob Manejo Orgânico, nas Condições da Baixada Fluminense-Seropédica/RJ*; Comunicado Técnico 102; Embrapa: Brasília, Brazil, 2007; pp. 1–4. Available online: <https://ainfo.cnptia.embrapa.br/digital/bitstream/CNPAB-2010/34502/1/cot102.pdf> (accessed on 2 February 2017).
44. Higa, T. Effective microorganisms: A biotechnology for mankind. In Proceedings of the First International Conference on Kyusei Nature Farming, Khon Kaen, Thailand, 17–21 October 1989; Parr, J.F., Hornick, S.B., Whitman, C.E., Eds.; U.S. Department of Agriculture: Washington, DC, USA, 1991; pp. 8–14.
45. Asia Pacific Natural Agriculture Network (APNAN). *Application Manual for APNAN Countries Asia-Pacific Natural Agriculture Network*, 1st ed.; APNAN: Bangkok, Thailand, 1995.
46. Freire, L.R.; Balieiro, F.C.; Zonta, E.; Anjos, L.H.C.; Pereira, M.G.; Lima, L.; Guerra, J.G.M.; Ferreira, M.B.C.; Leal, M.A.A.; Campos, D.V.B.; et al. *Manual de Calagem e Adubação do Estado do Rio de Janeiro*; Embrapa: Brasília, Brazil; EDUR: Seropédica, Brazil, 2013; p. 430.
47. Filho, O.M.C.; Drumond, M.A.; Languidey, P.H. Gliricidia Sepium—Leguminosa Promissora Para Regiões Semi-Áridas. *Embrapa—Circular Técnica* 35. 1997. Available online: <https://www.infoteca.cnptia.embrapa.br/infoteca/bitstream/doc/131682/1/CTE35.pdf> (accessed on 2 February 2017).
48. Elevitch, C.R.; Francis, J.K. Gliricidia sepium (gliricidia). In *Species Profiles for Pacific Island Agroforestry*; Elevitch, C.R., Ed.; Permanent Agriculture Resources (PAR): Honolulu, HI, USA, 2006. Available online: <https://raskisimani.files.wordpress.com/2013/01/gliciridia-gliciridia.pdf> (accessed on 2 February 2017).
49. Wandelli, E.V.; Costa, J.R.; Souza, S.G.A.; Perin, R. Cerca-Viva de Gliricidia Sepium. *Embrapa—Comunicado Técnico* 37; 2006; pp. 1–6. Available online: <https://www.infoteca.cnptia.embrapa.br/handle/doc/681499> (accessed on 5 March 2017).
50. Marin, A.M.P.; Menezes, R.S.C.; Silva, E.D.; Sampaio, E.V.S.B. Efeito da Gliricidia sepium sobre nutrientes do solo, microclima e produtividade do milho em Sistema agroflorestal no Agreste Paraibano. *Rev. Bras. Cienc. Solo* **2006**, *30*, 555–564. [[CrossRef](#)]
51. Paula, P.D.; Campello, E.F.C.; Guerra, J.G.M.; Santos, G.A.; Resende, A.S. Decomposição das podas das leguminosas arbóreas Gliricidia sepium e Acacia angustissima em um sistema groflorestal. *Cienc. Florest.* **2015**, *25*, 791–800. [[CrossRef](#)]
52. Sparrevik, M.; Adam, C.; Martinsen, V.; Cornelissen, G. Emissions of gases and particles from charcoal/biochar production in rural areas using medium-sized traditional and improved “retort” kilns. *Biomass Bioenergy* **2015**, *72*, 65–73. [[CrossRef](#)]
53. Lehmann, J.; Gaunt, J.; Rondon, M. Bio-char sequestration in terrestrial ecosystems—A review. *Mitig. Adapt. Strateg. Glob. Chang.* **2006**, *11*, 403–427. [[CrossRef](#)]
54. Asai, H.; Samson, B.K.; Haefele, S.M.; Songyikhangs, K.; Homma, K.; Kiyono, Y.; Inoue, Y.; Shiraiwa, T.; Horie, T. Biochar amendment techniques for upland rice production in Northern Laos. 1. Soil physical properties, leaf SPAD and grain yield. *Field Crops Res.* **2009**, *111*, 81–84. [[CrossRef](#)]
55. Hungria, M.; Campo, R.J.; Mendes, I.C. Benefits of inoculation of the common bean (*Phaseolus vulgaris*) crop with efficient and competitive *Rhizobium tropici* strains. *Biol. Fertil. Soils* **2003**, *39*, 88–93. [[CrossRef](#)]
56. Embrapa. Uso do Extrato Aquoso de Folhas de NIM Para o Controle de Spodoptera Frugiperda na Cultura do Milho—Circular Técnica 88. 2006, pp. 1–5. Available online: <https://www.embrapa.br/documents/1344498/2767891/uso-do-extrato-aquoso-de-folhas-de-nim-para-o-controle-de-spodoptera-frugiperda-na-cultura-do-milho.pdf/f1d204a5-fa0d-4818-b859-59d30d039605> (accessed on 10 April 2017).

57. Leite, L.F.C.; Araújo, A.S.F.; Costa, C.N.; Ribeiro, A.M.B. Nodulação e produtividade de grãos do feijão-caupi em resposta ao molibdênio. *Rev. Cienc. Agron.* **2009**, *40*, 492–497.
58. Carmo, H.F.; Silva, M.A.; Madari, B.E. Utilização do biochar como adionador de carbono no solo e seus efeitos químicos nas propriedades do solo, sob feijoeiro comum no Cerrado brasileiro. In Proceedings of the Anais do II Workshop Internacional Sobre Clima e Recursos Naturais nos Países de Língua Portuguesa, Bragança, Portugal, 15–19 November 2010; pp. 159–165.
59. Ferreira, E.P.B.; Martins, L.M.V.; Xavier, G.R.; Rumanjek, N.G. Nodulação e produção de grãos em feijão-caupi (*Vigna unguiculata* L. Walp.) Inoculado com isolados de rizóbio. *Rev. Caatinga* **2011**, *24*, 27–35.
60. Gualter, R.M.R.; Boodey, R.M.; Rumjanek, N.G.; Freitas, A.C.R.; Xavier, G.R. Eficiência agrônômica de estirpes de rizóbio em feijão-caupi cultivado na região da Pré-Amazônia maranhense. *Pesqui. Agropecu. Bras.* **2011**, *46*, 303–308. [[CrossRef](#)]
61. Mankiw, N.G. *Principles of Economics*, 6th ed.; Library of Congress Control Number: 2010941869; South-Western: London, UK, 2011; ISBN 13 978-0-538-45305-9.
62. Yamato, M.; Okimori, Y.; Wibowo, I.F.; Anshori, S.; Ogawa, M. Effects of the application of charred bark of Acacia mangium on the yield of maize, cowpea and peanut, and soil chemical properties in South Sumatra, Indonesia. *Soil Sci. Plant Nutr.* **2006**, *52*, 489–495. [[CrossRef](#)]
63. Bergamaschi, C. Ocorrência de Bactérias Diazotróficas Associadas às Raízes e Colmos de Cultivares de Sorgo. Master's Thesis, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, 2006; p. 83.
64. Latawiec, A.E.; Peake, L.; Baxter, H.; Cornelissen, G.; Grotkiewicz, K.; Hale, S.; Królczyk, J.B.; Kubon, M.; Łopatka, A.; Medynska-Juraszek, A.; et al. A reconnaissance-scale GIS-based multicriteria decision analysis to support sustainable biochar use: Poland as a case study. *J. Env. Eng. Land. Manag.* **2017**, *25*, 208–222. [[CrossRef](#)]
65. Glaser, B.; Wiedner, K.; Seelig, S.; Schmidt, H.-P.; Gerber, H. Biochar organic fertilizers from natural resources as substitute for mineral fertilizers. *Agron. Sustain. Dev.* **2015**, *35*, 667–678. [[CrossRef](#)]
66. Kammann, C.I.; Schmidt, H.P.; Messerschmidt, N.; Linsel, S.; Steffens, D.; Müller, C.; Koyro, H.-W.; Conte, P.; Joseph, S. Plant growth improvement mediated by nitrate capture in co-composted biochar. *Sci. Rep.* **2015**, *5*, 11080. [[CrossRef](#)] [[PubMed](#)]
67. Conte, P.; Marsala, V.; de Pasquale, C.; Bubici, S.; Valagussa, M.; Pozzi, A.; Alonzo, G. Nature of water-biochar interface interactions. *GCB Bioenergy* **2013**, *5*, 116–121. [[CrossRef](#)]
68. Chan, K.Y.; Van Zwieten, L.; Meszaros, I.; Downie, A.; Joseph, S. Agronomic values of greenwaste biochar as a soil amendment. *Aust. J. Soil Res.* **2007**, *45*, 629–634. [[CrossRef](#)]
69. Coelho, F.C.; Vieira, C.; Mosquim, P.R.; Cassini, S.T.A. Nitrogênio e molibdênio nas culturas do milho e do feijão em monocultivos e em consórcios: Efeitos sobre o feijão. *Revista Ceres* **1998**, *45*, 393–407.
70. Dobereiner, J. A importância da fixação biológica de nitrogênio para a agricultura sustentável. *Biotechnol. Cienc.* **1997**, *1*, 2–3.
71. Bashan, Y.; Bashan, L.E. Chapter two-how the plant growth-promoting bacterium Azospirillum promotes plant growth-a critical assessment. *Adv. Agron.* **2010**, *108*, 77–136.
72. Souza, J.E.B. Co-Inoculação de Rhizobium Tropici e Azospirillum Brasilense No Feijoeiro-Comum Visando Aumento de Produtividade e Redução de Custo de Produção. Master's Thesis, Universidade Federal de Goiás, Goiânia, Brazil, 2015; p. 83.
73. Bruun, E.W.; Ambus, P.; Egsgaard, H.; Hauggaard-Nielsen, H. Effects of slow and fast pyrolysis biochar on soil C and N turnover dynamics. *Soil Biol. Biochem.* **2012**, *46*, 73–79. [[CrossRef](#)]
74. Guereña, D.; Lehmann, J.; Hanley, K.; Enders, A.; Hyland, C.; Riha, S. Nitrogen dynamics following field application of biochar in a temperate North American maize-based production system. *Plant Soil* **2013**, *365*, 239–254. [[CrossRef](#)]
75. Graber, E.R.; Harel, Y.M.; Kolton, M.; Cytryn, E.; Silber, A.; David, D.R.; Tsechansky, L.; Borenshtein, M.; Elad, Y. Biochar impact on development and productivity of pepper and tomato grown in fertigated soilless media. *Plant Soil* **2010**, *337*, 481–496. [[CrossRef](#)]
76. Rondon, M.A.; Lehmann, J.; Ramírez, J.; Hurtado, N. Biological nitrogen fixation by common beans (*Phaseolus vulgaris* L.) increases with bio-char additions. *Biol. Fertil. Soils* **2007**, *43*, 699–708. [[CrossRef](#)]
77. Chintala, R.; Mollinedo, J.; Schumacher, T.E.; Malo, D.D.; Julson, J.L. Effect of biochar on chemical properties of acidic soil. *Arch. Agron. Soil Sci.* **2014**, *60*, 393–404. [[CrossRef](#)]

78. Gao, S.; DeLuca, T.H. Influence of Biochar on Soil Nutrient Transformations, Nutrient Leaching, and Crop Yield. *Adv. Plants Agric. Res.* **2016**, *4*, 1–16.
79. Monterumici, C.M. Processed vs. Non-Processed Biowastes for Agriculture: Effects of Post-Harvest Tomato Plants and Biochar on Radish Growth, Chlorophyll Content and Protein Production. *Int. J. Mol. Sci.* **2015**, *16*, 8826–8843. [[CrossRef](#)] [[PubMed](#)]
80. Novak, J.M.; Buscher, W.J.; Laird, D.L.; Ahmedna, M.; Watts, D.W.; Niandou, M.A.S. Impact of biochar amendment on fertility of a southeastern coastal plain soil. *Soil Sci.* **2009**, *174*, 105–112. [[CrossRef](#)]
81. Glaser, B.; Lehmann, J.; Zech, W. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal—A review. *Biol. Fertil. Soils* **2002**, *35*, 219–230. [[CrossRef](#)]



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