CHILEAN JOURNAL
of AGRICULTURAL RESEARCH

RESEARCH ARTICLE

Physiological response of 'Castillo el Tambo' coffee plants to biochar and chemical fertilization applications

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Received: 10 October 2022; Accepted: 20 December 2022; doi:10.4067/S0718-58392023000300307

ABSTRACT

The fertilizers costs are currently elevated, and it is necessary to evaluate complementary agronomic strategies. Biochar (BC) offers several potential benefits at a relatively low cost. The research aimed was to study the effect of the application of four different doses of biochar (BC) (0, 4, 8, and 16 t ha^{-1}) and four fertilization levels (FL) (0%, 33%, 66% and 100% of the nutritional requirements). We obtained the BC from coffee (*Coffea arabica* L.) pulp. The selected response variables were: Stomatal conductance (g_s), water use efficiency (WUE), root hydraulic conductivity (K), leaf photosynthetic pigments (Chl), and total dry weight (TDW) of coffee 'Castillo el Tambo'. The experiment was conducted under greenhouse conditions. The results showed that plants with 0 t ha^{-1} and 0% FL registered low values in the physiological parameters evaluated (g_s = 60.5 mmol m^{-2} s^{-1}; WUE = 3.03 g DM L^{-1} H_2O; Chl = 59.4 atLEAF readings; K_r = 9.7×10^{-6} kg s^{-1} MPa^{-1}; TDW = 9.7 g). On the other hand, we recorded a positive effect when 'Castillo el Tambo' coffee plants received 8 t ha^{-1} BC (K_r = 11.8×10^{-6} kg s^{-1} MPa^{-1}), especially at fertilization levels of 66% and 100% (g_s = 146.7 and 167.5 mmol m^{-2} s^{-1}; Chl = 72.8 and 66.6 Chl content; WUE = 4.3 and 5.0 g DM L^{-1} H_2O; TDW = 13.6 and 16.0 g, respectively). In conclusion, the use of BC manufactured with coffee pulp, mainly at a dose of 8 t ha^{-1}, can be an alternative to complement the chemical nutrition of coffee plants.

Key words: Assimilate partitioning, mineral nutrition, plant growth, root hydraulic conductivity.

INTRODUCTION

Coffee (*Coffea arabica* L.) is one of the most important crops in the world due to the beverage's popularity and accessibility for any social and economic population. It is also the second most commercialized product worldwide after oil. In Colombia, coffee is one of the main crops with a production of about 13 890 000 60-kg bags of green coffee in 853 700 ha and 11 218 000 bags exported in 2020 (Federación Nacional de Cafeteros de Colombia, 2022; Sanchez-Reinoso, 2022).

Coffee cultivation is responsible for the production of a large number of different residues such as fresh pulp. It is reported that 500 kg pulp can be obtained from 1000 kg processed coffee cherries (Reichembach and de Oliveira-Petkowicz, 2020). Wastewater from coffee production has a high potential for pollution to the environment (e.g., rivers or water streams) since it contains toxic substances such as alkaloids, tannins, and polyphenolics. The biological degradation of these substances is difficult as efficient and safe management practices are not carried out (Adugna et al., 2008). The use of coffee waste for the manufacture of value-added products can boost economic growth, generating social benefits and supporting environmental objectives (Duarte et al., 2020). In this regard, the transformation of agricultural residues (coffee pulp) through the process of pyrolysis could be an appropriate approach for their management and an alternative of great interest as a soil amendment. This practice can provide numerous benefits for agriculture as a complement in the preparation of substrates commonly used at the nursery stage of coffee cultivation or reduce the application of fertilizers of chemical synthesis in production systems.

The biochar (BC) is a heterogeneous substance, whose main characteristics are its C content in stable structures (condensed aromatics), labile substances such as volatile compounds (tars), ashes (mineral content), and moisture (Tomczyk et al., 2020). The use of biomass to obtain BC has been a much-studied topic since it seems to be a very promising alternative for the proper and efficient management of residues, allowing the return of the nutrients contained therein to the soil (Kwoczynski and Cmelík, 2021). Numerous benefits of the application of BC to the soil have been reported, for example: i) It increases the pH, cation exchange capacity, and nutrient availability, reduces exchangeable acidity, and contributes to the bioremediation of heavy metals and hydrocarbons in chemical variables (Singh et al., 2020); ii) regarding the physical properties of soil, it improves resistance to root penetration, reduces bulk density, and increases soil water availability for plants (Razzaghi et al., 2020), and iii) concerning the biological properties of soil, it favors the enzymatic activity of microorganisms, root nodulation, biomass, and microbial community (Singh et al., 2020). Finally, several studies have demonstrated the benefits of the use in soil with nutrient deficiencies. Asai et al. (2009) have reported that applications of 16 t ha^{-1} of BC manufactured from teak (*Tectona grandis* L. f.) and burma padauk (*Pterocarpus macrocarpus* Kurz) wood residues increased the soil hydraulic conductivity and yield in rice plants under conditions of P deficiency. Sorrenti et al. (2019) obtained similar results when the application of BC (made with peach and vine pruning wood) improved the nutritional status of nectarine trees, concluding that the beneficial effects of BC are evidenced as time passes, especially under limiting soil conditions such as low nutrient availability.

The loss of fertile land is a huge threat to the world's food supply (Song et al., 2020). Farmers use sometimes synthetic fertilizers at higher doses than recommended to improve crop productivity (Deshmukh and Badgujar, 2018). However, several organic components have been developed to reduce the excessive use of chemical fertilizers, with BC being a potential alternative that improves both, soil quality parameters and crop productivity (Alam et al., 2020). Nevertheless, the positive effect of the use of BC on plant physiology remains limited, especially in tropical or subtropical crops such as coffee (Sanchez-Reinoso et al., 2022).

The application of BC can cause several positive effects on the plant physiology. Qian et al. (2019) concluded that the application of BC produced with rice husk (1%, 5%, and 10% w/w) favored the net photosynthesis rate, chlorophyll index, and growth in soybean plants since it increased the uptake of nutrients such as P, especially at a high dose (10% w/w). Additionally, Zhang et al. (2020a) stated that the application of a mixture of BC from corn stalks and animal manure improved stomatal opening, photosynthesis, synthesis of photosynthetic pigments, and photosystem II (PSII) activity of sugar beet in soils with saline-alkaline conditions. Finally, Faloye et al. (2019) showed that the combined application of 20 t ha^{-1} BC of pod residues and 300 kg ha^{-1} inorganic fertilizer (NPK 15:15:15) improved the yield and water use efficiency of maize crops under water deficit conditions, minimizing the negative effects of water scarcity.

The agriculture currently needs integrated management of plant mineral nutrition. In this context, the use of residues from production systems has become an alternative to help crop production. The utilization of a by-product such as coffee pulp as BC can be an interesting agronomic strategy to improve the sustainability of this crop. Also, the effect of the use of BC on plant physiology has gained importance in recent years (Vijayaraghavan, 2021). However, the knowledge about the effects of this type of amendment on the physiology of coffee plants is still very limited. Therefore, this research was focused on the study of the potential use of coffee residues (pulp) as a source for BC manufacture, the subsequent analysis of the incorporation of this BC into the soil, and its effect on the physiological behavior of coffee plants with different doses of fertilizers. For this reason, the objective of this study was to evaluate the effect of the application of four different doses of BC obtained from coffee pulp and four levels of fertilization on the physiological responses of coffee (*Coffea arabica* L. 'Castillo el Tambo') plants on the variables of gas exchange, photosynthetic pigments, and accumulation and partitioning of assimilates.

MATERIALS AND METHODS

Growth conditions and plant material

The experiment was carried out between September 2018 and January 2019 in a greenhouse of the Faculty of Agricultural Sciences of the Universidad Nacional de Colombia, Bogotá campus (4°35'56'' N, 74°04'51'' W; 2556 m a.s.l.) The growth conditions in the greenhouse during the evaluation were: A natural photoperiod of 12:12 h with a photosynthetically active radiation (PAR) of 800-1200 µmol m^{-2} s^{-1} at noon (according to weather conditions), 25/20 °C day/night temperature, and relative humidity 60% to 80%. Three-month-old coffee (*Coffea* *arabica* L.) 'Castillo el Tambo' seedlings (2 mo-old) with four fully-expanded leaves were transplanted into 2 L pots with soil (a soil mass of 1.8 kg) from a coffee crop at the farm "Luxemburgo" located in the municipality of Chaparral Tolima (3°49'39.2" N, 75°34'07.1" W). The chemical and physical characteristics of the soil were: pH 5.56; effective cation exchange capacity (ECEC) 13.1 meq 100 g^{-1}; electrical conductivity 0.16 dS m^{-1}; total N: 0.27%, Ca: 9.95 meq 100 g^{-1}, K: 0.48 meq 100 g^{-1}, Mg: 2.56 meq 100 g^{-1}, Na: 0.14 meq 100 g^{-1}, Cu: 0.81 mg kg^{-1}, Fe: 20 mg kg^{-1}, Mn: 46 mg kg^{-1}, Zn: 4.10 mg kg^{-1}, B: 0.07 mg kg^{-1} and P: 8.70 mg kg^{-1}, and silt loam texture 26% clay, 54% silt, and 20% sand.

Treatment of biochar doses and fertilization levels

The biochar (BC) treatments were established at the time of transplanting the coffee plants by mixing the soil with four different BC doses. The BC doses were the equivalent to 0, 4, 8, and 16 t ha^{-1} (BC0 = 0 g $plant^{-1}$; BC4 = 3 g $plant^{-1}$; BC8 = 6 g $plant^{-1}$, and BC16 = 12 g $plant^{-1}$). The doses were selected based on the agronomic responses in a field experiment (Sanchez-Reinoso et al., 2022). The BC used was manufactured from coffee pulp under medium pyrolysis at 500 °C and showed the following characteristics: pH 9.42; electrical conductivity 19.4 dS m^{-1}; organic C (OC): 46.4%; ashes 20.8%; N: 2.81%; P: 1.11%; Ca: 0.97%; K: 4.37%; Mg: 0.43%; Cu: 53.5 mg kg^{-1}; Fe: 2795 mg kg^{-1}; Zn: 110 mg kg^{-1}; B: 99.3 mg kg^{-1}; CEC: 103 meq 100 g^{-1}; OC/N: 16.5.

Fertilization treatments were carried out 5 d after transplanting (DAT) using an 18-46-0 compound fertilizer (diammonium phosphate (DAP), Yara, Colombia) as a source of N and P, and urea as supplemental N (UREA, Yara, Colombia). The fertilization levels (FL) used were 0% (0 kg ha^{-1} DAP and 0 kg ha^{-1} UREA), 33% (10 kg ha^{-1} DAP and 5 kg ha^{-1} UREA), 66% (20 kg ha^{-1} DAP and 10 kg ha^{-1} UREA) and 100% (40 kg ha^{-1} DAP and 20 kg ha^{-1} UREA) of the nutritional requirements (F0 = 0 g $plant^{-1}$ DAP and 0 g $plant^{-1}$ UREA; F33 = 2 g $plant^{-1}$ DAP and 1 g $plant^{-1}$ UREA; F66 = 4 g $plant^{-1}$ DAP and 2 g $plant^{-1}$ UREA; and F100 = 8 g $plant^{-1}$ DAP and 4 g $plant^{-1}$ UREA). These doses were selected based on the physical and chemical analysis of the soil and the nutritional requirements (1.2 g N $plant^{-1}$ and 2 g P_2O_5 $plant^{-1}$) reported by Salamanca-Jimenez (2017). It was not necessary to apply additional P since the levels of this element found in the soil were valued as adequate for the growth stage in which the experiment was carried out.

The volume of water applied in each irrigation during the experiment was as follows: i) 40 mL $plant^{-1}$ between 0 and 20 DAT; ii) 50 mL $plant^{-1}$ between 21 and 50 DAT; iii) 60 mL $plant^{-1}$ between 51 and 65 DAT; iv) 75 mL $plant^{-1}$ between 66 and 80 DAT, and finally, v) 100 mL $plant^{-1}$ between 81 and 100 DAT. The irrigation application was carried out every 5 d. The treatments were set in a completely randomized design in a factorial arrangement; the first factor was the four BC doses (0, 4, 8, and 16 t ha^{-1}) and the second factor was the four levels of fertilization (0%, 33%, 66%, and 100% of the nutritional requirements). Each treatment was made up of five coffee plants as replicates, for a total of 80 plants throughout the experiment.

Leaf gas exchange parameters and root hydraulic conductivity

The stomatal conductance (g_s) was estimated on the second fully expanded leaf from the canopy superior portion using a steady-state porometer (SC-1; Decagon Devices, Pullman, Washington, USA). The water use efficiency (WUE) was determined using the ratio between the total dry weight of the plant and the total amount of water received by each seedling during the development of the experiment. The g_s and WUE were quantified at the end of the experiment 100 DAT.

The root hydraulic conductivity (K_r) was determined between 08:00 and 11:00 h only in the plants (5 mo-old) without chemical fertilization (0% nutritional requirements) but treated with all BC doses. Each plant was cut 5 cm above the soil surface; then, a 5 mm attachment was fitted to the top of the roots, and K_r readings were taken using a high-pressure flow meter (HCFM Gen 3; Dynamax, Houston, Texas, USA) connected to a N gas tank. Water was injected into the root system in the opposite direction of normal flow during transpiration. K_r (kg s^{-1} kPa^{-1}) was determined as the resulting slope between the water flow and the pressure of the root system of the coffee plant, taking measurements every 2 s at 22.5 ± 0.5 °C.

Plant growth (height, relative growth rate, DM accumulation, and assimilate partitioning)

Plant height at time 1 was determined at 0 DAT, whereas the height at time 2 was recorded at 100 DAT, starting from the base of the stem to the apical meristem. The relative growth rate (RGR) was estimated according to the following Equation 1:

$$RGR = \frac{[ln(Height\ at\ time\ 2) - ln(Height\ at\ time\ 1)]}{(Time_2 - Time_1)} \qquad Equation\ (1)$$

The DM of the organs and the total DM of the plant were quantified at 100 DAT. The plant material was dried in a compressed dry air oven (ThelcoMod 27, Chicago, Illinois, USA) at 80 °C for 48 h to obtain the total dry weight of the plant. Finally, the agronomic efficiency (AE) was calculated as the ratio between the total DM of the treatment and the total DM of the plants without fertilization (0 t ha^{-1} BC and 0% FL).

Chlorophylls and chlorophyll *a* fluorescence

The relative chlorophyll (Chl) content and the maximum quantum efficiency of photosystem II (PSII) (F_v/F_m) were also measured on the second fully expanded leaf from the plant canopy at 100 DAT. The Chl content was determined using a chlorophyll meter (atLEAF CHL STD, FT Green, Wilmington, Delaware, USA). The F_v/F_m ratio was evaluated through a continuous excitation fluorometer (Handy PEA, Hansatech Instruments, Kings Lynn, UK) using clips for dark adaptation for 30 min. At the end of the dark-adaptation period, variables such as minimum fluorescence (F_0), maximum fluorescence (F_m), and F_v/F_m were determined. The F_0 was measured with modulated low-intensity light (< 0.1 µmol m^{-2} s^{-1}) to avoid affecting the variable fluorescence. The F_m was recorded using a saturating light pulse (3000 µmol m^{-2} s^{-1}) for 0.8 s. The variable fluorescence (F_v) was calculated by the difference between F_0 and F_m. Finally, the maximum quantum efficiency of PSII was obtained by the F_v/F_m ratio.

Experimental design and data analysis

A completely randomized design with a factorial arrangement of the treatments was used. The first factor was the four doses of BC (0, 4, 8, and 16 t ha^{-1}) and the second factor was the four fertilization levels (0%, 33%, 66%, and 100% of the nutritional requirements) for a total of 16 treatments with five replicates. When there were significant differences in the ANOVA, the comparative Tukey test of means was used at $P \leq 0.05$. Data were analyzed using Statistix version 9.0 (analytical software, Tallahassee, Florida, US). Additionally, a principal component analysis was performed using the InfoStat 2016 software (Di Rienzo et al., 2016).

RESULTS

Leaf gas exchange parameters

Table 1 summarizes the effects of BC and FL on the different variables at 100 DAT. The g_s showed differences ($P \leq 0.001$) in the BC×FL interaction. In general, g_s was higher in coffee plants treated with 100% chemical fertilization compared to plants without fertilizer application. The increasing addition of BC caused an increment in g_s, mainly in plants with fertilizers at 66% and 100% of their requirements (total N [1.2 g N $plant^{-1}$] and P [2 g P_2O_5 $plant^{-1}$] consumption per plant in that phenological stage), reaching values of ~180 mmol m^{-2} s^{-1} at a dose of 16 t ha^{-1} combined with 100% of their nutritional requirements (Figure 1A). On the other hand, differences on the K_r were only obtained between the factor BC dose. An increase in BC improved the movement of water from the roots towards the shoot (9.7×10^{-6} kg s^{-1} MPa^{-1} with 0 t ha^{-1} *vs.* 11.6×10^{-6} kg s^{-1} MPa^{-1} with 16 t ha^{-1}) (Figure 1B).

Chlorophylls and chlorophyll *a* fluorescence

Differences were also found on the interaction between BC and FL on the Chl content (expressed as atLEAF readings) and F_v/F_m (Table 1). In general, the plants treated with 0 t ha^{-1} BC + 0% FL registered the lowest Chl (59.4 atLEAF readings) and F_v/F_m (0.37) values compared to the other treatments. These variables also increased gradually with higher doses of BC and FL. The F_v/F_m showed a greater progression mainly in the group of plants without fertilization and with different BC doses (0 t ha^{-1} BC = 0.37, 4 t ha^{-1} BC = 0.46, 8 t ha^{-1} BC = 0.53, and 16 t ha^{-1} BC = 0.51) (Figure 2).

Table 1. Summary of the ANOVA of the effect of the application of different biochar (BC) doses (0, 4, 8, and 16 t ha^{-1}) and fertilization levels (FL) (0%, 33%, 66%, and 100% of the nutritional requirements) on physiological variables at 100 d after transplanting (DAT). *, **, ***Significantly different at 0.05, 0.01, and 0.001 probability levels, respectively; ^{NS}: nonsignificant; PSII: photosystem II.

Variable	Abbreviation	Source of variation		
		BC	FL	BC × FL
Stomatal conductance	g_s	***	***	**
Chlorophyll content	Chl	**	***	*
Maximum quantum efficiency of PSII	F_v/F_m	**	***	*
Total dry weight	TDW	**	***	**
Plant height		**	NS	**
Leaf DM partitioning	LDMP	***	***	**
Stem DM partitioning	SDMP	***	***	***
Root DM partitioning	RDMP	**	***	***
Relative growth rate	RGR	***	***	**
Water use efficiency	WUE	**	***	***
Agronomic efficiency	AE	***	***	***

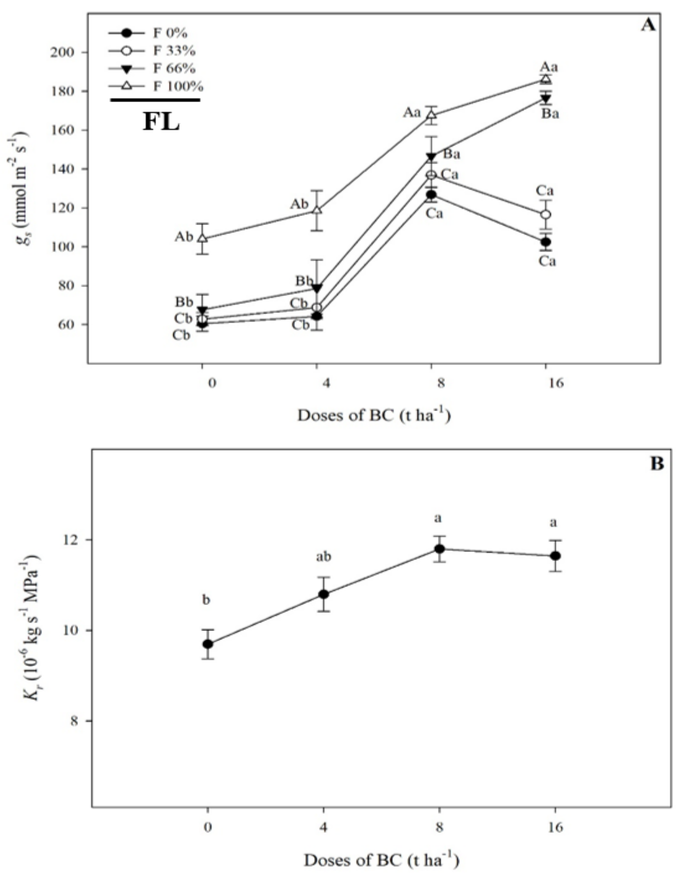

Figure 1. Effect of the application of different doses of biochar (BC) (0, 4, 8, and 16 t ha^{-1}) and levels of chemical fertilization (FL) (0%, 33%, 66%, and 100% of the nutritional requirements) on stomatal conductance (g_s) in leaves (A) and root hydraulic conductivity (K_r) (B) of coffee plants at 100 d after transplanting (DAT). Points represent the mean of five values (n = 5) ± standard error. Capital letters indicate significant differences between BC doses. Lower case letters indicate significant differences between levels of fertilization. Similar letters indicate that the means are not significantly different according to Tukey test at $P \leq 0.05$.

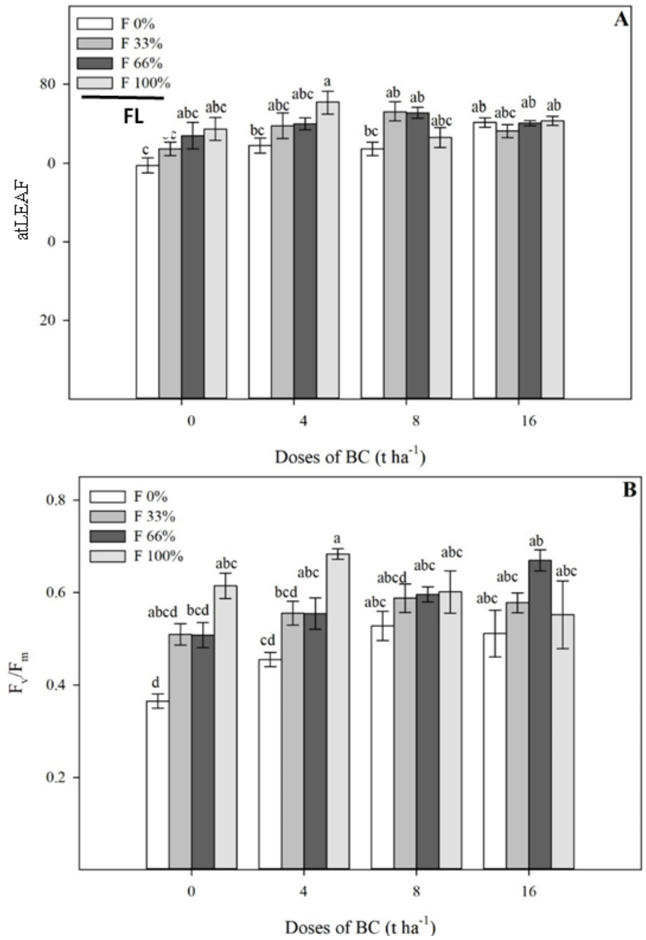

Figure 2. Effect of the application of different doses of biochar (BC) (0, 4, 8, and 16 t ha^{-1}) and levels of chemical fertilization (FL) (0%, 33%, 66%, and 100% of the nutritional requirements) on the relative chlorophyll content expressed as atLEAF readings (A) and the maximum quantum efficiency of photosystem II (PSII) (F_v/F_m ratio) (B) of coffee seedlings at 100 d after transplanting (DAT). Bars represent the mean of five plants ± standard error. Similar letters indicate that the means are not significantly different according to Tukey test at $P \leq 0.05$.

Growth: DM accumulation, assimilate partitioning, and relative growth rate

The growth parameters of coffee plants were conditioned to the treatments because of the ANOVA showed differences between the intensity of the added nutritional factors (Table 1). Regarding the total dry weight, plants with 0 t ha^{-1} BC + 0% FL registered the lowest biomass (9.7 g). In general, higher biomass was obtained when the nutrition of coffee plants was improved through higher BC doses and fertilization. Doses between 8 and 16 t ha^{-1} BC incremented *ca*. 60% the biomass accumulation as compared to plants with 0 t ha^{-1} BC and 0% FL, independently of chemical fertilization treatment (Figure 3A). Finally, Figure 3B shows that leaves, stems, and roots represented about 38%, 24%, and 38% of the total DM in plants without BC or chemical fertilization (0 t ha^{-1} BC+0% FL). However, a redistribution of DM towards the roots was observed when plants were exposed to the highest BC dose (16 t ha^{-1}), especially when 33% and 66% of the chemical fertilization was supplied. In this case, the leaves, stems, and roots represented about 34%, 24%, 40% of the total DM.

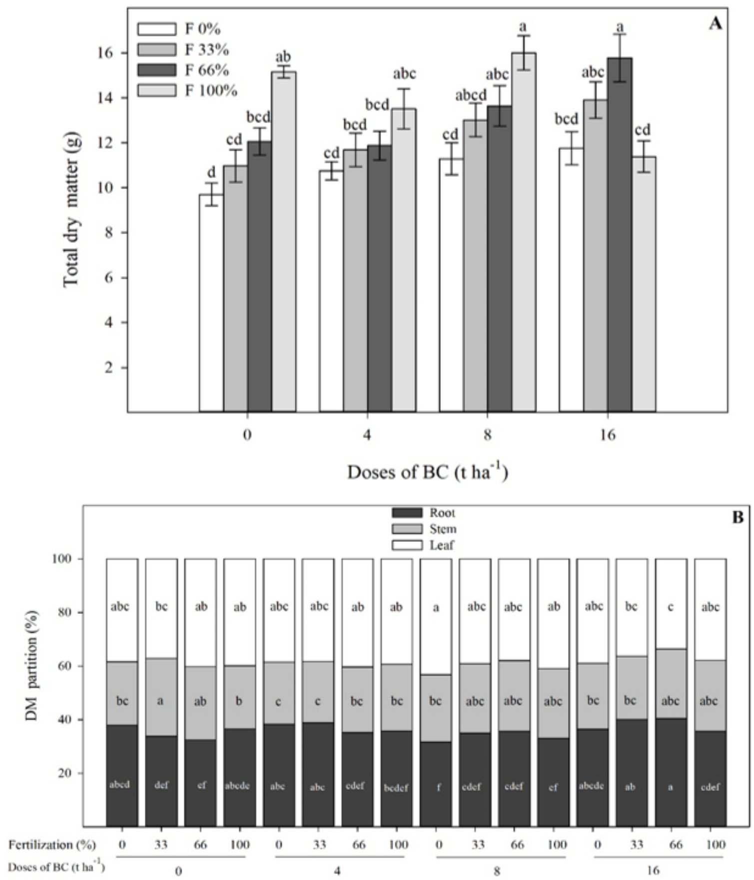

Figure 3. Effect of the application of different doses of biochar (BC) (0, 4, 8, and 16 t ha^{-1}) and levels of chemical fertilization (FL) (0%, 33%, 66%, and 100% of the nutritional requirements) on the total DM (A) and assimilate partitioning (B) of coffee plants at 100 d after transplanting (DAT). Bars represent the mean of five plants ± standard error. (A) Similar letters indicate that the means are not significantly different according to the Tukey test at $P \leq 0.05$. (B) Similar letters between white bars indicate that the means of the assimilate partitioning in leaves are not significantly different according to the Tukey test at $P \leq 0.05$. Similar letters between gray bars indicate that the means of the assimilate partitioning in stems are not significantly different according to the Tukey test at $P \leq 0.05$. Similar letters between black bars indicate that the means of the assimilate partitioning in roots are not significantly different according to the Tukey test at $P \leq 0.05$.

Plant height was also lower with 0 t ha^{-1} BC+0% FL (18.9 cm) compared to 4 and 8 t ha^{-1} BC at different levels of fertilization (FL). Higher levels of chemical fertilization, especially between 0 and 8 t ha^{-1} BC, caused a greater increase in this variable. In this range of BC doses, plants treated with 66% or 100% chemical fertilization showed an average height of 23.8 cm. However, the use of 16 t ha^{-1} BC caused growth inhibition at all levels of chemical fertilization, finding values close to those obtained in the FL treatments with 0 t ha^{-1} BC (which ranged between 21.3 and 22.3 cm) (Figure 4A). Figure 4B shows the analysis of plant growth expressed as RGR. Trends similar to those obtained in plant height were observed for this variable. Plants treated with 0 t ha^{-1} BC+0% FL also showed a lower RGR (2.91 mm cm^{-1} d^{-1}). In addition, a positive effect was found when the plants received increasing levels of fertilization, especially at 8 t ha^{-1} BC (values ranged between 3.12 and 3.21 mm cm^{-1} d^{-1}). Finally, the increase in fertilization levels in plants with 16 t ha^{-1} BC did not represent a significant change in RGR, whose values were on average 3.04 mm cm^{-1} d^{-1}.

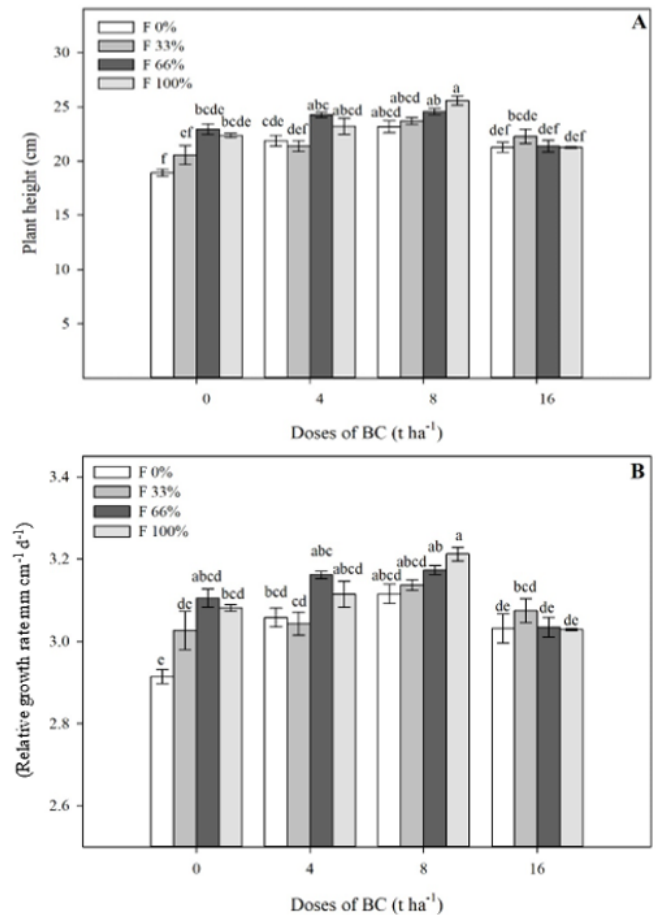

Figure 4. Effect of the application of different doses of biochar (BC) (0, 4, 8, and 16 t ha^{-1}) and levels of chemical fertilization (FL) (0%, 33%, 66%, and 100% of the nutritional requirements) on plant height and relative growth rate of coffee plants at 100 d after transplanting (DAT). Bars represent the mean of five plants ± standard error. Similar letters indicate that the means are not significantly different according to Tukey test at $P \leq 0.05$.

Water use efficiency and agronomic efficiency

A progressive increase in WUE was generally evidenced as the level of chemical fertilization was higher, regardless of the dose of BC applied (Figure 5A). Plants without chemical fertilization (0% FL) registered the lowest WUE values with the four doses of BC applied (WUE ranged from 3.03 to 3.67 g DM L^{-1} H_2O). In addition, WUE was favored at a higher dose of FL (4.22 and 5.00 g DM L^{-1} H_2O in plants with 4 and 8 t ha^{-1} BC and 100% FL) except for the group of plants with 16 t ha^{-1} BC (~ 3.56 g DM L^{-1} H_2O with 100% FL). A similar trend was also observed on AE which increased by 65% in plants with 100% FL in almost all BC amendments compared to plants without BC and FL (Figure 5B).

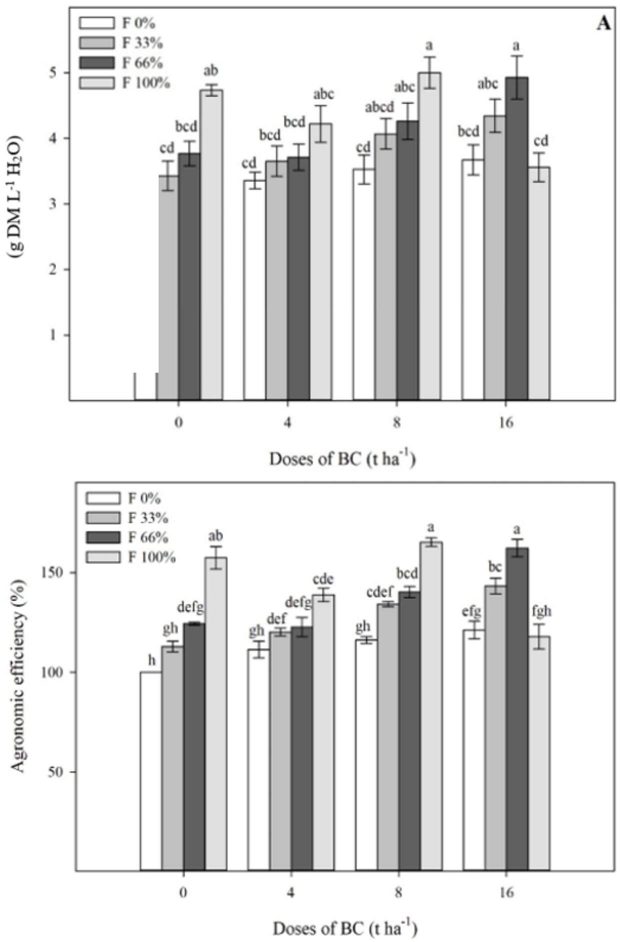

Figure 5. Effect of the application of different doses of biochar (BC) (0, 4, 8, and 16 t ha^{-1}) and levels of chemical fertilization (FL) (0%, 33%, 66%, and 100% of the nutritional requirements) on the water use efficiency (WUE) and agronomic efficiency of coffee plants at 100 d after transplanting (DAT). Bars represent the mean of five plants ± standard error. Similar letters indicate that the means are not significantly different according to Tukey test at $P \leq 0.05$.

Principal component analysis (PCA) biplot

The PCA shows that the variables were indicated by vectors, while the treatments were indicated by points (Figure 6). In general, PCA1 and PCA2 represented 66.7% and 14.3% of the variation of the different attributes analyzed, respectively. The vectors of WUE, TDW, F_v/F_m, plant height, and g_s have angles close to the origin, showing a high correlation between the variables regarding the physiological behavior of coffee plants. The plants with 0 and 8 t ha^{-1} BC at 100% FL and those with 16 t ha^{-1} BC at 66% FL were grouped on the extreme right sector of the biplot analysis (group V). On the contrary, the application of 0 t ha^{-1} BC and 0% FL (group I) was located in the sector opposite to group V, indicating a negative effect of BC doses and fertilization levels on the variables evaluated. Additionally, three differential effects were observed in the BC doses and FL in coffee plants: i) 0 t ha^{-1} BC+33% FL and 4 t ha^{-1} BC+0% FL (group II) behaved with the same tendency as group I; ii) 0 t ha^{-1} BC+66% FL, 8 and 16 t ha^{-1} BC+0% FL, 4 t ha^{-1} BC+33% FL, and 16 t ha^{-1} BC+100% FL (group III) showed a lower negative effect on the physiological variables evaluated. Finally, iii) plants with 4 t ha^{-1} BC+100% FL, 8 and 16 t ha^{-1} BC+33% FL, and 8 t ha^{-1} BC+66% FL (group IV) registered the best physiological response, showing a similar trend to group V.

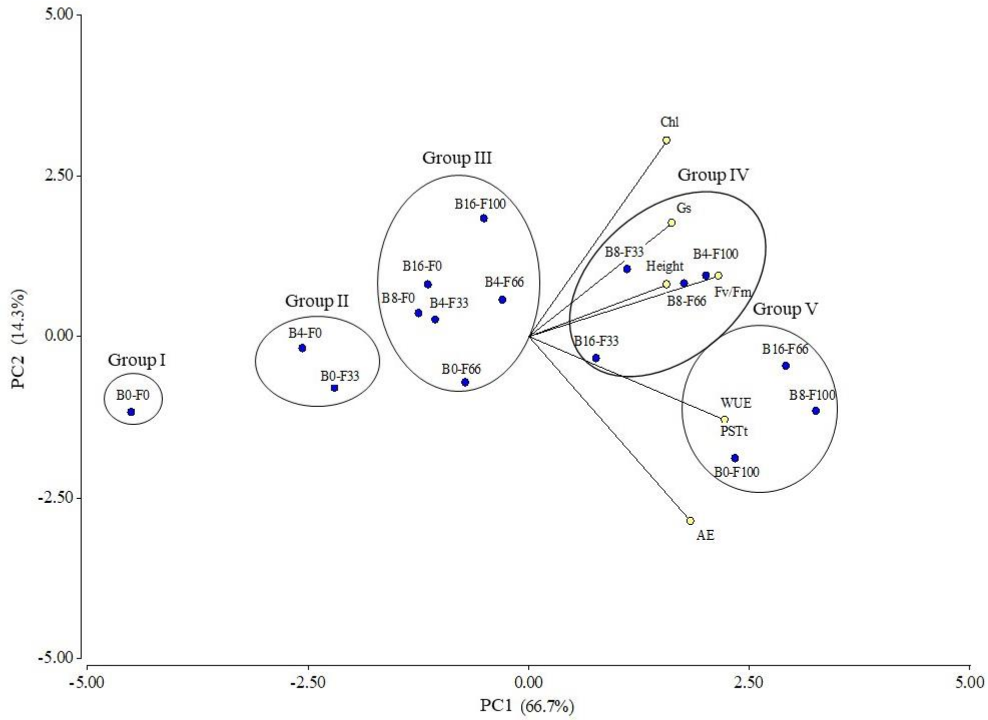

Figure 6. Principal component analysis (PCA) of different physiological variables in coffee (*Coffea arabica*) plants under different doses of biochar and levels of chemical fertilization. Chl: Chlorophyll content (At-leaf readings); g_s: leaf stomatal conductance; Height: plant height; F_v/F_m: maximum quantum efficiency of photosystem II (PSII); WUE: water use efficiency; TDW: total dry weight; AE: agronomic efficiency. B0: biochar dose 0 t h^{-1}; B4: biochar dose 4 t h^{-1}; B8: biochar dose 8 t h^{-1}; B16: biochar dose 16 t h^{-1}. F0: 0% of the nutritional requirements; F33: 33% of the nutritional requirements; F66: 66% of the nutritional requirements; F100: 100% of nutritional requirements.

DISCUSSION

The use of BC has been reported as an alternative for conserving nutrients in the soil and improving crop growth conditions (Domingues et al., 2017). However, the physiological responses of coffee plants to the application of BC with different FL have been little studied. The results of this research show that coffee plants responded to BC applications, mainly at 8 t ha^{-1}, and when seedless were well supplied with chemical nutrients (66% and 100% of the nutritional requirements), since physiological parameters, such as g_s, WUE, photosynthetic pigment content, DM accumulation and AE, were favored. Recent studies have reported that crop yield and agronomic performance can be improved with the application of BC especially when chemical fertilization is accompanied by organic or inorganic fertilizers (Arif et al., 2017). Positive results of the use of BC combined with other fertilizer sources have also been found in maize (*Zea mays* L.) (Tanure et al., 2019) and citrus trees (Zhang et al., 2021). These studies reported that BC applications (with doses between 2% and 50% w:v or from 10 to 50 t ha^{-1}) improved growth, nutritional status, and plant yield.

Nutrient deficiencies can generate stomatal limitations of photosynthesis (Morales et al., 2018). The results of this study confirmed that coffee plants without an adequate nutrient supply (0 t ha^{-1} and 0% FL) registered a greater stomatal closure (Figure 1A). However, coffee plants treated with 4, 8 and 16 t ha^{-1} BC registered a higher opening when the level of chemical fertilization was also higher. Similar trends were reported by Tanure et al. (2019), who observed that additions of BC made with eucalyptus residues (between 20 and 60 g kg^{-1} soil) caused high values of g_s and photosynthesis and a greater relative water content (RWC) in maize plants. These authors indicate that BC applied to the soil can improve the specific surface and increase porosity, favoring water retention and availability in the soil. Liu et al. (2016) also suggested that mixing sandy soils with BC reduces the infiltration rate, keeping water closer to the surface for longer.

The results obtained also showed that high g_s values are related to an increase in K_r, especially when 8 t ha^{-1} BC was added to plants (Figure 1B), indicating a greater water flow in coffee plants. Shashi et al. (2018) also reported the positive effects of applications between 5 and 20 t ha^{-1} BC made with rice husk on the water relations of maize plants, observing a progressive increase in the RWC with the addition of BC. Finally, Tanure et al. (2019) concluded that plants grown with BC applications obtained a high biomass production, generating a greater stomatal opening and higher WUE.

The Chl content (atLEAF readings) and the F_v/F_m were considerably lower in coffee plants with a 0% FL and without the BC application. However, increases in the content of photosynthetic pigments and the F_v/F_m were registered with higher doses of BC and FL, especially when 8 t ha^{-1} were added to plants (Figure 2). Farhangi-Abriz and Torabian (2018) found that applications of BC made with maple (*Acer pseudoplatanus* L.) residues (between 50 and 100 g kg^{-1} soil) favored the synthesis of chlorophyll *a*, *b*, total and the F_v/F_m in soybean plants. These authors indicate that BC applications improved the N metabolism of soybean plants because of factors such as nutrient input in BC ashes (which helps to improve N form and availability) and soil biota. It has been observed that higher contents of photosynthetic pigments in plants treated with BC are related to a higher concentration of foliar N compared to plants without BC application (Naeem et al., 2018).

The increase in DM in plants with BC application has been recorded in crop species such as sorghum (Deng et al., 2019). Such increase was also observed in this study when plants with an application of 4 and 8 t ha^{-1} BC and 100% FL showed higher values of biomass production compared to plants with 0 t ha^{-1} and 0% FL. Tanure et al. (2019) reported that high doses of BC improved soil fertility, nutritional status, and plant growth. Also, Saha et al. (2019) observed that green chireta (*Andrographis paniculata*) plants treated with 5 t ha^{-1} BC (made with residues of lemongrass, *Cymbopogon flexuosus*) together with 60 kg ha^{-1} N (urea), 20 kg ha^{-1} P (single super phosphate) and 40 kg ha^{-1} K (muriate of potash) showed an increase in the accumulation of fresh and DM due to higher microbial biomass, C content and dehydrogenase and phosphatase activity in the soil. On the other hand, Ye et al. (2019) reported that BC application rates greater than 10 t ha^{-1} did not contribute to a higher crop yield (in the short term), indicating that the benefits observed during the first year are mainly related to a liming effect of BC in the soil. These authors also mention that the N in BC is largely unavailable since it is mostly present as heterocyclic aromatic N. An initial immobilization of N in the soil can occur since the organic C in BC is easily mineralized, so it is advisable to add some form of available N (either organic or inorganic) together with BC. This agrees with what was observed in this study, showing that doses of 16 t ha^{-1} did not cause higher biomass in coffee plants (Figure 3A).

The results of this study show a series of important advantages for the cultivation of coffee based on the management of crop nutrition using BC. It is important to note that the incorporation of BC into the soil accompanied with fertilizers of chemical synthesis is a strategy that has been used in other species to improve plant growth and biomass accumulation (El-Naggar et al., 2019), obtaining similar results in coffee cultivation. Another important result of this study is that the use of BC combined with chemical fertilization can improve plant physiology through enhanced g_s and increased water status (better K_r and WUE). These results provide information to recommend the pyrolysis of coffee bean residues to obtain BC and favor the recycling of nutrients to soils in coffee-growing areas. These practices help the sustainability of the productive system since this amendment shows a positive impact on the plant-soil relationship (Lehmann and Joseph, 2015).

CONCLUSIONS

In conclusion, the use of biochar (BC) from coffee pulp combined with inorganic fertilization generates a positive effect on the physiological behavior of coffee plants. The application of 8 t ha^{-1} in combination with chemical fertilization levels of 66% or 100% favored DM accumulation, root hydraulic conductivity, and water use efficiency in 'Castillo el Tambo' coffee seedlings. The above results suggest that the application of BC manufactured with coffee pulp (mainly at 8 t ha^{-1}) can be a tool to complement the plant nutrition management of this crop since it can reduce the levels of chemical fertilizers in the medium term and reduce the cost production.

Author contribution

Conceptualization: H.R-D., L.L., A.D.S-R. Methodology: A.D.S-R., A.C-J. Writing-original draft: A.D.S-R. Writing-review & editing: H.R-D., L.L. Supervision: H.R-D. Project administration: H.R-D. Funding acquisition: A.D.S-R. All co-authors reviewed the final version and approved the manuscript before submission.

Acknowledgements
The authors would like to thank the Government of Tolima, the Administrative Department of Science, Technology and Innovation (COLCIENCIAS, 755-2016, for the training of high-level human capital for Tolima, Colombia) currently known as the Ministry of Science, Technology and Innovation (Minciencias), and the Soil Sciences Research Group - GRICIS endorsed by the Universidad del Tolima for the funding support.

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