Reyes-Herrera DF *et al.* (2023)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 51, Issue 3, Article number 12873
DOI:10.15835/nbha51312873
Research Article

Physiological responses of coffee (*Coffea arabica* L.) plants to biochar application under water deficit conditions

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Abstract

Water deficit is one of the main abiotic stressors in crop production. The development of strategies to improve plant tolerance to water deficits has gained importance. Biochar application can be considered an alternative to mitigate abiotic stress. The use of coffee pulp to produce biochar could be a novel strategy for improving drought tolerance in coffee crops. Coffer plants cv. 'Castillo' were grown in pots or PVC pipes filled with silt loam soil in two separate experiments to evaluate the effect of coffee pulp biochar application on physiological responses under water deficit conditions. Four different biochar doses (0, 4, 8, and 16 t · ha^{-1}) were used. A water deficit was imposed through progressive reduction irrigation (25%, 50%, 75%, and 90% of water lost via evapotranspiration). The leaf gas exchange, maximum quantum yield of PSII (F_v/F_m), biomass, and water status were measured. Reduced irrigation negatively affected the F_v/F_m, leaf gas exchange, biomass, and water status. Biochar (8 t ha^{-1}) increased photosynthesis in both well-irrigated plants (6 µmol m^{-2} s^{-1}) and with reduced irrigation (3.5 µmol m^{-2} s^{-1}) compared to 0 t ha^{-1} biochar (reduced irrigation: 1.8 µmol m^{-2} s^{-1} and well irrigated: 3.9 µmol m^{-2} s^{-1}). In conclusion, 8 t ha^{-1} biochar can be a recommended practice for coffee production, not only to capture carbon and reintroduce it to the soil, but also to alleviate the effects of moderate water deficit. In future investigations, biochar application can be evaluated as an alternative to soil management or coffee plant nutrition, and its interaction with drought stress scenarios.

Keywords: biochar application; coffee; drought; oxidative stress; stomatal conductance

Abbreviation: BC, biochar; WI, well-irrigated; RI, reduced irrigation; g_s, stomatal conductance; P_n, net photosynthetic rate; *Ci*, intercellular CO_2 concentration; WUE, water use efficiency; Chl, leaf relative chlorophyll content; F_v/F_m, maximum photochemical efficiency of PSII; K_r, root hydraulic conductivity; DQE, decrease in F_v/F_m; UAE, unit area efficiency.

Received: 07 Sep 2022. Received in revised form: 20 Jul 2023. Accepted: 21 Aug 2023. Published online: 31 Aug 2023.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Introduction

Coffee is one of the most important crops grown in Colombia and *Coffea arabica* L. is the most cultivated species in the country. In 2020, this species reached 834,400 tons, covering an area of 844,743.78 ha (Federación Nacional de Cafeteros de Colombia, 2021). Colombia is considered the third-largest producer in the world, with approximately 15 million 60-kg bags, after Brazil and Vietnam (Organización Internacional del Café, 2016).

Coffee cultivation generates several by-products such as fresh pulp (Montilla, 2006). For each million 60-kg bags of green coffee exported by Colombia, 162,900 t of fresh pulp was obtained (Rodríguez and Zambrano, 2010). If not processed properly, large quantities of these by-products can lead to environmental pollution (Echeverria and Nuti, 2017), as well as losses of organic matter that could instead be used to increase the amount of organic carbon in the soil. However, the appropriate handling of these products is frequently associated with high energy costs and is thus disposed of in ways that do not permit the efficient use of these carbon sources (Rodríguez-Frómeta *et al.*, 2020). Additionally, the presence of phytotoxic substances and organic acids in coffee by-products has been documented and can affect soil and water quality, as well as restrict crop growth if not properly handled (Dadi *et al.*, 2019). The accumulation of these pollutants has motivated the search for value-added products from coffee residues, with a focus on solving some of these environmental problems and reducing carbon emissions (Gonçalves *et al.*, 2013). In this context, the use of fresh coffee pulp for biochar (BC) production has been tested as a promising alternative because it can be incorporated into the soil to improve its physical, chemical, and microbiological characteristics, while increasing crop yields (Lehman, 2009; Jeffery *et al.*, 2016; Hussain *et al.*, 2019). BC is a carbon-rich product that originates from the pyrolysis of biomass, generally of plant origin. It is obtained via pyrolysis, that is, the transformation of organic matter exposed to high temperatures in an atmosphere with low oxygen availability (Sohi, 2012).

BC can be applied to crops (directly to the soil) as an amendment, together with fertilizers (Steiner *et al.*, 2007; Van Zwieten *et al.*, 2010). Biochar can also be used as an alternative to potting substrates (Zulfiqar *et al.*, 2019). Some studies have found that soil properties such as water retention (Amoakwah *et al.*, 2017), pH (Sorrenti *et al.*, 2016; Zulfiqar *et al.*, 2021), hydraulic conductivity, and nutrient availability increase with the application of BC (Ch'ng *et al.*, 2015; Ahmad *et al.*, 2017). Additionally, BC applications have shown positive effects on plant physiological variables, such as greater germination (Sun *et al.*, 2017), root growth, dry matter accumulation (Olmo *et al.*, 2014), and photosynthesis rate (Batool *et al.*, 2015).

In Colombia, coffee is generally planted on the slopes of the Andes Mountain range (Ocampo-López *et al.*, 2017). The Andean region is affected by climatic variability (ENSO phenomena), which generates reductions in rainfall patterns and increases in temperature (Ruiz and Pabón, 2013). Forecasts on water limitation in coffee production indicate that it will increase in several important coffee regions because of climate change (Bunn *et al.*, 2015; Tounekti *et al.*, 2018). Drought is the main environmental stress that negatively affects coffee production, decreasing the yield by up to 80% in very dry seasons (DaMatta *et al.*, 2018).

Cultivated plants show adverse effects in terms of growth and physiological parameters when subjected to water deficits (Lobell and Gourdji, 2012; Merlaen *et al.*, 2019). Plants modify their water relations to reduce water loss through processes such as transpiration and a decrease in the maximum photochemical efficiency of PSII (F_v/F_m), which can cause a reduction in the accumulation of biomass (Anjum *et al.*, 2011; Marques *et al.*, 2017; Zandalinas *et al.*, 2018). In coffee, severe and prolonged drought conditions negatively affect cell metabolism, which is associated with greater oxidative stress (Dubberstein *et al.*, 2018). Therefore, early leaf senescence is stimulated, resulting in a decrease in total leaf area (DaMatta *et al.*, 2018) and a subsequent reduction in yield (Dubberstein *et al.*, 2018).

Biochar favors water retention in soil because of its high specific surface area (Jeffery *et al.*, 2016; Rajapaksha *et al.*, 2016). Karhu et al. (2011) observed that the water retention capacity of agricultural soils increased with the incorporation of 9 t ha^{-1} of BC. Keshavarz-Afshar et al. (2015) found that the application of 1% and 2% (w/w) BC in *Silybum marianum* L. Gaertn plants under moderate and severe drought stress conditions improved the water retention capacity of the soil. However, the magnitude of this effect is insufficient to influence plant growth and development. In contrast, Saleem-Akhtar *et al.* (2014) found that tomato (*Solanum lycopersicum* L.) plants grown in a soil mixture with BC (5% w/w) under drought conditions showed higher stomatal conductance than plants without BC and under the same stress conditions. Zoghi et al. (2019) also found that application of 30 g kg^{-1} of BC to *Quercus castaneifolia* C.A.M. plants under reduced irrigation conditions (40% field capacity) increased the photosynthetic rate and stomatal conductance by 38% and 39%, respectively, causing an increase in plant height, diameter, and dry matter accumulation. Fischer *et al.* (2019) observed that BC can increase the yield of tomato crops, which is associated with an increase in water use efficiency.

Crop production is closely linked to root development, adequate nutrition, soil structure, and texture. These factors are mainly associated with a good plant water status and the amount of plant-available water in the soil (Sadeghian and Jaramillo, 2016; Morales *et al.*, 2020). Currently, climate change is a significant concern in coffee production, threatening the survival of this crop worldwide (Poltronieri and Franca-Rossi, 2016). These conditions affect bean yield and quality (Silva *et al.*, 2019), generating a need to modify or create agronomic practices to prevent risks (Poltronieri and Franca-Rossi, 2016). These strategies should focus on innovative actions that promote the efficient use of water stored in the soil (Silva *et al.*, 2019) and resource conservation. In this sense, this study sought to evaluate a strategy for utilizing the transformed byproduct of coffee production (pulp) as a soil amendment. The hypothesis of this study was that coffee pulp biochar application mitigates the negative effects of drought stress as a result of reduced irrigation levels in coffee plants, as evidenced by stomatal and non-stomatal limitations of photosynthesis. In addition, our study allowed evaluated a progressive drought condition and its effects on physiological variables of coffee plants The specific objective of this study was to evaluate the effects of the soil application of BC obtained from coffee pulp on the physiological behavior of coffee plants under water deficit conditions. The information obtained in this study can be used by community scientists in future investigations of the physiological behavior of coffee plants under drought stress and biochar application. In addition, coffee farmers can be included in coffee pulp biochar applications as a strategy for drought stress mitigation.

Materials and Methods

Plant material and growth conditions

Two separate experiments were conducted between March and August 2019 in a greenhouse at the Faculty of Agricultural Sciences of the Universidad Nacional de Colombia, Bogotá Campus (4°35'56″ " N and 74°04'51"″ W, altitude 2,556 masl). Three-month-old coffee plants (stage 14 according to the BBCH scale (Arcila-Pulgarín *et al.*, 2002)) of the Castillo variety were purchased from a local nursery and used in two experiments. Fifty-six uniform plants were selected from each batch. In the first experiment, 32 plants were transplanted into 3-L plastic pots to study root hydraulic conductivity. In the second experiment, 24 plants were transplanted into PVC pipes (Protucol PVC S.A.S., Bogotá, Colombia) with 8.84-L capacity (15 cm in diameter and 50 cm in height) to study the responses of roots to the treatments (Shashidhar *et al.*, 2012) and leaf gas exchange parameters. The soil used for both experiments was collected from a coffee farm located in the municipality of Chaparral, Department of Tolima, Colombia (3°49’39.2″ N, 75°34’07.1″ W). The physical and chemical characteristics of the soil were the following: silt loam texture: 20% sand, 54% silt and 26% clay; chemical characteristics: total N: 0.49%, Ca: 9.95%, K: 0.48%, Mg: 2.56%, 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}; pH: 5.56; effective cation exchange capacity (ECEC):13.1 meq/100 g; and electrical conductivity (EC):0.16 dS m^{-1}.

The growth conditions in the greenhouse during the evaluations were as follows: 25/20 °C day/night temperature, relative humidity of 60 to 80%, and a natural photoperiod of 12 h, with photosynthetically active radiation (PAR) of 800-1200 μmol m^{-2} s^{-1} at noon, depending on weather conditions. Five days after transplanting (DAT), the plants were fertilized with 7 g of an 18-46-0 NPK compound fertilizer (DAP®, Yara, Bogotá, Colombia) as an N and P source, and 3 g of a simple N fertilizer (UREA®, Yara, Bogotá, Colombia). In Colombia, it is recommended to apply 7-16 g of N, 4-6 g of P_2O_5, and 10 g of K_2O between the first 2-4 months (Salamanca-Jiménez, 2017). However, K was not applied because the levels found in the soil for this element were considered adequate for the growth stage in which the experiment was conducted (Sadeghian, 2008).

Biochar characteristics and treatments

The BC used was obtained from coffee pulp, which had a medium-level pyrolization at 500 °C for 20 min using a rotatory kiln (6 m long x 0.7 m internal diameter, Tecsol, Bogotá, Colombia). The BC had the following characteristics: pH: 9.42; electrical conductivity: 19.4 dS m^{-1}; organic carbon (OC):46.4%; ashes: 220.8%; 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}; Cationic Exchange Capacity: 103 meq/100g; OC/N ratio: 16.5. BC treatments were established at the time of transplanting the coffee plants by mixing the soil with four different doses of BC in both experiments. The doses of BC used were 0 g/plant (B0), 5 g/plant (B4), 10 g/plant (B8), and 20 g/plant (B16) for the trial in pots, and 0 g plant (B0), 17 g plant (B4), 34 g plant (B8), and 68 g plant (B16) for the trial in PVC pipes. These rates corresponded to field rates of 0, 4, 8, and 16 t ha^{-1}, which were selected based on agronomic responses observed in other cultivated species (Sánchez-Reinoso *et al*., 2020).

Water deficit treatment application

At the end of the acclimatization period of 20 DAT, half (4) of the pots and half (3) of the pipes of each BC rate were assigned to WI (well-irrigated) and half to RI (reduced irrigation). The reduced irrigation treatment was induced by a progressive decrease in irrigation water over four periods of 10 days each. Water reduction was approximately 25, 50, 75, and 90% of the water requirement of coffee plants, which was calculated by measuring the daily water loss due to evapotranspiration of each plant in its respective container. The volume of water applied during each irrigation period was as follows: i) 25% reduction between 20 and 30 DAT (WI pots: 55 mL/plant vs. RI pots: 40 mL/plant; WI pipes: 110 mL/plant vs. RI pipes: 85 mL/plant); ii) 50% reduction between 30 and 40 DAT (WI pots: 55 mL/plant vs. RI pots: 28 mL/plant; WI pipes: 110 mL/plant vs. RI pipes: 55 mL/plant); iii) 75% reduction between 40 and 50 DAT (WI pots: 55 mL/plant vs. RI pots: 15 mL plant; WI pipes: 110 mL/plant vs. RI pipes: 30 mL/plant); and iv) 90% reduction between 50 and 60 DAT (WI pots: 55 mL/plant vs. RI pots: 6 ml/plant; WI pipes: 110 mL/plant vs. RI pipes: 12 mL/plant). Irrigation was performed on days 0 and 5, at the beginning of each period. The experiments lasted for 60 d from DAT. The treatments were set up in a completely randomized factorial design, where the first factor was the water deficit condition (WI and RI), and the second factor was the BC dose (0, 4, 8, and 16 t ha^{-1}) for a total of eight treatments. The test was carried out in pots with four replicate plants per treatment for a total of 32 plants. The test was carried out in PVC pipes with three replicates per treatment for a total of 24 plants.

Leaf gas exchange parameters

Stomatal conductance (g_s) was measured in both experiments at the end of each irrigation period (30, 40, 50, and 60 DAT) on the second fully expanded leaf from the upper part of the canopy using a steady-state porometer (SC-1; Decagon Devices Inc., Pullman, WA). Total plant transpiration (E) was estimated only for the experiment in pots on the same dates as g_s using the gravimetric technique described by Díaz-Leguizamón *et al.* (2016), which consists of measuring the difference in the weight of plants in their pots every 24 h.

The net photosynthesis rate (P_n) and intercellular CO_2 concentration (*Ci*) were measured at the end of the last irrigation period (90%) in the test carried out in pipes only. Similar to g_s, measurements were taken on the second fully expanded leaf of the upper part of the canopy using a portable photosynthesis meter (LI-COR 6200, Lincoln, NE, USA). The chamber conditions during the measurements were as follows: PAR of 800 μmol m^{-2} s^{-1}, leaf temperature of 24 ± 5 °C, and a leaf-to-air vapor pressure difference of 1.8 ± 0.5 kPa. g_s and P_n were measured between 800 and 1100 h (*Rodrigues et al.*, 2018). Carboxylation efficiency was calculated as the ratio of P_n to *Ci* (Alvarado-Sanabria *et al.*, 2017). Finally, water use efficiency (WUE) was calculated using the method described by Raviv and Blom (2001), which estimates the ratio between the total dry weight of the plant and the total amount of water each seedling received during the development of the experiment.

Chlorophyll content and fluorescence

Leaf relative chlorophyll content (Chl) and maximum photochemical efficiency of PSII (F_v/F_m) were measured in the second fully expanded leaf from the upper part of the canopy at the end of each irrigation period in both experiments. The chlorophyll content was determined using a chlorophyll meter (At-leaf, FT Green, Wilmington, DE, USA). Chlorophyll fluorescence parameters were measured using a continuous excitation fluorometer (Handy PEA; Hansatech Instruments, Kings Lynn, UK). After the leaves were allowed to dark-adapt for 30 min using the instrument's leaf clips, minimum fluorescence (F_0), maximum fluorescence (F_m), and maximum photochemical efficiency of PSII (F_v/F_m) were recorded. The F_0 was recorded with low intensity-modulated light (<0.1 μmol m^{-2} s^{-1}) without affecting the fluorescence variable. F_m was measured using a saturating light pulse of 0.8 s (3000 μmol m^{-2} s^{-1}). Variable fluorescence was calculated as the difference between F_0 and F_m. The F_v/F_m ratio was obtained from the F_v and F_m. Additionally, the decrease in F_v/F_m (DQE) was calculated for each treatment according to the methodology described by de Oliveira et al. (2020), such that DQE (%) = [(control F_v/F_m – water deficit F_v/F_m)/(control F_v/F_m) × 100]. Finally, the treatments were classified into four categories: DQE ≤ 25, good tolerance; 26 ≥ DQE ≤ 41, moderate tolerance; 42 ≥ DQE ≤ 55, low tolerance; and DQE ≥ 56, sensitive.

Root hydraulic conductivity and root variables

Root hydraulic conductivity (K_r) and root variables were quantified only in plants grown in the pipes at the end of the last drought stress period (60 DAT). Hydraulic conductivity was estimated between 800 and 1100 h using a hydraulic conductance flow meter (Gen 3; Dynamax, Houston, TX, USA). Readings were performed by cutting the stems of the plants at a height of 5 cm above the ground, keeping the roots within the pipes. The stem diameter ranged between 4 and 5 mm, and the equipment attachment was adapted to this diameter to record measurements in the upper part of the roots. The stem hydraulic conductivity was measured using the transient measurement mode, which rapidly increased the applied pressure and simultaneously measured the corresponding flow (Tyree *et al.*, 1995). Degassed deionized water was forced through the root stem under increasing pressure until it reached 300 kPa. Instantaneous flow and pressure were recorded every 2 s. Hydraulic conductivity (kg s^{-1} kPa^{-1}) was calculated from the linear regression slope between the pressure and flow. The root volume was determined according to the water displacement method, whereas the root length was determined from the crown to the tip of the root and expressed in centimetres according to the techniques described by Shashidhar *et al.* (2012).

Leaf area and dry matter accumulation

At 60 DAT, all plants from both experiments were harvested and destroyed, and the total leaf area, dry matter of organs (leaves, stems, and roots), and total dry matter were measured. Leaf area was calculated from digital images obtained using a photographic camera (D3300; Nikon Corp., Tokyo, Japan). Subsequently, the photos were analyzed using Java image-processing software (Image J; National Institute of Health, Bethesda, MD, USA). The unit area efficiency (UAE) was also estimated and expressed as the quantum of yield produced over a unit land area for a specific period of crop growth as follows: UAE = (yield in dry weight/soil area) × (1/duration of the crop). Finally, the tissues were dried in a compressed dry air oven (Thelco Mod 27, Chicago) at 80 °C for 48 h to obtain the total dry weight of the plant.

Statistical analysis

Repeated measures analysis of variance was performed. When significant differences were observed, Tukey's comparative test of means was used at $P \leq 0.05$. Data were analysed using Statistix v. 9.0, software (analytical software, Tallahassee, FL, USA). Additionally, principal component analysis was performed using the InfoStat 2016 program (Di Rienzo *et al.*, 2016).

Results

Differences in g_s were observed in both experiments from 50 to 60 DAT (Figure 1). At 50 DAT, RI plants showed a significant decrease in g_s, especially with doses of BC < 8 t ha^{-1} of BC, compared to WI plants in the pot test (Figure 1A). The values of this variable also showed a reduction of approximately 53% and 21% for the RI treatments (65 and 110 mmol m^{-2} s^{-1} for B0 and B4 at 50 DAT, respectively) compared to WI plants (140 mmol m^{-2} s^{-1} in B0 plants) in plants grown in pipes (Figure 1B). The application of B8 in RI plants caused an increase of approximately 49% and 13% in g_s, registering values closer to those of WI plants and not treated with BC in both tests (pots: 59 vs. 88 mmol m^{-2} s^{-1}; pipes: 136 vs. 120 mmol m^{-2} s^{-1}, respectively) at 60 DAT. Overall, the different BC doses did not cause a significant variation in g_s in WI plants at different sampling points in either experiment.

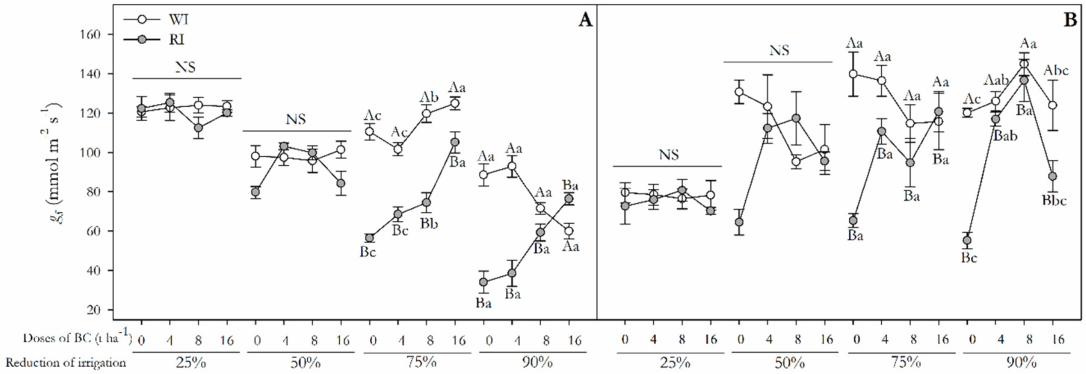

Figure 1. Effect of the application of different doses of biochar (0, 4, 8, and 16 t ha^{-1}) and reduced irrigation (25, 50, 75, and 90% of the evapotranspiration needs) on stomatal conductance (g_s) in leaves of coffee plants established in pots (A) and pipes (B) at four sampling points (30, 40, 50, and 60 days after transplanting (DAT))

WI: well-irrigated plants; RI: plants subjected to reduced irrigation. Bars represent ± standard error (pots n = 4; pipes n = 3). Capital letters refer to differences between irrigation level under each irrigation reduction period. Lowercase letters refer to differences between biochar doses under each irrigation reduction period. The same letters indicate that means are not statistically different according to Tukey's test at $P \leq 0.05$.

Trends similar to those registered for g_s were also obtained for E from 30 to 60 DAT in the pots (Figure 2A). In general, WI plants did not exhibit major variations in transpiration with the four BC doses throughout the experiment. At the end of the experiment (60 DAT), plants with RI and without BC (0 t ha^{-1}) showed a reduction in E of 34% compared to WI plants with the same BC dose (3.75 vs. 5.71 g H_2O $plant^{-1}$ day^{-1}, respectively). The BC application at B8 in RI plants favored *E*. Regarding the root hydraulic conductivity, coffee plants with RI and B0 registered a reduction of 45% (12.71 x 10^{-7} kg s^{-1} MPa^{-1}) compared to WI plants and the same BC dose (23.21 x 10^{-7} kg s^{-1} MPa^{-1}) (Figure 2B). Conversely, the increase in BC doses favored K_r in RI plants at 60 DAT, obtaining a higher value with B8 (17.56 x 10^{-7} kg s^{-1} MPa^{-1}). Overall, K_r did not show variations in WI plants, except for those treated with 16 t ha^{-1} BC, which showed a small reduction.

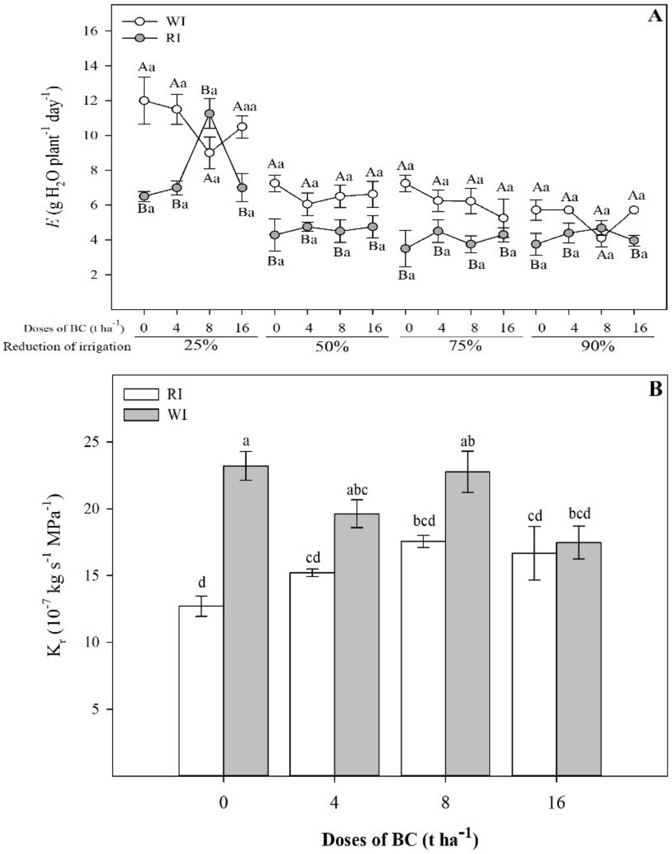

Figure 2. Effects of the application of different doses of biochar (0, 4, 8, and 16 t ha^{-1}) and reduced irrigation (90% of the evapotranspiration needs) in coffee seedlings.

(A) Total transpiration through the experiment of plants grown in pots at four sampling points (30, 40, 50, and 60 days after transplanting (DAT))

WI: well-irrigated plants; RI: plants subjected to reduced irrigation. Points represent the mean of four plants ± standard error. Capital letters refer to differences between stress conditions under each period of irrigation reduction; lowercase letters indicate differences between biochar doses under each period of irrigation reduction. (B) Root hydraulic conductivity (K) at 60 DAT of plants grown in pipes. Bars represent the mean of three plants ± standard error. the same letters indicate that means are not statistically different according to Tukey's test at $P \leq 0.05$.

In general, RI plants showed lower P_n and P_n/C_i values than WI plants, regardless of the tested BC dose (Figure 3). Plants grown in B0 and subjected to reduced irrigation had the lowest values for these parameters (P_n: 1.8 µmol m^{-2} s^{-1}; P_n/C_i:0 .006 µmol m^{-2} s^{-1}, respectively). However, the use of B8 showed an increase of 53% and 94% P_n in both WI plants (6 µmol m^{-2} s^{-1}) and RI (3.5 µmol m^{-2} s^{-1}) compared to B0 (RI: 1.8 µmol m^{-2} s^{-1} and WI: 3.9 µmol m^{-2} s^{-1}), respectively (Figure 3A). A similar trend was observed in carboxylation efficiency for all evaluated treatments (Figure 3B).

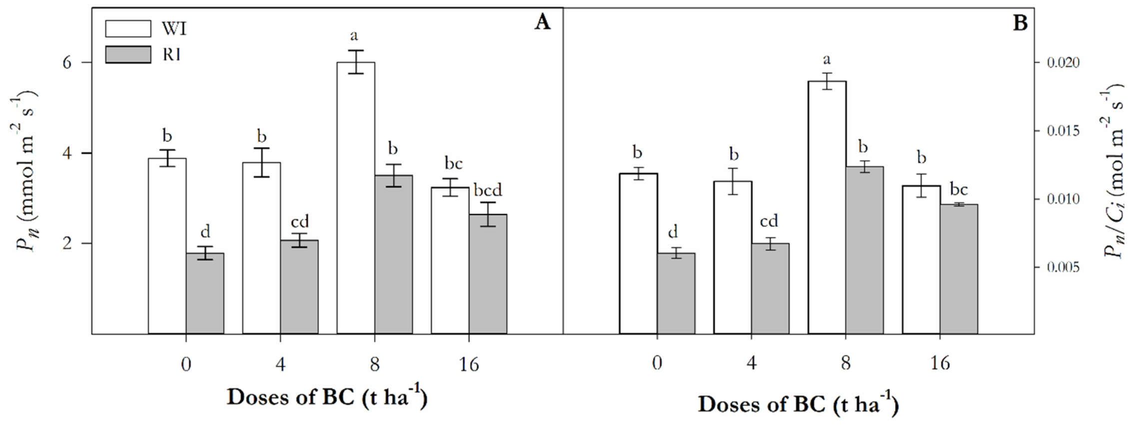

Figure 3. Effects of the application of different doses of biochar (0, 4, 8, and 16 t ha^{-1}) and reduced irrigation (90% decrease in irrigation) on net photosynthesis (P_n) (A) and carboxylation efficiency (P_n/C_i) (B) in coffee plants grown in PVC pipes at 60 DAT

Bars represent the mean of three plants ± standard error. WI: well-irrigated plants; RI: plants subjected to reduced irrigation. Bars with same letters indicate that means are not statistically different according to Tukey's test at $P \leq 0.05$.

A progressive reduction in relative chlorophyll content with a reduction in irrigation volume was mainly observed in plants without BC in both tests (Figure 4A and Figure 4B). Plants with reduced irrigation and a dose of B0 showed the lowest relative chlorophyll content, reaching an approximate reduction of 37% in both experiments at 60 DAT. However, the higher doses of BC (B8 and B16) caused a noticeable increase in the relative chlorophyll content in RI plants, registering values similar to the WI plants with the different doses of BC at the end of the experiments (60 DAT) (pots: 73.8 and 76.1 At-leaf readings; pipes: 67.2 and 71.2 At-leaf readings of B8 and B16, respectively). Likewise, the relative chlorophyll content in WI plants did not vary with different doses of BC in either experiment.

Differences in the F_v/F_m ratio began between the different treatment groups from 50 DAT in the pots and 40 DAT in the pipes (Figures 4C and 4D). At the end of the trial, RI plants grown with B0 showed 59% decline (F_v/F_m: 0.28) and 47% (F_v/F_m: 0.35) in pots and PVC pipes, respectively. Similarly, the progressive increase in BC in RI plants caused a slight increase in this ratio at this sampling point. In contrast, the F_v/F_m in well-irrigated plants treated with different doses of BC was ≈0.65 in both experiments. Finally, the DQE calculated for coffee plants grown in pots with 0 t ha^{-1} BC reached values close to 60% (sensitivity to drought); B4 registered a DQE of 44% (low tolerance to drought), and B8 and B16 had an average of 35% (moderate tolerance) (Figure 4E). In plants grown in pipes, the DQE for the B0 dose was significantly greater than that of the other doses (≈47% vs. ≈35%) (Figure 4F).

Differences were found between the BC treatments, reduced irrigation, and their interaction with dry matter accumulation in roots, shoots, and total and water use efficiency (Table 1). In general, RI plants and plants without BC showed lower total dry weight (TDW) than WI plants in both experiments. RI plants without BC (dose 0 t ha^{-1}) showed a lower total dry matter compared to their controls (pots: 5.0 g vs. 6.5 g; pipes 8.6 g vs. 9.9 g, respectively). However, the increase in the doses of BC favored biomass accumulation in RI plants, reaching values similar to those of WI plants at each dose. Likewise, no differences were found between the different doses of BC in WI plants in either test (Figure 5A and 5D). Dry matter accumulation in the shoots showed trends similar to those observed for the total biomass. RI plants and B0 had a lower weight compared to WI plants with the same BC dose for both tests (pots: 3.2 g vs. 4.6 g; pipes: 5.0 g vs. 6.7 g). It was also observed that different doses of BC caused a positive increase in the dry matter of the shoot in RI plants, whereas WI plants did not show variations (Figure 5B and 5E). The root dry weights showed different trends between the trials.

Table 1. Summary of the analysis of variance of the effect of the application of different doses of biochar (0, 4, 8, and 16 t ha^{-1}) and irrigation levels (100% evapotranspiration vs. reduced irrigation) on root hydraulic conductivity (K), total dry weight (TDW), root dry weight (RDW), shoot dry weight (SDW), water use efficiency (WUE), unit area efficiency (UAE), net photosynthetic rate (P_n), carboxylation efficiency (P_n/C_i), root volume, of coffee plants grown in two different types of containers (pots and PVC pipes) measured at a single sampling point in both experiments (at 60 DAT)

Pot test			
Variable	Source of variation		
	Biochar	Irrigation level	Biochar × Water stress
Root hydraulic conductivity	NS	***[z]	**
Total dry weight	**	***	NS
Root dry Weight	***	*	**
Shoot dry weight	NS	**	**
Water use efficiency	**	***	*
Unit area efficiency	**	***	*
Pipe test			
Variable	Source of variation		
	Biochar	Irrigation level	Biochar × Water stress
Photosynthesis	***	***	*
Carboxylation efficiency	***	***	**
Total dry weight	**	**	*
Root dry weight	***	***	NS
Shoot dry weight	***	***	**
Water use efficiency	**	***	**
Root volume	*	***	*
Unit area efficiency	**	**	**

[z] *, **, and *** are significantly different at the probability levels of 0.05, 0.01, and 0.001, respectively. NS, not significant.

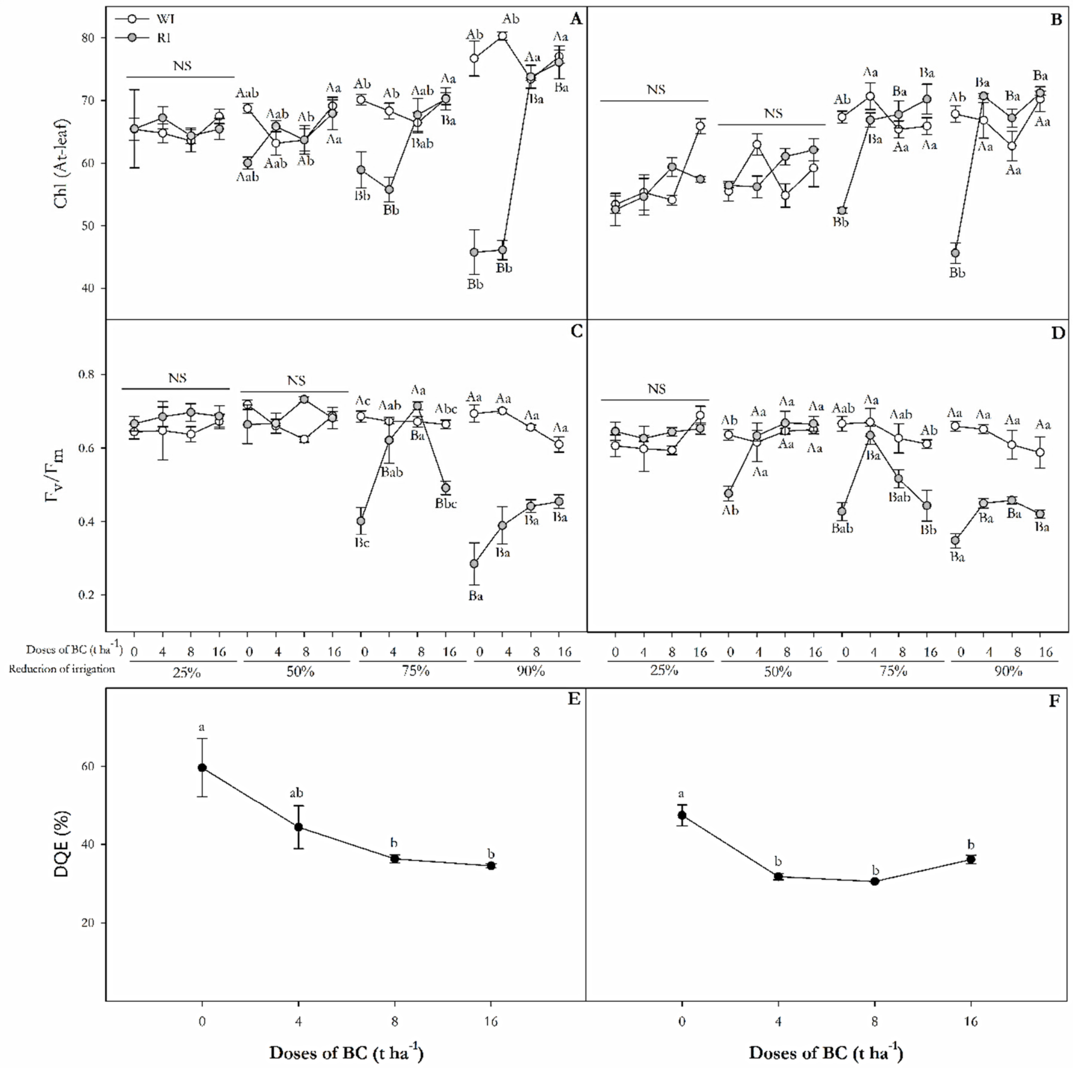

Figure 4. Effect of the application of different doses of biochar (0, 4, 8, and 16 t ha^{-1}) and reduced irrigation (25, 50, 75, and 90% of the evapotranspiration needs) on the relative content of chlorophyll expressed as At-leaf units (A and B), maximum photochemical efficiency of PSII (F_v/F_m) (C and D) and the decrease of the F_v/F_m ratio (DQE) at 60 DAT (90% decrease in irrigation) (E and F). Figures A, C, and E refer to plants grown in pots; figures B, D, and F represent those grown in pipes

WI: well-irrigated plants; RI: plants subjected to reduced irrigation. Points or bars represent the mean of four plants from the pot test and three plants from the pipe test ± standard error. In figures A, B, C, and D, the capital letters refer to differences between the stress conditions under each period of irrigation reduction; lowercase letters indicate differences between biochar doses under each period of irrigation reduction. In Figures E and F, the same letters indicate that the means are not statistically different according to Tukey's test at $P \leq 0.05$

WI plants grown in pots with the application of B4 and B8 registered an increase close to 90% in the root dry matter compared to plants without BC application (3.7 and 3.6 g vs. 1.9 g, respectively) (Figure 5C). In RI plants, greater dry matter accumulation in the root was observed with B8 and B16, with no differences between WI and RI plants. On the other hand, the incorporation of B16 showed the lowest values of dry matter in the root in WI and RI plants, compared to plants without BC under both irrigation conditions in the plants grown in pipes (B16 2.8 g and RI 3.0 g vs. B0 3.2 g and RI 3.5 g, respectively) (Figure 5F).

RI plants had higher WUE values than WI plants at 60 DAT in both experiments. However, the application of B8 induced an increase of 50% in the WUE in RI plants grown in pots (8.6 vs. 5.7 g dry matter L^{-1} H_2O) and 36% in plants grown in PVC pipes (6.0%).

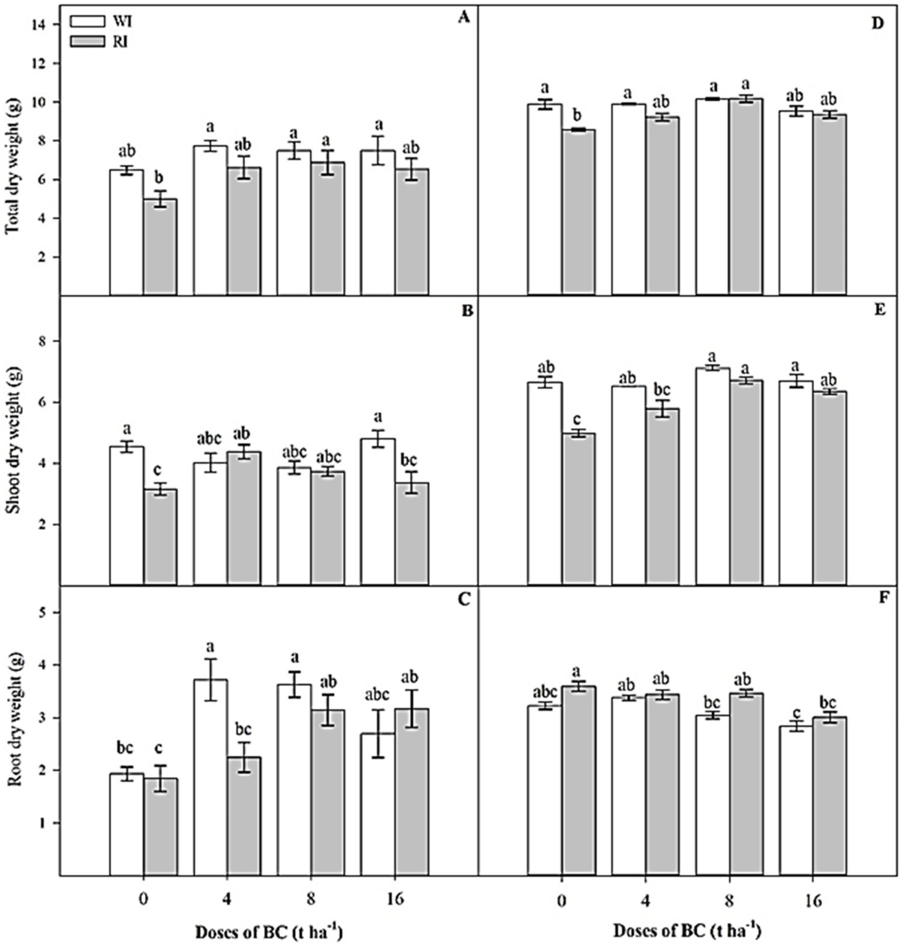

Figure 5. Effect of the application of different doses of biochar (0, 4, 8, and 16 t ha^{-1}) and reduced irrigation on the dry matter accumulation in different organs in coffee plants at 60 DAT (90% decrease in irrigation): A and D) Total dry weight; B and E) Dry weight of the shoot; C and F) Dry weight of the root. Figures A, C, and E represent the pot test; figures B, D, and F represent the test in pipes

WI: well-irrigated plants; RI: plants subjected to reduced irrigation. Bars represent the mean of four plants from the pot test and three plants from the pipe test ± standard error. The same letters indicate that means are not statistically different according to Tukey's test at $P \leq 0.05$.

RI plants showed higher values of WUE compared to WI plants at 60 DAT for both experiments. However, the application of B8 induced an increase of 50% in the WUE in RI plants grown in pots (8.6 vs. 5.7 g dry matter L-1 H2O) and of 36% in the plants grown in PVC pipes (6.0 vs. 4.4 g dry matter L-1 H2O) (Figure 6A and 6B, respectively). Likewise, RI caused a decrease in UAE in plants without BC application (pots: 281 vs. 391 µg cm^{-2} day^{-1}; pipes: 324 vs. 373 µg cm^{-2} day^{-1}). However, the application of B8 also caused a higher UAE in RI plants in both experiments compared to plants with B0 under stress (pots: 420 µg cm^{-2} day^{-1}; pipes: 383 µg cm^{-2} day^{-1}) (Figure 6C and Figure 6D).

The roots of WI-irrigated plants without the application of BC had an average biomass of 3.2 g, length of 39 cm, and volume of 21.3 mL (Figure 7). In contrast, RI plants with B0 showed shorter roots and lower volume, but a higher dry weight than WI plants (root length: 35.8 cm; root volume: 22.4 mL and RDW: 3.6

g). In contrast, the application of B8 increased root volume and weight in RI plants, reaching the highest values among all treatments (root volume: 27.6 mL and root length: 35 cm).

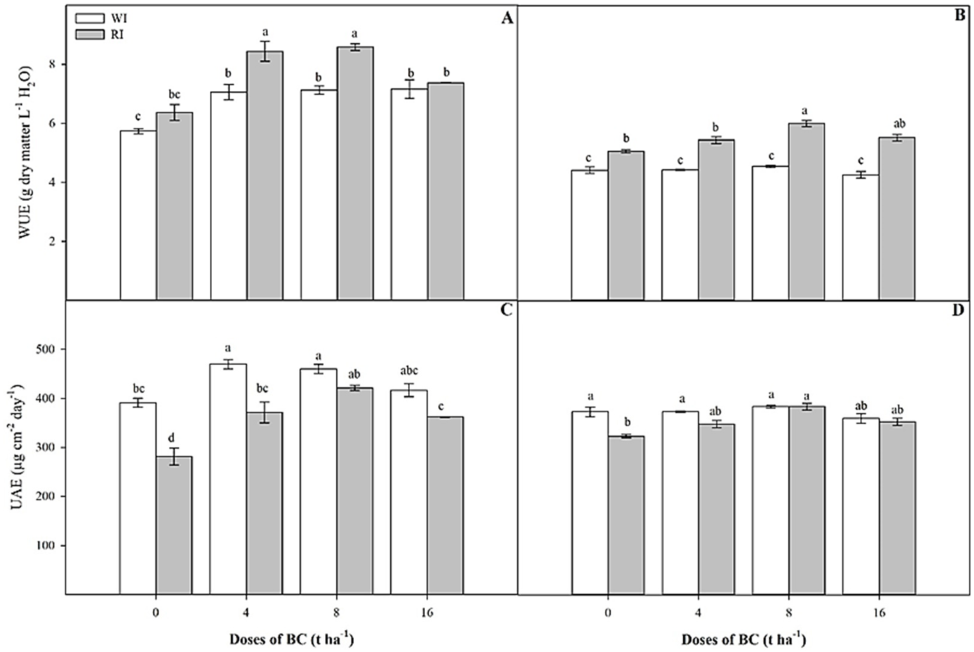

Figure 6. Effect of the application of different doses of biochar (0, 4, 8, and 16 t ha^{-1}) and reduced irrigation (25, 50, 75, and 90% of evapotranspiration needs) on the water use efficiency (WUE) (A and B) and the unit area efficiency (UAE) (C and D) in coffee plants at 60 DAT (90% decrease in irrigation)

WI: well-irrigated plants; RI: plants subjected to reduced irrigation. Figures A and C refer to plants grown in pots; figures B and D to plants grown in pipes. Bars represent the mean of four plants in the pot test and three plants in the pipe test ± standard error. Bars values indicated by same letters are not statistically different according to Tukey's test at $P \leq 0.05$.

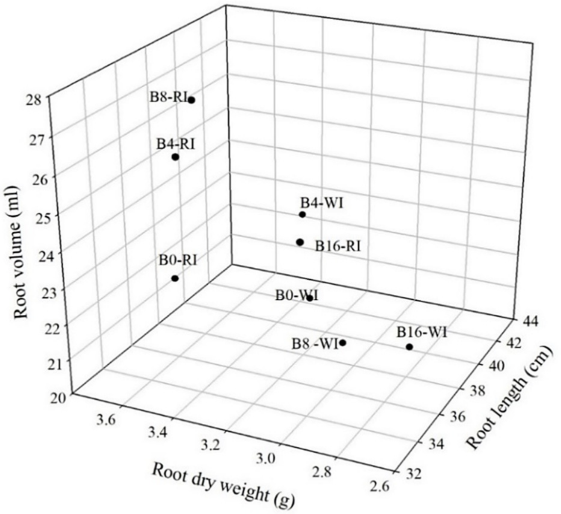

Figure 7. Three-dimensional graph (root length, root dry weight, and root volume) for coffee plants under water deficit conditions of the experiment carried out in PVC pipes at 60 DAT (90% decrease in irrigation). B: biochar dose; WI: well-irrigated plants; WI: reduced irrigation plants. Data correspond to the mean of three data points

PCA1 and PCA2 represented 61.9 and 20.8% of the variation in the studied attributes, respectively (Figure 8). Based on the above, the vectors UAE, TDW, g_s, shoot dry weight (ShDW), P_n, P_n/C_i, leaf dry weight (LDW), LA, and F_v/F_m showed angles close to the origin, indicating that there is a high correlation between the physiological behavior of coffee plants and these variables. The application of 8 t ha^{-1} BC to WI plants formed a unique group (V). In contrast, RI plants without the application of BC (group I) were located in the sector opposite to group V, indicating a negative effect of reduced irrigation on physiological variables. Three differential effects were also observed in the application of BC for the two irrigation conditions of coffee plants: i) RI plants with B4 and B16 behaved similarly to group I; ii) 8 t ha^{-1} BC (group III) had a less positive effect on the evaluated physiological variables of IR plants; and iii) WI plants with 0, 4, and 16 t ha^{-1} BC (group IV) had the best physiological response, showing behavior similar to that of group V.

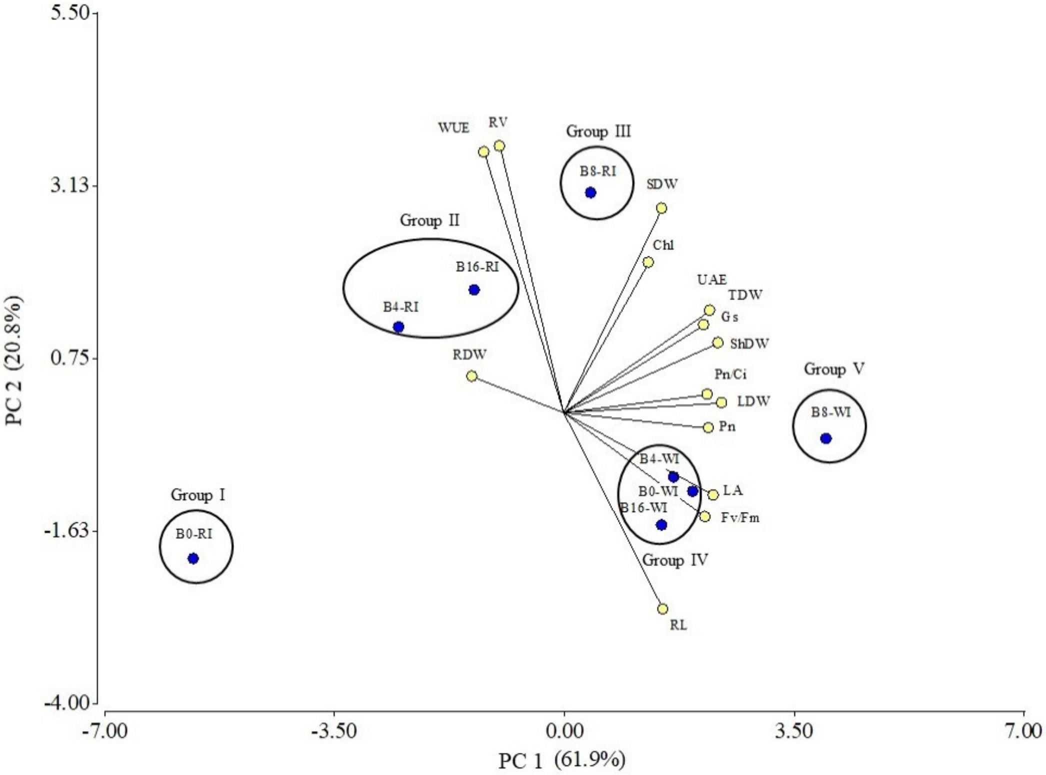

Figure 8. Principal component analysis (PCA) biplot of different physiological variables in coffee plants under conditions of well or reduced irrigation in the experiment carried out in PVC pipes measured at 60 DAT

The treatments are identified by points, while the variables are represented by vectors. RDW: root dry weight; RV: root volume; RL: root length; Chl: At-leaf chlorophyll content; SDW: stem dry weight; TDW: total dry weight; ShDW: shoot dry weight; g_s: stomatal conductance; UAE: unit area efficiency; LDW: leaf dry weight; P_n: net photosynthesis; P_n/C_i: Carboxylation efficiency; LA: leaf area; F_v/F_m: maximum photochemical efficiency of PSII; WI: well-irrigated plants; RI: plants subjected to reduced irrigation. B0: biochar dose of 0 t h^{-1}; B4: biochar dose of 4 t h^{-1}; B8: biochar dose of 8 t h^{-1}; B16: biochar dose of 16 t h^{-1}.

Discussion

Biochar has been reported to be an alternative for the relief of abiotic stress in plants (Ali *et al.*, 2017; Riaz *et al.*, 2019). However, little is known regarding the effect of BC application on the physiological behavior of coffee plants under water deficit conditions. In this study, the soil application of different doses of BC (0, 4, 8, and 16 t ha^{-1}) to plants of this species was evaluated under conditions of water deficit. It was found that the application of 8 t ha^{-1} of BC favored some physiological parameters such as P_n, g_s, WUE, and Chl content under reduced irrigation conditions. Recent studies have shown that BC application mitigates the effects of water deficit in crops, such as corn (Romdhane *et al*., 2019), wheat (Abbas *et al*., 2018), and soy (Hafeez *et al*., 2017). These authors observed that BC improved growth, photosynthesis, and yield. It also reduced oxidative stress, possibly due to improvements in the physicochemical characteristics (pH, electrical conductivity, and content of nutrients in the soil) and water content of the treated soils. From a physiological point of view, BC applications buffer the effects of water deficit on leaf photosynthesis because BC can stimulate chlorophyll synthesis and a less pronounced reduction in stomatal conductance (Wu *et al.,* 2023).

The reduction in the assimilation of CO_2 caused by drought may be due to stomatal limitations (Fracasso *et al*., 2016). Additionally, non-stomatal limitations have been recorded in the form of biochemical and structural damage under severe drought conditions (Keshavarz-Afshar *et al*., 2015; Drake *et al*., 2017). In the present study, a negative impact of RI on the gas exchange properties (P_n, g_s, and E) was observed when the BC applications were low (B0 and B4). However, it has been proven that BC applications can help mitigate the damage associated with water deficits in plants (Abbas *et al*., 2018; Abideen *et al*., 2020) because BC helps to retain more water in the soil (Kuzyakov, 2009; Tayyab *et al*., 2018), decreases resistance to root penetration (Busscher *et al*., 2010), and improves the availability of nutrients to plants (Liang *et al*., 2006). The results showed that the application of 8 t ha^{-1} of BC mitigated the negative effects of RI in coffee plants and even stimulated photosynthesis in WI plants (Figure 3). This agrees with the findings of Lyu *et al.* (2016), who observed that application of 9 t ha^{-1} of BC improved the soil water content, generating a positive effect on water potential and photosynthesis in *Pyrus ussuriensis* Maxim. plants. Abideen *et al.* (2020) also reported that an advantage of BC application is the greater availability of water in the soil, allowing a moderate increase in g_s and an increase in P_n.

Relative chlorophyll content values (At-leaf readings) and F_v/F_m ratio were considerably decreased by RI, especially in plants without BC application. However, increases in the content of photosynthetic pigments and F_v/F_m were registered with an increase in BC doses, indicating a positive effect on the mitigation of the water deficit impact. Keshavarz-Afshar *et al.* (2015) observed that edaphic applications of 1 and 2% (w/w) BC improved the synthesis of chlorophyll pigments through greater water retention in the soil of *Silybum marianum* L. plants under drought conditions. In contrast, Lyu *et al.* (2016) found that the kinetics of chlorophyll fluorescence was affected to a lesser extent in *P. ussuriensis* plants under drought stress and treated with BC. These authors also suggested that the better performance of plants treated with BC was due to a lower rate of decrease in the soil water level caused by BC, generating a positive effect on the leaf water potential. Gavili *et al.* (2019) reported that a higher level of photosynthetic pigments in soybean plants treated with BC was related to a higher concentration of foliar N than in plants without BC application. In addition, BC leads to a significant increase in chlorophyll content under normal and stressful conditions because BC-treated soils provoke Mg^{2+} uptake, which is considered a building block in the synthesis of chlorophyll. On the other hand, BC can improve N nutritional status, the photosynthetic electron transfer rate, and the proportion of open PSII reaction centers, causing full use of the light energy absorbed by leaves for photosynthesis and higher efficiency of photosystem II (Fv/Fm ratio) (Wang *et al.,* 2021).

In the present study, lower dry matter accumulation was observed in RI plants without BC application. Pranata-Erdiansyah *et al.* (2019) also observed a decrease in biomass in *Coffea canephora* L. plants under conditions of RI at 66% field capacity. However, the results obtained in this study showed that high BC application (> 8 t ha^{-1}) favored dry matter accumulation under RI conditions. Similar trends were also observed by Gavil *et al.* (2019), who reported that an edaphic application of 1.25% (w/w) BC significantly increased the dry (35%) and fresh (27%) matter yields compared to the control (0% BC) in soybean plants with and without water stress. Deng *et al.* (2019) also concluded that the benefits of BC application on plant growth in environments with water stress were due to an increase in the water retention capacity of the soil and an increase in the soil carbon content resulting from the dose of BC used. Additionally, BC application can optimize root length, root surface area, and root density and improve root cell viability, causing the root system to be a key pathway regulating aboveground plant growth under deficit irrigation conditions (Zhang *et al.*, 2023)

UAE and K_r also increased in RI plants, and B8. K_r can be influenced by factors such as root anatomy and architecture (Yin *et al.*, 2014). In this study, the application of BC (8 t ha^{-1}) increased the volume, length, and dry matter of the roots under reduced irrigation. Romdhane *et al.* (2019) and Abideen *et al.* (2020) observed higher root growth in maize and *Phragmites karka* (Retz.) plants treated with BC under drought conditions. These authors concluded that BC causes better dry matter accumulation in the roots because it can favor water and nutrient uptake. Finally, the greater water and nutrient uptake due to BC under water-deficit conditions can also explain why coffee plants with reduced irrigation and treated with B8 showed a higher UAE. Uzoma *et al.* (2011) also reported greater UAE in maize plants treated with BC from cattle manure.

The results obtained in this study are important for the coffee sector because they show a series of advantages in the use of BC from physiological and crop management approaches. It is necessary to note that the incorporation of BC to the soil is an immediate and efficient strategy to reduce the negative effects of periods of water deficit in coffee, as concluded in studies on other species (Lyu *et al.*, 2016; Hafeez *et al.,* 2017; Gavili *et al.*, 2019; Abidden *et al.*, 2020). The analysis of physiological responses such as leaf gas exchange, water use efficiency, photosynthetic pigments, maximum photochemical efficiency of PSII, root hydraulic conductivity, and root parameters helped to understand the relationship between the mechanisms of acclimatization of coffee plants to drought and the effects of different BC doses applied.

Conclusions

In conclusion, RI negatively affected different physiological processes, such as chlorophyll *α* fluorescence, leaf gas exchange, dry matter accumulation, and plant water status, indicating that coffee plants showed moderate susceptibility to water deficit, as evidenced by the DQE values. Additionally, BC produced from coffee pulp has a positive effect on several physiological parameters. This was mainly evidenced by the stimulation of photosynthesis and a better root system aimed at generating efficient use of water in coffee plants under optimal and reduced irrigation. These results allow us to recommend that the use of BC can be a promising agronomic management alternative to handle situations of moderate water deficit in coffee crops, mainly in variable climatic scenarios. The added advantage of using BC is given by the fact that it can be derived from the pulp generated from the wet-processing of coffee beans. This practice would help improve the sustainability of the coffee production system at multiple levels, since this BC amendment also has a positive impact on the plant-soil relationship, especially under water stress conditions (Lehman et al. 2009; Jeffery et al. 2016).

Authors' Contributions

Conceptualization: AD-SR, L-L, and HR-D; Data curation: AD-SR and HR-D; Formal Analysis: AD-SR and HR-D; Funding acquisition: AD-SR and HR-D; Investigation: DF-RH and AD-SR; Methodology: DF-RH, AD-SR, and HR-D; Project administration; AD-SR, L-L, and HR-D; Resources: AD-SR and HR-D; Software: ADS-R and HR-D; Supervision: AD-SR, L-L, and HR-D; Validation: DF-RH and AD-SR; Visualization: DF-RH; Writing - original draft: DF-RH and AD-SR; Writing - review and editing: L-L and HR-D. All authors have read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

The authors acknowledge the funding support of 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.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Abbas T, Rizwan M, Ali S, Adrees M, Mahmood A, Zia-ur-Rehman M, … Qayyum MF (2018). Biochar application increased the growth and yield and reduced cadmium in drought stressed wheat grown in an aged contaminated soil. Ecotoxicology and Environmental Safety 148:825-833. *https://doi.org/10.1016/j.ecoenv.2017.11.063*

Ahmad M, Lee SS, Lee SE, Al-Wabel MI, Tsang DC, Ok YS (2017). Biochar-induced changes in soil properties affected immobilization/mobilization of metals/metalloids in contaminated soils. Journal of Soils and Sediments 17:717-730. *https://doi.org/10.1007/s11368-015-1339-4*

Ali S, Rizwan M, Qayyum MF, Ok YS, Ibrahim M, Riaz M, … Shahzad AN (2017). Biochar soil amendment on alleviation of drought and salt stress in plants: a critical review. Environmental Science and Pollution Research 24:12700-12712. *https://doi.org/10.1007/s11356-017-8904-x*

Alvarado-Sanabria O, Garcés-Varón G, Restrepo-Díaz H (2017). Physiological response of rice plants (*Oryza sativa* L.) subjected to different periods of two night temperatures. Journal of Stress Physiology & Biochemistry 13:35-43.

Amoakwah E, Frimpong KA, Okae-Anti D, Arthur E (2017). Soil water retention, air flow and pore structure characteristics after corn cob biochar application to a tropical sandy loam. Geoderma 307:189-197. *https://doi.org/10.1016/j.geoderma.2017.08.025*

Anjum SA, Xie XY, Wang LC, Saleem MF, Man C, Le W (2011). Morphological, physiological and biochemical responses of plants to drought stress. African Journal of Agricultural Research 6:2026-2032. *https://doi.org/10.5897/AJAR10.027*

Arcila-Pulgarín J, Buhr L, Bleiholder H, Hack H, Meier U, Wicke H (2002). Application of the extended BBCH scale for the description of the growth stages of coffee (*Coffea* spp.). Annals of Applied Biology 141:19-27. *https://doi.org/10.1111/j.1744-7348.2002.tb00191.x*

Batool A, Taj S, Rashid A, Khalid A, Qadeer S, Saleem A, Ghufran M (2015). Potential of soil amendments (Biochar and Gypsum) in increasing water use efficiency of *Abelmoschus esculentus* L. Moench. Frontiers in Plant Science 6:733. *http://dx.doi.org/10.3389/fpls.2015.00733*

Beebe SE, Rao IM, Blair MW, Acosta-Gallegos JA (2013). Phenotyping common beans for adaptation to drought. Frontiers in Physiology 4:35. *https://doi.org/10.3389/fphys.2013.00035*

Bunn C, Läderach P, Rivera OO, Kirschke D (2015). A bitter cup: climate change profile of global production of Arabica and Robusta coffee. Climatic Change 129:89-101. *https://doi.org/10.1007/s10584-014-1306-x*

Busscher WJ, Novak JM, Evans DE, Watts DW, Niandou MAA, Ahmedna M (2010). Influence of biochar on physical properties of a Norfolk loamy sand. Soil Science 175:10-14. *https://doi.org/10.1097/SS.0b013e3181cb7f46*

Ch'ng HY, Ahmed OH, Majid NMA (2015). Improving phosphorus availability, nutrient uptake and dry matter production of *Zea mays* L. on a tropical acid soil using poultry manure biochar and pineapple leaves compost. Experimental Agriculture 52:447-465. *https://doi.org/10.1017/S0014479715000204*

Dadi D, Daba G, Beyene A, Luis P, Van der Bruggen B (2019). Composting and co-composting of coffee husk and pulp with source-separated municipal solid waste: a breakthrough in valorization of coffee waste. International Journal of Recycling of Organic Waste in Agriculture 8:263-277. *https://doi.org/10.1007/s40093-019-0256-8*

DaMatta FM, Avila RT, Cardoso AA, Martins SC, Ramalho JC (2018). Physiological and agronomic performance of the coffee crop in the context of climate change and global warming: A review. Journal of Agricultural and Food Chemistry 66:5264-5274. *https://doi.org/10.1021/acs.jafc.7b04537*

de Oliveira MMT, Shuhua L, Kumbha DS, Zurgil U, Raveh E, Tel-Zur N (2020). Performance of *Hylocereus* (Cactaceae) species and interspecific hybrids under high- temperature stress. Plant Physiology and Biochemistry 153:30-39. *https://doi.org/10.1016/j.plaphy.2020.04.044*

Deng B, Bada B, Tammeorg P, Helenius J, Luukkanen O, Starr M (2019). Drought stress and *Acacia seyal* biochar effects on sorghum gas exchange and yield: A greenhouse experiment. Agriculture and Natural Resources 53:573-580. *https://doi.org/10.34044/j.anres.2019.53.6.03*

Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW (2016). InfoStatversion 2016. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. Retrieved 2022 August 12 from *http://www.infostat.com.ar*

Díaz-Leguizamón JJ, Chingaté-Cruz OF, Sánchez-Reinoso AD, Restrepo-Díaz H (2016). The effect of foliar applications of a bio-stimulant derived from algae extract on the physiological behavior of lulo seedlings (*Solanum quitoense* cv. Septentrionale). Ciencia e Investigación Agraria 43:25-37. *http://dx.doi.org/10.4067/S0718-16202016000100003*

Drake JE, Power SA, Duursma RA, Medlyn BE, Aspinwall MJ, Choat B, … Tissue DT (2017). Stomatal and non-stomatal limitations of photosynthesis for four tree species under drought: A comparison of model formulations. Agricultural and Forest Meteorology 247:454-466. *https://doi.org/10.1016/j.agrformet.2017.08.026*

Dubberstein D, Rodrigues WP, Semedo JN, Rodrigues AP, Pais IP, Leitão AE, … Ramalho JC (2017). Mitigation of the negative impact of warming on the Coffee crop: The Role of Increased Air [CO_2] and Management Strategies. In: Shanker A (Ed). Climate Resilient Agriculture-Strategies and Perspectives. Intechopen LTD, UK. pp 57-85. *https://doi.org/10.5772/intechopen.72374*

Echeverria MC, Nuti M (2017). Valorisation of the residues of coffee agro-industry: perspectives and limitations. Open Waste Management Journal 10:13-22. *https://doi.org/10.2174/1876400201710010013*

Federación Nacional de Cafeteros de Colombia (2020). Estadístias caferetas. Retrieved 2022 August 12 from *https://federaciondecafeteros.org/wp/estadisticas-cafeteras/*

Fischer B, Manzoni S, Morillas L, García M, Johnson M, Lyon S (2019). Can biochar improve agricultural water use efficiency? In: Geophysical Research Abstracts; European Geosciences Union: Munich, Austria, Volume 21.

Fracasso A, Trindade L, Amaducci S (2016). Drought tolerance strategies highlighted by two *Sorghum bicolor* races in a dry-down experiment. Journal of Plant Physiology 190:1-14. *https://doi.org/10.1016/j.jplph.2015.10.009*

Gavili E, Moosavi AA, Haghighi AAK (2019). Does biochar mitigate the adverse effects of drought on the agronomic traits and yield components of soybean? Industrial Crops and Products 128:445-454. *https://doi.org/10.1016/j.indcrop.2018.11.047*

Gonçalves M, Guerreiro MC, Ramos PH, de Oliveira LCA, Sapag K (2013). Activated carbon prepared from coffee pulp: potential adsorbent of organic contaminants in aqueous solution. Water Science & Technology 68:1085-1090. *https://doi.org/10.2166/wst.2013.349*

Hafeez Y, Iqbal S, Jabeen K, Shahzad S, Jahan S, Rasul F (2017). Effect of biochar application on seed germination and seedling growth of *Glycine max* (L.) Merr. Under drought stress. Pakistan Journal of Botany 49:7-13.

Hussain S, Rao MJ, Anjum MA, Ejaz S, Zakir I., Ali MA, … Ahmad S (2019). Oxidative stress and antioxidant defense in plants under drought conditions. In: Hasanuzzaman M, Hakeem K, Nahar K, Alharby H (Eds). Plant Abiotic Stress Tolerance. Springer: Cham. Switzerland pp 207-219. *https://doi.org/10.1007/978-3-030-06118-0_9*

Jeffery S, Verheijen FG, Kammann C, Abalos D (2016). Biochar effects on methane emissions from soils: A meta-analysis. Soil Biology and Biochemistry 101:251-258. *https://doi.org/10.1016/j.soilbio.2016.07.021*

Karhu K, Mattila T, Bergström I, Regina K (2011). Biochar addition to agricultural soil increased CH4 uptake and water holding capacity–Results from a short-term pilot field study. Agriculture, Ecosystems & Environment 140:309-313. *https://doi.org/10.1016/j.agee.2010.12.005*

Keshavarz-Afshar R, Hashemi M, DaCosta M, Spargo J, Sadeghpour A (2015). Biochar application and drought stress effects on physiological characteristics of *Silybum marianum*. Communications in Soil Science and Plant Analysis 47:743-752. *https://doi.org/10.1080/00103624.2016.1146752*

Kuzyakov Y, Subbotina I, Chen H, Bogomolova I, Xu X (2009). Black carbon decomposition and incorporation into soil microbial biomass estimated by ^{14}C labeling. Soil Biology and Biochemistry 41:210-219. *https://doi.org/10.1016/j.soilbio.2008.10.016*

Lehman NL (2009). The ubiquitin proteasome system in neuropathology. Acta Neuropathologica 118:329-347. *https://doi.org/10.1007%2Fs00401-009-0560-x*

Liang B, Lehmann J, Solomon D, Kinyangi J, Grossman J, O'neill B, … Neves EG (2006). Black carbon increases cation exchange capacity in soils. Soil Science Society of America Journal 70:1719-1730. *https://doi.org/10.2136/sssaj2005.0383*

Lobell DB, Gourdji SM (2012). The influence of climate change on global crop productivity. Plant Physiology 160:1686-1697. *https://doi.org/10.1104/pp.112.208298*

Lyu S, Du G, Liu Z, Zhao L, Lyu D (2016). Effects of biochar on photosystem function and activities of protective enzymes in *Pyrus ussuriensis* Maxim. under drought stress. Acta Physiologiae Plantarum 38:1-10. *https://doi.org/10.1007/s11738-016-2236-1*

Marques MC, Nascimento CWA, da Silva AJ, da Silva-Gouveia-Neto A (2017). Tolerance of an energy crop (*Jatropha curcas* L.) to zinc and lead assessed by chlorophyll fluorescence and enzyme activity. South African Journal of Botany 112:275-282. *https://doi.org/10.1016/j.sajb.2017.06.009*

Merlaen B, De-Keyser E, Ding L, Leroux O, Chaumont F, Van Labeke MC (2019). Physiological responses and aquaporin expression upon drought and osmotic stress in a conservative vs. prodigal *Fragaria* x *ananassa* cultivar. Plant Physiology and Biochemistry 145:95-106. *https://doi.org/10.1016/j.plaphy.2019.10.030*

Montilla PJ (2006). Caracterización de algunas propiedades físicas y factores de conversión del café. BSc Dissertation, Universidad de Caldas. 107 p.

Morales F, Ancín M, Fakhet D, González-Torralba J, Gámez AL, Seminario A, … Aranjuelo I (2020). Photosynthetic metabolism under stressful growth conditions as a bases for crop breeding and yield improvement. Plants 9:88. *https://doi.org/10.3390/plants9010088*

Ocampo-López OL, Castañeda-Peláez K, Vélez-Upegui JJ (2017). Caracterización de los ecotopos cafeteros colombianos en el Triángulo del Café. Perspectiva Geográfica 22:89-108. *https://doi.org/10.19053/01233769.6100*

Olmo M, Alburquerque JA, Barrón B, Del Campillo MC, Gallardo A, Fuentes M, Villar R (2014). Wheat growth and yield responses to biochar addition under Mediterranean climate conditions. Biology and Fertility of Soils 50:1177-1187. *https://doi.org/10.1007/s00374-014-0959-y*

Poltronieri P, Rossi F (2016). Challenges in specialty coffee processing and quality assurance. Challenges 7:19. *https://doi.org/10.3390/challe7020019*

Pranata-Erdiansyah NP, Wachjar A, Sulistyono E, Supijatno S (2019). Growth response of seedlings of four Robusta coffee (*Coffea canephora* Pierre. Ex. A. Froehner) clones to drought stress. Pelita Perkebunan 35:1-11. *https://doi.org/10.22302/iccri.jur.pelitaperkebunan.v35i1.344*

Rajapaksha AU, Chen SS, Tsang DC, Zhang M, Vithanage M, Mandal S, … Ok YS (2016). Engineered/designer biochar for contaminant removal/immobilization from soil and water: potential and implication of biochar modification. Chemosphere 148:276-291. *https://doi.org/10.1016/j.chemosphere.2016.01.043*

Raviv M, Blom TJ (2001). The effect of water availability and quality on photosynthesis and productivity of soilless-grown cut roses. Scientia Horticulturae 88:257-276. *https://doi.org/10.1016/S0304-4238(00)00239-9*

Riaz M, Arif MS, Hussain Q, Khan SA, Tauqeer HM, Yasmeen T, … Haider MS (2019). Application of biochar for the mitigation of abiotic stress-induced damages in plants. In: Hasanuzzaman M, Fujita M, Oku H, Islam T (Eds). Plant Tolerance to Environmental Stress: Role of Phytoprotectants. Taylor & Francis Group, London pp 285-304.

Rodrigues WP, Silva JR, Ferreira LS, Machado-Filho JA, Figueiredo FA, Ferraz TM, Ramalho JC (2018). Stomatal and photochemical limitations of photosynthesis in coffee (*Coffea* spp.) plants subjected to elevated temperatures. Crop and Pasture Science 69:317-325. *https://doi.org/10.1071/CP17044*

Rodríguez N, Zambrano D (2010). Los subproductos del café: Fuente de energía renovable. Avances técnicos Cenicafe 393:1-8.

Rodríguez-Frómeta RA, Sánchez JL, García JMR (2020). Evaluation of coffee pulp as substrate for polygalacturonase production in solid state fermentation. Emirates Journal of Food and Agriculture 32:117-124. *https://doi.org/10.9755/ejfa.2020.v32.i2.2068*

Romdhane L, Awad YM, Radhouane L, Dal Cortivo C, Barion G, Panozzo A, Vamerali T (2019). Wood biochar produces different rates of root growth and transpiration in two maize hybrids (*Zea mays* L.) under drought stress. Archives of Agronomy and Soil Science 65:846-866. *https://doi.org/10.1080/03650340.2018.1532567*

Ruiz ADC, Pabón JD (2013). Efecto de los fenómenos de El Niño y La Niña en la precipitación y su impacto en la producción agrícola del departamento del Atlántico (Colombia). Cuadernos de Geografía: Revista Colombiana de Geografía. 22:35-54.

Sadeghian S (2008). Fertilidad del suelo y nutrición del café en Colombia: Guía práctica. Boletín técnico No 32:44.

Sadeghian S, Jaramillo A (2016). Nutrición de los cafetales en Colombia, en escenarios de la niña. Chinchiná, Colombia. Cenicafé pp 12.

Salamanca-Jimenez A (2017). Coffee crop fertilization in Colombia : A mini-review. International Potash Institute 50:22-30.

Saleem-Akhtar S, Li G, Andersen MN, Liu F (2014). Biochar enhances yield and quality of tomato under reduced irrigation. Agricultural Water Management 138:37-44. *https://doi.org/10.1016/j.agwat.2014.02.016*

Sánchez-Reinoso AD, Ávila-Pedraza EA, Restrepo-Díaz H (2020). Use of biochar in agricultura. Acta Biológica Colombiana 25:327-338. *https://doi.org/10.15446/abc.v25n2.79466*

Shashidhar HE, Gowda HV, Raveendra GM, Kundur PJ, Kumar GN, Suprabha N, ... Sonam R (2012). PVC tubes to characterize roots and shoots to complement field plant productivity studies. In: Shashidhar HE, Henry A, Hardy B (Eds). Methodologies for root drought studies in rice. International Rice Research Institute, Philippines pp 15-21.

Silva BM, de Oliveira GC, Serafim ME, Carducci CE, da Silva ÉA, Barbosa SM, ... Guimarães PTG. (2019). Soil management and water-use efficiency in Brazilian coffee crops. In: Castanheira DT (Ed). Coffee-Production and Research; IntechOpen: London, UK pp 1-20.

Sohi S (2012). Carbon storage with benefits. Science 338:1034-1035. *https://doi.org/10.1126/science.1225987*

Sorrenti G, Masiello CA, Toselli M (2016). Biochar interferes with kiwifruit Fe-nutrition in calcareous soil. Geoderma 272:10-19. *https://doi.org/10.1016/j.geoderma.2016.02.017*

Steiner C, Teixeira G, Lehmann J, Nehls T, de Macedo J, Blum W (2007). Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian soil. Plant and Soil 291:275-290. *https://doi.org/10.1007/s11104-007-9193-9*

Sun J, Drosos M, Mazzei P, Savy D, Todisco D, Vinci G, ... Piccolo A (2017). The molecular properties of biochar carbon released in dilute acidic solution and its effects on maize seed germination. Science of The Total Environment 576:858-867. *https://doi.org/10.1016/j.scitotenv.2016.10.095*

Tayyab M, Islam W, Khalil F, Ziqin P, Caifang Z, Arafat Y, ... Hua Z (2018). Biochar: An efficient way to manage low water availability in plants. Applied Ecology and Environmental Research 16:2565-2583. *http://dx.doi.org/10.15666/aeer/1603_25652583*

Tounekti T, Mahdhi M, Al-Turki TA, Khemira H (2018). Water relations and photo-protection mechanisms during drought stress in four coffee (*Coffea arabica*) cultivars from southwestern Saudi Arabia. South African Journal of Botany 117:17-25. *https://doi.org/10.1016/j.sajb.2018.04.022*

Tyree MT, Patiño S, Bennink J, Alexander J (1995). Dynamic measurements of roots hydraulic conductance using a high-pressure flowmeter in the laboratory and field. Journal of Experimental Botany 46:83-94. *https://doi.org/10.1093/jxb/46.1.83*

Uzoma KC, Inoue M, Andry H, Fujimaki H, Zahoor A, Nishihara E (2011). Effect of cow manure biochar on maize productivity under sandy soil condition. Soil Use and Management 27:205-212. *https://doi.org/10.1111/j.1475-2743.2011.00340.x*

Van Zwieten L, Kimber S, Morris S, Chan KY, Downie A, Rust J, … Cowie A (2010). Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. Plant and Soil 327:235-246. *https://doi.org/10.1007/s11104-009-0050-x*

Wang S, Zheng J, Wang Y, Yang Q, Chen T, Chen Y and Wang T (2021). Photosynthesis, chlorophyll fluorescence, and yield of peanut in response to biochar application. Frontiers in Plant Science 12:1000. *https://doi.org/10.3389/fpls.2021.650432*

Wu Y, Wang X, Zhang L, Zheng Y, Liu X, Zhang Y (2023). The critical role of biochar to mitigate the adverse impacts of drought and salinity stress in plants. Frontiers in Plant Science 14:1163451. *https://doi.org/10.3389/fpls.2023.1163451*

Yin J, Bassuk NL, Olberg MW, Bauerle TL (2014). Fine root hydraulic conductance is related to post-transplant recovery of two *Quercus* tree species. Journal of the American Society for Horticultural Science 139:649-656. *https://doi.org/10.21273/JASHS.139.6.649*

Zandalinas SI, Mittler R, Balfagón D, Arbona V, Gómez-Cadenas A (2018). Plant adaptations to the combination of drought and high temperatures. Physiologia Plantarum 162:2-12. *https://doi.org/10.1111/ppl.12540*

Zhang W, Wei J, Guo L, Fang H, Liu X, Liang K, Niu W, Liu F, Siddique KHM (2023). Effects of two Biochar types on mitigating drought and salt stress in tomato seedlings. Agronomy 13:1039. *https://doi.org/10.3390/agronomy13041039*

Zoghi Z, Hosseini SM, Kouchaksaraei MT, Kooch Y, Guidi L (2019). The effect of biochar amendment on the growth, morphology and physiology of *Quercus castaneifolia* seedlings under water-deficit stress. European Journal of Forest Research 138:967-979. *https://doi.org/10.1007/s10342-019-01217-y*

Zulfiqar F, Allaire SE, Akram NA, Méndez A, Younis A, Peerzada AM, Wright SR (2019). Challenges in organic component selection and biochar as an opportunity in potting substrates: a review. Journal of Plant Nutrition 42:1386-1401. *https://doi.org/10.1080/01904167.2019.1617310*

Zulfiqar F, Wei X, Shaukat N, Chen J, Raza A, Younis A, Siddique KH (2021). Effects of Biochar and biochar–compost mix on growth, performance and physiological responses of potted *Alpinia zerumbet*. Sustainability 13:11226. *https://doi.org/10.3390/su132011226*