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Balanced mixture of biochar and synthetic fertilizer increases seedling quality of *Acacia mangium*

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ABSTRACT

Biochar is a charcoal-like substance produced by pyrolisis of organic material from agricultural and forestry wastes, that can improve soil and substrates physical and chemical properties in the field and in plant nurseries. Despite some studies showing the advantages of its use in forestry nurseries, other studies show that biochar effects on seedling growth and quality are variable, and depends on the specific properties of the biochar and the particular species response to it. The objective of this study was to evaluate, through the analysis of the substrate and plant measurements, the effect of biochar produced from *Acacia mangium* Willd wood residues on the production of the same species seedlings. An experiment with complete factorial design, in a completely randomized arrangement, was established, with 9 treatments resulting from the combination of *A. mangium* biochar (BAM) and synthetic fertilizer (FS), with 3 dose levels for BAM: 0, 40, and 80 $\text{ton} \cdot \text{ha}^{-1}$ and 3 levels for the FS: 0% 50% and 100% of common fertilizer practice in the studied region of Colombia. The quality of the seedlings was estimated using the Dickson index (DQI), which considers the size and biomass allocation of the plant. The addition of BAM + FS increased the cationic exchange capacity, nitrogen, phosphorus, organic matter and carbon in the substrate without modifying the pH or the concentrations of calcium and potassium. Foliar analysis showed an increase in these cations in BAM treatments. An increase of over 130% was found in the DQI of seedlings with BAM + FS, but an intermediate dose of both elements was required to achieve this improvement in seedling quality. Neither of the two fertilizer alternatives alone resulted in good seedling quality.

1. Introduction

Residues from human activities, including agricultural systems, generate loads that overcome the natural mechanisms of recycling and system self-purification (Kratz et al., 2012). Thus, it is important to establish strategies to mitigate their negative externalities. Among them, "forest residues" are those that come directly from management practices applied to forest plantations such as thinning, pruning, and felling (Hakkila et al., 1997). The production of this type of waste is considered to be close to 86.6 million tons / year and from these 168 million metric tons of carbon dioxide are released into the atmosphere each year in the word (EPA, 2014). These wastes also create problems in the different tree crops when, after the different silvicultural activities, the wood material is disseminated throughout the crop increasing fire risk and niches for pathogens due to the substrate provided, and affecting the mobility of workers inside the plantations. However, it is important to note that these residues may be at the same time an important input of nutrients to the system if they are properly managed (e.g. De Moraes Goncalvez et al., 2004). One alternative for mitigating the negative effects of the accumulation of forest residues is the incorporation of these residues into substrates for the seedlings production of forestry nurseries.

Depending on the management provided in the nursery, the plants can develop greater growth, vigor and health, representing advantages for reforestation and for the wood industry (Briggs

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et al., 2012). In this regard, one of the main objectives of forest nurseries is to obtain high quality plants in the initial stages of development. For this purpose, it is important to understand the correlations between different morphological traits and to identify the best cultivation period and practices (Dionisio et al., 2019b). Moreover, to achieve high quality plants, it is necessary to improve the physical, chemical and biological properties of their substrate (Buamscham et al., 2012). The quality of the substrate, understood as the ability to supply nutrients, air, and water to the seedling, is essential for plant development. So, the substrate must have properties such as efficient water absorption and retention, free drainage properties, high air-filled porosity, high nutrient supply and anchorage for the plants (Dunnett and Kingsbury, 2008). Relevant variables for substrate selection are related with its functions (plant support, aeration, nutrient retention and moisture retention) but also with its availability and costs. Likewise, important factors such as environmental conditions and plant management, morphology and physiology are fundamental to predict the success of seedling establishment in the field.

The estimation of physiological parameters of nursery plants, such as the content of nutrients in leaf tissues or the allocation of biomass to key organs such as roots, allows us to understand the differences in their growth and to predict to a certain extent their subsequent survival in the field (Prieto et al., 2003). The quality of the seedlings has usually been established from different allometric measurements such as the dry weight of different plant parts (root and shoot), number of branches, plant height and root collar diameter (South, 2000). However, indices that combine some of these variables have also been used, and proven to serve as predictors for establishing seedling development. The Dickson Quality Index - DQI (Dickson et al. 1960) is one of these indices for evaluating seedling quality as a function of total dry matter, shoot height, stem base diameter, and shoot (i.e. the sum of the stem and leaves) and root dry matter (Binotto et al., 2010).

Biochar is a charcoal-like substance produced by pyrolisis of organic material from agricultural and forestry wastes (Kelsi, 2010). It can increase carbon sequestration and, under certain conditions, improve soil and substrates physical and chemical properties in the field and in plant nurseries, respectively. In this way, if its biological benefits on any stage of the production system are proven, biochar can be used as a sustainable alternative to decrease -and take advantage of- the residues of an agroecosystem. Used in nurseries, biochar may act as a matrix where nutrients are slowly released and, because of its low bulk density and high porosity, facilitates water relations that improve seedling growth (Buamscham et al., 2012; Altland and Krause, 2012; Briggs et al., 2012; Marimon-Junior et al., 2012). Biochar from wood could be an alternative to materials like perlite, given its low density, high porosity, and content of significant amounts of elements such as K, P and Mg. (Angst et al., 2013). Although the influence of biochar on nursery substrates has been poorly studied for tree seedling production, there are studies such as those of Elad et al. (2010), Dumroese et al. (2011) and Tian et al. (2012), analyzing nutrient leaching, seedling growth and systemic resistance to diseases, that have shown favorable responses. However, the effects of biochar on treeś seedlings growth and quality differ among species and biochar characteristics (e.g. Fornes & Belda, 2019; Aung et al, 2018). So, despite the potential advantages of biochar use, its particular effects should be tested on different study systems since positive and negative effects on soil and crop productivity have been reported (Aung et al, 2019).

Based on this background, the objective of this study was to determine the effect of biochar derived from *Acacia mangium* Willd pruning residues, on the growth and quality of seedlings of this species produced in nursery conditions. Comparisons were made with the standard production system using synthetic fertilizers (FS). Our hypothesis was that biochar alone, or a combination of biochar plus a low FS input, may optimize *A. mangium* seedling growth and quality, compared to the conventional technology, thus making possible to take advantage of residues produced in one stage of the production system, at the same time as reducing external inputs, to optimize the whole production chain.

2. Materials and methods

2.1. Study site and experimental design

The study was carried out in a nursery for *Acacia mangium* seedling production belonging to the company Green Cooperation, in the township of Planas, department of Meta, Colombia (4° 05′- 5° 08 ’N, and between 74° 05 ’and 73° 30′ W).

The experiment was set up in a complete factorial design, with 9 treatments, 3 replications and 3 fertilizer materials evaluated: *A. mangium* biochar (BAM), synthetic fertilizer (FS), and BAM + FS mixture, with 3 levels of doses for BAM – 0, 40, and 80 ton·ha^{-1}- and 3 levels for FS -application at 0%, 50% and 100% of the conventional dose applied (Table 1). BAM levels were based on Wolf et al. (2013) and Jeffery et al. (2011) who estimated an average of 50 ton · ha^{-1} for application of this material for an increase of 18–28% in crop yield on a global scale. Particularly, the application recommended for the acacia biochar was ≅ 47 ton · ha^{-1} (Eyles et al., 2013).

(Table 1) The evaluated biochar was produced from thinning and pruning wood residues of an *A. mangium* plantation in a process of slow pyrolysis with residence burning times from 11 to 14 h at temperatures between 350° C and 400° C in a pyrolysis oven. The biochar was produced according to the methodology of Jouiad et al. (2015). Once the biochar was produced, it was passed through 4.75 mm – 1 mm sieves to produce a particle size of approximately 1–5 mm. The synthetic fertilizer (FS) used was Triple Fifteen: 15% of total nitrogen, 15% of soluble phosphorus, neutral ammonium citrate and 15% of water-soluble potassium. This fertilizer is used as part of the fertilization plan at the plantation using an application dose of 100 g / plant when seedlings are planted in the field. Accordingly, this fertilizer was applied to the substrate and mixed in the respective cultivation bags with the following equivalence 50%: 50 g per plant and 100%: 100 g per plant.

The study was carried out in a greenhouse with mean temperature between 27.2 and 28 °C. Seedings of *A. mangium* were established according to the ISTA methodology (2013). Seeds came from the same plantation where the study was carried out and from a single tree. The seeds were treated with hot water in order to eliminate pathogens, and dried under open air conditions. Five seeds were sown in each polyethylene bag (10 cm high × 5 cm diameter) for a total of 27 bags, comprising the 9 treatments with their three repetitions. The percentage of germination was calculated at day 18 (which was 100%) according to the ISTA methodology (2013). Then, the seedlings were spiked to leave only one growing seedling per bag. During the whole experiment period, sprinkler irrigation was applied at a rate of 1 m^3 of water per week per cultivation bed (1.5 m by 3 m long), which was a water supply previously estimated to guarantee no water deficit for the growing seedlings.

Table 1.
Treatments applied in the study combining different levels of FS: Synthetic Fertilizer (0%, 50% and 100% of the conventional dose applied in the field) and BAM: Biochar of *Acacia mangium* Willd (the equivalent of 0, 40, and 80 ton·ha^{-1}).

FS (%)BAM (ton· ha^{-1})	**0**	**50**	**100**
0	B_0S_0	B_0S_{50}	B_0S_{100}
40	$B_{40}S_0$	$B_{40}S_{50}$	$B_{40}S_{100}$
80	$B_{80}S_0$	$B_{80}S_{50}$	$B_{80}S_{100}$

2.2. Variables measured in the substrate

The substrate used in the experiment for seedlings growth was soil (Oxisols) taken from the studied region where *A. mangium* plantations grow and where seeds were collected. It was characterized physically and chemically (Table 2) in order to establish a baseline for comparisons between the treatments. After this characterization, it was disinfected with hot water and hydrogen peroxide. Likewise, the BAM was characterized (table 3). The proposed treatments were mixed and accommodated in the bags, which were placed on the cultivation beds. At the end of the experiment (i.e. 12 weeks after sowing), the physico-chemical properties of the substrate were determined again. The properties measured were cation exchange capacity (CEC), pH, total N, plant-available P, exchangeable bases and organic matter.

The determination of the CEC and interchangeable bases was carried out by the NH_4O Ac 1 N method at soil pH (permanent CEC of the soil). The analytical methods were carried out according to the methodology described by Motta (1990).

The pH was analyzed according to the protocols of the National Soil Laboratory of the Agustín Codazzi Geographical Institute, Colombia. It was measured in deionized water in a 1:1 ratio, in 20 g of soil / 20 g of water.

The percentage of organic matter (OM) was determined in the laboratory using the Walkley and Black method by means of the conversion factor 1.742 applied to the measured organic carbon content (OM = Organic Carbon*1.742). The amount of organic matter allows the measurement of the levels of mineralization and humidification, soil structure and inference of humidity retention in the soil.

The total N was calculated by Kjeldahl digestion. It is important to note that, of the total nitrogen in soils, approximately 98% is part of the organic matter and the rest is in the form of nitrates, nitrites, ammonia and to a lesser extent, in the form of N_2 and nitrogen oxides.

Phosphorus analysis was performed using the lactate methodology. This technique allows the extraction of phosphorus from an extraction solution based on lactic acid, acetic acid and ammonia (Egner, 1941).

2.3. Variables measured in the seedlings

At the end of the experiment, the total plant height (i.e. the length of the aereal portion of the plant, L), the stem basal diameter (BD), and the dry weight of the aerial (AW, (leaves plus stem) and belowground (roots) organs (RW) were measured. From these variables the Dickson's seedling quality index (DQI, equation (1)) was calculated, selected for integrating different allometric variables and being satisfactorily used in other species (eg. Kim et al., 2003).

$$DQI = \frac{TSW}{(L/BD) + (AW/RW)} \tag{1}$$

where: DQI: Dickinson's quality index, TSW: total seedling dry weight (g), L: length of the aerial part (cm), BD: stem basal diameter (mm), AW: dry weight of the aerial part (g), RW: root dry weight (g).

Likewise, all the leaves of each seedling were collected, oven dried at 60 °C and weighed. Larger elements such as N, P, K, Ca and Mg were analyzed in the foliar samples. The N and P were analyzed by the Kjeldahl and colorimetry methodologies, respectively, while the atomic absorption method was applied to the interchangeable bases (Datei Spectrometer, Germany).

2.4. Statistical analysis

The response variables obtained from the substrate and seedlings were modeled linearly using a simple factorial design in a completely randomized arrangement. The statistical analysis involved a one-way analysis of variance for the established design. Bonferroni correction was used to adjust the p value in both soil (CEC, N, P, OM and OC) and leaf variables (N, P, K, Ca and Mg). When differences were found in the analysis of variance, Tukey tests were applied for pairwise comparison of means. The necessary assumptions to apply the analysis of variance were fulfilled; the normality of residuals was corroborated with the Shapiro-Wilks test and homogeneity of variance with the Bartlett test. It should be noted that initially an analysis of multivariate variance by group of variables was applied; however, the assumptions for such an analysis were not met (multivariate normality of residuals with Royston's test and homogeneity of variance and covariance matrices with Box's M test).

To complement the analysis, the relative change between each fertilization treatment and the control (B_0S_0) was estimated and graphically displayed. For that purpose, the mean values of each variable at the end of the experiment were used. The comparisons with the control have only a descriptive purpose and no additional statistical analysis was applied. The significance of differences with this treatment, or between any other pair of treatments, can be seen with the Tukey test.

3. Results

3.1. Physico-chemical properties of the substrate in response to the treatments

Significant differences (ANOVA, $p < 0.001$) were found among some of the treatments in CEC, N, P, organic matter (OM) and organic carbon (OC) (Table 4). The EC, pH and the content of Ca and Mg were the only variables that did not respond significantly to the treatments (ANOVA, $p > 0.05$). .

The treatment with the highest average CEC was $B_{40}S_{50}$ followed by $B_{80}S_{100}$ (Table 4), being the increase in CEC of $B_{40}S_{50}$ of around 750% (7.5 times higher) relative to the control B_0S_0 (Fig. 1), which presents the lowest CEC of all studied treatments. In both fertilized treatments with the highest CEC, the amount of BAM and FS was balanced, that is, they were the two treatments with a low dose of both materials ($B_{40}S_{50}$) and a high dose of both materials ($B_{80}S_{100}$), although surprisingly the one with the highest CEC resulted from the low dose of materials ($B_{40}S_{50}$). The fertilized treatments with the lowest CEC were B_0S_{50}, which did not differ significantly from the control (Table 4, Fig. 1) and $B_{80}S_{50}$.

Regarding the variables N, P, OM and OC, the highest averages were found in the $B_{80}S_{100}$ treatment (Table 4). The lowest values of N were observed in the control, followed by treatments with no BAM or low BAM dose (B_0S_{50}, B_0S_{100} and $B_{40}S_{100}$), whereas the lowest values of P and OM were observed in $B_{40}S_0$ (Table 4).

Table 2
Analysis of the chemical properties of the initial soil where the *Acacia mangium* seeds were sown. EC = electrical conductivity; CEC = cation exchange capacity.

pH-logH+	ECdS/m	N total %	P%	K%	Ca%	Mg%	Na%	CECcmol+* kg^{-1}	Organic Matter%	Organic Carbon%
3.4	0.37	0.0033	0.024	–	0.047	0.0065	2.27	1.5	0.59	0.31

All elements in g * 100 g^{-1} (i.e. % of dry weight of the substrate).

Table 3
Analysis of the chemical properties of BAM. OM = organic matter; OC = organic carbon; EC = electrical conductivity; CEC = cation exchange capacity.

CEC	OM	OC	pH	EC	N total	P	K	Ca	Mg	Na	Fe
11.45cmol+* kg^{-1}	96.71%	44.57%	7.10-logH+	0.37dS/m	0.84%	0.04%	0.27%	0.21%	0.04%	0.029%	2432ppm

(All elements and OM and OC in g * 100 g^{-1} or %)

Table 4
Estimated mean and standard deviation (SD) for physico-chemical variables of the substrate at the end of the experiment (12 weeks) in the 9 treatments that arose from the combination of biochar and synthetic fertilizer (see Table 1 for the meaning of each treatment). Different letters within the columns indicate significant differences in the corresponding variable in at least two treatments (ANOVA, Tukey tests, $p < 0.05$).

Treatments	CEC*Mean	SS	N**Mean	SD	P**Mean	SD	OM*Mean	SD	OC*Mean	SD
B_0S_0	1.51e	0.728	0.0003f	0.0041	0.024 cd	0.0051	0.59bc	0.109	0.31 cd	0.062
B_0S_{50}	2.49de	0.618	0.018e	0.0025	0.021 cd	0.0068	0.62c	0.112	0.33 cd	0.064
B_0S_{100}	6.96c	0.756	0.016e	0.0092	0,032c	0.0061	0.58bc	0.123	0.31 cd	0.070
$B_{40}S_0$	4.72d	0.596	0.022c	0.0041	0.011d	0.0135	0.45e	0.100	0.27d	0.058
$B_{40}S_{50}$	12.79a	0.667	0.027b	0.0268	0.12a	0.0057	0.59bc	0.084	0.35c	0.049
$B_{40}S_{100}$	6.82c	0.596	0.017e	0.0060	0.081b	0.0057	0.47 cd	0.079	0.28 cd	0.045
$B_{80}S_0$	4.50d	0.615	0.033b	0.0012	0.030c	0.0087	1.156b	0.066	0.66b	0.036
$B_{80}S_{50}$	2.49 cd	0.618	0.02c	0.0067	0.13a	0.0080	1.154b	0.053	0.67b	0.031
$B_{80}S_{100}$	9.82b	0.640	0.043a	0.0009	0.13a	0.0158	1.46a	0.039	0.84a	0.022

* cmol + * kg-1 **% by weight of substrate. CEC = cation exchange capacity; OM = organic matter content, OC = organic carbon.

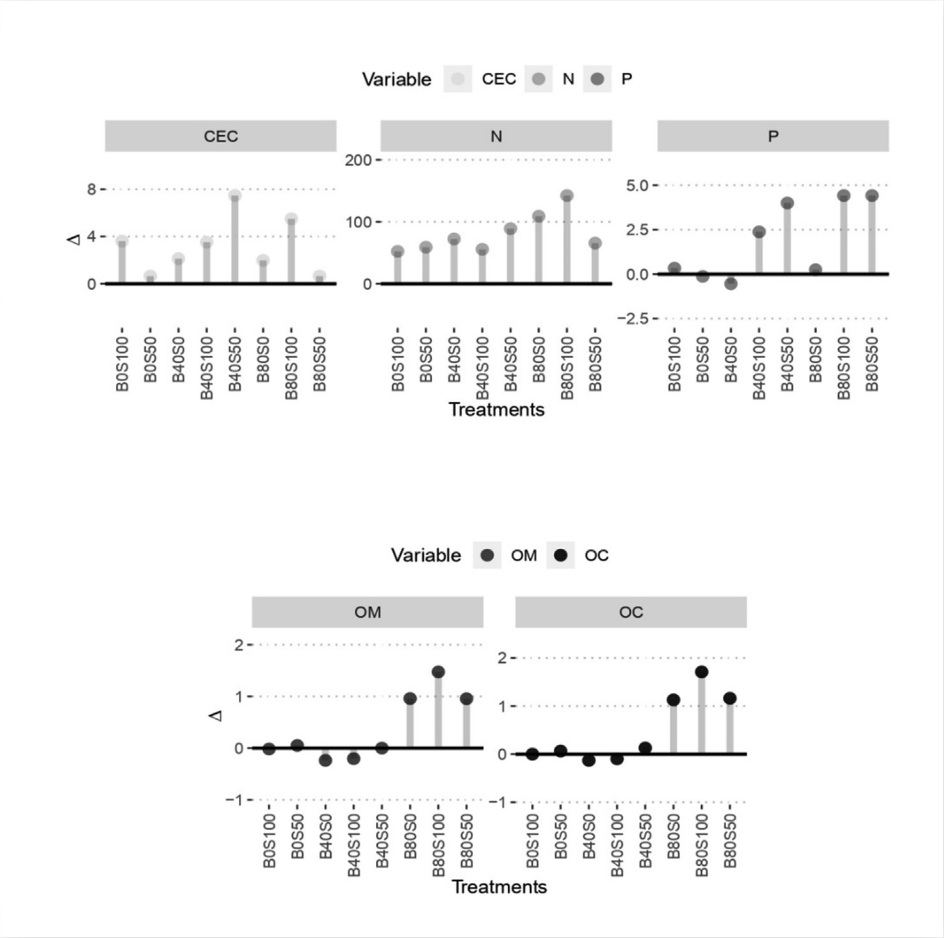

Fig. 1. Relative mean change (△) in each treatment in relation to the control (B_0S_0) at the end of the experiment, estimated as the ratio between the value in the treatment and the control (e.g. a change of 2 is equal to an increase of 200%). See Table 1 for the meaning of each treatment.

Regarding the differences with the Control, the highest positive change in N concentration -observed in $B_{80}S_{100}$- was in the order of 140 times (14200%) (Fig. 1). This treatment presented increases of 157% and 375% in relation to treatments with synthetic fertilizer but with no BAM (B_0S_{50} and B_0S_{100}).

There were two fertilized treatments that did not differ from the control in their low P levels, B_0S_{50} and $B_{40}S_0$, presenting both even lower mean average values than B_0S_0. They were treatments with a low dose of a unique fertilizer (BAM or FS). In contrast, treatments with mixtures of both fertilizers ($B_{40}S_{50}$, $B_{80}S_{50}$ and $B_{80}S_{100}$) had a positive difference of P level in the order of 400% respect to the control (Fig. 1). The greatest difference in OM respect to the Control was in the order of 150% ($B_{80}S_{100}$, Fig. 1), followed by $B_{80}S_0$ and $B_{80}S_{50}$, without effect in $B_{40}S_{50}$ (Fig. 1). The same pattern was followed by the OC, which is evident considering that the OM was deduced from the OC.

3.2. Seedling response to the treatments

Treatment effects (ANOVA, $p < 0.0001$) were observed in the concentration of all the measured nutrients (N, P, K, Ca, Mg) in the leaf tissues. However, not all the treatments had the same impact on the chemical elements analyzed.

The highest N concentration was found in $B_{80}S_0$ (with high BAM and without FS), while the lowest was found in another biochar treatment but a high dose of SF, $B_{80}S_{100}$. However, an equally high leaf concentration of N was found in the two treatments with FS and without BAM (B_0S_{50} and B_0S_{100}) and in $B_{40}S_{100}$. Unlike this result, in the case of P, all the treatments without BAM had the lowest values (B_0S_0, B_0S_{50} and B_0S_{100}, although also $B_{40}S_0$, with a low dose of BAM and without FS), regardless of the amount of FS added (Table 5). The highest levels of P were observed in the treatments with intermediate doses of BAM and the addition of FS ($B_{40}S_{50}$ and $B_{40}S_{100}$), presenting the treatments with high doses of BAM ($B_{80}S_{50}$ and $B_{80}S_{100}$) intermediate levels of P in leaves (Table 5).

In general, the treatments without BAM also had less foliar K, Ca and Mg than those with biochar (Table 5). But, within these, the treatments with the highest concentration of each cation differed. K was highest in the treatments with the highest dose of BAM and FS ($B_{80}S_{50}$ and $B_{80}S_{100}$). Ca was highest in both treatments with BAM (medium and high dose) and high dose of FS ($B_{40}S_{100}$ and $B_{80}S_{100}$), and Mg increased in treatments with intermediate doses of BAM and variable doses of FS ($B_{40}S_{50}$ and $B_{40}S_{100}$) (Table 5). Therefore, it is not possible to identify a unique combination of BAM and FS that favored all nutrients at leaf level.

The quality of the seedlings, estimated through the DQI, is a variable that integrates the growth and the allocation of biomass within the plants. The highest value was observed in $B_{40}S_{50}$, a treatment with intermediate levels of BAM and FS (Table 6). A homogeneous group with the lowest averages of DQI was formed by the plants of treatments $B_{80}S_0$, $B_{40}S_0$, B_0S_0, B_0S_{50}, and B_0S_{100}, that are characterized by being no mixtures, but having one or another type of fertilizer, or none (Table 6). A wide difference (7.5 times higher) was found in favor of $B_{40}S_{50}$ with respect to the average DQI observed in the control (B_0S_0).

Table 6

Mean and standard deviation (SD) of the estimated Dickson's seedling quality index for seedlings of *A. mangium* subjected to 9 fertility treatments (see Table 1). The treatments are ordered from highest to lowest based on their average value. Different letters denote significant differences between treatments (Tukey's test, $p < 0.05$).

Treatments	DQI	Groups*	SD
B40S50	0.756	A	0.00791
B80S50	0.326	B	0.24180
B40S100	0.241	BC	0.24180
B80S100	0.211	C	0.21134
B80S0	0.026	D	0.02662
B40S0	0.019	D	0.08124
B0S0	0.015	D	0.00810
B0S50	0.006	D	0.00017
B0S100	0.005	D	0.00244

4. Discussion

4.1. Chemical properties of the substrate

Having found two treatments with mixtures of BAM and FS ($B_{40}S_{50}$ and $B_{80}S_{100}$) associated with the highest mean CEC, it seems that there exists a synergy of the two compounds. In this regard, the biochar would play an exchange role of the elements given by the FS, taking advantage of the elements contributed by the FS and capturing them through the high reaction of its surface. It should be noted that these two treatments have balanced doses of both elements (intermediate FS and BAM in $B_{40}S_{50}$, and high FS and BAM in $B_{80}S_{100}$), although the highest CEC was observed in $B_{40}S_{50}$. When the biochar is included in the soil, oxidation reactions occur in contact with water and oxygen, and these lead to an increase in the negative charge on the surface of the material, thus increasing the CEC (Joseph et al., 2010). Also, the increase in CEC with the addition of only FS could be due to the increase in the concentration of NH_4^+ and K^+ ions in the substrate solution (Amaral and Anghinoni 2001).

Although P inputs in wood biochar are usually low (0.01–0.1%) (Spokas et al., 2009), a variable amount of P can be added to the substrate from biochar (Gul et al., 2015). In this regard, in the present study, the amount of P was higher in some treatments containing BAM ($B_{40}S_{50}$, $B_{80}S_{50}$ and $B_{80}S1_{00}$), suggesting that the BAM itself contributed with P to the substrate. Research in wood biochars has established that significant amounts of P are easily soluble and available for the plant (Gundale and Deluca, 2006). In the case of the $B_{40}S_{50}$ treatment, there would be a causal relationship between the intake of P (represented in the foliar analysis) and the amount of the element in the substrate.

Table 5

Mean and standard deviation (SD) of different nutrients in foliar samples of *A. mangium* seedlings submitted to 9 fertility treatments (see Table 1 for details of the treatments).

Treatments	N*	SD	P*	SD	K*	SD	Ca*	SD	Mg*	SD
B_0S_0	2.25e	0.123	0.023f	0.062	0.18c	0.0082	0.14de	0.0153	0.043c	0.0118
B_0S_{50}	2.70abcd	0.123	0.026f	0.062	0.053c	0.0082	0.13de	0.0153	0.026c	0.0118
B_0S_{100}	2.83abc	0.123	0.0043f	0.062	0.053c	0.0082	0.096e	0.0153	0.026c	0.0118
$B_{40}S_0$	2.47cde	0.123	0.021f	0.062	0.24c	0.0082	0.17d	0.0153	0.04c	0.0118
$B_{40}S_{50}$	2.57bcde	0.123	0.36a	0.062	1.4b	0.0082	0.56b	0.0153	0.22a	0.0118
$B_{40}S_{100}$	2.88ab	0.123	0.33b	0.062	1.3b	0.0082	0.64a	0.0153	0.23a	0.0118
$B_{80}S_0$	3.01a	0.123	0.10e	0.062	0.23c	0.0082	0.23c	0.0153	0.03c	0.0118
$B_{80}S_{50}$	2.43de	0.123	0.24c	0.062	1.8a	0.0082	0.57b	0.0153	0.17b	0.0118
$B_{80}S_{100}$	1.56f	0.123	0.21d	0.062	1.7a	0.0082	0.63a	0.0153	0.16b	0.0118

*% by dry weight. Means with the same letters in the columns are not significantly different according to the Tukey test with significance of 5%

Something similar to that seen with P occurs with N. Although DeLuca and Aplet (2008) argue that biochars from wood generally contain low N content, in the present study treatments without BAM (B_0S_0, B_0S_{50} and B_0S_{100}) presented the lowest concentration of N, suggesting a direct or indirect contribution of the biochar on the greater content of this element. According to this, the highest N concentrations in the substrate were observed in the treatments with BAM ($B_{40}S_{50}$, $B_{80}S_0$ and $B_{80}S_{100}$). The high N concentration in BAM-FS mixture treatments, such as $B_{40}S_{50}$ and $B_{80}S_{100}$, could be due to the high retention of N from the FS by the biochar, rather than a contribution of N in itself from wood lignin. However, the high concentrations of N observed in treatments without FS, such as $B_{80}S_0$, suggests that the biochar itself has the ability to supply N. Although with less contribution, $B_{40}S_0$ (with intermediate BAM and without FS) also showed a higher concentration of N than the three treatments without BAM.

In general terms, biochar can be considered as organic matter that has undergone an accelerated stabilization process, differentiating it from the material it comes from. This would be the main reason why treatments with higher amounts of BAM ($B_{80}S_0$, $B_{80}S_{50}$ and $B_{80}S_{100}$) had the highest average value of OM and OC. The high doses of biochar used (80 ton $\cdot$ ha^{-1}) for these treatments would contribute to a greater aggregation and improved soil and substrate structure, because the organic matter in the biochar can act as a nucleus for agglomeration of mineral and organic particles (Awad et al., 2013), improving structure and water retention (Brewer et al., 2014). These treatments, as discussed in the preceding paragraphs, were also those with the highest concentration of N ($B_{80}S_0$), P ($B_{80}S_{50}$) or both elements at the same time ($B_{80}S_{100}$), although $B_{40}S_{50}$ (with a lower dose of BAM) also showed a high concentration of P and N.

4.2. Seedling quality and physiology (foliar analysis)

The highest DQI was found in $B_{40}S_{50}$, a treatment with a mixture of BAM and FS at intermediate doses. When analyzing which elements have been favored (or at least, differentiated) both in the substrate and at the leaf level in this treatment (which could explain the best performance of the seedlings), it is clear that at the substrate level this treatment has very high CEC and a high content of P (not exclusively limited to this treatment). At foliar level, this treatment was again characterized by high levels of P and Mg and intermediate levels of N, K and Ca.

The other treatments with high DQI (although lower than in $B_{40}S_{50}$) were $B_{40}S_{100}$ and $B_{80}S_{50}$, mixtures with high doses of FS or BAM that have high concentrations of P in substrate ($B_{80}S_{50}$) or in leaf tissue ($B_{40}S_{100}$) as well as high or intermediate levels of N, Ca, K, and Mg in leaves. According to these results, P could be considered as a key element for seedling development, accompanied by significant levels of other nutrients (although not necessarily the highest). As a distinctive feature of $B_{40}S_{50}$ the high CEC could help explain the superior performance of plants by allowing a greater availability of all nutrients.

Like this research, Arteaga-Crespo (2013) found in an evaluation of *A. mangium* biochar for conditioning substrates for the production of *Tlipariti elatum*, that the highest values of DQI were obtained at 50% of BAM dose. It has been proven that high values of this index result in robust plants with balanced aerial and radical fractions, which will exhibit better behavior in the field (Oliet et al., 2000). Sáenz et al. (2010) proposed that the minimum value in quality seedlings should be around 0.2 and they established the following ranges to interpret the DQI: <0.2 as low-quality seedlings; 0.2–0.5 medium quality; and>0.5 as high quality. In this study, we found that high doses of FS ($B_{40}S_{100}$ and $B_{80}S_{100}$) were not the most adequate for producing seedlings of the best quality (they presented medium DQI values), and even resulted in low quality seedlings (DQI < 0.2 in B_0S_{100}) when they were not accompanied with BAM. The intermediate FS values were also not adequate when they were not accompanied with BAM (B_0S_{50}) or BAM was used in an unbalanced proportion ($B_{40}S_{100}$). The total absence of FS resulted in poor seedling quality (B_0S_0, $B_{40}S_0$ and $B_{80}S_0$). In contrast, the combination of intermediate doses of BAM and FS of $B_{40}S_{50}$ were those that resulted in an ICD of good quality seedlings (DQI > 0.5).

Considering the distinctive substrate variables of $B_{40}S_{50}$, as already mentioned, this treatment was highlighted with the highest CEC and probably the highest anion exchange capacity (AEC) as well as high concentrations of P and N. At the level of foliar analysis, this treatment was characterized by the highest level of P, suggesting a possible greater extraction of this element in the substrate. Although seedlings of all treatments were grown on a similar original soil (with a low pH of 3.4), the amount of P from both BAM and FS in treatments with high levels of these fertilizers may have played a key role in the P content found in the leaf analysis. In this regard, it is worth mentioning that in all the treatments except $B_{40}S_{100}$, although in different magnitude, the P decreased (during the study period with respect to the initial values of the substrate (values in Table 4 vs Table 1). Borchard et al (2012) showed that oxygen that is in the functional groups of biochar is responsible for the adsorption of elements such as P, which has probably been more readily available in $B_{40}S_{50}$ for plants due to a potentially high AEC. P is of great importance in plant metabolism, playing a vital role in energy transfer, respiration, and photosynthesis (Grant et al., 2011)

The treatments with the highest total N content in the substrate were $B_{40}S_{50}$, $B_{80}S_{50}$ and $B_{80}S_{100}$ (see previous section), however, this was not directly translated at the level of foliar N or DQI. The treatment with the highest foliar N was $B_{80}S_0$ that was indistinguishable from treatments with low N in substrate such as B_0S_{50}, B_0S_{100} and $B_{40}S_{100}$. Although it has been widely reported that biochar applications in soils and substrates not only provide nitrogen but can increase the availability for plants to take up this nutrient (Ward et al., 1997), the process would only be obvious in $B_{80}S_0$, not in other biochar treatments such as $B_{80}S_{50}$ and $B_{80}S_{100}$.

On the other hand, the fact that treatments with low N levels in the substrate show high levels of foliar N suggests a high efficiency in the use of N in them, two of which did not contain BAM (B_0S_{50} and B_0S_{100}). However, these two treatments had very low DQI, in contrast to $B_{40}S_{100}$ with similar leaf N (but with BAM in the substrate). This result suggests that, although N is a key element for plant development and high levels of leaf N are indicative of high photosynthetic capacity (Marschener et al., 2012; Kammann and Graber 2015), this variable by itself is not enough to determine the development of a balanced seedling in terms of growth and quality.

Although no differences were observed between treatments at the substrate level in elements such as Ca, Mg or K, these differed between treatments at the leaf level, indicating an uneven availability for the plants. Thus, plants from treatments $B_{40}S_{100}$ and $B_{40}S_{50}$ were the most efficient in translocating Mg to leaf tissues, while Ca was in a higher foliar concentration in treatments $B_{40}S_{100}$ and $B_{80}S_{100}$, and K, in $B_{80}S_{50}$ and $B_{80}S_{100}$. In all cases, the highest concentrations of cations in leaves were associated with BAM treatments, so the biochar could have a considerable effect on the uptake of the different nutrients by the plant. The efficiency in the absorption of different nutrients by the roots is fundamental in this sense. Yamato et al (2006) have established that the inclusion of biochar in the soil could be crucial to the supply of available nutrients thanks to the immediate availability of the salts found on the surface of the biochar. This “short-term” entry of available nutrients into the soil could result in a higher plant productivity (Jindo et al., 2012). Although there were significant differences between them, treatments with high levels of cations in their leaves ($B_{40}S_{100}$, $B_{80}S_{50}$ and $B_{80}S_{100}$) represented high DQI (Table 6). This demonstrates the importance of the availability of these elements for plants, probably as a consequence of BAM. It should be noted that two of these treatments ($B_{80}S_{100}$ and $B_{40}S_{100}$) had a high CEC that could favor this process, although it should be noted that $B_{80}S_{50}$ did not stand out in this variable.

Organic residues produce biochar with basic pH through heating by pyrolysis, due to the removal of functional groups that can generate acidity and the increase in ashes that lead to increased salts content (e.g., Ahmad et al. 2013; Cantrell and Martin, 2012; de Moraes Goncalves et al., 2004; Dionisio et al., 2019; ISTA, 2013; Kratz et al., 2012; Marschner et al., 2012; Li et al. 2011). The nutrients contained in salts such as KOH, NaOH, $MgCO_3$ and $CaCO_3$, can be separated from the biochar matrix, causing an increase in pH and increasing the availability of these elements to be adsorbed by plants (Cao and Harris, 2010). From the results of the present study, no significant differences were found in the pH between treatments. This parameter even decreased with respect to the initial value that was already very low due to the intrinsic characteristics of the soils in the study region (Amézquita et al., 2013). Therefore, we infer that there was no supply of elements that leads to basicity, perhaps due to the low pyrolysis temperatures.

5. Conclusions

In the analysis of chemical properties of the substrate, such as CEC, organic matter, carbon, and nutrients, such as N and P, we found an improvement both in the properties and in the content (and probably, availability) of nutrients in some combinations of BAM + FS. Also, an increase in the absorption of elements such as Ca, Mg and K by plants was found in the treatments with BAM + FS which was reflected in better seedling qualities (higher DQI). However, only in a single combination of BAM and FS did the DQI attain a threshold (>0.5) that produced good quality seedlings. This treatment, $B_{40}S_{50}$, characterized by high CEC, N and P in the substrate, was composed of intermediate concentrations of both fertilizers (40 ton $\cdot$ ha^{-1} of BAM and 50% of FS), so we infer that, in addition to the biological advantages, there would be an advantage in both economic and environmental terms due to the 50% decrease in the use of FS. Biochar-only treatments resulted in low DQI values. This suggests that biochar alone would not represent an alternative for increasing the physiological characteristics of *A. mangium* seedlings, but that it should be complemented with the addition of other fertilizers in balanced amounts.

CRediT authorship contribution statement

Giovanni Reyes Moreno: Conceptualization, Investigation, Data curation, Statistical analysis, Manuscript writing and editing. **Maria Elena Fernández:** Conceptualization, Original draft writing, review and editing of final manuscript version. **Enrique Darghan Contreras:** Data curation, statistical analysis.

Declaration of interest statement

None.

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