JEE Journal of Ecological Engineering

Journal of Ecological Engineering 2024, 25(3), 282–290
https://doi.org/10.12911/22998993/182868
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Received: 2023.12.30
Accepted: 2024.01.13
Published: 2024.01.30

Effects of Different Biochar Types on the Growth and Functional Traits of Rice (*Oryza sativa* L.)

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ABSTRACT

Biochar's impact on plant growth is complex, varying with type and application rate. This study explored how four biochars (rice husk, cocoa shell, peanut shell, carob) affect rice (*Oryza sativa* L.) at five rates (0–5%). Biochar significantly enhanced rice growth, but the optimal type and rate varied. Total dry mass increased by an average of 20% in biochar-treated groups compared to controls, with rice husk and cocoa shell biochar at 5% application rate achieving the highest yields. The effects on plant components differed. While leaf mass fraction responded favorably to all biochar types, stem and root mass fractions remained largely unchanged. Additionally, root strength, as measured by root dry matter content, increased with all biochars, particularly rice husk biochar which boosted it by 8%. All biochar types also enhanced leaf mass per area, a key indicator of photosynthetic efficiency. These findings highlight the importance of tailoring biochar application strategies to specific crops and soil conditions. Optimizing biochar application based on its influence on root strength, leaf mass allocation, and growth across different crop species and soil conditions can unlock its full potential for sustainable development.

Keywords: sustainable development, soil reclamation, restoration, biomass, waste.

INTRODUCTION

Derived from biomass combustion, biochar serves as a circular economy solution addressing food security and environmental concerns, with its multifaceted applications spanning soil improvement, waste reduction, climate change mitigation, and energy generation (Jatav *et al*., 2021; Phillips *et al*., 2022; Singh *et al*., 2022; Kumar *et al*., 2023). These versatile applications, either individually or in synergy, hold the potential for substantial socio-economic benefits. In response to climate change's impact on agriculture – manifesting as heightened temperatures and reduced rainfall – farmers worldwide are observing crop vulnerabilities (Malhi *et al*., 2021). In this context, biochar emerged just 15 years ago as a global strategy for climate mitigation, gaining prominence as a soil amendment that curbs greenhouse gas emissions and facilitates CO_2 removal (Mohan *et al*., 2018; Lehmann *et al*., 2021; Adebajo *et al*., 2022). In comparison to other organic options, biochar stands out as the most effective soil amendment due to its remarkable capacity to enhance the chemical, physical, and microbiological aspects of soil, including boosting nutrient availability and retention because to its high charge density (Lehmann & Joseph, 2009; Ding *et al*., 2016; Olmo *et al*., 2016).

The most frequent biochar application rates to soils depend on objectives, soil conditions, target crops, biochar qualities, and intended outcomes. However, application rates range from 5 to 15 t ha^{-1} (up to 0.5% by soil mass) either to 300 to 600 t ha^{-1} (2 to 10% by mass), with 5% being most usual (Sohi *et al*., 2010; Lehmann *et al*., 2021). In terms of biochar sources, there is a variety of feedstocks like poultry litter, acacia bark, corn cobs, paper pulp, green waste, wood, peanut hull, pine chip or biosolids (Jeffery *et al*., 2011; Ali *et al*., 2021). For instance, the beneficial effects of cocoa shell biochar (CSB) on maize and cayenne pepper production are associated with reduced soil acidity, while peanut shell biochar (PSB) enhances various soil parameters in low fertility soils, such as saline-sodic paddy soils, due to biochar's porous structure, large specific surface area, and nutrient absorption capacity (Wu *et al*., 2015; Ding *et al*., 2016; Hagemann *et al*., 2017; Cornelissen *et al*., 2018; Ariani *et al*., 2021; Dominchin *et al*., 2021; Singh Karam *et al*., 2022). When compared to limestone, 2% rice husk biochar (RHB) had a favorable impact on wheat plant development and Cd accumulation (Niu *et al*., 2022). The amount of soil organic carbon (SOC), soil pH, soil cation exchange capacity (CEC), and available P, K, and N increases as a result of RHB applications (Abrishamkesh *et al*., 2015; Asadi *et al*., 2021). RHB burned at 700 °C enhanced soil pH by decreasing aluminum concentration in acidic soils (Singh Karam *et al*., 2022). Nevertheless, soil texture may determine the RHB's effects. A RHB rate of 1%, for example, had no effect on alkalinity in tropical Alfisols (Gamage *et al*., 2016). In general, biochar makes soil less acidic, increases soil fertility, reduces the amount of fertilizer used, and helps root growth and nitrogen use (Hussain & Ravi, 2022).

Plant traits, encompassing morphological, physiological, and phenological characteristics, are vital in responding to environmental changes and determining ecological strategies, thereby influencing ecosystem properties (Kleyer *et al*., 2019; Xiang *et al*., 2017). Plant allocation patterns can serve as indicators or predictors of plant responses, such as biochar-induced changes in soil characteristics, with biomass allocation favouring roots in response to below-ground limitations and favoring shoots in response to above-ground limitations (Pérez-Harguindeguy *et al*., 2013). Research findings indicate that high rates of biochar addition can stimulate fine root proliferation, as evidenced by increased specific root length, decreased root diameter, and root tissue mass density, with potential implications for plant fitness and performance regardless of fertilization levels (Xiong *et al.*, 2016). Unraveling the intricate relationship between biochar type, application rate, and rice performance, this study tested the hypothesis that while moderate biochar application boosts rice growth and functional traits, exceeding optimal levels could diminish or even negate these benefits. By evaluating four biochar types (rice husks, cocoa shells, peanut shells, and carob) on functional traits of rice (*Oryza sativa* L.), this study aimed to unlock biochar's full potential for sustainable agriculture.

MATERIALS AND METHODS

Soil characteristics and climate conditions

The soil used for the experiment is classified as Fluvisol, coming from rice areas of Ecuador in the arable layer (20 cm). The soil is of clay silt loam type with a texture with 16.8% sand, 49.6% silt and clay 33.6%. The bulk density of the soil was 1.26 g/cm^3. The experiment was developed with rice plants (*Oryza sativa* L.) variety INIAP-11, under semi-controlled conditions in a greenhouse of the Escuela Superior Politécnica Agropecuaria de Manabí Félix López (ESPAM MFL) in Calceta, Ecuador. The average temperature in the greenhouse during the experiment was 25.7 ± 4.3 °C (mean ± SD) and the relative humidity was 80.2 ± 1.2%.

Biochar production

The biochar was produced from *Ceratonia siliqua* L. residues (CRB), *Theobroma cacao* L. husk (CHB), *Oryza sativa* L. husk (RHB) and *Arachis hypogaea* L. husk (PHB). The biochar was made using a laboratory-scale pyrolysis apparatus based on the design of the Anila Stove. The pyrolysis time depended on the raw material used. The carob tree was one of the materials that obtained the maximum temperatures and the one that needed more time for pyrolysis, because it is a dense wood. The maximum temperatures reached during pyrolysis ranged between 350 °C and 550 °C and the process time with temperatures > 300 °C fluctuated between 1.5 and 2 h, depending on the raw material unlike carob that required more than 2 h, being a dense wood. To develop the experiment was needed 3780 g for each type of raw material. The different types of biochar were ground in a stainless-steel mill model sk100 (Retsch, Germany) with a < 2 mm mesh.

Pot experiment

The test was developed in unperforated pots, with a capacity of 5 kg, with a volume (6 L) with a height of 25 cm and 18.5 cm wide. The pots were filled with 4000 g (soil + biochar). An A×B factorial design was used for the experiment. There were four distinct types of biochar (Factor A) and four different application rates (Factor B) of 1.0, 1.5, 3.0, and 5.0% of biochar to each of the treatments. In addition, there was a control treatment. One rice plant was transplanted per pot for 90 days.

Biomass and functional traits

At the end of the experiment the aerial part of the plant was cut, which was separated into stem, leaves and fruits. The fresh mass of leaves and stem was obtained. The roots were separated from the soil, washed, and dried to obtain fresh mass. Then all the parts were dried at 70 °C for 48 h in a stove (Faithful, USA), after which the dry mass of all the organs was obtained. The total dry mass (TDM) was obtained with the sum of the root dry mass (RDM), stem dry mass (SDM) and leaf dry mass (LDM).The root mass fraction (RMF), leaf mass fraction (LMF) and stem mass fraction (SMF) were calculated with the dry mass of root, stem and leaves divided by the total plant dry mass (Poorter *et al*., 2012; Puglielli *et al*., 2015). The dry matter content (DMC) of root (RDMC), stem (SDMC) and leaf (LDMC) was obtained as dry mass (mg) divided by fresh mass (g) (Garnier *et al*., 2001; Shipley & Vu, 2002; Hodgson *et al*., 2011). The leaf mass per area (LMA) was calculated as the ratio of dry mass to leaf area (g m^{-2}). Before drying, three leaves were scanned for each rice plant at a resolution of 600 dpi (Pérez-Harguindeguy *et al*., 2013; de la Riva *et al*., 2016; Xiong *et al*., 2016; Féret *et al*., 2019).

Statistical analysis

The data obtained were statistically analyzed at a probability level of 5% using one-way ANOVA comparing all the treatments obtained from the interaction between factors plus the absolute control. Tukey's HSD post hoc test was used to perform the multiple comparison of means. A two-way ANOVA was then used, where the combined effects of biochar types and application rates were analyzed. Statistical analysis was performed using InfoStat software. To demonstrate the effect of the factors against the control, an orthogonal contrast was performed to measure statistical significance (Di Rienzo *et al*., 2008).

RESULTS

Effect of biochar on total dry mass

Total plant dry mass (including roots, stems, and leaves) was analyzed to see how biochar treatment affected it. The total dry mass of plants in the control group and those treated with biochar differed significantly (Fig. 1a, Table 1). Analysis of the effects of biochar application rate on total dry mass (Fig. 1b) confirmed this finding. Not all biochars had the same results, though (Fig. 1c). There was a linear relationship between the amount of carob and cocoa biochar used and the increase in rice's dry mass (Fig. 1c). After being exposed to a high concentration of cocoa biochar, TDM increased by 54.5% when compared to the control and by 14% when compared to carob biochar (Fig. 1c). At each application rate, peanut and rice biochar had the least effect on total dry mass. Leaf, stem, and root dry mass were significantly affected by biochar type (Table 1). The optimum cocoa biochar application rate for maximizing dry mass yield was found to be 5%.

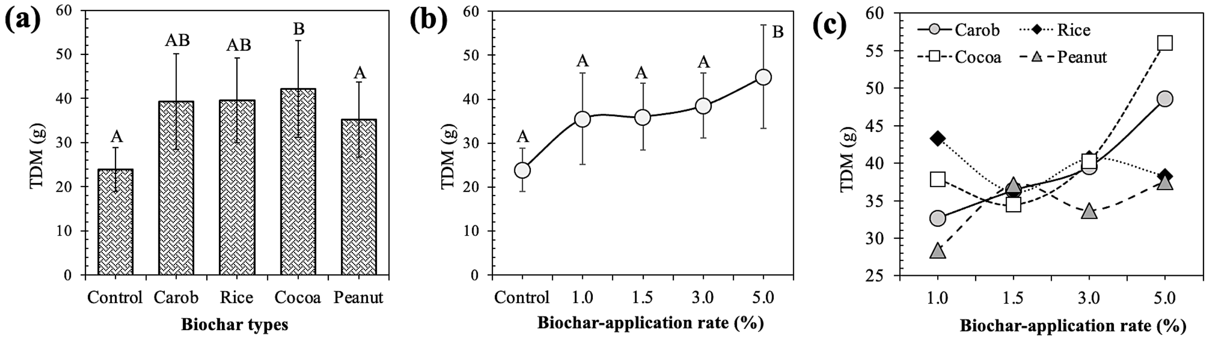
Effect of biochar on mass fraction

Although the stem and root mass fractions did not increase in the biochar-treated versus control groups, the leaf mass fraction did (Table 2, Fig. 2a). The mass fractions were significantly influenced by the biochar application rate (Fig. 2b). Positive effects of biochar treatments and doses on LMF relative to control are seen in Figs. 2a and 2c. However, biochar treatments had no effect on SMF or RMF when compared to the control (Table 2).

Table 1. ANOVA results for the effect on biochar type (treatment) and application rate on dry mass (leaf, stem, root, total)

Variables	Biochar type	App. rate	Biochar x app. rate	Control vs treat.	% VC
Leaves dry mass (LDM)	0.0111**	<0.0001***	0.0015 **	0.0016 **	23.10
Stem dry mass (SDM)	0.0539	0.0001***	0.0012**	0.0019**	29.39
Root dry mass (RDM)	<0.0001***	0.036	0.0318	<0.0001***	22.25
Total dry mass (TDM)	0.0029**	<0.0001***	0.0017 **	0.0001***	21.46

Note: %VC – percent of variation coefficient. *P* values are shown: * $P<0.05$; ** $P<0.01$; *** $P<0.001$.



Biochar types: p-value (≤0.0029)
Tukey α = 0.05

Biochar-application rate: p-value (<0.0001)
Tukey α = 0.05

Interaction biochar * application
p-value (0.0017)

Figure 1. Effect of biochar types and application-rate on total plant dry mass

Increases in LMF were observed at low treatment rates for carob and peanut biochar (Fig. 2c). The greatest average LMF of any treatment was 0.11, which was achieved with high-rate rice biochar. LMF average value as high as 0.11 were also found in carob and peanut at a dosage rate of 1%. The average SMF value was highest for cocoa biochar at 0.76 with the application of cocoa biochar at a rate of 5%, whereas the lowest average SMF value of 0.75 was observed when carob biochar was applied at a rate of 1.5% (Fig. 2d, e, f). The range of RMF varies from 0.15 to 025, with maximum for cocoa biochar at 1% and minimum for cocoa biochar at 5% and control treatment (Fig. 2g, h, i).

Effect of biochar on dry matter content and LMA

Although all types of biochar influenced LMA, LDMC and RDMC (Table 3, Fig. 3), no change was observed in SDMC. All forms of biochar showed increases in RDMC in response to both low and high treatment (Fig. 3g). Comparatively, rice biochar increased RDMC values by up to as 8%. Approximately 16.7 mg/g RDMC was achieved with a carob and cocoa application rate of up to 1.5%. However, at 5.0% rice biochar application rate, RDMC was the highest at 17 mg/g (Fig. 3). The effect of dosing different types of biochar on LMA is presented in Figure 4, where statistical significance is observed specially using rice, carob, and peanut biochar. An application-rate of peanut biochar of 1.5% and rice biochar of 5.0% both reached the highest value of LMA up to an average of 37.8 g m^{-2}.

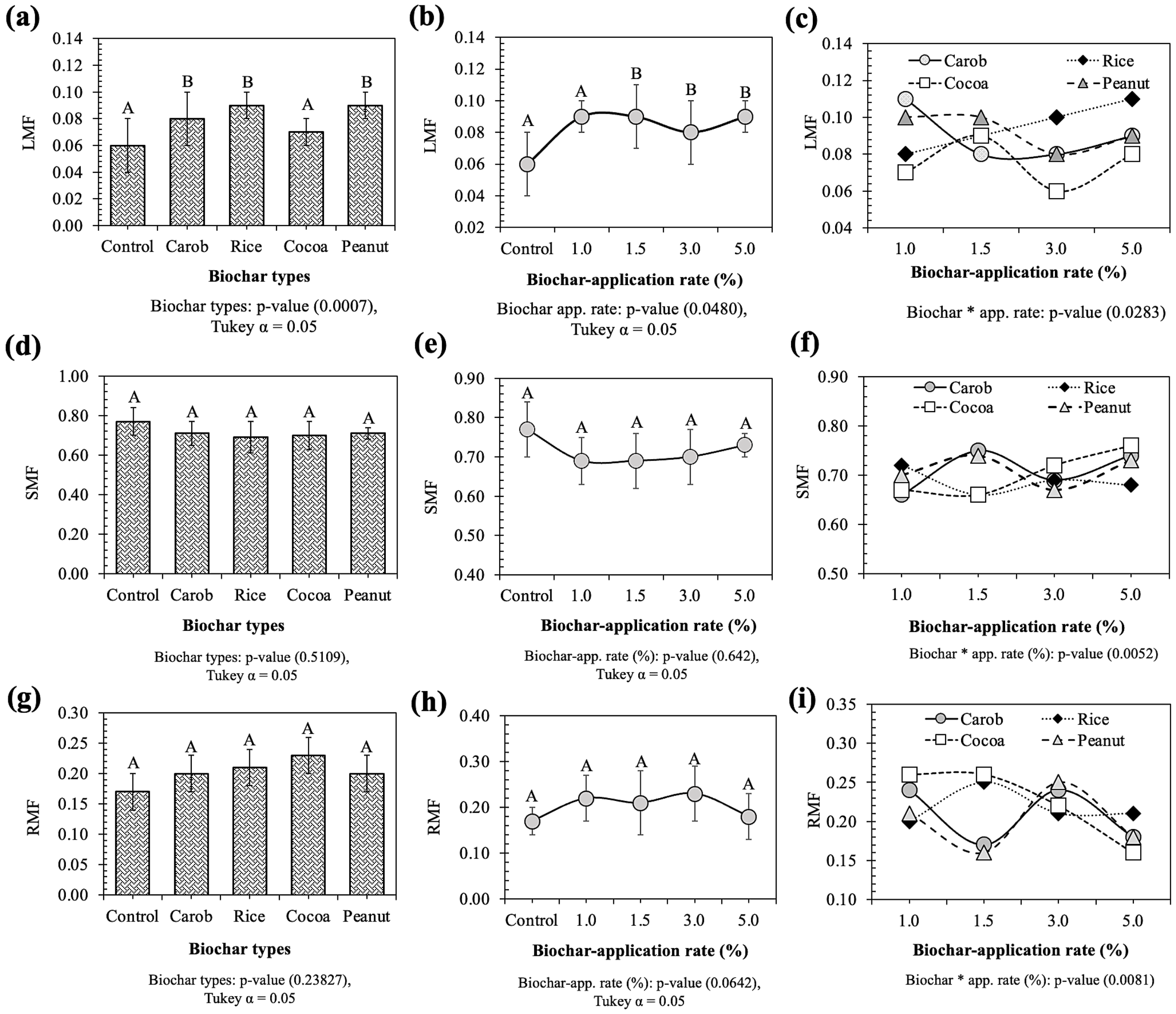
DISCUSSION

Consistent with previous research, our results show that distinct types of biochars had significant and positive effects on total plant dry mass, with differences observed between the control group and biochar-treated rice plants. The results show a positive linear relationship between increased dry mass and carob and cocoa biochar application rates, with cocoa biochar at a high concentration producing the highest increase (up to 43.2%). Peanut and rice biochar had less of an impact. The best application rate for maximizing rice dry mass yield was discovered to be 5% for cocoa biochar. Liu *et al.* (2021) found that adding wheat straw biochar at a 2% application rate increased the total dry mass by 12.9% when compared to the non-biochar treatment. Besides, research has indicated that the use of rice biochar can improve soil qualities, increase rice yield, and improve nutrient uptake in paddy soils (Xiong *et al.*, 2016; Yao *et al.*, 2021). On the other hand, soil type, biochar addition, and irrigation practices were

Table 2. ANOVA results for the effect of biochar type (treatment) and application rate on mass fraction (leaf, stem, root, total)

Variables	Biochar type	App. rate	Treatm. x app. rate	Control vs treatment	% VC
Leaf mass fraction (LMF)	0.0007**	0.0480 *	0.0283 *	<0.0001***	23.07
Stem mass fraction (SMF)	0.5238 NS	0.0706 NS	0.0081 **	0.144 NS	9.55
Root mass fraction (RMF)	0.2382 NS	0.0056 **	0.0052 **	0.7535 NS	28.02

Note: %VC – percent of variation coefficient, *P* values are shown: * $P<0.05$; ** $P<0.01$; *** $P<0.001$.



Biochar types: p-value (0.0007), Tukey α = 0.05

Biochar app. rate: p-value (0.0480), Tukey α = 0.05

Biochar * app. rate: p-value (0.0283)

Biochar types: p-value (0.5109), Tukey α = 0.05

Biochar-app. rate (%): p-value (0.642), Tukey α = 0.05

Biochar * app. rate (%): p-value (0.0052)

Biochar types: p-value (0.23827), Tukey α = 0.05

Biochar-app. rate (%): p-value (0.0642), Tukey α = 0.05

Biochar * app. rate (%): p-value (0.0081)

Figure 2. Effect of biochar types and application-rate on leaf (LMF), stem (SMF) and root mass (RMF) fraction

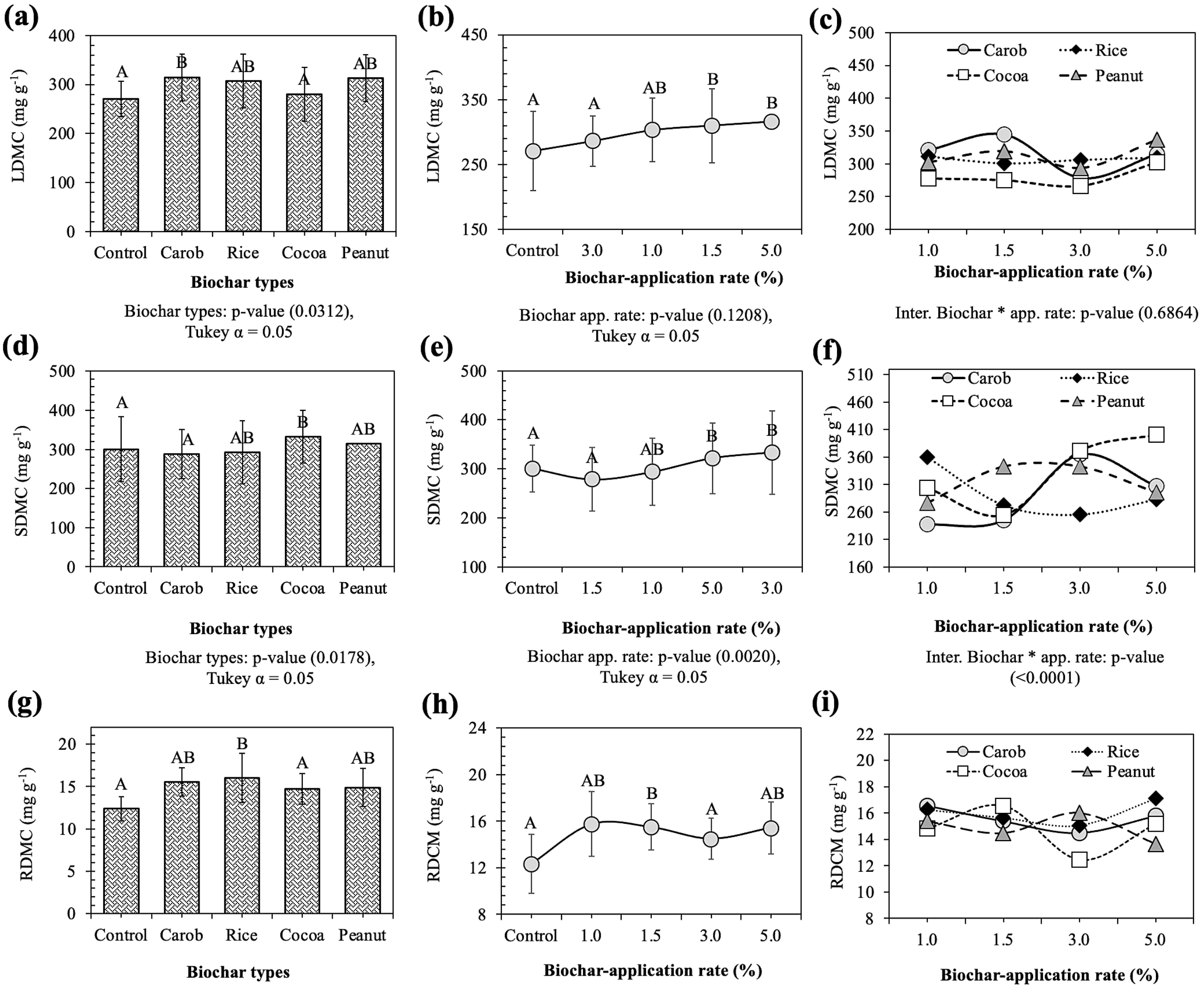
Table 3. ANOVA results for the effect of biochar types and application-rate on leaf, stem, and root dry matter content and leaf mass per area

Variables	Treatment	App. rate	Treatment x app. rate	Control vs treat.	% VC
Leaf mass per area (LMA)	0.2118 NS	0.5126 NS	0.8764 NS	<0.0001***	21.42
Leaf dry matter content (LDMC)	0.0327	0.2671 NS	0.5911 NS	<0.0001 ***	19.69
Stem dry matter content (SDMC)	0.1237 NS	0.001**	<0.0001***	0.4272 NS	23.62
Root dry matter content (RDMC)	0.0239	0.0454	0.0015 **	0.0003 **	12.65

Note: %VC – percent of variation coefficient, P values are shown: * P<0.05; ** P<0.01; *** P<0.001.

found to have a significant impact on the plant total dry mass (Liu *et al.*, 2021).

In the present study, the application of carob and peanut biochar at low rates increased LMF values to as high as 0.11. High-rate rice biochar had the highest average LMF of 0.11, whereas cocoa biochar had the highest average SMF of 0.76. Optimal application rates vary among different biochar varieties, highlighting the context-specific nature of biochar effects on plant mass fractions. According to literature, biochar treatments at high rates tend to increase root biomass and root mass fraction compared to organic amendments and no amendment treatments (Farrar *et al.*, 2021). In general, the partitioning perspective on biomass allocation among organs focuses on size-independent ratios such as mass fractions (Kleyer *et al.*, 2019). LMF, SMF, and RMF, state variables characterizing plant allocation (Poorter *et al.*, 2012), indicate that plants in nutrient-poor environments tend to allocate a



Biochar types: p-value (0.0312), Tukey α = 0.05

Biochar app. rate: p-value (0.1208), Tukey α = 0.05

Inter. Biochar * app. rate: p-value (0.6864)

Biochar types: p-value (0.0178), Tukey α = 0.05

Biochar app. rate: p-value (0.0020), Tukey α = 0.05

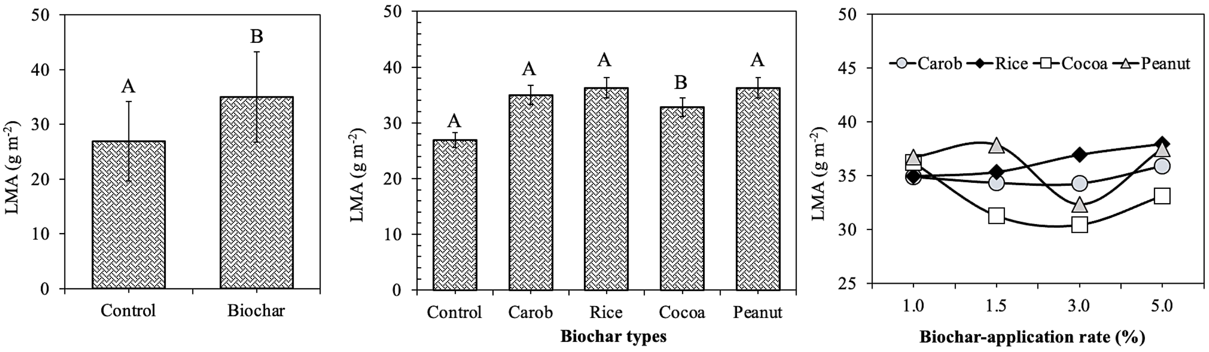
Inter. Biochar * app. rate: p-value (<0.0001)

Biochar types: p-value (≤0.0239), Tukey α = 0.05

Biochar app. rate: p-value (0.0454), Tukey α = 0.05

Inter. Biochar * app. rate: p-value (0.0015)

Figure 3. Effect of biochar types and application-rate on leaf, stem, and root dry matter content



Orthogonal contrast: Biochar vs Control, p-value (<0.0001)

Biochar types: p-value (0.0007), Tukey α = 0.05

Interaction Biochar * app. rate: p-value (0.0081)

Figure 4. Effect of biochar types and application-rate on leaf mass per area (LMA)

higher proportion of new biomass to their roots and maintain a greater distribution of biomass in roots compared to stems, with the root-mass fraction serving as a simple expression of biomass distribution in roots. The changes in allocation when nutrients are limiting are the strongest of all allocation responses, with a large increase in roots at the expense of stem and of leaf biomass (Pérez-Harguindeguy *et al.*, 2013). With increasing total biomass, plants increase SMF at the expense of LMF and to a lesser extent RMF (Poorter *et al.*, 2012). This suggests a positive relationship of plant height with

SMF, a negative one with LMF, and no correlation with RMF (Kleyer *et al*., 2019). In line with previous research, the current study revealed that biochar treatments have the ability to boost LMF but had no effect on stem and root mass fractions.

In response to both low and high treatment, all types of biochar exhibited elevated levels of RDMC. The application of rice biochar at a rate of 5.0% resulted in the highest RDMC value of 17 mg/g. Plant traits like leaf and stem dry matter content are stable along gradients such as nutrients or disturbance, but not along a light gradient (Mason *et al*., 2011). References point out that rice roots are able to take up some biochar nanoparticles in soil and then transport them into plant shoots (Wu *et al*., 2015). In this sense, biochar is useful for enhancing the quality of acidic soil (Singh Karam *et al*., 2022). The alkaline effect of biochar expands the range of nutrient absorption and utilization and providing the required nutrients for rice growth as much as possible (Mansoor *et al*., 2021). Biochar application offers nutritional benefits to both plants and soils, as it contains abundant essential minerals such as calcium, magnesium, copper, and iron, supporting plant growth and development; however, biochar derived from nutrient-poor feedstocks may provide limited short-term soil fertility benefits, resulting in marginal enhancements in crop growth (Alburquerque *et al*., 2014).

High leaf mass per unit area (LMA) is advantageous under adverse growing conditions, favoring slow tissue turnover and often considered an adaptation to drought, as it scales linearly with photosynthetic capacity and leaf biomass investment (de la Riva *et al*., 2016; Puglielli *et al*., 2015). In this study, the results showed a positive effect on LMA values by the addition of all types of biochars under all application rates. In these terms, leaf density and the leaf volume to area ratio are favored by biochar treatments (Xiong *et al*., 2016). The application of biochar has been shown to have significant effects on various root variables, including root length, diameter, surface area, and dry weight, as demonstrated by Olmo *et al.* (2016) and Huang *et al.* (2021). Additionally, biochar addition has been found to increase both shoot and root biomass, as reported by Prendergast-Miller *et al.* (2014). Furthermore, the allocation of biomass is influenced by biochar addition, with an increase in leaf allocation observed, as highlighted by Alburquerque *et al.* (2014). These findings collectively emphasize the impact of biochar application on root development, biomass accumulation, and biomass allocation patterns in plants.

Adding biochar improved rice's functional traits, according to the study hypothesis, and the effect was favorable at high concentrations. However, due to the wide range of biochars, soils, and fertilizer management strategies used in most reference experiments, comparing results across studies is challenging (Liu *et al*., 2016).

CONCLUSIONS

Biochar, a charcoal-like material, significantly increased the total dry weight of rice plants, with carob and cocoa biochars showing the strongest linear effect. The optimal application rate for maximizing dry mass was 5%. Biochar also enhanced root strength, with rice biochar leading to a 22.8% increase in root dry matter content (RDMC). Carob and cocoa biochars similarly boosted RDMC but stem dry matter content (SDMC) remained unchanged across all biochars. This suggests biochar's impact on plant traits depends on type and application rate. While biochar's potential as a fertilizer substitute remains unclear, its effectiveness in enhancing soil quality and crop productivity warrants further research into its mechanisms and optimal management practices for sustainable agriculture.

Acknowledgements

Authors are grateful to ESPAM-MFL for its invaluable support.

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