



Productivity and nutritional performance of red lettuce in crop succession as affected by biochar

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Abstract

The use of biochar as a soil conditioner has been widely investigated due to its potential to mitigate nutrient loss in agricultural soils, offering a sustainable alternative to intensive mineral fertilization. This study was conducted to evaluate the impact of increasing biochar rates on the productivity and morphological characteristics of red lettuce (*Lactuca sativa* L. cv. Scarlet) grown in a lowfertility Oxisol, in a succession system following arugula. The experiment was conducted in a greenhouse, in a completely randomized design (CRD), consisting of six treatments corresponding to rates of 0 %, 0.8 %, 1.6 %, 2.4 %, 3.2 %, and 4.0 % biochar incorporated into the substrate, with five replicates. The biochar was derived from the pyrolysis of sugarcane bagasse. Plants were harvested 30 days after transplanting and evaluated for morphological and biomass parameters. Total leaf number, stem diameter, and total fresh mass did not differ significantly among treatments. However, a significant increase was observed in marketable fresh mass and leaf dry mass at biochar rates between 1.6 % and 4.0 % compared with the 0 % and 0.8 % treatments. The results indicate that biochar, even in lowfertility soils and crop succession systems, improves marketable biomass and leaf dry mass accumulation in red lettuce, although the effect on primary morphological characteristics was limited, suggesting that the material enhances nutrient retention essential for productivity.

Keywords: Agronomic performance, Soil fertility, *Lactuca sativa*

Introduction

Lettuce (*Lactuca sativa* L.), belonging to the Asteraceae family, is a globally cultivated vegetable of great economic importance (Alomari *et al.*, 2024). Since lettuce is consumed fresh, mainly in salads, growers must adopt adaptable cultivars and cultivation methods that ensure high productivity and nutritional quality. Intensive cultivation, however, often relies on excessive mineral fertilization, which can lead to soil degradation, increased acidity, longterm loss of organic matter, low nutrientuse efficiency, and nitrate accumulation in plant tissue (Sylvestre *et al.*, 2019). Given these challenges, the search for more sustainable agricultural practices that improve soil fertility and ecological balance is imperative (Sossa *et al.*, 2024).

In this context, biochar, a carbonrich solid material produced by pyrolysis of biomass under

limited oxygen conditions, has been used as a promising soil conditioner (Visiy *et al.*, 2022). The application of biochar is intended to improve the total organic carbon stock in the soil, as well as to enhance physicochemical properties such as cation exchange capacity (CEC), water retention, and porosity. Biochar contains highly recalcitrant carbon that resists decomposition, contributing to the maintenance of organic matter and soil fertility over several cultivation cycles (Carter *et al.*, 2013).

The impact of biochar varies widely according to feedstock, application rate, and soil type. In soils of semiarid regions, for example, biochar can be an effective conditioner for degraded soils (Asri, 2022). Optimizing biochar use often requires combining it with mineral or organic fertilizers to ensure satisfactory productivity, as pure biochar may have a low content of essential

nutrients (AkotoDanson *et al.*, 2018). Combined application can generate synergistic effects, improving nutrient use efficiency, reducing leaching losses, and increasing the bioavailability of N, P, K, Ca, and Mg (Sossa *et al.*, 2024).

The longterm effect of biochar in crop succession, as in the present study following arugula cultivation, is a practice that can intensify production but imposes greater nutritional stress on the soil. In a previous experiment, conducted in the same pots and soil, it was observed that biochar significantly influenced only the number of leaves and stem diameter of arugula, while biomass remained stable. This finding underscores the need to investigate whether lettuce, which is more nutrient responsive, exhibits a more evident response to the residual effect and new application of biochar (Visiy *et al.*, 2022).

Moreover, the biochar source and its pretreatment are crucial. Materials such as corncob biochar or tomato waste biochar (Asri, 2022; Visiy *et al.*, 2022) and cashew nut shell biochar (Sossa *et al.*, 2024) have been investigated, but the use of sugarcane bagasse biochar enriched with microsilica is of particular interest, because its alkaline composition and nutrient enrichment can directly influence soil pH and cation availability.

Considering that the soil used has low fertility (Oxisol, B horizon), the aim of this study was to improve understanding of the agronomic effectiveness of biochar in a crop succession system. Therefore, this work was carried out to evaluate the influence of increasing rates of enriched biochar on the morphological and productive characteristics of red lettuce (*Lactuca sativa* L. cv. Scarlet) grown in a lowfertility substrate. It is hypothesized that biochar improves nutrient availability and, consequently, the productive performance and biomass accumulation of lettuce, overcoming the null effect observed in the preceding arugula crop.

Material and methods

Study site and experimental design

The experiment was conducted in a greenhouse at the Olericulture Educational Production Unit (UEP), belonging to the Instituto Federal Institute of Education, Science and Technology of South of Minas Gerais - IFSULDEMINAS, Campus Inconfidentes, in the municipality of Inconfidentes, Minas Gerais. The site is located at an altitude of 940 m, 22°18'47" S latitude and 46°19'54.9" W longitude, with a subtropical climate with dry winters and hot summers (Cwa), and average annual temperature of 19 °C and rainfall of 1,800 mm.

The experiment used a completely randomized design (CRD), consisting of six treatments corresponding to different concentrations of biochar applied to the soil (T1: 0 %; T2: 0.8 %; T3: 1.6 %; T4: 2.4 %; T5: 3.2 %; and T6: 4.0 %), with five replicates, totaling 30 experimental units.

Soil preparation and fertilization

The experiment was set up in 10 liter plastic pots filled with 6 dm³ of a sample of a lowfertility Oxisol, taken from the B horizon. This soil had been previously used for arugula cultivation, characterizing a crop succession system. A composite soil sample was analyzed, yielding the following chemical characteristics: pH in water = 5.29; P = 28.8 mg dm⁻³; K = 138.7 mg dm⁻³; Al = 0.30 cmolc dm⁻³; Ca = 2.8 cmolc dm⁻³; Mg = 0.48 cmolc dm⁻³; H+Al = 3.26 cmolc dm⁻³; sum of bases (SB) = 3.64 cmolc dm⁻³; CEC = 6.89 cmolc dm⁻³; base saturation (V) = 52.75 %; organic matter (O.M.) = 18.6 g dm⁻³; Al saturation (m) = 7.62 %; Ca/Mg = 5.78; Mg/K = 1.36; Zn = 2.4 mg dm⁻³; Fe = 33.0 mg dm⁻³; Mn = 21.0 mg dm⁻³; Cu = 1.9 mg dm⁻³; B = 0.2 mg dm⁻³; Prem = 11.71 mg L⁻¹. The

soil was classified as medium-textured based on physical analysis, with 40 % sand, 42 % silt, and 18 % clay.

Liming and fertilization protocols followed the guidelines established by the Instituto Agronômico (Aguiar *et al.*, 2014). To correct soil acidity, 7.5 g of lime (relative neutralizing value [RNV] of 80 %, containing 10 % MgO and 38 % CaO) was incorporated per pot. Fertilization consisted of applying rates equivalent to 40, 150, and 60 kg ha⁻¹ of N, P₂O₅, and K₂O, respectively, before arugula sowing. Additionally, shortly before transplanting the lettuce seedlings, each pot received supplementary fertilization consisting of 0.95 g of urea (0.40 g N), 0.50 g of single superphosphate (0.10 g P₂O₅), and 0.38 g of potassium chloride (0.23 g K₂O). These rates were estimated to supply approximately 60, 20, and 40 kg ha⁻¹ of N, P₂O₅, and K₂O, and to raise base saturation to 80 %.

Characteristics and application of biochar

The biochar used in this study was based on microsilica and char derived from the pyrolysis of sugarcane bagasse. This material underwent a refining process and was enriched with highly oxidative sterilizing inorganic compounds, as well as macro and micronutrients, including potassium, iron, manganese, copper, calcium oxide, zinc, and magnesium. The physicochemical properties of the biochar included an average moisture content of 5 %, average bulk density of 0.5 g cm⁻³, and a fine black powder appearance. The material is alkaline, which favors nutrient availability to the crop.

Biochar was applied directly to the soil, after being weighed according to the treatment rate (T1 to T6), and thoroughly mixed into the substrate before seedling planting. The application was intended both to supply macro and micronutrients and to contribute to water retention and the control of harmful microorganisms. The applied amount of 80 g to 400 g per pot corresponded

to increasing concentrations in the substrate. The treatments were distributed in a completely randomized design, enabling evaluation of the effect of progressive rate increases on the variables of interest.

Lettuce cultivation and evaluation

Seedlings were produced in 288-cell plastic trays using a 1:1 mixture of vermicompost and a commercial substrate for vegetable seedling production. Seeds of the red lettuce cultivar Scarlet (Sakata Seed Sudamerica Ltda.), recognized for its hardiness and adaptability, were used. This cultivar has large plants, broad leaves, light purplebrown coloration, and high crispness, with an average cycle of 60 days. Upon reaching the transplanting stage (three expanded leaves), the seedlings were transplanted to the pots. Two seedlings were transplanted into a single hole in the center of each pot; this density was chosen to ensure sufficient biomass for all proposed analyses.

Harvest occurred 30 days after transplanting, when morphological and productive parameters of the plants were evaluated. The total number of leaves was counted to determine vegetative development and divided into marketable leaves—those suitable for consumption—and nonmarketable leaves, discarded due to damage, inadequate size, or poor shape. Stem diameter (mm) was measured as an indicator of the plant's structural vigor, while plant height (cm) enabled evaluation of vertical growth.

Yield parameters evaluated included total fresh mass, marketable fresh mass, and nonmarketable fresh mass (g), allowing estimation of marketable yield. All fresh masses were determined on a semianalytical balance (BEL Equipamentos, model MARK ES 1501, capacity 1500 g, resolution 0.1 g).

For dry mass determination, leaves (petiole and blade) and stem were separated and placed

in a forced air circulation oven at $65\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ until the samples reached constant weight. After drying, the material was again weighed on the same balance, obtaining leaf dry mass and stem dry mass.

The collected data were subjected to statistical analysis using the SISVAR 5.0 software (version 4.3; Universidade Federal de Lavras, Lavras, MG). The F test was used for analysis of variance, and means were compared using the Scott-Knott test at 5 % probability. The response of the analyzed variables as a function of biochar rates was also examined by regression analysis.

Results and discussion

Increasing biochar rates applied to the low fertility soil did not significantly affect the morphological variables evaluated, with the exception of the number of nonmarketable leaves (Table 1).

In Table 1, the analysis of variance for most morphological variables—total leaf number ($p = 0.5657$), marketable leaf number ($p = 0.2683$), stem diameter ($p = 0.9058$),

and plant height ($p = 0.7016$)—indicated no significant differences among treatments.

The absence of a significant difference in parameters such as height and stem diameter, which are indicators of structural vigor, contrasts with some studies where marked improvements with biochar were observed (Jaborova *et al.*, 2021). For example, Jaborova *et al.* (2021) reported a 25 % to 39 % increase in lettuce leaf length with biochar rates of 2 % and 3 %. Biomass stability, as observed in the previous experiment with arugula, may extend to these lettuce characteristics, suggesting that, under the specific conditions of this succession soil, biochar had a limited effect on macroscopic vegetative development or that the optimal rate for these variables was not reached.

The only morphological variable that showed a significant difference was the number of nonmarketable leaves ($p < 0.05$). Biochar rates of 0.8 %, 3.2 %, and 4.0 % resulted in more nonmarketable leaves (mean values of 3.00, 2.50, and 2.60, respectively) than the 0 %, 1.6 %, and 2.4 % treatments. Thus, there was no clear increasing or decreasing trend as a function of biochar rates (Table 1). Although

Table 1. Morphological and structural variables of red lettuce as a function of biochar rates.

Treatment ¹	Total leaf number	Marketable leaf number	Nonmarketable leaf number	Stem diameter (mm)	Plant height (cm)
0.0 %	19.3	17.5	1.9 b	19.38	21.39
0.8 %	20.0	17.0	3.0 a	18.85	22.15
1.6 %	19.9	17.8	2.0 b	18.06	22.80
2.4 %	20.8	18.8	1.6 b	19.50	23.15
3.2 %	20.2	17.7	2.5 a	18.71	22.55
4.0 %	18.9	16.3	2.6 a	18.87	22.50
CV (%) ²	8.50	9.12	31.33	11.04	7.83
F value ³	0.79 ^{ns}	1.38 ^{ns}	2.69*	0.30 ^{ns}	0.59 ^{ns}

¹Means followed by the same letter in the column do not differ by the Scott-Knott test at 5 % probability.

²CV: coefficient of variation.

³F value for treatments; ^{ns}: not significant; *: significant at 5 % probability.

Source: authors (2026).

an increase in leaf number is generally positive, this difference in nonmarketable leaves may indicate that, at these rates, increased vegetative vigor led to an increase in damaged or poorly formed leaves.

Table 2 presents the results related to shoot biomass production. There were no significant differences among treatments for total fresh mass ($p = 0.1152$), stem dry mass ($p = 0.2108$), or marketable fresh mass ($p = 0.0978$). Treatments significantly influenced leaf dry mass production ($p < 0.05$): biochar rates of 1.6 %, 2.4 %, 3.2 %, and 4.0 %, with means ranging from 15.62 g to 16.57 g, resulted in greater production when compared to the 0 % and 0.8 % treatments. Differences were also observed among treatments for nonmarketable fresh mass ($p < 0.05$), in which the 0 % and 2.4 % treatments resulted in lower values than the other rates (Table 2).

Overall, despite the limited effect on individual morphological characteristics, biochar application resulted in an increase in dry biomass accumulation and marketable yield of red lettuce,

especially from the 1.6 % rate onward. The increase in leaf dry mass is a robust indicator of physiological efficiency and carbon accumulation capacity. This response can be attributed to the ability of biochar to improve nutrient availability and water retention in the soil (Alomari *et al.*, 2024). The biochar, being enriched with micronutrients and having an alkaline pH, may have facilitated cation uptake and improved the edaphic environment, compensating for the soil's originally low fertility. Synergy between biochar and fertilization, even at relatively low fertilizer rates, is important to maximize productivity (Sossa *et al.*, 2024).

Nonmarketable fresh mass also differed significantly ($p < 0.01$). The higher rates (0.8 %, 1.6 %, 3.2 %, and 4.0 %) formed the upper group, with 15.0 g to 18.0 g, while the 0 % and 2.4 % treatments formed the lower group. This result aligns with the increase in the number of nonmarketable leaves (Table 1) and may be a side effect of greater total biomass accumulation at high rates. Similar results were observed by Cho *et al.* (2024), who found that very high biochar rates can be detrimental, possibly due to

Table 2. Biomass and productivity variables of red lettuce.

Treatment ¹	Total fresh mass (g)	Stem dry mass (g)	Nonmarketable fresh mass (g)	Marketable fresh mass (g)	Leaf dry mass (g)
0 %	114.0	0.79	9.0 b	104.5	12.86 b
0.8 %	133.5	1.41	18.0 a	114.5	14.67 b
1.6 %	154.5	1.05	15.0 a	139.5	16.57 a
2.4 %	152.5	1.00	11.5 b	141.0	16.42 a
3.2 %	155.5	0.90	17.0 a	138.5	16.54 a
4.0 %	150.5	0.63	18.0 a	132.5	15.62 a
CV (%) ²	18.22	49.61	28.28	18.26	11.27
F value ³	1.99 ^{ns}	1.55 ^{ns}	4.02*	2.12 ^{ns}	3.54*

¹Means followed by the same letter in the column do not differ by the ScottKnott test at 5 % probability.

²CV: coefficient of variation.

³F value for treatments; ^{ns}: not significant; *: significant at 5 % probability.

Source: authors (2026).

soil alkalization or nutrient adsorption. However, in this study, the 2.4 % (T4) rate showed atypical results, keeping nonmarketable mass in the lower group despite resulting in the highest marketable yield.

The positive response of leaf dry mass yield at higher rates is consistent with the literature, where other studies with this crop indicate that it benefits from biochar application in terms of yield increase (Jabborova *et al.*, 2021; Visiy *et al.*, 2022). Moreover, sugarcane bagasse biochar, enriched with potassium and calcium oxide, may have acted as a K source, which is essential for improving lettuce quality at maturity (Hong *et al.*, 2022).

In summary, intermediate to high biochar rates (1.6 % to 4.0 %) outperformed the low or nobiochar treatments (0 % and 0.8 %) in marketable biomass production, indicating that the incorporation of this conditioner optimizes resource use in soil under crop succession.

For the regression analysis, only the variables number of nonmarketable leaves, nonmarketable fresh mass, and leaf dry mass, which showed significant treatment effects, were analyzed. The F values, significance probabilities (p), coefficients of determination (R^2), and equations are described in Table 3. For the number of nonmarketable leaves, a quadratic behavior was observed, with the maximum estimated at 1.92 % biochar. The proximity of this value to the tested rates indicates that this may be the optimal dose for this parameter. For total

fresh mass, nonmarketable fresh mass, and leaf dry mass, there was a tendency toward a linear increase as a function of rates. However, this increase was gradual, as indicated by the low slope coefficients relative to the intercepts (Table 3).

Conclusion

The application of biochar to a lowfertility substrate, in a crop succession system, positively affects some productive parameters of red lettuce cv. Scarlet. Although morphological variables such as plant height and stem diameter remain unchanged at all biochar rates, the accumulation of agronomically valuable leaf biomass was enhanced. Biochar rates from 1.6 % up to 4.0 % resulted in increased leaf dry mass compared to the 0 % and 0.8 % treatments. The 2.4 % biochar rate yielded the best combination of the highest leaf dry mass production with the lowest nonmarketable leaf number and fresh mass, and can be recommended as the most suitable agronomic rate. This result is consistent with the regression analysis, which estimates the highest number of nonmarketable leaves at approximately 1.9 % biochar.

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Table 3. F values, significance probability, coefficient of determination, and fitted equations for the variables analyzed as a function of biochar rates.

Variable	Equation	F	p	R^2
Nonmarketable leaf number	$Y = -0.42 x^2 + 1.62 x + 16.73$	5.17	0.032	77.4
Nonmarketable fresh mass (g)	$Y = 1.56 x + 11.36$	6.89	0.015	41.7
Leaf dry mass (g)	$Y = 0.80 x + 13.76$	13.66	0.001	48.2

Source: authors (2026).

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