International Journal of Agriculture and Biosciences 2026 15(4): 1484-1495.
https://doi.org/10.47278/journal.ijab/2026.060

RESEARCH ARTICLE
eISSN: 2306-3599; pISSN: 2305-6622

Lettuce Nutrition and Minerals Acquisition by the Addition of Biochar and Nitrogen Fertilization under Greenhouse Conditions

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ABSTRACT

This study examined the interactive effects of biochar and urea fertilization on the growth performance, yield, and nutritional composition of romaine lettuce (Lactuca sativa L. var. Romaine) under greenhouse conditions. Although biochar enhances soil structure and nutrient retention, its low nitrogen content often limits plant productivity. A randomized complete block design with ten treatments combining varying rates of biochar (1%, 2%, 3%) and urea (0, 75, 150 kg N ha^{-1}) was implemented using silt loam soil. Results indicated that the application of biochar alone at 2% increased shoot fresh weight and leaf number compared with the control. The combined application of biochar and urea significantly improved most growth and yield parameters compared with control and biochar-only treatments. The 2% biochar + 150 kg N ha^{-1} urea treatment produced the tallest plants (21.75 cm) and the greatest shoot fresh weight (163 g), while 1% biochar + 150 kg N ha^{-1} resulted in the highest shoot dry weight (26.75 g). Root dry weight peaked with 2% biochar + 75 kg N ha^{-1} (21.25g). Root length remained unaffected. Biochemical analyses indicated enhanced crude fat (up to 5.03%) and crude fiber (up to 7.50%) under combined treatments, whereas higher nitrogen levels reduced total phenolic and flavonoid contents and suppressed DPPH radical scavenging activity. Macronutrient accumulation (N, P, K, Ca, Mg) was significantly elevated under biochar–urea integration, with optimal responses varying by treatment. In contrast, when biochar was applied without urea, plant nitrogen concentration decreased progressively with increasing biochar rates. Overall, these findings demonstrate that balanced integration of biochar and nitrogen fertilization can maximize lettuce yield and nutritional quality.

Keywords: Lactuca sativa, Nitrogen immobilization, Lettuce sustainable production, Poor soils, Biochar.

Article History
Article # 25-822
Received: 24-Dec-25
Revised: 19-Jan-26
Accepted: 23-Feb-26
Online First: 13-Mar-26

INTRODUCTION

Lettuce (Lactuca sativa L.) is one of the most widely consumed leafy vegetables worldwide and contributes appreciably to dietary fiber, iron, and vitamin C intake (Shi et al., 2022; John et al., 2025). Lettuce nutritional value further characterized by notable carbohydrate content and high total ascorbic acid (ascorbic acid + dehydroascorbic acid), which influence flavor and confer multiple health benefits (Chadwick et al., 2016; Medina-Lozano et al., 2021; Shi et al., 2022). Optimal growth and the attainment of these nutritional qualities require a balanced supply of essential macro- and micronutrients (Giannothanasis et al., 2025). One of the essential nutrients required for lettuce growth and quality development is nitrogen, as it is critically involved in vegetative development, chlorophyll biosynthesis, and the accumulation of biomass and nutritionally important compounds. Lettuce, like other leafy vegetables including kale, parsley, and collard, is classified as a heavy feeder, with its fast growth creating a substantial requirement for nitrogen fertilization to ensure sufficient soil nitrogen availability during the brief cultivation period (Guillermo and Barrientos, 2026). High nitrogen requirements in lettuce can be met through the

Cite this Article as: Al-Issa TA, Alomari LM and Omari HJ, 2026. Lettuce nutrition and minerals acquisition by the addition of biochar and nitrogen fertilization under greenhouse conditions. International Journal of Agriculture and Biosciences 15(4): 1484-1495. https://doi.org/10.47278/journal.ijab/2026.060

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application of inorganic fertilizers, which provide readily available nitrogen forms that support rapid vegetative growth. Among these, urea is one of the most widely used nitrogen fertilizers worldwide, containing 46% nitrogen and offering very high bioavailability, making it an efficient source to meet the substantial nitrogen demands of lettuce (Skorupka and Nosalewicz, 2021). Global urea production reached approximately 209 million tons in 2019 and continues to grow steadily, reflecting its widespread adoption and importance in modern agriculture (FAO, 2019). However, to achieve high yield and quality in lettuce production, other essential nutrients are also required, which can be supplied in sufficient amounts without relying on the high concentrations found in inorganic fertilizers. This allows for the use of alternative additives that are not classified as inorganic to meet the crop's nutritional needs such as biochar. Biochar is a carbon-rich material produced through pyrolysis, a process in which biomass is thermally decomposed at elevated temperatures under oxygen-limited conditions (Lehmann and Joseph, 2012). Biochar consists of a mixture of char and ash, characterized by a very high carbon content, typically ranging from 70 to 95% (Luostarinen et al., 2010). Due to its availability, renewability, and high carbon content, agricultural waste represents an ideal feedstock for biochar production and its utilization not only provides a sustainable raw material but also helps mitigate food waste, which remains one of the most pressing global challenges today (Ionov et al., 2026). A number of benefits can be provided by the use of biochar, including improving soil texture, structure, particle size distribution, porosity, density, plant growth (Vijayaraghavan, 2021), increased soil carbon sequestration (Lehmann, 2007) and improved soil fertility (Sohi et al., 2010). Biochar improves soil fertility indirectly by increasing the soil's ability to retain nutrients (Qian et al., 2015). Under the soil organic carbon depleted conditions of sunken greenhouse soils, biochar exhibits high stability and minimal decomposition, thereby serving as a long-term carbon reservoir that enhances soil carbon sequestration and potentially improves the structural and functional resilience of nutrient- and water-limited soils (Zhao et al., 2020). The amount of the minerals in biochar depends on the type of biomass used that they were made of. Biomass can be grouped into four main categories: woody biomass, human and animal waste, aquatic biomass, and agricultural residues (Pradeep et al., 2025). Woody biomass is found globally and plays a crucial role in meeting future energy demands, encompassing components such as branches, leaves, bark, and other tree residues (Vassilev et al., 2012). Animal and human waste biomass encompasses materials such as animal manure, food and fruit residues, paper, plastic, pulp, municipal solid waste, sewage sludge, and similar organic matter (Kim et al., 2020). Agricultural biomass generally comprises crop residues and harvested products, such as straw, flowers, and shells, whereas aquatic biomass consists of algae and microorganisms found in water (Chen et al., 2026). Biochar produced from these biomass types has been shown to contain high amounts of essential minerals, including N, P, K, Mg, Ca, and Na, as observed in biochar derived from plant wastes (Singh et al., 2010; Wu et al., 2016; Alomari et al., 2024), in mixed and non-mixed animal wastes biochar (Song & Guo 2012; Subedi et al., 2016; Wu et al., 2016), and in sludge wastes biochar (Lu et al., 2013: Zhang et al., 2015: Wu et al., 2016; Ho et al., 2017). Biochar properties vary substantially with the production process employed, which in turn influences their effectiveness in field-scale agricultural applications and their capacity for carbon sequestration (Sohi et al., 2010; Kookana et al., 2011; Meyer et al., 2011). Pyrolysis temperature strongly controls biochar's physicochemical properties, such as surface area, pH, and surface functional groups, and consequently determines its performance as a soil amendment (Ding et al., 2014). As pyrolysis temperature increased, surface area, the degree of carbonization, pH, and volatile matter increased, whereas cation exchange capacity (CEC) and the abundance of surface functional groups decreased (Tomczyk et al., 2020). Consequently, biochar can be used to increase crop field's production. Alomari et al., (2024) demonstrated that biochar application significantly enhanced lettuce root fresh and dry weight as well as root length, which in turn increased the accumulation of phosphorus, potassium, magnesium, and calcium in the leaves. Furthermore, the combined application of biochar and poultry manure produced an additional increase in lettuce fresh and dry biomass. Other studies reported that adding biochar to the soil increased lettuce leaves number (Trupiano et al., 2017), and lettuce plant height (Upadhyay et al., 2014). Biochar, on the other hand, does not include any significant amounts of existing nitrogen, although it does contain some decomposable carbon (Taylor, 2010). Mohawesh et al. (2019) reported that increasing biochar concentrations in tomato cultivation did not result in a corresponding rise in nitrogen content of the fruits. Applying biochar together with nitrogen fertilizer represents an effective and sustainable strategy to improve soil fertility, boost crop yields, and mitigate environmental impacts. Numerous studies have reported divergent findings concerning the combined application of nitrogen and biochar, with some indicating a reduction in plant nitrogen content, whereas others have observed an enhancement of nitrogen levels in the plant. A three-year field study demonstrated that combining biochar with nitrogen fertilizer enhanced soil properties, stimulated enzyme activity, shaped microbial communities, and created a stable "soil nutrient bank," collectively boosting maize yield even under reduced nitrogen inputs (Lu et al., 2026). Additionally, Biochar combined with a nitrogen fertilizer scored a significant amount of nitrogen level in oilseed rape compared to control and biochar-only treatments (1%B+0U, 2%B+0U, and 3%B+0U) (Liao et al., 2020). However, contrasting results have also been reported, as the application of biochar combined with nitrogen fertilizer was found to significantly increase N_2O emissions and gross nitrification, indicating elevated nitrifier activity, while simultaneously reducing nitrogen uptake by timothy grass and ultimately lowering biomass yield (Bhattarai et al., 2025). Moreover, Shareef and Zhao, (2016) reported that biochar can immobilize nitrogen, which might be due to the high carbon to nitrogen ratio.

Hence, the objective of this study is to evaluate the combined effects of biochar and nitrogen fertilizer on lettuce by assessing how this integrated management influences the plant's nutrient uptake, overall biomass production, and key quality attributes, including nutritional composition and phytochemical content, in order to provide insights into sustainable strategies for optimizing lettuce yield and quality under different fertilization regimes.

MATERIALS & METHODS

Experimental Site and Design

The experiment was carried out at the Agricultural Research Center, Faculty of Agriculture, Jordan University of Science and Technology (JUST), Al-Ramtha ciry, Irbid Governorate, Jordan (32.4950°N, 35.9912°E) (Fig. 1), under greenhouse conditions from November 2020 to January 2021.

Four-week-old romaine lettuce seedlings were transplanted into plastic pots (20 cm diameter), each filled with 4 kg of silt loam soil (2% clay, 74% silt, and 24% sand) with an initial soil pH of 7.5. The soil was thoroughly mixed with different rates of biochar and urea to create 10 treatments. These treatments, applied on a dry weight basis, included: T0 (control (just soil)), T1 (1% biochar + 0 kg/ha urea), T2 (1% biochar + 75 kg/ha U), T3 (1% biochar + 150 kg/ha urea), T4 (2% biochar + 0 kg/ha urea), T5 (2% biochar + 75 kg/ha urea), T6 (2% biochar + 150 kg/ha urea), T7 (3% biochar + 0 kg/ha urea), T8 (3% biochar + 75 kg/ha urea), and T9 (3% biochar + 150 kg/ha urea). The specific urea rates (0, 75, 150 kg N ha^{-1}) were selected to reflect a proportional adjustment from the reference fertilizer application of 175 kg N ha^{-1} used in previous lettuce trials (Cantú et al., 2017), accounting for the additional nutrient contribution and soil amendment effects of biochar, thereby avoiding potential over-fertilization while assessing combined effects on soil fertility and crop performance. The biochar rates of 1%, 2%, and 3% were chosen to avoid excessive increases in soil pH, since both the biochar and the soil were already alkaline, and higher rates could disrupt nutrient availability and plant growth. The experemental design of the study was the randomized complete block design (RCBD) with 4 blocks. According to FAO data, Jordan produced approximately 563,547.98 tons of tomatoes, and given this substantial production, biochar was produced from tomato plant residues through slow pyrolysis at 300–350°C for use in this study (FAO, 2025). The collected feedstock was air-dried under ambient greenhouse conditions prior to pyrolysis. Following pyrolysis, the resulting biochar was ground to a uniform particle size of 2–3 mm before experimental application. The average temperature in the greenhouse was maintained at 20°C, with an average relative humidity of 75%. The chemical properties of the soil, and biochar are shown in Table 1.

Table 1: Soil and biochar chemical analysis

Biochemistry analysis	Soil	Biochar
N (mg/ kg)	0.072	1.393
P (mg/ kg)	1.42	620.82
K (mg/ kg)	54.95	132.9
Mg (mg/ kg)	228.0	137.1
Ca (mg/ kg)	1512.76	725.9
Na (mg/ kg)	419.65	108.3
OM (%)	3.422	-
C:N	27.63	39.11

Data reproduced from Alomari et al. (2024) Journal of Ecological Engineering 25(6):12–28, CC BY 4.0 (https://doi.org/10.12911/22998993/186723).

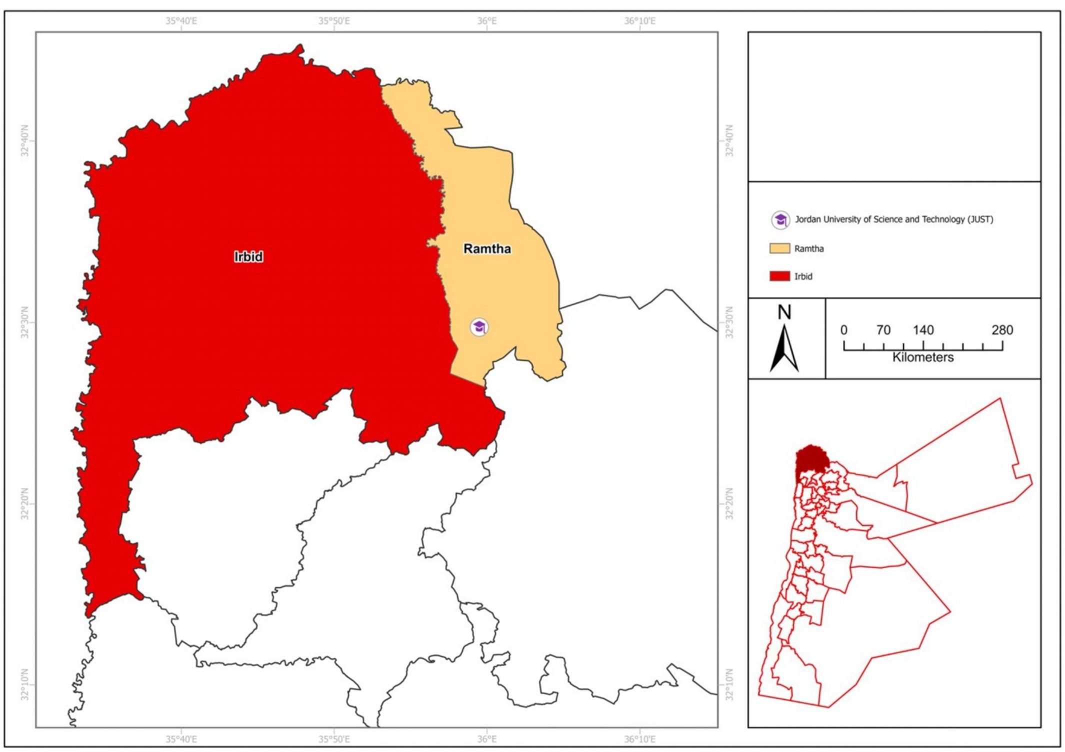

Fig. 1: Location map of the study area.

Study Measurements

Lettuce head height was measured from the soil surface to the tallest leaf, root length was determined using a tape measure, and the number of leaves was counted. Shoot and root fresh weights were recorded, and dry weights were obtained after oven-drying at 70°C for 7 days. The mineral contents (N, P, K, Mg, Ca, and Na) of lettuce shoots were analyzed after ashing in a muffle furnace, except for nitrogen. Total nitrogen was determined using the Kjeldahl method, following the procedures outlined by the Association of Official Agricultural Chemists (AOAC, 1995). Total phosphorus in the plant shoots was measured using the dry ashing method, followed by spectrophotometric analysis, as described by Chapman and Pratt (1961). Sodium and potassium concentrations were directly measured following the procedures outlined by AOAC (1995). Magnesium and calcium contents were determined by titration, following the method described by Heald (1965). Total crude fiber was determined in the laboratory using the method described by Van Soest (1966). Crude fat content was determined using the ether extraction method, as described by Thiex et al. (2003). The total phenolic content of the solvent extracts was measured using the Folin–Ciocalteu reagent, with gallic acid as the standard for the calibration curve, following the method of Slinkard and Singleton (1977). The total flavonoid content of lettuce plants was measured using the colorimetric method described by Zhishen et al. (1999). The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of lettuce plants was assessed following the method of Brand-Williams et al. (1995).

The media and soil analyses employed in this study were previously reported by Alomari et al. (2024), and the same batches were utilized here, with differences only in the treatments rate and experimental design. Total nitrogen was determined using the Kjeldahl method following the guidelines of the Association of Official Agricultural Chemists (AOAC, 1995). Available phosphorus was measured via the Olsen method (pH 8.5, 0.5 M $NaHCO_3$) as described by Kuo (1996). Sodium and potassium contents were quantified using flame photometry (BWB-xp), with concentrations calculated from standard curves prepared from known Na and K solutions (AOAC, 1995). Exchangeable magnesium and calcium were extracted using 1 M NH_4-acetate at pH 7, according to Soil and Plant Analysis Council (1999). Organic matter content was assessed using the loss-on-ignition method as outlined by Motsara & Roy (2008).

Statistical Analysis

The collected data were analyzed using the General Linear Model (GLM) procedure in SAS version 9.2 (2002). Mean comparisons were conducted using the least significant difference (LSD) test at a 5% probability level ($p \leq 0.05$).

RESULTS & DISCUSSION

Plant Growth Characteristics

The results (Fig. 2) show that lettuce plants treated with varying concentrations of biochar and urea exhibited differences in plant height, number of leaves, shoot fresh weight, and shoot dry weight. These findings indicate that the combined application of biochar and urea significantly improves lettuce growth parameters. Fig. 2A revealed that lettuce plants treated with 2% biochar combined with 150 kg/ha urea exhibited the greatest plant height, followed by treatments with 2% biochar + 75 kg/ha urea and 1% biochar + 150 kg/ha urea. These treatments significantly outperformed all other combinations and the control, which recorded the lowest plant height.

Similarly, the number of leaves were significantly increased in plants receiving combined biochar and nitrogen compared to the control and biochar-only treatments. All treatments incorporating urea with biochar exhibited the highest number of leaves, with no significant differences among the combinations (Fig. 2B). This effect can be attributed to the nitrogen in the urea applied, which is essential for promoting vegetative growth. In addition, the presence of biochar likely reduced fertilizer nitrogen loss rate (Huang et al., 2014; Deniel et al., 2018). The results of the current study were comparable to those obtained by Gashaw & Haile (2020) who indicated that applications of nitrogen fertilizers scored higher leaves number compared to other treatments.

Shoot fresh weight was significantly increased by the combined application of biochar and nitrogen. Fig. 2C revealed that the combination of 2% biochar + 150 kg/ha urea resulted in the highest shoot fresh weight, followed closely by 3% biochar + 150 kg/ha urea and 1% biochar + 150 kg/ha urea, indicating that higher nitrogen availability significantly enhanced plant biomass production. Among treatments without nitrogen fertilization, 2% biochar resulted in the highest shoot fresh weight compared with the control and the 1% and 3% biochar treatments. These results align with Alomari et al. (2024), which shows that the application of biochar alone (3%) did not increase lettuce fresh weight compared to the control. Similarly, Fig. 2D illustrates that all treated plants with biochar and urea combinations showed increased root fresh weight compared to control. Among the biochar-only treatments, 2% biochar produced the highest root fresh weight, exceeding the 0%, 1%, and 3% treatments.

The results also revealed that the highest root fresh weight was observed in the treatment with 2% biochar and 75 kg/ha urea (2%B+75U), while control plants showed the lowest weight (34.0 g). Our results agreed with those obtained by Shi et al. (2022) who reported that the addition of biochar and urea to maize increased root fresh weight to those were treated with urea alone and control. This indicates that the biochar-mineral urea combination has the potential to improve nitrogen utilization efficiency (El Sharkawi et al., 2018). However, Yeshiwas et al. (2018) reported that applying higher rates of nitrogen and an organic fertilizer (farmyard manure) does not lead to a significant increase in lettuce root weight.

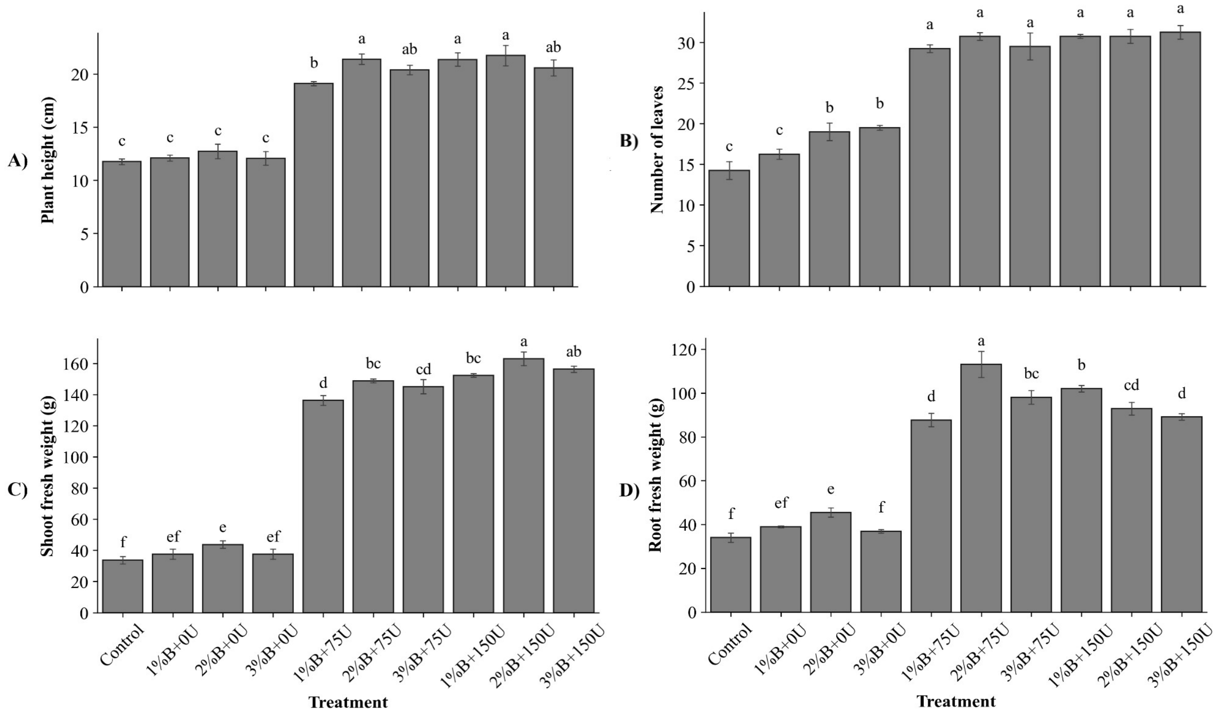

Fig. 2: Plant height (A), number of leaves (B), shoot fresh weight (C), and shoot dry weight (D) of romaine lettuce grown under greenhouse conditions for 8 weeks. Plants were treated with varying levels of biochar (1%, 2%, 3%) and urea (0, 75, 150 kg N ha^{-1}). Columns with the same letter within each parameter are not significantly different according to LSD at P ≤ 0.05 (LSD values: plant height = 1.77 cm, number of leaves = 2.49, shoot fresh weight = 9.07 g, shoot dry weight = 3.05 g; n = 4 replicates).

Surprisingly, root length was not significantly affected by any of the treatments, as shown in Table 2. The observed values for root length across all treatments are relatively consistent ranging from 30.0 and 35 cm, with no substantial deviations from the control. This may indicate that the factors governing root elongation, such as genetic traits, soil structure, or water availability, were not altered significantly by the treatments. Furthermore, the biochar's role in nutrient retention or nitrogen availability might not directly affect root elongation but rather other growth parameters like shoot biomass or overall plant height. Yin et al. (2024) reported that biochar application markedly reduced root length, nutrient uptake, and translocation in rice, wheat, and corn seedlings, with rice experiencing the strongest inhibitory effects among the three crops.

Table 2: Root length, shoot dry weight, and root dry weight of romaine lettuce grown under greenhouse conditions for 8 weeks. Plants were treated with different levels of biochar (1%, 2%, 3%) and urea (0, 75, 150 kg N ha^{-1})

Treatments	Root length (cm)	Shoot dry weight (g)	Root dry weight (g)
Control	33.50	10.15^{d}	4.77^{e}
1% biochar + 0 urea	32.50	10.19^{d}	5.00^{e}
2% biochar + 0 urea	33.25	12.50^{d}	9.74^{d}
3% biochar + 0 urea	35.00	13.00^{d}	9.39^{d}
1% biochar + 75 kg/ha urea	31.50	20.75^{c}	19.25^{abc}
2% biochar + 75 kg/ha urea	30.00	21.25^{bc}	21.25^{a}
3% biochar + 75 kg/ha urea	31.50	21.50^{bc}	17.75^{bc}
1% biochar + 150 kg/ha urea	32.25	26.75^{a}	20.75^{ab}
2% biochar + 150 kg/ha urea	30.75	24.00^{ab}	20.25^{ab}
3% biochar + 150 kg/ha urea	30.75	23.50^{bc}	16.75^{c}
LSD	NS	3.05	3.17

Values followed by the same letter within each column are not significantly different according to LSD at P≤0.05 (n = 4 replicates).

The results presented in Table 2 also revealed that treatments combining biochar and nitrogen showed a significant difference in shoot dry weight compared to biochar only treatments and the control. The 1% biochar + 150 kg/ha urea granted the highest dry weight compared to all other treatments, while the lowest dry weight was recorded in the control. These findings suggest that the interaction between biochar and nitrogen is crucial for improving biomass accumulation, likely due to enhanced nutrient and water availability in the soil. Our study findings are consistent with those reported by Reibe et al. (2015) and Baiga and Rajashekhar (2017).

Our study also revealed that the root dry weight of lettuce plant treated with 2% and 3% of biochar and the combination of biochar and urea showed significant increased values compared to the control. Among these, the 1% biochar + 75 kg/ha urea treatment resulted in the highest root dry weight, whereas the lowest was the control plants (Table 2).

Biochemical Responses

Table 3 presents the effects of different biochar levels (1%, 2%, and 3%) combined with urea application rates (75, and 150 kg/ha) on crude fat content, crude fiber content, total phenols, total flavonoids, and radical scavenging activity (DPPH) of lettuce plants.

The crude fat content of lettuce was significantly influenced by the treatments. The control treatment exhibited the lowest crude fat content, while the highest was observed in the treatment with 3% biochar and 150 kg/ha urea, highlighting the positive role of biochar and

Table 3: Crude fat, crude fiber, total phenols, total flavonoids content (mg/100 g) and DPPH radical scavenging activity of romaine lettuce grown under greenhouse conditions for 8 weeks. Plants were treated with varying levels of biochar (1%, 2%, 3%) and urea (0, 75, 150 kg N ha^{-1})

Treatments	Crude fat content (%)	Crude fiber content (%)	Total phenols (mg/100g)	Total flavonoids (mg/100gm)	DPPH (%)
Control	3.21[d]	4.91[b]	1884.8[bc]	1258.0[bcd]	39.55[ab]
1% biochar + 0 urea	4.37[abc]	5.77[b]	2034.5[ab]	1289.7[ab]	40.80[ab]
2% biochar + 0 urea	3.90[bcd]	4.99[b]	1858.8[bc]	1481.9[a]	42.10[a]
3% biochar + 0 urea	3.66[cd]	5.27[b]	2178.5[a]	1369.5[ab]	42.83[a]
1% biochar + 75 kg/ha urea	4.65[ab]	7.34[a]	1615.5[de]	1052.5[de]	26.05[d]
2% biochar + 75 kg/ha urea	3.97[bcd]	7.26[a]	1805.3[bcd]	1207.9[bcd]	35.98[bc]
3% biochar + 75 kg/ha urea	4.06[bc]	7.50[a]	1696.0[cde]	1273.3[bc]	38.48[abc]
1% biochar + 150 kg/ha urea	4.25[abc]	7.49[a]	1513.1[e]	963.8[e]	32.88[c]
2% biochar + 150 kg/ha urea	4.28[abc]	7.36[a]	1693.4[cde]	1066.8[cde]	35.48[bc]
3% biochar + 150 kg/ha urea	5.03[a]	7.11[a]	1614.7[de]	950.3[e]	35.95[bc]
LSD	0.85	1.03	235.7	207.2	5.87

Mean values followed by the same letter within each column are not significantly different according to LSD test at P≤0.05 (n = 4 replicates).

nitrogen fertilization on fat accumulation in lettuce. This can be attributed to the synergistic effects of biochar and higher nitrogen availability, which could enhance lipid biosynthesis pathways (Shanmuganathan et al., 2023).

Crude fiber content increased significantly with the combined application of biochar and urea; however, the control and all three biochar-only treatments did not differ significantly from one another. The highest fiber content was observed in plants treated with 3% biochar + 75 kg/ha urea, whereas the lowest was the control. These results suggest that biochar and urea enhance structural carbohydrate accumulation, possibly due to improved soil nutrient availability and plant metabolism. This finding contradicts earlier studies by Gasim (2001) and Adam (2004), which suggested that nitrogen fertilizers had no significant effect on crude fiber content. However, our results aligned with those obtained by Safdar (1997), Tariq (1998), and Amin (2011) which indicated a relationship between nitrogen application and increased plant fiber content. These discrepancies may be attributed to differences in experimental conditions, soil types, or crop species, emphasizing the importance of context-specific studies to fully understand the effects of nitrogen on plant fiber content.

The phenolic content ranged from 1513.12 mg to 2178.44 mg per treatment, highlighting the influence of the treatments on phenol accumulation. Interestingly, treatments combining biochar with urea fertilizer displayed a reduction in total phenols content, while other nitrogen combined biochar treatments had no significant differences from control, Stumpf et al. (2019) reported that phenolic compounds appeared less susceptible to changes in nitrogen supply, it is reasonable to infer that nitrogen availability plays a limited role in modulating these specific biochemical traits. Similarly, the addition of higher nitrogen levels (150 kg/ha urea) led to a marked decline in flavonoid content across all biochar concentrations. The highest flavonoid concentration was observed in plants grown with 2% biochar and no nitrogen fertilization (2%B + 0U). The results suggest that using biochar alone, particularly at higher concentrations (2% & 3%), can significantly enhance the flavonoids content of lettuce. The results also showed that the highest DPPH radical scavenging activity was observed in control and treatments with biochar only, particularly at 3% biochar concentrations, whereas, the treatment with 1% biochar + 75 kg/ha urea recorded the lowest value, indicating a significant inhibitory effect of excessive nitrogen fertilization on antioxidant capacity. These findings highlight the importance of balancing biochar and nitrogen applications to optimize both plant growth and nutritional quality. Further research is required to investigate the interaction between biochar and nitrogen in affecting secondary metabolite pathways and their production. In the contrary, nitrogen fertilization had an inhibitory effect on the accumulation of secondary metabolites in the lettuce leaves. This decline can be attributed to a dilution effect resulting from rapid biomass accumulation and potential inhibition of secondary metabolite biosynthesis. These results indicate a potential trade-off between yield and antioxidant quality, as higher nitrogen fertilization, while promoting biomass accumulation, corresponded with a reduction in total phenolic content, flavonoid levels, and DPPH radical scavenging activity in lettuce leaves. This highlights the need to optimize nitrogen rates to balance crop productivity with nutritional and phytochemical quality. Interestingly, these findings contrast with those of Ma et al. (2015), who reported that increasing nitrogen fertilizer application rates led to a significant enhancement in the phytochemical profile of wheat grain; specifically, the highest nitrogen level (N300) resulted in the greatest total phenolic content, total flavonoid content, and antioxidants activity across both study locations (Zhengzhou and Wenxian) compared to the control group. Additionally, Mahlangu et al. (2016) reported that total phenolic content and antioxidants capacity in lettuce peaks at intermediate N levels and subsequently declines at higher concentrations.

Minerals Analysis

Results of this study showed that the effect of different biochar and urea application rates on the nitrogen, phosphorus, potassium, sodium, calcium, and magnesium content in lettuce leaves was highly significant (Table 4).

The results indicated that biochar combined with urea significantly increased nitrogen levels compared to biochar only treatments and the control. The highest nitrogen content was observed in the 1% biochar + 150 kg/ha urea treatment. The 2% and 3% biochar + 150 kg/ha urea treatments were slightly lower, but still significantly higher than the control. Huang et al. (2018) studied the impact of repeated seasonal biochar applications on nitrogen uptake

Table 4: Nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), and sodium (Na) content of romaine lettuce grown under greenhouse conditions for 8 weeks. Plants were treated with varying levels of biochar (1%, 2%, 3%) and urea (0, 75, 150 kg N ha^{-1})

Treatments	Nitrogen (%)	Phosphorus (mg/g)	Potassium (mg/g)	Sodium (mg/g)	Calcium (mg/g)	Magnesium (mg/g)
Control	1.10^{d}	2.48^{f}	20.57^{f}	10.10^{cd}	1.86^{e}	5.48^{d}
1% biochar + 0 urea	1.03^{e}	3.60^{a}	30.88^{d}	10.69^{abc}	2.23^{d}	8.99^{a}
2% biochar + 0 urea	0.98^{ef}	3.20^{c}	24.93^{e}	10.68^{abc}	3.14^{b}	8.68^{a}
3% biochar + 0 urea	0.95^{f}	2.56^{ef}	20.96^{f}	9.11^{d}	3.77^{a}	7.54^{bc}
1% biochar + 75 kg/ha urea	1.22^{c}	3.20^{c}	36.79^{bc}	11.62^{ab}	2.65^{c}	8.42^{ab}
2% biochar + 75 kg/ha urea	1.30^{ab}	3.03^{d}	37.90^{bc}	11.85^{a}	2.36^{cd}	9.11^{a}
3% biochar + 75 kg/ha urea	1.30^{ab}	2.93^{d}	35.91^{c}	11.77^{ab}	3.42^{ab}	6.71^{c}
1% biochar + 150 kg/ha urea	1.34^{a}	3.52^{ab}	41.65^{a}	10.25^{cd}	1.47^{f}	9.12^{a}
2% biochar + 150 kg/ha urea	1.30^{ab}	3.39^{b}	39.08^{ab}	10.61^{bc}	2.23^{d}	8.50^{ab}
3% biochar + 150 kg/ha urea	1.26^{bc}	2.70^{e}	35.30^{c}	10.38^{c}	3.65^{a}	8.80^{a}
LSD	0.07	0.15	3.02	1.21	0.36	1.03

Values followed by the same letter within each column are not significantly different according to LSD at $P \leq 0.05$ (n = 4 replicates).

and utilization in rice. They found that in the first four seasons, biochar had little to no effect on fertilizer nitrogen uptake. By contrast, in the fifth and sixth seasons, biochar increased soil nitrogen content by 14–26% but decreased the uptake of fertilizer nitrogen by the plants by 19–26%. Depending on biochar properties and soil conditions, the co-application of biochar with nitrogen fertilizers has been reported to enhance nitrogen retention via physio-chemical adsorption and microbial immobilization mechanisms, leading to improved nitrogen use efficiency and reduced nitrogen leaching losses (Ghorbani et al., 2022), albeit potentially at the expense of short-term plant nitrogen uptake due to reduced nitrogen availability. Nitrogen immobilization is influenced by multiple factors, including biochar feedstock, its C:N ratio, the C:N ratio of co-applied substrates, and the availability of carbon sources for soil microorganisms (Zavalloni et al., 2011). Mechanistically, this involves adsorption of NH_3^- or organic nitrogen onto biochar, cation and anion exchange reactions, and enhanced immobilization resulting from labile carbon provided by the biochar (Clough et al., 2013). A meta-analysis of 124 published studies found that biochar application generally reduced soil inorganic nitrogen (NO_3^--N by 12% and NH_4^+-N by 11%), primarily due to the C:N ratio of the biochar and soil pH promoting nitrogen immobilization, although co-application with organic fertilizers often enhanced soil NH_4^+-N compared to controls (Gao et al., 2019). High-temperature biochar have greater NO_3^- adsorption capacity than low-temperature ones, and the feedstock species also affects the adsorption efficiency (Yao et al., 2012; Mizuta et al., 2004). Kameyama et al. (2012) reported that sugarcane bagasse biochar produced at ≥700°C showed significant NO_3^- adsorption, which was attributed to their high pH and associated basic functional groups rather than to surface area or microporosity. The C:N ratio of biochar dictates whether it induces nitrogen immobilization or mineralization. A threshold C:N of approximately 20 has been reported: biochar with C:N ratios above 20 tend to promote nitrogen immobilization, whereas those below 20 favor nitrogen mineralization. (Chan & Xu, 2009). In this study, the soil already had a relatively high C:N ratio of 27:1, and the biochar applied had an even higher C:N ratio of 39:1 (Table 1). This combination further increased nitrogen immobilization, which explains why plant nitrogen uptake decreased as biochar application increased.

Phosphorus content ranged from 2.48 mg/g in the control to 3.60 mg/g in the 1% biochar + 0 urea treatment. Biochar alone followed by 1% and 2% + 150 kg/ha urea significantly increased phosphorus levels compared to the control and all other treatments. However, as shown in Table 4, increasing the biochar rate was associated with a decrease in phosphorus content. This trend may be attributed to the rise in soil pH with higher biochar additions, which can reduce phosphorus availability through precipitation or adsorption processes. Biochar are capable of adsorbing phosphorus because of their porosity, high surface area, abundant surface functional groups, and elevated mineral content (Luo et al., 2023). Notably, several previous studies reported an increase in phosphorus in plants in response to the application of biochar and nitrogen (Uzoma et al., 2011; Güneş et al., 2014; Trupiano et al., 2017). A study of Deniel et al. (2018) investigated the effects of rice husk biochar addition on rice growth performance and fertilizer nitrogen recovery. The result showed that biochar application at a rate of 5 Mg/ha followed 10 and 20 Megagram/ha respectively increased soil available phosphorus compared to the control treatment. Based on a meta-analysis of 124 studies, biochar application markedly enhanced soil phosphorus by increasing surface-available phosphorus by 45% and microbial biomass phosphorus by 48%, with the greatest effects observed for biochar produced from manure or other low C:N feedstocks, generated at low pyrolysis temperatures, or applied at higher rates (Gao et al., 2019).

Potassium content was significantly impacted by biochar and urea treatments comparing to control which had the lowest potassium content. Our results revealed a distinct trend in which increasing biochar application led to a reduction in potassium levels in the plants, a pattern that persisted even when urea was applied. Notably, among all treatment combinations, the application of 1% biochar together with 150 kg/ha urea produced the highest potassium content, suggesting that moderate biochar levels in combination with nitrogen fertilization may partially mitigate the negative effect of higher biochar rates on potassium availability. Our results align with those obtained by Coelho et al. (2018) who reported that biochar and urea treatments increased potassium content in corn compared to biochar application alone. Cheng et al., (2025) reported that in wheat, the control (no fertilizer) and conventional urea treatments maintained higher total potassium during the grain-filling (25.5–27.5 mg/g) and maturity stages (25.7–26.27 mg/g) compared to starch-coated alkali-modified biochar-urea and starch-coated biochar-urea treatments, likely due to potassium adsorption by biochar. In contrast, starch-coated biochar-urea showed the highest available potassium at maturity (217.33 mg/ kg), demonstrating the superior potassium release capacity of unmodified biochar, while the control experienced a significant decline (126 mg/ kg) reflecting continuous soil potassium depletion).

The analysis of the effect of biochar and urea treatments on the Sodium (Na) content in lettuce leaves revealed statistically significant differences across the treatments. The sodium concentration ranged from a low of 9.11 mg/g in the 3% biochar + 0 urea treatment to a high of 11.85 mg/g in the 2% biochar+75 kg/ha urea treatment. The control treatment measured 10.10 mg/g. specifically, the 3% biochar+0 urea treatment resulted in the lowest sodium accumulation, being significantly lower than most other treatments and the control. Conversely, the highest sodium levels were observed primarily in treatments receiving 75 kg/ha urea, with 2% biochar+75 kg/ha urea and 3% biochar+75 kg/ha urea being the only treatments significantly higher than the control. Treatments with the highest urea rate (150 kg/ha urea) tended to have moderate sodium content, similar to the control and the 0 urea treatments, showing that the combined application rate influences Na accumulation. Several studies have reported that biochar generally reduces sodium accumulation and soil salinity, although the magnitude of the effect varies depending on experimental conditions, biochar properties, and soil characteristics. Biochar application in saline-sodic soil has been shown to reduce sodium accumulation in rice plants while increasing dry biomass, grain yield, and overall grain quality, highlighting its potential to alleviate salt stress and enhance rice productivity (Jin et al., 2018). Rostamian et al. (2015) reported that rice husk-derived biochars and activated carbons showed increased sodium sorption with higher surface area and pore volume, with KOH-activated carbon exhibiting the highest sorption, indicating that biochar properties strongly influence sodium accumulation in plants grown in saline soils. Interestingly, Alomari et al. (2024) reported an increase in sodium content in romaine lettuce with biochar application, although the increase was not statistically significant compared to the control.

The analysis of the effect of the treatments on Calcium (Ca) content in lettuce leaves revealed a wide range of values and clear, statistically significant differences. The calcium content spanned from the lowest concentration of 1.47 mg/g in the 1% biochar+150 kg/ha urea treatment to the highest concentrations of 3.77 mg/g (3% biochar+0 urea) and 3.65 mg/g (3% biochar+150 kg/ha urea). A distinct pattern emerges when examining the biochar rate: in both the 0 urea and 150 kg/ha urea groups, increasing the biochar rate from 1% to 3% led to a significant increase in calcium content, with the 3% biochar treatments resulting in the highest accumulation. Conversely, the highest urea rate (150 kg/ha) combined with the lowest biochar rate (1%) resulted in the lowest calcium content, which was significantly lower than the control and all other treatments. This decline could be attributed to possible soil acidification caused by excessive nitrogen fertilization suppressing calcium uptake (Xing et al., 2024). This indicates that the positive effect of biochar on calcium accumulation is highly dependent on both the biochar rate and the nitrogen (urea) level. The positive effect of biochar on calcium accumulation is further supported by studies showing that calcium-enriched biochar, containing up to 6.9% Ca, can increase soil calcium availability and enhance plant mineral uptake (Kováčik et al., 2022). This effect is further corroborated by findings from eucalyptus biochar application, which not only increased soil calcium availability but also promoted calcium uptake and enhanced total plant biomass in upland rice (Butphu et al., 2020).

The treatments had a substantial and generally positive effect on the Magnesium (Mg) content in lettuce leaves, with most biochar and urea combinations significantly increasing Mg concentration compared to the control. The control treatment had the lowest Mg content at 5.48 mg/g. The highest Mg accumulation was observed across multiple treatments, primarily those with 1% biochar+0 urea, 2% biochar+75 kg/ha urea, 1% biochar+150 kg/ha urea, and 3% biochar+150 kg/ha urea. All of these were statistically grouped as the highest-yielding treatments. The 3% biochar+75 kg/ha urea treatment, however, resulted in the lowest increase among the treated plots at 6.71 mg/g, which was still significantly higher than the control. This effect could be due to the ability of biochar to enhance soil cation exchange capacity (CEC), thereby increasing nutrients availability including calcium and magnesium (Domingues et al., 2020). The application of biochar (BC) significantly enhanced the magnesium (Mg) content in lettuce shoots, increasing it from 573.13 mg/ kg in the control group to 941.88 mg/ kg, representing an increase of approximately 64.3%, which is consistent with the findings of Woldetsadik et al., (2017), who reported that biochar application significantly increased Mg concentrations in lettuce tissues across two consecutive growing cycles, demonstrating positive residual effects in both sandy loam and clay soils. Overall, the application of both biochar and urea effectively doubled the magnesium concentration in the lettuce leaves compared to the untreated control, though the highest rate of biochar (3%) combined with 75 kg/ha urea was less effective than other combinations.

Practical Implications for Lettuce Growers

Based on the findings of this study, the following recommendations can be provided to lettuce producers to optimize yield and quality:

- Biochar can enhance lettuce fresh weight, but its benefits are markedly greater when applied alongside nitrogen fertilization, indicating that integrated nutrient management maximizes productivity.
- Optimal Application Rates: For maximum vegetative growth and biomass, a combination of 2% biochar and 150 kg N ha^{-1} is recommended. However, if the primary goal is to increase shoot dry weight, a lower biochar rate of 1% with 150 kg N ha^{-1} is sufficient and more cost-effective.
- Enhancing Mineral Content: The combined application of biochar and urea is a practical strategy to produce

lettuce with higher concentrations of essential minerals (N, P, K, Ca, and Mg) and increased crude fiber and fat content, thereby improving the nutritional profile of the crop.

- Balancing Yield and Bioactive Compounds: Growers targeting the "functional food" market (high antioxidants) should be cautious with high nitrogen rates. While 150 kg N ha^{-1} maximizes yield, it reduces total phenols and flavonoids. To maintain high antioxidant activity, a moderate nitrogen level or higher biochar-to-nitrogen ratios should be considered.
- Sustainability and Soil Health: Utilizing biochar derived from agricultural waste (such as tomato residues) offers a sustainable way to improve nutrient retention in silt loam soils, reducing fertilizer leaching and promoting circular economy practices in greenhouse production.

Conclusion

The results demonstrate that biochar is agronomically effective for lettuce production when integrated with nitrogen fertilization. Biochar applied alone improved lettuce biomass and, therefore, is recommended as a standalone amendment for short-cycle leafy vegetables grown in silt loam soils. On the other hand, a combination of 2% biochar with 150 kg N ha^{-1} is the most reliable strategy to maximize plant height and fresh biomass, whereas 1% biochar with 150 kg N ha^{-1} provides a more cost-efficient option when the objective is to increase shoot dry matter. From a nutritional perspective, biochar–urea integration enhances the accumulation of essential minerals (N, P, K, Ca, and Mg) and structural components such as crude fiber and fat, improving overall crop nutritional value. However, high nitrogen inputs consistently reduced phenolic compounds, flavonoids, and antioxidant activity, indicating a clear yield–quality trade-off. Therefore, growers targeting premium or functional-food markets should avoid excessive nitrogen fertilization and instead adopt moderate nitrogen rates combined with higher biochar proportions to preserve antioxidant capacity. Practically, the use of biochar derived from agricultural residues offers a sustainable tool to improve nutrient retention and fertilizer use efficiency in greenhouse systems, particularly under alkaline soil conditions. Adoption of biochar–nitrogen co-application allows producers to fine-tune management according to production goals, either maximizing biomass or improving phytochemical quality, while contributing to waste recycling and long-term soil health. Future optimization should prioritize rate adjustment rather than blanket application, especially in systems where quality attributes are economically important.

DECLARATIONS

Funding: This research was funded by Jordan University of Science and Technology, Irbid, Jordan.

Acknowledgement: The authors would like to express their sincere gratitude to the Deanship of Scientific Research at Jordan University of Science and Technology (JUST) for funding this research. The authors also sincerely thank Mr. Mohamed Metwaly for his valuable contribution in designing the location map of the study area.

Conflict of Interest: The authors declare that there is no conflict of interest regarding the publication of this paper.

Data Availability: All the data is available in the article.

Ethics Statement: This study did not require ethical review, as it did not involve sensitive human data or animal subjects.

Author's Contribution: Taha A. Al-Issa: Visualization; funding acquisition; project administration; final approval of the article. Laith M. Alomari: Research concept and design; collection and/or assembly of data; writing the article; critical revision of the article; final approval of the article. Hussam J. Omari: Data analysis and interpretation; writing the article; critical revision of the article; final approval of the article.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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