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INTEGRATIVE ASSESSMENT OF BIOCHAR IMPACTS ON SOIL CHEMISTRY, PLANT WATER RELATIONS, AND PHOTOSYNTHETIC PIGMENT CONTENT IN *FRAGARIA* × *ANANASSA*

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Background. Biochar is widely promoted as a soil amendment that can improve fertility, retain nutrients, and enhance crop performance, yet its effects vary strongly with soil type, application rate, and time since application. Evidence for perennial fruit crops grown on fertile soils without mineral fertilizer remains limited. This study evaluated how biochar affects soil chemical properties, leaf physiology, and fruit production of strawberry under field conditions.

Materials and Methods. A two-year field experiment (2024–2025) was conducted with biochar applied at 0, 2, 5, and 10 tonnes per hectare, each treatment replicated in three plots, without synthetic fertilizer. One year after application, composite soil samples were analyzed for acidity, soil organic matter, mineral nitrogen, phosphorus, potassium, sulfur, calcium, and magnesium, plant-available and total trace elements, following national standard methods. Leaf relative water content and photosynthetic pigment concentrations were measured in both years, and individual fruit weight and yield were recorded.

Results. Biochar effects on bulk soil chemistry were limited and selective. Phosphorus and potassium, already abundant, and soil acidity remained unchanged. The clearest responses occurred in soluble pools: ammonium nitrogen, water-soluble calcium, and plant-available zinc and mercury declined, while water-soluble magnesium rose sharply, with several effects strongest at the lowest rate. Biochar improved leaf water status in



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both years and markedly increased chlorophyll *b* content in the second year, particularly at the highest application rate. In contrast, chlorophyll *a* and carotenoids levels remained largely unchanged. Compared with the control, fruit weight declined in the 5 t/ha treatment during the second growing season.

Conclusions. Biochar produced measurable but modest effects on the soil-plant system that did not translate into yield gains within two years, indicating that benefits on fertile soils emerge slowly and depend on application rate and management.

Keywords: biochar, strawberry, soil amendment, nitrogen immobilization, chlorophyll, water-soluble cations, crop yield, field experiment

INTRODUCTION

In recent decades, biochar has garnered increasing scientific and practical interest as a multifunctional carbon-rich material with significant potential for environmental remediation and agricultural enhancement. Biochar is defined as a carbonaceous solid produced through the pyrolytic (oxygen-limited conditions, 300–1000 °C) decomposition of diverse organic feedstocks, including lignocellulosic biomass, agricultural residues, animal manure, municipal sewage sludge, and dedicated energy crops (Kloss *et al.*, 2012; Lehmann & Josef, 2024; Mazurak *et al.*, 2025). When applied as a soil amendment, biochar can induce a range of beneficial effects. Its high specific surface area and cation exchange capacity enhance soil nutrient retention and water-holding capacity (Jeffery *et al.*, 2011; Razzaghi *et al.*, 2020; Zhu, 2025), while also contributing to the accumulation of soil organic carbon (Yan *et al.*, 2025). By reducing nutrient leaching (Luo *et al.*, 2024) and, in some cases, supplying plant-available nutrients, biochar can improve crop nutrition (Atkinson *et al.*, 2010; Biederman & Harpole, 2013). Furthermore, it reduces soil bulk density, thereby facilitating root penetration and improving soil aeration (Toková *et al.*, 2020). Notably, biochar is highly stable in the soil environment, which means that its positive effects can persist not only in the year of application but also for decades, making it a valuable tool for long-term soil fertility improvement and carbon sequestration (Ippolito *et al.*, 2020).

Biochar application has also been shown to alleviate the phytotoxic effects of various environmental contaminants, including organic pollutants (Sil *et al.*, 2021; Khomenchuk *et al.*, 2022), heavy metals (Khan *et al.*, 2022; Sarraf *et al.*, 2024), and excessive salinity (Umer, 2025). By binding toxic compounds and altering soil pH, biochar creates a more favorable rhizosphere environment. As a result, plants cultivated on degraded or contaminated soils amended with biochar often exhibit improved growth performance, enhanced yield (Rathinapriya *et al.*, 2025), and reduced accumulation of heavy metals and other harmful substances. These properties position biochar as a promising tool not only for sustainable agriculture but also for phytoremediation.

Strawberry (*Fragaria × ananassa* Duchesne) is a high-value horticultural crop of significant economic importance worldwide, with production highly dependent on water availability and fertilizer management. Research has demonstrated that biochar application can increase the relative water content in strawberry leaves, which is closely associated with improved soil moisture retention (Orlandella *et al.*, 2025). In addition, biochar-enhanced soil nutrient status often leads to elevated leaf nutrient concentrations and increased antioxidant capacity in plant tissues (Ye *et al.*, 2024).

While the beneficial effects of biochar are well documented, its performance varies significantly depending on both its intrinsic properties and soil characteristics, leading to

variability in outcomes. Considering these factors, the present study aims to determine the impact of biochar soil amendment on the physiological traits and yield of strawberry cultivated in heavy clay soils under Ukrainian agroecological conditions. This work seeks to provide insights into the potential of biochar to enhance crop productivity in conventional horticultural systems.

MATERIALS AND METHODS

Experimental site and soil characteristics. The field experiment was conducted during 2024–2025 at the experimental site near Sukhovolia village, Lviv Oblast, Ukraine (49°49'45.4"N, 23°50'47.8"E). The soil type was classified as Luvisol according to the World Reference Base (2006) (IUSS, 2006), characterized by heavy clay texture with the following initial properties (0–20 cm depth): pH 6.0 (aqueous extract), pH 5.1 (1 M KCl extract), soil organic matter (SOM) 0.9 %, electrical conductivity (EC) 60.7 $\mu\text{S}/\text{cm}$, available phosphorus 436.5 mg/kg and potassium 76.3 mg/kg, and exchangeable magnesium 0.1 mEq/100 g soil.

Plant material and experimental design. The strawberry *Fragaria* $\times$ *ananassa* Duch. cv. 'Clery' was used as the experimental crop. 'Clery' is an early-ripening short-day cultivar producing medium-to-large fruit (Garzoli *et al.*, 2020). Certified bare-root frigo transplants were planted on April 6, 2024. The experimental design was a randomized complete block with four treatments (see next section) and three replicates (blocks). Each plot measured 3 $\times$ 4 m (12 m²) and comprised three rows of 15 plants (45 plants per plot) spaced 20 cm within rows and 90 cm between rows, corresponding to a planting density of ~50,000 plants/ha.

Biochar application and soil preparation. Biochar from hardwood feedstock obtained via slow pyrolysis at 450 °C was used. Four application rates were tested: 0 (control), 2, 5, and 10 t/ha dry weight. Biochar was surface-applied and incorporated into the top 15 cm by rotary tillage one week before transplanting. No synthetic fertilizers were used. Standard cultural practices were applied uniformly across all treatments.

Soil sampling and analysis. Soil samples were collected one year after the biochar amendment. From each of three replicate plots per treatment, five subsamples were taken at 0–20 cm depth following an X pattern, air-dried for 7 days, and composited at plot level (n = 3 per treatment). A 1 kg subsample was sieved through a 2 mm mesh and submitted for physicochemical analysis (DSTU ISO 11464:2007).

The following parameters were assessed: clay content by the pipette method; EC conductometrically (1:5 suspension); pH potentiometrically in water (1:2.5) and KCl extract (1 M) (DSTU ISO 10390:2007; DSTU 7910:2015); organic matter by Tyurin dichromate oxidation (DSTU 4289:2004); hydrolyzed nitrogen by Cornfield alkaline hydrolysis (DSTU 7863:2015); mineral nitrogen (NH_4^+ + NO_3^-) colorimetrically after KCl extraction; available P and K by the Kirsanov method (0.2 M HCl + 0.03 M $(\text{NH}_4)_2\text{SO}_4$) with molybdenum blue colorimetry and flame photometry, respectively (DSTU 4405:2005); available S turbidimetrically (DSTU 8347:2015); exchangeable Ca and Mg by 1 M ammonium acetate extraction (DSTU 7945:2015) and AAS (DSTU 7945:2015). Plant-available Mn, Cu, Cr, Zn, Pb, and Hg were determined in DTPA extracts (pH 7.3) by AAS with electrothermal atomization (Varian AA240Z, USA); total metals were determined following nitric acid digestion (Anton Paar Microwave digestion system, Austria) as described in O. Makar *et al.* (2021).

Leaf sampling. Leaf samples were collected for physiological and biochemical analysis at post-harvest periods (early July 2024, 2025). Fully expanded, healthy leaves from the middle canopy stratum were selected from 10 randomly chosen plants per plot to ensure representative sampling. Following collection, leaf samples were immediately sealed in polyethylene bags, kept in insulated cool boxes with ice packs, and transported to the laboratory for analysis.

Leaf water status was assessed by measuring the relative water content (RWC) as described in Makar *et al.* using the formula: $RWC (\%) = [(FW - DW) / (TW - DW)] \cdot 100$, where FW, TW, and DW represent fresh, turgid, and dry weights, respectively (2019).

Photosynthetic pigments were determined spectrophotometrically (Lichtenthaler, 1987). Fresh leaf tissue (300 mg) was ground with quartz sand and $CaCO_3$, extracted in 25 mL of 80 % acetone under dim light, filtered, and absorbance measured at 663, 647, and 470 nm (ULAB 102, China). Chlorophyll *a*, chlorophyll *b*, total carotenoids, total chlorophyll, and Chl *a/b* ratio were calculated per Lichtenthaler and Wellburn (1983) and expressed as mg/g fresh weight (Wellburn, 1994).

Yield assessment. Fruits were harvested twice weekly at full ripeness (uniform red coloration) throughout the fruiting period in both years. All marketable fruits per plot were counted and weighed individually (Radwag balance, Poland); damaged or malformed fruits were recorded separately and excluded from calculations. Total plot yield was expressed as g/plot to account for variation in plant establishment. Data were compiled by treatment and year for statistical analysis.

Statistical analysis. Data for soil physicochemical parameters (**Table 1**), leaf water status, photosynthetic pigments, and yield were analyzed by one-way ANOVA with biochar rate as the main factor; significant differences ($p < 0.05$) were further assessed by Tukey's HSD test. Normality and homogeneity of variance were verified using Shapiro–Wilk and Levene's tests. These data are based on three independent field replicates ($n = 3$) and are presented as means $\pm$ SD. Trace element concentrations (**Table 2**) were obtained from a single composite sample per treatment measured in triplicate and are therefore presented as instrument-computed means without inferential statistics. All analyses were performed in GraphPad Prism 11.0.0 ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Soil physicochemical properties. Soil analysis one year after biochar application revealed selective and moderate changes in physicochemical properties (**Table 1**). Contrary to expectations for alkaline hardwood-derived biochar, pH slightly declined from 5.1 (control) to 4.9–5.0 in amended soils. This unexpected acidification may reflect enhanced nitrification generating protons during ammonium oxidation, or the application of low-ash, low-alkalinity biochar.

Organic matter remained unchanged at 0.9 % across the control, 2, and 5 t/ha treatments, with only a marginal increase to 1.1% at 10 t/ha (+22 %). This modest response falls well below theoretical expectations (~0.5–1.5 % increase assuming 60–80 % biochar carbon content and complete mixing), likely due to dilution in dense clay soil (bulk density ~1.4–1.5 g/cm³), incomplete biochar incorporation, and potential underestimation by the Tyurin dichromate oxidation method, which may incompletely oxidize the recalcitrant aromatic carbon structures of biochar.

Table 1. Effect of biochar amendment on soil physicochemical properties and nutrient status in a field experiment with strawberry, measured one year after application

Parameter	Biochar application rate			
	0 t/ha (Control)	2 t/ha	5 t/ha	10 t/ha
Electrical conductivity ($\mu\text{S}/\text{cm}$)	60.7 $\pm$ 10.2	56.4 $\pm$ 10.2	62.2 $\pm$ 6.1	57.4 $\pm$ 7.3
pH (aqueous extract)	6.0 $\pm$ 0.3	5.9 $\pm$ 0.2	5.9 $\pm$ 0.2	5.9 $\pm$ 0.3
pH (salt extract)	5.1 $\pm$ 0.2 ^a	5.0 $\pm$ 0.3 ^{ab}	4.9 $\pm$ 0.2 ^b	4.9 $\pm$ 0.3 ^b
Soil organic matter (%)	0.9 $\pm$ 0.3 ^a	0.9 $\pm$ 0.3 ^a	0.9 $\pm$ 0.4 ^a	1.1 $\pm$ 0.3 ^b
Mineral N ($\text{NH}_4^+ + \text{NO}_3^-$) (mg/kg)	27.3 $\pm$ 2.7 ^a	22.9 $\pm$ 3.7 ^b	24.5 $\pm$ 2.9 ^{ab}	25.6 $\pm$ 3.8 ^{ab}
NO_3^- N (mg/kg)	16.8 $\pm$ 0.6	15.4 $\pm$ 0.6	16.1 $\pm$ 0.7	16.9 $\pm$ 0.6
NH_4^+ N (mg/kg)	10.5 $\pm$ 1.1 ^a	7.5 $\pm$ 0.8 ^b	8.4 $\pm$ 0.9 ^{ab}	8.7 $\pm$ 1.0 ^{ab}
Available phosphorus (mg/kg)	436.5 $\pm$ 14.3	442.4 $\pm$ 17.7	385.1 $\pm$ 30.8	391.8 $\pm$ 42.3
Available potassium (mg/kg)	76.3 $\pm$ 12.2	83.8 $\pm$ 7.7	60.8 $\pm$ 11.6	81.8 $\pm$ 9.5
Available sulfur (mg/kg)	10.0 $\pm$ 1.7	11.1 $\pm$ 0.7	10.8 $\pm$ 0.7	8.9 $\pm$ 0.9
Exchangeable Mg (mEq/100 g)	0.1 $\pm$ 0.05 ^a	0.4 $\pm$ 0.2 ^b	0.2 $\pm$ 0.1 ^{ab}	0.1 $\pm$ 0.05 ^a
Exchangeable Ca (mEq/100 g)	2.0 $\pm$ 0.3 ^a	1.2 $\pm$ 0.3 ^b	1.7 $\pm$ 0.3 ^{ab}	1.7 $\pm$ 0.3 ^{ab}

Note: values are means $\pm$ SD (n = 3). Different superscript letters within rows indicate significant differences (Tukey's HSD, $p < 0.05$); parameters without superscripts showed no significant treatment effects

Soil nitrogen availability. Biochar affected mineral N availability in a dose-dependent manner that did not follow a linear pattern. Total mineral N ($\text{NH}_4^+ + \text{NO}_3^-$) was significantly reduced only at 2 t/ha (22.9 vs. 27.3 mg/kg in control, -16 %), while reductions at 5 and 10 t/ha were not statistically significant. The effect was selective for ammonium, which declined from 10.5 to 7.5 mg/kg at 2 t/ha (-29 %, $p < 0.05$), with intermediate values at higher rates; nitrate remained stable across treatments.

This selectivity reflects biochar's cation-exchange behavior, whereby negatively charged surface sites preferentially retain cationic NH_4^+ while showing minimal affinity for anionic NO_3^- (Aghoghovwia *et al.*, 2022). Contributing mechanisms include electrostatic adsorption of NH_4^+ onto oxygen-containing functional groups formed through surface oxidation, microbial N immobilization during decomposition of biochar with a high C:N ratio (Davys *et al.*, 2023), and physical occlusion of NH_4^+ within biochar pores. Notably, maximum immobilization occurred at the lowest application rate, with progressive recovery at higher rates. This pattern may result from ash-derived cations (K^+ , Ca^{2+} , Mg^{2+}) competing with NH_4^+ for exchange sites at higher application rates, enhanced nitrification, or spatial variability given the modest sample size. Biochar-N interactions typically follow a biphasic pattern, with initial immobilization transitioning to enhanced retention as biochar matures (Davys *et al.*, 2023). The present study captured this early phase; however, no yield reductions were observed, suggesting that immobilization was insufficient to induce N deficiency. The absence of synthetic fertilizer provided a conservative test of biochar's intrinsic N dynamics.

Phosphorus (P), potassium (K), and base cation availability. Available P was markedly elevated across all treatments (385.1–442.4 mg/kg P_2O_5 ; control, 436.5 mg/kg)

with no significant differences, placing all values within the excessively high provision class (>250 mg/kg P_2O_5) of the Ukrainian Kirsanov gradation. A modest downward trend at higher rates may indicate weak P sorption by biochar, as reactive functional groups can promote sorption rather than release when soil P already exceeds plant demand (Zhang *et al.*, 2025). Available K showed a comparable pattern with no significant treatment effects (60.8–83.8 mg/kg; control, 76.3 mg/kg). Together, these results indicate that biochar's principal short-term effect on macronutrient cycling in this nutrient-rich soil operated through nitrogen transformations rather than P or K dynamics (Xiu *et al.*, 2025).

Water-extractable Mg increased fourfold at 2 t/ha (0.4 vs. 0.1 mEq/100 g in the control) before returning to control levels at higher rates (0.1–0.2 mEq/100 g), suggesting that low-rate application released soluble Mg from ash, while at higher rates this release was offset by precipitation, enhanced leaching, or re-sorption onto the expanded biochar surface. Water-extractable Ca showed a contrasting response, declining significantly at 2 t/ha (1.2 meq/100 g, -40 % relative to the control), with smaller, non-significant reductions at 5 and 10 t/ha. Despite biochar's high Ca content, this decline is most plausibly attributable to Ca precipitation with the abundant soil phosphate pool: at P_2O_5 exceeding 380 mg/kg, the soil solution is supersaturated with respect to calcium phosphate phases, so released Ca rapidly precipitates from the water-extractable fraction (Lindsay, 1979; do Nascimento *et al.*, 2018). Enhanced Ca leaching (Blanco-Canqui, 2017; Gelardi *et al.*, 2021) and complexation with biochar functional groups (Uchimiya *et al.*, 2011; Lehmann, Joseph, 2024) may also contribute. The divergent responses of Mg and Ca at 2 t/ha are chemically coherent: both cations are released from ash, but Ca phosphates are far more thermodynamically stable than Mg phosphates under typical soil pH, causing released Ca to precipitate preferentially while Mg remains in solution.

Trace element bioavailability and environmental implications. Plant-available trace element concentrations were assessed by DTPA extraction (Table 2). Biochar effects were element-specific: DTPA-extractable Zn decreased significantly at 2 t/ha (1.00 mg/kg, -32 %) and 5 t/ha (1.10 mg/kg, -26 %) relative to the control (1.48 mg/kg), returning to near-control levels at 10 t/ha. Hg showed more consistent immobilization, decreasing by 30–50 % across all treatments (0.0005–0.0007 vs. 0.0010 mg/kg in the control). DTPA-extractable Mn, Cu, Cr, and Pb showed no significant treatment effects. Notably, the 2 t/ha rate produced the strongest response for several parameters, including maximal Zn immobilization, peak water-extractable Mg, and the largest decline in water-extractable Ca.

Multiple mechanisms may operate simultaneously: electrostatic adsorption onto negatively charged surfaces, complexation with oxygen-containing functional groups, precipitation as carbonates or phosphates, physical occlusion within micropores, and redox-mediated transformation (Beesley *et al.*, 2011; He *et al.*, 2019; Wei *et al.*, 2025). The strong Hg response likely reflects its high affinity for sulfur- and nitrogen-containing functional groups; however, given the very low baseline concentrations ($\sim 10^{-4}$ mg/kg), the practical significance of these reductions should be interpreted cautiously.

In contrast, total trace element concentrations showed a high spatial variability and no systematic relationship to biochar rate (Table 2).

The absence of progressive increases with dose indicates that the amendment introduced no substantial trace element contamination, an important consideration given concerns about element loading from some biochar feedstocks, particularly manure- and sludge-derived chars. The high variability – for example, a 2.6-fold range in total

Cu – reflects the heterogeneous trace-element distribution typical of soils with variable parent material and management history. With only three replicate plots per treatment, this variability limits the power to detect modest changes in total element pools and may partly explain the lack of significant effects.

Table 2. Plant-available (DTPA-extractable) and total trace element concentrations in biochar-amended soil one year after application

Biochar dose Elements	0 t/ha (Control)	2 t/ha	5 t/ha	10 t/ha
DTPA-extractable elements / bulk element concentrations (mg/kg)				
Manganese (Mn)	14.6 / 365.9	16.8 / 447.6	13.6 / 318.6	15.4 / 377.1
Copper (Cu)	0.48 / 28.8	0.44 / 29.2	0.56 / 20.9	0.50 / 53.8
Chromium (Cr)	0.007 / 9.3	0.005 / 10.0	0.006 / 13.5	0.007 / 12.0
Zinc (Zn)	1.48 / 21.9	1.00 / 28.1	1.10 / 27.5	1.42 / 21.8
Lead (Pb)	0.56 / 8.1	0.59 / 7.0	0.61 / 10.2	0.66 / 11.4
Mercury (Hg)	0.0010 / 0.034	0.0007 / 0.038	0.0007 / 0.034	0.0005 / 0.045

Note: values are instrument-computed means of three readings from a single composite sample per treatment (plant-available (DTPA-extractable) / total concentrations, mg/kg). As these determinations were based on a composite sample rather than on independent field replicates, no inferential statistics were applied, and the data are presented as indicative trends. Measurement uncertainty was within the analytical sensitivity of the instrument

Overall, under the high-fertility conditions of this experiment, biochar exerted limited and selective effects on soil chemistry one year after application. The most pronounced responses occurred in dynamic, water-soluble pools rather than in total concentrations. Several effects, notably maximal responses at 2 t/ha, suggest that biochar's short-term influence was governed more by the receiving soil's chemical environment than by the amendment dose. Whether these subtle soil chemical changes translate into measurable plant responses is examined in the following sections addressing leaf water status, photosynthetic pigments, and fruit yield.

From soil to plant: biochar effects on strawberry growth and physiological responses

Biochar effects on leaf water status. Leaf water status responded clearly to biochar amendments in both experimental years (**Fig. 1**). In 2024, leaf relative water content (RWC) in biochar-amended plants ranged from 69 % to 78 %, peaking at the 5 t/ha rate (78 %) compared with 70 % in the control. In 2025, when growing conditions appeared more challenging, as indicated by the lower control RWC (63 %), the beneficial effect of biochar was more uniform across rates: RWC in all biochar treatments ranged narrowly from 72 % to 74 % compared with 63 % in the control.

Across both years, biochar amendment consistently improved leaf water status relative to the unamended control, with treated plants maintaining 6–14 percentage points higher RWC. The magnitude of this effect was greater in 2025 than in 2024, particularly relative to the control, suggesting that biochar's beneficial influence on plant water relations becomes more pronounced under conditions of greater water stress.

This interpretation is consistent with documented improvements in soil water-retention capacity and plant-available water following biochar amendment, particularly in the longer term as the amendment equilibrates with the soil matrix (Lentz *et al.*, 2019). The improved leaf water status of biochar-treated plants may, in turn, have contributed to the enhanced fruit development observed in 2025 (see below).

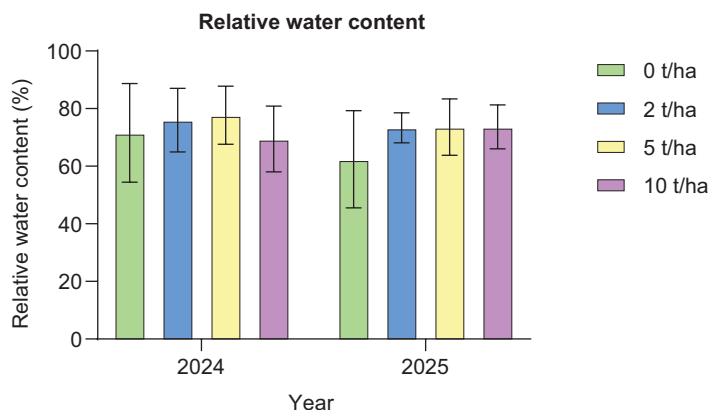


Fig. 1. Leaf relative water content (RWC) in strawberry plants grown under different biochar application rates (0, 2, 5, and 10 t/ha) in a field experiment over two growing seasons (2024 and 2025). Values represent means $\pm$ SD from three replicate plots per treatment

Photosynthetic pigments. Leaf concentrations of Chl *a*, Chl *b*, and carotenoids showed pigment-specific and year-specific responses to biochar (**Fig. 2**).

In 2024, no significant differences were detected for any pigment across treatments. In 2025, substantial effects emerged, particularly for Chl *b*, which increased significantly in all biochar treatments relative to the control (0.40 mg/g): 0.62 mg/g at 2 t/ha ($p < 0.01$), 0.59 mg/g at 5 t/ha ($p < 0.01$), and 0.72 mg/g at 10 t/ha ($p < 0.0001$), representing 62–80 % increases. Chl *a* and carotenoids responded more modestly, with only the 2 t/ha treatment differing significantly from the control in both cases ($p < 0.05$). Importantly, the Chl *b* dose-response did not mirror the soil water-extractable Mg pattern (which peaked at 2 t/ha), indicating that the pigment response cannot be attributed solely to Mg availability.

The delayed emergence of biochar effects is consistent with well-documented weathering dynamics: progressive oxidation through wetting-drying cycles and microbial colonization gradually develops oxygen-containing functional groups and increases cation exchange capacity, with plant responses typically becoming pronounced 12–24 months after application (Joseph *et al.*, 2021; Schmidt *et al.*, 2021). The 62–80 % Chl *b* increases substantially exceed the average 16.1 % total chlorophyll increase reported in a global meta-analysis of 347 comparisons (He *et al.*, 2020), possibly reflecting the low baseline chlorophyll concentrations and favorable nutritional context of the second year.

Several mechanisms may account for the Chl *b* enhancement. First, nitrogen immobilized during the first year is typically released as biochar weathers and microbial communities equilibrate (Yan *et al.*, 2024); as a structural component of the chlorophyll tetrapyrrole ring, gradual N release could partly explain the timing of the response. Second, biochar treatments maintained higher leaf relative water content (**Fig. 1**), particularly in 2025, supporting chlorophyll biosynthesis while reducing stress-induced

degradation (Blanco-Canqui, 2017; Gelardi *et al.*, 2021). Third, although soil water-extractable Mg did not parallel the Chl *b* dose-response, Mg availability in all biochar treatments was equal to or greater than the control, providing a generally improved supply of this central chlorophyll-coordinating atom. A recent study on alfalfa reported a comparable 37.5 % Chl *b* increase at an intermediate biochar rate (Yan *et al.*, 2024), consistent with the broader pattern that biochar's nutritional benefits vary by feedstock, soil, and species.

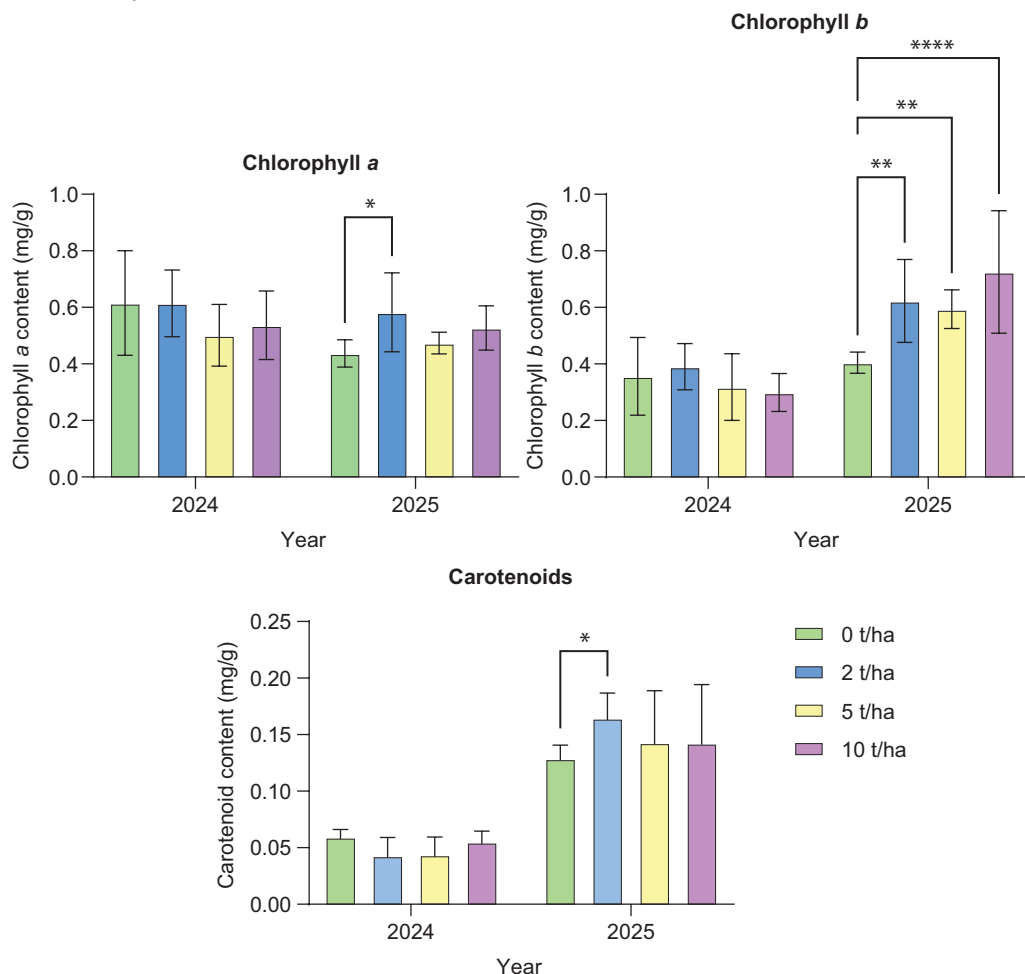


Fig. 2. Leaf concentrations of chlorophyll *a*, chlorophyll *b*, and carotenoids in strawberry plants grown under different biochar application rates (0, 2, 5, and 10 t/ha) in a field experiment over two growing seasons (2024 and 2025). Values represent means $\pm$ SD from three replicate plots per treatment ($n = 3$). Asterisks indicate significant differences compared with the control within each year based on one-way ANOVA followed by Tukey's HSD test (* $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$)

Fruit production and yield responses. Despite the significant Chl *b* enhancement in 2025, biochar produced no detectable effect on individual fruit weight or total yield. Individual fruit weight was not significantly affected by biochar application in 2024, with mean values ranging from 9.1 to 9.9 g across treatments (**Fig. 3**). In 2025, however, fruit weight

in the 5 t/ha treatment (8.5 g) was significantly lower than in the control (11.5 g; $p < 0.001$), the 2 t/ha treatment (10.7 g; $p < 0.01$), and the 10 t/ha treatment (10.4 g; $p < 0.05$), while no significant differences were observed among the other three treatments.

In contrast, total plot yield did not differ significantly among treatments in either year (Fig. 3). Yield increased from 2024 (140–210 g/plot) to 2025 (260–480 g/plot) across all treatments, likely reflecting plant establishment and the natural reproductive cycle of the strawberry, but the high within-treatment variability in the second season masked any potential biochar effects.

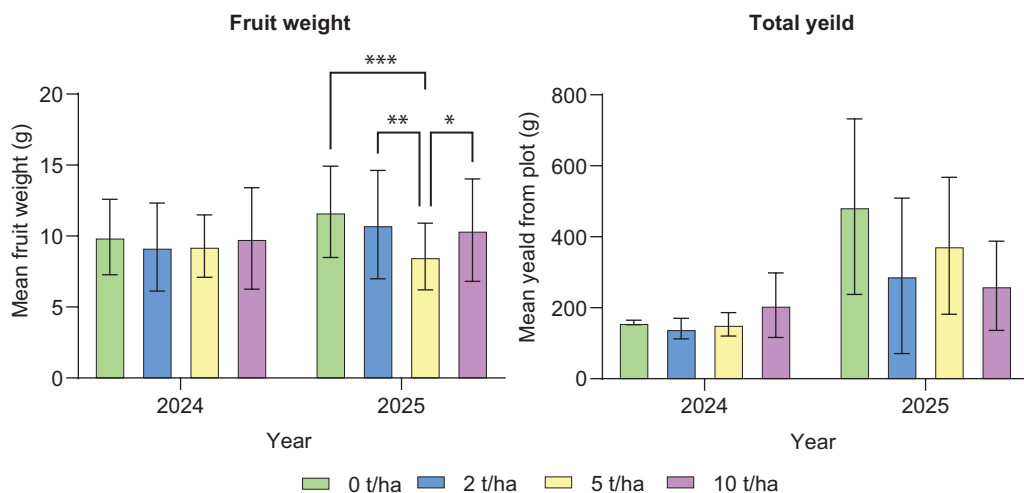


Fig. 3. Mean individual fruit weight and total plot yield under different biochar application rates (0, 2, 5, and 10 t/ha) over two growing seasons (2024 and 2025). Data are means $\pm$ SD ($n = 3$). Asterisks indicate significant differences from the control (Tukey's HSD: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

This disconnect between physiological responses and crop performance reflects a common challenge in biochar research. Strawberry yield is determined by multiple interacting processes, including flower initiation, pollination success, fruit set, and achene-driven receptacle expansion, rather than by photosynthetic capacity alone. Enhanced Chl *b* may therefore improve carbon fixation without increasing fruit weight, particularly if additional photosynthate is allocated to vegetative growth or reserves. High inherent variability in strawberry production, combined with limited replication, further reduced statistical power to detect moderate effects.

The two-year observation period may have been insufficient to capture the full temporal development of biochar effects on a perennial crop. Trials spanning 5–10 years have frequently reported delayed yield responses absent in initial years, and the second-year Chl *b* enhancement may represent an early indicator of emerging benefits. The exclusion of synthetic fertilizer, while valuable for isolating biochar effects, may also have limited the productivity response. In high-fertility soils, biochar's primary benefits derive from improved nutrient retention rather than direct supply; without fertilizer inputs to retain, these benefits were likely minimal, while transient nitrogen immobilization may have offset other effects. Studies combining biochar with fertilization frequently report synergistic effects on productivity.

The literature on biochar effects on strawberry yield is mixed (Song *et al.*, 2023; Orlandella *et al.*, 2025). Some studies report 15–30 % yield increases in sandy or

degraded soils, whereas others find negligible responses in high-fertility soils. A meta-analysis of biochar effects on crop yield reported an average increase of approximately 10–15 %, with the strongest effects in acidic, coarse-textured soils under nutrient or water limitation. The present results align with this pattern: adequate initial fertility, heavy clay texture, and the short observation period likely combined to minimize detectable yield responses despite measurable changes in soil chemistry and plant physiology. Future research should examine biochar effects over extended timeframes, evaluate interactions with fertilization, and identify conditions under which biochar provides economically viable benefits for strawberry production.

CONCLUSIONS

This two-year field study evaluated biochar amendment (2, 5, and 10 t/ha) effects on soil properties, leaf physiology, and fruit production of strawberries grown without synthetic fertilizer. One year after application, biochar produced limited and selective effects on bulk soil chemistry: available P and K were unaffected, consistent with the soil's excessive P status, while pH remained stable. The most pronounced responses occurred in dynamic, water-soluble pools, including transient ammonium immobilization, shifts in water-extractable Mg and Ca, and selective reduction of plant-available Zn and Hg. Several effects were strongest at 2 t/ha, indicating that biochar's short-term influence was governed more by the receiving soil chemistry than by the application dose.

At the plant level, biochar improved leaf water status across both years and produced a marked, delayed increase in Chl *b* in the second season, with the strongest effect at 10 t/ha. This delayed, dose-dependent pigment response is consistent with progressive biochar weathering. However, these physiological changes did not translate into measurable gains in fruit weight or yield.

Overall, biochar exerted measurable but modest effects on the soil-plant system that did not propagate into agronomic productivity over two seasons, consistent with the broader finding that biochar benefits are most pronounced in nutrient-poor, acidic, or coarse-textured soils. Realizing agronomic benefits in high-fertility soils may require longer observation periods, integration with fertilization, and site-specific optimization of application rate.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Animal Rights: this article does not contain any studies with animal subjects performed by any of the authors.

Human Rights: this article does not contain any studies with human subjects performed by any of the authors.

AUTHOR CONTRIBUTIONS

Conceptualization, [S.T.; N.R.]; methodology, [S.T.; N.R., A.B., Ye.Z.]; validation, [N.R.]; formal analysis, [S.T.; N.R.]; investigation, [S.T.; Ye.Z., A.B.]; resources, [S.T.; N.R.]; data curation, [N.R.]; writing – original draft preparation, [S.T.]; writing – review and editing, [S.T.; N.R.]; visualization, [S.T.] supervision, [N.R.]; project administration, [S.T.; N.R.].

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КОМПЛЕКСНА ОЦІНКА ВПЛИВУ БІОВУГІЛЛЯ НА ХІМІЧНИЙ СКЛАД ҐРУНТУ, ВОДНИЙ РЕЖИМ РОСЛИН І ВМІСТ ФОТОСИНТЕТИЧНИХ ПІГМЕНТІВ У *FRAGARIA* × *ANANASSA*

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Обґрунтування. Біовугілля широко популяризують як меліорант для ґрунту, здатний підвищувати його родючість, утримувати поживні речовини та покращувати продуктивність сільськогосподарських культур, проте його ефект суттєво залежить від типу ґрунту, норми внесення та часу, що минув після цього. Дані щодо багаторічних ягідних культур, які вирощують на родючих ґрунтах без використання мінеральних добрив, залишаються обмеженими. Це дослідження оцінювало вплив біовугілля на хімічні властивості ґрунту, фізіологію листя та плодоношення суниці садової в польових умовах.

Матеріали та методи. Дворічний польовий експеримент (2024–2025 рр.) проводили із внесенням біовугілля у дозах 0, 2, 5 та 10 т на гектар без використання синтетичних добрив; кожен варіант обробки мав потрібну повторність на дослідних ділянках. Через рік після внесення змішані зразки ґрунту проаналізували на кислотність, вміст органічної речовини, мінерального азоту, фосфору, калію, сірки, водорозчинного кальцію та магнію, а також рухомих і валових форм мікроелементів відповідно до національних стандартних методів. Відносний вміст води в листі, водний дефіцит і концентрацію фотосинтетичних пігментів вимірювали протягом обох років, а масу окремої ягоди та загальну врожайність фіксували впродовж усього періоду плодоношення.

Результати. Вплив біовугілля на загальний хімічний склад ґрунту був обмеженим і вибірковим. Вміст фосфору та калію, які й без нього були в надлишку, а також кислотність ґрунту залишилися без змін. Найчіткіші реакції зафіксовано у пулі розчинних елементів: вміст амонійного азоту, водорозчинного кальцію, а також рухомих форм цинку та ртуті знизився, тоді як рівень водорозчинного магнію різко зріс, причому кілька ефектів виявилися найбільш вираженими за найнижчої норми внесення. У рослин біовугілля покращило водний режим листя в кожен із років і помітно підвищило концентрацію хлорофілу *b* на другий рік (найсильніше – за найвищої норми внесення), тоді як вміст хлорофілу *a* та каротиноїдів змінився незначно. Маса ягід була нижчою на другий рік проведення експерименту на дослідних ділянках із нормою внесення 5 т/га.

Висновки. Біовугілля мало помітний, проте помірний вплив на систему ґрунт–рослина, проте це не вплинуло на урожайність протягом двох років. Можна зробити висновок, що позитивний вплив біовугілля на родючі ґрунти проявляється поступово та залежить від норм внесення й агротехніки.

Ключові слова: біочар, суниця садова, меліорант, іммобілізація азоту, хлорофіл, водорозчинні катіони, врожайність культур, польовий експеримент