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Evaluation of plants' response to activated biochar through visual and proximal sensing monitoring

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ABSTRACT

This study presents two laboratory-scale greenhouse experiments evaluating the effects of biochar addition on lettuce (*Lactuca sativa*) and spinach (*Spinacia oleracea*). Two biochar types, derived from pyrolysis at 550 °C of softwood (SW) and sewage sludge (SS), were applied at two doses (2 and 15 g/L), as both unaltered material and after physical activation with CO_2 at 900 °C for 2 h. Visual monitoring included plant growth geometrical parameters, chlorophyll content (SPAD), and soil properties. Proximal sensing was based on continuous non-destructive monitoring of plant health through the normalized difference vegetation index (NDVI). Overall, the biochar application increased biomass and vegetative growth compared to control plants. Specifically, 15 g/L achieved larger fresh biomass (SS: +294% spinach, +87% lettuce; SW: +58% spinach, +19% lettuce) and SPAD (SS: +48% spinach, +14% lettuce; SW: +55% spinach, +15% lettuce) compared to 2 g/L. Activation further improved leaf number (up to +47%) and fresh biomass (+57%) in lettuce, and also in spinach (+32% leaves and +121% fresh biomass). Biochar increased soil electrical conductivity in lettuce (+6.4% SS, +1.3% SW) and moisture (SS: +5.8% spinach, +7.5% lettuce; SW: +10.2% spinach, +1.1% lettuce), while pH remained stable. Integrating NDVI trends with SPAD and geometric plant modeling supported the assessment of vegetative growth. In conclusion, biochar, particularly that derived from SS, improved plant growth and soil properties, demonstrating its potential as a sustainable growing media to enhance productivity and resilience in controlled cropping systems.

1. Introduction

Growing awareness of environmental challenges and resource depletion has increased the demand for sustainable agricultural practices, which aim to ensure food security, preserve ecosystems, and reduce the environmental footprint of farming activities [1]. Circular economy promotes valorization of agricultural residues to close material loops and minimize waste [2] thus enhancing the resilience and productivity of agroecosystems through the adoption of biofertilizers and renewable energy [3]. Biochar, a stable carbon-rich material obtained through the pyrolysis of biomass, has gained increasing attention in agriculture. Biochar addition to soil can enhance physical, chemical, and biological properties, increase water retention, cation exchange capacity and microbial activity, improve nutrient availability and plant growth [4], [5], and it is recognized for its role in long-term carbon sequestration, mitigating climate change [6], [7], [8], [9], [10]. The agronomic effectiveness of biochar depends on its feedstock, production conditions, and dose, requiring site-specific evaluation for optimal outcomes on plant growth.

This study considered lettuce and spinach as model crops due to their short but distinct growth cycles (shorter in lettuce than in spinach), contrasting nutrient requirements, and integrated production practices

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[11], their increasing relevance in Italian agricultural production statistics [12], and their major contribution to dietary nitrate exposure in the Italian population [13]. A bibliographic search was conducted on Scopus over the last ten years with the keywords “lettuce” OR “spinach” AND “biochar”, and 135 references were selected. Among these, 17 references (Table 1) detailed the physicochemical properties of biochar. Biochar application generally enhances plant growth and productivity, although responses depend on biochar type, dose, and experimental conditions. In detail, in both lettuce and spinach, compared to control, biochar increased leaf number (from +30% to +85%) [11,13–17], plant height and leaf length (from +9% to +42% and from +25% to +27%, respectively) [14,16,18], fresh and dry biomass (from +25% to over +300%) [11,19–24], SPAD and total chlorophyll (from +27% to +90%) [10,17,18]. Yield and fresh leaf weight exceeded the control by more than +100% under optimized or combined treatments [17,19,24]. Neutral or negative effects were occasionally reported, with no significant differences in growth parameters [12,13,21]. Reported biochars derive from pyrolysis of a wide range of feedstocks, including wood [13,24], animal by-product, manure, wheat straw, maize straw, rice husk and cotton stalks [14,21,25–28,30–32], sewage sludge, food waste and municipal solid waste [18,19,23,24]. Biochars derived from these feedstocks were produced under variable pyrolysis conditions, including temperatures of 300–400 °C [18,24,30]–500 °C [14,23], and up to 700 °C [23], with residence times ranging from 30 min to 11 h and often under N_2 atmosphere [14,19,25]; however, some studies did not specify pyrolysis conditions [13,21]. This resulted in a wide range of chemical properties. Total N content ranged from <0.1 wt% in wood- and straw-derived biochar [14,24] to 5 wt% in sewage sludge- and cotton stalk-derived biochar [15,16]. Total P and K contents were also variable, with higher K levels in biochar from municipal solid waste and food waste [16,22]. Alkaline pH, typically between ~7.2 and 10.2 [17,25], is reported. Electrical conductivity ranged from <0.1 dS m^{-1} to >10 dS m^{-1}, especially in manure- and waste-derived biochar [14,17]. Ash content varied from <1 wt% in biochar derived from compost [14] to 50 wt% in poultry manure biochar [26]. Full details on the performed literature review are in Table I of Supplementary Material.

Table 1

Overview of previous studies on effect of biochar in agriculture on lettuce and spinach plants (L: lettuce, S: spinach, SPAD: Soil Plant Analysis Development).

Ref.	Plant	Effects of biochar (compared to control plants)
[14]	S	increased number of leaves (+77%) and SPAD (+27%)
[15]	S	biomass increase was 61.66 %, 81.81 %, and 101.41 % with the addition of 3 % sawdust char, 3 % reed cane char, and 5 % sludge char, respectively
[16]	L	variable effects on Chlorophyll *a* (−10.4%), chlorophyll *b* (+2.5%), carotenoid content (−20.0%)
[17]	L	no significant differences in leaf area, dry yield, and number of leaves
[18]	L	increase in plants' height (+9.2%), number of leaves (+84.6%) and biomass dry weight (+25.3%)
[19]	S	increase in number of leaves (+9.7%)
[20]	L	increase in fresh leaf weight (+33.2%), leaf length (+25.2%) and number of leaves (+18.4%)
[21]	L	increase in fresh leaf weight (+106.0%), number of leaves (+32.4%), and SPAD (+30.7%)
[22]	S	increase in shoot length (+42.1%), root length (+43.5%), shoot fresh weight (+58.15%), root fresh weight (+100-150%), total chlorophyll (+90.7%)
[23]	S	increase in shoot fresh weight (169.9%, 364.8%), dry weight (62.3%, 309.1%), root fresh weight (145.9%, 369.1%), and dry weight (296.1%, 441.5%) respectively with 0.5% and 1.5% biochar
[24]	S	increase in plant height (+36%), leaf length (27%), number (48%) and width (22%)
[25]	S	2% biochar improved aggregates stability (19.85%) and mitigated soil acidity
[26]	S	pots amended with biochar had higher yields than the control pots (+4.5%)
[27]	L	decrease in yield (−3.6%)
[28]	S	increase in leaves fresh weight (+6%) and dry weight (+19%), in roots fresh weight (+18%) and dry weight (+3%)
[29]	S	increase in biomass fresh weight (+90% at 5 t ha^{-1}, +81% at 10 t ha^{-1}) with maize biochar, and in biomass dry weight (+45% at 5 t ha^{-1} and +220% at 10 t ha^{-1}) with rice husk biochar
[30]	S	increase in biomass fresh weight (+106% in spring, +341% in autumn), and of K content in leaves (+72% in spring, +36% in autumn)
[31]	S	increase in fresh leaf biomass (+38%)

The references in Table 1 indicate that biochar addition promotes plant health and growth with effects highly context-dependent, underscoring as key knowledge gap the need for targeted, crop-specific evaluations [11,18,21,26]. Another knowledge gap identified concerns biochar activation. This is commonly implemented when biochar is used for pollutant adsorption from water [6] and gas flows [7], [8], as catalytic support in electrochemical and energy applications [9], and in the production of new materials [10]. To the best of our knowledge, the effect of biochar activation on lettuce and spinach has not been investigated yet. A pot experiment exploring grass growth [32] showed that steam-activated biochar increased available nitrogen by 50%, while in another study various post-processing treatments applied to biochar resulted in an average 14% increase in plant growth compared to untreated biochar [33].

This study aims to explore the impact of biochar as soil amendment on lettuce and spinach cultivated in 1L pots in a lab-scale greenhouse. The novelty relies on the following items addressing a current gap in the literature where limited information is available on the agronomic application of CO_2-activated biochars: two different standard biochars (deriving from a woody biomass and sewage sludge produced by slow pyrolysis in a pilot-scale rotary kiln at the UK Biochar Research Centre) were applied to lettuce and spinach as unaltered material and after physical activation with CO_2, considering two doses of biochar for each plant and treatment. A further element of novelty is the integration of conventional visual monitoring with a low-cost proximal sensing approach, which is still uncommon in greenhouse-based biochar studies. Plant response was assessed integrating visual and proximal sensing techniques. Visual monitoring included a weekly manual acquisition of plant response (number and dimensions of leaves, and Soil Plant Analysis Development-SPAD), soil moisture, electrical conductivity and pH, and of fresh and dry biomass at the end of the tests (44 days for lettuce and 135 days for spinach). A low-cost proximal sensing system installed in the greenhouse enabled continuous non-invasive acquisition of reflectance values in the red, green, and near-infrared regions (550, 660, and 850 nm) to gather data on plant health through the normalized difference vegetation index (NDVI). The integration of visual monitoring and proximal sensing is increasingly important for a comprehensive crop assessment. Previous studies have applied proximal sensing in greenhouse to evaluate wood distillate effects on strawberry plants through leaf gas exchange and vegetation reflectance indices [34], while another used hyperspectral leaf imaging for in vivo phenotyping of soybean [35]. However, the use of low-cost proximal sensing systems specifically to evaluate the agronomic performance of activated biochar remains largely unexplored.

2. Materials and methods

2.1. Materials

This study involved two standard biochars produced at the UK Biochar Research Centre of the University of Edinburgh (https://www.biochar.ac.uk) from the pyrolysis of softwood pellets and sewage sludge at 550 °C, identified as SW and SS (Fig. 1). Full details on pyrolysis conditions are in Table II of Supplementary Materials. Physicochemical properties of the biochars are as follows. SW has higher carbon (85.5 wt%) and lower ash (1.3 wt%), nitrogen (<0.1 wt%) and phosphorus (0.06 wt%), while SS has lower carbon (29.5 wt%) and higher C stability (84.4%), ash (58.9 wt%), nitrogen (3.75 wt%) and phosphorus (2.29 wt%). Both are slightly alkaline (pH 7.91–8.17), and SS had higher

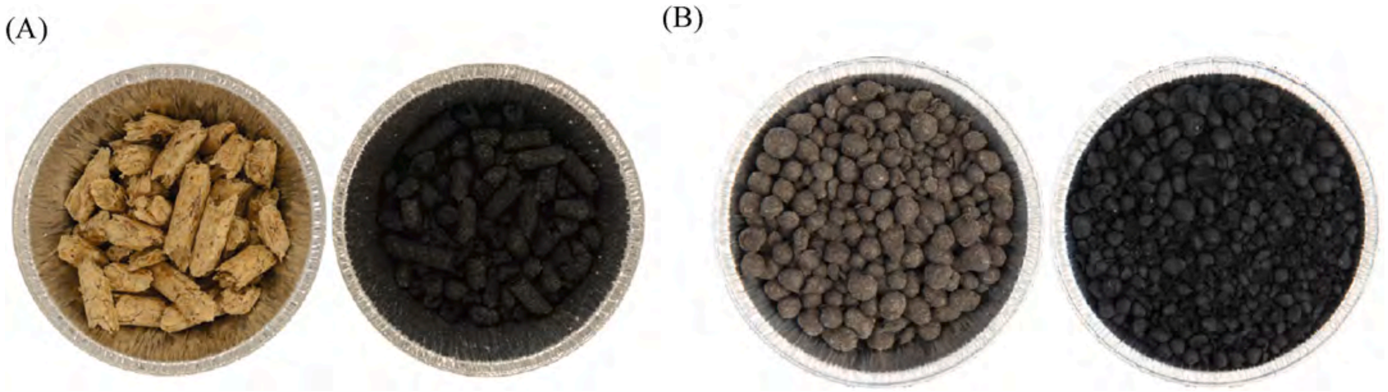

Fig. 1. Parent biomass and biochars considered in this study: (A) Soft wood pellets (SW) biochar, (B) Sewage sludge (SS) biochar.

electrical conductivity (280.8 dS/m) compared to SW (0.09 dS/m). Physical activation with CO_2 at 900 °C for 2 h was performed at the Institute for Chemicals and Fuels from Alternative Resources (ICFAR) of Western University, Canada (https://www.icfar.ca). Activation significantly increased the biochar specific surface area (SW: from 19.2 to 263.4 m^2/g; SS: from 15.7 to 109.2 m^2/g) and total pore volume (SW: from 0.012 to 0.209 cm^3/g; SS: from 0.010 to 0.169 cm^3/g). Details on the characteristics of biochar are in Table III of Supplementary material.

2.2. Experimental setup and monitoring

Experiments were conducted in a laboratory-scale greenhouse, located in Turin, on lettuce (*Lactuca sativa*, October-December 2024, duration 44 days) and spinach plants (*Spinacia oleracea*, November 2024-March 2025, duration 135 days). 3 weeks-old lettuce seedlings were transplanted into 1 L pots filled with a commercial peat-based medium (TS3, Turco Silvestro, Albenga, Italy; blond peat to black peat ratio 15:85, pH 6.0, electrical conductivity 0.56 dS m^{-1}, total porosity 90%, bulk density 150 kg m^{-3}). An automated drip irrigation device was installed in each pot (WD-01BDE Dual path, 30 PSI, 0.8 L/min for 1 min per 2 times per day for lettuce and 1 min once a day for spinach). Biochar was added to the medium at two doses (2 g/L and 15 g/L) [36], and plants were named SW2, SW2a, SW15, SW15a for softwood biochar, SS2, SS2a, SS15, SS15a for sewage sludge biochar, and C (control, i.e. no biochar). Three replicates were involved for each treatment, resulting in 54 plants (27 lettuce and 27 spinach) positioned randomly inside the greenhouse.

Visual monitoring was as follows. Chlorophyll content was measured as Soil Plant Analysis Development (SPAD) using a MC-100 chlorophyll concentration meter Apogee Instruments Inc., Logan, UT, USA (measurement area: 63.6 mm^2; resolution: 1 μmol m^{-2}; wavelengths: 931 nm and 653 nm). SPAD was measured weekly on two leaves from each plant. Soil electrical conductivity (EC), moisture, and pH were checked with a Brumong B0D8KJ9F27 probe (pH range: 3.5–9, accuracy: ±0.5; EC range: 0–3000 μS/cm, accuracy: ±100 μS/cm). At harvest, the following data were measured: number of leaves, biomass weight (fresh and dry for leaves and dry for roots) using a Kern PLU 4200-2F balance (Kern & Sohn GmbH, Germany) and a TCN 30 PLUS (LSI Lastem, Italy) oven. Geometry of plants was acquired at harvest measuring (2 replicates for each treatment) the plant height and ideal diameter, and spinach leaf dimensions (lettuce leaves dimensions were not recorded because they grew mostly vertically and are curled) (Fig. 2). These data were then processed using RStudio software to approximate individual plants to cylindrical geometries, thereby enabling a standardized geometric representation for comparative analysis. This approach allowed the evaluation of variations in plant dimensional development in the tested conditions.

Proximal sensing monitoring was based on a low-cost automated sensor system described in another study [36], consisting of a MAPIR Survey 3W multispectral camera (12 MP; 550 nm,660 nm, and 850 nm) attached on the roof of the greenhouse in nadiral position. A Raspberry Pi with remote SSH access remotely managed MAPIR camera and stored data. Images were acquired hourly and used to calculate the normalized difference vegetation index (NDVI), allowing continuous, non-destructive monitoring of plant vigor throughout the growing period.

2.3. Preliminary sensitivity assessment

The collected experimental data underwent statistical evaluation via Pearson correlation analysis and one-way ANOVA to assess the increases observed in the tests after biochar treatments and to evaluate the influence of chemical parameters and biochar types on the results. All analyses were conducted using a significance level of $\alpha = 0.05$.

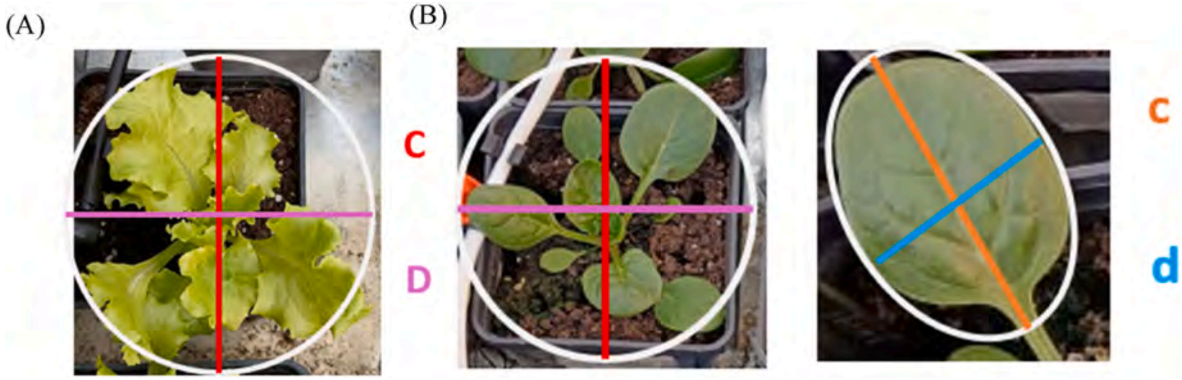

Fig. 2. Measurement of geometrical parameters in (A) lettuce and (B) spinach plants.

3. Results

3.1. Visual monitoring

The development of lettuce and spinach plants at harvest (Fig. 3) displayed significant variations in their growth across the different treatments with biochar in relation to the control plants. For each measured parameter, results are first presented as an overall comparison between biochar and control, followed by the general effect of activated biochar compared to the control, and finally detailing differences according to biochar type, application rate, and activation, always in comparison with the control. The effect of biochar on number of leaves compared to control was as follows. Overall, it was positive in lettuce (+36.2 ± 2.5%) and spinach (+30.4 ± 3.2%) (Fig. 4), consistently with literature ([14], [18], [19], [21]), and 15 g/L of biochar led to more leaves (+52.8%) than 2 g/L (+38.9 ± 0.9%) [13], [17], [18], [20]. Activated biochar increased the leaves number in lettuce (+47.1 ± 0.5%) and in spinach (+31.9 ± 1.4%) compared to the control. Considering the types and doses of biochar, the number of leaves increased compared to control for SW2a (+63.2 ± 0.5%), SS2a (+58.6 ± 0.7%), SS2 (+47.1 ± 1.1%), SS15 (+40.2 ± 0.7%) and SS15a (+37.9 ± 0.9%), SW15a (+28.7 ± 0.2%), SW15 (+5.8 ± 0.4%) and SW2 (+8.1 ± 0.7%).

Biochar supplement increased, compared to control, also biomass weight (Fig. 5). Overall, lettuce improved fresh (+45.9 ± 0.5%) and dry (+53.8 ± 0.5%) biomass, confirming previous studies ([18], [21], [22], [23], [28], [30], [37]). The increase was higher for spinach (fresh weight +155.5 ± 1.8%, dry +86.9 ± 1.2%). Considering biochar types, fresh biomass improvement compared to control was observed for lettuce (SS: +46.0 ± 0.1%, SW: +26.4 ± 0.2%) and spinach (SS: +268 ± 0.87%, SW: +43 ± 0.2%). Higher biochar dose led to heavier biomass in lettuce (fresh +32.3 ± 0.4% for 2 g/L and +45.8 ± 0.6% for 15 g/L; dry +39.8 ± 0.4% for 2 g/L and +53.1 ± 0.6% for 15 g/L) and spinach (fresh +139.6 ± 1.79% for 2 g/L and +174.3 ± 1.8% for 15 g/L; dry +82.2 ± 1.3% for 2 g/L and +92.4 ± 1.16% for 15 g/L). The greater biomass increase observed with SS biochar compared to SW biochar could be related to its higher ash content (58.9 wt%), greater nutrient availability (total N 3.75 wt%), and higher electrical conductivity (280.8 dS/m), which likely improved nutrient supply to plants. In contrast, SW biochar, characterized by a lower ash content (25 wt%), negligible N content (<0.1 wt%), and lower electrical conductivity (0.09 dS/m), showed a relatively weaker stimulatory effect on plant growth compared to sewage sludge biochar. Considering activation and biochar type compared to control, the largest biomass was recorded with SS15a in lettuce (+105.9 ± 0.2% fresh, +110.4 ± 0.1% dry), and with SS15 in spinach (+407.9 ± 0.6% fresh, +278.5 ± 0.3% dry). Full details on the monitoring of leaf number, fresh and dry biomass weights are in Fig. I of Supplementary Material.

Fig. 6 shows the positive relationship between leaf number and fresh biomass weight in lettuce and spinach plants. In lettuce, the association was weak (biochar: intercept = 17.1, slope = 0.796, $R^2 = 0.226$; control: intercept = 5.29, slope = 0.941, $R^2 = 0.330$), while spinach showed a stronger connection, particularly with biochar supplement (biochar: intercept = −10.3, slope = 1.67, $R^2 = 0.764$; control: intercept = −1.47, slope = 0.546, $R^2 = 0.576$). Overall, these results indicate that biochar enhanced biomass accumulation per unit of leaf development, especially in spinach, while in lettuce leaf number was a less reliable predictor of fresh biomass weight.

Overall, compared to control, dry weight of roots increased in lettuce (+132.6 ± 1.2%), and decreased in spinach (−27.6 ± 0.5%) with biochar supplement, suggesting a potential trade-off between aboveground and belowground growth, although responses varied across species and treatments. In lettuce, the two types of biochar had a similar dry root weight, with an overall increase ranging from +113.4 ± 0.7% (SW) to 151.7 ± 0.9% (SS), and opposite effects of biochar doses for SS (+133.7 ± 1.2% for 2 g/L; +169.7 ± 0.2% for 15 g/L) and SW (+140.3 ± 1.0% for 2 g/L; +86.6 ± 0.3% for 15 g/L). Another study showed greater root development in lettuce plants treated with biochar, compared to the control: root length by +43.5% and fresh weight by +100-150% [22]. In spinach, SS recorded a slight increase of dry root weight (+5.7 ± 0.5%), while SW led to a decrease (−65.1 ± 0.5%). Again, the dose effects were not aligned for SS (−32.6 ± 0.3% for 2 g/L; (+44.0 ± 0.1% for 15 g/L) and no differences in dry root weight were measured for SW. Activated biochar improved substantially dry root weight in lettuce (+189.7 ± 0.5%) compared to unaltered biochar, whereas no significant changes were ascribable to biochar activation in spinach. Full details on root weight are in Fig. II of Supplementary Material.

In both crops, biochar application resulted in an overall increase in SPAD chlorophyll values compared to control, confirming other studies including SPAD measurements ([14], [21]) and chlorophyll *a* and *b*

Fig. 3. Plants at harvest: (A) lettuce and (B) spinach.

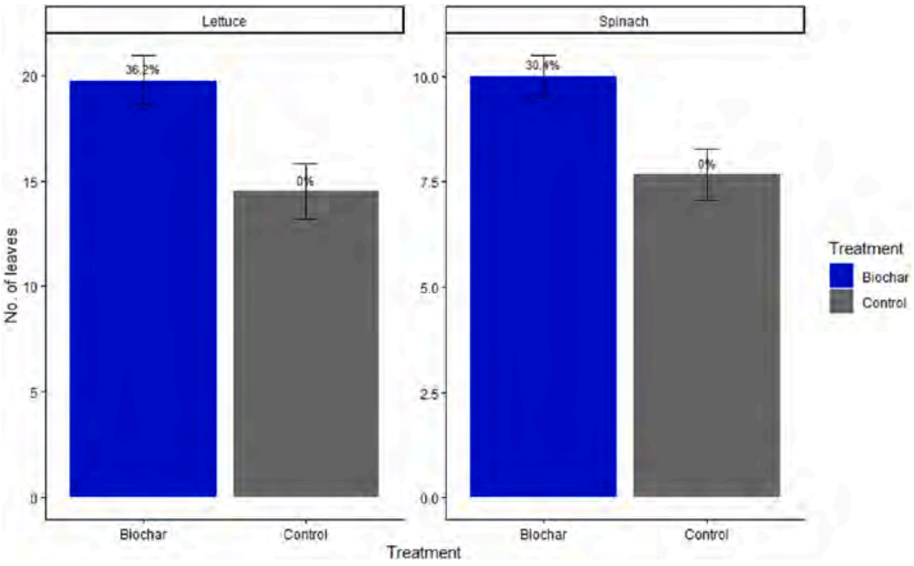

Fig. 4. Average number of leaves at the end of tests in lettuce and spinach supplemented with biochar (blue) and in control plants (grey). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

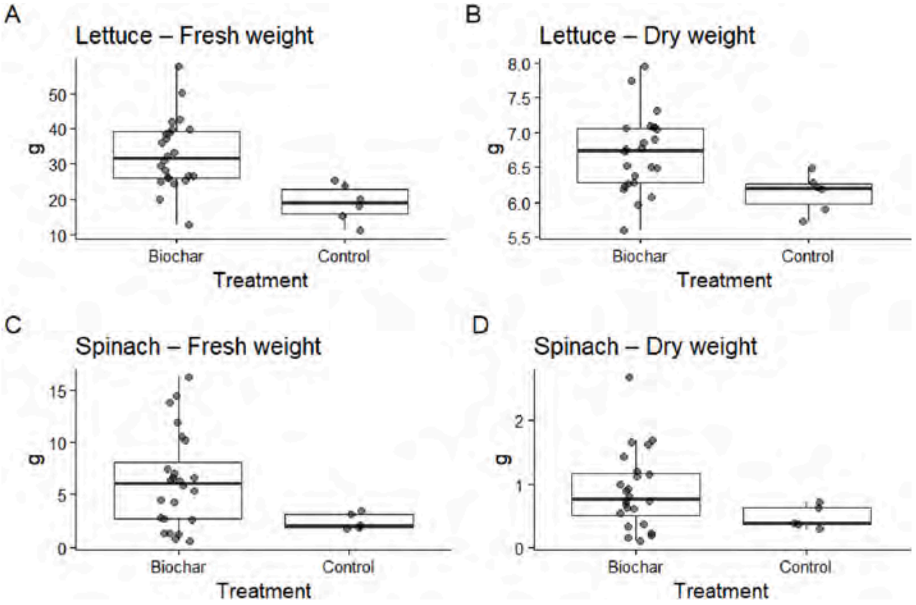

Fig. 5. Average values of fresh and dry biomass weight at the end of tests in lettuce (A, B) and spinach (C, D) supplemented with biochar and in control plants.

contents in leaves ([16], [22]). In lettuce, SPAD increased with biochar application (+8.2% ± 0.9%) compared to control, with variable effects observed for single treatments ranging from a slight decrease (−7.5 ± 0.4% for SW15a) to an increase (+16.4 ± 0.9% SS15a). Biochar at different doses produced comparable responses (+8.6% ± 1.1% for 2 g/L; +7.7% ± 1.2% for 15 g/L) compared to control. Considering the effect of biochar types on SPAD (Fig. 7), limited differences were observed in lettuce (SS: +9.8% ± 1.0%; SW: +6.6% ± 1.3%) and stronger effect on spinach (SW: +17.4 ± 3.23%; SS: (+10.8 ± 3.52%), particularly in the second part of the test. In the last measurement before the end of the test, SPAD values in spinach plant supplemented with biochar were 42.9 ± 0.8% higher than control.

Biochar had effects also on soil characteristics at the end of the tests for both lettuce and spinach (Fig. 8), improving EC and water retention, compared to control, without substantially affecting pH that was stable across treatments (6.5–6.7).

3.2. Geometric analysis of the plants

Leaf number and biomass alone did not adequately capture plant spatial development, due to variability in leaf size and architecture. Therefore, plant geometry was characterized by measuring plant height, canopy diameter, and (for spinach) leaf dimensions, and subsequently approximated using a cylindrical model. (section 2.2). The aim was to obtain a modelled representation to compare, from a geometric point of view, the growth of plants included in the tests. Data were subsequently grouped according to the relevant classification criteria (with biochar vs control, biochar types, activated or unaltered biochar), and the resulting comparisons are presented in Fig. 9. Considering only the comparison of plants supplemented with biochar (overall average) with control plants, the modelled volume showed an increase of 38.8 ± 1.2% for lettuce and of 195.7 ± 1.5% for spinach. However, comparing the effect of activated biochar, unaltered biochar and the control group, a more marked difference can be seen in lettuce, with an increase of +35.6 ± 0.4% and

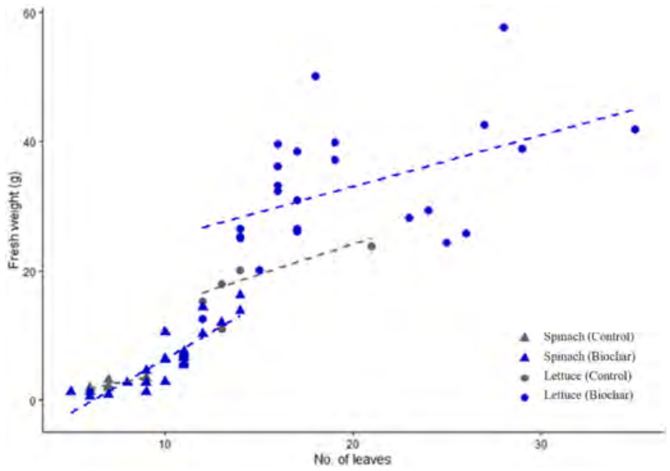

Fig. 6. Trend of biomass fresh weight vs number of leaves in lettuce (circle) and spinach (triangle) with biochar (blue) and in control plants (grey). Points represent single plants, dashed lines linear regression trends. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

+50.1 ± 0.9% compared to the control group for unaltered and activated biochar respectively. Comparing the effect of biochar type, the modelled volume of lettuce plants was similar (+56.4 ± 1.4% for SS, +51.5 ± 3.2% for SW), while spinach plants reacted differently (+300.5 ± 4.3% for SS, +104.4 ± 3.1% for SW). Finally, spinach showed larger increase in modelled volume compared to the control with little difference due to biochar activation (+185.6 ± 1.3% for unaltered biochar, +193.7 ± 2.2% for activated biochar), while for lettuce the increase was smaller (+35 ± 0.9% for unaltered biochar, +50.1 ± 1.8% for activated biochar).

3.3. *Proximal monitoring*

The NDVI trends (Fig. 10) highlighted a differential response to biochar application to lettuce and spinach. In lettuce, NDVI values showed a similar temporal pattern in both treatments, with biochar-amended plants slightly higher (+3.6 ± 0.01%) than control during early growth stages (weeks 1–3). Toward the end, NDVI decreased in both treatments, and the difference between control and biochar became negligible. Other studies reported a decline in NDVI values over time, indicating physiological stress in plants [38], [39]. In contrast, spinach displayed a more marked response to biochar, with consistently higher NDVI values across monitoring period. Biochar increased NDVI by +10.2 ± 0.02% compared to control, with the largest differences in the mid-to-late growth stages. These results suggest that biochar had a less noticeable effect on canopy vigor in lettuce, while it showed a superior response in photosynthetic activity and biomass development in spinach, probably reflecting species-specific responses to improved soil conditions. A comparative analysis was carried out between two complementary indicators of leaf photosynthetic status, NDVI and SPAD, to better interpret crop responses to biochar application (Fig. 10). In lettuce, the combined analysis revealed a divergence between the two measurements from week 4 onward: while SPAD values continued to increase in both treatments, reaching higher values in biochar-amended plants (+8.2% ± 0.9% compared to control), NDVI showed a clear decline over the same period (−45.5 ± 0.8% for control, −50.0 ± 1.8% for biochar from week 4 to 5). This contrasting behavior suggests that, at later growth stages, NDVI may be more strongly influenced by changes in canopy structure or senescence rather than by leaf chlorophyll content alone, as indicated by SPAD. The vertical growth of the lettuce plants and the orientation of the leaves with regard to the light source, may also have influenced the NDVI values in the final stages of the experiment by altering the plants' reflectance. Indeed, NDVI integrates variations in red and NIR reflectance at the canopy scale, thus, it can be

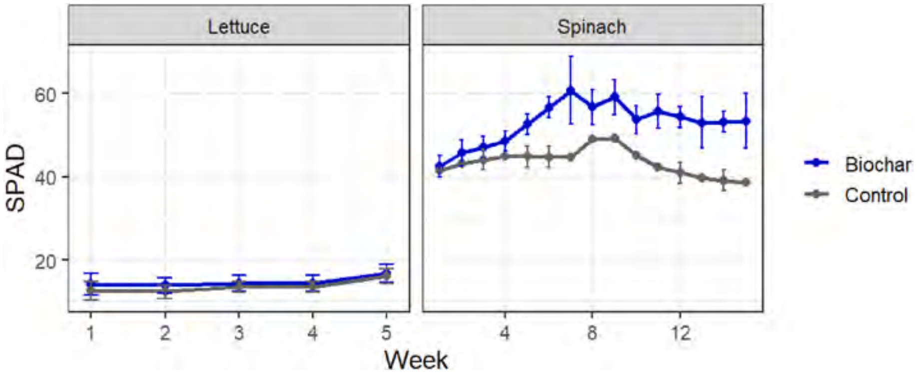

Fig. 7. Trend in SPAD chlorophyll content measurement for lettuce and spinach plants for Control (without biochar addition) in grey, and plants treated with biochar in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

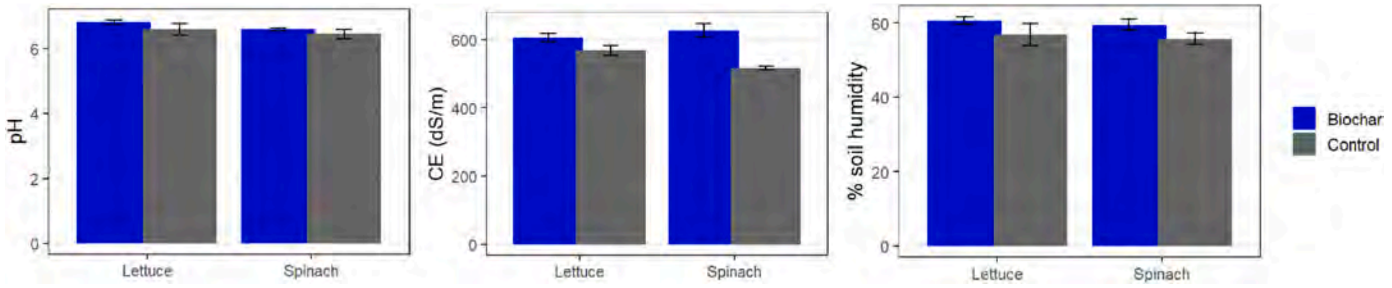

Fig. 8. Soil parameters: pH, Electrical conductivity (CE), soil humidity for lettuce and spinach plants, considering treatment with biochar (blue) and treatment without the addition of biochar: Control (grey). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

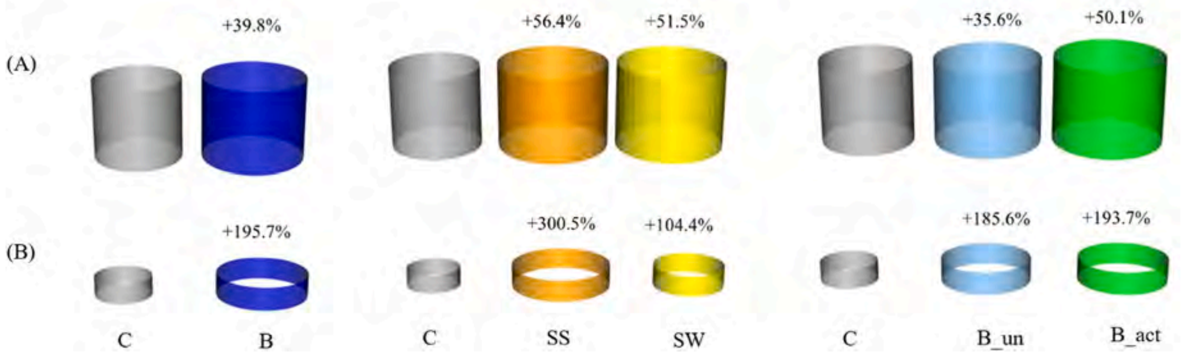

Fig. 9. Display of the percentage variation in the modelled volume of (A) lettuce and (B) spinach plants supplemented with biochar (B, overall average, blue), SS (orange), SW (yellow), unaltered biochar (B_un, overall average, light blue), activated biochar (B_act, overall average, green) compared to control plants (C, grey). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

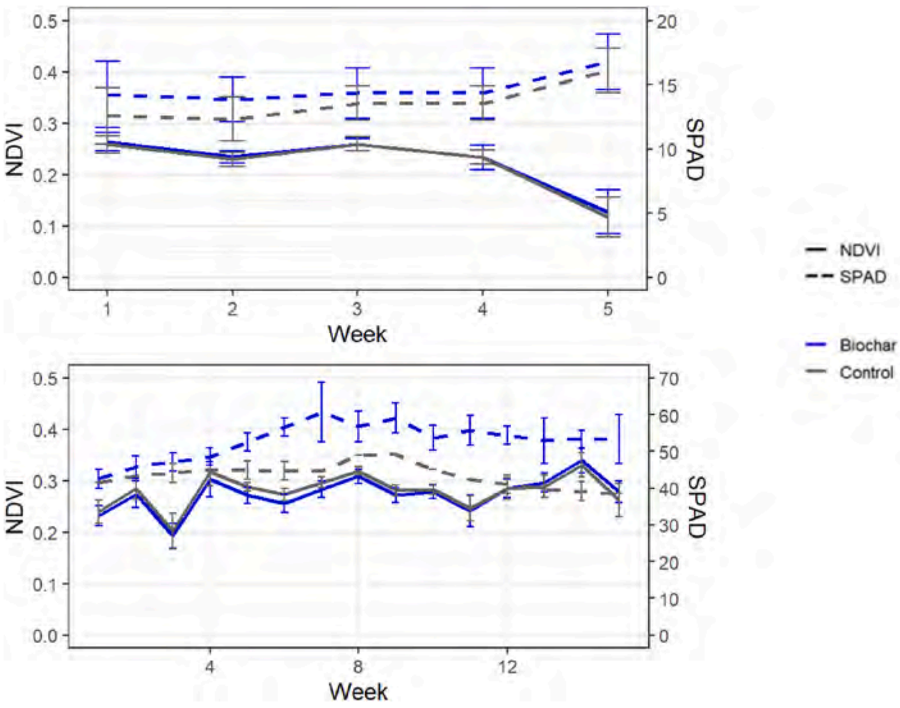

Fig. 10. Trends in SPAD chlorophyll content (dotted line) and NDVI (continuous line) values measured in lettuce and spinach plants supplemented with biochar (blue) compared to control (grey) plants. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

affected by leaf area and orientation and canopy structure [40], [41]. In spinach, SPAD measurements highlighted more evident differences between control and biochar treatments throughout the experiment, with biochar consistently associated with higher SPAD values (+14.1 ± 3.4%), in agreement with the NDVI trends but without emphasizing such a marked difference in treatment (biochar vs control).

3.4. Preliminary sensitivity assessment

Pearson correlation analysis on biochar-amended samples revealed only weak associations between biomass and the measured chemical parameters. Simple linear regressions indicated marginal positive relationships between biomass and EC and potassium content (all $p = 0.093$), each explaining approximately 6% of the observed variance ($R^2 = 0.06$), while no strong correlations were detected with other chemical variables. The response to biochar differed between species. In lettuce, the effect was marginal (one-way ANOVA: $F(2,27) = 3.12$, $p = 0.060$), and differences among biochar types, activation status, and dose were not significant when considering only biochar-amended treatments (all $p > 0.21$), although a weak biochar×dose interaction was observed ($F(1,16) = 3.90$, $p = 0.066$). In contrast, spinach showed a clear and significant response. Species-specific analysis ($F(2,26) = 9.97$, $p < 0.001$) and analysis restricted to biochar-amended pots both confirmed the effect, with biochar type remaining significant ($F(1,16) = 1\ 2.54$, $p = 0.0027$). Sewage sludge biochar consistently outperformed soft wood biochar, particularly under non-activated conditions ($p = 0.0033$). A combined ANOVA including both crops confirmed a strong species effect ($F(1,41) = 71.75$, $p < 0.001$) and a significant overall biochar effect ($F(2,41) = 7.91$, $p = 0.001$), with no significant plant species×biochar interaction ($p = 0.56$).

4. Conclusions

This study demonstrated that biochar application significantly enhanced growth, biomass accumulation, and chlorophyll content in both lettuce and spinach, with species-specific responses. Lettuce showed up to +56.9% increase in fresh biomass and +62.1% increase in dry biomass with activated biochar, while spinach exhibited the largest response with SS biochar addition, reaching +268% in fresh weight and +159% in dry weight compared to control. Root biomass increased notably in lettuce (+132.6%) but showed more variable trends in spinach. Soil electrical conductivity and moisture improved under biochar amendments, particularly with SS biochar, whereas pH remained largely stable. The relationship between NDVI and SPAD highlighted a stronger and more consistent positive response to biochar in spinach, while in lettuce the divergence between the two indices at later stages suggests structural canopy effects influencing NDVI. Plant volume modeling was included to overcome the limitations of single measurements, such as leaf number or biomass weight. Also, the value of combining visual and proximal sensing techniques is evident. Individual parameters, such as leaf number, biomass weight, or NDVI do not adequately capture plant development, whereas integrating multiple measurements (SPAD, NDVI dynamics, and geometric plant modeling) enabled a more accurate assessment of vegetative growth. Future research should explore the integration of geometric modeling and proximal sensing as a promising non-destructive approach for biomass estimation, enabling the scale-up to greenhouse and field conditions. In conclusion, biochar - particularly if nutrient-rich, as SS - enhances plant performance and soil quality, while integrating visual and proximal sensing with geometric modeling provides a robust framework for sustainable crop monitoring and precision agriculture.

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CRediT authorship contribution statement

I. Orlandella: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **K.N. Smith:** Formal analysis, Investigation, Visualization, Writing – review & editing. **E. Belcore:** Conceptualization, Data curation, Methodology, Validation, Writing – review & editing. **R. Ferrero:** Funding acquisition, Methodology, Supervision, Validation, Writing – review & editing. **M. Pugliese:** Funding acquisition, Methodology, Supervision, Validation, Writing – review & editing. **F. Berruti:** Conceptualization, Visualization, Writing – review & editing. **M. Piras:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing. **S. Fiore:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing.

Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biombioe.2026.109585.

Data availability

Data will be made available on request.

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