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Dual-scale experimental analysis: effect of biochar on lettuce growth and health

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ABSTRACT

This study aimed to evaluate the effects of biochar on lettuce growth under laboratory and pilot scale greenhouses conditions integrating visual and proximal sensing monitoring approaches. Monitoring happened (i) visually, recording at harvest the number of leaves, chlorophyll content, fresh and dry biomass; and (ii) via a remote sensing system collecting multispectral data, enabling calculation of the Normalized Difference Vegetation Index (NDVI). Three different tests were performed synchronously at both scales, and the plant pathogen *Fusarium oxysporum* was also added at pilot scale. Overall, biochar addition increased lettuce plants' growth and physiological performance. The results of visual monitoring were: in test 1 (Nov-Dec 2024) +39% number of leaves and +161% biomass fresh weight at laboratory scale, and +12% number of leaves and +32% biomass fresh weight at pilot scale; in test 2 (March-April 2025) +11% number of leaves and +52% biomass fresh weight at laboratory scale, and a decrease in both (−16% and −44%) at pilot scale; in test 3 (May-July 2025) +16% number of leaves and no variation in fresh biomass weight at laboratory scale, and +28% number of leaves and +47% fresh biomass weight at pilot scale. Chlorophyll content increased with biochar (up to +12% at laboratory scale and up to +68% at pilot scale). In some cases, plants supplemented with biochar were less affected by Fusarium wilt, although results varied according to the type of biochar and applied dosage. Overall, biochar contributed to improved plant performance in the majority of experimental scenarios, although its effects were context-dependent and influenced by scale and seasonal conditions. The integration of visual and proximal sensing supported scalable, non-destructive crop monitoring.

1. Introduction

Sustainable agriculture integrated with circular economy offers a transformative pathway toward resilient food systems and environmental stewardship [1]. Sustainable agriculture combines resource efficiency with circular economy principles and digital technologies, such as precision agriculture and real-time monitoring, enables smarter use of water [2]. Biofertilizers enhance soil fertility through microbial processes, reduce chemical inputs, improve nutrient availability, crop yields and plant resilience [3]. Biochar is a stable, carbon-rich material obtained through the thermal decomposition of biomass via pyrolysis, with physical and chemical properties shaped by feedstock and pyrolysis conditions [4–7]. Biochar is valued as soil amendment for its ability to reduce soil bulk density, enhance water-retention capacity, and improve nutrient availability and to suppress plant diseases [8]. In addition, biochar promotes beneficial microbial activity, thereby supporting soil fertility [9] and sustainable crop growth [10,11]. Biochar has been reported to improve water retention and rhizosphere quality while providing potential co-benefits for carbon sequestration [4,6,11]. The effects of biochar are highly dependent on the specific characteristics of the soil and climate in which it is applied; when introduced into alkaline soils, biochar can buffer pH, potentially reducing the need for conventional chemicals [12]. In recent literature, biochar has been broadly tested in the agronomic sector showing promising effects on plant

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development in different crops: coffee [13], rice [14], soybean [15], tomato [16–18], hot pepper [19], spinach [20]. Given that this study focuses on the cultivation of lettuce, a specific literature review was conducted to deepen the understanding of biochar effect of this specific crop. A Scopus search using the keywords "lettuce AND biochar," identified English-language articles published in the last 10 years. Records were screened for relevance based on title, abstract, and full text when necessary. Studies were included only if they evaluated biochar application in lettuce and provided a direct comparison with a non-amended control treatment; studies not meeting these criteria were excluded. This screening process resulted in 24 relevant studies, which are summarized in Table I of the Supplementary Materials.

The selected references reported biochar derived from various types of biomasses, applied at doses in the range 1% and 100% by volume (median value 21.8%-vv), 0.5% and 100% by weight (median value 17.2%-ww), and 1% and 40% tons per hectare (median value 12 t ha^{-1}). Heterogeneous biochar application metrics limit direct comparison among studies. Overall, compared to control samples, biochar showed a positive effects on lettuce growth and soil properties, with most studies relying on periodic visual and biometric assessments, revealing knowledge gaps and the need for further research. First, no studies compared trials considering the same crop supplemented with the same biochar dose and involving different seasonal or climatic conditions. Second, there is a lack of investigations carried out at different experimental scales within the same trial, considering the same biochar, dose, and crop.

In recent years, the use of proximal-sensing sensors for monitoring plant growth in greenhouse has increased. In controlled environment, like greenhouses, proximal sensing is especially useful for automatic continuous crop conditions' monitoring. Remote sensing in crop management enables site-specific monitoring of soil and plant conditions, supporting data-driven decisions that improve productivity while reducing environmental impacts [21].One of the most used indicators for evaluating photosynthetic performance and vegetative vigor is the normalized difference vegetation index (NDVI), related to the plants' ability to photosynthesize [22]. This approach improves measurement objectivity and consistency over time, facilitates real-time decision--making and improves the efficiency of crop management procedures. Despite extensive studies on biochar as a soil amendment, limited research has evaluated its effects simultaneously at laboratory and pilot greenhouse scales while integrating proximal sensing techniques. One investigated the effect of nitrogen excess on pepper plants under two temperature conditions, measuring plant health indicators as NDVI [23]. Another explored the effect of acidic or limed soil on wheat plants via hyperspectral images [24]. NDVI was used in this study as a rapid proximal indicator of canopy development and vegetative vigor. However, in small greenhouse-grown lettuce plants, NDVI values may be affected by incomplete canopy closure, substrate/and background exposure, leaf overlap, changing plant geometry, sensor spectral response, illumination variability, and radiometric-calibration uncertainty. Therefore, NDVI was interpreted as a complementary monitoring variable rather than as a direct and independent measure of biochar-induced physiological response [25]. The main goal of this study lies in the investigation of biochar effect on the growth of lettuce plants, considering four biochar materials having different characteristics at two doses in a multi-scale experimental design. Laboratory- and pilot-scale trials in greenhouse replicated three times along the year (winter, spring and summer) are compared integrating visual monitoring with proximal sensing monitoring. Also, trials including pathogens were performed at pilot scale, to assess the effect of biochar in plant response under biotic stress conditions. The novelty of this study lies in combining biochar treatment, greenhouse lettuce growth monitoring, and low-cost NDVI-based proximal imaging within the same experimental framework.

2. Materials and methods

2.1. Materials

This study involved four standard biochar produced at the UK Biochar Research Centre of the University of Edinburgh (www.biochar.ac.uk) from the pyrolysis at 550°C of soft wood pellets (SWP), wheat straw pellets (WSP), sewage sludge (SS) and rice husk (RH). The materials were identified as SWP550, WSP550, SS550, RH550 and due to the availability of the different biochars, not all materials were included in each experiment: SWP550 and SS550 were tested in test 1, SWP550 and RH550 in test 2, and SWP550, RH550, and WSP550 in test 3. The four biochars exhibited distinct physicochemical characteristics depending on their feedstock origin. SWP550 showed the highest carbon content (85.5%), with low ash content (1.25%) and a neutral pH, indicating a carbon-rich and stable material. In contrast, WSP550 was characterized by lower carbon content (68.3%), higher ash fraction (21.3%), alkaline pH (9.9), and greater potassium content (1.56%), suggesting a higher contribution of mineral components. SS550 differed markedly due to its high ash content (58.9%), lower carbon concentration (29.5%), and greater amounts of nitrogen (3.75%) and phosphorus (2.29%), together with high electrical conductivity (280.8 dS m^{-1}), reflecting the nutrient-rich nature of sewage-sludge-derived biochar. RH550 showed intermediate carbon content (48.7%), high ash fraction (47.9%), and alkaline pH (9.7). Full details about the biochars are in the Supplementary Materials (Tables II and III, Fig. I). Considering the differences in composition, their carbon, ash, and nutrient contents highlight the strong dependence on feedstock type and indicate distinct potential roles when applied as soil amendments [26].

2.2. Experimental setup and tests

Six experimental trials were conducted in greenhouse: three at laboratory scale and three at pilot scale (Fig. 1) in different periods of the year: test 1 in November-December 2024, test 2 in March-April 2025, test 3 in June-July 2025. Lettuce (*Lactuca sativa* L.) plantlets were produced at Centre Agroinnova of the University of Turin (Grugliasco), in greenhouse conditions at average daily temperature of 23-24°C, with daily watering. Relative humidity was maintained at 60–70% and a natural photoperiod was considered. The cultivar selected was 'lattuga gentile' (Four, Italy), which is susceptible to race 1 of *F. oxysporum* f. sp. lactucae [27]. Seeds were sown into 60-holes trays filled with steamed (90°C for 30 min) peat mix substrate (TS3, Turco Silvestro, Albenga, Italy; blond peat: black peat 15:85, pH 6.0, salinity 0.56 dS m^{-1}, porosity 90%, density 150 kg m^{-3}). Two-week-old plantlets were transplanted in plastic pots (1 L), on a substrate composed by peat. To ensure standardized and reproducible disease development, environmental conditions in the greenhouse during the trials were kept at an average day/night temperature of 24/18°C. Each pot was equipped with an self-activating drip irrigation grid (WD-01BDE Dual path, 30 PSI, 0.8 L/min for 1 min twice per day at laboratory-scale and 1 L/min for 1 min twice per day at pilot-scale). Control pots (without biochar) were included in the trials.

The three experimental periods were characterized by different seasonal environmental conditions. Meteorological data obtained from the University of Turin Observatory showed a progressive increase in temperature and solar radiation from the first to the third experiment. Mean air temperature ranged from 5.6 to 8.8°C during Test 1 (November–December), increased to 11.1–15.3°C during Test 2 (March–April), and reached 18.6–26.0°C during Test 3 (May–July). Similarly, monthly cumulative global solar radiation increased from 168 to 206 MJ m^{-2} during Test 1 to 369–494 MJ m^{-2} during Test 2 and 623–731 MJ m^{-2} during Test 3. These differences in environmental conditions can be considered when comparing plant responses among experimental periods, as seasonal factors may contribute to variations in growth and yield. However, the aim of this study was to assess biochar effects across

Fig. 1. Experimental setup: (A) Laboratory scale greenhouse at DIATI, Politecnico of Torino, Italy; (B) Pilot scale greenhouse at AGRINNOVA, University of Torino, Italy.

different greenhouse scales, while the reported meteorological data provide additional context for the interpretation of the experimental results. The pathogen *Fusarium oxysporum* f. sp. *lactucae* race 1 strain MYA3040 (ATCC), originally isolated from wilted lettuce plants in Italy (Agroinnova collection; [28]), established in Italian lettuce fields as described by Ref. [27], was used. For inoculum preparation, the strain was cultured on potato dextrose broth (PDB) on a rotary shaker at 25°C for 7 days. Liquid cultures were filtered through double-layered cheesecloth to remove mycelial fragments. The conidial concentration of the resulting microconidial suspension was precisely determined and confirmed using a hemocytometer. The tests at pilot scale also involved the artificial inoculation at transplanting. To guarantee uniform infection pressure and homogeneous distribution of the pathogen within the root zone, 150 mL/pot of the water suspension containing 10^5 conidia/mL of the pathogen was applied via soil drenching around the base of each plantlet immediately after transplanting Fare clic o toccare qui per immettere il testo. The pathogen assay was performed only at the pilot scale because adequate spatial separation between inoculated and non-inoculated treatments could not be ensured in the laboratory greenhouse.

Based on a previous study [29], biochar was applied at two doses (2 g/L and 15 g/L) and was mixed steam-treated peat based growing medium (TS3, Turco Silvestro, Albenga, Italy; blond peat: black peat 15:85, pH 6.0, salinity 0.56 dS m^{-1}, porosity 90%, density 150 kg m^{-3}). In each trial, a completely randomized design (CRD) was adopted; test 1 included three replicates per treatment, whereas test 2 and 3 included five replicates per treatment to increase the robustness of the experimental results. At least two biochar types, each applied at two doses, were tested using 1 L pots as experimental units. The samples were identified as SW2, SW15, RH2, RH15, WS2, WS15, C (control, i.e. no biochar) and C_in (control with pathogen inoculation).

2.3. *Visual and proximal sensing monitoring*

The trial tests integrated two monitoring activities: visual monitoring, based on data acquired at the end of the tests, and proximal sensing monitoring through low-cost systems installed in the greenhouses (Fig. 2). Proximal sensing monitoring was performed at lab-scale in tests 1 and 2 and at pilot-scale in test 2. The duration of the tests depended on the period of the year and it was equal to 4 weeks for test 1, 8 weeks for test 2, and 6 weeks for test 3.

Visual monitoring was carried out under blinded conditions, where the assessor was unaware of the specific pot treatments. Visual monitoring was based on parameters related to the growth of lettuce plants at the harvest, i.e. number of leaves, fresh and dry biomass weight of leaves, and on chlorophyll content recorded weekly through Soil Plant Analysis Development (SPAD) values 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) at laboratory-scale and at pilot-scale the leaf relative chlorophyll content was estimated in vivo with a chlorophyll meter SPAD-502 Plus (Konica Minolta, Inc., Osaka, Japan). SPAD values represent the mean of biological replicates. Due to the use of different chlorophyll meters, comparisons between scales were based on response trends rather than absolute SPAD values. The fresh weight of the leaves was measured using a Kern PLU 4200-2F (Kern & Sohn GmbH, Germany) technical balance**.** The dry weight of the leaves was measured after 24-h at 80°C in a TCN 30 PLUS (LSI Lastem, Italy) oven. Throughout the monitoring period, soil pH and electrical conductivity (EC) were logged weekly using a Brumong B0D8KJ9F27 probe (pH range: 3.5–9, accuracy: ±0.5; EC range: 0–3000 µS/cm, accuracy: ±100 µS/cm)**.** A stem section for each plant, at harvesting, was observed to confirm the presence of vascular browning, a classic symptom of Fusarium wilt of

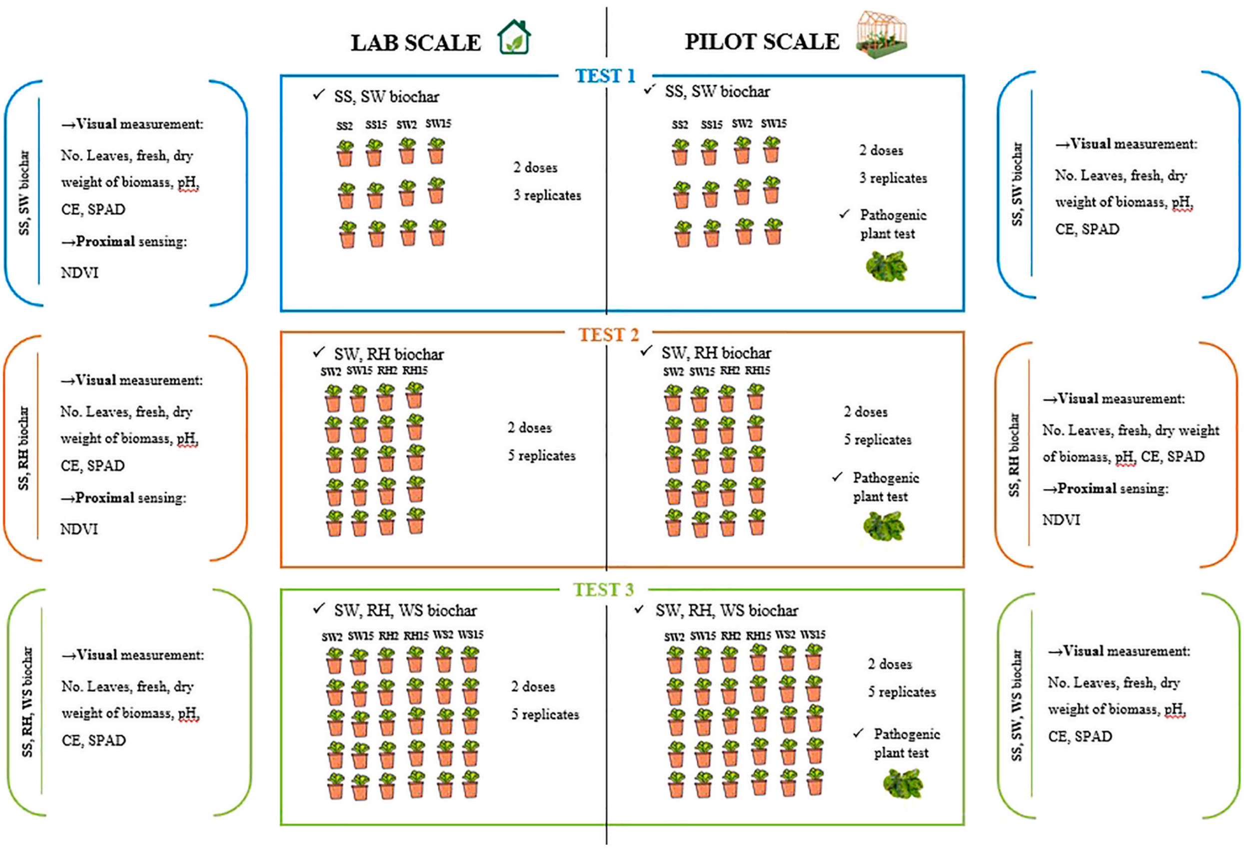

Fig. 2. Experimental design of the tests performed in greenhouse at laboratory and pilot scale.

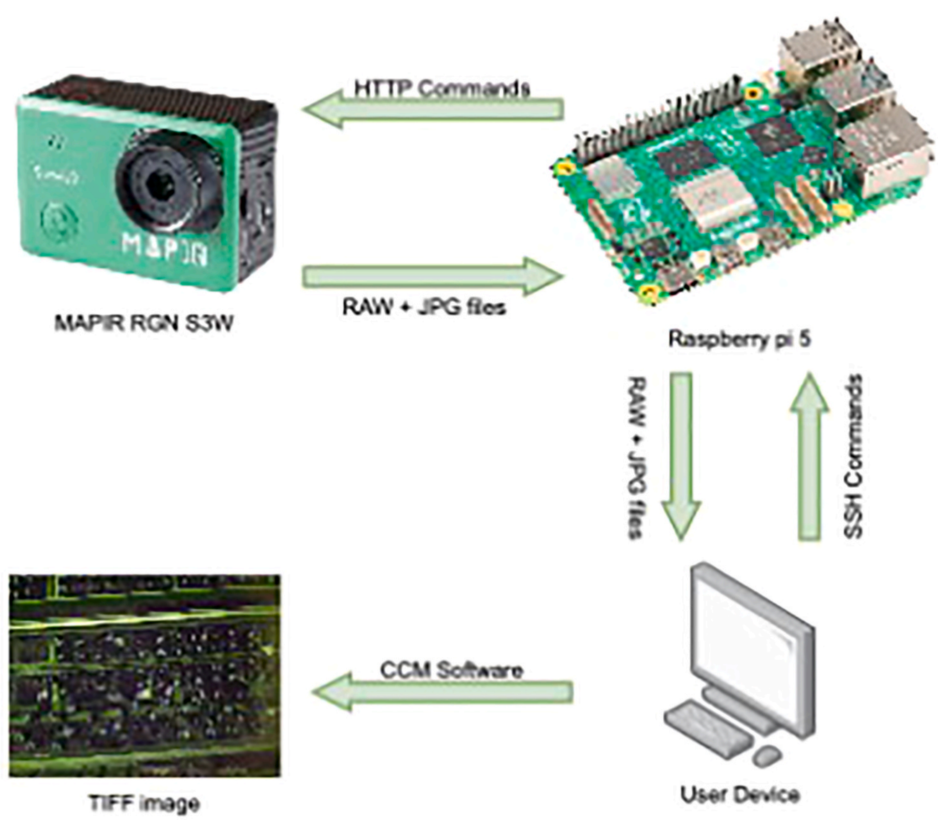

Fig. 3. Outline of the proximal sensing monitoring systems installed in the greenhouses.

lettuce. The disease incidence (DI) index was evaluated as the percentage of plants with symptoms. The disease severity (DS) index was calculated by dissecting each plant longitudinally using the following 5-class ordinal scale: 0 = no symptoms, healthy plant; 1 = initial leaf chlorosis and mild vascular browning; 2 = severe leaf chlorosis and initial symptoms of wilting of foliage; 3 = severe leaf chlorosis and severe wilting; 4 = plant dead [30]. Vascular stem sections (approximately 5 mm) from symptomatic plants were surface-sterilized (1% NaOCl for 1 min), rinsed in sterile water, and plated onto Komada's selective medium to confirm that the symptoms were caused by *F. oxysporum* f. sp. *lactucae*. Morphological identification of the emerging colonies confirmed the presence of the inoculated pathogen. Percentage increases were calculated for each biological replicate relative to the corresponding control replicate. Data are reported as mean ± standard deviation (SD) of the calculated percentage increases. Proximal sensing monitoring was performed using low-cost systems installed in the greenhouses and including a MAPIR RGN S3W camera, a Raspberry Pi 5 and a NT9666x Wi-Fi module (Fig. 3). Featuring an 87° field of view, the camera's 12 MP sensor captures data every hour across three spectral bands. The resulting images consist of spectral reflectance values in the red, green, and near-infrared regions (550, 660, and 850 nm) of the electromagnetic spectrum. All samples were captured in a single nadiral shot using 10 × 10 cm calibration pane. In laboratory case, the camera has been positioned at 85 cm above the pot surface, corresponding to an approximate working distance of 65 cm from the lettuce canopy. At this distance, the horizontal field of view covered 87° per image, corresponding to an imaged ground area of approximately 123x 92 cm. In pilot case, the camera was placed at 1.9 m from the lettuce, with a foot print of 3.58×2.66. In both cases, the camera optical axis has been oriented perpendicular to the pot surface to minimize geometric distortion and changes in viewing angle among treatments.

Image acquisition has been performed under natural greenhouse lighting conditions. To limit radiometric variability, the camera settings have been kept constant throughout the experiment whenever possible. In particular, exposure time, ISO/gain and white balance have been set to manual fixed values. Before image acquisition, radiometric correction were performed using a calibrated reflectance panel with known reflectance values. The reference-panel used for calibration are provided by MAPIR. Inside of the greenhouse have been installed several extra panels used for geometric scaling of the pictures.

A Python script controlled MAPIR cameras through Wi-Fi module, storing images on Raspberry Pi with remote SSH accessp and images were acquired at twice daily intervals throughout the monitoring period. Images were radiometrically calibrated, normalized for illumination, and selected daily to ensure consistent multispectral data across lab and pilot greenhouses.

The data acquired via proximal sensing were calibrated by correcting raw images to 16-bit TIFF format, and applying radiometric calibration using a known-spectral panel to obtain surface reflectance values, wich was then used to calculate the Normalized Difference Vegetation Index (NDVI) from the near-infrared and red bands according to eq. (1) [22]:

$$NDVI = \frac{NIR - Red}{NIR + Red} \tag{1}$$

where NIR and Red correspond to the near-infrared and red spectral bands extracted from the calibrated TIFF files, where the leaves of each plant were manually segmented to achieve consistency of the final results and associated to the applied biochar type and dose.

Image quality has been visually checked before analysis. Images have been excluded when they showed evident blur, overexposure, underexposure, strong shadows, condensation, dust on the lens, incorrect framing, missing calibration information, or incomplete visibility of the plant canopy.

Radiometric calibration has been performed using the proprietary MAPIR reflectance calibration target and the MAPIR Camera Control processing workflow. Before plant-image acquisition, the calibration panel has been collected under the same illumination conditions and acquisition geometry used for the lettuce images. The MAPIR calibration target consists of spectrally characterized reference surfaces, whose reflectance values are used by the software to relate image digital numbers to band-specific reflectance [31,32]. The calibration targhet is composed of four panels at 18%, 50%, 80% and 94% reflectance level respectively. the raw images has been ralibrated using aempirical linear method [33]. Specifically, the acquired images have been corrected of the vignetting and white balanced, then converted to calibrated 16-bit TIFF files. the calibration workflow has been applied using the MAPIR calibration open software [31,32]. The calibrated images were used to compute the NDVI. No lens-distortion correction has been applied beyond the standard corrections implemented in the MAPIR processing workflow; this point is therefore acknowledged as a limitation of the proximal-sensing protocol. This limitation is particularly relevant for low-cost or consumer-grade imaging sensors, for which standardized radiometric characterization, including dark-current, flat-field/vignetting, gain and spectral-response assessment, is recommended when quantitative reflectance retrieval is required [34].

2.4. Sensitivity analysis

The experimental dataset was statistically analyzed to evaluate correlations among treatments and experimental scales. First, all treatment conditions were compared using a one-way ANOVA (Analysis of Variance), then the two experimental factors “biochar treatment” and “experimental scale”, were analyzed using a two-way ANOVA with replication, as previously reported [35,36]. Microsoft Excel was used for statistical analysis, and results were considered significant at a 95% confidence level ($p < 0.05$). Additionally, Pearson's correlation analysis was performed as an exploratory analysis to evaluate statistical associations among treatment responses for the selected variables, and correlation coefficients were used to identify similarities or differences in response patterns among treatments.

Segmentation has been manually done by expert operators, under a blind condition. It is possible to consider a potential operator-dependent uncertainty. The uncertainty was mainly associated with boundary pixels, while NDVI values were calculated over the whole segmented leaf area, reducing the influence of a small number of potentially misclassified pixels. Since the same segmentation procedure was applied consistently across all treatments, any residual segmentation error was expected to be non-differential rather than treatment-specific.; therefore, NDVI results are interpreted as exploratory trends.

3. Results and discussion

3.1. Visual monitoring

3.1.1. Test 1

The results of Test 1 (November-December 2024) at laboratory scale (Fig. 4) showed a positive effect of biochar addition on the growth of lettuce plants (+36 ± 0.4% number of leaves, +46 ± 0.5% fresh biomass, +87 ± 0.5% dry biomass) compared to control samples and confirming the findings of previous studies [37,38]. Higher dose (15 g/L) had better results than the lower (2 g/L); the number of leaves, the fresh weight of the biomass, and the dry weight increased (+36 ± 0.4%, 46 ± 0.6%, and 53 ± 0.6%, respectively), compared to the control plants. Considering the effect of biochar type, the best performance was achieved in plants supplemented with SS (+39 ± 0.1% number of leaves, +87 ± 0.2% fresh biomass, +96 ± 0.2% dry biomass at 15 g/L, and +53 ± 0.1% number of leaves, +32 ± 0.1% fresh biomass, +37 ± 0.1% dry biomass at 2 g/L). On another hand, SW exhibited limited effect (+17 ± 0.1% number of leaves, +19 ± 0.1% fresh biomass, +26 ± 0.1% dry biomass at 15 g/L, and +36 ± 0.3% number of leaves, +46 ± 0.3% fresh biomass, +56 ± 0.3% dry biomass at 2 g/L) compared to

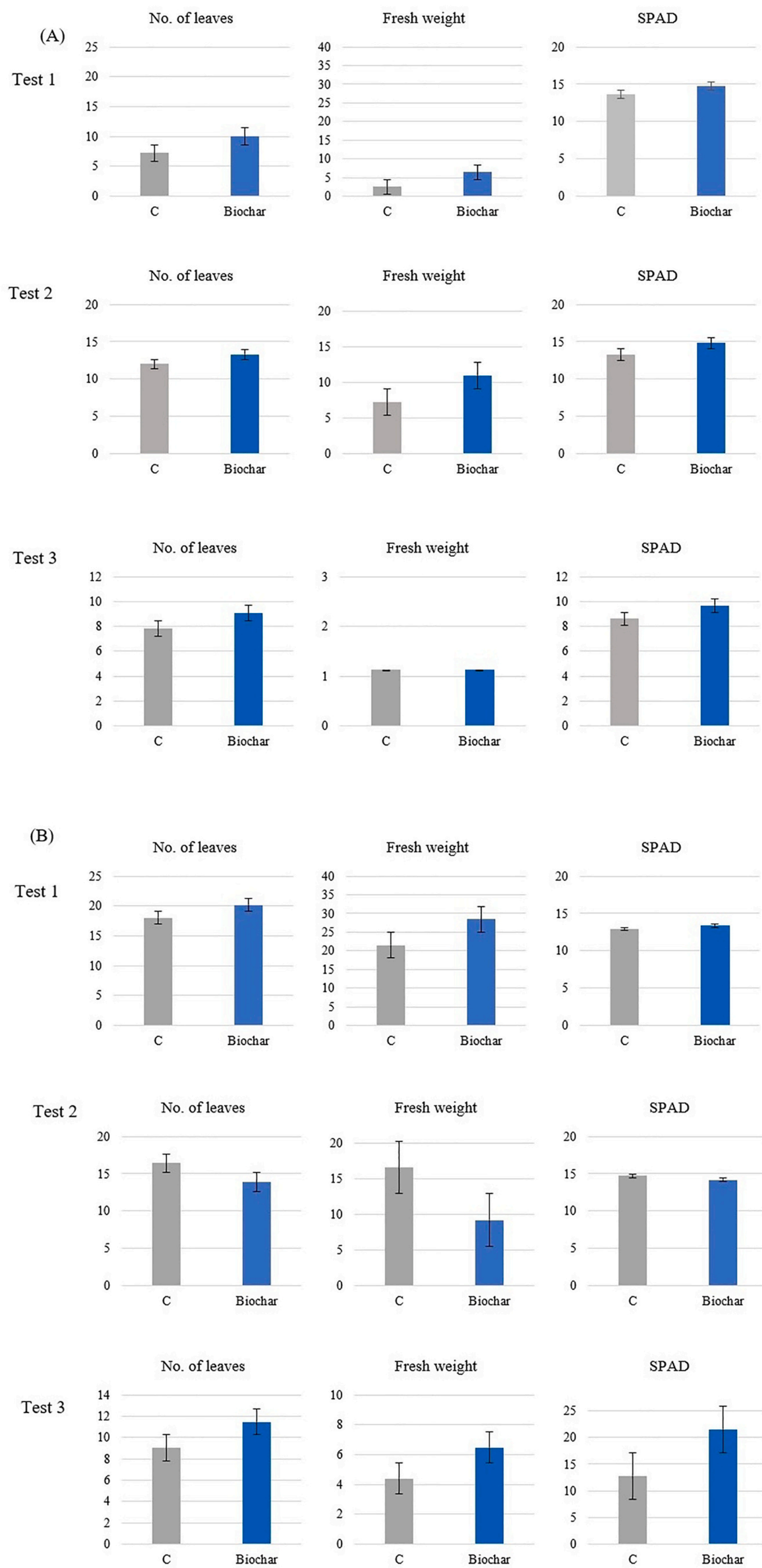

Fig. 4. Results of Test 1 (November – December 2024), Test 2 (March – April 2025), and Test 3 (May–July 2025) at laboratory (A) and pilot scale (B): trend of numbers of leaves, fresh weight, and chlorophyll content of leaves. Comparison of control (C) and biochar treatment (Biochar).

control. Measurement of chlorophyll showed an increase in plants with very marked biochar: on average 14.7 ± 0.9 (+8 ± 0.9% respect control), there were no particular differences between different dose and different type of biochar. Concerning the two soil parameters measured: pH remained similar to control pots (+1 ± 0.17%), while EC on average reached higher values in plants with biochar (+4 ± 32%) compared to control. At the pilot scale, biochar showed a positive effect on plant growth (+12 ± 0.3% number of leaves, +32 ± 0.3% fresh biomass, +26 ± 0.3% dry biomass) and lower dose performed better that the higher one (+19 ± 0.1% number of leaves, +35 ± 0.3% fresh biomass, +25 ± 0.3% dry biomass). Analyzing the different types of biochar, the best result was obtained for SW at higher (+26 ± 0.1% number of leaves, +74 ± 0.4% fresh biomass, +73 ± 0.5%) and lower dose (+20 ± 0.1% number of leaves, +49 ± 0.6% fresh biomass, +43 ± 0.5%). Chlorophyll content showed a slight increase (+3 ± 0.1%) in plants supplemented with biochar. Overall, regarding soil parameters, pH did not show significant changes with biochar, recording an average value of 6.83 ± 0.01 (−1.8 ± 0.1% compared to control), while EC exhibited an increment (+7 ± 0.1%). Full details about trend of numbers of leaves, fresh weight, and chlorophyll content of leaves, dry weight of biomass, pH and EC of the soil are in the Supplementary Materials (Fig. II, Fig. III).

Test 1 at pilot scale evaluated biochar effects in presence of pathogens (Fig. 5). Both leaf number and fresh leaf weight decreased markedly under pathogen stress (−37.1 ± 2.6% and −73.6 ± 0.5% respectively). Considering the different types of biochar, RH showed the lesser decrease of number of leaves compared to the control (−37.2 ± 2.6%); with regard to fresh biomass, no substantial differences between the different types of biochar were recorded. The soil parameters showed moderate variations among treatments: pH decreased slightly in all inoculated and biochar-amended pots compared with the control (−5.5 ± 0.1%). In contrast, EC increased markedly in the amended treatments (+58.1 ± 8.9%) compared with the control. In the various treatments, the best results were achieved by RH and SW with +71.6 ± 8.1% and +71.9 ± 4.1% respectively. A disease reduction was observed in plants supplemented with soft wood biochar at dose of 2 g/pot (SW2) compared to the untreated control. Disease incidence was reduced to −66.7 ± 7.1%, while disease severity to −42.9 ± 47.1% by the application of SW2 compared to the untreated control. Sewage sludge biochar at dose of 2 g/pot (SS2) did not reduced Fusarium wilt disease on lettuce.

3.1.2. Test 2

During Test 2 (March–April 2025) at laboratory scale, one SW2 replicate and two SW15 replicates were excluded from further analyses due to premature plant mortality before the end of the experimental period. Overall, the number of leaves increased (+10.7 ± 1.6%), and fresh weight doubled (+51.9 ± 2.6%) in the pots where biochar has been added (Fig. 4), consistently with literature [39,40]. This result was also confirmed by the dry weight of leaves, where plants treated with biochar showed an increase of +51.5 ± 0.3%. In this case, no significant differences were found between the two different biochar doses applied: +12.5 ± 1.7% (2 g/L) and +8.9 ± 1.5% (15 g/L) for numbers of leaves, and +52.1 ± 2.3% (2 g/L) and +50.9 ± 3.7% (15 g/L) for dry weight. The higher dose showed better results in fresh weight, with an increase of +71.3 ± 3.1% compared to the control. Considering the different treatments, SW revealed better results for fresh weight both in lower and higher dose: +63.7 ± 2.1% and +122.1 ± 3.4% respectively. This result is also confirmed by the trend in dry weight of leaves with an increase from +58.6 to +74.3%. Chlorophyll content was higher in plants treated with biochar (+11.7 ± 1.5%), with greater increase for 15 g/L (+13.8 ± 1.7%) than 2 g/L (+9.6 ± 1.3%). Considering the different treatments, RH performed better (+17.4 ± 1.7%) than SW (+6.1 ± 1.5%). The soil parameters showed consistent trends in terms of biochar dosage and treatment, and in general pH and EC values did not show significant differences compared to the control. At pilot scale, test 2 generally showed a decrease in growth parameters with biochar compared to control, which was also associated with an overall poor plant health status observed throughout the experiment. In detail, the number of leaves decreased by −15.6 ± 0.3%, and the fresh and dry biomass declined by −13.9 ± 5.2% and −44.4 ± 5.2% respectively. Chlorophyll content displayed a slight decrease in presence of biochar (−3.6 ± 0.1%). The soil parameters (pH and EC) showed no significant differences in the biochar treatments compared to the control. Full details about the dry weight of biomass, pH and EC of the soil are in the Supplementary Materials (Fig. IV, Fig. V).

In the pathogen-inoculated trial of test 2 at pilot-scale, clear differences were observed among treatments in terms of lettuce growth and physiological parameters (Fig. 5). Given that test 1's low dose of biochar was insufficient to successfully counteract the negative impact of pathogens on plants (Fig. 5), we decided to continue using both doses of biochar to further investigate how this might affect tests 2 and 3, following evidence from other crop systems [36,41]. Overall, the presence of the pathogen (C_in) markedly reduced plant performance compared to control (C), confirming its inhibitory effect on growth (−28.1 ± 4.4% number of leaves). However, the application of biochar seemed to mitigate these negative impacts to varying extents. In general, except for the higher dose of SW biochar (SW15), the decrease was smaller (−5.7 ± 1.5%) than that observed for the plants without biochar and with pathogen inoculation (C_in) (−28.1 ± 4.4%). A significant increase was observed in both fresh biomass and chlorophyll contents in plants with biochar: +145.5 ± 2.7% and +5.2 ± 0.5% respectively. Considering the different types of biochar, SW15 displayed the smallest increase (+38.6 ± 3.6%), On average, the other biochar treatments (SW2, RH2, RH15) showed an increase in biomass weight from +148.9 3.6% to +211.3 ± 1.8% compared to control, and a clear increase even compared to treatment with fungicide without biochar (Fung) (+145.5 ± 2.7%). In general, soil parameters showed a slight decrease in pH (−4.3% ± 2.6) and an increase in electrical conductivity (+38.3 ± 0.6%). A disease reduction was observed on plants supplemented with rice husk biochar at dose of 2 g/pot (RH2) and 15 g/pot (RH15). Disease incidence was reduced to −60.0 ± 49.0% and −60.0 ± 49.0%, while disease severity to −77.8 ± 12.2% and −77.8 ± 12.2% by, respectively, the application of RH2 and RH15 compared to the untreated control. Soft wood biochar at dose of 2 g/pot (SW2) and of 15 g/pot (SW15) was not able to confirm in test 2 the Fusarium wilt disease reduction observed in test 1.

3.1.3. Test 3

In test 3 (May-July 2025) at laboratory-scale, one plant in the SW15 replicate was not included in the data analysis for technical problems. Overall, the number of leaves (Fig. 4) showed an increase with biochar (+16.1 ± 1.2%), and the fresh weight improvement was insignificant compared to the control (+0.4 ± 0.5%). However, biochar responses were treatment- and dose-dependent, as not all treatments resulted in improved plant performance. When analyzed by dose, a slight increase in fresh weight was observed at 2 g/L (+3.7 ± 0.4%), whereas a decrease occurred at 15 g/L (−2.9 ± 0.6%). The only treatment that increased compared to the control was RH at 15 g/L (+34.1 ± 0.9%). These results were confirmed by dry weight values, with the only increase being +25.4 ± 0.1% for RH15. Chlorophyll content showed an overall increase in plants with biochar (12.4%), with better results (13.9%) for 2 g/L compared to control. Looking at the treatments in detail, the best results were related to RH with an increase in both doses of +17.5 ± 4.5%. The soil parameters did not show significant differences within biochar treatments, as pH was −0.5 ± 0.3% and EC was +1.2 ± 10.8% compared to control. Test 3 at pilot scale showed positive effect of biochar (+27.5 ± 0.1% number of leaves, +46.9 ± 0.4% fresh biomass, +68.2 ± 0.1% chlorophyll) compared to control samples. Conversely, dry biomass showed an overall decrease of −28.9 ± 0.2%. This contrasting response between fresh and dry biomass suggests that biochar effects may vary depending on the growth parameter considered, although the underlying mechanisms cannot be determined from the available measurements. In general, no significant difference was

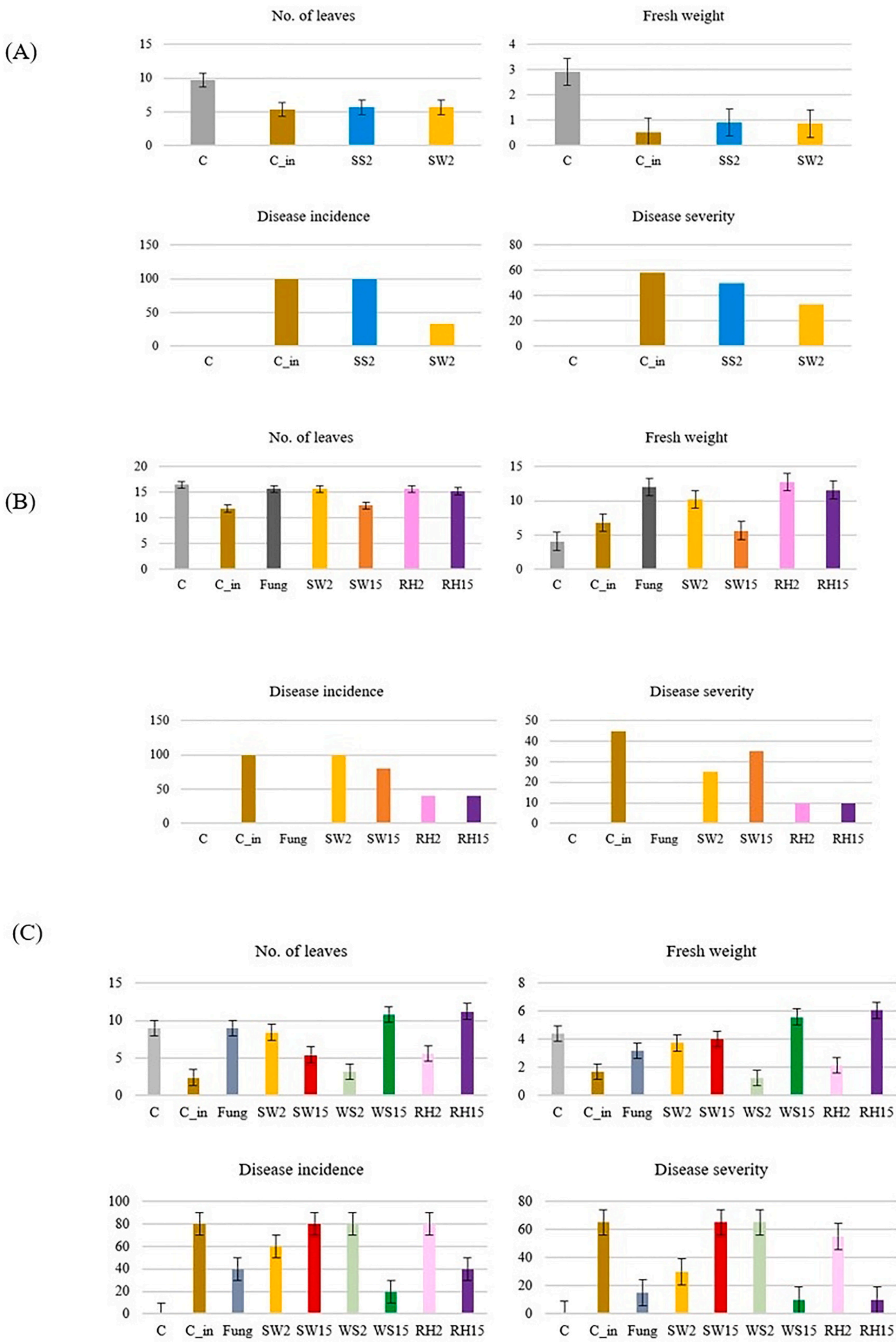

Fig. 5. Results of pathogen line of (A) Test 1 (November – December 2024), (B) Test 2 (March – April 2025), and (C) Test 3 (May – July 2025) at pilot-scale greenhouse: trend of number of leaves, fresh weight of leaves, disease incidence, disease severity. Comparison of healthy control (C), inoculated control (C_in), fungicide treatment control (Fung), soft wood biochar at dose of 2 g/pot (SW2), soft wood biochar at dose of 15 g/pot (SW15), wheat straw biochar at dose of 2 g/pot (WS2), wheat straw biochar at dose of 15 g/pot (WS15), rice husk biochar at dose of 2 g/pot (RH2), rice husk biochar at dose of 15 g/pot (RH15).

found between the two biochar doses, except for the measurement of fresh biomass, where 2 g/L yielded twice the result of 15 g/L compared to control: +61.5 ± 0.3% and +32.4.2 ± 1.2% respectively. Considering the different treatments, there were no particular differences in the number of leaves; however, in terms of fresh biomass, RH showed increases of +82% and +97% for the two doses compared to the control. Similarly, in chlorophyll content, the best increase was recorded for WS at both doses, with +82% and +87% compared to control. Soil parameters showed a constant pH value (+0.72 ± 0.2%); the EC value was significantly lower in plants treated with biochar (−61.7 ± 3.34%) compared to control. Full details about the dry weight of biomass, pH and EC of the soil are in the Supplementary Materials (Fig. VI, Fig. VII).

Overall, in test 3 with pathogens inoculation (Fig. 5), the results showed a decrease in the pots treated with biochar in all plant development parameters: number of leaves (−17.4 ± 0.3%), fresh weight of biomass (−13.7 ± 0.4%), and chlorophyll content (−2.3 ± 0.3%). However, this decrease compared to control is less marked than in the samples inoculated without the addition of biochar (C_in), which showed −73.3 ± 4.8% numbers of leaves, −61.5 ± 2.3% fresh weight and −43.1 ± 9.0% chlorophyll content. The less pronounced reduction observed in biochar-treated plants suggests a potential positive effect of biochar in mitigating the impact of Fusarium infection. Considering the different types of biochar, the only treatments that showed an increase of chlorophyll content values compared to control were WS15 (+33.7 ± 8.4%) and RH15 (+36.3 ± 9.9%). The same increase, compared to control, was shown by the sample without biochar treated with fungicide (Fung) (+36.0 ± 6.7%). Concerning the numbers of leaves and fresh biomass, WS15 (+20.0 ± 4.7% for number of leaves, and +27.0 ± 2.0% for fresh weight) and RH15 (+24.4 ± 4.3% for number of leaves, and +37.4 ± 2.1% for fresh weight) treatments were the only ones to show an increase compared to control also respect to samples treated with fungicide (+1.1 ± 4.4% number of leaves, and −27.9 ± 2.2% fresh weight). A disease reduction was observed on plants supplemented with rice husk biochar at dose of 15 g/pot (RH15), wheat straw biochar at dose of 15 g/pot (WS15) and soft wood biochar at dose of 2 g/pot (SW2). Disease incidence was reduced to −50.0 ± 40.0%, −75.0 ± 40.0%, −25.0 ± 40.0%, while disease severity to −85.0 ± 49.0%, −85.0 ± 40.0% and −54.0 ± 43.6% by, respectively, the application of RH15, WS15 and SW2 compared to the untreated control. Soft wood biochar at dose of 2 g/pot (SW2) was able to confirm in test 3 the Fusarium wilt disease reduction observed in test 1, while rice husk biochar at dose of 15 g/pot (RH15) was able to confirm in test 3 the results observed in test 2.

3.2. Proximal sensing monitoring

Fig. 6 presents an example of a calibrated image from the pilot-scale tests, illustrating the outcome after the calibration process and providing the basis for the subsequent calculation and analysis of the NDVI.

The temporal evolution of NDVI values is in Fig. 7, as a function of biochar type and compared with the control group. Across all cases, NDVI values in test 1 at laboratory scale (Fig. VIII, Supplementary material) exhibited no significant differences in treatment with biochar (+1.8 ± 4.3%). This finding suggests that, under the conditions of this study, the spectral response of the plants was not substantially influenced by the presence or type of biochar. Test 2 at pilot-scale showed a slight decrease in NDVI values of plants treated with biochar respect to control (−2.6 ± 0.2%). At laboratory scale, test 2 demonstrated a slight increase of NDVI with biochar (+5.2 ± 0.1.%). Finally, the Test 3 at laboratory-scale showed no significant differences among plants, with an increase of +1.3 ± 0.1% in plants with biochar. In week 5 a decline in NDVI values was observed, accompanied by an overall increase in standard deviation. Similar decreases at later growth stages have been reported in literature and may reflect physiological changes in plant canopies [42]. However, the simultaneous increase in variability could also be attributed to factors affecting image quality, such as lens contamination, progressive dust accumulation, or suboptimal radiometric calibration. While the latter cannot be confirmed with certainty, it remains a plausible contributing factor. In Fig. 7, the NDVI trends for test 2 at laboratory-scale experiment are presented. NDVI values were slightly lower than those typically reported in the literature [25,43,44], ranging between approximately 0.1 and 0.5. Over the 11-week observation period, a marginally better performance was observed in the biochar-treated samples. However, the high standard deviation associated with these measurements limits the interpretability and reliability

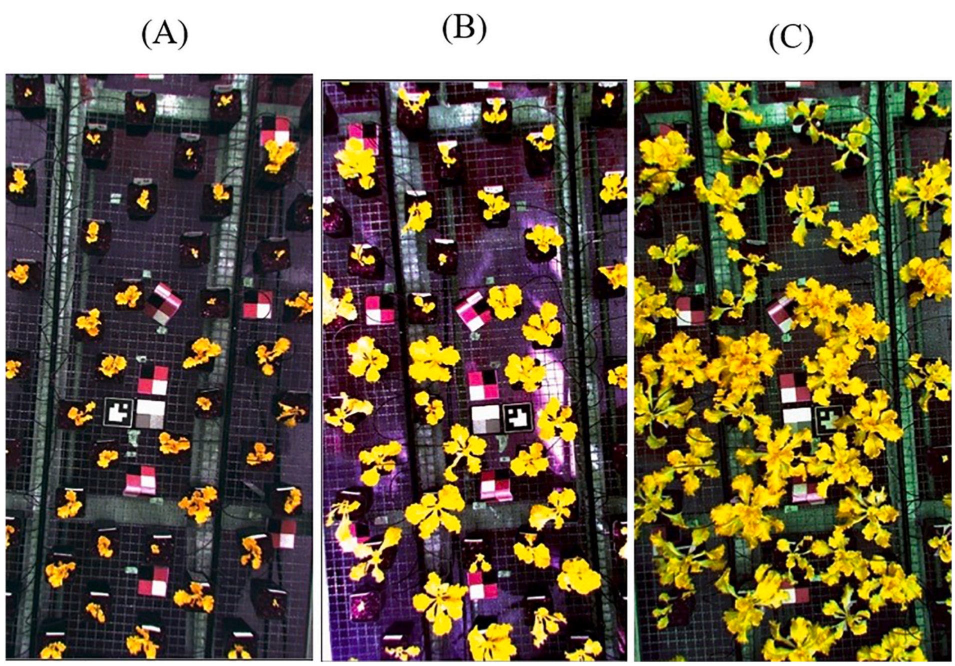

Fig. 6. Example of calibrated images acquired during a trial at pilot-scale greenhouse after (A) 20 days from transplant, (B) 35 days and (C) 50 days; RGN false color visualization.

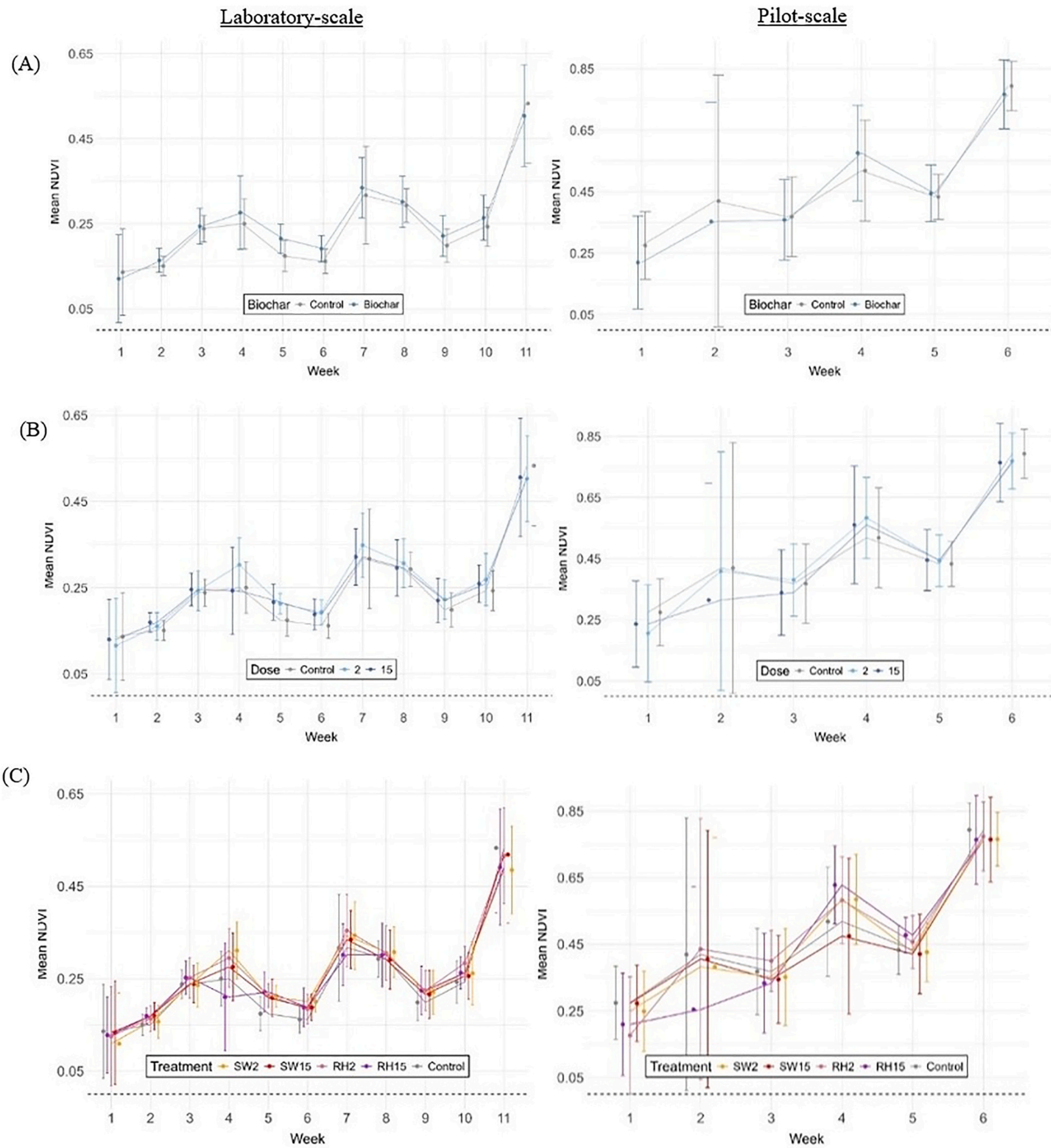

Fig. 7. NDVI value patterns for Test 2 at laboratory and pilot-scale greenhouse: (A) biochar-treated plants and control plants; (B) biochar-treated plants and control plants at two distinct dosages (2 g/L and 15 g/L); (C) biochar-treated plants and control plants at various dosages (SW2: soft wood biochar 2 g/L, SW15: soft wood biochar 15 g/L, RH2: rice husk biochar 2 g/L, RH15: rice husk biochar 15 g/L, C: control plants).

of the results, again indicating a lack of statistically significant differences in vegetation response due to the presence or absence of biochar treatments over time. Despite the acquisitions being conducted at different times, both experiments show a decline in NDVI values around the fifth week, suggesting a possible reduction in photosynthetic activity. A joint examination of the two datasets also reveals a clear increase in photosynthetic performance, as indicated by higher NDVI values, between the tenth and eleventh weeks, although differences among treatment types or biochar application rates remain negligible. In test 2 at pilot-scale (Fig. 7), the NDVI values appear to be more consistent with those typically reported in the literature, starting at approximately 0.25 during the early growth stages and reaching nearly 0.85 at later stages. Overall, the standard deviation was generally larger at laboratory-scale, especially in week two, likely due to lower spatial resolution and smaller leaves causing segmentation errors. Variability decrease progressively over time with canopy growth. Similarly NDVI trends at pilot scale mirrored lab results, with a drop around week five, and no significant differences among biochar treatments.

The interpretation of weekly NDVI trends should therefore be considered with caution, because changes in NDVI may not reflect only variations in plant physiological status but also changes in canopy structure, leaf-area development, plant geometry and the progressive reduction of visible substrate background as lettuce plants grew. In small potted plants, incomplete canopy closure can increase the contribution of soil or substrate pixels to the spectral signal, whereas later growth stages may produce higher NDVI values simply because of greater canopy cover and leaf overlap. For this reason, NDVI was interpreted here as a complementary indicator of canopy development rather than as a direct proxy of biochar-induced physiological response. This interpretation is consistent with previous work showing that NDVI-based monitoring of crop development is closely related to leaf area index and canopy structure, and therefore benefits from validation against independent structural or biomass-related measurements [39].This outcome supports previous findings in the literature, which describe biochar primarily as a soil conditioner capable of improving soil quality, water retention, and, consequently, promoting leaf growth and cell turgor [3,8,45,46]. However, such effects are mainly detectable in the mid-infrared spectral region, where water absorption features are most prominent. Since the sensor used in this study does not cover those wavelengths, it was not possible to assess whether these physiological responses could be effectively monitored through proximal sensing in this spectral range.

3.3. Sensitivity analysis

Pearson's correlation analysis was first conducted on the complete dataset of numbers of leaves, SPAD and pH, including all three laboratory-scale tests and subsequently extended to all biochar-based treatments and the control (C, RH2, RH15, SW2, SW15, WS2, WS15, SS2, SS15). For the number of leaves, several moderate to strong positive correlations emerged among treatments derived from the same feedstock, such as RH2–RH15 ($r = 0.68$) and SW2–SW15 ($r = 0.69$), suggesting similar growth responses within the same biochar typology. Conversely, weaker or negative correlations (e.g., WS2–RH2, $r = -0.07$; SS15–C, $r = -0.37$) indicate divergent morphological outcomes. SPAD values showed analogous patterns, with strong correlations among treatments sharing compositional properties, including RH2–RH15 ($r = 0.74$) and SW2–SW12 ($r = 0.78$), reflecting consistent effects on chlorophyll content. In contrast, negative correlations in SW15–WS2 ($r = -0.28$) or weaker associations involving SS15 ($r < 0.35$ with several treatments) suggest more heterogeneous physiological responses. pH values exhibited a higher variability, with both positive relationships (e.g., WS2–SS15, $r = 0.62$) and negative ones (e.g., WS2–SW15, $r = -0.28$), highlighting a stronger sensitivity of soil acidity to specific biochar properties rather than to application rate alone. Overall, the correlation patterns show that the number of leaves and chlorophyll content exhibit coherent responses across tests for each specific biochar, pH remains more treatment-sensitive and dependent on distinct characteristics of the applied biochar. To statistically evaluate the effect of the different treatments on plant response at laboratory scale, a one-way ANOVA was first performed on the experimental dataset to identify significant differences among treatments for the selected response variables. Subsequently, a two-way ANOVA was carried out to assess not only the individual effects of treatment type and test factor, but also their potential interaction. ANOVA was frequently used in previous studies to statistically evaluate group mean differences as well as variability within and between those groups [36]. For statistical comparisons, only datasets with common treatments across experiments were considered, for this reason the statistical analysis was conducted on the subset of laboratory tests characterized by comparable biochar application conditions, corresponding to Test 2 and Test 3, and including the following treatment groups: C, SW2, SW15, RH2, and RH15. The response variables considered were the number of leaves, pH, and SPAD values. NDVI analyses were performed separately using Test 2 data only, as this was the only trial including measurements at both laboratory and pilot scales. Results from the two-way ANOVA enabled the identification of statistically significant effects and interaction patterns between treatments, providing insights into differential plant responses under the tested biochar.

ANOVA results showed significant treatment effects: leaf number differences were confirmed by F-value > F crit and low p-values, while SPAD and pH also reflected treatments and block effects. Interaction terms were not significant (F-value ≤ F crit), indicating no setuptreatment-interaction. The statistical analysis demonstrated that the number of leaves was significantly affected by treatment when evaluated through one-way ANOVA (p value $= 1.11{*}10^{-3} < 0.05$), indicating clear differences among treatments. The two-way ANOVA confirmed a significant effect both for the factor “treatment” (p value $= 1.58{*}10^{-4} < 0.05$) and for the factor “test condition” (p value $= 2.2249{*}10^{-10} < 0.05$), while no significant interaction was detected between the two factors (p value $= 1.71{*}10^{-1} > 0.05$). This suggests that treatments influence leaf development independently of test conditions. For SPAD values, one-way ANOVA showed again a significant treatment effect (p value $= 7.086{*}10^{-6} < 0.05$). Similarly, the two-way ANOVA indicated that both the treatment factor (p value $= 2.81{*}10^{-8} < 0.05$) and test condition factor (p value $= 6.08{*}10^{-7} < 0.05$) were statistically significant when accounting for variability between the two laboratory test sets, whereas the interaction between the different treatments over time was not significant (p-value $= 7.83{*}10^{-1} > 0.05$). Regarding pH, the one-way ANOVA detected a significant effect of treatment (p value $= 1.23{*}10^{-2} < 0.05$), while the two-way ANOVA revealed a strong influence of the test factor (p value $= 6.34{*}10^{-7} < 0.05$) and differences among treatments (p value $= 3.99{*}10^{-2} < 0.05$) and no significant interaction (p value $= 3.97{*}10^{-1} > 0.05$). Although all parameters exhibited statistically detectable effects, morphological responses (leaf number) showed the clearest treatment-driven differences, whereas physiological indicators (SPAD and pH) were influenced simultaneously by treatment and test conditions. Statistical analyses were carried out on the complete dataset from Tests 1, 2, and 3, encompassing both experimental scales, using one-way ANOVA to assess treatments effects on lettuce fresh and dry biomass, andtwo-way ANOVA to evaluate the influence of scale and the treatment interaction. For fresh weight of leaves, the one-way ANOVA revealed no significant differences among treatments ($F = 2.30051 < F$ crit $= 3.9361$; $p = 0.13249$), indicating that the treatments alone did not significantly affect the final biomass. In contrast, the two-way ANOVA showed a highly significant effect of treatment ($F = 30.54701 > F$ crit $= 1.7946$; $p = 8.33{*}10^{-25}$) and of scale ($F = 13.80250 > F$ crit $= 3.9819$; $p = 4.12{*}10^{-4}$). The interaction between treatment and scale was also statistically significant ($F = 2.70146 > F$ crit $= 1.7946$; $p = 2.32{*}10^{-3}$), suggesting that treatment effects were partially dependent on the experimental scale. For dry weight of leaves, the one-way ANOVA already indicated significant differences among treatments ($F = 23.03503 > F$ crit $= 3.9361$; $p = 5.58{*}10^{-6}$). The two-way ANOVA further confirmed this trend, revealing highly significant effects of both treatment ($F = 25.44072 > F$ crit $= 1.7946$; $p = 1.56{*}10^{-22}$) and scale ($F = 190.20983 > F$ crit $= 3.9819$; $p = 2.22{*}10^{-21}$). The interaction term was also significant ($F = 21.91813 > F$ crit $= 1.7946$; $p = 9.80{*}10^{-21}$), highlighting that the treatment impact on dry biomass varied across scales. The strong scale effect on dry weight suggests differences in growth efficiency between laboratory and pilot setups. ANOVA outcomes indicate that both treatment and scale affect lettuce biomass, particularly dry weight underscoring the importance of scale effects when extrapolating laboratory results to pilot or industrial conditions. The NDVI values were analyzed across the different treatments (control and biochar) for dataset of Test 2 and the two experimental scales (laboratory and pilot). A one-way ANOVA performed on the NDVI values revealed highly significant differences between grouped observations ($F = 56.4995 > F$ crit $= 4.0427$; $p = 1.19 \times 10^{-9}$). However, because this analysis did not account for experimental scale, a two-way ANOVA was subsequently performed to separately evaluate the effects of treatment and scale. In this case, treatment alone did not show a significant effect ($F = 0.0681 < F$ crit $= 2.6059$; $p = 9.91 \times 10^{-1}$), whereas experimental scale exhibited a statistically robust influence ($F = 47.6169 > F$ crit $= 4.0847$; $p = 2.58 \times 10^{-8}$). The interaction term was not significant ($F = 0.0453 < F$ crit $= 2.6059$; $p = 9.96 \times 10^{-1}$), indicating that no treatment × scale interaction was detected. Overall, the two-way ANOVA indicates that the observed variability in NDVI was primarily explained by experimental scale. This strong scale dependence underscores the importance of considering system architecture when extrapolating spectral indices from laboratory to pilot-scale. ANOVA details in Supplementary Materials (Tables IV, V, and Table VI).

4. Conclusions

The findings of this study suggest promising prospects for investigating the potential use of biochar to other crop species and under different cultivation conditions, including greenhouse production systems and future open field application. Previous research has already demonstrated the beneficial effects of biochar in rooftop cultivation systems within the broader framework of urban agriculture, indicating its potential to improve productivity under spatially constrained conditions [47]. Furthermore, another study reported that the combined application of vermicompost and biochar in open-field environments can achieve superior agronomic outcomes compared to conventional mineral fertilizers [48]. In our experiments, the application of biochar led to highly encouraging results across various agronomic parameters, such as biomass weight (at laboratory-scale +46%, +52%, +0.4%, for test 1, 2 and 3; at pilot-scale +32%, −14%, +47% for test 1, 2 and 3) leaf number (at laboratory-scale +36%, +11%, +16%, for test 1, 2 and 3; at pilot-scale +12%, −16%, +28% for test 1, 2 and 3), and chlorophyll values (at laboratory-scale +8%, +12%, +12%, for test 1, 2 and 3; at pilot-scale +3%, −4%, +68% for test 1, 2 and 3), suggesting a potential role in supporting plant growth and physiological performance, although these effects were influenced by specific season, cultivation scale, biochar feedstock, and application rate. While soil parameters such as pH and EC showed no substantial variation across treatments, the pathogen-inoculation tests revealed an excellent response in plants amended with biochar, with a reduction of Fusarium wilt, in accordance with previous studies in greenhouse and field conditions [29]. The integration of visual and proximal monitoring proved useful in detecting the positive effects of biochar on plant performance, although some limitations emerged, particularly the low sensitivity of NDVI in distinguishing between biochar treatments. Specifically, near-infrared monitoring alone does not allow the detection of potential biochar-related enhancements in photosynthetic activity. Future work will focus on improving data quality and on integrating spectral information with metric parameters to refine the analysis and interpretation of vegetation responses. Overall, proximal sensing monitoring technologies could be readily transferred to the full scale through the integration of drone-based platforms, enabling rapid, non-destructive assessment of crop status over large areas. Also, water stress conditions are becoming common because of climate change, and biochar application can reduce water stress. A recent study [49] evaluated plants' water stress under different irrigation regimes using remote sensing techniques to evaluate changes in spectral and thermal profiles through various spectral indices compared to NDVI.

In conclusion, biochar represents an additional promising variable to investigate, as its application could be integrated into open-field trials to support proximal and visual monitoring approaches, enabling the assessment of crop status in relation to water availability and irrigation requirements. Collectively, these results highlight the potential versatility of biochar amendments under greenhouse cultivation conditions, providing a basis for further investigation of their long-term effects on soil quality, crop productivity, and sustainable agricultural management across diverse cultivation scenarios.

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CRediT authorship contribution statement

Ilaria Orlandella: Data curation, Formal analysis, Investigation, Validation, Visualization, Writing – original draft. **Kyra Nancie Smith:** Data curation, Investigation, Visualization. **Elena Belcore:** Data curation, Validation, Visualization, Writing – original draft. **Renato Ferrero:** Methodology, Software, Validation, Writing – review & editing. **Massimo Pugliese:** Conceptualization, Methodology, Supervision, Validation, Writing – review & editing. **Marco Piras:** Conceptualization, Methodology, Project administration, Supervision, Validation, Writing – review & editing. **Silvia Fiore:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmf.2026.100068.

Data availability

Data will be made available on request.

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