Journal of Cleaner Production 551 (2026) 147960

Contents lists available at ScienceDirect

Journal of Cleaner Production

journal homepage: www.elsevier.com/locate/jclepro

Hydroponic rocket (*Eruca sativa* Mill.) microgreens grown with digestates from anaerobic digestion of maize co-digested with oxidized and non-oxidized biochars: nutrient dynamics and plant performance

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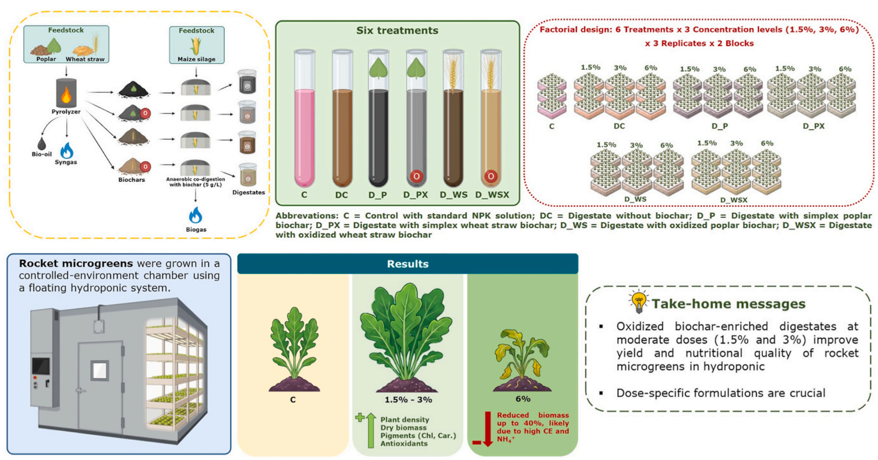
HIGHLIGHTS

- Biochar co-digested silage maize showed reduced phytotoxicity.
- Biochar addition during anaerobic digestion improved nutrient use efficiency.
- Resulting digestates enhanced rocket microgreen growth and physiological traits.
- PLS-R modeling identified key nutrients for hydroponic solution optimization.
- Moderate doses (<3%) promoted anti-oxidant accumulation in microgreens.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Organic nutrient inputs
Hydroponic systems
Microgreens
Biochar co-digested digestates
Chemical composition

ABSTRACT

Sustainable food production increasingly relies on circular strategies aimed at recovering and reusing nutrients from organic residues. This study assessed the agronomic performance and suitability of digestates obtained from maize silage anaerobic digestion co-digested with oxidized and non-oxidized biochars as nutrient supplements for the hydroponic cultivation of rocket (*Eruca sativa* Mill.) microgreens.

Microgreens were cultivated for 18 days in a floating hydroponic system under controlled conditions. Six treatments were tested: a standard nutrient solution (control), and five solutions enriched by maize-silage digestates, either without biochar or co-digested with unoxidized or oxidized biochars derived from poplar and wheat straw. Digestates were applied at three concentration levels (1.5%, 3%, 6% v/v) in the standard nutrient solution. Plant responses were evaluated in terms of dry biomass, pigments, sugars, and antioxidants. Linear mixed-models and the Partial Least Squares Regression (PLS-R) were used to identify key response drivers.

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https://doi.org/10.1016/j.jclepro.2026.147960
Received 10 October 2025; Received in revised form 31 January 2026; Accepted 7 March 2026
Available online 10 March 2026

Digestates co-digested with oxidized biochar, applied at moderate doses (1.5–3%) significantly increased plant performance, pigment content and antioxidants, with fresh biomass increasing up to 74% and. In contrast, high application rates (6%), particularly of unoxidized wheat-straw-derived biochar co-digested digestate, reduced biomass by *ca* 40%, primarily due to increased electrical conductivity (EC) and associated osmotic stress. Multivariate analysis highlighted EC and nutrient balance as major factors influencing growth and quality traits.

Overall, oxidized biochar co-digested digestates applied at moderate doses proved compatible with hydroponic microgreen production, enhancing crop performance and nutritional quality while supporting nutrient recycling within circular soilless cultivation systems.

1. Introduction

The world population, expected to exceed 9.7 billion by 2050 (FAO, 2017), is placing unprecedented pressure on food, water, and energy resources (Falcon et al., 2022). Within this context, conventional linear models based on the “take, make, dispose” approach are proving to be increasingly unsustainable due to resource scarcity and significant environmental impacts (Rashid et al., 2023). To address these challenges, circular economy strategies, particularly those focused on the recovery and recycling of nutrients from organic residues, have gained increased attention as effective tools to reduce reliance on synthetic fertilizers and to improve resource efficiency in agriculture (Nkoa, 2014).

Among the innovations aligned with circular principles, soilless cultivation methods have emerged as promising approaches to optimize resource use in food production. Hydroponic cultivation, particularly with floating systems, is being adopted with the objective of optimizing water and nutrient efficiency, reducing production times, and enhancing the yields of short-cycle leafy vegetable crops (Waiba et al., 2020). These characteristics render hydroponics especially suitable for the production of sprouts, microgreens and baby leaves, all fast-growing crops with both high nutritional value and commercial appeal (Bulgari et al., 2017; Di Gioia et al., 2021).

Microgreens, rich in vitamins, antioxidants, and bioactive compounds that often exceed those found in mature vegetables have gained particular attention (Di Gioia et al., 2023; Xiao et al., 2012). The short cultivation cycle (less than 14 days) and low space requirements render microgreens ideal candidates for controlled environmental agriculture, where resource efficiency is critical (Seth et al., 2025). Although the absolute yields per harvest are modest, the rapid turnover across multiple cycles ensures high productivity relative to production time (Seth et al., 2025). However, the application of organic inputs in hydroponics remains technically challenging, especially when using recycled resources derived from organic waste (Asaduzzaman et al., 2022; Bergstrand et al., 2020; Weimers et al., 2022; Williams and Nelson, 2016).

Digestate, a by-product of anaerobic digestion (AD), exemplifies both the potential and complexity of introducing organic nutrients into hydroponic systems. Anaerobic digestion enables the conversion of organic matter into biogas, a renewable energy source, while simultaneously producing digestate, a nutrient-rich by-product with significant agronomic value (Peng et al., 2020). Both the solid and liquid fractions of digestate contain levels of macro- and micronutrients necessary to sustain the requirements of plants, and bioactive compounds (phytohormones, amino acids and vitamins) that contribute to plant growth and stress resilience (Jacob et al., 2025; Pelayo Lind et al., 2021).

Nonetheless, the direct use of digestate in soilless systems is limited by excessive concentrations of ammonium (NH_4^+), sodium (Na^+) and volatile organic acids, combined with a high electrical conductivity (EC). These factors can cause phytotoxicity, root damage and nutrient imbalances in sensitive crops (Nkoa, 2014). In hydroponic systems, where nutrient balance and solution stability are critical, the use of raw or poorly stabilized digestate may compromise crop development (Bergstrand et al., 2020). These concerns are amplified in microgreen cultivation, attributable to their high surface-to-volume ratio and rapid metabolic activity which render microgreens highly sensitive to environmental stressors and nutrient imbalances from exposure to inadequately treated organic inputs (Ebert, 2022).

Rocket (*Eruca sativa* Mill.) microgreens represent an ideal model crop to assess the agronomic viability and potential risks associated with the use of digestate in hydroponic systems, attributable to their fast growth, combined with a heightened sensitivity to nutritional imbalances and the tendency to accumulate nitrates under non-optimal conditions (Bulgari et al., 2017; Di Gioia et al., 2023). Given these challenges, innovative approaches to improving digestate quality are essential towards unlocking its potential as a sustainable nutrient source in soilless cultivation.

Biochar is a carbon-rich material derived from the pyrolysis of biomass under oxygen-limited conditions (Rosi et al., 2024; Varkolu et al., 2025). Studies have demonstrated that biochar, when applied directly either as a soil amendment or as a substrate component in soilless systems, can enhance plant growth and nutrient content in various crops. In rocket, biochar applications reduced pollutant accumulation and supported healthier plant development without compromising quality (Parlavecchia et al., 2020). Results have also shown that up to 20% v/v biochar as a sustainable alternative of peat improved plant performance and reduced nitrate accumulation, thereby addressing important quality and safety concerns (Bini et al., 2025).

The use of biochar together with the digestate ameliorate the quality and reduce the phytotoxic-inducing risks of digestate. The physicochemical properties of biochar permit the adsorption of excess ammonium, heavy metals, and phytotoxic compounds such as organic acids through mechanisms such as ion exchange, electrostatic attraction, and surface complexation (Ahmad et al., 2014; Liang et al., 2014). Biochar can be combined with digestate in several ways: (i) as digestate-derived biochar, obtained by pyrolyzing the solid fraction of digestate (Yan et al., 2023); (ii) as biochar-enriched digestate, where biochar is co-applied with digestate to stabilize nutrients and enhance agronomic value (Hammerschmiedt et al., 2021), and (iii) as an additive during anaerobic digestion, where biochar improves nutrient retention and process efficiency (B. Chen et al., 2024; Viaene et al., 2024). Recently, modifying the AD process with biochar addition has emerged as a promising strategy to produce higher-quality digestate for improved agronomic value and reduced phytotoxic risk. Biochar has also been shown to improve both methane yield and the chemical composition of the resulting digestate (Rosi et al., 2024). The physicochemical properties of biochar, including high surface area, porosity, and cation exchange capacity (CEC), allow it to interact with nutrients and microbial communities during digestion (Rosi et al., 2024). Biochar efficacy is dependent on numerous factors, including the origin of the feedstock, pyrolysis conditions, and post-treatment oxidation, which can modify the abundance and type of functional groups on its surface (Rosi et al., 2024). Oxidized biochar, obtained through activation processes such as ozone treatment, has shown enhanced surface reactivity and functionalization, to improve digestion efficiency and nutrient availability (Rosi et al., 2024). These biochar-mediated modifications are relevant for hydroponic systems, where plants are directly exposed to the nutrient. Previous studies have shown that biochar addition during anaerobic digestion can alter the chemical profile of the liquid digestate fraction, affecting ionic strength, nutrient balance, and the availability of potentially phytotoxic compounds (B. Chen et al., 2024; Paritosh and

Vivekanand, 2019; Rosi et al., 2024; Viaene et al., 2024). Such changes may influence plant growth responses in soilless cultivation by moderating salinity stress and nutrient imbalances, which are among the main constraints limiting the use of digestate-based nutrient solutions in hydroponics (Bergstrand et al., 2020; Nkoa, 2014).

To the best of our knowledge, while the agronomic potential of biochar as a direct input is well established, the impact resulting from the incorporation into anaerobic digestion (i.e. where biochar is added directly to the feedstock before digestion to act as an additive during the AD process) on the digestate quality and subsequent crop performance remains largely unexplored. This knowledge gap is particularly relevant for soilless cultivation systems, where plants are directly exposed to nutrient solutions and lack the buffering capacity of soil, making them highly sensitive to nutrient imbalances and potentially phytotoxic compounds.

Given the ongoing transition from conventional hydroponic fertigation regimes to more sustainable organic-based systems, the organic nutrient sources are progressively integrated. Hence, the primary objective of the study was to assess the agronomic compatibility and physiological effects of introducing digestate-derived nutrients into an existing hydroponic fertigation regime.

Specifically, this work aimed to determine: (i) whether digestates obtained from anaerobic digestion co-digested with biochar can be safely and effectively used as nutrient supplements in hydroponic microgreen production; (ii) whether biochar type and oxidation state influence digestate quality and plant responses; (iii) whether digestate concentration determines a threshold between beneficial and stress-inducing effects, and (iv) whether multivariate modelling approaches, specifically partial least squares regression (PLS-R), represent an effective strategy to identify both the key nutritional drivers of plant responses and optimal nutrient solution concentrations. With respect to objective (ii), a specific focus was placed on comparing digestates derived from anaerobic digestion co-digested with simplex and oxidized biochar produced from poplar and wheat straw.

The underlying hypotheses for each of the above-mentioned aims, were respectively, as follows: (i) biochar-modified digestates are able enhance growth and quality traits of rocket microgreens at appropriate inclusion rates in hydroponic nutrient solutions; (ii) biochar type and oxidation state will influence the agronomic performance of digestate-based nutrient solutions in hydroponic systems; (iii) digestate concentration determines degree of phytotoxic effects in hydroponic systems, which will be primarily driven by salinity and nutrient imbalances rather than ammonium toxicity; and (iv) plant growth, physiological traits, and secondary metabolite accumulation are driven by complex, nonlinear interactions among nutrients, which can be effectively explored using multivariate modelling approaches.

Through this multidisciplinary approach, combining waste valorization, biochar technology, multivariate statistical analysis, and soilless cultivation, the present research was anticipated to contribute to the development of more sustainable nutrient management strategies for hydroponic systems based on organic residue recovery.

2. Material and methods

2.1. Experimental set-up

The hydroponic cultivation of rocket (*Eruca sativa* Mill.) was carried out using 750 mL square polypropylene pots (13 × 13 cm base, 4.5 cm depth) as illustrated (Fig. 1C). In each pot, a square polystyrene raft was positioned on the nutrient solution surface. Each raft contained 16

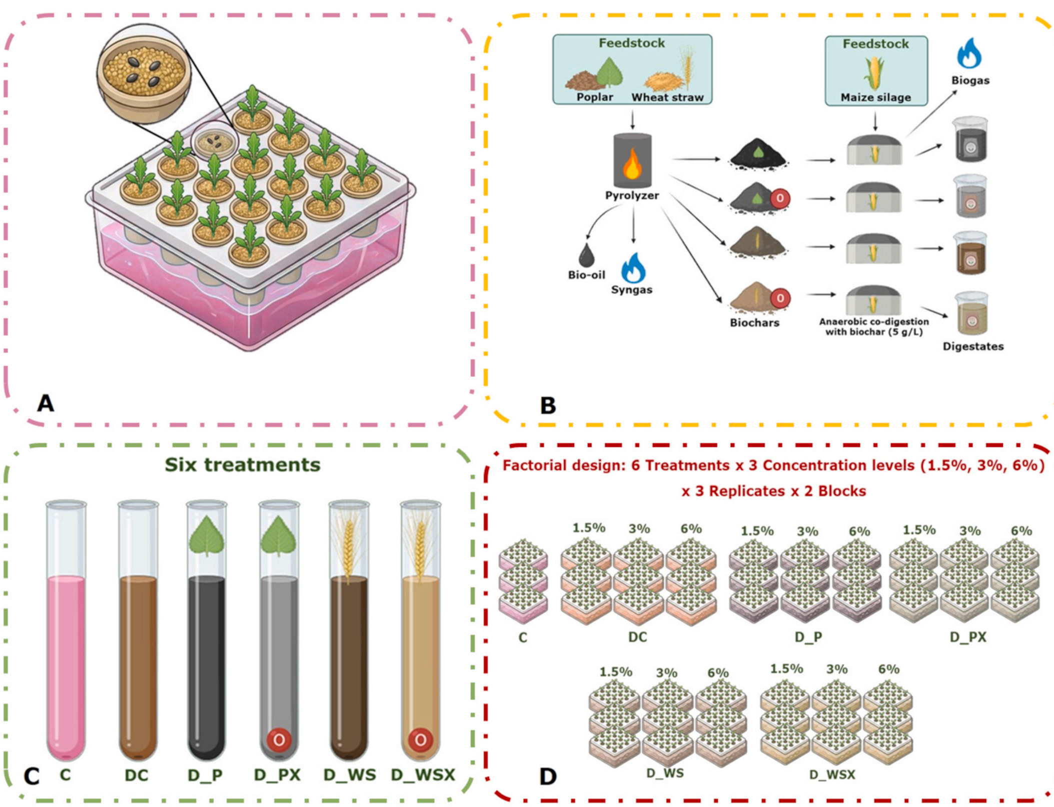

Fig. 1. Schematic overview of the experimental workflow and hydroponic cultivation system. The diagram illustrates: (A) the floating hydroponic microgreen system, including square polypropylene pots, polystyrene rafts with 16 truncated-conical planting holes filled with vermiculite, and rocket (*Eruca sativa* Mill.) seedlings grown under controlled conditions; (B) the production of poplar- and wheat-straw-derived biochars (oxidized and non-oxidized) and their addition to maize silage during anaerobic digestion; (C) the six treatments (C, DC, D_P, D_PX, D_WS, D_WSX); (D) the factorial experimental design consisting of six treatments applied at three digestate concentration levels (1.5%, 3%, and 6% v/v), with three replicates per treatment and two independent experimental blocks.

evenly distributed holes with a truncated-conical geometry (2.5 cm in diameter and 4 cm in height), securely placed within the pot opening to permit stable flotation (Fig. 1A).

Each hole was filled with vermiculite as a growth substrate. The truncated-conical geometry of the planting holes, combined with the fine particle size of vermiculite, ensured substrate stability and prevented loss into the nutrient solution, and is commonly adopted in floating hydroponic microgreen systems. Rocket seeds, purchased from Blumen (Italy), were sown directly into the holes (four seeds per hole) on the rafts, totaling 64 seeds per raft (Fig. 1A). The experiment was conducted in two independent runs (considered as blocks), with three replicates per treatment in each run.

The nutrient solution was prepared by dissolving 7 g of commercial NPK fertilizer [NPK(Mg) 17-16-11 (2) plus micronutrients] (KB Concime Universale, Scott® s.r.l., Treviso, Italy) in 5 L of deionized water, supplemented with 5 mL of acidifying agent (CIFOVIR 1®, CIFO s.r.l., San Giorgio di Piano, Italy; N 5%, P_2O_5 17%, pH 2.5, EC 2.5 dS m^{-1}). For each pot, 450 mL of nutrient solution was used. Nutrient solution volumes were sufficient to sustain plant growth over the entire experimental period without replenishment. EC and pH of the nutrient solutions were measured using a calibrated portable multiparameter meter. Nutrient concentrations (macro- and micronutrients) were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) analysis of freshly prepared nutrient solutions, prior to plant cultivation. All measurements were performed in triplicate for each treatment.

Poplar-derived and wheat straw-derived biochar, both in non-oxidized (P and WS, respectively) and oxidized forms (PX and WSX, respectively), were produced as described by Rosi et al. (2024). Biochar was obtained through the pyrolysis of poplar wood and wheat straw, respectively, at 550 °C under oxygen-limited conditions (Fig. 1B). Oxidation was carried out using oxone ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$), following the protocol described by Rosi et al. (2024), in order to increase the abundance of surface functional groups. All biochar samples were characterized for pH, EC, cation exchange capacity (CEC), and surface area using standard methods (Ahmad et al., 2014). Anaerobic digestion was performed at laboratory scale under controlled conditions using maize silage as the feedstock. Biochar was added directly to the maize silage prior to digestion at a rate corresponding to 5 g of biochar per liter of digestate. This dosage was selected based on previous studies indicating that relatively low biochar additions (1–10 g L^{-1}) are sufficient to influence microbial activity, nutrient retention, and digestate quality during anaerobic digestion, while minimizing solid carryover into the liquid fraction. The digestates used in the hydroponic experiment were obtained from this biochar-amended anaerobic digestion process (Paritosh and Vivekanand, 2019; Viaene et al., 2024; Zhang et al., 2022). The chemical characterization of the liquid digestates used in the experiment is reported in Supplementary Table 1.

The experimental design (Fig. 1C) included six treatments (Treatments): C, representing the control with standard hydroponic solution without digestate; DC, digestate from maize silage without biochar; D_P, digestate from maize silage with simplex poplar biochar; D_WS, digestate from maize silage with simplex wheat straw biochar; D_PX, digestate from maize silage with oxidized poplar biochar, and D_WSX, digestate from maize silage with oxidized wheat straw biochar. Biochar particle size was <0.25 mm, as reported by Rosi et al. (2024). Each digestate was sieved through a 1 mm^2 mesh to remove the solid fraction, which was then discarded. The liquid fraction was then incorporated into the hydroponic nutrient solution at three concentration levels (CL): 1.5%, 3%, and 6% v/v (namely, L15, L30, and L60, respectively) (Fig. 1D). Although the exact amount of biochar potentially present in the liquid fraction was not quantified, the experimental design aimed to assess indirect effects of biochar addition during anaerobic digestion, rather than direct biochar–plant interactions. Digestates were added to a standard hydroponic nutrient solution to evaluate their agronomic compatibility and effects on plant performance under non-limiting nutrient conditions, rather than to replace conventional fertilization.

Seed germination was initiated by placing the pots in a dark environment at 18 °C for 48 h. Plants were subsequently grown under controlled conditions with a 14-h light/10-h dark photoperiod, air temperatures of 20 °C during the day and 15 °C at night, and a light intensity of 180 μmol m^{-2} s^{-1}. The cultivation period lasted 18 days from sowing.

At harvest, samples were collected for the determination of photosynthetic pigments, sugars, polyphenols, anthocyanins and ascorbic acid. Photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids) were selected as indicators of plant physiological status, reflecting photosynthetic performance and potential stress-related responses to different nutrient regimes (Lichtenthaler and Buschmann, 2001; Taiz et al., 2023). Polyphenols, anthocyanins, and ascorbic acid were analyzed as key antioxidant compounds associated with secondary metabolism and nutritional quality (Gill and Tuteja, 2010). In microgreens, these parameters are particularly relevant because they directly contribute to product quality at harvest (Kyriacou et al., 2016; Xiao et al., 2012), and are also sensitive to variations in nutrient availability and the chemical composition of the growing solution. Moreover, antioxidant-related traits are known to respond to changes in nutrient balance and salinity, making them suitable indicators for assessing plant responses to digestate-based nutrient solutions (Gill and Tuteja, 2010).

Analyses were conducted by an external laboratory using standardized protocols. Chlorophyll *a* and *b* contents were determined by extracting fresh microgreens with N N-dimethylformamide for 24 h. Thereafter, the chlorophylls were measured at 647 and 664 nm and quantified (Moran, 1982). Carotenoids were extracted in 99.9% acetone in a 1:4 ratio and the samples incubated at 4 °C in the dark for 24 h. Then, carotenoids were quantified after measuring the absorbance at 510 nm (Gitelson et al., 2002). Ascorbic acid was measured by mixing 0.02 mL of extract with 0.05 mL 5% metaphosphoric acid and measuring the absorbance at 525 nm (AOAC International, 2000).

Total phenols were determined according to the Folin–Ciocalteu spectrophotometric method (Emmons et al., 1999) at 734 nm and then expressed as gallic acid equivalents (GAE) (mg gallic acid g^{-1}). Anthocyanins were extracted in 1% methanolic HCl and the absorbance measured at 535 nm and then expressed as cyanidin-3-glucoside equivalents (Klein and Hagen, 1961).

Sucrose content and reducing sugars were determined according to Rorem et al. (1960) and Miller (2002), respectively. Total sugars were quantified using the anthrone method according to Yemm and Willis (1954). We used the anthrone method to directly quantify total soluble sugars, as it detects reducing sugars, sucrose, and other soluble carbohydrates in a single assay. It was considered that summing the sucrose and reducing sugar contents may result in an underestimation of the total sugar content if additional carbohydrate fractions were present.

For each compound analyzed in the leaf tissue, both the concentration (expressed as the mass of the compound of interest on a gram fresh mass basis) and the total content (the mass of the compound of interest per raft) were determined. The total content per raft was calculated by multiplying the compound concentration by the total fresh biomass per raft. This parameter was included to provide a more comprehensive evaluation of metabolite accumulation, including both biochemical concentration and biomass productivity. Such an approach allows for an improved assessment of the actual yield of nutritionally or functionally relevant compounds per cultivation unit.

2.2. Statistical analyses

Statistical analyses were performed using RStudio (version 4.3.1) (R Core Team, 2024). For each parameter, the assumptions of normality and homogeneity of variances were evaluated using the Shapiro–Wilk test and Levene's test, respectively from the "car" package (Fox and Weisberg, 2019). When these assumptions were not met, then in order to normalize the distribution of the response variables, a Box–Cox transformation was applied (Venables and Ripley, 2002). Following transformation, linear mixed-effects models were fitted using the lmer function from the lme4 package in R (Bates et al., 2015). The data were analyzed using a two-way factorial analysis of variance (ANOVA) according to the following linear model:

$$\text{parameter} = \mu + \text{Block} + \text{Treatment} + \text{Level} + \text{Treatment} \times \text{Level} + \varepsilon$$

where μ is the overall mean, Block is the effect of the two independent runs, Treatment is the effect of the digestate type, Level is the effect of the digestate concentration, Treatment × Level is the interaction between treatment and level, and ε is the random error.

When the analysis of variance (ANOVA) revealed statistically significant effects of the examined factors on the measured variables, the Tukey's Honestly Significant Difference (HSD) test was subsequently applied for pairwise comparisons. In cases where significant interactions between factors were detected, a simple effects analysis was conducted to examine the influence of one factor within the specific levels of the interacting factor. This strategy was chosen to retain the structure of the interaction, offering a clearer biological interpretation rather than generalized multiple comparisons.

To evaluate the contribution of each factor to the total variability, the proportion of explained variance was calculated by relating the sum of squares of each factor to the total sum of squares accounted for by the model, excluding the residual error. This quantification aimed to identify the relative influence of each factor and the interaction on the dependent variables, highlighting those that played the most substantial role in shaping the experimental outcomes.

The relationships between nutrient concentrations in the hydroponic solutions and plant physiological responses were analyzed using the Partial Least Squares Regression (PLS-R) (Mevik et al., 2024; Mevik and Wehrens, 2007). The analysis was performed with the objective of modeling complex multivariate data characterized by high collinearity among independent variables and multiple dependent variables. The independent variables (X matrix) included the concentrations of macro- and micronutrients detected in the different digestate treatments. Instead, the dependent variables (Y matrix) comprised plant physiological parameters such as fresh biomass weight, dry biomass weight, number of plants per raft, pigment concentrations, as well as sugar, polyphenol, anthocyanin and ascorbic acid contents.

Prior to the analysis, both the X and Y matrices were mean-centered and standardized (auto-scaled) to ensure comparability among variables with different units and scales. The optimal number of latent components was determined by minimizing the Root Mean Square Error of Prediction (RMSEP) through leave-one-out cross-validation (LOOCV). Model quality was assessed by examining the coefficient of determination (R^2) and RMSEP for each dependent variable. Variables with R^2 values above 0.60 were considered to exhibit strong predictive performance, while those with lower R^2 and higher RMSEP indicated reduced predictive reliability.

Variable Importance in Projection (VIP) scores were calculated to identify the most influential independent variables contributing to the model. VIP scores greater than 1.0 were interpreted as indicative of a significant contribution to the prediction of plant responses. The linear and quadratic regression coefficients derived from the PLS model were also examined to explore both linear and non-linear relationships between nutrients and plant traits.

The entire analysis was performed using the "pls" package (Mevik et al., 2024; Mevik and Wehrens, 2007). The model structure followed the general formula:

$$Y = Xb + E$$

where Y represents the matrix of dependent variables, X the matrix of independent variables, b the matrix of regression coefficients (including intercepts), and E the matrix of residuals. Intercept values were calculated as part of the PLS regression output, but were not explicitly included in the interpretation as focus was placed on the nutrient-derived coefficients and VIP scores. This methodological approach permitted the comprehensive evaluation of nutrient influence on plant physiological responses under controlled hydroponic conditions, supporting both predictive modeling and the biological interpretation of observed effects.

Although nutrient concentrations in hydroponic solutions are inherently dynamic due to plant uptake, the present study focused on the physiological and nutritional characteristics of microgreens at the time of harvest. The time of harvest represents the commercially and nutritionally relevant stage for human consumption. Accordingly, correlations between nutrient solution composition and plant responses were evaluated at this specific stage to specifically assess how different nutrient regimes influenced final crop quality, as opposed to temporal uptake dynamics.

3. Results

Digestate-based treatments increased Ca, K, Mg, and P concentrations compared to the C, while slightly reducing ammoniacal nitrogen (NH_4^+–N). Digestates derived from biochar-codigested substrates generally showed lower macronutrient concentrations than DC. Nitrate nitrogen (NO_3^-–N) was below the detection limit (0.1 mg L^{-1}) in all nutrient solutions. Macronutrient concentrations increased with digestate concentration (C < L15 < L30 < L60), whereas NH_4^+–N followed the opposite trend (Supplementary Table 2).

Increasing digestate concentration resulted in a significant increase in Fe, Mn, Mo, Na, Pb, Se, and Zn concentrations in the nutrient solution (C < L15 < L30 < L60), whereas B followed a distinct pattern, with the highest concentration observed at L30 and comparable values at C, L15, and L60 (Supplementary Table 2). With respect to the treatments, all digestate-based solutions showed significantly higher concentrations of Fe, Mn, Mo, Na, Pb, Se, and Zn compared to the C, although treatment-specific differences were observed among digestate types. In contrast, B concentrations were generally comparable among treatments, with the exception of D_PX, which exhibited higher values.

EC is an integrative indicator of ionic strength, was substantially higher in digestate-based treatments compared to the C, with the highest value observed in D_WS. EC also increased significantly with digestate concentration, reaching the maximum at L60 (Supplementary Table 2).

Across digestate concentration levels (C–L60), nutrient concentrations (P, Fe, Mn, Mo, Se, and Zn) and EC increased in a dose-dependent manner, with the highest values consistently observed at L60 (Supplementary Table 2).

Differences observed among digestate-based treatments reflected the indirect effects of biochar addition during anaerobic digestion on the chemical composition of the resulting liquid fraction. In particular, digestates co-digested with different biochar types showed distinct nutrient profiles and EC, which translated into different nutrient supply regimes for microgreens.

3.1. Impact of treatments and digestate concentration levels on biomass parameters

The ANOVA revealed significant effects of Treatment, Level, and their interaction on the number of plants per raft, while the Block factor was not significant (Supplementary Table 3). The highest number of plants per raft was recorded at L30, particularly in D_WSX (34.0 ± 0.7), whereas the lowest values occurred at L60, especially in D_WS (15.5 ± 0.8) (Fig. 2A). At L15 and L30, most treatments were statistically comparable to the C except D_WS, which consistently showed reduced establishment. At L60, all digestate-based treatments exhibited significantly lower plant establishment than the C, with no significant differences among digestate types.

Fresh biomass weight was significantly affected by Treatment, Level and their interaction (Supplementary Table 3). The highest biomass

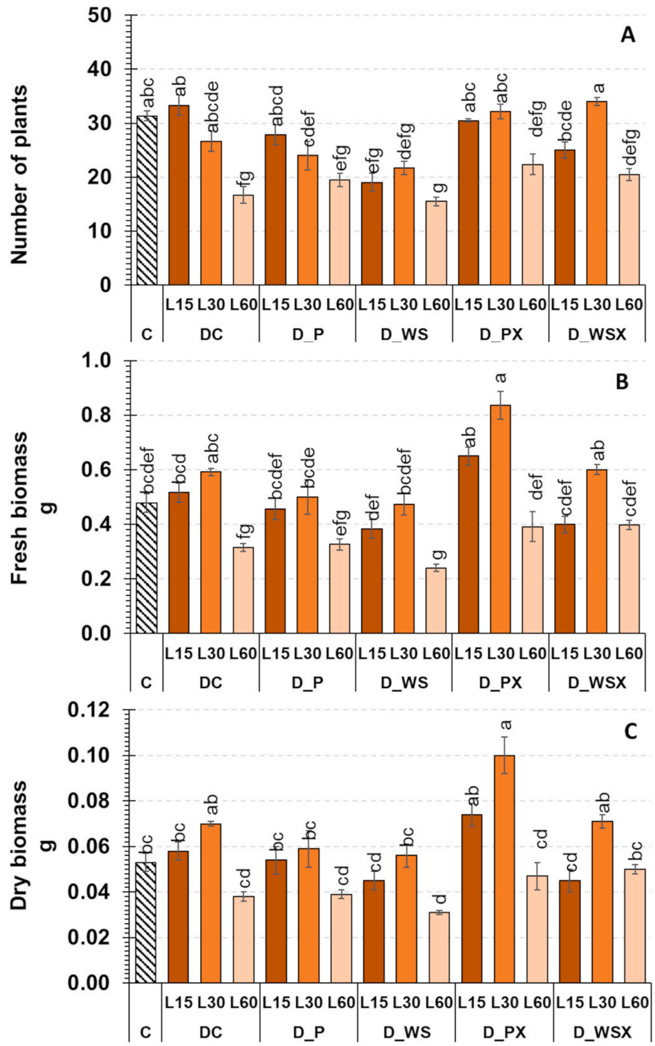

Fig. 2. Effects of treatments and digestate concentration levels on rocket microgreen biomass parameters: (A) number of plants per raft, (B) fresh biomass weight, and (C) dry biomass weight. Bars represent mean values ± standard error. Different letters indicate statistically significant differences among treatments and concentration levels according to Tukey's post hoc test ($p < 0.05$). Treatment abbreviations: C = control (standard hydroponic solution); DC = digestate from maize silage without biochar; D_P = digestate with simplex poplar biochar; D_WS = digestate with simplex wheat straw biochar; D_PX = digestate with oxidized poplar biochar; D_WSX = digestate with oxidized wheat straw biochar. Digestate concentration levels in nutrient solution: L15 = 1.5%, L30 = 3%, L60 = 6%.

across all treatments and levels was recorded in D_PX at L30, whereas the lowest was found in D_WS at L60 (Fig. 2B). At L15, no significant differences from the C were observed. At L30, D_PX was significantly higher biomass than the C (+74.3%), whereas the other treatments were statistically comparable. At L60, all digestate-based treatments resulted in significantly lower biomass than the C, with D_WS exhibiting the strongest reduction.

Dry biomass weight was significantly affected by Treatment, Level and Treatment × Level interaction (Supplementary Table 3). The highest dry biomass value was recorded in D_PX at L30 (0.100 ± 0.008 g), while the lowest was observed in D_WS at L60 (0.047 ± 0.006 g) (Fig. 2C). At L15, no significant differences from the C were observed. At L30, only D_PX showed a significantly higher dry biomass than (+88.7%). At L60, all digestate-based treatments resulted in significantly lower dry biomass than the C, with D_WS consistently exhibiting the lowest dry biomass.

The ratio of dry to fresh biomass was significantly affected by digestate Level, while Treatment and their interaction were not significant (Supplementary Table 3). The ratio of dry to fresh biomass increased progressively with digestate concentration, following the trend C ≤ L15 < L30 < L60 (Table 1).

3.2. Chlorophyll and carotenoids in response to treatments and digestate concentration levels

The ANOVA highlighted significant effects of Treatment, Level, and their interaction on both the concentrations and total amounts of chlorophyll *a* (Chl_a), chlorophyll *b* (Chl_b), and carotenoids (Supplementary Table 4).

Overall, Chl_a concentration was significantly higher in digestate-based treatments compared to the C across most treatment–level combinations, with total Chl_a content per raft showing a similar pattern, particularly at L30, and reductions observed at L60 in specific treatments. The highest Chl_a concentration was observed in D_WS at L15 (1.676 mg g^{-1} FW), while the lowest was recorded in D_WSX at L30 (0.729 mg g^{-1} FW) (Fig. 3A). Similarly, total Chl_a content per raft ranged from a maximum of 0.749 mg per raft in D_PX at L30 to a minimum of 0.324 mg per raft in D_WS at L60 (Fig. 3B).

Chl_b concentrations were higher in digestate-based treatments than in the C across all levels (Fig. 3C). The highest Chl_b concentration was observed in D_WS at L15 (0.586 mg g^{-1} FW), while the lowest was recorded in C (0.252 mg g^{-1} FW). Total Chl_b content per raft varied across treatments and digestate levels, with higher values generally observed at L30 in specific digestate-based treatments (Fig. 3D). The highest total Chl_b content per raft was found in D_PX at L30 (0.224 mg), while the lowest was in D_WS at L60 (0.117 mg) (Fig. 3D.

Overall, carotenoid concentrations were higher in digestate-based treatments than in the C across most treatment–level combinations (Fig. 3E). The highest carotenoid concentration was observed in D_WS at L15 (0.31 mg g^{-1} FW), while the lowest was recorded in D_PX at L15 (0.188 mg g^{-1} FW). The highest carotenoid content per raft was measured in D_PX at L30 (0.158 mg), and the lowest in D_WS at L60 (0.071 mg) (Fig. 3F). Across digestate levels, total carotenoid content was highest at L30, intermediate at L15, and lowest at L60 and in the C (Table 2).

3.3. Effect of treatments digestate concentration levels on sucrose, reducing sugars, and total sugar content

Sucrose concentration and total content were significantly affected by Treatment, Level, and their Interaction (Supplementary Table 5). Overall, sucrose concentration showed marked variability across treatment–level combinations (Fig. 4A). The highest sucrose concentration was recorded in D_WS at L15 (0.273 mg g^{-1} FW) corresponding to a significant 84% increase compared to the C, while the lowest was found in D_WSX at L30 (0.077 mg g^{-1} FW) (Fig. 4A). Similarly, total sucrose content per raft ranged from a maximum 0.113 mg in D_PX at L15 to a minimum of 0.047 mg in D_WSX at L60 (Fig. 4B).

Reducing sugar concentrations and total content per raft were significantly affected by Treatment, Level, and their interaction (Supplementary Table 5). Overall, reducing sugar concentrations were higher in digestate-based treatments than in the control across most treatment–level combinations (Fig. 4C). The highest concentration was recorded in D_WS at L15 (3.612 mg g^{-1} FW), corresponding to a significant 105% increase compared to the control (1.761 mg g^{-1} FW), whereas the lowest value was found in D_WSX at L15 (1.548 mg g^{-1} FW) (Fig. 4C). Similarly, total reducing sugar content per raft ranged from the lowest value in the control to a maximum in D_PX at L30 (1.998 mg, corresponding to a 137% increase relative to the control) (Fig. 4D).

Total sugar concentrations and total content per raft also showed significant variation due to Treatment, Level, and their interaction (Supplementary Table 5). Overall, total sugar concentrations (Fig. 4E)

Table 1
Mean values (standard error) for the number of rocket microgreen plants, fresh biomass weight, dry biomass weight, and dry-to-fresh biomass ratio across treatments and digestate concentration level (CL). Different letters indicate statistically significant differences among treatments according to the Tukey's HSD test at $p < 0.05$.

Source of variation	Levels	number of plants		Fresh biomass weight		Dry biomass weight		Dry to fresh biomass	
		n°	sig	g	sig	g	sig	%	sig
Treatment	C	31.33 (0.92)	a	0.479 (0.034)	ab	0.053 (0.004)	abc	11.16 (0.22)	b
	DC	25.56 (1.92)	bc	0.474 (0.031)	b	0.055 (0.004)	b	11.73 (0.14)	ab
	D_P	23.78 (1.37)	c	0.428 (0.030)	bc	0.051 (0.004)	bc	11.78 (0.15)	ab
	D_WS	18.72 (0.92)	d	0.366 (0.029)	c	0.044 (0.003)	c	12.11 (0.20)	a
	D_PX	28.33 (1.28)	ab	0.625 (0.051)	a	0.074 (0.006)	a	11.79 (0.15)	ab
	D_WSX	26.5 (1.5)	abc	0.466 (0.026)	b	0.055 (0.003)	b	11.83 (0.20)	ab
CL	C	31.33 (0.92)	a	0.479 (0.034)	b	0.053 (0.004)	b	11.16 (0.22)	c
	L15	27.13 (1.12)	a	0.481 (0.023)	b	0.055 (0.003)	b	11.49 (0.12)	c
	L30	27.7 (1.13)	a	0.600 (0.029)	a	0.071 (0.004)	a	11.80 (0.13)	b
	L60	18.9 (0.74)	b	0.334 (0.016)	c	0.041 (0.002)	c	12.25 (0.12)	a

Treatment abbreviations: C = control (standard hydroponic solution); DC = digestate from maize silage without biochar; D_P = digestate with simplex poplar biochar; D_WS = digestate with simplex wheat straw biochar; D_PX = digestate with oxidized poplar biochar; D_WSX = digestate with oxidized wheat straw biochar. Digestate levels in nutrient solution: L15 = 1.5%, L30 = 3%, L60 = 6%.

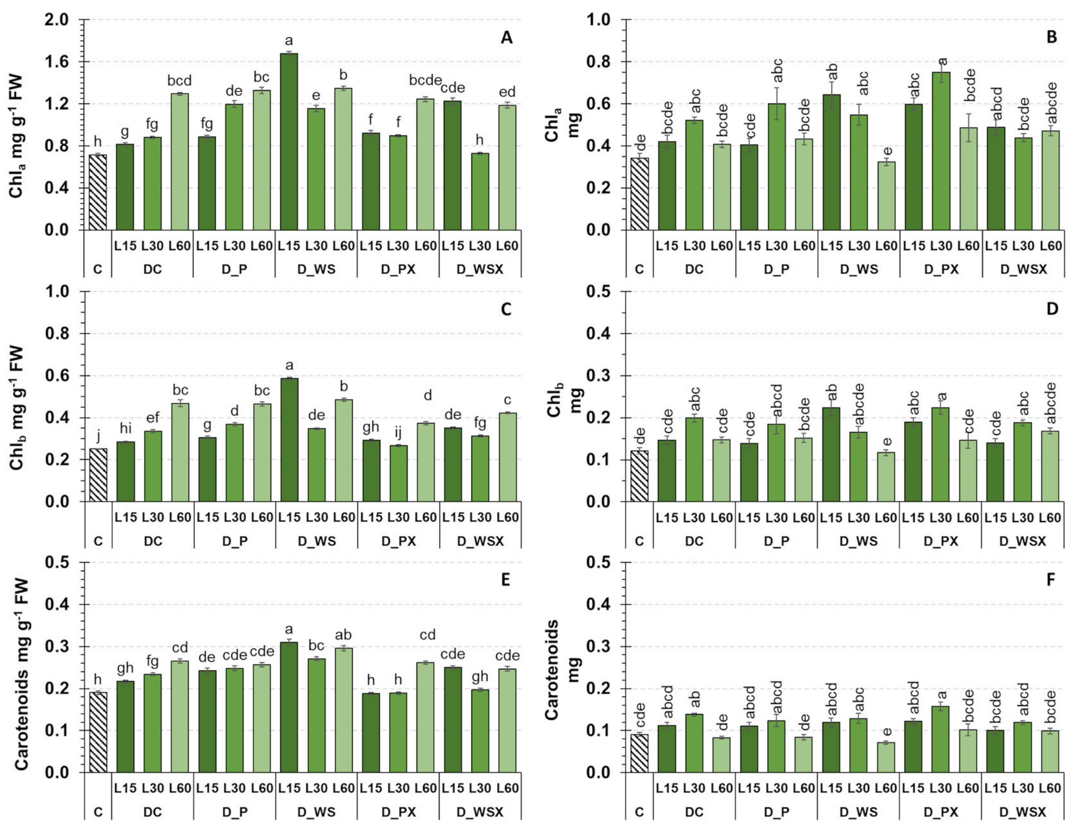

Fig. 3. Effect of treatments and digestate concentration levels on rocket microgreen pigment composition: concentration (mg g^{-1} FW) of (A) chlorophyll *a* (Chl_a), (C) chlorophyll *b* (Chl_β), and (E) carotenoids, and total content per raft (mg) of (B) Chl_a, (D) Chl_β, and (F) carotenoids. Bars represent mean values ± standard error. Different letters indicate statistically significant differences among treatments and concentration levels according to Tukey's post hoc test ($p < 0.05$). Treatment abbreviations: C = control (standard hydroponic solution); DC = digestate from maize silage without biochar; D_P = digestate with simplex poplar biochar; D_WS = digestate with simplex wheat straw biochar; D_PX = digestate with oxidized poplar biochar; D_WSX = digestate with oxidized wheat straw biochar. Digestate levels in nutrient solution: L15 = 1.5%, L30 = 3%, L60 = 6%.

and total sugar content per raft (Fig. 4F) varied markedly across treatment–level combinations. The highest concentration overall was recorded in D_WS at L15 (8.374 mg g^{-1} FW), while the lowest concentration was observed in D_P at L30 (3.669 mg g^{-1} FW). For total sugar content per raft, the maximum was in D_PX at L30 (3.901 mg), whereas the minimum was found in D_PX at L15 (1.457 mg).

Although all interactions between treatment and digestate level were statistically significant for sucrose, reducing sugars, and total sugars, the main effects are reported in Table 3 for completeness, but are not further discussed in the text.

3.4. *Effect of treatments on phenols, anthocyanins, and ascorbic acid content*

The concentration and total content per raft of free and total polyphenols, as well as anthocyanins and ascorbic acid, were significantly affected by Treatment, digestate Level, and their interaction (Supplementary Table 6).

Free polyphenol concentration and total content per raft varied significantly across treatment–level combinations (Fig. 5A and B). The highest free polyphenol concentration was detected in D_WS at L15

Table 2
Mean values (standard error) for the concentration and total amount per raft of pigments in rocket microgreens across different treatments and digestate concentration level (CL). Different letters indicate statistically significant differences among treatments according to the Tukey's HSD test at $p < 0.05$.

Source of variation	Levels	Concentration Chl_a		Tot. Cont. Chl_a		Concentration Chl_b		Tot. Cont. Chl_b		Concentration Carotenoids		Tot. Cont. Carotenoids	
		mg g^{-1} FW	sig	mg	sig	mg g^{-1} FW	sig	mg	sig	mg g^{-1} FW	sig	mg	sig
Treatment	C	0.72 (0.01)	d	0.34 (0.02)	c	0.25 (0)	e	0.121 (0.008)	b	0.19 (0.005)	e	0.091 (0.005)	c
	DC	1 (0.05)	c	0.45 (0.02)	b	0.36 (0.02)	c	0.164 (0.008)	a	0.238 (0.005)	c	0.111 (0.006)	ab
	D_P	1.14 (0.05)	b	0.48 (0.03)	b	0.38 (0.02)	b	0.158 (0.01)	ab	0.249 (0.003)	b	0.106 (0.007)	b
	D_WS	1.39 (0.05)	a	0.5 (0.04)	b	0.47 (0.02)	a	0.168 (0.013)	a	0.292 (0.005)	a	0.106 (0.008)	b
	D_PX	1.02 (0.04)	c	0.61 (0.04)	a	0.31 (0.01)	d	0.187 (0.011)	a	0.213 (0.009)	d	0.128 (0.008)	a
	D_WSX	1.05 (0.06)	c	0.47 (0.02)	b	0.36 (0.01)	bc	0.165 (0.007)	a	0.231 (0.006)	c	0.106 (0.004)	b
CL	C	0.72 (0.01)	c	0.34 (0.02)	b	0.25 (0)	d	0.121 (0.008)	c	0.19 (0.005)	d	0.091 (0.005)	c
	L15	1.1 (0.06)	b	0.51 (0.02)	a	0.36 (0.02)	b	0.168 (0.008)	ab	0.242 (0.008)	b	0.113 (0.004)	b
	L30	0.97 (0.03)	b	0.57 (0.03)	a	0.33 (0.01)	c	0.192 (0.007)	a	0.228 (0.006)	c	0.133 (0.005)	a
	L60	1.28 (0.01)	a	0.42 (0.02)	ab	0.44 (0.01)	a	0.146 (0.006)	b	0.265 (0.004)	a	0.088 (0.004)	c

Treatment abbreviations: C = control (standard hydroponic solution); DC = digestate from maize silage without biochar; D_P = digestate with simplex poplar biochar; D_WS = digestate with simplex wheat straw biochar; D_PX = digestate with oxidized poplar biochar; D_WSX = digestate with oxidized wheat straw biochar. Digestate levels in nutrient solution: L15 = 1.5%, L30 = 3%, L60 = 6%. Chlorophyll *a* = Chl_a, chlorophyll *b* = Chl_b, Total Content = Tot. Cont.

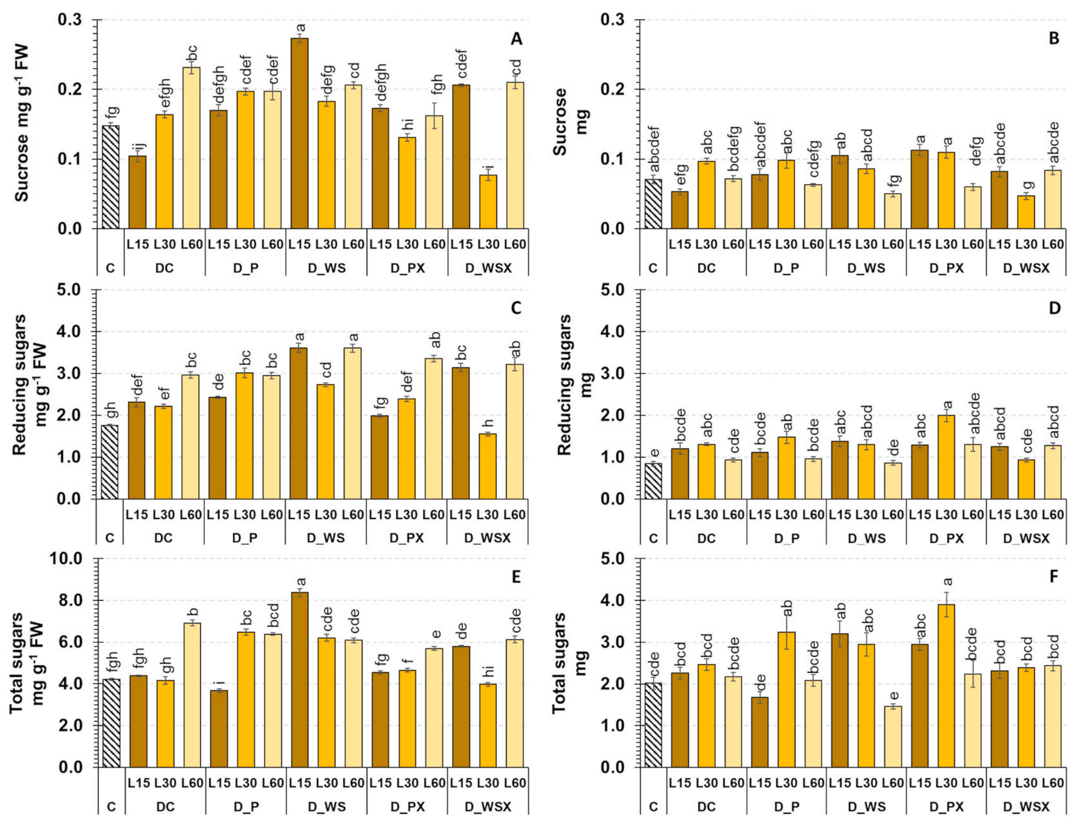

Fig. 4. Effect of treatments and digestate concentration levels on rocket microgreen sugar composition: concentration (mg g^{-1} FW) of (A) sucrose, (C) reducing sugars, and (E) total sugars, and total content per raft (mg) of (B) sucrose, (D) reducing sugars, and (F) total sugars. Bars represent mean values ± standard error. Different letters indicate statistically significant differences among treatments and concentration levels according to Tukey's post hoc test ($p < 0.05$). Treatment abbreviations: C = control (standard hydroponic solution); DC = digestate from maize silage without biochar; D_P = digestate with simplex poplar biochar; D_WS = digestate with simplex wheat straw biochar; D_PX = digestate with oxidized poplar biochar; D_WSX = digestate with oxidized wheat straw biochar. Digestate levels in nutrient solution: L15 = 1.5%, L30 = 3%, L60 = 6%.

(145.445 μg GAE g^{-1} FW), while the lowest in D_WSX at L30 (98.321 μg GAE g^{-1} FW). Similarly, the highest free polyphenol content was detected in D_PX at L30 (92.136 μg GAE) (Fig. 5B), while the lowest in D_WS at L60 (33.993 μg GAE).

Total polyphenol concentration and content also varied significantly across treatments and digestate levels (Fig. 5C and D). The highest total polyphenol concentration was observed in D_WS at L15 (503.864 μg GAE g^{-1} FW), whereas the lowest was recorded in the C (320.369 μg GAE g^{-1} FW). Similarly, total polyphenol content ranged from a maximum of 325.063 μg GAE in D_PX at L30 to a minimum of 114.767 μg GAE in D_WS at L60.

Anthocyanin concentration and total content per raft showed significant variability across treatments and digestate levels (Fig. 5E and F). The highest anthocyanin concentration was observed in D_WSX at L30 (11.696 μg C3GE g^{-1} FW), whereas the lowest was recorded in D_WS at L15 (7.073 μg C3GE g^{-1} FW). Similarly, total anthocyanin content ranged from a maximum of 9.486 μg C3GE in D_PX at L30 to a minimum of 1.965 μg C3GE in D_WS at L60.

Ascorbic acid concentration and total content per raft were likewise significantly affected by treatment and digestate level (Fig. 5G and H).

Table 3
Mean values (± standard error) for the concentration and total amount per raft of sucrose, reducing sugars, and total sugars across in rocket microgreens different treatments, levels, and blocks. Data are expressed in mg g^{-1} FW for concentrations and mg for total amounts. Significance levels (sig) are indicated for each parameter, showing differences among treatments (C, DC, D_P, D_WS, D_PX, D_WSX) and digestate concentration level (CL) (L15, L30, L60). Different letters indicate statistically significant differences among treatments according to the Tukey's HSD test at $p < 0.05$.

Source of variation	Levels	Concentration of sucrose		Tot. Cont. sucrose		Concentration of reducing sugars		Tot. Cont. reducing sugars		Concentration of total sugars		Tot. Cont. total sugars	
		mg g^{-1} FW	sig	mg	sig	mg g^{-1} FW	sig	mg	sig	mg g^{-1} FW	sig	mg	sig
Treatment	C	0.148 (0.004)	c	0.071 (0.006)	ab	1.761 (0.016)	d	0.842 (0.057)	c	4.218 (0.054)	e	2.019 (0.146)	b
	DC	0.166 (0.013)	c	0.074 (0.005)	b	2.497 (0.092)	c	1.149 (0.06)	b	5.156 (0.311)	cd	2.301 (0.075)	b
	D_P	0.188 (0.006)	b	0.08 (0.005)	ab	2.798 (0.076)	b	1.182 (0.077)	b	5.508 (0.321)	b	2.331 (0.213)	b
	D_WS	0.221 (0.01)	a	0.08 (0.007)	ab	3.314 (0.111)	a	1.182 (0.08)	b	6.885 (0.27)	a	2.536 (0.228)	b
	D_PX	0.155 (0.007)	c	0.094 (0.007)	a	2.578 (0.144)	c	1.531 (0.108)	a	4.964 (0.134)	d	3.029 (0.219)	a
	D_WSX	0.164 (0.015)	c	0.071 (0.005)	b	2.637 (0.196)	bc	1.152 (0.052)	b	5.302 (0.236)	bc	2.382 (0.076)	b
CL	C	0.148 (0.004)	b	0.071 (0.006)	ab	1.761 (0.016)	c	0.842 (0.057)	b	4.218 (0.054)	c	2.019 (0.146)	c
	L15	0.185 (0.011)	ab	0.086 (0.005)	a	2.697 (0.116)	b	1.248 (0.046)	a	5.354 (0.311)	bc	2.481 (0.129)	b
	L30	0.15 (0.008)	b	0.087 (0.005)	a	2.379 (0.097)	b	1.402 (0.079)	a	5.095 (0.202)	b	2.989 (0.15)	a
	L60	0.201 (0.006)	a	0.066 (0.003)	b	3.218 (0.062)	a	1.068 (0.051)	ab	6.24 (0.089)	a	2.078 (0.096)	c

Treatment abbreviations: C = control (standard hydroponic solution); DC = digestate from maize silage without biochar; D_P = digestate with simplex poplar biochar; D_WS = digestate with simplex wheat straw biochar; D_PX = digestate with oxidized poplar biochar; D_WSX = digestate with oxidized wheat straw biochar. Digestate levels in nutrient solution: L15 = 1.5%, L30 = 3%, L60 = 6%. Total Content = Tot. Cont.

The highest ascorbic acid concentration was observed in D_WS at L30 (0.204 mg g^{-1} FW), whereas the lowest was recorded in D_WS at L60 (0.071 mg g^{-1} FW). Similarly, total ascorbic acid content ranged from a maximum of 0.146 mg in D_PX at L30 to a minimum of 0.017 mg in D_WS at L60.

Although Treatment × Level interactions were statistically significant for all antioxidant-related parameters, the main effects are reported in Table 4 for completeness and are not further discussed.

3.5. Modeling nutrient influence on plant traits using partial least squares

The PLS analysis was used to explore the relationships between the nutrient composition of the growing medium and plant traits (Supplementary Table 7). The model accounted for multicollinearity among nutrients and captured both linear and nonlinear responses through latent variables.

For plant growth parameters, NH_4^+–N and EC showed positive linear effects on plant number, fresh weight, and dry weight, while P and Mn exhibited pronounced quadratic responses, indicating optimal concentration ranges. Fresh weight was strongly influenced by K and Mg through nonlinear effects. Pigment-related variables (Chl_a, Chl_b and carotenoids) were mainly associated with Fe and NH_4^+–N through linear effects, while quadratic terms for P, Zn, K, and Mn suggested threshold responses at higher nutrient availability.

Secondary metabolites (free and total polyphenols, anthocyanins, and ascorbic acid were influenced by both linear and quadratic nutrient effects. In particular, Mn, Se and P showed notable quadratic influences on total polyphenols, while the accumulation of ascorbic acid was significantly affected by K and NH_4^+–N through quadratic responses. Overall, the prevalence of quadratic coefficients highlighted the importance of balanced nutrient supply to avoid suboptimal or excessive concentrations.

For most variables, the model demonstrated strong predictive performance, with R^2 values exceeding 0.60 for key parameters such as fresh weight ($R^2 = 0.701$), dry weight ($R^2 = 0.680$), Chl_b concentration ($R^2 = 0.618$), anthocyanin concentration ($R^2 = 0.647$), and total anthocyanin content ($R^2 = 0.736$) (Fig. 6). Higher RMSEP values for total polyphenols (0.749) and dry weight over fresh weight (0.704) indicated lower accuracy for these traits were less reliable, likely reflecting more complex physiological regulation.

The VIP scores provided identified EC (VIP = 0.340), N (VIP = 0.281), and Se (VIP = 0.269) as the most influential predictors of plant responses. Conversely, nutrients such as Ca (VIP = 0.194) and Mo (VIP = 0.206) had comparatively lower importance.

4. Discussion

The novelty of the present work was evident from: (i) the comparison between oxidized and unoxidized biochar forms used as AD additives, (ii) the evaluation of poplar-derived biochar, a scarcely studied feedstock despite its availability from short-rotation forestry and its favourable structural and chemical properties, and (iii) the assessment of the resulting digestates in a hydroponic microgreen system, a cultivation model particularly sensitive to nutrient imbalances and thus suitable for detecting both beneficial and stress-related effects.

The present study demonstrated the multifactorial impacts of digestate-based treatments, application levels, and their interactions on the morpho-physiological, biochemical, and metabolic traits of hydroponically grown rocket microgreens. The variability observed across treatments reflected both compositional differences among digestates and the physiological plasticity of rocket microgreens in adapting to diverse nutrient and stress conditions. These findings emphasized the complexity of nutrient dynamics in hydroponic systems, where nutrient composition and dosing critically influence plant performance and metabolic regulation (Möller and Müller, 2012; Sani et al., 2025). Similar results were reported by Truzzi et al. (2025), who showed that vermicompost derived from solid and liquid digestates enhanced root biomass, leaf morphology, and secondary metabolite synthesis in both wild rocket (*Diplotaxis tenuifolia* L.) and parsley (*Petroselinum crispum* Mill.). These findings confirmed that digestate origin and application methods significantly influenced morphological and biochemical traits in leafy vegetables under controlled conditions.

Our study demonstrated that plant biomass and establishment were strongly affected by digestate type and concentration. Moderate application rates (1.5% and 3%), particularly in oxidized biochar-enriched digestates (D_PX and D_WSX), consistently improved plant density, fresh and dry biomass (Table 1), pigment content (Table 2), and antioxidant accumulation (Tables 3 and 4). Notably, D_PX at 3% increased fresh biomass by approximately 74% compared to the C, demonstrating that moderately applied, biochar co-digested digestates optimized nutrient supply to crop needs in hydroponic culture (Pelayo Lind et al., 2021).

In contrast, unoxidized wheat straw biochar (D_WS) at the highest concentration (6%) reduced growth by up to 40%, likely due to excessive EC combined with nutrient imbalance and a relative limitation of readily available nitrogen (Table 1). Such stress conditions, well-documented in previous studies, can disrupt early plant development through osmotic stress or ion toxicity (Nkoa, 2014; Pelayo Lind et al., 2021). These results were consistent with prior hydroponic research

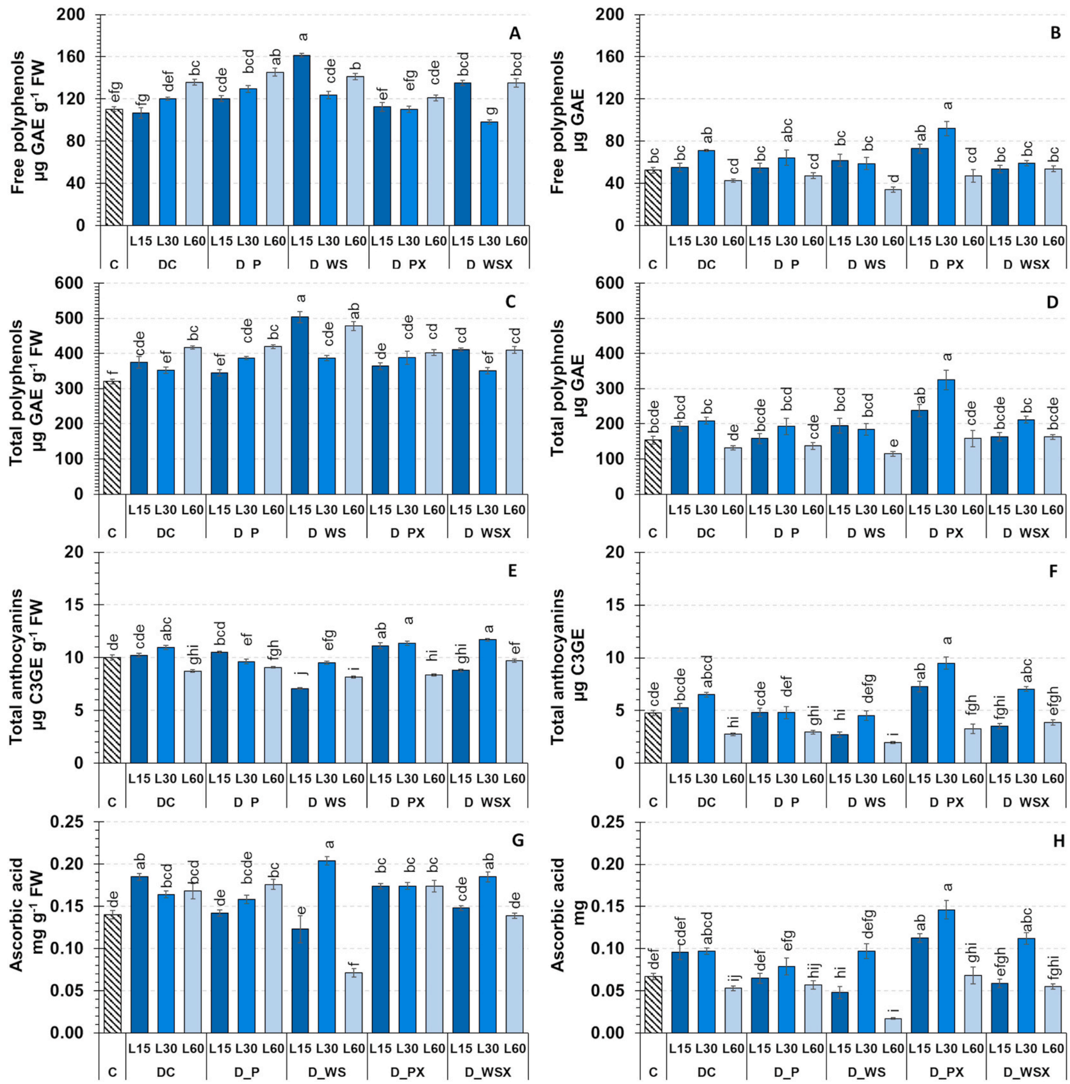

Fig. 5. Effect of treatments and digestate concentration levels on rocket microgreen antioxidant compounds: concentration (mg g^{-1} FW) of (A) free polyphenols, (C) total polyphenols, (E) total anthocyanins, and (G) ascorbic acid, and total content per raft (mg) of (B) free polyphenols, (D) total polyphenols, (F) total anthocyanins, and (H) ascorbic acid. Bars represent mean values ± standard error. Different letters indicate statistically significant differences among treatments and concentration levels according to Tukey's post hoc test ($p < 0.05$). Treatment abbreviations: C = control (standard hydroponic solution); DC = digestate from maize silage without biochar; D_P = digestate with simplex poplar biochar; D_WS = digestate with simplex wheat straw biochar; D_PX = digestate with oxidized poplar biochar; D_WSX = digestate with oxidized wheat straw biochar. Digestate levels in nutrient solution: L15 = 1.5%, L30 = 3%, L60 = 6%.

using wheat-straw-derived biochar, which demonstrated that the application increased both EC and pH, and also altered micronutrient availability, resulting in dose-dependent effects on plant growth and nutrient uptake (Kunnen et al., 2024). The inhibitory effects observed in our study at 6% D_WS may, therefore, have reflected similar salt accumulation and nutrient imbalance–related stress responses. This interpretation was further supported by the observed increase in dry-to-fresh biomass ratio at higher digestate concentration (Fig. 2), suggesting osmotic adjustment or shifts in biomass allocation toward structural tissues. These findings highlighted the delicate balance between nutrient supply and osmotic stress, especially in microgreens, which are particularly sensitive to salinity and nutrient imbalances (Ebert, 2022; Gruda et al., 2013). Overall, growth inhibition observed at the highest digestate concentration was primarily attributable to salinity-driven osmotic and ionic stress combined with relative nitrogen limitation, rather than to ammonium toxicity. It is worth noting that such salinity-related stress could potentially be mitigated under a nutrient replacement approach, in which digestate-derived nutrients either partially or fully substitute mineral fertilization. However, the present experimental design intentionally evaluated digestate supplementation on top of a standard nutrient solution, reflecting a realistic transitional scenario in hydroponic systems. Within this framework, the results identified critical application thresholds beyond which digestate addition may induce osmotic stress or relative nutrient limitations.

Photosynthetic pigment responses were treatment-dependent. D_WS increased Chl_b and carotenoids, but failed to raise total pigment content per plant due to biomass inhibition (Fig. 3), suggesting stress-induced compensatory mechanisms, as was reported previously by Lichtenthaler and Babani (2004). Conversely, D_PX and D_WSX increased both pigment concentrations and total content, particularly at 3%, suggesting improved photosynthetic capacity and plant vigor. These improvements corroborated previous findings from hydroponic lettuce systems where biochar co-digested digestates improved chlorophyll content, antioxidant production and overall plant vigor (Rosli et al., 2023). In addition, poplar-derived biochar has been shown to maintain endive plant performance and nutrient supply when used as a peat alternative in soilless systems (Sabatino et al., 2020), supporting its compatibility with controlled environment agriculture. The enhanced

Table 4
Mean values (standard error) for the concentration and total amount per raft of rocket microgreen anti-oxidant compounds across treatments (C, DC, D_P, D_WS, D_PX, D_WSX), and digestate concentration level (CL) (L15, L30, L60). Different letters indicate statistically significant differences among treatments according to the Tukey's HSD test at $p \leq 0.05$.

Source of variation	Levels	Concentration free polyphenols		Tot. Cont. free polyphenols		Concentration of total polyphenols		Tot. Cont. total polyphenols		Concentration anthocyanin		Tot. Cont. anthocyanin		Concentration ascorbic acid		Tot. Cont. ascorbic acid	
		µg GAE g^{-1} FW	sig	µg GAE	sig	µg GAE g^{-1} FW	sig	µg GAE	sig	µg C3GE g^{-1}	sig	µg C3GE	sig	mg g^{-1} FW	sig	mg	sig
Treatment	C	110.386 (2.458)	e	52.479 (2.75)	b	320.369 (6.925)	c	153.546 (11.821)	b	10.025 (0.26)	ab	4.762 (0.252)	b	0.14 (0.005)	b	0.067 (0.004)	bc
	DC	120.939 (3.401)	cd	56.197 (3.133)	ab	381.529 (8.884)	b	177.672 (9.791)	b	9.976 (0.243)	ab	4.841 (0.407)	b	0.172 (0.004)	a	0.082 (0.006)	b
	D_P	131.775 (3.03)	b	55.485 (3.249)	b	383.927 (8.27)	b	162.641 (10.621)	b	9.745 (0.166)	b	4.188 (0.31)	b	0.159 (0.004)	b	0.067 (0.004)	bc
	D_WS	141.989 (4.069)	a	51.524 (3.986)	b	456.229 (13.856)	a	164.172 (12.136)	b	8.254 (0.25)	c	3.065 (0.305)	c	0.133 (0.014)	b	0.054 (0.009)	c
	D_PX	114.48 (2.202)	de	70.713 (5.481)	a	384.759 (8.106)	b	240.297 (20.776)	a	10.293 (0.346)	a	6.672 (0.682)	a	0.174 (0.003)	a	0.109 (0.009)	a
	D_WSX	122.936 (4.521)	c	55.541 (1.69)	b	390.6 (8.111)	b	179.325 (7.657)	b	10.065 (0.302)	ab	4.802 (0.405)	b	0.157 (0.005)	b	0.076 (0.007)	b
CL	C	110.386 (2.458)	c	52.479 (2.75)	b	320.369 (6.925)	c	153.546 (11.821)	bc	10.025 (0.26)	b	4.762 (0.252)	b	0.14 (0.005)	b	0.067 (0.004)	b
	L15	127.249 (3.905)	b	59.665 (2.254)	ab	399.668 (11.566)	ab	189.326 (8.451)	b	9.552 (0.278)	b	4.715 (0.329)	b	0.155 (0.005)	ab	0.076 (0.005)	b
	L30	116.314 (2.343)	c	69.081 (3.157)	a	373.047 (5.581)	b	224.336 (12.341)	a	10.636 (0.183)	a	6.467 (0.379)	a	0.177 (0.004)	a	0.106 (0.005)	a
	L60	135.709 (2.045)	a	44.93 (1.896)	c	425.511 (6.135)	a	140.802 (6.076)	c	8.812 (0.111)	c	2.959 (0.155)	c	0.146 (0.008)	ab	0.05 (0.004)	c

Treatment abbreviations: C = control (standard hydroponic solution); DC = digestate from maize silage without biochar; D_P = digestate with simplex poplar biochar; D_WS = digestate with simplex wheat straw biochar; D_PX = digestate with oxidized poplar biochar; D_WSX = digestate with oxidized wheat straw biochar. Digestate levels in nutrient solution: L15 = 1.5%, L30 = 3%, L60 = 6%. Total Content = Tot. Cont.

performance of D_PX and D_WSX in our study further emphasized the benefits of oxidized biochar in stabilizing nutrients and reducing phytotoxic risks during hydroponic cultivation. Such nutrient efficiency is central to the advantages of hydroponic systems, especially for fast-growing leafy crops such as microgreens and baby leaves (Di Gioia et al., 2021).

Sugar metabolism exhibited similar patterns. D_WS increased reducing sugars at both 1.5% and 6%, but total sugar remained low due to the inhibition on biomass which was evidenced from comparing the concentration to the total experimental content (Fig. 4). This result was consistent with stress-induced carbohydrate reallocation toward osmoprotective functions rather than growth under stress (Braun et al., 2014; Lal et al., 2022). In contrast, D_PX and D_WSX treatments at 3% promoted both higher sugar concentrations and biomass accumulation, suggesting enhanced sucrose synthesis, efficient phloem transport, and carbon partitioning to growing tissues. These results are supported by previous reports on oxidized biochar improving nutrient availability while mitigating phytotoxic effects (Ahmad et al., 2014; Rosi et al., 2024). Beyond nutrient dynamics, the capacity of biochar to adsorb organic pollutants (Parlavecchia et al., 2020) adds value in closed-loop systems where contamination risks from digestate residues may persist.

The antioxidant profile (comprising polyphenols, anthocyanins, and ascorbic acid) was significantly influenced by treatment type and concentration, showing distinct dose–response patterns (Fig. 5). D_WS treatments increased polyphenol concentrations at both 1.5% and 6%, consistent with mild abiotic stress activating the phenylpropanoid pathway (Lee et al., 2021; Libutti et al., 2023). However, the highest total antioxidant yield per plant occurred in D_PX treatments at both 1.5% and 3%. This suggested that moderate stress, combined with enhanced nutrient availability, may have stimulated secondary metabolism without compromising growth. Our results were shown to support the concept that controlled stress can increase the antioxidant capacity of microgreens, contributing to their superior nutritional profile compared to mature vegetables (Di Gioia et al., 2023; Xiao et al., 2012).

Anthocyanin and ascorbic acid dynamics mirrored those of polyphenols. D_PX at 3% produced the highest concentrations of components, highlighting a synergistic effect of biochar oxidation and digestate application on antioxidant biosynthesis. This suggested an enhanced flux through the flavonoid biosynthesis pathways under improved nutrient availability and moderated oxidative signaling (Fan et al., 2025; Smirnoff and Wheeler, 2024). Conversely, D_WS at 6% reduced ascorbic acid, likely due to metabolic disruption under sustained stress, in which excessive oxidative load impaired ascorbate regeneration and redox homeostasis. These findings highlighted the sensitivity of microgreens to nutrient imbalances in organic-amended hydroponics (Bergstrand et al., 2020; Di Gioia et al., 2023; Nkoa, 2014).

The PLS regression analysis elucidated complex interactions between nutrient availability and plant parameters. Key macro- and micronutrients, especially NH_4^+–N, K, P, Mn and Se, exhibited both linear and quadratic effects on multiple responses (Supplementary Table 7). Mn and P showed non-linear associations with polyphenol accumulation, suggesting optimal concentration thresholds, beyond which effects either plateau or decline. Similarly, ascorbic acid displayed non-linear correlations with NH_4^+–N and K, indicating that excessive macronutrients may disrupt redox homeostasis or carbon allocation. Although NH_4^+–N emerged as an influential variable in the PLS model, its role should be interpreted in the context of relative availability rather than toxicity. In the present study, increasing digestate concentration was associated with reduced ammoniacal nitrogen levels compared to the C, suggesting that nitrogen limitation, rather than ammonium excess, may have contributed to growth inhibition under high digestate application rates. These patterns were consistent with previous findings (Park et al., 2019), reinforcing the critical role of nutrient balance in sensitive hydroponic crops.

The PLS model showed strong predictive power for fresh weight (R^2

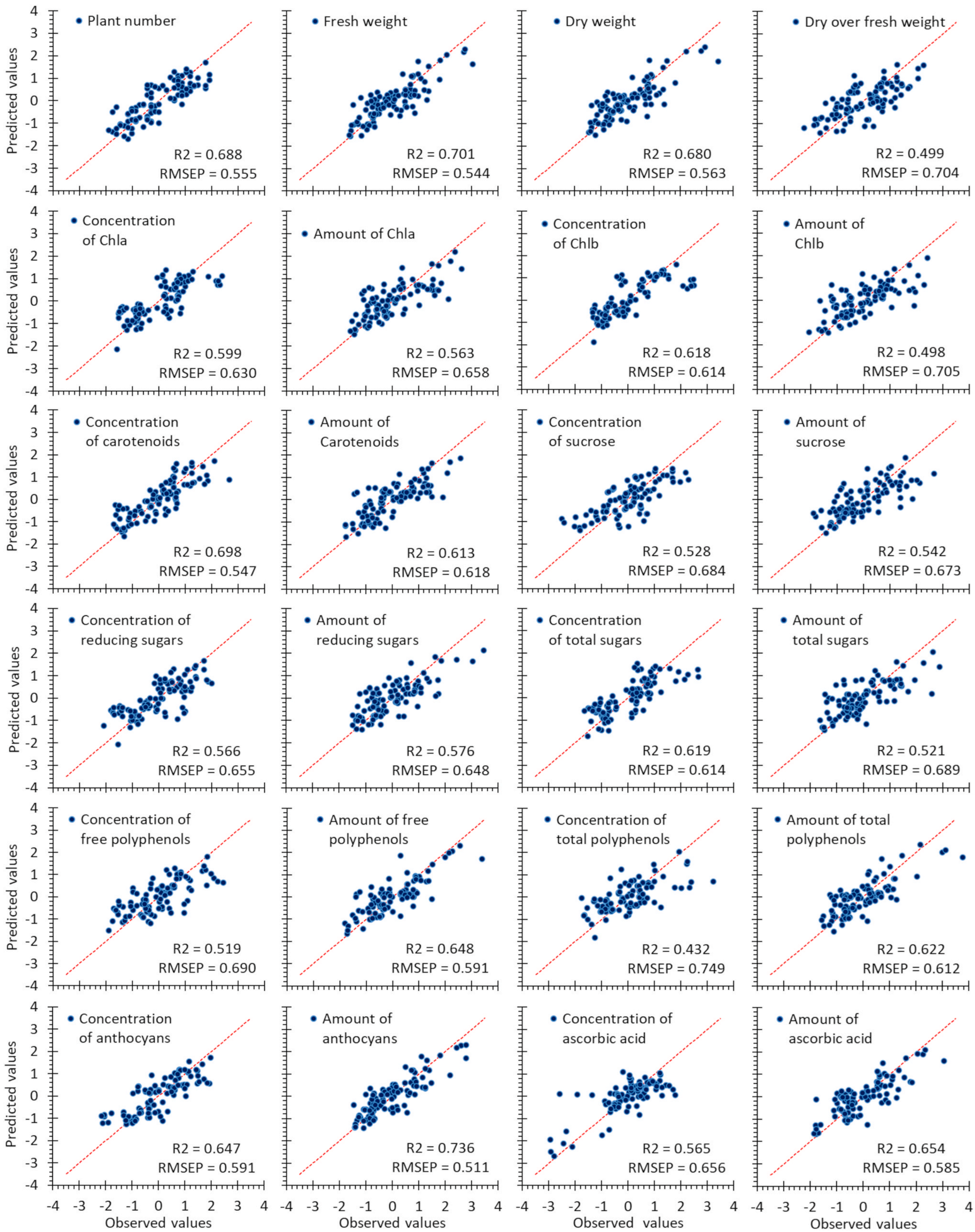

Fig. 6. Scatter plots comparing observed vs. predicted values for key plant physiological parameters based on the Partial Least Squares (PLS) regression model. The solid red diagonal line represents the ideal 1:1 correlation between observed and predicted values. Each subplot displays the coefficient of determination (R^2) and the Root Mean Square Error of Prediction (RMSEP), indicating the predictive performance of the model for each parameter. Higher R^2 values suggest a better fit of the model, while lower RMSEP values indicate higher predictive accuracy.

= 0.701), dry weight (R^2 = 0.680), Chl_b (R^2 = 0.618), and anthocyanins (R^2 = 0.736), confirming its effectiveness in capturing complex nutrient–trait relationships. Lower R^2 values for total sugars (0.521) and polyphenols (0.580) indicated that additional factors, including genotype and environmental variability, played a significant role in regulating these traits, corroborating previous findings by Damerum et al. (2015).

The VIP scores highlighted EC, NH_4^+–N, and Se as the most influential predictors (Fig. 7). EC was the primary driver of biomass and pigment traits, underscoring the critical need to manage salinity in organic-amended hydroponics, where ionic strength fluctuates (Conversa et al., 2021). The positive influence of Se on antioxidant traits emphasized its role in stress tolerance and redox regulation (Farag et al., 2022; Liu et al., 2022).

Beyond trait-specific insights, the use of multivariate modeling also provides a powerful tool to interpret plant responses to complex nutrient solutions. By identifying key drivers such as EC, NH_4^+–N, and Se, this approach supported the optimization of hydroponic formulations, enabling the enrichment of beneficial components while limiting or mitigating phytotoxic factors. These insights illustrated both the challenges and the opportunities of using organic nutrients like digestate-based nutrients in hydroponics, given their inherent variability and potential salinity risks (Bergstrand et al., 2020). In this context, Pelayo Lind et al. (2021) demonstrated that biogas digestate can serve as a sole nutrient source in soilless cultivation when managed appropriately. That study showed that through controlled nitrification to reduce ammonium levels and regulate pH, it was possible to attain hydroponic Chinese cabbage (bok choy) yields comparable to mineral fertilizers. This supported broader applications of biochar co-digested digestates in hydroponics where nutrient transformations and delivery are carefully managed.

Overall, our results highlighted the critical importance of digestate quality and precise application strategies to optimize hydroponic crop performance. Our results corroborated those of Bini et al. (2025), showing that moderate biochar additions improve plant growth and nutrient quality in soilless systems, while excessive amounts can negatively affect growth by increasing salinity and pH. Similarly, our study demonstrated that low to moderate doses of oxidized biochar-enriched digestates (D_PX and D_WSX) enhanced microgreen biomass and antioxidant content, whereas higher doses or unoxidized biochar (D_WS) induced stress. The stress was likely attributable to nutrient imbalances and salinity. Although our work used biochar-modified digestates in hydroponics, whereas Bini et al. (2025) applied biochar directly as a

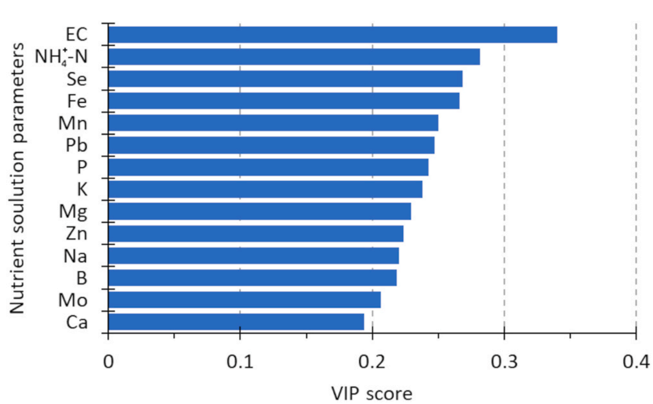

Fig. 7. Variable Importance in Projection (VIP) scores of the nutrient solution parameters derived from the Partial Least Squares (PLS) regression model. Higher VIP scores indicate greater importance in explaining the variance of the dependent variables. Nutrient solution parameters: electrical conductivity (EC) and ammoniacal nitrogen (NH_4^+-N), selenium (Se), iron (Fe), manganese (Mn), lead (Pb), phosphorous (P), potassium (K), magnesium (Mg), zinc (Zn), sodium (Na), molybdenum (Mo) and calcium (Ca).

substrate amendment, both approaches emphasized the importance of careful biochar processing and dosage to maximize benefits and avoid phytotoxicity.

Careful selection and processing of biochar can mitigate negative effects such as nutrient imbalances and salinity stress, thereby improving nutrient uptake and plant growth in controlled environments. The present results are consistent with circular economy principles, where resource recovery from organic waste, including modified anaerobic digestion, supports sustainable agriculture (J. Chen et al., 2024; Falcon et al., 2022).

Despite the robustness of our experimental design, this study demonstrated six distinct limitations that should be considered when interpreting the results. First, the experiment was conducted under controlled hydroponic conditions using a single crop species (rocket microgreens) and a short cultivation cycle, which may limit generalization of the findings to other crops, growth stages and production systems, respectively. Second, the digestates were obtained from a specific anaerobic digestion process (maize silage co-digested with biochar at fixed rates) under defined operational conditions, meaning that variability in feedstock composition, biochar characteristics and digestion parameters in real-world applications could yield different outcomes. Third, while our analysis included a broad range of morphological, biochemical, and metabolic traits, we did not directly assess root development, microbial interactions in the rhizosphere and long-term effects of repeated applications, which are likely to influence nutrient uptake and plant performance. Fourth, plant nutrient uptake and full nutrient balances were not directly assessed, as nutrient concentrations in the nutrient solution were not monitored throughout the cultivation cycle. Given the short growth period of microgreens and the focus on harvest-stage quality traits, the study aimed to evaluate agronomic and physiological responses to different nutrient regimes rather than nutrient uptake kinetics or absolute nutrient use efficiency. This approach reflects common hydroponic practice, where nutrient solutions are typically adjusted and replenished rather than discarded after each cycle. Accordingly, the present results should be interpreted as indicative of the implications of introducing digestate-derived nutrients into an existing fertigation regime, rather than as an optimization of nutrient-efficient fertilization strategies. Fifth, the study did not evaluate the potential risks related to residual contaminants in digestates, which could be relevant for food safety in closed-loop hydroponic systems. Finally, this study did not address the economic feasibility or life-cycle environmental implications associated with the production and use of biochar-enriched digestates in hydroponic systems. Factors such as biochar production costs, energy requirements, digestate handling, and potential trade-offs between agronomic benefits and environmental burdens were beyond the scope of this work and warrant further investigation.

Future research should therefore, not only focus on these limitations, but also focus on elucidating the mechanisms underlying the biochar-induced changes in nutrient dynamics and root physiology. Moreover, long-term impacts on crop yield and quality under diverse hydroponic conditions also necessitate further assessment. Integrating genotype-specific responses and environmental factors will be essential towards developing tailored fertilization protocols that maximize both sustainability and productivity, while ensuring agronomic reliability and product safety.

5. Conclusions

The present study demonstrated that oxidized biochar-enriched digestates represent effective and safe organic nutrient sources for hydroponic rocket microgreens. When applied at moderate concentrations (1.5–3%), these amendments significantly improved plant growth, pigment accumulation, and antioxidant profiles, indicating enhanced nutrient availability and reduced phytotoxic effects. Conversely, high concentrations of unoxidized digestates, particularly those derived from wheat straw, negatively affected plant performance, primarily due to excessive EC and the onset of osmotic and ionic stress coupled with relative nitrogen limitation.

Overall, oxidized biochar forms proved superior to unoxidized ones, confirming a greater agronomic suitability. Between oxidized variants, poplar-derived digestate (D_PX) generally sustained higher biomass and antioxidant accumulation than wheat-derived digestate (D_WSX), although both showed clear advantages in comparison to the respective simplex forms. Moderate doses (1.5–3%) were consistently more effective than C or high dose (L60), as they balanced nutrient supply with minimal stress, whereas excessive application levels induced salinity-related stress and nutrient imbalance.

The significant interaction between digestate type and application level highlighted the need for dose-specific nutrient management strategies. PLS-R proved valuable for identifying which nutrients should be prioritized for dose optimization, thereby supporting targeted and efficient fertilization strategies. The detection of quadratic responses further emphasized the importance of precise input calibration, even for potentially beneficial elements.

The integration of oxidized biochar into digestate-based fertilizers presents a promising strategy for supplementing conventional hydroponic fertilization with recovered nutrients, while maintaining crop performance and enhancing nutritional quality in circular, soilless cultivation systems. When combined with multivariate modelling approaches, this strategy may support the development of more sustainable hydroponic practices by identifying optimal application ranges and minimizing stress-related effects. Future research should focus on validating these results under commercial-scale conditions, across a broader range of crops, and over multiple production cycles.

CRediT authorship contribution statement

Stefania Truschi: Writing – original draft, Visualization. **Luca Rosi:** Writing – review & editing, Visualization, Supervision, Funding acquisition. **Marco Cenni:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Roberto Vivoli:** Resources, Investigation, Data curation. **Marco Napoli:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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.

Acknowledgements

The authors thank Dr. Anne Whittaker Ph.D., for her valuable assistance with English language revision.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2026.147960.

Data availability

Data will be made available on request.

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