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Effect of Circular-Economy-Derived Experimental Biostimulants on Growth and Development of Soilless Strawberry Plants in Greenhouse Conditions

Samreen Nazeer[1] | Anna Agosti[1] | Leandra Leto[1] | Ali Amini[2] | Andrea Remelli[2] | Andrea Di Fazio[1] | Andrea Turolla[2] | Angela Paterna[3] | Estella Rao[3] | Martina Cirlini[1] | Benedetta Chiancone[1,3]

[1]Università di Parma, Parma, Italy | [2]Department of Civil and Environmental Engineering (DICA), Politecnico di Milano, Milano, Italy | [3]National Research Council (CNR), Institute of Biophysics, Palermo, Italy

Correspondence: Samreen Nazeer (samreen.nazeer@unipr.it) | Benedetta Chiancone (benedetta.chiancone@unipr.it)

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Keywords: biostimulants | *Fragaria vesca* | fruit quality | nutrient uptake | plant growth | sustainable agriculture

ABSTRACT

Increasing awareness of the adverse effects of agrochemicals is driving farmers to adopt more sustainable strategies for enhancing plant growth. Biostimulants have gained significant attention in both academic research and the horticultural industry as promising tools to promote growth, improve stress tolerance, and enhance crop performance, while reducing environmental impacts. This study, conducted from March to July 2024, evaluated the effects of different experimental biostimulants (EBs) on the growth, development, and productivity of wild strawberry plants (*Fragaria vesca* L., cv. "Malga") cultivated under greenhouse soilless conditions. Despite their diverse origins, all tested EBs were sustainably derived from circular economy processes: (i) a suspension of freeze-dried microalgae (*Tetraselmis chuii*) (MA), (ii) a suspension of a lyophilized microbial mixed culture of purple non-sulfur bacteria (PNSB), and (iii) a hydro-alcoholic extract of pulverized hop biomass (HE). The EBs were applied weekly to the substrate: MA and PNSB at concentrations of 5% and 10%, and HE at 15 and 30 mL/L. Morphological and physiological parameters were recorded weekly until plant uprooting, while biochemical analyses were performed on leaves and fruits at the end of the trial. Application of EBs significantly enhanced plant growth and yield. MA treatments resulted in the greatest plant height, antioxidant activity, and total phenolic content; PNSB promoted the highest total root length and both fresh and dry biomass; and HE at 30 mL/L produced the greatest fruit number per plant. These results underline the potential of biostimulants to optimize strawberry production in controlled environments and contribute to more sustainable agricultural practices.

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1 | Introduction

The excessive use of chemical fertilizers in modern agriculture has significantly contributed to climate change, primarily through the release of greenhouse gases such as nitrous oxide, which has a global warming potential nearly 300 times greater than that of carbon dioxide (Mahankale 2024). These fertilizers have also been linked to soil and organic matter degradation, biodiversity loss, and water pollution through runoff (Bishnoi 2018).

Wild strawberry (*Fragaria vesca* L.), a high-value fruit crop valued for its richness in vitamins, antioxidants, and minerals, is widely cultivated worldwide (Fierascu et al. 2020). Despite its importance, open-field strawberry cultivation faces major challenges, including pests, diseases, and unpredictable weather conditions, all of which negatively affect yield and fruit quality (Swain et al. 2023). Soilless cultivation systems provide a promising solution by allowing precise control over water and nutrient availability (Wallach 2019).

To address both the environmental issues associated with excessive fertilizer use and the specific challenges of strawberry cultivation, organic fertilizers and biostimulants have emerged as sustainable alternatives. Organic fertilizers improve soil structure, enhance water retention, and supply nutrients in a slow-release form, thereby reducing environmental impacts while supporting crop productivity and agricultural sustainability (Rouphael and Colla 2020). Biostimulants, on the other hand, represent innovative tools to improve strawberry production in greenhouse systems.

Algae-based biostimulants enhance plant resilience by providing bioavailable nutrients, phytohormones, and stress-mitigating compounds (Li et al. 2024). Applications of microalgae (MA) in strawberry cultivation have been shown to increase fruit yield, quality, and antioxidant content by stimulating the biosynthesis of secondary metabolites (Žunić et al. 2024). Several strains, including *Chlorella vulgaris*, *Chlorella pyrenoidosa*, *Acutoseamua dimorphus*, and *Spirulina platensis*, have demonstrated strong potential for enhancing plant growth and yield (Dineshkumar et al. 2019). However, no studies have investigated the effect of the marine microalga *Tetraselmis chuii* (T. chuii) on strawberry plants. *T. chuii* is of particular biotechnological and nutritional interest due to its rich biochemical profile and functional properties. It exhibits a rapid growth rate, can be cultivated efficiently on a large scale under controlled conditions, and thus represents a sustainable and scalable resource (Čmiková et al. 2025). Recognized for its safety and nutritional benefits, *T. chuii* has been approved by the European Commission as a novel food and acknowledged as a functional food ingredient, (Cruz and Vasconcelos 2023) further supporting its potential applications in human health, wellness, and nutraceutical development.

Purple non-sulfur bacteria (PNSB) are phototrophic microorganisms that have recently attracted attention for their remarkable metabolic versatility and their ability to produce valuable compounds such as 5-aminolevulinic acid (5-ALA) and indole-3-acetic acid (IAA), both of which play important roles in supporting plant growth and improving crop yield and quality in sustainable systems (Sakarika et al. 2020). For example, application of PNSB to *Chenopodium formosanum* Koidz enhanced plant height, leaf chlorophyll content, shoot and root dry weight, root length, and root volume (Sundar et al. 2024).

Hop leaves are another promising resource, as they contain phenolic constituents including flavanols, prenylated flavonoids, and proanthocyanidins, all associated with antioxidant, antimicrobial, and phytoestrogenic activities (Sabbatini et al. 2024). Traditionally considered an agricultural byproduct, hop vegetative biomass remains underexploited as a raw material for value-added applications. To the best of the authors' knowledge, no studies have explored the use of hop extracts as biostimulants in crop production. Nevertheless, their richness in bioactive compounds makes them worthy of investigation in strawberry cultivation.

The main objective of this study was to evaluate the potential of experimental biostimulants (EB) derived from circular processes on the morpho-physiological, biochemical, and organoleptic quality traits of strawberry plants cultivated in greenhouse soilless systems. Three products were tested at different concentrations: a suspension of freeze-dried microalgae (*T. chuii*), a suspension of a lyophilized microbial mixed culture of PNSB, and a hydro-alcoholic extract obtained from pulverized hop vegetative biomass (HE).

2 | Materials and Methods

2.1 | Plant Material

The study was conducted using wild strawberry plants (*Fragaria vesca* L.) cv. Malga. This cultivar is recognized for its robust fruit production and for fruits with a pleasant aroma, firm texture, and juicy flesh. Moreover, cv. Malga fruits are particularly appreciated for their balanced flavor, which combines sweetness with a subtle tartness, making it an ideal choice for research purposes (Nour 2021).

2.2 | Plant Growing Conditions and Experimental Design

The experiment was carried out in a greenhouse from 25 March 2024 to 1 July 2024, lasting 14 weeks (hereafter referred to as WAT, weeks after transplanting). Wild strawberry plants, purchased from a commercial nursery, were transplanted into pots ($60 \times 17 \times 15$ cm) filled with a substrate specifically formulated for strawberry cultivation, consisting of peat moss (*Sphagnum* acid peat), perlite, clay, and sand (Techno Grow, Calvisano, BS, Italy). The substrate was not sterilized, as per the manufacturer's specifications, since it was free of major soilborne pathogens at the time of purchase. Plants were irrigated as needed, based on substrate moisture, plant response, and climatic conditions, but were not fertilized.

2.3 | Experimental Biostimulants Preparation

2.3.1 | Microalgae (MA)

Tetraselmis chuii (strain CCAP 66/21b) was cultivated in F/2 medium (Guillard 1975) prepared with 20 nm-ultrafiltered

clean seawater. Cultures were maintained under sterile conditions for 4 weeks in a climatic chamber at 19°C ± 1°C, with continuous aeration. Illumination was provided by a combination of warm and white light, following a 14:10 h light/dark photoperiod. Algal growth was monitored throughout the culture period by measuring optical density (OD) (Paterna et al. 2022). Cells were harvested by centrifugation using a Sorvall × Pro Series centrifuge (Thermo Scientific) at 10,000 g for 10 min at 4°C in 50 mL Falcon tubes, yielding fresh biomass pellets. Residual salts were removed by washing the pellets three times with distilled water. The washed biomass, obtained as a by-product from extracellular vesicle isolation in microalgal cultures, was freeze-dried (ALPHA 1-2 LDplus, Martin Christ, Germany).

2.3.2 | Purple Non Sulfur Bacteria (PNSB)

A mixed culture of PNSB was enriched from an environmental sample collected from a pond in a peri-urban wetland near Milan (Northern Italy) and cultivated according to Amini et al. (2025). Briefly, after enrichment, PNSB were grown in batch cultures in flat-plate flasks illuminated with monochromatic 850 nm radiation (40 W/m^2 incident light intensity) at 30 ± 2°C and pH 6.8–7.2. Deproteinated cheese whey, a dairy by-product, was fermented in a 1-L AnSBR and used as a carbon source at 1.8 g Chemical Oxygen Demand (COD)/L in modified Ormerod medium. Once the substrate was depleted and stationary phase reached, the mixed culture was collected and centrifuged at 5000 rpm for 10 min at room temperature. The pellet was pooled in Petri dishes, frozen at −80°C, and lyophilized for 48 h (Coolsafe PRO 55–4, LaboGene).

2.3.3 | Vegetative Hop Biomass Extracts (HE)

At the end of the growing season, hop vegetative biomass (leaves, branches, and discarded cones) was collected at Azienda Agricola Ludovico Lucchi (Campogalliano, Emilia-Romagna, Italy; 44°41′25.36″ N, 10°50′20.03″ E). The biomass was dried at 35°C using a low-temperature dryer (PKT 2002, Packtin srl, Reggio Emilia, Italy) until the final moisture content reached 0.5%. Dried biomass was ground with a hammer mill (Rapide 200Z, Nuova S.r.l., Villanova sull'Arda, Italy) and sieved (1000 μm) to obtain fine powder. Extraction was performed with ethanol:water (80:20 v/v) at a 1:20 solid-to-liquid ratio. The suspension was agitated for 2 h at room temperature (200 strokes/min) using a digital shaker (HS 501, IKA-Werke GmbH & Co., Staufen, Germany). The liquid extract was separated from solid residues by centrifugation at 5000 rpm for 10 min at room temperature (centrifuge 4206, Alc International, Pévy, France) (Martelli et al. 2020).

2.4 | Experimental Design

The treatments were as follows: (i) MA at 5% (MA5), (ii) MA at 10% (MA10), (iii) PNSB at 5% (PNSB5), (iv) PNSB at 10% (PNSB10), (v) HE at 15 mL/L (HE15), (vi) HE at 30 mL/L (HE30), and (vii) a control treated only with distilled water (C). Three plants were grown per pot, with two pots per treatment. Biostimulants were freshly prepared each week. For MA and PNSB, 50 g (5%) or 100 g (10%) of lyophilized powder were suspended in distilled water and adjusted to 1000 mL. For HE, 15 mL (HE15) or 30 mL (HE30) of extract were diluted in 1000 mL of distilled water.

2.5 | Data Collection

Plants were monitored every 2 weeks, and the following parameters were recorded: plant height (cm), measured with a tape from the crown base to the longest leaf, and the number of leaves (n°). Physiological parameters including leaf flavonoid content (FLV, F660nm/F325nm), chlorophyll content (CCLa, T850nm/T720nm), and soil plant analysis (SPAD, T650nm/T940nm) were also assessed using a Multiple Pigment Meter (MPM-100S, Bio Scientific Ltd., Herts, England).

At harvest, fruits were characterized for fresh weight (g) using an electronic scale (KERN EMB 1000-2, Vicenza, Italy), while berry length and width were determined with a digital vernier caliper (Moore & Wright MW110-15DFC Fractional, Burgess Hill, UK). Fruit yield was expressed as the total number of fruits harvested per plant. Firmness (kg/cm^3) was measured with a "Geotester Pocket Penetrometer" equipped with a 3 mm plunger, placing the fruit horizontally on a stand. Skin color was evaluated according to the CIE Lab color space, where L* indicates lightness, a* the red/green coordinate, and b* the yellow/blue coordinate, using a portable colorimeter (CM 2600d, Minolta Co., Osaka, Japan).

Leaf and fruit nutrient contents (K^+, NO_3^-, Ca^{2+}) were measured with a LAQUAtwin pocket meter (Danileyko et al. 2023).

For biochemical analyses, fruits were crushed to obtain juice for pH determination (LLG-pH meter 5, Hyde Manchester, UK) and total soluble solids (TSS, °Brix) using an optical portable refractometer (Hanna Instruments, Padova, Italy). Extracts were prepared by mixing 1 g of fruit with 20 mL of a 70:30 methanol–water solution and leaving the mixture at room temperature for 2 h, following a procedure partially adapted from Contessa et al. (2013).

Total phenolic content (TPC) was determined using Folin–Ciocalteu's reagent with modifications. Briefly, 250 μL of extract were mixed with 1 mL of aqueous Folin–Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA, 1/10 v/v) and 2 mL of sodium carbonate solution (20% w/v), and incubated in the dark for 30 min. Absorbance at 760 nm was measured with a spectrophotometer (JASCO V-530, Easton, MD, USA). Quantification was based on a calibration curve prepared with gallic acid (10–100 mg/kg). Each sample extract was analyzed in duplicate, with the instrument software programmed to perform three consecutive readings per replicate. Results were expressed as mg GAE/g.

Antioxidant capacity was assessed using the DPPH radical scavenging assay (Aldrich, St. Louis, MO, USA). Five standard Trolox solutions (0.1–1 mmol/L) were prepared to construct a calibration curve, and a blank (100 μL extraction solution) was analyzed under identical conditions. Radical inhibition percentage (I%) was calculated as: I% = [(AbsB-AbsS)/AbsB] *100, where AbsB is the

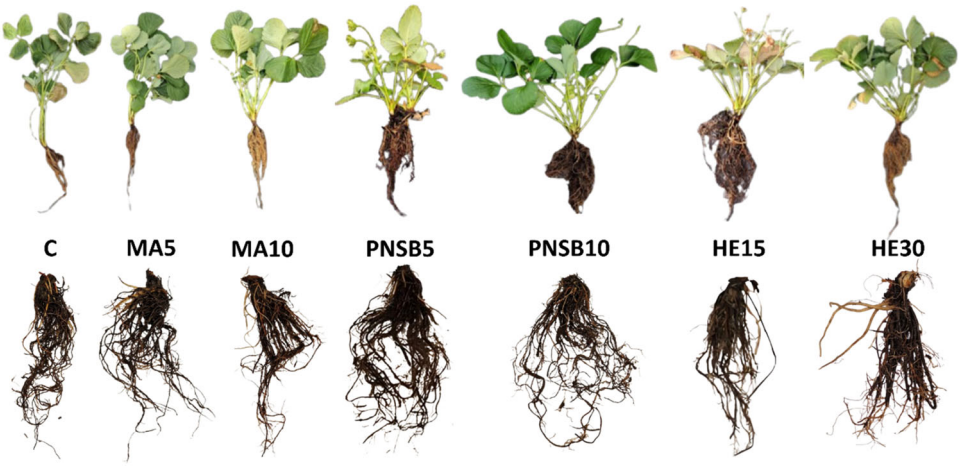


FIGURE 1 | Features of whole plants and root apparatus from different treatments, at uprooting time.

absorbance of the blank and AbsS the absorbance of the sample/Trolox solution. Results were expressed as mg/g Trolox Equivalent Antioxidant Capacity (TEAC). All analyses were performed in duplicate, with three consecutive measurements for each sample.

At 14 weeks after transplanting (14 WAT), plants were uprooted (Figure 1), and fresh and dry weights of the whole plant (PFW, PDW) and roots (RFW, RDW) were measured using a digital balance (KERN EMB 1000-2, Vicenza, Italy). Fresh weights were recorded immediately, while dry weights were obtained after oven-drying at 70°C for 48 h.

For a representative sample of six plants per treatment, root traits were further analyzed (Figure 1), including total root length (TRL, cm), length per volume (L/V, cm/m^3), total projected area (TPA, cm^2), total surface area (TSA, cm^2), average diameter (AD, mm), and root volume (RV, cm^3), using a root scanner (WinRhizo STD 4800, software). Roots were carefully washed with distilled water, gently dried, and evenly arranged on the scanner tray. A thin water layer was added to improve contrast and prevent curling. The scanner lid was then closed to avoid displacement, resolution set at 600 dpi, and scans acquired. Images were analyzed and data recorded.

2.6 | Statistical Analysis

All experimental data were first tested for normality and homogeneity of variance. Repeated-measures Analysis of Variance (ANOVA) was applied to plant height, leaf number, flavonoid content, chlorophyll content, and SPAD, followed by Tukey's HSD test for multiple comparisons at $p \leq 0.05$, using IBM SPSS Statistics 29.0.1.0 (SPSS Inc., Chicago, IL, USA).

One-way ANOVA was performed for fruit traits (fresh weight, length, width, firmness, yield, and skin color L*, a*, b*), root traits (TRL, L/V, TPA, TSA, AD, RV), nutrient content in leaves and fruits (K^+, NO_3^-, Ca^{2+}), and biochemical parameters (pH, TSS, TPC, DPPH), also followed by Tukey's HSD test at $p \leq 0.05$.

Plant responses to experimental biostimulants at different concentrations were summarized using a heatmap generated with the MetaboAnalyst 6.0 web tool. Data were log10-transformed, and Euclidean distance with hierarchical clustering was applied. Results were visualized with a color scale (red = increased values, blue = decreased values).

3 | Results

3.1 | Morphological Parameters

Plant height increased progressively from 2 WAT to 14 WAT, but significant differences were observed among treatments. The tallest plants ($p < 0.001$) were consistently those treated with MA10, whereas the lowest height values were recorded for HE30 and PNSB5 (Figure 2a, Table SM1).

Although HE30 plants displayed the lowest plant height, they consistently developed the highest number of leaves throughout the experiment. In contrast, control (C) plants produced significantly fewer leaves than all treated groups (Figure 2b, Table SM1).

3.2 | Physiological Parameters

Periodic measurements of leaf flavonoid content revealed dynamic responses to the biostimulants. At 4 WAT, control plants showed a significantly higher flavonoid content ($p < 0.001$) compared to all treated plants, regardless of biostimulant type. However, 2 weeks later, the trend reversed: the highest flavonoid levels were recorded in MA5- and HE30-treated plants, while the lowest values occurred in control and HE15 plants (Figure 3a).

By contrast, leaf chlorophyll content and SPAD index were not significantly influenced by treatments at any monitoring time, and values remained statistically comparable. Nonetheless, both parameters showed a slight decline from the first assessment to the end of the trial (Figure 3b,c).

3.3 | Fruit Quality Attributes

From 8 WAT until the end of the experiment, ripe fruits were harvested. The number of fruits per plant was not

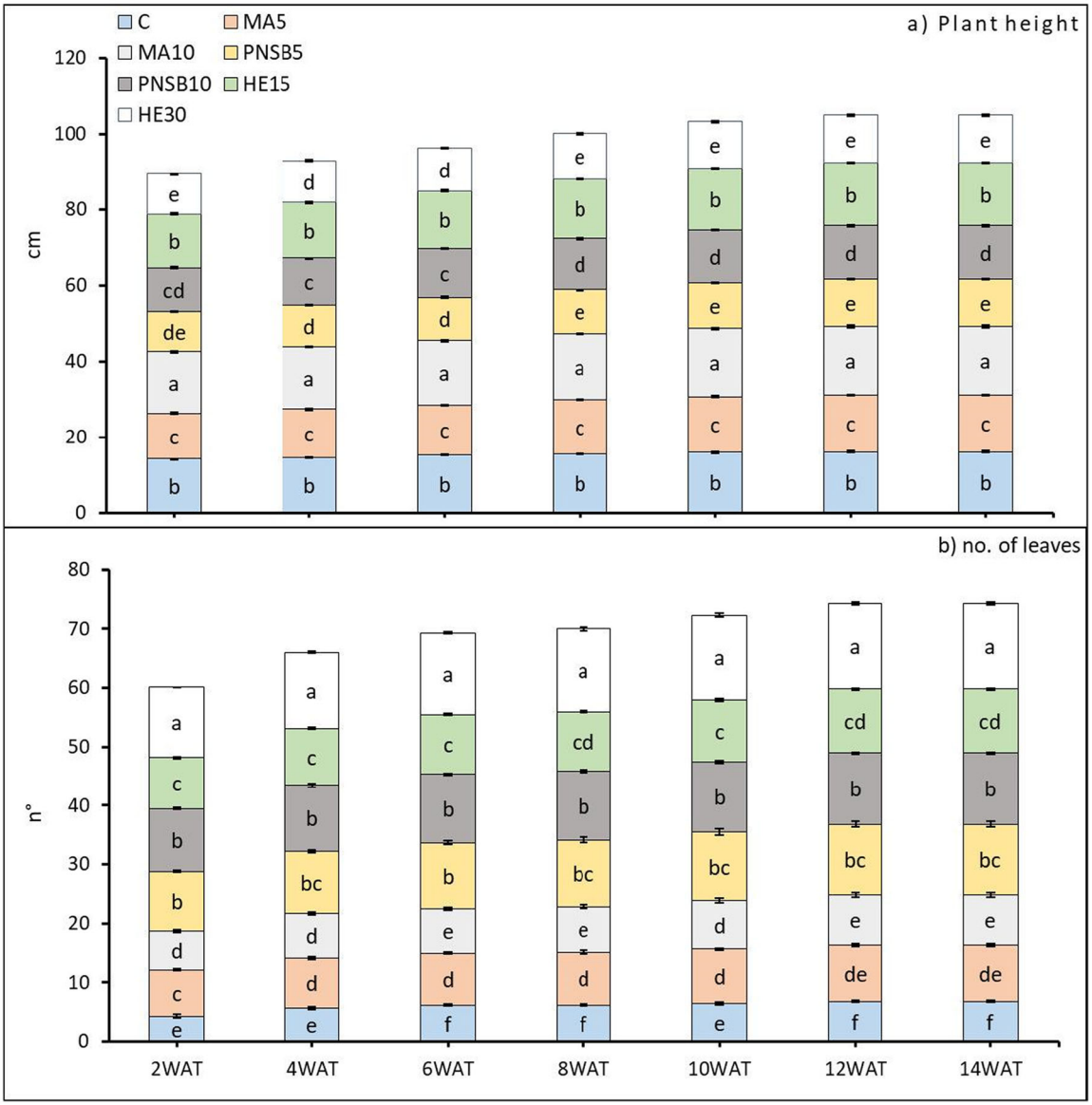


FIGURE 2 | (a) Plant height and (b) no. of leaves of strawberry plants under different biostimulants application at different monitoring times. Within each date, different letters indicate values statistically different. Repeated measure Analysis of Variance (ANOVA). Tukey's HSD test for multiple comparison at $p \leq 0.05$, using IBM SPSS Statistica 29.0.1.0 software (SPSS Inc., Chicago, IL, USA). C, control; HE15, hop extract at 15 mL/L; HE30, hop extract at 30 mL/L; MA5, microalgae at 5%; MA10, microalgae at 10%; PNSB5, purple non-Sulphur bacteria at 5%; PNSB10, purple non-Sulphur bacteria at 10%; WAT, weeks after transplanting.

significantly affected by treatments, averaging three fruits per plant (Table 1). Hop extract treatments (HE15 and HE30) significantly increased fruit fresh weight, with fruits being nearly twice as heavy as those from control plants. Fruit weight in MA- and PNSB-treated plants was comparable to controls (2.63 ± 0.06 g for MA, 2.77 ± 0.48 g for PNSB, and 1.8 ± 0.03 g for C) (Table 1). Fruit length was significantly greater in all treatments except HE30 compared to the control, with PNSB5 fruits being twice as long as control fruits (Table 1). The widest fruits were produced by MA10 plants (2.23 ± 0.04 cm), while control fruits ranked among the narrowest (Table 1). Fruit firmness was highest in C and HE15 plants (0.27 ± 0.02 kg/cm^3), whereas MA10, PNSB5, and PNSB10 fruits were significantly softer (0.09 ± 0.03, 0.08 ± 0.02, and 0.14 ± 0.02 kg/cm^3, respectively) (Table 1).

Fruit color parameters also varied by treatment. Control fruits showed the highest lightness (L*), while among treated groups, MA10 and HE15 produced the brightest fruits. HE15 fruits also had the highest redness (a*), followed by C and PNSB10 fruits. Fruits from MA5 and PNSB5 treatments had the highest yellowness (b*), whereas HE15 fruits—although rich in redness—showed the lowest b* values (Table 1).

Fruit pH was lowest in control plants. All biostimulants increased fruit pH, with the highest value observed in PNSB10 fruits (Table 1).

Biostimulants strongly influenced total soluble solids (TSS). Control fruits had the lowest TSS (4.27 ± 0.03 °Brix), whereas HE15 and HE30 nearly doubled this value (10.3 °Brix and 10.8 °Brix, respectively). PNSB treatments yielded intermediate TSS

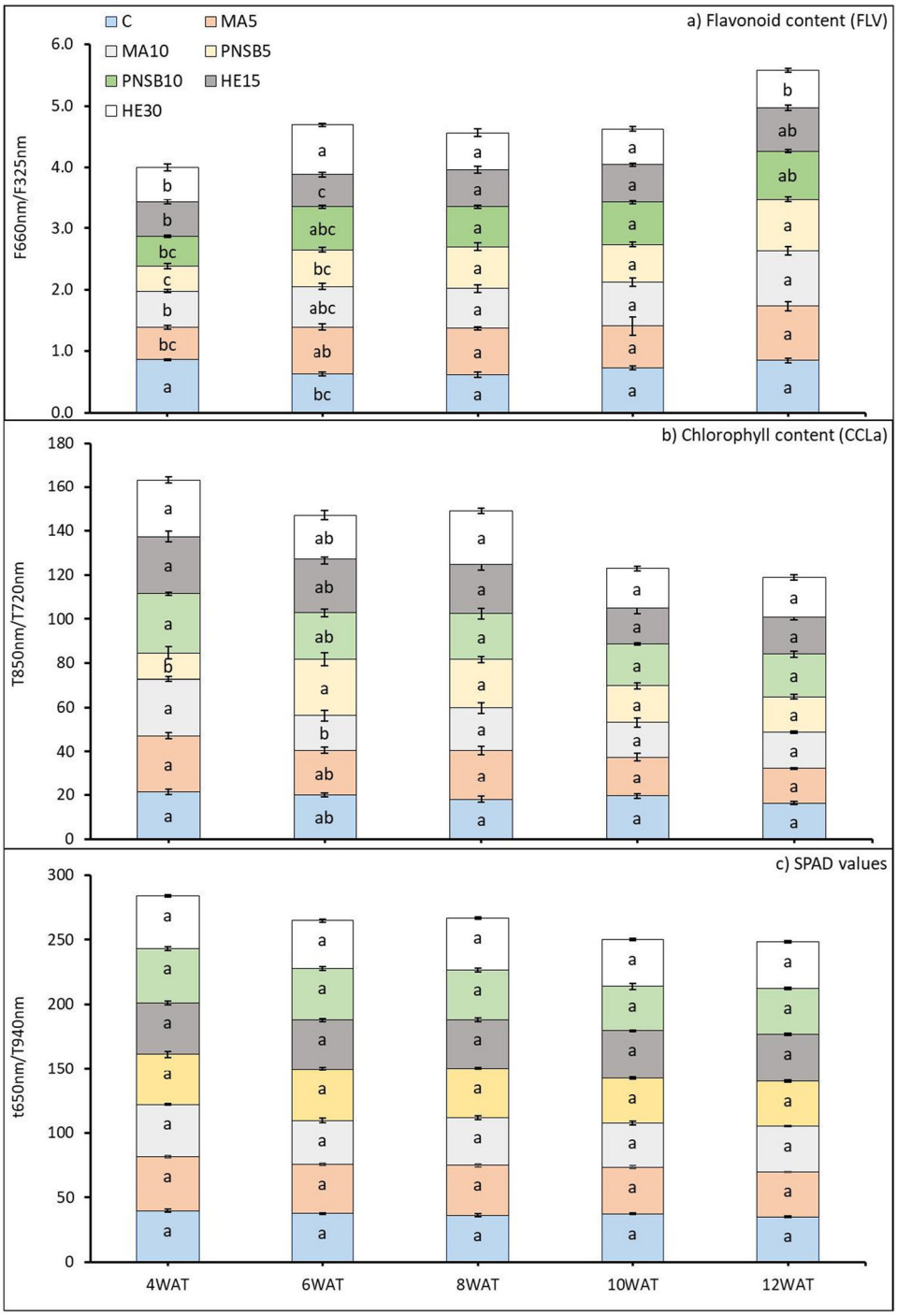


FIGURE 3 | (a) Flavonoid content, (b) chlorophyll content and (c) SPAD values of strawberry leaves under different biostimulants application at different points. Within each date, different letters indicate values statistically different. Repeated measure Analysis of Variance (ANOVA). Tukey's HSD test for multiple comparison at $p \leq 0.05$, using IBM SPSS Statistica 29.0.1.0 software (SPSS Inc., Chicago, IL, USA). Abbreviations - C, control; HE15, hop extract at 15 mL/L, HE30; hop extract at 30 mL/L; MA5, microalgae at 5%, MA10; microalgae at 10%, PNSB5, purple non-Sulphur bacteria at 5%, PNSB10; purple non-Sulphur bacteria at 10%,; WAT, weeks after transplanting.

values, with the highest concentration producing the greatest effect (Table 1).

3.4 | Biochemical Analysis

Total phenolic content (TPC) was particularly enhanced by MA5, which produced the highest values among all treatments (Table 1). A similar trend was observed for antioxidant activity (DPPH assay), with MA5 fruits showing a 16.5% higher activity compared to control fruits (Table 1). PNSB treatments also increased antioxidant capacity, though not significantly.

The similarity in trends for TPC and antioxidant activity confirms their correlation, suggesting that (poly)phenols are the main contributors to antioxidant potential.

TABLE 1 | Effect of experimental biostimulants on fruit quality attributes of strawberries grown under soilless (pots) greenhouse conditions.

	TNF (n°)	Fresh weight (g)	Length (cm)	Width (cm)	Firmness (kg/cm^3)	L*	a*	b*	pH	TSS (g/L)	TPC (mg/g)	DPPH (mg/g)
C	7.00 ± 0.15 d	1.83 ± 0.03b	1.57 ± 0.02c	1.35 ± 0.02 cd	0.27 ± 0.02a	55.20 ± 0.45a	34.49 ± 0.26b	34.07 ± 0.44b	3.37 ± 0.01c	4.27 ± 0.03g	3.36 ± 0.19b	7.60 ± 0.33b
MA5	9.00 ± 0.13c	2.28 ± 0.06b	2.28 ± 0.06b	1.23 ± 0.03d	0.18 ± 0.03ab	38.82 ± 0.35 d	28.82 ± 0.35 d	35.01 ± 0.43a	3.97 ± 0.01b	5.23 ± 0.03e	4.56 ± 0.09a	10.90 ± 0.49a
MA10	10.00 ± 0.11b	3.00 ± 0.02b	1.36 ± 0.13d	2.23 ± 0.04a	0.09 ± 0.03b	44.06 ± 0.21b	32.14 ± 0.25c	32.11 ± 0.39b	4.03 ± 0.03b	4.80 ± 0.10f	3.40 ± 0.19b	7.71 ± 0.87b
PNSB5	9.00 ± 0.13c	3.24 ± 0.02b	3.26 ± 0.02a	1.44 ± 0.02 cd	0.08 ± 0.02b	34.81 ± 0.29 d	31.48 ± 0.60c	36.86 ± 0.85a	3.97 ± 0.00b	5.87 ± 0.03 d	2.94 ± 0.14b	7.88 ± 0.86b
PNSB10	9.00 ± 0.13c	2.30 ± 0.10b	2.18 ± 0.04b	1.26 ± 0.02 d	0.14 ± 0.02b	41.70 ± 0.24c	34.67 ± 0.61b	32.34 ± 0.47b	4.29 ± 0.04a	9.83 ± 0.03c	3.23 ± 0.12b	9.07 ± 0.15ab
HE15	9.00 ± 0.13c	4.08 ± 0.77a	2.27 ± 0.08b	1.82 ± 0.05b	0.27 ± 0.02a	43.46 ± 0.57b	38.51 ± 0.34a	27.17 ± 0.15c	4.04 ± 0.02b	10.33 ± 0.09b	2.78 ± 0.24b	7.73 ± 0.32b
HE30	12.00 ± 0.00a	4.27 ± 0.48a	1.68 ± 0.07c	1.54 ± 0.07c	0.16 ± 0.02ab	37.17 ± 1.18 d	33.15 ± 0.50b	19.44 ± 0.14 d	4.12 ± 0.01b	10.77 ± 0.09a	3.14 ± 0.08b	7.67 ± 0.15b

Note: One-way ANOVA, Tukey's test ($p \leq 0.05$) (IBMSPSS Statistics 29.0.1.0 software, SPSS Inc.; Chicago, IL).
Abbreviations: a*, redness or blueness; b*, yellowness; C, control, DPPH, 2,2-diphenyl-1-picrylhrdrazl (antioxidant); HE15, hop extract at 15 mL/L; HE30: hop extract at 30 mL/L; L*, lightness; MA5, microalgae at 5%; MA10, microalgae at 10%, PNSB5, purple non-Sulphur bacteria at 5%; PNSB10, purple non-Sulphur bacteria at 10%, TNF, total number of fruits; TPC, total phenolic content; TSS, total soluble solids.

3.5 | Leaf and Fruit Macronutrient Content

Potassium (K^+) content differed markedly between tissues, with fruits containing on average 135.5% more K^+ than leaves. The greatest increase (+252.4%) occurred in fruits from HE15 plants. In leaves, the highest K^+ levels were detected in MA5-treated plants, while the lowest were in controls (Figure 4a). In fruits, the highest K^+ levels were recorded in HE15 and HE30 treatments, whereas control fruits contained the least. Specifically, HE-treated fruits had +132.68% more K^+ than control fruits (Figure 4a).

For nitrate (NO_3^-), leaves and fruits showed comparable concentrations. HE30 produced the highest NO_3^- levels in leaves, while MA5 significantly increased NO_3^- in both leaves and fruits. In contrast, control plants had the lowest NO_3^- levels in leaves and among the lowest in fruits, along with HE15 and HE30 (Figure 4b).

Calcium (Ca^{2+}) content was higher in leaves (+92.5%) than in fruits, with the maximum difference (+211.8%) recorded in PNSB5-treated plants. The highest Ca^{2+} levels were found in leaves of MA10- and PNSB5-treated plants and in fruits from MA10 and PNSB10 plants. In both tissues, control plants had the lowest Ca^{2+} levels (Figure 4c).

3.6 | Root Attributes

At the end of the trial, root analysis revealed that PNSB treatments significantly improved all measured parameters. PNSB10 plants exhibited the greatest TRL, L/V, TPA, TSA, and RV values (Table 2). MA10 also produced favorable root traits, though less pronounced. Conversely, HE30 treatment led to significant reductions in several root parameters, including TRL, L/V, TPA, and TSA (Table 2).

3.7 | Fresh and Dry Weight of Different Plant Parts

Biostimulants influenced both plant and root biomass. Plant fresh weight increased by 124.5% in MA5 and 111.9% in PNSB10 compared to controls, with both treatments producing the statistically highest values. Similar trends were observed for plant dry weight (Table 2).

Root biomass followed the same pattern, with PNSB10 producing the highest fresh and dry weights (Table 2).

3.8 | Heatmap Summary of Treatment Effects

To provide an integrated overview, a heatmap was generated as described by Campana et al. (2025) (Figure 5). Intensely red cells indicate higher values of the corresponding parameter for a given treatment, while blue cells represent lower values. This visualization highlights the overall effects of each biostimulant. Among treatments, PNSB10 showed the strongest performance, particularly in root development. MA5 treatment was especially effective for fruit quality, enhancing TPC and antioxidant activity. Regarding vegetative growth, responses varied: MA10

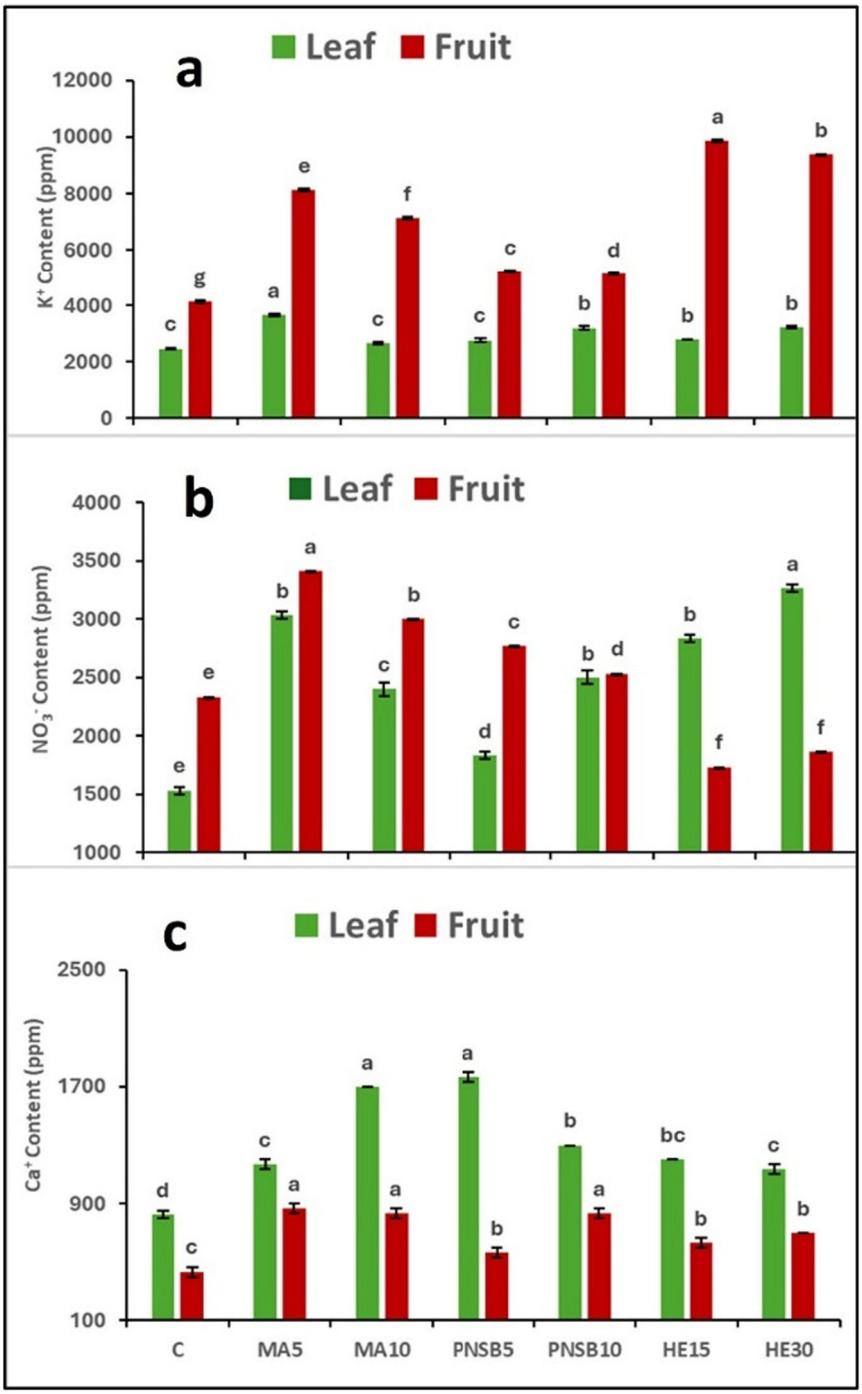


FIGURE 4 | Influence of experimental biostimulants on (a) potassium, (b) nitrate and (c) calcium content in leaf and fruit of strawberry plants. Within leaf and within fruit, different letters indicate values statistically different. One-way Analysis (ANOVA), Tukey's test ($p \leq 0.05$) (IBM SPSS Statistics 29.0.1.0 software, SPSS Inc.; Chicago, IL). C, control; MA5, microalgae at 5%; MA10, microalgae at 10%; PNSB5, purple non-Sulphur bacteria at 5%; PNSB10, purple non-Sulphur bacteria at 10%; HE15, hop extract at 15 mL/L; HE30, hop extract at 30 mL/L; WAT, week after transplanting.

promoted the greatest plant height, while HE30 induced an increase in leaf number.

4 | Discussion

This study highlights the effects of different experimental biostimulants on the morpho-physiological responses and fruit quality parameters of strawberry plants cultivated soilless under controlled conditions. To the best of our knowledge, this is the first investigation using these specific experimental biostimulants. Therefore, the results are discussed in comparison with products of similar origin.

Regarding microalgae-based products, several studies have explored their use in agriculture. Jalalian et al. (2024) showed that *Chlamydomonas* extract influenced strawberry morphological, physiological, and biochemical characteristics. Similarly, the

TABLE 2 | Effect of biostimulants on root quality attributes and fresh and dry biomass of strawberry plants grown under soilless (pots) greenhouse conditions.

	TRL (cm)	L/V (cm/m^3)	TPA (cm^2)	TSA (cm^2)	AD (mm)	RV (cm^3)	PFW (g)	PDW (g)	RFW (g)	RDW (g)
C	74.24 ± 3.58bc	323.27 ± 14.98b	8.46 ± 0.78bc	8.89 ± 0.40bc	0.61 ± 0.06d	0.95 ± 0.15b	6.76 ± 0.43e	1.44 ± 0.22c	5.83 ± 0.11c	1.00 ± 0.01e
MA5	79.86 ± 8.63bc	251.54 ± 8.52b	7.65 ± 0.28bc	10.42 ± 0.96b	0.72 ± 0.03cd	1.04 ± 0.10b	15.18 ± 0.18a	4.79 ± 0.11a	5.95 ± 0.63c	0.80 ± 0.04e
MA10	113.60 ± 5.03b	339.30 ± 29.67b	8.60 ± 0.54bc	13.29 ± 0.56ab	0.93 ± 0.04abc	2.28 ± 0.17a	11.97 ± 0.09b	3.74 ± 0.25b	6.61 ± 0.19bc	1.84 ± 0.02c
PNSB5	90.45 ± 1.16b	252.74 ± 13.81b	9.13 ± 0.72ab	10.09 ± 0.78b	1.05 ± 0.10ab	2.21 ± 0.39a	8.47 ± 0.20c	1.53 ± 0.15c	7.44 ± 0.13ab	1.42 ± 0.18d
PNSB10	198.47 ± 26.75a	493.63 ± 30.18a	11.41 ± 0.59a	17.19 ± 1.58a	0.81 ± 0.03bcd	2.56 ± 0.19a	14.33 ± 0.31a	4.83 ± 0.14a	8.42 ± 0.15a	3.19 ± 0.10a
HE15	94.23 ± 13.60b	264.51 ± 16.08b	8.59 ± 0.55bc	10.99 ± 1.41b	0.69 ± 0.05cd	0.99 ± 0.11b	7.14 ± 0.05de	3.33 ± 0.23b	6.23 ± 0.07bc	2.26 ± 0.02b
HE30	30.50 ± 3.19c	90.73 ± 9.06c	6.39 ± 0.48c	4.74 ± 0.17c	1.15 ± 0.09a	0.93 ± 0.08b	7.88 ± 0.13cd	3.39 ± 0.12b	5.77 ± 0.21c	1.12 ± 0.04de

Note: One-way ANOVA, Tukey's test ($p \leq 0.05$) (IBMSPSS Statistics29.0.1.0 software, SPSS Inc.; Chicago, IL).
Abbreviations: AD, average diameter; C, control; HE15, hop extract at 15 mL/L; HE30, hop extract at 30 mL/L; L/V, length per volume; MA5, microalgae at 5%; MA10, microalgae at 10%; PDW, plant dry weight; PFW, plant fresh weight; PNSB10, purple non-Sulphur bacteria at 10%; RDW, root dry weight; RFW, root fresh weight; RV, root volume; PNSB5, purple non-Sulphur bacteria at 5%; TPA, total projected area; TSA, total surface area; TRL, total root length.

application of *Chlorella fusca* (Chk0059) was found to enhance strawberry growth and physiological parameter (Kim et al. 2023). Beyond microalgae (MA), research has also focused on the agricultural applications of PNSB. For example, treatment with PNSB improved growth, development, and morpho-physiological attributes of Djulis (*Chenopodium formosanum* Koidz) (Sundar et al. 2024). Hop extract has never been tested as a biostimulant in strawberry cultivation, but extracts from other plants, such as *Calendula officinalis*, *Salvia officinalis*, *Tagetes* sp., and *Taraxacum officinale*, have been shown to promote plant growth and improve root morphological attributes (Furmanczyk et al. 2023).

In the present study, the greater height of plants treated with 10% MA is consistent with previous findings demonstrating the beneficial effects of *T. chuii* and its potential in agriculture, comparable to results obtained with *Spirulina platensis* and *Chlorella vulgaris (*Miranda et al. 2024; Dineshkumar et al. 2018). Increased plant height may be attributed to the supply of essential macronutrients (e.g., nitrogen, phosphorus, potassium), micronutrients, and plant growth regulators (auxins, gibberellins, cytokinins), as well as amino acids, polysaccharides, and vitamins present in MA extracts, all of which are readily available for plant uptake (Miranda et al. 2024). Conversely, the significantly lower height recorded for plants treated with hop extract at 30 mL/L suggests a phytotoxic or inhibitory effect at this concentration, likely due to the complex mixture of secondary metabolites, including allelochemicals such as phenolic compounds, terpenes, alkaloids, and saponins, contained in HE (Tlak Gajger and Dar 2021). Studies on *Albizia lebbeck* extracts similarly reported inhibitory effects on shoot length and overall growth in several crops (*Brassica juncea* (L.) Czern., *Cucumis sativus* L., *Phaseolus mungo* L., *Raphanus sativus* L., and *Vigna unguiculata*) (Uddin et al. 2007).

Interestingly, although HE30 reduced plant height, the same plants developed the highest number of leaves, suggesting that this extract does not exert a negative effect on overall strawberry growth.

With respect to physiological parameters, leaf flavonoid content at 4 WAT was significantly higher in control plants than in treated ones, possibly reflecting early stress in the absence of biostimulant support. A comparable study confirmed that microalgae-based biostimulants can significantly enhance flavonoid content in strawberries relative to untreated controls (Žunić et al. 2024). No differences were observed in chlorophyll content or SPAD values, suggesting that while biostimulants influence secondary metabolites (such as flavonoids), they did not alter the primary photosynthetic machinery or overall leaf greenness under these conditions. Similar findings were reported in potatoes, where biostimulant application did not improve chlorophyll content or SPAD values (Wadas and Dziugieł 2020).

The twofold increase in fruit fresh weight following HE treatments at both 15 and 30 mL/L indicates a significant stimulation of fruit development. This effect may be due to hop bioactives such as xanthohumol and hop bitter acids, known for their antioxidant and antimicrobial properties (Huu et al. 2022). These compounds may improve plant health and metabolic

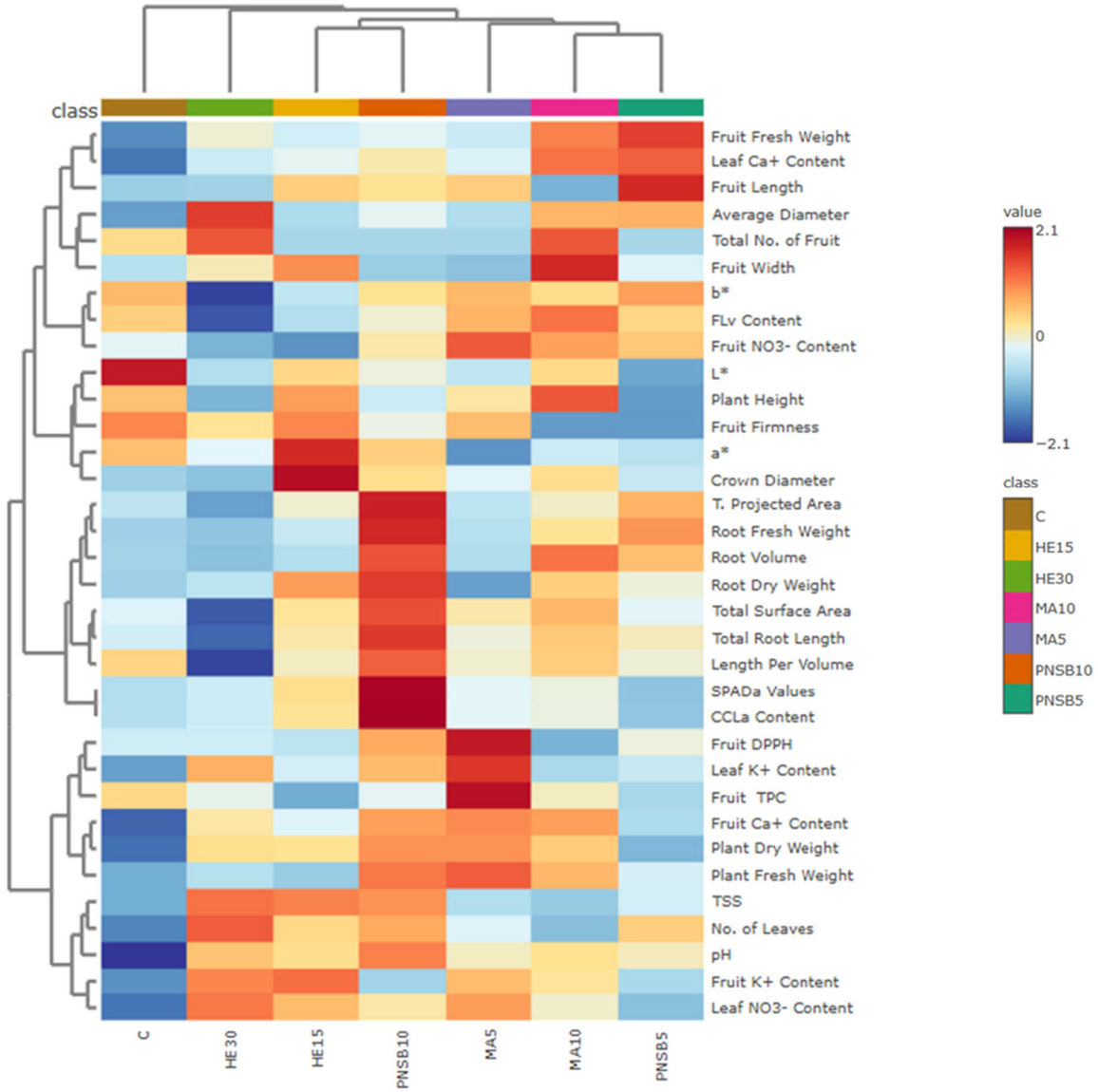


FIGURE 5 | Heat map displaying the all-measured parameters (morpho-physiological, biochemical, and quality parameters of fruit and leaves) of the strawberry plants, grown under control soilless condition. C, control; HE15, hop extract at 15 mL/L; HE30, hop extract at 30 mL/L; MA5, microalgae at 5%; MA10, microalgae at 10%; PNSB5, purple non-Sulphur bacteria at 5%; PNSB10, purple non-Sulphur bacteria at 10%.

efficiency, resulting in greater fruit biomass. Similarly, the twofold increase in fruit length following 5% PNSB treatment highlights the strong potential of PNSB in enhancing growth and fruit development. Xuan et al. (2024) reported analogous results in canary melons, where phosphorus-solubilizing PNSB strains improved fruit weight, firmness, and color intensity. PNSB, especially *Rhodopseudomonas palustris*, are known for producing phytohormones such as IAA and 5-ALA, which promote cell elongation and division. Moreover, their ability to solubilize phosphorus, fix atmospheric nitrogen, enhance photosynthetic efficiency, and improve stress tolerance contributes directly to fruit growth and elongation (Morrison and Bose 2024).

Fruit firmness was higher in C and HE15 fruits, likely due to the bioactive compounds in hop extracts, in line with previous studies where biostimulant and plant extract applications improved fruit firmness and other quality traits in apples (Mosa et al. 2023). Conversely, the reduced firmness in MA10- and PNSB5-treated fruits suggests a distinct physiological response, potentially due to resource reallocation toward vegetative growth or yield, or alterations in cell wall metabolism (Vicente et al. 2007).

Fruit color parameters revealed distinct treatment effects on pigment biosynthesis. HE15 strongly enhanced redness (a*), likely by stimulating anthocyanin synthesis via phenolic compounds (Sunil and Shetty 2022). In contrast, MA-treated fruits exhibited lower redness than controls, which contrasts with reports of increased redness in apples following microalgae treatments (Di-Vaio et al. 2021). MA5 and PNSB5 produced the highest yellowness (b*), suggesting enhanced carotenoid accumulation, while HE15 induced a metabolic shift favoring anthocyanin over carotenoid biosynthesis. These results confirm that biostimulant effects on fruit color are concentration-dependent and compound-specific (Trejo-Téllez et al. 2023).

PNSB10-treated fruits consistently exhibited higher pH values, while control fruits had the lowest pH, indicating that all biostimulants influenced fruit acidity. Similar trends were observed in cherries treated with *Ascophyllum nodosum*-based extracts, which increased fruit pH, width, and diameter (Correia et al. 2015).

The increase in total soluble solids (TSS) observed with hop extract treatments, particularly HE15 and HE30, indicates enhanced sugar accumulation. This effect may be linked to flavonoids and bitter acids in hops, which can stimulate photosynthesis and carbon assimilation (Karabín et al. 2016). Comparable results were reported in olive, where combined application of moringa leaf extract and seaweed extract improved fruit TSS (Al-Saif et al. 2023), and in apples, where biostimulant mixtures enhanced soluble solids (Mosa et al. 2023).

The MA5 treatment, particularly at the lowest concentration, significantly increased TPC and antioxidant activity in strawberries. This enhancement is likely due to bioactive compounds in the microalgae extract that stimulate secondary metabolism and phenolic compound synthesis (Cichoński and Chrzanowski 2022). MA5 also increased fruit antioxidant activity by 16.5%, further supporting its role in promoting secondary metabolism. Conversely, the lower TPC and DPPH values under MA10 may reflect metabolic overload or feedback inhibition. Graziani et al. (2020) similarly reported improved antioxidant activity and TPC in pple plants, cv. Annurca, treated with microalgae.

Nutrient content in leaves and fruits was also strongly influenced by treatments. HE30 increased NO_3^- levels in leaves, while MA5 significantly enhanced K^+ levels. Control plants consistently had the lowest NO_3^-, K^+, and Ca^{2+} concentrations, confirming the positive impact of biostimulants on nutrient uptake and accumulation. In fruits, MA5 most effectively increased K^+, while both MA5 and HE15 induced the highest NO_3^- accumulation. These results align with studies in wheat, strawberry, orange, and hot pepper, where biostimulants improved leaf and fruit macronutrient content (Kilic 2024).

At the root level, MA and PNSB at high concentrations significantly promoted root length, surface area, and volume. In contrast, HE30 inhibited root growth, reducing length and surface area. The positive effects of MA and PNSB are likely related to their production of plant growth-promoting substances (e.g., auxins), improved nutrient availability, and enhanced plant vigor (Wu et al. 2025). The inhibitory effect of HE30 suggests phytotoxicity or imbalances in growth regulators at this concentration. Similar results were reported in wheat, where phosphate-solubilizing bacteria improved root traits including length, surface area, and branching (Yahya et al. 2022), and in sugar beet, where microalgae extracts enhanced root length, fine root diameter, and tip number (Barone et al. 2018).

Fresh and dry biomass of both leaves and roots increased with MA5 and PNSB10 treatments, likely due to improved nutrient uptake, enhanced photosynthesis, and bioactive compounds such as phytohormones, amino acids, and antioxidants. These compounds enhance cell division, water retention, and metabolic efficiency (Jogawat et al. 2021). The strong root development in PNSB10-treated plants supports superior water and nutrient uptake, resulting in enhanced growth. In contrast, the lower biomass in controls or less effective treatments may be explained by limited nutrient availability, weaker root systems, and reduced physiological activity. Nookongbut et al. (2018) similarly showed that *R. palustris* PNSB increased shoot and root dry weight and photosynthetic rates in rice, consistent with enhanced nutrient bioavailability and uptake promoted by biostimulants.

5 | Conclusions

This study evaluated the effects of experimental biostimulants—microalgae, purple non-sulfur bacteria, and hop extracts—derived from circular economy processes. The findings demonstrated significant improvements in plant growth, yield, and fruit nutritional quality, underscoring the potential of converting biomass traditionally regarded as waste into valuable agricultural inputs.

The differential responses observed highlight the importance of optimizing both dosage and application timing. Future research should further explore the interactions between biostimulants, soil microbiota, and plant physiology to refine their sustainable use.

Overall, these biostimulants represent a promising and eco-friendly strategy to enhance strawberry production and soil health while reducing dependence on synthetic inputs. They provide a valuable tool to address the challenges of greenhouse cultivation and to promote more resilient and sustainable farming systems.

Author Contributions

Conceptualization: Andrea Turolla, Angela Paterna and Benedetta Chiancone. Methodology: Samreen Nazeer, Andrea Turolla, Angela Paterna, Martina Cirlini and Benedetta Chiancone. Validation: Andrea Turolla, Angela Paterna, Estella Rao, Martina Cirlini and Benedetta Chiancone. Formal Analysis: Samreen Nazeer, Anna Agosti, Andrea Turolla, Angela Paterna, Martina Cirlini and Benedetta Chiancone. Investigation: Samreen Nazeer, Anna Agosti, Andrea Remelli, Leandra Leto, Andrea Remelli, Andrea Di Fazio, Estella Rao, Ali Amini. Resources: Andrea Turolla, Angela Paterna, Martina Cirlini and Benedetta Chiancone. Data Curation: Samreen Nazeer, Anna Agosti, Leandra Leto, Martina Cirlini and Benedetta Chiancone. Writing – Original Draft Preparation: Samreen Nazeer, Anna Agosti, Andrea Turolla, Angela Paterna, Estella Rao, Martina Cirlini and Benedetta Chiancone. Writing – Review and Editing: Andrea Turolla, Angela Paterna, Estella Rao, Martina Cirlini and Benedetta Chiancone. Visualization: Andrea Turolla, Angela Paterna, Estella Rao, Martina Cirlini and Benedetta Chiancone. Supervision: Andrea Turolla, Angela Paterna, Estella Rao, Martina Cirlini and Benedetta Chiancone. Project Administration: Andrea Turolla, Angela Paterna, Estella Rao, Martina Cirlini and Benedetta Chiancone. Funding Acquisition: Andrea Turolla, Angela Paterna, Estella Rao, Martina Cirlini and Benedetta Chiancone.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Table SM1: Influence of experimental biostimulants on strawberry plant morphological parameters.