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Biochar Enhances Plant Growth, Fruit Yield, and Antioxidant Content of Cherry Tomato (*Solanum lycopersicum* L.) in a Soilless Substrate

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Abstract: Biochar soil amendment can improve growing medium water and nutrient status and crop productivity. A pot experiment was conducted using *Solanum lycopersicum* var. *cerasiforme* plants to investigate the effects of biochar amendment (20% application rate) on a soilless substrate, as well as on plant growth, fruit yield, and quality. During the experiment, substrate characteristics, plant morphological traits, and root and leaf C/N content were analyzed at three sampling points defined as early stage (36 days after germination), vegetative stage (84 days a. g.), and fruit stage (140 days a. g.). Fruit morphological traits, titratable acidity, lycopene, and solid soluble content were measured at the end of the experiment. Biochar ameliorated substrate characteristics (N_{av} increase of 17% and C_{tot} increase of 13% at the beginning of the study), resulting in a promotion effect on plant root, shoot, and leaf morphology mainly at the vegetative and fruit stages. Indeed, at these two sampling points, the biochar-treated plants had a greater number of leaves (38 and 68 at the vegetative and fruit stages, respectively) than the untreated plants (32 and 49, respectively). The biochar also increased leaf area with a rise of 26% and 36% compared with the values measured in the untreated plants. Moreover, the amendment increased twofold root length, root surface area, and root, stem, and leaf biomasses in comparison with untreated plants. Regarding plant productivity, although fruit morphology remained unchanged, biochar increased flower and fruit numbers (six times and two times, respectively), acidity (75%), lycopene (28%), and solid soluble content (16%). By unveiling promoting changes in morphological traits, fruit number, and antioxidant content occurring in cherry tomato plants growing in a biochar-treated soilless substrate, it could be possible to highlight the importance of biochar for future applications in the field for enhancing plant production and fruit quality in a sustainable agriculture framework.

Keywords: amendment; fruit quality; physicochemical characteristics; plant morphology; sustainable agriculture

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1. Introduction

Global demand for crops is connected to a strong environmental impact mainly due to habitat fragmentation and land clearing as well as fertilization uses polluting both water and terrestrial ecosystems [1]. Therefore, in the future, it will be crucial to use new methods and technologies to achieve great agricultural yields with low global environmental impacts.

Among several new strategies, biochar could be used in a sustainable agriculture context because, so far, it has been demonstrated that its application in soil significantly

changes the most physicochemical properties together with plant traits [2]. Biochar is a solid material obtained from a pyrolysis process that performs a thermochemical transformation of biomass at high temperatures and in total or partial absence of oxygen, dramatically reducing greenhouse gas emissions [3]. In particular, biochar has been shown to increase both soil carbon and water content as well as macro aggregates, electrical conductivity, pH, total nitrates/nitrites, ammonia, nitrogen [4–6], extractable phosphorus, and cation-exchange capacity [7]. Furthermore, due to its skeletal-sponge structure, biochar reduces soil leaching of ammonium [6] and improves rhizosphere microbial communities and activities with particular regard to both cellulose-degrading and nitrogen-fixing bacteria [8]. All these findings highlight that biochar enhances important functions, such as soil carbon sequestration and nitrogen soil retention, becoming a good technological product for future sustainable agriculture [1]. No less important is the possibility to use biochar as an alternative container substrate component to commonly used substrates, such as peat moss, vermiculite, perlite, bark, and compost, which are costly both economically and environmentally [9,10]. Indeed, the biochar influence in soilless substrates has been studied and reviewed on several plant species, causing changes in different soil properties [11,12]. Biochar caused pH adjustment [13] and higher cation-exchange capacity with greater potassium retention and availability in a soilless substrate in [10]. Tomato and marigold plants showed improved growth when biochar was incorporated in a peat moss substrate in [14], and a promoting physiological effect and higher fruit yield were observed in pea plants grown in a similar substrate in [15].

Although changes in soil characteristics due to the biochar application seem to have a generally positive trend, with a mean yield increase of 10%, averaging different crops, soils, and climates [16], the results of the biochar effects on crop development are still inconsistent [17]. This is due to various factors that characterize biochar, such as the starting parental material, pyrolysis conditions, and soil physicochemical characteristics [18].

Tomato plants (*Solanum lycopersicum* L.) in the Mediterranean region are optimally grown in passive solar greenhouses on well-drained, sandy loamy soils with pH values ranging between 6 and 7. Tomato is a plant species of great commercial importance worldwide [19]. Indeed, tomato is the most consumed non-starchy vegetable with a global production of about 164 million tons (t) of fresh fruit harvested on a 4.7 million hectare (ha) surface [20]. From a health point of view, a large body of research supports an inverse relationship between consuming tomatoes and tomato products and the risk of certain cancers as well as cardiovascular disease, osteoporosis, ultraviolet-light-induced skin damage, and cognitive dysfunction [21]. Indeed, tomatoes are the most significant source of dietary lycopene, a powerful antioxidant, and in general, secondary metabolites, such as cis-lycopene, trans-lycopene, β-carotene, and other carotenoids, which are directly involved in these protective actions [22].

To date, there is still poor information on the effects of biochar on tomato plant growth, fruit yield, and antioxidant content [23–28]. In particular, Tartaglia et al. [24] and Guo et al. [25] recently demonstrated that biochar addition may facilitate the reduction of farming input, being a sustainable practice for enhancing tomato plant growth, fruit quality, and yield. Other authors [26–28] investigated the biochar supplementation in tomato plants grown in salt-affected or contaminated soils. She et al. [26] highlighted how biochar amendments have the potential to ameliorate salt stress and enhance tomato production. Both Almaroai et al. [27] and Alam et al. [28] agreed that biochar application minimizes the uptake of the toxic element, thus alleviating the health risk. Finally, Kavitha et al. [17] reviewed contradictory results and often-incomplete datasets in various studies concerning the effects of environmental factors, such as water availability, mineral nutrients (nitrogen, phosphorus, potassium, and calcium), and plant growth regulators, on antioxidant content in tomato fruits.

Given the above-mentioned multiple effects that biochar can have on soil characteristics, we hypothesized that biochar-derived changes in resource supply may play a crucial role in enhancing cherry tomato plant growth, fruit yield, and antioxidant content. To test

this hypothesis, after assessing the effects of biochar on substrate physicochemical characteristics, morphological parameters of roots, shoots, and fruits together with the number of fruits and their antioxidant content were investigated in a time-course pot experiment. The identification of possible relationships between any alterations of soilless substrate physicochemical characteristics, plant growth, and fruit yield may further contribute to elucidating the mechanisms of biochar actions and its use. In addition, the data from this study could be used as a basis for researching the long-term effects of biochar in the field and sustainable agriculture to increase vegetable crop yield and quality.

2. Materials and Methods

2.1. Experimental Design

Seeds of *Solanum lycopersicum* L. var. *cerasiforme* (*S. l. cerasiforme*; cherry tomato average/early-ripening variety, small, round, bright red fruit in bunches, by Sementi Dotto) were obtained from a commercial nursery (Varese, Italy). Six seeds of cherry tomato were sown each in 9 L cylindrical pots (h 24 cm, lower Ø 21 cm, and upper Ø 26 cm), filled with a commercial soilless substrate. After germination, only one seedling was left to grow for each pot.

Two treatments were set up: (i) the control condition (C) characterized by a mixture of peat, silica sand, and bark humus (1:2:1), and (ii) the biochar treatment prepared with the mix of peat, silica sand, bark humus, and biochar (1:2:1:1). Thirty pots for each treatment were then placed in a growth chamber under the following conditions: day/night temperature of 22/17 °C, air humidity around 60%–70%, 16 h light/8 h dark cycle with a light intensity of about 500 μmol·m^{-2}·s^{-1} at pot height (light meter sensor—HD2302.0—Delta Ohm; Caselle di Selvazzano, Italy). All potted seedlings were watered to saturation with 1.2 L tap water every 2 days, and no fertilizer was added.

Ten biochar-treated plants (B) and 10 untreated plants (C) were collected at each of three sampling points defined as early stage (Es, 36 days after plant germination), vegetative stage (Vs, 84 days after plant germination), and fruit stage (Fs, 140 days after plant germination) for a total of 60 cherry tomato plants.

2.2. Biochar and Substrate Analysis

The biochar was provided by Romagna Carbone s.n.c. (Bagnacavallo, Italy), produced from orchard pruning biomass, and slow pyrolyzed at a temperature of 500 °C with a residence time of 3 h. The main physicochemical properties of the biochar were determined as described in a previous study [15] and are presented in Table 1.

Table 1. Biochar physicochemical characteristics. Each value represents the mean (n = 8) ± 1 SE.

Parameter	Unit	Biochar
pH	–	9.7 ± 0.1
EC	dS·m^{-1}	7.5 ± 0.4
CEC	cmol(+)·kg^{-1}	21.3 ± 0.3
N_{tot}	g·kg^{-1}	9.1 ± 0.2
N_{av}	mg·kg^{-1}	30 ± 0.4
P_{tot}	mg·kg^{-1}	1221.9 ± 21.3
P_{av}	mg·kg^{-1}	217 ± 3.0
C_{tot}	g·kg^{-1}	778.1 ± 0.1
C_{org}	g·kg^{-1}	705.6 ± 0.1
H	g·kg^{-1}	45.3 ± 0.2
H/C_{org}	–	0.76

pH: 1:5 v/v biochar/water solution; EC = electrical conductivity 1:5 v/v biochar/water solution; CEC = cation exchange capacity; N_{tot} = total nitrogen; N_{av} = available nitrogen; P_{tot} = total phosphorus; P_{av} = available phosphorus; C_{tot} = total carbon; C_{org} = organic carbon; H = hydrogen.

Substrate physicochemical characteristics, in the C and B conditions, were determined at the beginning of the experiment (T0) and the Es, Vs, and Fs sampling points.

Eight substrate samples were collected on the surface, in the middle (18 cm), and at the bottom of the pots (32 cm). Once released from roots, substrate samples were mixed in one bulk sample, air-dried until constant weight, passed through a 2 mm sieve, and stored at 4 °C in the dark until processed.

pH and EC were assessed according to Conyers and Davey [29] and Rhoades [30], respectively. Cation exchange capacity (CEC) was assessed according to Mehlich [31] using $BaCl_2$. Total nitrogen (N_{tot}) and total carbon (C_{tot}) were determined by dry combustion [32] using a CHN elemental analyzer (Carlo Erba Instruments, Mod 1500, Series 2, Cornaredo, Italy). Available nitrogen (N_{av}) was determined by a modified Kjeldahl procedure using Devarda's alloy [33], as a reducing agent to convert NO_3 and NO_2 into NH_4^+, and subsequent Kjeldahl digestion. Total phosphorus (P_{tot}) content was determined by spectrophotometry (UV-1601 Shimadzu, Kyoto, Japan) according to the test method described by Bowman [34]. Available phosphorus (P_{av}) was extracted by a $NaHCO_3$ solution at pH 8.5 and evaluated by spectrophotometry according to Olsen et al. [35].

2.3. Plant Analysis

At each sampling point (Es, Vs, and Fs), 10 replicates were used for measuring morphological traits of leaves, stems, and roots, while 6 replicates were used for determining the C/N content of leaves and roots. In particular, all leaves were detached from the branches and counted to have leaf number (no.). Afterward, to measure leaf area (cm^2), leaves were scanned (400 dpi) using a portable scanner (Epson Perfection V600), and scanned images were processed with WinRHIZO software (Pro V. 2007d, Regent Instruments Inc., Ville de Québec, QC, Canada).

Roots were washed and scanned with a calibrated flatbed scanner coupled to a lighting system for image acquisition (Expression 10000 XL, Epson America Inc., Long Beach, CA, USA); scanned images were analyzed by WinRHIZO software to measure root length (m) and surface area (cm^2).

Afterward, the plant tissues were separately oven-dried at 70 °C until constant weight and weighed to obtain the dry mass (g) of leaves, stems, and roots.

Moreover, the determination of total carbon (C_{tot}) and nitrogen (N_{tot}) content in roots and leaves of C and B plants was performed by a CHN elemental analyzer (PerkinElmer, 2400 Series, II CHNS/O elemental analyzer, Waltham, MA, USA). First, roots and leaves were harvested at the Es, Vs, and Fs sampling points; finely ground in liquid nitrogen with mortar and pestle; and then dried at 80 °C to eliminate humidity traces. The analyzer was calibrated by the atropine standard, with a calibration repetition every 10 samples.

2.4. Fruit Analysis

Before fruit ripening, flower number was determined with a count (no.) for 10 replicates for each treatment. To have homogeneity in fruit collection, tomato fruits were harvested from 10 plants for each treatment at point 5 of the ripening color chart [36], and fruit number (no.) was measured.

To evaluate fruit dry mass (g), tomato fruits were oven-dried, at 70 °C for 48 h, and weighed.

Morphometric fruit parameters, such as polar and equatorial diameters (cm), epicarp thickness (mm), and right and left mesocarp thickness (mm), were determined by scanning fruits and analyzing the images with ImageJ software (open source https://imagej.nih.gov/ij/ accessed on 1 March 2016).

Furthermore, fruits were homogenized (VWR Collection, VDI 12) to evaluate other parameters. Both trans- and cis-lycopene contents were determined by extracting 6 g of homogenate with 60 mL of hexane–methanol–acetone (2:1:1 volume) with 2.5% of BHT for 30 min at 4 °C in dark conditions. Subsequently, 10 mL of distilled water was added, and the polar phase (hexane) recovered. The polar phase was subjected to spectrophotometric reads at 472 nm (maximum absorbance peak of the trans-lycopene) and 502 nm (maximum absorbance peak of the cis-lycopene). The titratable acidity, expressed as a percentage of

citric acid, was measured according to the titration method at pH 8.1 with NaOH (0.1 N) [37]. The total soluble solid content, expressed as °Brix, was measured by a refractometer (HI 96813, Hanna Instruments, Woonsocket, RI, USA), after homogenate centrifugation at 13,000× *g* for 20 min at 8 °C [38].

2.5. Statistical Analysis

Square root or log transformations were applied to ensure normal distributions and equal variances. Leaf, stem, and root dry mass; leaf number; leaf area; root length; and root surface area were analyzed with a two-tailed T-test. Flower and fruit number, fruit dry mass, polar and equatorial diameter, epicarp thickness, right and left mesocarp thickness, trans- and cis-lycopene, titratable acidity, and total soluble solids were analyzed with a one-way ANOVA by Bonferroni post hoc test. Both statistical tests were applied at a significance level of 95%. Both statistical analyses were performed using SPSS 17.0 software package (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Substrate Physicochemical Characteristics

As shown in Table 2, at the beginning of the experiment (T0), the only differences between the untreated (C) and biochar-treated (B) substrates were found in available nitrogen (N_{av}) and total carbon (C_{tot}) contents. In detail, the concentrations of both N_{av} and C_{tot} were higher in the B treatment than in the C condition.

At the early-stage sampling point (Es), only the available phosphorous content (P_{av}) was 11% lower in the B condition than the C untreated substrate (42.6 mg·kg^{-1}) (Table 2).

At the vegetative-stage sampling point (Vs), the total carbon content (C_{tot}) was 14% higher in the B substrate compared with the C condition (20 g·kg^{-1}) (Table 2).

At the fruit-stage sampling point (Fs), in the B condition, on one side there was a 13% increase in substrate cation exchange capacity (CEC) and a 19% rise of P_{tot}, while on the other side, a 17% decrease in N_{av} concentration was observed in comparison with the C substrate (Table 2).

In the C substrate, a decrease of 11% was registered in CEC from the Es to the Fs sampling point (Table 2). Moreover, over time, within each treatment, there were decreases in nutrient concentrations. In the C condition, N_{tot}, N_{av}, and C_{tot} contents were lower at the Vs and Fs points in comparison with Es. In detail, N_{tot} decreased by 31% and 25% in Vs and Fs in comparison with Es (16 g·kg^{-1}), respectively (Table 2). N_{av} was 27% and 20% lower in Vs and Fs compared with Es (150 mg·kg^{-1}) (Table 2). In Vs and Fs, C_{tot} also decreased by 21% and 23%, respectively (Table 2). In the same treatment (C substrate), regarding the P_{tot} and P_{av} concentrations, the highest values were found at the Es sampling point (545 and 42.6 mg·kg^{-1}, respectively), followed by values measured at the Vs sampling point (440 and 38.7 mg·kg^{-1}, respectively) and Fs (347 and 23.9 mg·kg^{-1}, respectively) (Table 2).

In the B condition, N_{tot} and P_{av} contents were lower by 29% and 27% at the Fs sampling point in comparison with Es, respectively (Table 2). P_{tot} was 19% and 23% lower in Vs and Fs compared with Es (534 mg·kg^{-1}) (Table 2). In the B treatment, regarding the N_{av} and C_{tot} concentrations, the highest values were found at the Es sampling point (140 mg·kg^{-1} and 25.6 g·kg^{-1}, respectively), followed by values measured at the Vs sampling point (100 mg·kg^{-1} and 23.0 g·kg^{-1}, respectively), and the lowest contents were measured at Fs (100 mg·kg^{-1} and 20.1 g·kg^{-1}, respectively) (Table 2).

Table 2. Physicochemical characteristics of untreated (C) and biochar-treated (B) substrates were determined at the beginning of the experiment (T0) and early stage (Es, 36 days after plant germination), vegetative stage (Vs, 84 days after plant germination), and fruit stage (Fs, 140 days after plant germination). Letters a and b indicate a statistically significant difference ($p < 0.05$) between the two treatments inside each sampling point. Letters x, y, and z indicate a statistically significant difference ($p < 0.05$) among the Es, Vs, and Fs sampling points for each treatment. ($n = 8 \pm$ SE).

		Parameter							
		pH	**EC (dS·m^{-1})**	**CEC (cmol(+)·kg^{-1})**	**N_{tot} (g·kg^{-1})**	**N_{av} (mg·kg^{-1})**	**P_{tot} (mg·kg^{-1})**	**P_{av} (mg·kg^{-1})**	**C_{tot} (g·kg^{-1})**
T0	C	6.6 ± 0.1 a	0.9 ± 0.3 a	18 ± 0.9 a	13 ± 1.2 a	120 ± 5 b	457 ± 17 a	40.4 ± 2.2 a	23.0 ± 0.6 b
	B	6.7 ± 0.1 a	0.9 ± 0.3 a	19 ± 0.9 a	15 ± 1.4 a	140 ± 5 a	484 ± 18 a	42.4 ± 2.3 a	26.0 ± 0.6 a
Es	C	7.4 ± 0.1 ax	0.8 ± 0.3 ax	18 ± 0.9 ax	16 ± 1.5 ax	150 ± 17 ax	545 ± 20 ax	42.6 ± 1.5 ax	25.6 ± 0.6 ax
	B	7.4 ± 0.1 ax	1.1 ± 0.4 ax	19 ± 0.9 ax	14 ± 1.3 ax	140 ± 6 ax	534 ± 20 ax	37.9 ± 1.3 bx	25.6 ± 0.6 ax
Vs	C	7.4 ± 0.1 ax	0.9 ± 0.3 ax	17 ± 0.8 axy	11 ± 1.1 ay	110 ± 5 ay	440 ± 17 ay	38.7 ± 1.4 ay	20.1 ± 0.5 by
	B	7.5 ± 0.1 ax	1.2 ± 0.4 ax	18 ± 0.9 ax	12 ± 1.2 axy	110 ± 4 ay	432 ± 16 ay	38.7 ± 1.3 ax	23.0 ± 0.6 ay
Fs	C	7.5 ± 0.1 ax	1.0 ± 0.4 ax	16 ± 0.8 by	12 ± 1.2 ay	120 ± 5 ay	347 ± 13 bz	23.9 ± 0.8 az	19.8 ± 0.5 ay
	B	7.5 ± 0.1 ax	1.3 ± 0.4 ax	18 ± 0.8 ax	10 ± 0.9 ay	100 ± 4 bz	412 ± 15 ay	27.6 ± 0.9 ay	20.1 ± 0.5 az

EC = electrical conductivity; CEC = cation exchange capacity; N_{tot} = total nitrogen; N_{av} = available nitrogen; P_{tot} = total phosphorous; P_{av} = available phosphorous; C_{tot} = total carbon.

3.2. Cherry Tomato Plant Characteristics

3.2.1. Morphological Traits

Leaf number and area, root length, and surface area increased throughout the experiment for both the untreated and biochar-treated plants (Figure 1). Except as observed at Es, both at the Vs and Fs sampling points, the biochar-treated plants (B) showed higher values of these parameters compared with the untreated plants (C) (Figure 1). In detail, at the Vs and Fs sampling points, the B plants had a greater number of leaves (38 and 68, respectively) than the C cherry tomato plants (32 and 49, respectively) (Figure 1a). The biochar also increased the leaf area with a rise of 26% and 36% compared with the values measured in the C plants (15,775 and 17,949 cm^2 at Vs and Fs, respectively) (Figure 1b). In the B plants, at Vs and Fs, the amendment use increased 1.6- and 2-fold both the root length and root surface area in comparison with the untreated plants of *S. l. cerasiforme* (Figure 1c,d). In the C plants, the values for root length were 51 and 153 m (Figure 1c), and the values for root surface area were 385 and 1474 cm^2, respectively, at the Vs and Fs sampling points (Figure 1d).

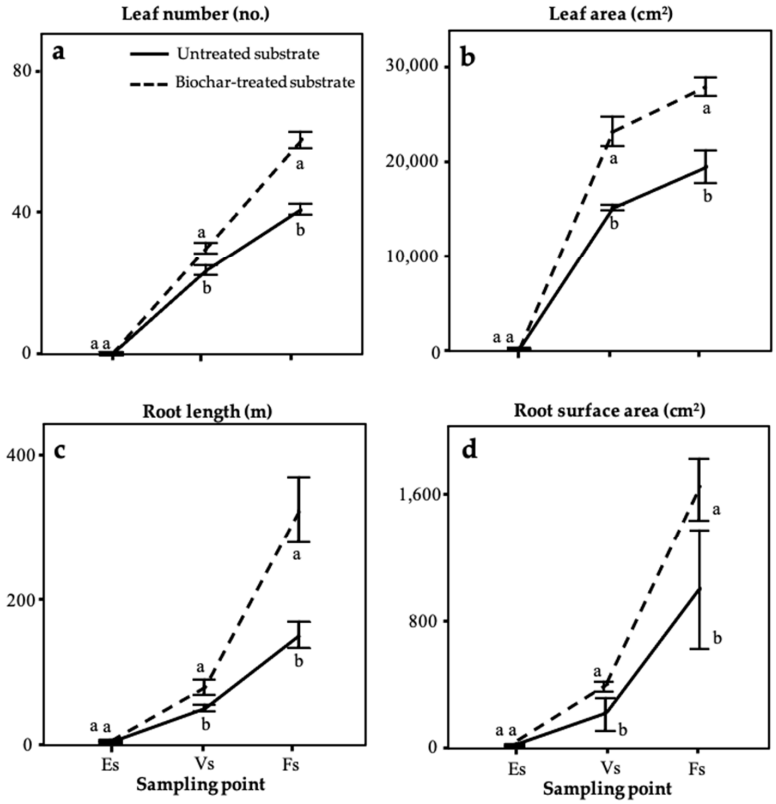

Figure 1. Leaf number (**a**) and area (**b**), root length (**c**), and surface area (**d**) were measured at the early stage (Es, 36 days after plant germination), vegetative stage (Vs, 84 days after plant germination), and fruit stage (Fs, 140 days after plant germination) of *Solanum lycopersicum* var. *cerasiforme* plants grown on the two different substrates (solid line, plants in the untreated substrate; dashed line, plants in the biochar-treated substrate). Letters indicate statistically significant differences between the two treatments inside each sampling point ($p < 0.05$) ($n = 10 \pm$ SE).

As reported in Figure 2, while leaf dry mass showed a linear growth (Figure 2a), stem and root biomass showed an exponential growth (Figure 2b,c). Nevertheless, they had the same trend, and at the Es sampling point, the different organ dry biomass did not show significant differences among the untreated and biochar-treated plants (Figure 2a–c). Differently, at Vs and Fs, the leaf, stem, and root dry masses were higher in the biochar-treated plants than in the untreated ones (Figure 2a–c). Specifically, at both sampling points (Vs and Fs) and for all the plant organs (leaves, stems, and roots), the biochar amendment

determined a twofold increase in dry biomasses in comparison with the untreated plants (Figure 2a–c).

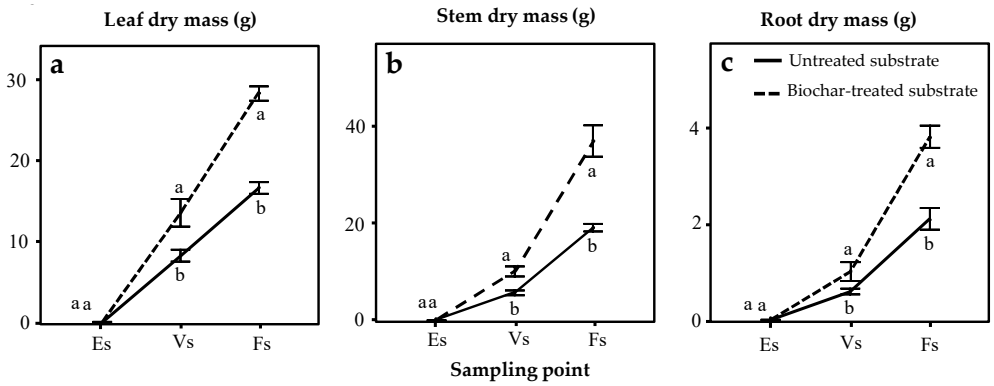

Figure 2. Leaf (**a**), stem (**b**), and root (**c**) dry mass measured at the early stage (Es, 36 days after plant germination), vegetative stage (Vs, 84 days after plant germination), and fruit stage (Fs, 140 days after plant germination) of *Solanum lycopersicum* var. *cerasiforme* plants grown on the two different substrates (solid line, plants in the untreated substrate; dashed line, plants in the biochar-treated substrate). Letters indicate statistically significant differences between the two treatments inside each sampling point ($p < 0.05$) ($n = 10 \pm$ SE).

3.2.2. Root and Leaf Carbon and Nitrogen Content

At the root level, the biochar positively affected only total nitrogen content (N_{tot}) at the Fs sampling point only (Table 3). Meanwhile, in leaves, the amendment negatively affected total carbon content (C_{tot}) only at the Es sampling point (Table 3). In detail, root N_{tot} increased by 31% in the biochar-treated cherry tomato plants (B) in comparison with the untreated plants (C) (1.28%). Moreover, the biochar induced a 10% decrease in leaf C_{tot} compared with the C plants (36.04%) (Table 3).

Table 3. Measurements of total nitrogen (N_{tot}) and total carbon (C_{tot}) were performed on roots and leaves of untreated (C) and biochar-treated (B) *Solanum lycopersicum* var. *cerasiforme* plants at the early stage (Es, 36 days after plant germination), vegetative stage (Vs, 84 days after plant germination), and fruit stage (Fs, 140 days after plant germination). Letters a and b indicate a statistically significant difference ($p < 0.05$) between the two treatments inside each sampling point. Letters x, y, and z indicate a statistically significant difference ($p < 0.05$) among the Es, Vs, and Fs sampling points for each treatment. ($n = 6 \pm$ SE).

		Characteristic			
		Root N_{tot} (%)	**Root C_{tot} (%)**	**Leaf N_{tot} (%)**	**Leaf C_{tot} (%)**
Es	C	3.39 ± 0.07 ax	37.85 ± 0.70 az	6.10 ± 0.03 ax	36.04 ± 1.45 ax
	B	3.68 ± 0.10 ax	37.77 ± 0.25 ay	6.93 ± 0.01 ax	32.49 ± 1.90 bx
Vs	C	2.22 ± 0.10 ay	40.53 ± 0.15 ay	3.64 ± 0.45 ay	35.37 ± 3.55 ax
	B	2.08 ± 0.22 ay	40.21 ± 0.70 ay	4.50 ± 0.40 ay	36.19 ± 2.85 ax
Fs	C	1.28 ± 0.005 az	42.87 ± 0.30 ax	1.45 ± 0.27 az	34.97 ± 1.50 ax
	B	1.68 ± 0.16 bz	41.32 ± 0.75 ax	1.88 ± 0.30 az	35.93 ± 1.90 ax

As shown in Table 3, at the root level, in both untreated and biochar-treated plants, there was a decrease in N_{tot} and an increase in C_{tot} over time.

In detail, in the untreated (*S. l. cerasiforme*) plants, N_{tot} content decreased by 35% at the Vs sampling point and by 62% at the Fs sampling point compared with the Es sampling point (3.39%). Instead, the increase in root C_{tot} of the C plants was 7% and 13% at the Vs and Fs sampling points compared with Es (37.85%), respectively (Table 3).

In the biochar-treated plants, root N_{tot} decreased by 43% and 54% at the Vs and Fs sampling points compared with the Es sampling point, respectively. These same plants had

9% higher root C_{tot} content at the Fs sampling point than at Es (Table 3). Moreover, at the root level, across the entire duration of the experiment, the untreated and biochar-treated plants had the same reduction in N_{tot} (97%).

In the leaves of both plants grown in the untreated and biochar-treated substrate, no significant difference was recorded for C_{tot} content over time. Instead, there was a significant decrease in the N_{tot} concentrations measured at the three sampling points (Table 3). Specifically, in both untreated and biochar-treated *S. l. cerasiforme* plants, the leaf concentration of N_{tot} was reduced 1.6 and 4 times at the Vs and Fs sampling points, respectively, compared with that reported at Es (in the C plants, the concentration was 6.10%, and in the B plants, it was 6.93%) (Table 3).

3.2.3. Fruit Characteristics

Both flower and fruit number, trans- and cis-lycopene, titratable acidity, and total soluble solid content were higher in the biochar-treated *S. l. cerasiforme* than in the untreated plants (Figure 3a,b,i–l). On the contrary, for fruit dry mass, polar and equatorial diameter, epicarp thickness, and right and left mesocarp thickness, no difference was detected (Figure 3c–h).

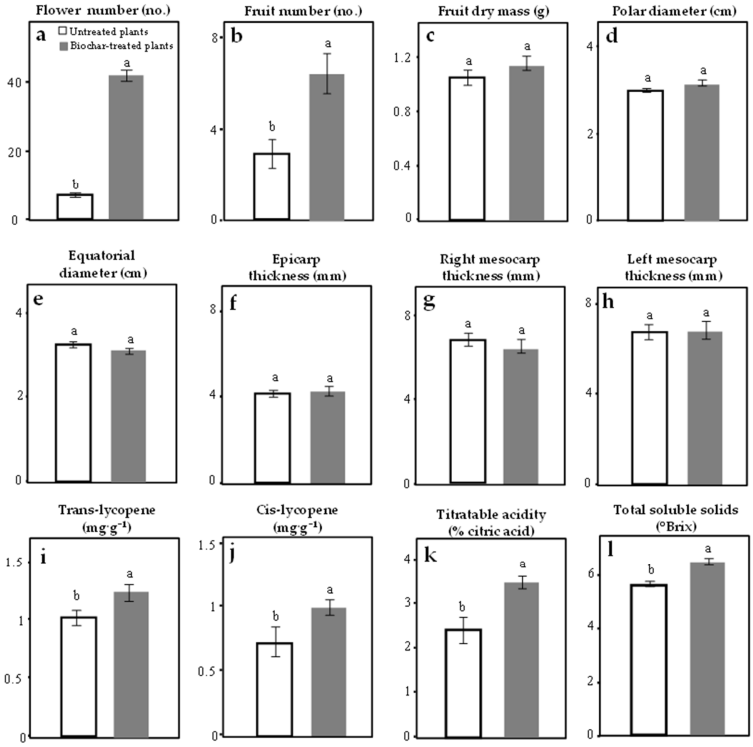

Figure 3. Flower number (**a**), fruit number (**b**), dry mass (**c**), polar (**d**) and equatorial diameter (**e**), epicarp thickness (**f**), right mesocarp (**g**) and left mesocarp thickness (**h**), trans-lycopene (**i**), cis-lycopene (**j**), titratable acidity (**k**), and total soluble solid content (**l**) measured for *Solanum lycopersicum* var. *cerasiforme* plants grown on the two different substrates (white box, plants in the untreated substrate; gray box, plants in the biochar-treated substrate). Letters indicate statistically significant differences ($p < 0.05$) ($n = 10 \pm$ SE).

Moreover, the biochar increased the number of flowers and fruits of *S. l. cerasiforme* plants treated with the soil conditioner six times and two times, respectively, compared with the untreated plants (Figure 3a,b). In addition, cherry tomato plants grown on the B substrate had 30%, 25%, 75%, and 16% higher trans- and cis-lycopene and titratable acidity and total soluble solid content, respectively, when compared with plants grown on the C substrate (Figure 3i–l).

4. Discussion

As reported in several works, biochar, used as an amendment, could have a promoting effect on plant growth and productivity as an indirect consequence of its positive effect on growth medium parameters (e.g., water holding capacity and pH enhancement, increased nutrient availability) [39–41]. Indeed, first, when biochar is applied to a growth substrate, it can retain water, thanks to its particularly porous internal structure [42]. Second, when biochar is used, research findings indicate significant increases in pH, organic carbon, and exchangeable cations [43]. Growing medium pH is an important characteristic in terms of nutrient availability and, in turn, plant growth. Growing substrates with a high cation exchange capacity can hold or bind nutrient cations; thus, nutrients are retained rather than leached and, therefore, more available for uptake by plants [44]. Biochar addition to growth media is currently being considered as a means to avoid the leaching of nutrients, which can deplete fertility, hasten substrate acidification, raise the cost of fertilizer for farmers, and reduce the yield of crops [45]. Thus, the use of biochar as an amendment may improve the nutrient supply to plants. Moreover, because of its porous nature, high surface area, and ability to adsorb soluble organic matter and inorganic nutrients, biochar also provides a suitable habitat for microbes important in releasing plant-growth-promoting substances [46].

Tomato plants grow optimally in both the field and greenhouse, achieving maximum production levels in soilless culture [11]. One of the most used substrate constituents for soilless vegetable cultivation is peat [12]. However, peat is a limited resource with huge demand, and its extraction results in deleterious environmental impacts [47]. Therefore, there is a growing interest in replacing peat with other soilless substrates, and biochar could entirely or partly substitute peat as a plant-growing constituent to produce vegetables [10]. Indeed, biochar used as a growth medium for soilless cultivation appears to offer a concrete opportunity to increase the economic and environmental sustainability of intensive cropping systems through the replacement of nonrenewable materials [11]. However, a few pieces of research [48–52] have been conducted to evaluate/investigate the potential of biochar amendment in enhancing tomato plant growth and yield in view of sustainable agriculture.

Therefore, we aimed to assess the potential use of biochar as a growing medium for soilless *S. l. cerasiforme* production by monitoring substrate physicochemical properties and plant characteristics at three different sampling points (early stage, Es; vegetative stage, Vs; and fruit stage, Fs). We found an increase in cation exchange capacity (CEC) in the biochar-treated substrate at the Fs sampling point only, and this might probably be due to an oxidation increase in this specific growth stage. Indeed, Liang et al. [53] demonstrated that biochar particles are subjected to oxidation processes, contributing to the increase in both surface charge density and, in turn, CEC. Moreover, biochar is known to have the potential to reduce nutrient/cation leaching in growing media, consequently leading to an increase in CEC [43,54,55].

Besides that, the higher content of available nitrogen (N_{av}) measured at the Fs sampling point in the untreated substrate could be attributable to a higher microbial activity that normally characterizes the biochar-treated substrate, which leads to a major reduction of N_{av} [17]. According to this, the high total carbon (C_{tot}) content in the biochar-treated substrate at the Es sampling point could be attributable to the early and high microbial activity producing organic acids in the growing medium [56]. Although many studies report a positive biochar effect on phosphorous availability (P_{av}) [57,58], in our study, lower content in the biochar-treated substrate, at the Es sampling point, was detected. This effect could be due to the biochar's ability to bring P_{av} into the substrate, which is dependent on the amount and form of phosphorous available in the substrate and biochar type [59]. Furthermore, our results follow those of other studies highlighting that the biochar amendment was a modest source of phosphorous for plant production [12,60]. Nevertheless, this observation was confirmed only at the early stage, since at the Fs sampling point, the biochar application led to an increase in total phosphorous (P_{tot}).

Over time, in both the untreated and biochar-treated substrates, there was a decrease in total and available concentrations of nutrients (carbon, nitrogen, and phosphorus). This decrease found between the Es and Fs sampling point might be related to the potential increase in uptake and accumulation of these nutrients by cherry tomato plants for enhancing growth and development. Indeed, our findings showed that the biochar-treated plants had higher values of all morphological traits measured for all organs than those plants grown in the untreated condition. In particular, these ameliorations were observed later in the cherry tomato plant phenology during Vs and Fs. These findings are in line with studies reporting a lack of biochar influence on the early stages of plant growth for *Triticum aestivum*, *Raphanus sativus*, and *Sorghum bicolor* (respectively, [61,62]). For instance, Free et al. [63] showed that maize's early growth was not significantly affected by biochar produced from a range of feedstock sources.

Massa et al. [11] and Choi et al. [64] observed a biomass increase for tomato plants when peat and pine bark were replaced by biochar by up to 40% in a soilless growing medium. In our study, although the 20% addition of biochar replaced peat, humus, and silica sand for 5%, 5%, and 10% respectively, the better cherry tomato plant growth observed at the Vs and Fs sampling points likely resides in positive interaction between biochar and plant nutrition, as also proposed in previous studies on radish, pepper, and tomato [45,48,65]. Indeed, biochar's ability to improve crop growth is reported to be a consequence of a potential extra nutrient budget [23] and/or an indirect consequence of improved physicochemical and biological characteristics of the biochar-treated growing medium [45]. Considering biochar properties and abilities [11,37], its addition to the soilless substrate might have led to more nutrients potentially available to the plants than in the untreated substrate. This higher nutrient content due to biochar presence [66,67] may be related to the observed increment of root length and surface area [68]. In particular, at the Fs sampling time, the improvement of root growth could be attributable to the fruit ripening stage during which plants use most of their energy to generate new roots for a major nutrient uptake [68,69].

Several mechanisms have been suggested to explain the positive effects of biochar on crop growth and yield, varying from physicochemical and biological changes [23,70] to macro- and micronutrient immobilization [71] in the growing substrate [37]. For instance, improvements in fruit yield and quality, such as higher fruit acidity, antioxidant compound content, organoleptic qualities, and their content in phytochemical compounds, are all well-documented effects in tomatoes grown on substrates with higher nutrient availability in the root zone due to biochar application [23,72–74]. Accordingly, in the current study, the biochar-treated plants showed a higher number of flowers and fruits, although the mean fruit biomass and morphology remained unchanged. Additionally, higher values of trans- and cis-lycopene, total soluble solids, and titratable acidity were found in the biochar-treated plants when compared with the untreated ones. In our research, the promotion of fruit quantity and quality could be attributable to the high P_{tot} content in the biochar-treated substrate and the high N_{tot} concentration in roots of the biochar-treated plants measured at the Fs sampling point. Indeed, according to other reports, there might be a relationship between the phosphorous and nitrogen contents in both growing substrates and plant tissues and the promotion of fruit production by improving the vegetative and reproductive properties of tomato plants [75–77]. Our results about cherry tomato fruit quality are also in agreement with those obtained by Almaroai et al. [27], Akhtar et al. [69], and Hameeda et al. [77], who reported increased values of lycopene, titratable acidity, and total soluble solids when biochar was used as a soil amendment.

Other important factors, besides the substrate nutrient concentration, related to the biochar improvement of fruit yield and tomato quality are the increase in substrate microbial biomass, nutrient uptake, plant tissue potassium concentration [45], and leaf photosynthetic activity [78,79]. Therefore, although in the current study these traits were not measured, we might speculate that the higher carbon availability observed in the biochar-treated substrate at the beginning of the experiment may have enhanced the microbial activity,

resulting in greater nitrogen demand and promoting immobilization and recycling of nitrate (NO_3^-) [65]. In addition, the higher N_{tot} and potassium cation concentration in the root zone of the biochar-treated plants may have led, respectively, to the increase in lycopene content [11], acidity, and soluble solid content of tomato bulks [79].

5. Conclusions

In the present study, the biochar addition to a soilless substrate significantly improved *Solanum lycopersicum* var. *cerasiforme* plant development through the enhancement of morphological and chemical traits during the vegetative and fruit stages. Moreover, the biochar-treated cherry tomato plants had a higher fruit number and antioxidant content as compared with the untreated plants. The improvement of these plant characteristics was coupled with the increase in nutrient availability in the biochar-based substrate. Therefore, the present work concurs with previous scientific reports underscoring that biochar amendment has a great potential for improving tomato yield and fruit quality through the enhancement of the growing medium's physical and chemical characteristics. In conclusion, we may assert that wood-based biochar can be considered a material to be added to soilless cultivations in the framework of sustainable agriculture, particularly for reducing the use of peat in growing media. Finally, although our findings refer to a short-term potted experiment, they give hints toward biochar use for long-term open-field tomato cultivation.

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