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Biochar Increases Soil Moisture and Improves Tomato Resilience Under Field Conditions: Results from a Two-Year Field Study in Tuscany (Italy)

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Abstract

Biochar, a carbon-rich by-product of wood pyrolysis, improves soil structure, water retention, and plant growth. A two-year field experiment (2024–2025) was conducted in Poggibonsi (Tuscany, Italy) on tomato cv. "Canestrino" under contrasting climatic conditions. A single biochar application (15 t ha^{-1}) was evaluated for its effects on soil properties, water dynamics, plant water status, and ecophysiological and tissue-level responses. From the results, it emerged that biochar improved soil quality by increasing organic matter (+7.7%) and the C/N ratio (+10.6%), while reducing bulk density (1.42 to 1.25 Mg m^{-3}). Soil water content was higher in amended plots, particularly in 2024 (32.84% vs. 24.87%), with a smaller increase in 2025 (24.66% vs. 24.08%). Improved soil water availability enhanced plant water status, as shown by less negative leaf water potential under stress conditions. Microscopic analyses confirmed better xylem integrity in treated plants, with reduced formation of tyloses and improved hydraulic functionality during drought. Agronomic responses reflected climatic variability: yield increased in biochar in 2024, whereas in 2025 drought stress reduced productivity in both treatments, with no significant differences. Overall, biochar improved soil moisture retention, plant water status, and ecophysiological performance, with effects dependent on seasonal rainfall patterns and environmental stress intensity.

Keywords: biochar; ecophysiology; tomato; water use efficiency; drought stress; soil moisture; sustainable agriculture

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1. Introduction

Agriculture currently accounts for approximately 70% of global freshwater withdrawals [1,2], and demand is projected to double by 2050, while freshwater availability may decline by up to 50% [3]. In a context where a rapid reversal of climate change is unlikely, scientific and technological innovation is essential to ensure food security and the sustainability of agricultural systems [4]. Drought is one of the main limiting factors in Mediterranean regions, where increasing temperatures and irregular precipitation are exacerbating water scarcity, together with soil degradation and loss of fertility. In these systems, reduced water availability directly limits crop productivity while increasing production costs and economic vulnerability [3,5]. Adaptation strategies include the adoption of more efficient agronomic practices, crop genetic improvement, and reduced food losses along the supply chain [6,7]. In this context, increasing water-use efficiency is crucial to address growing water scarcity and protect agricultural productivity in many regions worldwide [8,9]. This is particularly important in the Mediterranean region, where climate variability and limited water availability increasingly threaten tomato cultivation. As the second most important horticultural crop worldwide, grown for both fresh consumption and processing, tomatoes play a significant role in regional and global agri-food systems [10]. Strengthening tomato tolerance to drought and heat stress while maintaining yield stability is crucial to secure long-term productivity, sustainable water use, and environmental balance. In 2022, global tomato production reached 186.1 million tons from 4.7 million hectares, with China, the United States, India, and Turkey among the leading producers (https://www.fao.org/faostat; last accessed 20 March 2026). In Mediterranean areas, including Italy, regions such as Tuscany combine high-value supply chains with traditional local cultivars. However, increasing temperatures, more frequent heatwaves, and irregular rainfall are intensifying drought stress, negatively affecting growth, fruit set, and yield. These challenges highlight the need for strategies that enhance crop resilience and sustainable production under climate change.

In this context, biochar has recently emerged as a promising soil amendment for sustainable agricultural systems. According to the International Biochar Initiative (IBI), biochar is a solid material produced from the thermochemical conversion of biomass in an oxygen-limited environment. Biochar was initially utilized by pre-Columbian indigenous peoples in the Amazon region between 500 and 9000 years ago as one of several soil amendments that led to the creation of 'terra preta,' a nutrient-rich agricultural soil with a higher pH than the region's typically acidic and poorly productive soils [11,12].

In addition to its contribution to climate change mitigation through long-term carbon sequestration and reduced soil greenhouse gas emissions, biochar can improve tomato ecophysiological performance by enhancing soil water retention [13–15], nutrient availability, and root activity. These effects contribute to higher chlorophyll content, improved photosynthetic efficiency and stomatal conductance, as well as greater water-use efficiency under drought conditions. Biochar application has also been associated with increased accumulation of antioxidant compounds, including flavonoids, which play a key role in mitigating oxidative stress and improving plant tolerance to environmental constraints [16,17]. Similar relationships between enhanced antioxidant defense systems, improved physiological performance, and increased drought tolerance have been reported in sunflower, where stress-adaptive responses were evaluated through physiological and biochemical indicators closely linked to plant water status and oxidative stress regulation [18]. Despite these advances, reported effects of biochar remain highly variable, largely influenced by biochar type, soil properties, pedoclimatic conditions, and management practices. Notably, there is a scarcity of multi-year, open-field studies in Mediterranean environments specifically targeting tomato cultivation. Comprehensive evaluations assessing the impact of biochar on soil moisture dynamics, plant resilience to abiotic stress, and yield stability. Many studies have explored the impact of biochar on tomato cultivation. However, there remains considerable fluctuation and unpredictability regarding its effects on tomato yield. While certain studies have documented a significant increase in tomato yields through the conversion of agricultural residues into biochar [15,17], other research has reported neutral or highly variable effects on tomato productivity depending on environmental and agronomic conditions [19,20]. Mediterranean horticulture, addressing these knowledge gaps, is critical for developing effective, climate-resilient soil management strategies. Although tomato cultivation is widespread, local varieties such as "Canestrino di Lucca" are especially valued for their outstanding organoleptic properties and cultural significance. Within this framework, this study presents the results of a two-year open-field experiment conducted in Tuscany (Italy) under organic management on coarse-textured soils, where biochar was applied at a rate of 15 t ha^{-1} [21] in tomato (*Solanum lycopersicum*) cultivation. The study, conducted over two years under open-field conditions on sandy loam soil within an organic farming system, aimed to evaluate the effects of biochar application on tomato cultivation. Biochar was applied only during the first year, allowing assessment of its effects both one and two years after application and providing insight into its medium-term impacts. The investigation addressed ecophysiological responses as well as productive traits and included microscopic analyses of plant tissues to further explore structural modifications associated with the treatment. Over the two growing seasons, plant water status was assessed using pressure chamber measurements, providing direct information on plant ecophysiological responses to water availability. In addition, chlorophyll and flavonoid contents were monitored as indicators of the plant's capacity to adapt to environmental stress. Plant responses to biochar-induced changes in water and nutrient availability can be assessed using both destructive and non-destructive approaches [22]. Traditional destructive analyses, such as spectrophotometric determination of pigments, flavonoids, and antioxidant compounds, provide detailed biochemical information but require tissue sampling and prevent repeated measurements on the same plant. Therefore, the present study employed non-destructive ecophysiological measurements to investigate the effects of biochar on tomato performance, allowing continuous assessment of plant responses while preserving plant integrity.

The study also evaluated the effects of these factors on yield. Overall, the integration of these aspects provides valuable insights for improving sustainability, water use efficiency, and stability in tomato production systems in the Mediterranean region.

2. Materials and Methods

2.1. Study Area and Design of Experiments

The field experiment was conducted over two consecutive growing seasons (2024 and 2025) at the organic farm Biortaggi Valdelsa, located in Poggibonsi, central Italy (43.471630 N, 11.121613 E). The study site is located at an elevation of 90 m a.s.l. in a bend of the Foci stream that flows into the Elsa River about 400 m further north (Figure 1). The soil, classified as Fluventic Haplusteps, coarse, mixed, mesic [23] developed on quaternary sandy fluvial deposits. The topsoil texture (0–30 cm) is sandy loam, with a particle-size distribution of 53.0% sand, 28.1% silt, and 18.9% clay. Soil pH is alkaline with a value of 8.5 due to the calcareous parent material.

The study evaluated the response of tomato, cv. "Canestrino di Lucca", grown under open-field conditions. Transplanting took place on 20 April 2024, and 25 April 2025. At the time of transplanting, the seedlings were 35–40 days old from sowing. The plants were spaced 45 cm along the row and 1 m spacing between rows. Training was carried out using the traditional method, using one vertical stake per plant. The plants were trained to single stems, periodically removing axillary shoots, and progressively tied during growth. Apical pinching was performed when 5–7 flower clusters were achieved, depending on seasonal conditions.

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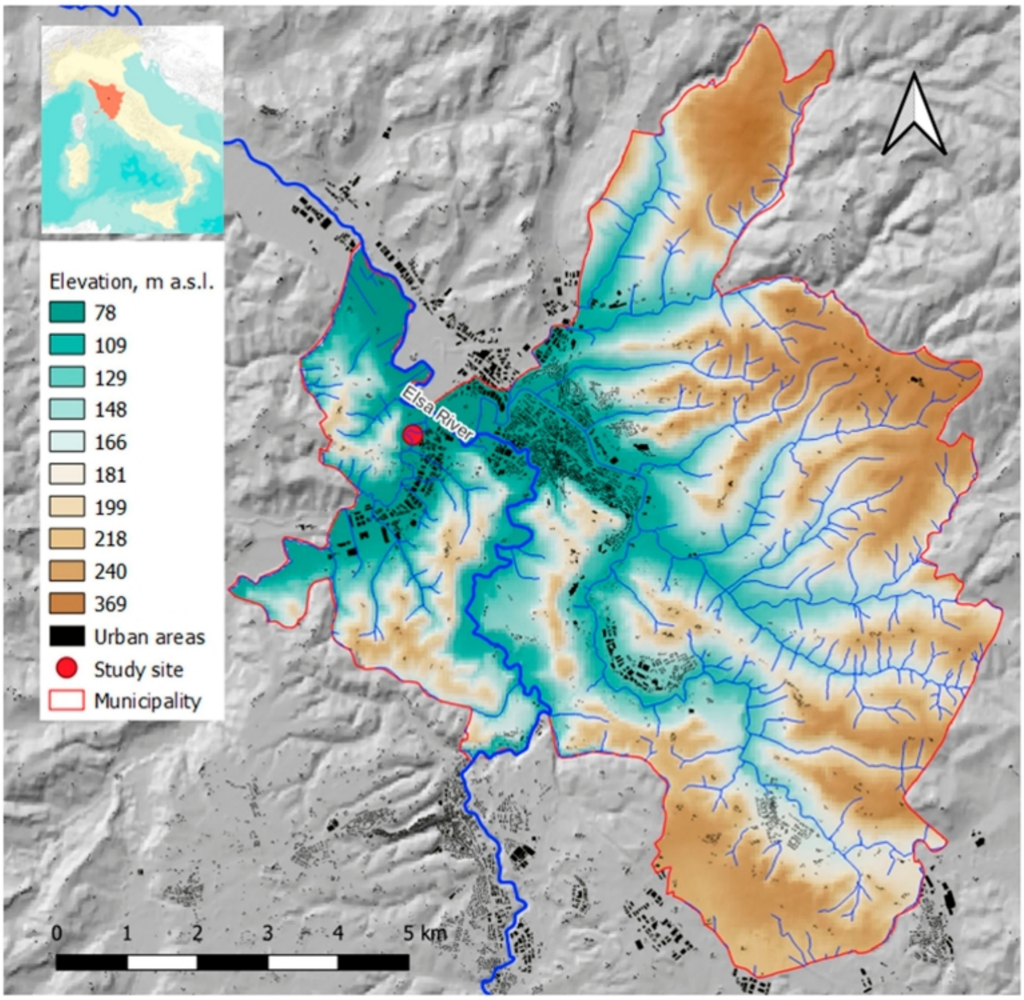

Figure 1. Location of the study site in the Municipality of Poggibonsi in central Tuscany.

Irrigation was supplied via a drip irrigation system and was maintained identically for both the control and biochar treatments throughout the two years. During the establishment phase (first 2–3 weeks after transplanting), 10–15 mm of water was applied per irrigation event, with a frequency of 1–2 applications per week. During the vegetative growth and flowering stages, irrigation was increased to 20–25 mm week^{-1}, distributed in 2–3 applications. During fruit swelling, irrigation volumes were further increased to 25–35 mm week^{-1}. As fruit ripening approached, irrigation was moderately reduced.

A strip-plot design with two treatments and three replicates was adopted: (i) soil amended with biochar (B) and (ii) untreated soil (control, C) (Figure 2). Biochar was manually applied before transplanting in April 2024 at a rate of 15 t ha^{-1} (fresh weight) and mechanically incorporated into the top 30 cm of soil using a power tiller. In 2025, no additional biochar was applied, and both the treated and control plots were maintained as established in the previous year. Considering a moisture content of 30%, the corresponding biochar dry matter application rate was approximately 10 t ha^{-1}. The selected application rate followed the Guidelines on Practical Aspects of Biochar Application to Field Soils in Various Soil Management Systems, which identifies 15 t ha^{-1} as a moderate and agronomically realistic rate, widely tested under field conditions and well below excessively high doses reported to potentially impair crop performance [21].

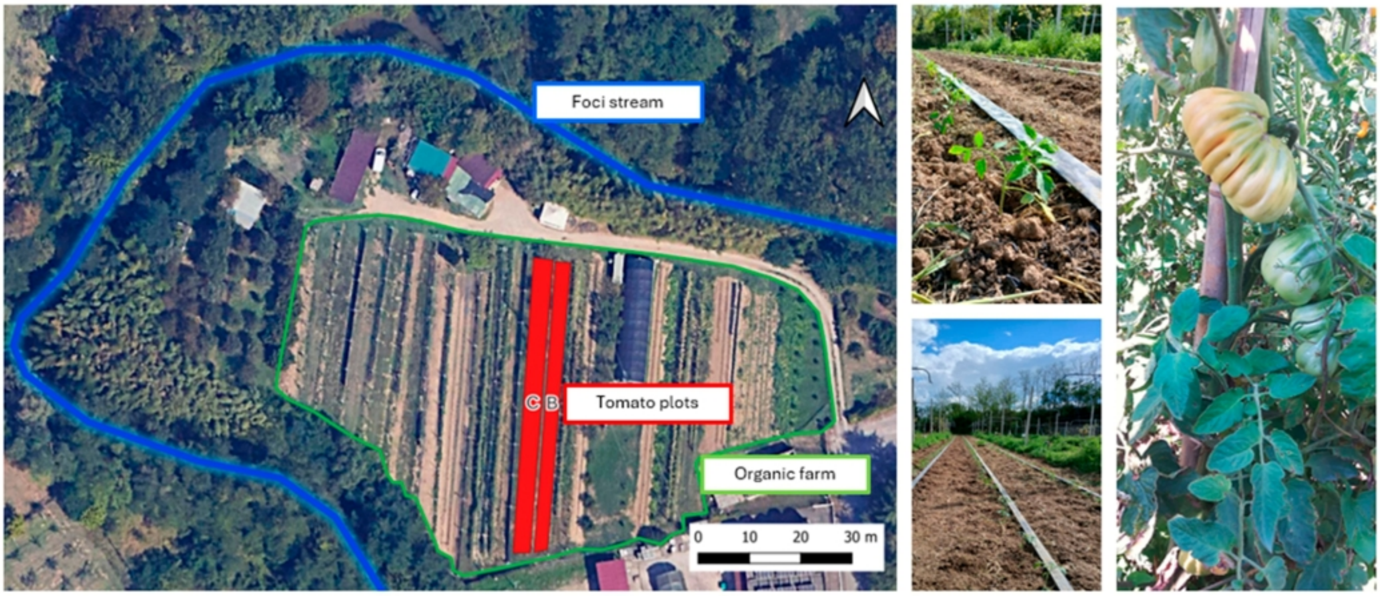

Figure 2. Experimental plots with the two treatments (C, control; B, biochar) at the organic farm Biortaggi Valdelsa.

This experimental approach allowed the assessment of the residual effects of the biochar applied in 2024 over time, while preserving the original distinction between treated and control plots.

Consequently, comparable soil and management conditions were maintained throughout the two years, in accordance with the agronomic practices adopted in the experiment.

Each treatment consisted of three replicates, with 30 plants per replicate (90 plants per treatment in total). Plots were arranged to minimize spatial variability and edge effects. All agronomic practices, including fertilization, irrigation, pest management, and crop maintenance, were uniformly applied to all treatments and in both experimental years.

The biochar used was a commercial plant-derived product, classified as Class I under Italian Legislative Decree 75/2010 and authorized for use in organic farming in accordance with Regulation (EU) 2019/2164. It was produced from orchard pruning residues through slow pyrolysis at approximately 600 °C. About 70% of the material consisted of particles smaller than 1 cm. The total content of different elements (Mg, P, K, Na, Ca) was determined after acid digestion with a microwave oven, according to EPA 305258 [24], with an ICP-OES (iCAP 6000 Series, Thermo Scientific, Waltham, MA, USA) based on the EPA 6010D 2014 standard [25]. For total carbon (C_{tot}) and total nitrogen (N_{tot}) of biochar, samples were oven-dried at 105 °C for 24 h, acid digested with a microwave oven (CEM, MARSXpress, Matthews, NC, USA) according to the EPA method 305258 [24] and determined using a CHN Elemental Analyzer (Carlo Erba Instruments, Milan, Italy; mod 1500 series 2). The pH was measured in a water solution (1:5 ratio) using a pH meter [26]. The polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) analysis was carried out via the US-EPA 3550C59 method [27]. The main physicochemical properties of the biochar are summarized in Table 1.

Table 1. Biochar physicochemical properties.

Parameter	Value
Particle size distribution	
Fraction > 0.5 mm	12%
Fraction < 2 mm	19%
Fraction < 5 mm	43%
N_{tot}	0.5%
K_{tot}	3020 mg kg^{-1}
P_{tot}	0.34%
Ca_{tot}	9920 mg kg^{-1}
Mg_{tot}	852 mg kg^{-1}
Na_{tot}	291 mg kg^{-1}
Phytotoxicity test and growth	Suitable
Water holding capacity	150%
C_{tot}	65%
Salinity	110 mS m^{-1}
Ash content	5.5 ± 0.5%
Molar H/C ratio	0.2
PAHs (∑16 US EPA)	≤4 mg/kg s.s
PCB	<0.25
pH	9.8

Abbreviations: N_{tot}: total nitrogen; K_{tot}: total potassium; P_{tot}: total phosphorus; Mg_{tot}: total magnesium; Na_{tot}: total sodium; C_{tot}: total carbon; PAHs, polycyclic aromatic hydrocarbons; PCB, polychlorinated biphenyls. PAHs (∑16 US EPA) refer to the sum of the 16 priority PAHs identified by the U.S. Environmental Protection Agency.

2.2. Meteorological Data and Experimental Conditions

The area is characterized by the Mediterranean climate. Meteorological data were obtained from the Regional Hydrological Service of Tuscany (SIR) monitoring network (https://sir.toscana.it/; last accessed 20 March 2026). Daily meteorological records for the growing seasons (May–August 2024 and 2025) were used to characterize site-specific environmental conditions, including air temperature and precipitation. Daily maximum temperatures were extracted from the dataset to describe seasonal thermal patterns.

A threshold of 30 °C was considered indicative of potential heat stress conditions in tomato crops [28].

2.3. Soil Physicochemical Analysis

Soil chemical characterization was conducted at its conclusion (3 September 2025), following the second-year tomato growing cycle, to assess the effect of the biochar application on key soil properties. To account for spatial variability, three subplots were established within each replicate, for both the control and treated areas. A total of 6 soil samples (3 per treatment) were collected at a depth of 0–15 cm, focusing on the layer most affected by biochar incorporation. These samples were combined to form a representative composite sample for each plot, which was subsequently air-dried and sieved to <2 mm prior to laboratory analysis. Chemical and physical parameters were determined using standardized international and national procedures, with results expressed on a dry weight basis. Soil pH was measured in a 1:2.5 soil-to-distilled water suspension according to ISO standard methods [29]. Particle size distribution was determined using official Italian methods [30]. Total nitrogen (N), soil organic carbon (SOC), and soil organic matter (SOM) were quantified via dry combustion using an elemental analyzer [31,32], and the C/N ratio was subsequently calculated. Soil bulk density (BD, Mg m^{-3}) was determined in both thesis (B and C) in five locations per treatment at 0–15 cm depth using a cylindrical core of

100 cm^3 volume (V). Samples were dried in an oven at 105 °C for 48 h, and weighed (DW) for calculating the bulk density (Mg m^{-3}) as

$$BD = DW/V$$

To evaluate the influence of biochar on water dynamics, rainfall data from May to August were retrieved from the "Poggibonsi" weather station (TOS01004965; 43.471° N, 11.129° E; 88 m a.s.l.; Gauss-Boaga: E 1,672,211 m, N 4,815,389 m). Soil volumetric water content (VWC) was monitored using Water Scout SM100 sensors (Spectrum Technologies, Haltom City, TX, USA) installed at a depth of 15 cm. Eight sensors were deployed (four per treatment) and connected to four master data acquisition units. VWC measurements were recorded continuously at 2 h intervals from May to the end of August to assess the effect of biochar on soil water retention capacity.

2.4. Plant Leaf Water Potential, Chlorophyll and Flavonoid Indices

To evaluate the effect of biochar application on tomato performance, ecophysiological measurements were conducted during the 2024 and 2025 growing seasons, between late June and August. Measurements were carried out at specific plant phenological stages identified according to the BBCH scale (Biologische Bundesanstalt, Bundessortenamt and Chemical Industry) [33] for tomato.

BBCH scale codes are assigned to the crop only when at least 50% of the plants have reached that developmental stage. BBCH codes were assigned when at least 50% of the plants in the field had reached the corresponding developmental stage [33], ensuring uniformity and representativeness of the phenological assessment across the crop.

The ecophysiological measurements included monitoring leaf chlorophyll and flavonoid content, as well as leaf water status. For each treatment and the corresponding control, three sub-plots were defined, within which representative plants were selected for ecophysiological measurements. Leaf chlorophyll (Chl) and flavonoid (Flav) content were monitored in situ using a portable Dualex® sensor (Force-A, Orsay, France), an optical leaf-clip device that determines absolute chlorophyll content (µg cm^{-2}) by analyzing the light transmittance of the leaf blade [34].

For each plot, three plants per replicate were randomly selected. On each plant, three fully expanded leaves of similar size and light exposure were chosen for measurements. Three measurements were performed on each leaf, resulting in a total of 27 measurements per replicate and 81 measurements per treatment.

Leaf water potential (Ψ_leaf) was measured between 12:00 and 14:00 using a pressure chamber (PMS Instrument Company, Albany, OR, USA). Midday water potential is widely considered a standard reference parameter for assessing plant water status under field conditions, as it provides a reliable estimate of plant water stress by integrating the effects of soil moisture availability and atmospheric evaporative demand during peak transpiration periods [35]. At each sampling date, three plants per replicate were randomly selected, and one fully expanded sun-exposed leaf was collected from each plant, resulting in 9 measurements per treatment. Measurements were performed immediately after excision to minimize water loss. The method follows the pressure chamber technique described by Scholander et al. [36].

Ecophysiological measurements were carried out during the growing season, with four samplings in both 2024 and 2025 (Table 2).

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Table 2. Ecophysiological measurements at different growth stages in 2024–2025.

Year	Transplanting Date	Sampling Date	DAT	Principal Growth Stage (PGS)	BBCH Code	Dualex Sensor	Pressure Chamber
2024	20 April 2024	5 June 2024	46	6—Flowering	65	x	
2024		15 July 2024	86	7—Fruit development	71	x	x
2024		5 August 2024	107	7—Fruit development	75	x	
2024		26 August 2024	128	8—Maturity of fruit	89	x	x
2025	25 April 2025	6 June 2025	42	6—Flowering	65	x	
2025		17 July 2025	83	7—Fruit development	71	x	x
2025		30 July 2025	96	7—Fruit development	75	x	x
2025		6 August 2025	103	8—Maturity of fruit	89	x	x

Abbreviations: DAT, days after transplanting; PGS, principal growth stage; BBCH, Biologische Bundesanstalt, Bundessortenamt and Chemical industry scale; x, indicates that measurements were performed using the corresponding instrument.

2.5. Tomato Production

Marketable tomato yield was assessed based on the total production across multiple harvest dates.

In 2024, harvests were conducted on 15 July and 5 August, while in 2025, on 23 July, 30 July and 26 August, in both control and biochar-treated plots. For each treatment, 15 healthy and productive plants (5 per plot) were randomly selected to represent the average performance. All fruits from the selected plants were harvested, counted, and weighed directly in the field using a portable electronic scale with an accuracy of ±0.01 kg. Yield was calculated based on the planting layout (0.45 × 1 m) and expressed as tons per hectare (t ha^{-1}).

2.6. Anatomical Organization of Tomato Root Collar Tissue

Plant collars were collected in late August. This sampling strategy allowed the monitoring of plant development from the seedling stage through to the final ripening stage of the crop cycle. Regardless of the collection time, both control and biochar-treated plants were sampled, washed with running water, and manually fragmented into small pieces using a Gillette Platinum razor blade. The material was fixed in 3% glutaraldehyde in 0.066 M cacodylate buffer (pH 7.2) for two hours and then washed in buffer for thirty minutes. Dehydration was carried out using an increasing ethanol gradient, ranging from 10% for 5 min to 30% for 10 min, 50% for 15 min, 70% for one hour, and finally 100% for one hour, with three changes every 20 min. The samples were then infiltrated with Technovit® 7100 resin, following an ethanol–resin gradient: 1:3 for two hours, 1:1 for 16–18 h, 3:1 for 24 h and 100% pure resin for 24 h with two changes. All infiltration steps were performed with a rotary stirrer to ensure uniform resin penetration. Polymerisation was carried out at 25–30 °C for 2–3 h by adding hardener to the infiltration solution.

Sections of 2–3 µm were obtained with an LKB Ultrotome NOVA microtome (LKB, Bromma, Sweden), spread in water, adhered to slides and dried at 20 °C. Staining was performed with 0.05% Toluidine Blue O (TBO) in benzoate buffer at pH 4.4 for approximately five minutes, followed by washing with water, drying at 27 °C and mounting on slides. TBO, a polychromatic cationic dye, interacts with cellular anionic groups, producing differentiated staining: pink-purple for carboxylated polysaccharides such as pectic acids, bright green or green-blue for polyphenols such as lignin and tannins, and purple or greenish blue for nucleic acids [37]. The observations were made using a Zeiss Axiovert (Carl Zeiss, Oberkochen, Germany) inverted optical microscope with 5× and 40× objectives and recorded using an AxioCam 208 Color video camera (Carl Zeiss, Oberkochen, Germany).

2.7. Statistical Analysis

Statistical analyses were performed in Python (v3.9; Python Software Foundation) using SciPy [38], with NumPy [39] and Pandas [40] libraries for data handling and processing. For all variables (yield, ecophysiological parameters, soil characteristics, and climate data), descriptive statistics were first calculated to characterize data distribution. Differences between treatments (B and C) and between years (2024 vs. 2025) were initially assessed using Student's *t*-test for independent samples in accordance with standard statistical procedures in agronomic research [41]. All parameters were analyzed using one-way ANOVA to evaluate treatment effects. All ecophysiological measurements (chlorophyll and flavonoids) were first averaged at the plot level, which represented the true experimental unit of the study. Accordingly, statistical analyses were performed using plot means as independent replicates ($n = 3$ per treatment). For variables with factorial structure (Year × Treatment × BBCH growth stage), a linear mixed-effects model (LMM) was applied [38]. Year, Treatment and BBCH stage were included as fixed factors, while replicate was treated as a random effect. All two- and three-way interactions were tested.

When significant effects were detected, Tukey's HSD test was used for pairwise comparisons of means [42]. The model was implemented using the statsmodels package (in Python v3.9) [43]. All statistical analyses were based on prior verification of normality and homoscedasticity assumptions. The level of statistical significance was set at $p < 0.05$.

3. Results

3.1. Meteorological Data and Experimental Conditions

A comparison of daily maximum temperatures from May to August shows similar seasonal patterns in 2024 and 2025, characterized by a progressive increase from late spring to a summer peak, followed by a slight decline toward the end of August (Figure 3). Mean maximum temperatures were comparable between years, with values of 31.9 °C in 2024 and 31.8 °C in 2025 ($\Delta = -0.1$ °C). Some differences in intra-seasonal variability were observed between the two years. In 2025, higher temperatures prevailed during May–early June and intermittently through July, accompanied by a more pronounced day-to-day variability. In contrast, 2024 was characterized by comparatively lower early-season temperatures, with warmer conditions shifting toward the second half of July and persisting through most of August. In both years, daily maximum temperatures frequently exceeded 30 °C.

3.2. Short- and Medium-Term Soil Responses Following a Single Biochar Application

At the end of the two-year experiment, soil samples from the C and B showed comparable physicochemical characteristics, particularly in terms of pH. Notably, as biochar was applied only during the first year, this consistency facilitated a clearer interpretation of its effects both immediately after application and in the following year, enabling the assessment of short and medium-term impacts. Conversely, soil organic matter (SOM) content was higher in B than in C (+7.7%). Additionally, the C/N ratio showed a significant increase in B (+10.6%). However, no significant differences were observed for total nitrogen (N_{tot}) ($p = 0.423$), indicating that the increases in organic matter did not translate into a proportional increase in nitrogen (Table 3).

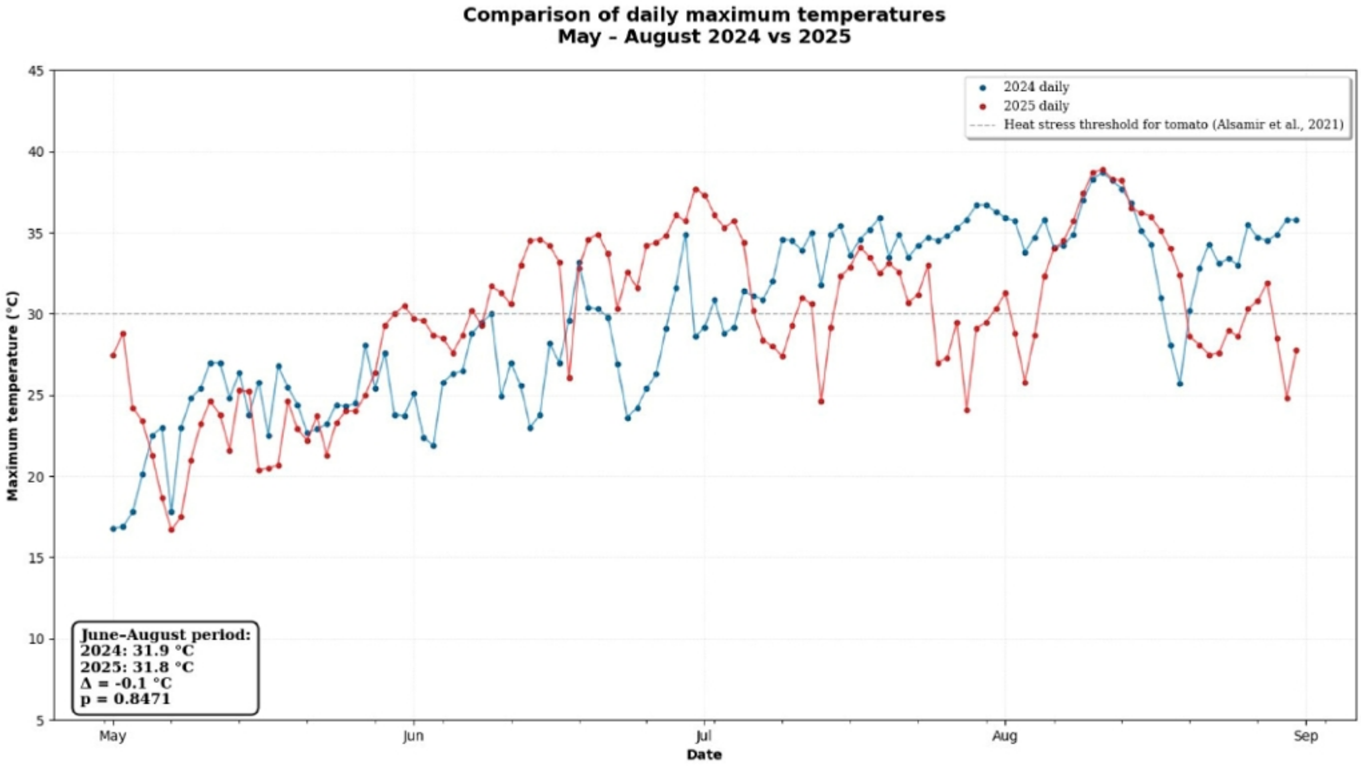

Figure 3. Daily max temperature: May–August 2024 vs. 2025. The dashed line indicates the heat stress threshold for tomato [28].

Table 3. Soil properties for biochar-amended (B) and control plots (C). Values are expressed as mean ± standard deviation. SOM: soil organic matter; N_{tot}: total nitrogen; C/N: carbon to nitrogen ratio. * $p < 0.05$; n.s = not significant.

Parameter	Unit	B	C	Variation (%)	*p*-Value
pH	-	8.15 ± 0.03	8.15 ± 0.03	0.0	n.s
SOM	g kg^{-1}	37.48 ± 0.69	34.81 ± 0.69	+7.7	0.032 *
N_{tot}	g kg^{-1}	2.17 ± 0.05	2.21 ± 0.05	−1.6	0.423 ns
C/N Ratio	-	9.95 ± 0.17	9.00 ± 0.17	+10.6	0.028 *

A consistent effect of biochar application was observed across both years, with treated soils exhibiting higher volumetric water content from May to August (VWC, $p < 0.001$ in 2024 and $p < 0.05$ in 2025).

In 2024, precipitation was concentrated primarily in May and June, with cumulative monthly values of 69.8 mm and 57.2 mm, respectively (Figure 4). Conversely, July and August saw lower precipitation, amounting to 36.4 mm and 33.4 mm, respectively. Throughout the monitored period, soil VWC in the biochar plot (B) was consistently higher than in the control plot (C), with an average difference of approximately 8 percentage points (32.84% versus 24.87%).

In 2025, despite a slightly lower total precipitation amount and a more uneven distribution of rainfall events, a similar trend was observed (Figure 4). Precipitation was highest in May (49.8 mm) and July (69.4 mm), while it was moderate in June (41 mm) and particularly low in August (24.4 mm). Again, higher soil moisture levels were observed in the biochar-treated plot compared to the control; however, the mean difference between the two treatments was less marked (24.66% in the treatment versus 24.08% in the control), although being statistically significant.

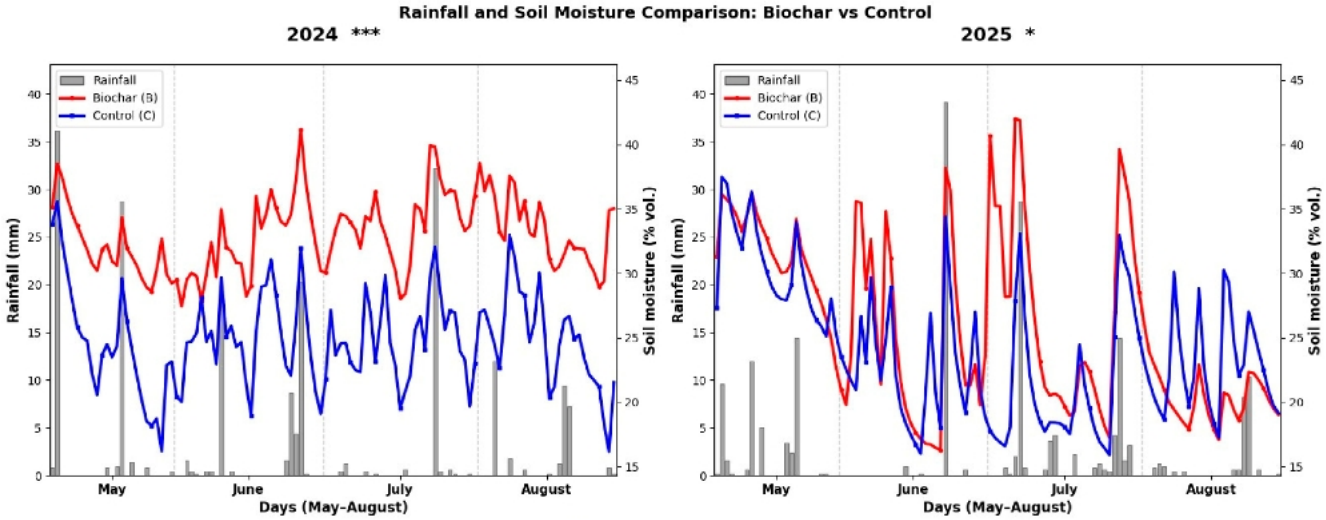

Figure 4. Rainfall events and soil moisture in May–August 2024 and 2025. *** $p < 0.001$; * $p < 0.05$.

The greater variability observed in 2025 and the more dynamic soil response to rainfall events contribute to explaining the smaller difference between treatments compared to 2024, consistent with the lower significance level (*, $p < 0.05$).

Biochar-amended soil exhibits a significantly lower bulk density than control (**), with mean values of approximately ~1.25 Mg/m^3 compared to ~1.42 Mg/m^3 (Figure 5). This reduction in bulk density indicates a notable alleviation of soil compaction in the B plots. In addition, the B samples displayed lower variability in bulk density, whereas the control samples showed a wider interquartile range and greater overall data dispersion, suggesting more heterogeneous soil structure.

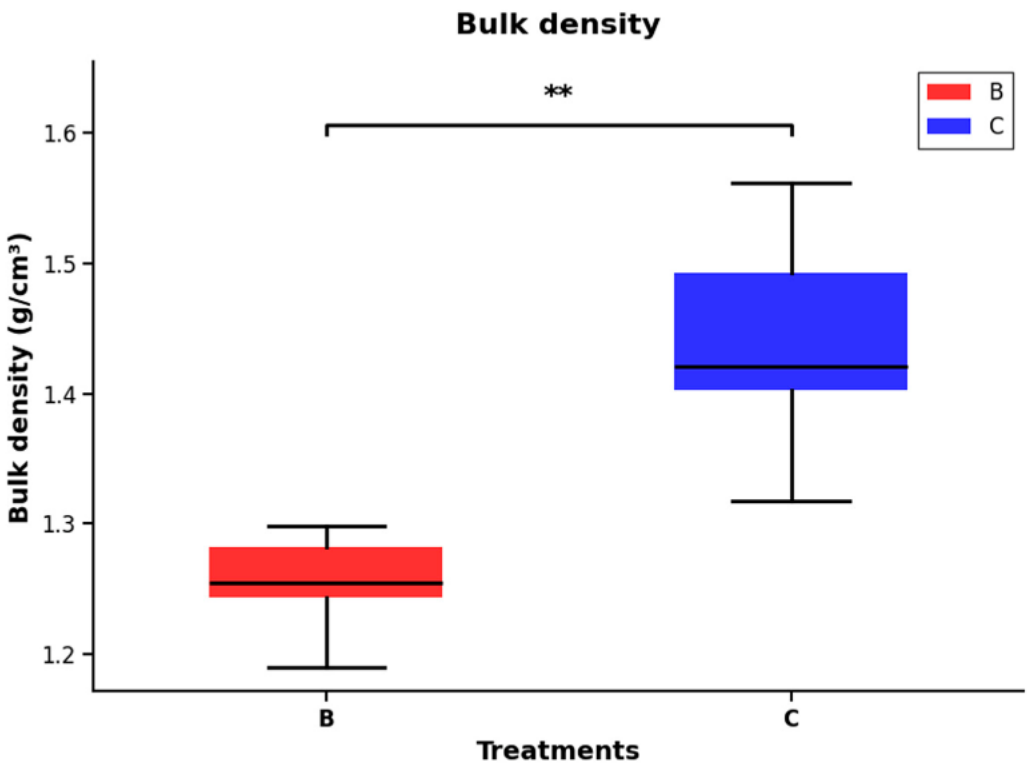

Figure 5. Bulk density of the control and biochar-amended soil. Bars represent mean values ± standard deviation. ** $p < 0.01$.

3.3. Plant Leaf Water Potential, Chlorophyll and Flavonoid Indices

Leaf water potential (Ψ_leaf) was influenced by both phenological stage (BBCH) and year of observation, with differences between treatments (Figure 6). In 2024, at BBCH stage 71 (Development of fruit), C showed more negative Ψ_leaf values than B (approximately −1.03 vs. −0.98 MPa), with statistically significant differences ($p < 0.05$). At BBCH stage 89 (Ripening of fruit), Ψ_leaf values were similar between the two treatments (≈−0.83 MPa), with no significant differences (n.s.). In 2025, Ψ_leaf showed more negative values compared to 2024. At the BBCH stage 71 (Development of fruit), C recorded significantly (**) lower values than treatment B (≈−1.05 vs. −0.77 MPa; $p < 0.01$). At the BBCH stage 75 (Development of fruit), both treatments showed the lowest values of the entire period (≈−1.20 MPa), with no significant differences (ns). Finally, at BBCH stage 89 (Ripening of fruit), C showed significantly (***) more negative values than the B (≈−1.13 vs. −0.98 MPa; $p < 0.001$), although the magnitude of these differences was not marked from an ecophysiological perspective. Overall, C showed systematically more negative Ψ_leaf values than B, particularly in 2025, indicating more negative plant water status under these conditions, suggesting more negative plant water status, especially in the early (BBCH 71) and late (BBCH 89) stages.

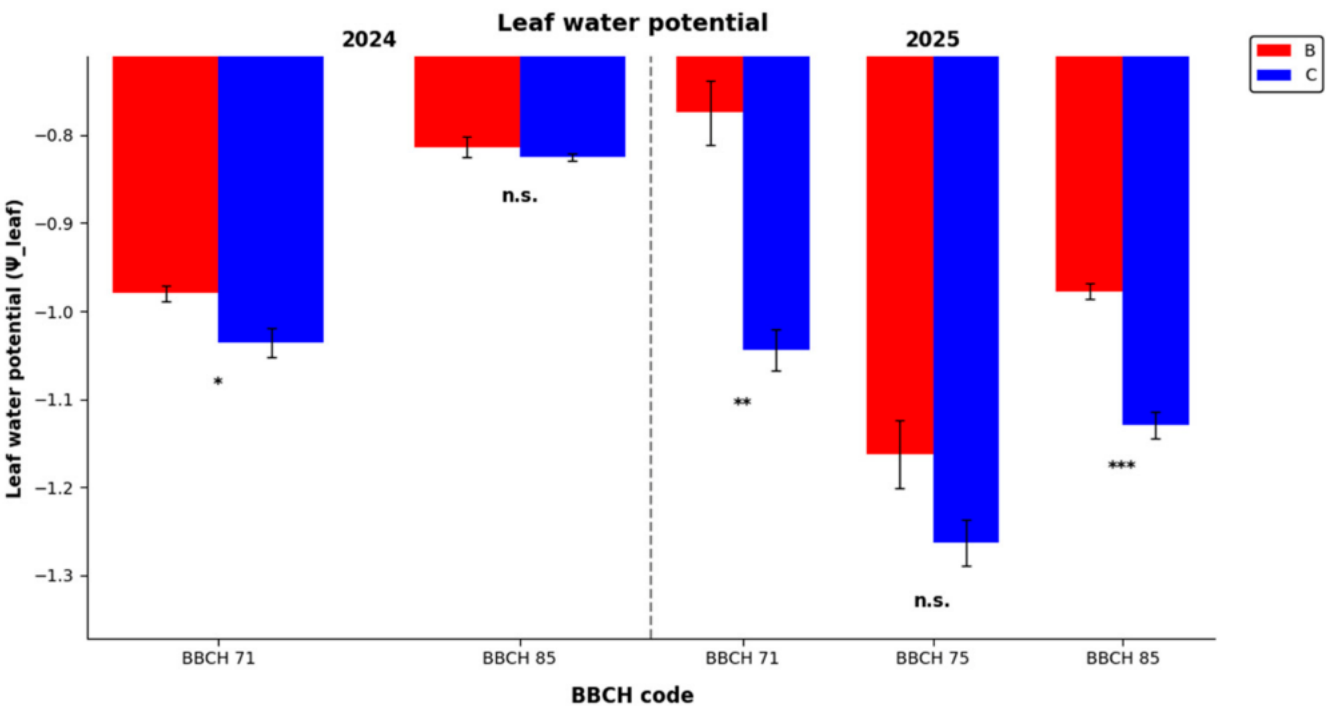

Figure 6. Leaf water potential (Ψ_leaf) at different phenological stages in 2024 and 2025. Bars represent mean values ± standard deviation. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; n.s. = not significant.

Flavonoid concentrations showed significant variations depending on the phenological stage. In 2024, values peaked at BBCH stage 65 for both treatments, with no significant differences between the two groups. Subsequently, a progressive reduction was observed, more marked in condition B than in C (Figure 7).

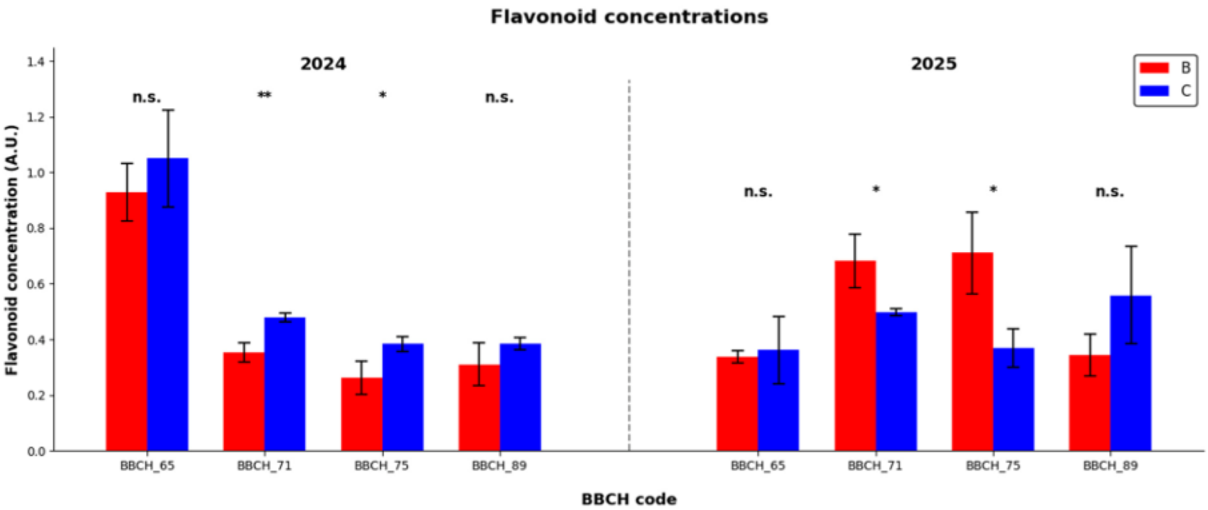

Figure 7. Flavonoid concentrations at different phenological stages in 2024 and 2025. Bars represent mean values ± standard deviation. Asterisks indicate significant differences between (B and C); ** $p < 0.01$; * $p < 0.05$; n.s. = not significant.

At BBCH stages 71 and 75, the differences between B and C are statistically significant (** and *, respectively), highlighting higher concentrations in condition C. Conversely, at BBCH stage 89, no significant differences were observed between the two treatments. In 2025, the overall trend appeared differently. As in 2024, at BBCH stage 65, no significant differences emerged in 2025; however, at BBCH stages 71 and 75, significantly higher concentrations were observed in condition B than in C (*), indicating a reversal of the trend compared to the previous year.

Finally, at stage BBCH 89, no significant differences were observed between the two conditions, confirming a convergence of flavonoid levels in the final stages of ripening. Overall, the results highlight a marked interaction between year and phenological stage, with a reversal of the pattern between conditions B and C in the two years considered.

Higher chlorophyll concentrations were observed in the biochar treatment at specific stages. In 2024, significant differences (**) were observed at BBCH stage 65 between B and C, with higher values for B. At BBCH stage 71, however, no significant differences were observed (Figure 8).

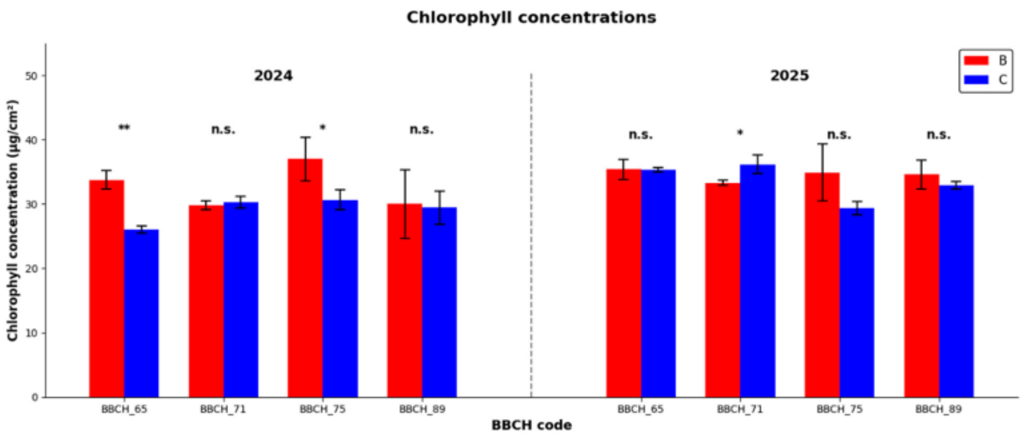

Figure 8. Chlorophyll concentrations at different phenological stages in 2024 and 2025. Bars represent mean values ± standard deviation. Asterisks indicate significant differences between (B and C) differences between treatments ** $p < 0.001$; * $p < 0.05$; n.s. = not significant.

Subsequently, significant differences (*) were observed at BBCH stages 75: Initially, B showed higher values, but this trend reversed at BBCH stage 89, where no significant differences were observed between B and C. In 2025, the overall trend was more homogeneous, with reduced differences between treatments. The only stage when highly significant differences (*) were observed was at BBCH stage 71, with C showing higher chlorophyll contents.

Additional statistical analyses using linear mixed models (LMMs), data shown in Supplementary Table S1, revealed significant Year × BBCH interactions for leaf water potential (Ψ_leaf), flavonoid, and chlorophyll concentrations. A significant Treatment × BBCH interaction was observed only for chlorophyll concentration (p = 0.036). The three-way interaction (Year × Treatment × BBCH) was significant for Ψ_leaf (p = 0.004) and flavonoid concentration (p = 0.003), whereas it was not significant for chlorophyll concentration. No significant Year × Treatment interaction was detected for any parameter.

3.4. Tomato Production

A significant year × treatment interaction was observed for yield (p = 0.001), demonstrating that the effect of biochar on crop productivity was strongly dependent on seasonal conditions. Yield showed clear differences between treatments in 2024, with treatment B producing significantly higher yields (*) than treatment C (Figure 9). In 2025, production decreased markedly in both treatments, and no significant differences (n.s.) were observed between them. Additional statistical analyses using linear mixed models (LMMs), with results shown in Supplementary Table S1, revealed a significant Year × Treatment interaction (p = 0.001). These patterns indicate that treatment effects were not consistent across years.

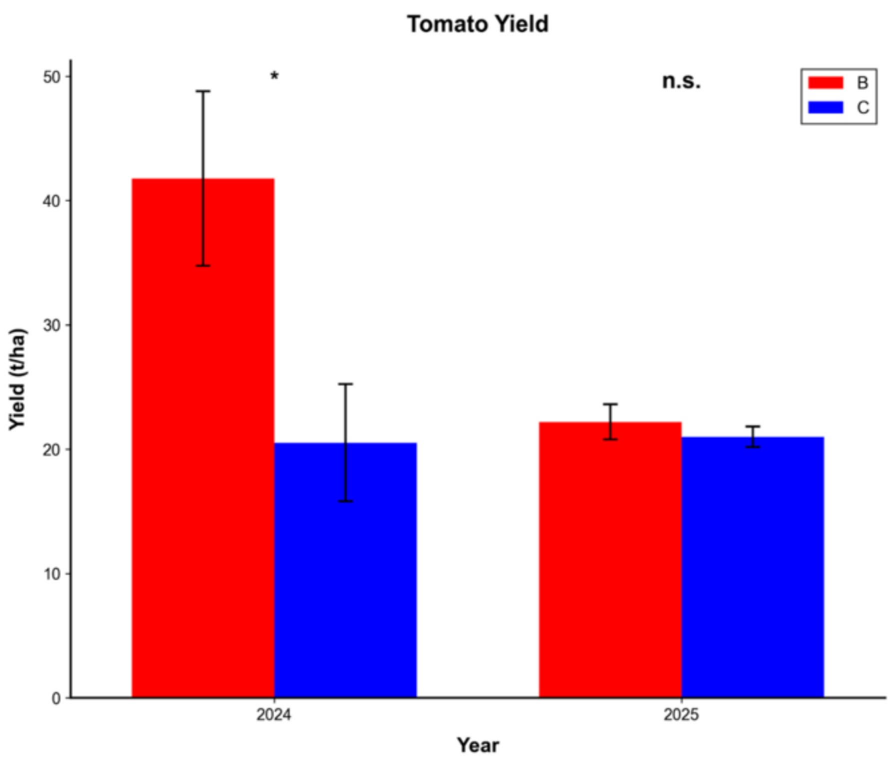

Figure 9. Tomato production (t/ha) by year and treatment. Bars represent mean values ± standard deviation. * $p < 0.05$, n.s. = not significant.

3.5. Anatomical Organization of Tomato Root Collar Tissues

Morphological examination of fresh root collar cross-sections collected in late August revealed the primary tissue systems, providing the structural framework for subsequent microscopic analysis. From the center to the periphery, the following anatomical regions were identified: the pith (P), where parenchyma cells had a characteristic globular appearance; a continuous xylem ring (X) of lignified tissue containing vascular bundles; a narrow vascular cambium (VC); the cortex (CX) with a characteristically deep green pigmentation; and the epidermis (E). This macroscopic organization establishes anatomical context and serves as a baseline for high-resolution microscopic observations (Figure 10).

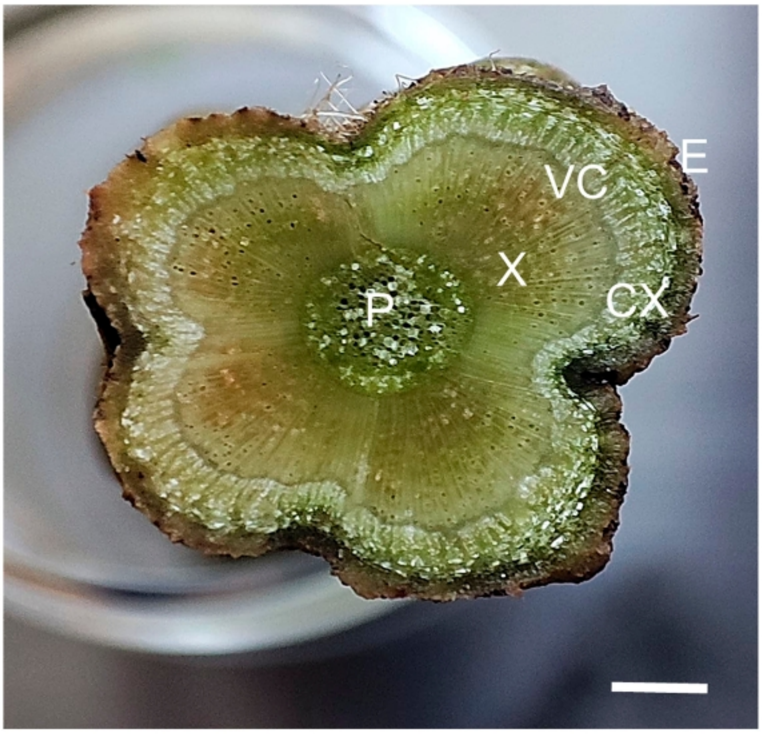

Figure 10. Macroscopic anatomy of the root collar. P: pith; X: xylem ring; VC: vascular cambium; CX: cortex; E: epidermis. Scale bar: 1 mm.

Subsequently, a more detailed qualitative microscopic analysis was conducted on cross-sections obtained at the root collar level in late August from both control and biochar-treated plants. Observation of the overall xylem structure revealed no evident macroscopic differences between the control (Figure 11a) and biochar-treated plants (Figure 11b) except for some inclusions (arrows in Figure 11a) with both groups displaying a tissue organization consistent with their developmental stage. However, a more detailed investigation of the conducting vessels revealed significant differences: in the control samples, even at low magnification (Figure 11c), circular structures and amorphous aggregates were observed adjacent to the vessel cell walls (arrows). Conversely, such formations were almost entirely absent in the vessels of plants treated with biochar (Figure 11d). These aggregates were likely identified as tyloses (Ty)—parenchyma cell protrusions that traverse the pits of the secondary cell wall to enter the vessel's lumen (arrow). Tyloses are known to contribute to hydraulic compartmentalization and may be involved in embolism prevention and the spread of pathogens. Additionally, the control samples exhibited potential salt deposits (sd, arrow in Figure 11c) enclosed within membranous structures, which may be associated with stress-related conditions. In biochar-treated samples, both tyloses and hypothetical salt deposits were absent (Figure 11d). High-magnification observation of the control samples further highlighted prominent wall pitting (arrow in Figure 11e), which might facilitate parenchymal cell expansion and the subsequent formation of tyloses. In contrast, in the biochar-treated samples (Figure 11f), the pits remained intact, and no tyloses or other occlusions were detected within the large xylem vessels.

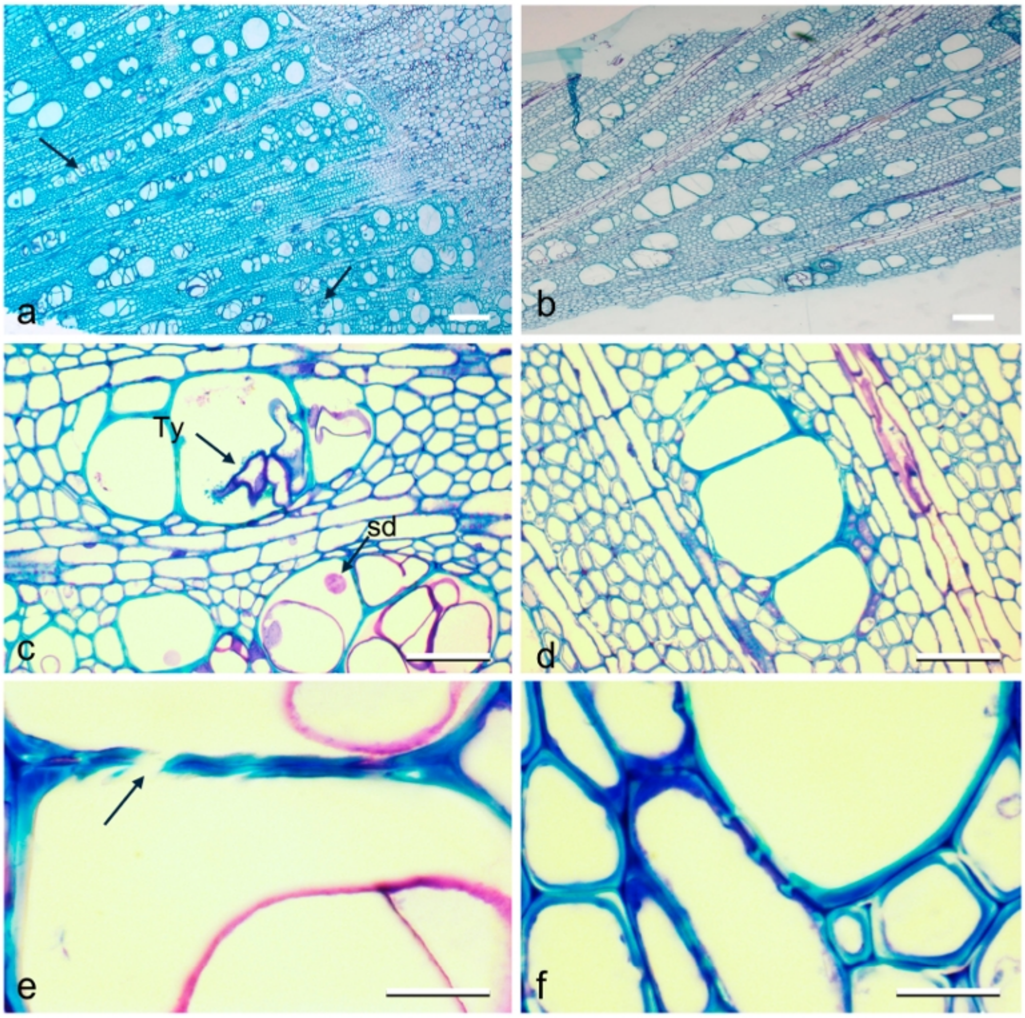

Figure 11. Histological analysis of tomato collar sections. (**a**) Detailed examination of xylem in control samples, revealing inclusions (indicated by arrows). (**b**) Xylem in biochar-treated plants. (**c**) Magnification of xylem vessels, highlighting putative tyloses (ty) and salt deposits (sd). (**d**) Corresponding magnification in biochar-treated samples. (**e**) A wider view focusing on altered xylem piths (indicated by an arrow); (**f**) corresponding focus on biochar-treated samples. Bars: in (**a**,**b**): 200 µm; (**c**,**d**): 40 µm; (**e**,**f**): 50 µm.

4. Discussion

The two-year field experiment, conducted in consecutive seasons on the same field, allowed us to observe the interactions between soil, plants, water, and crop productivity under varying climatic conditions while also assessing the persistence of biochar effects over time. Since biochar was applied only at the beginning of the experiment, it was possible to evaluate its effects both immediately after application and in the subsequent year, thereby providing insight into its short- and medium-term effects under real field conditions. The contrasting weather patterns observed between 2024 and 2025 highlighted the strong context dependency of biochar performance previously reported in the literature [44,45]. Although the open-field setting inevitably introduced environmental variability, it increased the practical relevance of the results by reflecting real farming conditions [46].

The absence of pH changes after adding alkaline biochar (pH 9.8) to a calcareous sandy loam soil (initial pH 8.5) is explained by the strong carbonate buffering system typical of such soils. The $CaCO_3$–CO_2–HCO_3^- equilibrium efficiently neutralizes added alkalinity, keeping pH within the naturally buffered range (7.5–8.5) [47]. Additionally, biochar represented less than 0.5% of total soil mass, and its surface functional groups undergo protonation or Ca^{2+} exchange rather than releasing free OH^- into the bulk solution. This result is consistent with meta-analyses showing that biochar has minimal or no pH effect on neutral-to-alkaline soils [44,48].

In contrast, statistically significant differences were observed for parameters related to soil organic matter. Specifically, the biochar-amended treatment (B) exhibited higher values of SOM compared to the control (C). This outcome aligns with numerous studies reporting that biochar predominantly contributes to the stabilization and accumulation of soil carbon fractions [49–51]. Additionally, the C/N ratio was significantly higher in treatment B, whereas total nitrogen did not differ significantly between treatments. This observation highlights that biochar's influence on soil nitrogen dynamics is highly context-specific, depending on biochar properties (e.g., feedstock, pyrolysis temperature), soil type, and environmental conditions [52]. Such findings underscore the dual role of biochar: while it enhances soil carbon storage and improves soil physical structure, its effects on nutrient cycling, particularly nitrogen availability, may be less predictable and require careful consideration in agronomic planning [51]. Biochar also improved soil physical properties, reducing bulk density, with average values of approximately 1.25 g/cm^3 in B compared to 1.42 g/cm^3 in C and increasing porosity, thereby creating more favorable conditions for root growth and water retention [53].

The improvement in soil structure was reflected in soil water dynamics. Throughout the study, biochar-amended soils generally maintained higher volumetric water content, confirming the well-documented capacity of biochar to enhance water retention through its porous structure and high specific surface area [53,54]. However, the magnitude of this effect varied considerably between years. In 2024, characterized by a relatively favorable rainfall distribution, differences in soil moisture between treatments were substantial. Conversely, in 2025, rainfall became more irregular and early heat waves increased evaporative demand, reducing the difference in soil moisture between treatments. These results indicate that biochar effectiveness depends not only on its intrinsic properties but also on climatic conditions, rainfall distribution, and biochar aging [44]. Under severe and prolonged stress, biochar improved soil water availability but could not completely compensate for increased atmospheric and soil water demand. Plant water status measurements supported these observations. Leaf water potential (Ψ_leaf) measurements at BBCH 71 in both years indicated significantly more negative values in control plants compared with treated plants, although the magnitude of these differences was limited from an ecophysiological perspective. These findings suggest that in untreated plants, water stress may develop earlier, potentially affecting key physiological processes such as nutrient transport and photosynthetic activity, which are known to be highly sensitive to reductions in plant water status [55]. These processes are in fact known to occur before completing stomatal closure, which occurs at more negative leaf water potential (Ψ_leaf) values (−0.7/−0.9 MPa). A similar trend was also observed in 2025 at BBCH 89. However, plants in 2025 experienced overall greater water stress due to combined heat and drought conditions, particularly during early summer. Under these harsher conditions, the buffering capacity of biochar remained evident but was insufficient to fully offset increased atmospheric and soil water demand [19].

This attenuation is likely due to lower and more irregular rainfall, which limited the capacity of the soil to store and retain water. These results highlight that the effectiveness of biochar in regulating soil moisture depends not only on its intrinsic properties but also on rainfall timing and distribution, with stronger effects under alternating wet–dry cycles [44,48,56]. As for flavonoids in tomato leaves, results collected over the course of the two-year experimental period suggest that they were strongly influenced by weather conditions. In 2024, following a rainy spring and a drier summer with temperatures approaching heat waves, no significant differences in flavonoid levels were observed during the BBCH 65, likely because water reserves were still sufficient. However, in the subsequent BBCH 71 and 75, flavonoid values were higher than in treatment B, suggesting that water deficit stimulated leaf flavonoid production as a protective mechanism [57]. In 2025, characterized by more variable rainfall and earlier heat waves, flavonoids at the same phenological stages were higher in B-treated plants. In this case, the increased soil water availability may have allowed plants to maintain active metabolic activity and carbon flow to secondary metabolism during fruit development [58]. Conversely, control plants may have allocated resources to survival rather than flavonoid biosynthesis. Under combined thermal and water stress, photosynthesis and carbon assimilation are known to be reduced, resulting in a reallocation of resources to maintenance and primary defense processes rather than secondary metabolism [59,60]. Furthermore, high-temperature stress can inhibit the activity of key enzymes of the phenylpropanoid pathway, such as phenylalanine ammonia-lyase (PAL), limiting flavonoid biosynthesis in leaf tissues [61,62]. Leaf chlorophyll concentrations, particularly during the 65 BBCH (B) phenological stage in 2024, showed significantly higher values, an effect that persisted in the subsequent phenological stage (75 BBCH), consistent with the improvement in soil water status induced by biochar [48]. Conversely, during the 89 BBCH stage, a reversal of this trend was observed, with a reduction in chlorophyll concentrations in treatment B. This phenomenon could be attributed to a dilution effect linked to increased fruit production, which was higher in treatment B. During this stage, the diversion of resources to storage organs may have reduced the availability of elements essential for chlorophyll synthesis, leading to a decrease in chlorophyll levels, in accordance with the source-sink equilibrium concept [63]. No significant differences were observed in 2025, except for BBCH 71, where chlorophyll levels were higher in plot B.

The significant Year × BBCH interactions for plant analysis (Ψ_leaf, flavonoids, and chlorophyll) indicate that the trend of these parameters across the phenological stages varied between the two study years. The treatment effect was limited, with a significant Treatment × BBCH interaction observed only for chlorophyll concentration. For Ψ_leaf and flavonoids, the significance of the Year × Treatment × BBCH interaction highlights a response dependent on both the year and the phenological stage. The absence of the Year × Treatment interaction for all parameters indicates that the treatment effect was not constant between the two years. In 2024, biochar significantly increased yield (~100%; $p < 0.05$), indicating that improvements in soil physical properties, water availability, and physiological performance were effectively translated into greater fruit production [44,45]. In contrast, yields declined substantially in both treatments during 2025, and no significant differences were detected. These results suggest that under moderate stress conditions, biochar can enhance crop productivity, whereas under severe drought and heat stress, its benefits may be insufficient to sustain yield advantages [64]. These observations are consistent with the results, as evident in Table S1 of the Supplementary Materials, which showed a significant Year × Treatment interaction ($p = 0.001$), indicating that yield responses to the treatment were not stable across years but strongly dependent on environmental conditions.

A qualitative morphological analysis revealed, in line with the findings regarding leaf and soil water content, that plants treated with biochar exhibited a more efficient and patent xylem structure compared to the control group. This observation suggests that biochar application reduced the reliance on typical adaptive responses to water stress, such as the formation of occlusive structures (e.g., tyloses) intended to compartmentalize vessels and prevent embolism [65]. This distinction in vascular anatomy is particularly relevant for tomato crops, which are notably sensitive to drought and undergo significant morpho-anatomical and physiological alterations when facing water deficits [66]. Consequently, maintaining an open vascular lumen enhances hydraulic conductance [65], aligning with the observed improvement in soil water availability and transpiration, particularly during late phenological stages [67]. In addition to general vessel characteristics, the control exhibited tyloses (Ty) and amorphous aggregates exclusively within their xylem vessels. Indeed, Gupta et al. [68] described tyloses as parenchyma cell protrusions that, while acting as a defense mechanism to compartmentalize vessels and restrict pathogen spread, also contribute to xylem occlusion. This process might inhibit efficient water transport in tomatoes, increasing their susceptibility to drought stress. Furthermore, Zahedi et al. [67] noted that drought stress frequently induces occluding vessels in tomato plants as an adaptive morphological response. Although these structures may offer structural defense, they represent an anatomical trade-off that limits water status maintenance. Conversely, the absence of these formations in biochar-treated samples suggests that biochar application improved soil moisture regimes, thereby preventing the induction of occlusive mechanisms [69].

Moving beyond vascular dynamics, our findings on overall anatomical preservation and development align with broader observations regarding root and stem structures, where biochar is known to promote seedling development and tissue preservation. For instance, in tomato plants, biochar derived from olive pruning increased root dry weight and improved root system architecture [69]. This is strongly supported by Calcan et al. [70], who reported that biochar application significantly reduced soil bulk density by approximately 50%, thereby enhancing tomato plant root development and facilitating improved water and nutrient uptake [70]. These findings collectively reinforce the concept of enhanced structural robustness observed in our study. From a more cautious perspective, biochar should not be considered a uniform soil amendment with constant and generalizable effects. Instead, it acts as a context-dependent modifier of the soil–plant system whose performance varies with environmental conditions, soil characteristics, and climatic variability. In this sense, as evidenced in the present study, its primary contribution may lie less in consistently increasing crop productivity across diverse environments and more in enhancing system resilience, improving soil physical properties, and promoting water retention under moderately stressful conditions [44,51,53,64].

In the present study, indeed, the results showed a variable plant response across the different conditions considered, confirming the strongly context-dependent nature of biochar effects.

5. Conclusions

Overall, this study highlights the potential of biochar as a soil amendment capable of improving, in particular, soil water retention and carbon content, as well as reducing the effects associated with water stress.

However, the relatively short experimental duration (two growing seasons under contrasting climatic conditions) limits conclusions regarding the long-term stability and persistence of these effects, particularly in relation to nutrient cycling and soil organic matter dynamics. The results emphasize the clear need for an optimization of application rates finely tuned to specific soil and environmental variations. In this context, the relatively high cost associated with field-scale application rates (e.g., ~15 t ha^{-1}) may limit short-term profitability, particularly in the absence of consistent yield increases. However, potential agronomic benefits—such as improved soil structure, enhanced water retention, and partial mitigation of stress effects—may translate into indirect or long-term advantages, including yield stability and reduced vulnerability to climate variability. Therefore, the cost–benefit balance of biochar should be evaluated not only in terms of immediate yield responses but also within the framework of more sustainable and resilient production systems. These findings highlight the importance of an integrated interpretation that simultaneously considers soil properties, inter-annual climatic variability, crop phenological stage, and economic sustainability. Further long-term, multi-site, and dose-optimization studies are needed to better quantify the persistence, variability, and overall cost-effectiveness of biochar effects in tomato-based agroecosystems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae12060737/s1, Table S1: Effects of growing year, BBCH phenological stage, treatment, and their interactions on leaf flavonoid content (a.u), leaf chlorophyll content (μg cm^{-2}), leaf water potential (LWP, MPa), and fruit yield (t ha^{-1}) in tomato (*Solanum lycopersicum* L.) during the 2024 and 2025 growing seasons.

Author Contributions: Conceptualization A.B., S.B., C.P., F.U. and G.C.; methodology A.B., S.B., C.P. and C.F. (Claudia Faleri); software, A.B., S.B. and F.U.; validation, A.B., S.B., G.C., C.P., F.P.V., F.U., V.C. and C.F. (Claudia Faleri); formal analysis, A.B., S.B., G.C., C.P., F.U. and C.F. (Claudia Faleri); investigation, A.B., S.B., G.C., C.P., F.P.V., F.U.,C.F. (Carolina Fabbri), G.M.L. and C.F. (Claudia Faleri); resources, A.B., S.B., G.C., C.P., F.P.V., F.U. and C.F. (Carolina Fabbri); data curation, A.B., S.B., G.C., C.P., F.P.V., F.U., V.C., G.M.L. and C.F. (Claudia Faleri); writing—original draft preparation, A.B., S.B., G.C., C.P., F.P.V., F.U. and C.F. (Carolina Fabbri); writing—review and editing, A.B., S.B., G.C., C.P., F.P.V., F.U., V.C. and C.F. (Claudia Faleri); visualization, A.B., S.B., G.C., C.P., F.P.V., F.U. and C.F. (Carolina Fabbri); supervision, A.B., S.B., G.C., C.P., F.P.V., F.U., G.M.L. and C.F. (Claudia Faleri); project administration, A.B., S.B. and C.P.; funding acquisition, A.B., S.B., C.P., G.M.L., F.U. and C.F. (Carolina Fabbri). All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The original data presented in the study are openly available in Biancalani, A. (2026). dataset_tomato_biochar at Zenodo. https://doi.org/10.5281/zenodo.20049191 (accessed on 5 May 2026).

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Conflicts of Interest: Author Carolina Fabbri was employed by the company Yanmar R&D Europe. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

B	Biochar
BBCH	Biologische Bundesanstalt, Bundessortenamt and Chemical Industry
C	Control
CX	Cortex
E	Epidermis
P	Pith
PAL	Phenylalanine ammonia-lyase
Pu	Punctuation marks
SOM	Soil Organic Matter
Ty	Tylosis
VBg	Vascular bundles in groups
VC	Vascular cambium
X	Xylem

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