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Effect of Biochar and Wood Distillate on Vegeto-Productive Performances of Tomato (*Solanum lycopersicum* L.) Plants, var. Solarino, Grown in Soilless Conditions

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Abstract: Nowadays, tomato, a commercially important crop, is increasingly cultivated in a soilless cultivation system to counteract climate change. Normally, this system uses cocopeat as a substrate, but its high cost and environmental impact have led to growing interest in alternatives like biochar (BC). In addition, biostimulants, such as wood distillate (WD), a pyrolysis by-product, are increasingly being used to improve fruit yield and quality. BC was used to partially replace (2% and 5%) cocopeat in growth bags for soilless tomato cultivation. Moreover, WD (3 mL/L) was distributed in the substrate every two weeks. The effect of BC and WD on tomato plant growth, fruit quality, and substrate microbial community was investigated. The integration of BC and WD into a soilless growing system for tomato cultivation can improve the fruit quality and influence the microbial activity of the substrate. Replacing part of the cocopeat in the substrate with BC and using an agri-waste-derived biostimulant represent a step forward in making agriculture more sustainable.

Keywords: agriculture; biochar; fruit quality; horticulture; microbial community; substrate modification; sustainability

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1. Introduction

Tomato (*Solanum lycopersicum* L.), a flowering plant that belongs to the *Solanaceae* family, is one of the most consumed vegetables in the world, with an estimated production of 186 million tons per year [1] and is known to be a rich source of important compounds, such as carotenoids, phenols, flavonoids, and ascorbic acid [2]. Since the open field condition is hampered by factors such as high temperature, drought, and high incidence of pests and diseases, the possibility of growing indeterminate tomato varieties in the greenhouse is becoming a good solution for farmers. According to Palmitessa et al. [3], Italy is a leader in the production of fresh tomatoes in Europe, with a cultivated area of 17,000 ha in open field and 7610 ha in greenhouses where the currently average production of fresh tomatoes is about 7–9 kg m^{-2}, numbers destined to increase in the future. Furthermore, in recent years, growing tomatoes in soilless conditions, in a greenhouse, has proven to be the most efficient method for producing high quality fruits with a good yield [4]. Since the goal for the future is to maximize plant productivity by minimizing the use of synthetic resources, these systems are becoming increasingly popular as a sustainable alternative to traditional soil-based agriculture [5]. Soilless tomato growing can overcome local water shortages and poor soil structure and composition by using controlled irrigation systems and optimizing the composition and quantity of water and nutrients [6,7]. Other than

fertigation management, in soilless agriculture, the selection of the type and composition of the growing substrate is a key factor for optimizing and supporting plant growth and production [8]. Nowadays, there are several growth substrates on the market, such as cocopeat, perlite, vermiculite, etc. Cocopeat (or coconut fiber) is the most used material [9]; however, it is quite expensive, and farmers are concerned about the real benefit/cost ratio of using cocopeat. Cocopeat is, in fact, produced in several tropical countries (Philippines, Indonesia, India, Vietnam, Sri Lanka) from where it is exported to Europe, affecting the environmental costs due to its transportation from the origin sites [10]. To solve this problem, there is an increasing interest in the evaluation of new materials, more environmentally sustainable, economical, and easily available [11], such as biochar. In recent years, interest in the use of biochar as a substrate component for soilless cultivation and for containers for the nursery sector has risen sharply [12,13]. Biochar is a soil amendment, similar to charcoal, obtained from the pyrolysis of organic matter of different origins, mainly pruning residues and fruit and plant waste, in oxygen-limited conditions and temperatures between 250 °C and 1000 °C [14–16]. Its addition in the soil can improve its physical properties, such as aggregation, water-holding capacity, and nutrient retention [17]; however, it can also stimulate the biological activity of the substrate, probably thanks to an enrichment of the organic matter [18]. Other than in soil, some studies have reported the use of biochar in soilless agriculture, as a way of reducing the cocopeat percentage in the growing box, but also of further reducing the water and nutrient use [19–21]. Moreover, it has been demonstrated that in soilless systems, biochar can be a source of phosphorus (P) and potassium (K), with a consequent increase in the availability of these minerals for plants [22]. During the pyrolysis process, through the distillation of the condensed gasses, a reddish-brown aqueous liquid is produced, known as wood distillate, wood vinegar, or pyroligneous acid [23,24]. This product is often discarded as waste; however, it contains compounds of interest like esters, alcohols, acids, sugars, and phenols co-related to the antioxidant and antimicrobial activities which makes it able to act as a biostimulant for crops, for increasing fruit production and quality [25]. The application of wood distillate is also interesting from the nutritional point of view; in fact, it has been shown that in tomato, the use of this product could increase the content of polyphenols in fruits [26]. Among the most studied effects related to the application of wood distillate to plants of agronomic interest, there is also the increased chlorophyll content and photosynthetic efficiency [27,28]. As for the use of biochar, the effectiveness of wood distillate depends on its concentrations, the type of culture, and the mode of application. Moreover, previous studies have shown that biochar can affect the microbial community in the substrate [29,30].

In the present study, the aim was to assess the effects of BC and WD treatments on the plant growth and fruit quality of tomato, cv. Solarino, cultivated in a soilless system, under controlled conditions. Additionally, with the goal of evaluating the variation in microbial activity, with a focus on metabolic phenotyping, in response to the addition of BC and WD to the substrate.

2. Materials and Methods

2.1. Plant Materials

The greenhouse experiment was carried out during the spring–summer season of 2023 from the second half of May to the end of July, for a total of 10 weeks. The variety used was Solarino (Rijk Zwaan-RZ), sown in a local nursery in April 2023. After 6 weeks, the plantlets were transplanted at the farm site of this experiment. Plants were monitored weekly until the end of July 2023, when they were uprooted. The site description and the experimental design are reported below.

2.2. Site Description

The experimental greenhouse, used to carry on this experiment, belongs to the farm Soc. Agricola Anzola Achille and Stefania, located in Boretto (44.8986, 10.5274; RE, Italy). Although, in this area, open field grown tomatoes for industrial processing are the typical

crop, since 1929, the Anzola family has produced different varieties of tomatoes for the fresh market in soilless and controlled conditions. Soilless tomato cultivation was carried out in growth bags, supplied by Ageon Srl of Borgo San Dalmazzo (Cuneo, Italy), a company specialized in products and services for soilless cultivation.

2.3. Characteristics of Biochar and Wood Distillate

Biochar (BC) and wood distillate (WD) used in this experiment were two commercial products provided by the company BioDea© Esperia Srl (Arezzo, Italy) [25].

BC is derived from the charring process of materials of plant origin from forestry and agriculture, such as olive pruning residues, marc, bran, fruit kernels, and shells. The characteristics of biochar (black silt) (Table 1) and wood distillate (Table 2) are summarized in tabular form.

Table 1. Characteristics of the biochar used in this study [31].

Particle Diameter (μm)	<500
Nitrogen (%)	<0.5
Potassium (g Kg^{-1})	3.020
Phosphorous (g Kg^{-1})	0.340
Calcium (g Kg^{-1})	9.920
Magnesium (g Kg^{-1})	0.852
Sodium (g Kg^{-1})	0.291
Total carbon (%)	65%
Water-holding capacity (max, %)	210
pH	9.85
Ash content (%)	7

Table 2. Characteristics of the wood distillate used in this study [31].

pH	3.5–4.5
Density	1500 g L^{-1}
Acetic acid	3.02 g Kg^{-1}
Total phenolic content	23–26 g Kg^{-1}
Heavy metal content	<1 mg Kg^{-1}

2.4. Preparation of Substrates and Experimental Design

The growth bags used in this study measured 100 × 24 × 12 cm, each containing a 2 kg slab of dried coconut fiber. In order to prepare the mixture of substrates of biochar and coconut fiber to be used in this experiment, the plastic covering on each growth bag was carefully cut open to access the coconut fiber slab inside. The slab was then manually shredded to ensure an even texture and uniform mixing with biochar at the specific concentrations outlined below. This mixing process aimed to create a homogeneous substrate blend, facilitating the consistent distribution of biochar throughout the growth substrate.

Once the coconut fiber and biochar were thoroughly mixed, the growth bags were sealed and arranged in their final positions in randomized block design, as reported in Figure 1. The substrate mixture in each bag was then hydrated gradually to reach optimal moisture levels before transplanting, thanks to an automatic drip system, linked to a central fertigation system for precise hydration and nutrient delivery. Finally, after 6 weeks in the nursery, six tomato plants were transplanted into each bag. Half of the plants grown on a BC-enriched substrate were also treated with a 3 mL/L solution of WD, distributed as 50 mL per each plant, every two weeks.

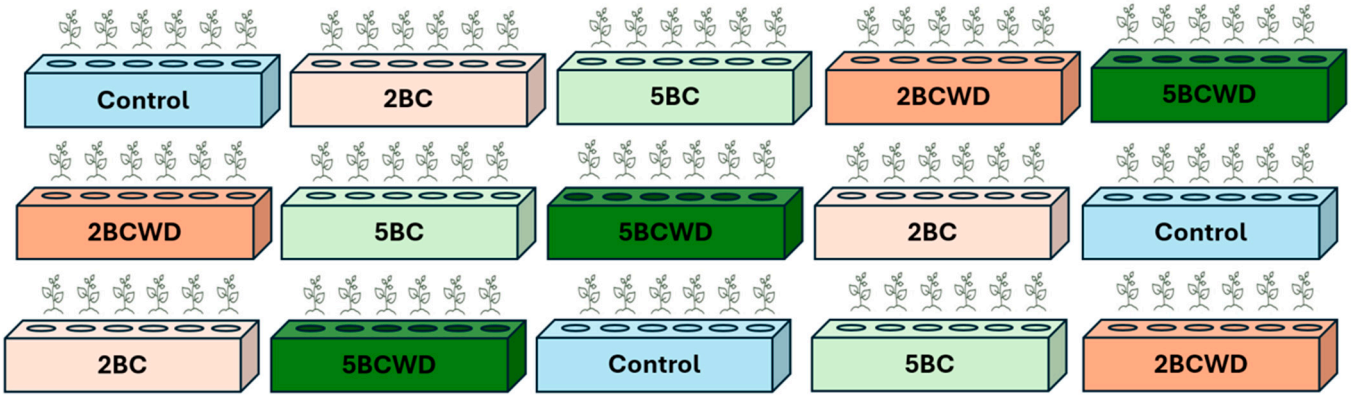

Figure 1. Representation of the experimental design. Per treatment, three growth bags with six plants each were disposed in a completely randomized block. Control: 100% coconut fiber-CF; 2BC: 2% of biochar and 98% of CF (*w*/*w*); 5BC: 5% of biochar and 95% of CF (*w*/*w*); 2BCWD: 2BC treated with WD; 5BCWD: 5BC treated with WD.

The experimental design was organized with the following theses (Figure 1): (1) control-C: 100% coconut fiber-CF; (2) 2BC: 2% of biochar and 98% of CF (*w*/*w*); (3) 5BC: 5% of biochar and 95% of CF (*w*/*w*); (4) 2BCWD: 2BC treated with WD; (5) 5BCWD: 5BC treated with WD. Per each thesis, a total of 18 plants, divided in three bags, were placed; with a total of 90 plants studied.

2.5. Fertigation

Plants were daily fertigated from two hours after sunrise to two hours before sunset for a maximum; the number of the fertigations varied during the season depending on plant development and the environmental conditions (light and temperature). The nutrient solution composition was the following: $Ca(NO_3)_2$ 137 g L^{-1}, KNO_3 12.5 g L^{-1}, Fe-EDDHA 1.9 g L^{-1}, KH_2PO_4 20 g L^{-1}, $MgSO_4$ 750 g L^{-1}, K_2SO_4 0.07 g L^{-1}, $MnSO_4$ 1.19 g L^{-1}, $ZnSO_4$ 0.5 g L^{-1}, $Na_2B_4O_7$ 0.14 g L^{-1}, $CuSO_4$ 0.03 g L^{-1}, and Na_2MoO_4 0.06 g L^{-1}. The pH and the EC of the solution were 5.5 and 4.5 mS cm^{-1}, respectively. The fertigation schedule was set up by the farm's owners, using a centralized and automatic system designed to avoid plant water and nutrient stress.

2.6. Vegetative and Productive Data Collection

Plants were monitored weekly, and the following vegetative parameters were registered per each plant and per each thesis: plant height (measured from stem base to the top, with a tap measurer), number of leaves, and diameter of the stem, setting the measure to 5 cm from the ground (with a digital caliber). Plant height measurements were taken from the day of transplanting until the day of plant topping, realized after 6 weeks after transplanting, to block the growing and improving the product quality; meanwhile, the data recording on number of leaves was stopped after 7 weeks from the transplanting, when plant defoliation started to promote fruit ripening.

Fruits were harvested when they reached the color of mature grade, according to common practice; per each treatment and per each plant, fruits were numbered, weighted, and measured (length and width with a digital caliper; Moore & Wright MW110-15DFC Fractional, Burgess Hill, UK). The fresh weight was measured in grams (g) using an electronic scale (KERN® EMB 1000-2, Vicenza, Italy). Fruit length and width were recorded by measuring the equatorial and longitudinal diameter of fifty fruits per treatment, using a digital caliper. Once the fruits were harvested, fifty fruits per each treatment were selected for further biochemical analyses.

For dry weight determination, ten tomatoes per plant were collected and weighed on an electric balance to obtain W1. After that, oven dry weight (W2) was determined after 48 h, in an oven, at 70 °C. To calculate the dry matter (%), the dry samples were weighed, and the following Equation (1) was applied:

$$Dry\ matter(\%) = \frac{(\text{weight of oven dry sample (W2)})}{(\text{weight of sample before drying (W1)})} \times 100 \quad (1)$$

2.7. Characterization of Tomato Fruits

For the characterization of tomato fruits in response to the use of BC and WD, 50 fully ripe fruits per treatment, chosen as a representative sample from the harvested batch, were carefully analyzed. The parameters of color, texture, total soluble solid content (TSS), titratable acidity (TA), electrical conductivity (EC), pH, total phenolic content (TPC), antioxidant activity (DPPH assay), and lycopene content were measured on whole tomato fruit or juice, obtained by crushing fruits and eliminating the seeds, using a sieve. More detailed descriptions of the analysis carried out are reported in paragraphs below.

2.7.1. Colorimetric Analysis

The colorimetric properties of both tomato skin and juice were evaluated using a portable colorimeter (CM 2600d, Minolta Co.; Osaka, Japan). Each sample was analyzed for the CIE L*, a*, and b* colorimetric parameters, which included L* (lightness), a* (redness or blueness), and b* (yellowness or greenness). Color readings were taken directly on the fruit surface using the colorimeter, with a consistent positioning and controlled ambient lighting to ensure accuracy. After skin color analysis, the fruits were crushed to extract juice. A 15 mL sample of juice was placed on a transparent glass plate, and CIE values were measured at room temperature. Each measurement was performed in triplicate to ensure reliability, and the average values for each treatment were calculated for further analysis.

2.7.2. Texture Profile

A penetration test was performed through a TA. XT2i Texture Analyser, equipped with a 30 kg load cell (Stable Micro Systems, Godalming, UK) and P/3 stainless steel with a flat-end cylindrical probe was used. The test velocity was set at 1 mm/s for a total distance of 10 mm for the tomato pericarp, at ambient temperature (25 ± 1 °C). The firmness of the pericarp was determined by measuring the maximum force in Newtons (N). The fruit was positioned in a horizontal orientation on an appropriate stand for the procedure, and the measurements were carried out on the fruit's equatorial region.

2.7.3. Total Soluble Solid Content (TSS)

To measure the TSS content, 50 fruits were squeezed, and a few drops of the liquid obtained were analyzed with an optical portable refractometer (Hanna instruments, Padova, Italy). Values obtained were reported as Brix degrees.

2.7.4. Titratable Acidity (TA)

The titratable acidity (TA) of the tomato juice was determined using the titration method [32]. About 5 g of the prepared tomato juice was taken and diluted with 100 mL of distilled water; phenolphthalein was used as an indicator. The TA of the tomato juice was calculated by titrating 5 g of tomato juice against 0.1 N NaOH. The acid content of the tomato fruit sample was calculated based on the volume of NaOH used for neutralizing the acid content in the sample using the following Equation (2):

$$\frac{(\text{Vol NaOH (mL)} \times 0.1(\text{normality NaOH}) \times 0.064)}{\text{g Juice}} \times 100 \quad (2)$$

where 0.064 is the citric acid milliequivalent factor.

Subsequently, the values of total soluble solids (TSSs) and titratable acidity (TA) were used to calculate the sugar/acid ratio (TSS/TA).

2.7.5. Electrical Conductivity (EC)

The electrical conductivity of tomato juice samples was measured by an electrical conductivity meter (Portamess® 913 X Cond, Loughborough, UK). The electrodes were completely immersed in the tomato juice sample and measured the EC until constant readings were obtained. This measurement was performed in triplicate, at room temperature (25 °C).

2.7.6. pH Determination

The pH of the juice was determined by a portable pH meter (Model LLG-pH meter 5, Hyde Manchester, UK) [33]. Each sample was tested in three replicates and only averaged values were taken for further data analysis.

2.7.7. Total Phenolic Content Determination

The total phenolic content (TPC) was evaluated by resorting to Folin–Ciocalteu's phenol reagent [34], with some modifications. In brief, 250 µL of extract, obtained from 1 g of tomato pulp with 20 mL of methanol/acetone (70:30 v/v), were mixed with 1 mL of an aqueous solution of Folin–Ciocalteu's phenol reagent (Sigma-Aldrich, St. Louis, MO, USA) (1/10 v/v), and 2 mL of an aqueous solution of sodium carbonate (20%, w/v), and kept in the dark for 30 min. The absorbance at 760 nm was measured using a spectrophotometer (JASCO V-530 spectrophotometer, Easton, MD, USA). To evaluate the quantity of polyphenols contained in the considered samples, a calibration curve was constructed, using gallic acid as a reference in a concentration range of 10–100 mg/kg (5 points). All the analyses were repeated twice on each sample extract. In addition, to achieve more accurate results, the instrumental software (Spectra Manager 1.54, JASCO Inc. Easton, MD, USA) was set up to perform three consecutive measurements on each analyzed sample. The same approach was then applied to the other assays used to determine the antioxidant capacity of extracts. Concerning total polyphenolic content, the results obtained for analyzed samples were expressed as mg GAE/kg FW.

2.7.8. Evaluation of Antioxidant Activity (AO) by 2,2 Diphenyl 1 Picrylhydrazyl (DPPH) Assay

The antioxidant capacity of the fruits was determined by applying the DPPH radical scavenging assays [35], with slight modifications. A total of 100 µL of sample extract or standard solution was added to 2.9 mL of a DPPH (Sigma-Aldrich, St. Louis, MO, USA) ethanolic solution (0.05 mM), and kept in the dark for 30 min. After that, the absorbance at 517 nm was measured (JASCO V-530 spectrophotometer, Easton, MD, USA). To evaluate the antioxidant capacity, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Sigma-Aldrich, St. Louis, MO, USA) was used as a reference, preparing five different standard solutions (0.1–1 mM) that were utilized for the calibration curve construction. In addition, a blank constituted of 100 µL of extraction solution was also analyzed under the same experimental conditions applied to the samples. Antioxidant capacity was calculated on the basis of radical inhibition percentage (I%), as follows: $I\% = [(AbsB - AbsS)/AbsB] \times 100$, where AbsB was the absorbance of the blank and AbsS was the absorbance of sample/Trolox standard solution. The results were then expressed as mM Trolox equivalent antioxidant capacity (TEAC). All the analyses were conducted in three consecutive measurements on each sample.

2.7.9. Lycopene Content

Lycopene was extracted using the protocol reported by [36], with some modifications, starting from 1 g of tomato pulp homogenized with 10 mL of hexane/acetone/ethanol (2:1:1 v/v) solution in a plastic test tube and continuously stirred in dark conditions for

2 h with a stirrer. The absorbance of supernatant (hexane layer) containing lycopene was read at 473 nm and 502 nm using a spectrophotometer (JASCO V-530 spectrophotometer, Easton, MD, USA). Absolute hexane was used as the blank. The lycopene concentration was expressed as mg/kg using the following Formula (3):

$$c = \frac{A}{\varepsilon \times \mathrm{d}} \quad (3)$$

where ε (1280) is 1 g lycopene molar extinction coefficient in 100 mL; A is the absorbance at 473 nm and 502 nm; and d is the length of quartz cuvette in cm.

2.8. Community-Level Physiological Profiling (CLPP)

Microbial metabolism in samples treated with biochar, wood distillate, and substrate control (without any treatment) at the end of the harvest, was analyzed using the Biolog system with Biolog EcoPlate™. This system is specifically designed for microbial community analysis and microbial ecology research. Each plate contains 31 of the most useful carbon sources in triplicate. At the end of harvesting, the plant was removed from the pot and the soil near the roots was collected. Five grams of samples were added to 50 mL Ringer solution (VWR, UK), and shaken at room temperature (22 °C) for 30 min at 200 rpm. After 20 min settling, the supernatant was serial diluted for three times in Ringer. A total of 100 µL of the third dilution was inoculated into each well of EcoPlates in triplicates and incubated at 30 °C for 72 h. The plates were analyzed by the Microplate Reader (wavelength data: OD590) at T0, and after 24, 48, and 72 h to observe the dynamic utilization of different carbon sources from microbes. For comparative analysis, we took the measurement performed after 24 h (T24) as we observed the maximum signal development at this point. The analysis of data was performed using average well color development (AWCD) as a parameter that enables an integral fingerprinting to be captured of the carbon sources used by [37]. The value of AWCD was calculated according to Equation (4), as follows:

$$\mathrm{AWCD} = \frac{(\mathrm{C} - \mathrm{R})}{n} \quad (4)$$

where C is the OD value of each well with a carbon source; R is the OD value of the control well (water); and n is the number of wells with carbon sources, and the value of n is 31. Using the Shannon index (H), it was evaluated that resulted from H = −ΣPi ln(Pi), where Pi = ODi/ΣODi, which is the proportional color development of the well over total color development of all wells of a plate. The number of substrates oxidized (substrate richness, SR) was calculated as the sum of the number of cells where the ODi value reached 0.15 after 24 h [38].

2.9. Statistical Data Analysis

One-way analysis of variance, using the general linear model (GLM), with IBM SPSS Statistics 29.0.1.0 software (SPSS Inc.; Chicago, IL, USA), was used to analyse the following parameters: plant height, number of leaves, stem diameter, color, texture, total soluble solid content (TSS), titratable acidity (TA), electrical conductivity (EC), pH, total phenolic content (TPC), antioxidant activity (DPPH assay), and lycopene content. Mean separation was carried out resorting to Tukey's test ($p \leq 0.05$). Phenotypic raw data from Biolog analysis were elaborated with Rstudio (R version 4.3.1, Package "pheatmap" version 1.0.12) for statistical analysis and data visualization of assays, using the default algorithms for clustering. To reduce the noise levels, all absorbance values of carbon source utilization were referred against the negative control well (A1) and subsequently, all divided by the respective AWCD. Negative values were set to 0. Normalized data were used for statistical analysis.

3. Results

3.1. Vegeto-Productive Plant Performances

At the end of this experiment, plants did not suffer the treatment with biochar and wood distillate. Plants of all treatments grew normally and all the phenological phases were observed. Looking at the plant growth parameters (height, number of leaves, and stem diameter) the effect of the treatment was detected only on some specific monitoring dates. For example, at flowering and fruit setting (week 2 and week 5), the 5BCWD plants showed a height statistically higher than those of the control (Table 3).

Table 3. Influence of biochar and wood distillate on plant height, during the first eight weeks of tomato plant cultivation.

Treatment	Height (cm)							
	Week 1	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**
Control	12.5 ± 0.7^{ab}	32.0 ± 0.9^{b}	49.7 ± 1.3^{a}	103.3 ± 2.6^{a}	115.2 ± 3.4^{b}	153.8 ± 3.6^{a}	199.5 ± 3.9^{ab}	229.0 ± 4.2^{a}
2BC	12.4 ± 0.3^{b}	32.5 ± 0.5^{b}	50.8 ± 1.6^{a}	94.8 ± 1.8^{a}	115.3 ± 2.3^{b}	156.5 ± 2.5^{a}	196.8 ± 2.4^{b}	222.3 ± 5.7^{a}
5BC	13.15 ± 0.4^{a}	33.8 ± 1^{ab}	53.5 ± 1.1^{a}	101.3 ± 2.9^{a}	115.2 ± 2.5^{b}	156.8 ± 4.9^{a}	187.6 ± 4.1^{c}	222.3 ± 8.9^{a}
2BCWD	12.0 ± 0.6^{b}	31.5 ± 0.5^{b}	50.6 ± 1.4^{a}	98.0 ± 1.7^{a}	119.2 ± 1.9^{ab}	158.3 ± 3.8^{a}	204.0 ± 2.9^{ab}	242.5 ± 7.9^{a}
5BCWD	13.3 ± 0.4^{a}	35.0 ± 1.2^{a}	51.1 ± 1.7^{a}	98.8 ± 3.5^{a}	124.6 ± 2.4^{a}	161.2 ± 3.0^{a}	208.6 ± 4.4^{a}	239.5 ± 3.7^{a}

One-way ANOVA, Tukey's test, $p \leq 0.05$. Within week, different letters indicate values statistically different. Control: 100% coconut fiber-CF; 2BC: 2% of biochar and 98% of CF (w/w); 5BC: 5% of biochar and 95% of CF (w/w); 2BCWD: 2BC treated with WD; 5BCWD: 5BC treated with WD. Week 1 and 2: formation of side shoots; Week 3: inflorescence emergence; Week 4: flowering; Week 5 and 6: development of fruits; Week 7 and 8: ripening of fruit and seeds.

For all the monitoring times, the n° of leaves recorded was not statistically different among the treated plants; the only exception was evidenced at week 6, in which all treated plants (2BC, 5BC, 2BCWD, and 5BCWD) presented a n° of leaves significantly higher than the control (25 ± 0.26 vs. 20 ± 0.90) (Table 4). The weekly recording of the stem diameter evidenced, as could be expected, its continuous growth (Figure 2). Moreover, the stem diameter, measured every week for all the duration of this experiment, increased with a comparable trend for all the plants, treated and non-treated, reaching a final value of 1.92 ± 0.05 for control plants and 1.93 ± 0.07 for treated plants.

Table 4. Influence of biochar and wood distillate on leaves number during the first six weeks of tomato plant cultivation.

Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Control	6.67 ± 0.21^{a}	8.00 ± 0.00^{a}	12.83 ± 0.40^{a}	12.50 ± 0.61^{a}	20.33 ± 1.30^{a}	21.83 ± 0.90^{b}
2BC	7.00 ± 0.21^{a}	8.00 ± 0.21^{a}	12.17 ± 0.42^{a}	12.50 ± 0.44^{a}	21.50 ± 0.71^{a}	24.50 ± 0.66^{a}
5BC	6.67 ± 0.21^{a}	7.83 ± 0.51^{a}	12.67 ± 0.40^{a}	12.83 ± 0.61^{a}	20.83 ± 0.74^{a}	24.50 ± 0.57^{a}
2BCWD	6.67 ± 0.21^{a}	8.67 ± 0.00^{a}	11.67 ± 0.47^{a}	13.00 ± 0.34^{a}	22.33 ± 0.76^{a}	24.67 ± 0.50^{a}
5BCWD	6.60 ± 0.21^{a}	7.80 ± 0.54^{a}	11.20 ± 0.76^{a}	12.40 ± 0.60^{a}	22.00 ± 0.47^{a}	25.40 ± 0.50^{a}

One-way ANOVA, Tukey's test, $p \leq 0.05$. Within week, different letters indicate values statistically different. Control: 100% coconut fiber-CF; 2BC: 2% of biochar and 98% of CF (w/w); 5BC: 5% of biochar and 95% of CF (w/w); 2BCWD: 2BC treated with WD; 5BCWD: 5BC treated with WD. Week 1 and 2: formation of side shoots; Week 3: inflorescence emergence; Week 4: flowering; Week 5 and 6: development of fruits.

Furthermore, in terms of final production, the yield per plant, considered both as the number of red fruits harvested per plant and the total weight of fruits harvested per plant, no statistically significant differences were observed among treatments (Table 5).

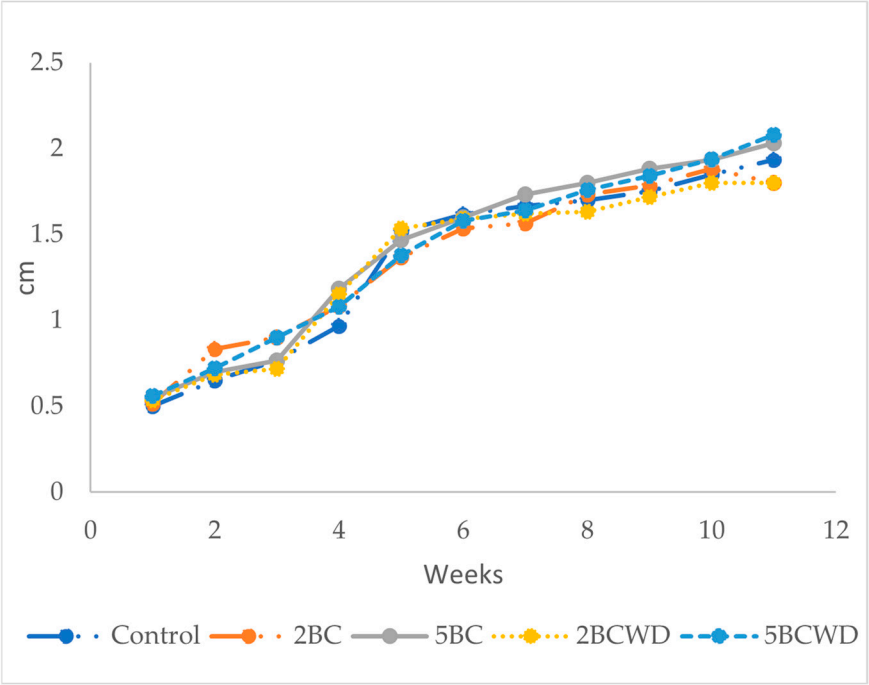

Figure 2. Trends of tomato plant stem diameter growth in response to various treatments, during 12 weeks of monitoring. Control: 100% coconut fiber-CF; 2BC: 2% of biochar and 98% of CF (*w*/*w*); 5BC: 5% of biochar and 95% of CF (*w*/*w*); 2BCWD: 2BC treated with WD; 5BCWD: 5BC treated with WD. Week 1 and 2: formation of side shoots; Week 3: inflorescence emergence; Week 4: flowering; Week 5 and 6: development of fruits; from Week 7 to week 12: ripening of fruit and seeds.

Table 5. Influence of biochar and wood distillate on productive and fruit morphological parameters, at the end of plant cycle.

Treatment	Yield × Plant^{-1}	Weight × Plant^{-1}	Fruit Length	Fruit Width	Fruit Fresh Weight	Fruit DMC
	(n°)	(g)	(cm)	(cm)	(cm)	(%)
Control	80.2 ± 10.9 ^{a}	1095.4 ± 148.7 ^{a}	3.8 ± 0.13 ^{a}	2.4 ± 0.03 ^{a}	13.7 ± 0.4 ^{a}	7.7 ± 0.2 ^{a}
2BC	89.0 ± 7.4 ^{a}	1277.2 ± 106.8 ^{a}	3.7 ± 0.05 ^{a}	2.40 ± 0.04 ^{a}	14.8 ± 0.5 ^{a}	8.0 ± 0.2 ^{a}
5BC	87.0 ± 6.4 ^{a}	1244.2 ± 92.2 ^{a}	3.9 ± 0.06 ^{a}	2.4 ± 0.05 ^{a}	14.3 ± 0.4 ^{a}	7.9 ± 0.4 ^{a}
2BCWD	79.5 ± 6.7 ^{a}	1107.6 ± 92.8 ^{a}	3.8 ± 0.06 ^{a}	2.4 ± 0.02 ^{a}	13.3 ± 0.4 ^{a}	7.4 ± 0.2 ^{a}
5BCWD	97.0 ± 5.2 ^{a}	1291.0 ± 72.3 ^{a}	3.7 ± 0.05 ^{a}	2.4 ± 0.05 ^{a}	13.9 ± 0.4 ^{a}	8.0 ± 0.9 ^{a}

One-way ANOVA. Within each parameter different letters indicate values statistically different. Control: 100% coconut fiber-CF; 2BC: 2% of biochar and 98% of CF (*w*/*w*); 5BC: 5% of biochar and 95% of CF (*w*/*w*); 2BCWD: 2BC treated with WD; 5BCWD: 5BC treated with WD.

The recorded morphological parameters of tomato fruits and the statistical analysis of data are reported in Table 5. Statistical analysis did not evidence any difference for all the morphological parameters considered; in fact, tomato fruits harvested from treated plants did not differ either in size nor in fresh and dry weight from those of non-treated plants.

3.2. Characterization of Tomato Fruits

All the parameters useful for fully characterizing tomato fruits are represented in Tables 6 and 7. In particular, in this study, color, texture profile, pH, TSS, TA, TSS/TA, EC, TPC, AO and lycopene content were evaluated.

Table 6. Influence of biochar and wood distillate on the tomato fruit and juice color parameters.

Treatment	L* Skin	a* Skin	b* Skin	L* Juice	A* Juice	b* Juice
Control	35.79 ± 0.46 ^{a}	19.43 ± 0.55 ^{b}	18.66 ± 0.76	34.36 ± 0.24	8.13 ± 0.42 ^{c}	9.11 ± 0.25 ^{ab}
2BC	34.40 ± 0.40 ^{b}	21.45 ± 0.42 ^{ab}	20.16 ± 0.36	34.36 ± 0.19	9.36 ± 0.20 ^{b}	9.40 ± 0.19 ^{a}
5BC	35.05 ± 0.34 ^{ab}	22.65 ± 0.49 ^{a}	20.21 ± 0.38	35.10 ± 0.28	9.3 ± 0.23 ^{b}	8.75 ± 0.26 ^{b}
2BCWD	34.44 ± 0.14 ^{b}	20.99 ± 0.32 ^{b}	19.35 ± 0.38	34.63 ± 0.16	11.25 ± 0.23 ^{a}	8.50 ± 0.15 ^{b}
5BCWD	34.25 ± 0.27 ^{b}	21.32 ± 0.42 ^{b}	19.04 ± 0.36	34.46 ± 0.21	9.63 ± 0.15 ^{b}	8.36 ± 0.26 ^{b}

One-way ANOVA, Tukey's test, $p \leq 0.05$. Within each parameter different letters indicate values statistically different. Control: 100% coconut fiber-CF; 2BC: 2% of biochar and 98% of CF (w/w); 5BC: 5% of biochar and 95% of CF (w/w); 2BCWD: 2BC treated with WD; 5BCWD: 5BC treated with WD.

Table 7. Influence of biochar and wood distillate on the tomato fruit quality and biochemical parameters.

Treatment	Firmness	TSS	TA	TSS/TA	EC	pH	TPC	AO	Lycopene
	(N)	(°Brix)	(% Citric Acid)		(mS)		(mg GAE/kg)	(mM TEAC)	(mg/kg)
Control	7.25 ± 0.21 ^{a}	9.15 ± 0.18 ^{a}	0.66 ± 0.05 ^{a}	13.79 ± 0.27 ^{c}	0.74 ± 0.03 ^{a}	4.65 ± 0.02 ^{a}	643.88 ± 9.97 ^{b}	1.72 ± 0.15 ^{b}	7.25 ± 0.18 ^{a}
2BC	7.42 ± 0.15 ^{a}	8.54 ± 0.08 ^{a}	0.54 ± 0.03 ^{b}	15.77 ± 0.16 ^{b}	0.68 ± 0.01 ^{b}	4.62 ± 0.03 ^{a}	742.14 ± 22.77 ^{a}	1.99 ± 0.13 ^{b}	6.32 ± 0.12 ^{ab}
5BC	7.18 ± 0.19 ^{a}	9.03 ± 0.15 ^{a}	0.53 ± 0.02 ^{b}	16.93 ± 0.29 ^{a}	0.68 ± 0.02 ^{b}	4.64 ± 0.03 ^{a}	751.38 ± 35.91 ^{a}	2.11 ± 0.14 ^{b}	6.54 ± 0.21 ^{ab}
2BCWD	6.48 ± 0.23 ^{b}	8.83 ± 0.18 ^{a}	0.51 ± 0.02 ^{b}	17.27 ± 0.35 ^{a}	0.65 ± 0.006 ^{b}	4.70 ± 0.04 ^{a}	773.98 ± 40.47 ^{a}	2.30 ± 0.11 ^{ab}	5.46 ± 0.20 ^{b}
5BCWD	6.39 ± 0.26 ^{b}	8.95 ± 0.17 ^{a}	0.52 ± 0.01 ^{b}	17.15 ± 0.35 ^{a}	0.71 ± 0.01 ^{ab}	4.69 ± 0.03 ^{a}	749.90 ± 29.10 ^{a}	2.79 ± 0.27 ^{a}	6.18 ± 0.23 ^{ab}

One-way ANOVA, Tukey's test, $p \leq 0.05$. Within each parameter different letters indicate values statistically different. Control: 100% coconut fiber-CF; 2BC: 2% of biochar and 98% of CF (w/w); 5BC: 5% of biochar and 95% of CF (w/w); 2BCWD: 2BC treated with WD; 5BCWD: 5BC treated with WD.

The statistical analysis of data on color parameters of tomato skin and juice showed differences in L*, a* and b*, respectively (Table 6). In terms of tomato skin color, the control fruits had a significantly higher lightness (L*) than those subjected to other treatments, with only the 5BC fruits having a comparable L* level. Conversely, 5BC fruits exhibited values of a* statistically higher than those from other treatments, thus, the 5BC treatment determined a more intense red color, a feature particularly appreciated by producers and consumers. Regarding the color of the tomato juice, the interpretation of the data is more complex; specifically, the 2BCWD samples exhibited the highest value of a*, with 2BC, 5BC, and 5BCWD showing intermediate values. The b* parameter of the juice seems to be higher in 2BC and control, instead, other treatments (5BC, 2BCWD, and 5BCWD) are characterized by lower values. There were no significant differences observed among all samples for the b* parameter of the skin and the L* parameter of the tomato juice (Table 6).

As shown in Table 7, the control, 2BC, and 5BC fruit samples showed significantly higher hardness values compared to the 2BCWD and 5BCWD; in particular, these two samples showed a hardness reduction of 10.62% compared to the control samples.

There were no statistically significant differences found in the pH of fruits from plants grown in different substrates and subjected to various treatments (Table 7). Likewise, none of the treatments tested influenced the TSS values, that ranged from 8.54 (2BC) to 9.15 (control). Nevertheless, differences in TA and the TSS/TA ratio were evident. Notably, it appears that the control fruits contained a higher percentage of citric acid compared to other treatments (Table 7). Moreover, 5BC, 2BCWD, and 5BCWD exhibited higher values of the TSS/TA ratio of 16.93 ± 0.29, 17.27 ± 0.35, and 17.15 ± 0.35, respectively (Table 7). The EC results showed significant differences only in the control group compared to the treated samples (Table 7).

The amount of total phenolic content (TPC), expressed as mg GAE/Kg, and antioxidant activity (AO), expressed as mM TEAC, are presented in Table 7. The concentration of TPC is significantly different between the control fruits, for which the lowest value was recorded (643.88 ± 9.97 mg GAE/Kg), and the fruits from all the other treatments (Table 7). The statistical analysis performed on the data obtained on the antioxidant activity (AO) of the fruits showed a higher scavenging activity of tomato fruits grown in substrates

amended with 5% of biochar and treated with WD (5BCWD) compared to control fruits, of 2.79 ± 0.27 and 1.72 ± 0.15 mM TEAC, respectively.

Lycopene content is one of the major parameters controlling the fruit color and the nutritional quality of tomato fruits. In this experiment, the tomato fruit lycopene level, considering both the trans- and cis-forms, ranged from 7.25 ± 0.18 mg/kg for the control to 5.46 ± 0.20 mg/kg for 2BCWD fruits (Table 7).

3.3. Community-Level Physiological Profiling (CLPP)

The results of the Biolog Ecoplate system are reported in Figure 3, which shows a heatmap of the 31 compounds present in Ecoplate and highlights which compounds were more or less metabolized by the microbial community present in each sample. As for other morphological and chemical analyses, the differences from the control sample are not always recorded. To obtain more complete information on the utilization of carbon sources, 31 substrates were divided into 6 groups according to their chemical class including amines, amino acids, carbohydrates, carboxylic acids, phenolic compounds, and polymers. Samples formed two different clusters based on metabolized compounds.

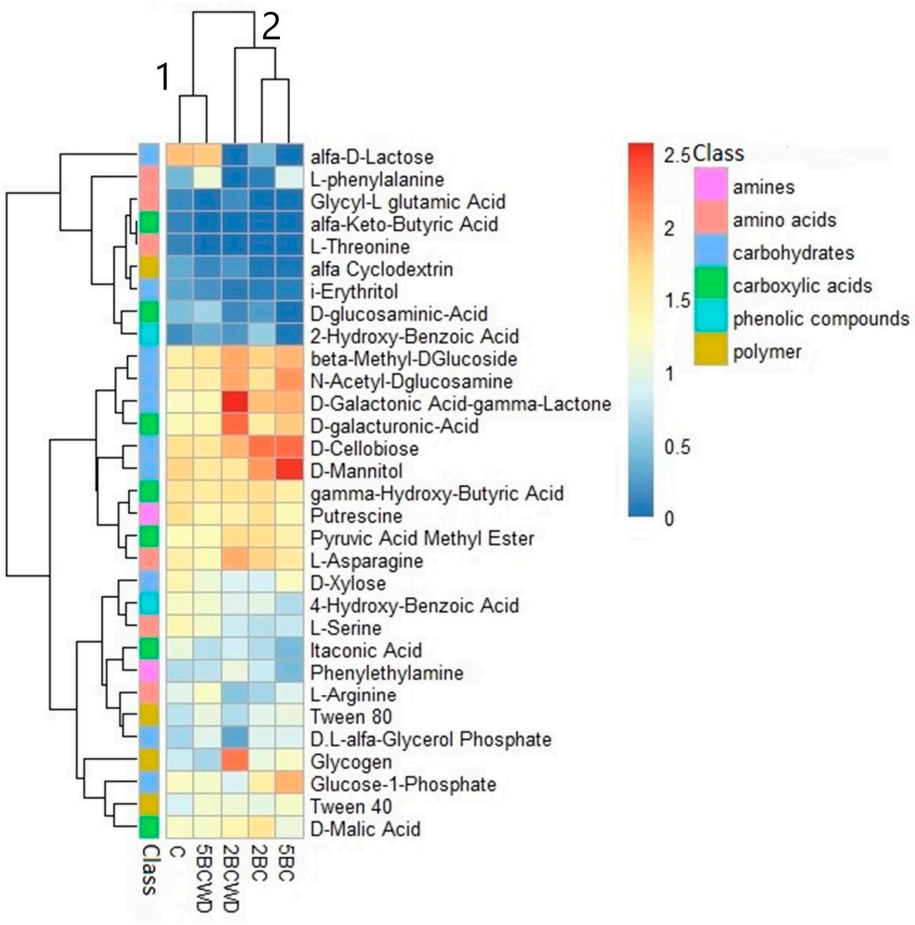

Figure 3. Heatmap displaying the metabolic profiling of microbial community in different soil samples exposed to the biochar (2BC and 5BC) and wood distillate (2BCWD and 5BCWD) with respect to the control sample (C). The substrate utilization patterns are clustered based on the Euclidean distance measure, calculated on the normalized Ecoplates data, and the color intensity represents the efficiency of strains in metabolizing the substrates supplied. The substrates are grouped into 6 chemical classes. The two metabolic clusters occurring after grouping of the samples are reported in the figure as cluster 1 and cluster 2.

At first sight, the control and 5BCWD (cluster 1) used more α-D-Lactose and less glycogen when compared to all the other treatments (cluster 2). The 5BCWD samples differed from control ones in the utilization of L-phenylalanine, L-arginine, Tween 80, and Tween 40, similar to other treated samples. The higher degree of substrate utilization was recorded for D-galacturonic acid, D-galactonic acid γ-lactone, L asparagine, and glycogen by sample 2BCWD. Samples treated with only biochar metabolized at high levels D-cellobiose, D-mannitol, and glucose-1-phosphate. Different from control, these treated samples show the lowest degree of utilization of 4-hydroxy-benzoic acid, L-serine.

The AWCD and richness indices were higher for the 5BCWD treatments than the control, while all the other treatments showed the lowest value. The other indices (the Shannon Index and Shannon evenness) are similar for all the samples (Table 8).

Table 8. Metabolic functional diversity of microorganisms in different treated samples.

Index	AWCD	SR	H′	E
Control	1.48 ± 0.14^{a}	17.10 ± 0.15^{ab}	3.27 ± 0.01^{a}	1.14 ± 0.02^{a}
2BC	1.06 ± 0.03^{b}	13.90 ± 0.14^{b}	3.17 ± 0.04^{a}	1.18 ± 0.02^{a}
5BC	1.03 ± 0.04^{b}	16.25 ± 0.35^{ab}	3.69 ± 0.05^{a}	1.11 ± 0.01^{a}
2BCWD	0.69 ± 0.01^{c}	14.15 ± 0.21^{b}	3.13 ± 0.03^{a}	1.17 ± 0.01^{a}
5BCWD	1.50 ± 0.01^{a}	20.20 ± 0.28^{a}	3.26 ± 0.06^{a}	1.04 ± 0.06^{a}

One-way ANOVA, Tukey's test, $p \leq 0.05$. Within each parameter different letters indicate values statistically different. Control: 100% coconut fiber-CF; 2BC: 2% of biochar and 98% of CF (*w*/*w*); 5BC: 5% of biochar and 95% of CF (*w*/*w*); 2BCWD: 2BC treated with WD; 5BCWD: 5BC treated with WD. AWCD: average well color development; SR: substrate richness; H′: Shannon index; E: Shannon evenness.

4. Discussion

The evaluation of the influence of biochar, as an amendment used in field cultivation, has been an interesting topic for years, and many studies available in the literature have been focused on this topic [15,39]; however, few are the studies investigating the possibility of replacing part of the non-renewable and cost-effective cocopeat with biochar, in soilless cultivation systems [8,11]. In this study, biochar was added, at two concentrations, in the growing bags used to cultivate indetermined tomato plants, and its effect, combined with wood distillate, on plant vegeto-productive performances, was evaluated.

The plant height was influenced by the biochar and wood distillate only at some specific phenological phases; at the flowering time, plants treated with 5BCWD showed a higher height than control plants. In the literature, controversial results are present, since some authors did not evidence any positive influence of biochar on plant height [8], while others, such as Graber et al. [29], showed that biochar treatment positively affected the height of tomato plants. Plants treated with biochar and wood distillate produced during their growth a number of leaves comparable with non-treated ones; in correspondence of the fruit ripening, treated plants showed a number of leaves higher than those of the control; these results are in line with what was observed by Simiele et al. [30] even if, in this study, the treated plants presented a higher number of leaves during the flowering period.

In the literature, it is reported that the addition of wood distillate determines an increase not only in the plant height, but also in the stem diameter [40]; these results are not confirmed in this study, in which the treated and non-treated plants showed stems with a comparable diameter.

The overall fruit yield, both in terms of number and weight of fruit per treatment, was not affected by the four treatments tested in this study. The total productions of 2BC, 5BC, 2BCWD, and 5BCWD are fully comparable to the final production of the control. The results of this study are consistent with those of Graber et al. [29] and Massa et al. [41], which showed that the impact of biochar on yield was not significant. However, there are also studies, such as Simiele et al. [30], showing that the use of biochar can improve the overall yield in terms of the number of fruits harvested. In addition, many authors [42,43], have reported that the application of WD can also positively affect plant productivity (total number of fruits and yield).

In this study, treatments with biochar and wood distillate showed no effect on fruit morphological parameters, such as longitudinal and polar diameter, as previously reported by Petruccelli et al. [44] on the 'Rio Grande' variety.

In this experiment, the fruit weight was not affected by any of the treatments applied, with values absolutely comparable with those of the control. Only the fruits harvested from poplar biochar-treated plants were found to have an average weight higher than fruits grown in the other treatments. In agreement with Petruccelli et al. [44], no significant differences were found in dry matter content. The substantially comparable behavior of treated plants, both in terms of morphological and productivity response, with those from the control confirm the validity of biochar as partial replacement of cocopeat fiber.

Color is one of the most important quality characteristics influencing tomato appearance and consumer acceptance [45,46]. According to Ain Najwa et al. [47], the skin color of tomato fruits, except for the redness value, showed a significant effect depending on different percentages of biochar applied. In this study, both skin and juice color were analyzed, and the statistical analysis showed that the effect of biochar and WD treatment is different depending on the tomato fruit part taken into consideration.

The firmness of the tomato fruits is associated with a better taste experience and a longer shelf life [48]. In this study, it was more the combination of BC and WD that influenced the fruit firmness, resulting in fruits slightly softer than those of the control. When only biochar was used, no differences in texture were observed; results in line with what was reported by Suthar et al. [49], who observed that firmness values in tomato fruits treated with 1% biochar and 3% biochar were comparable with the control group.

TSS and TA affect organoleptic fruit quality, in terms of sweetness and acidity. In this study, the TSS content of fruits harvested from treated plants did not differ from those of non-treated; results that are in accordance with previous studies which reported no differences in tomato fruit soluble solids content among different treatments [8]. Other authors reported, instead, that the addition of biochar determined an increase in the TSS of fruits [49]. The TA was strongly influenced by the treatment tested, with higher values in the control fruits; in contrast with results reported by Simiele et al. [30]. To better characterize the organoleptic quality of tomato fruits, it is possible to resort to the TSS/TA ratio [50]. In this study, the maximum sugar/acid ratio was achieved in fruits from plants grown with biochar and with wood distillate. According to Gao et al. [51], the sugar/acid ratio of tomato fruits can increase significantly with a higher content of soil organic matter, typical of substrates treated with organic fertilizers, such as biochar; in fact, in this condition, the resulting improved activity of soil bacteria can break down the organic matter and release nitrogen, phosphorus, and potassium [52] which has a positive effect on the enzymatic activity in the soil and leads to an increase in the sugar/acid ratio of the fruit.

Phenolic compounds originate from secondary metabolism and play a crucial role in various aspects of plant life such as growth, adaptation to the environment, and overcoming stress conditions [53]. Several factors affect the content of phenolic compounds in tomato fruits, such as genotypes, production system, water availability, and salinity [54,55]. In this study, the concentration of total phenolics differed significantly among treatments, with a higher content in treated fruits rather than in those from control. This result is completely in line with the literature; in fact, Benzon et al. [26] showed that the application of WD at concentrations of 0.2% and 0.4% on tomato plants increased the TPC in the fruits. As expected, there were also differences in the antioxidant activity of the fruits; namely, fruits from 5BCWD-treated plants presented an antioxidant activity higher than the other fruits tested. This result is consistent with several studies that report a positive effect of wood distillate on the antioxidant properties of the fruits. This positive effect is due to the composition of WD, a complex of numerous compounds such as esters, organic acids, phenols, alcohols, alkanes, etc. [43,56].

The lycopene content of tomato fruits is influenced by various factors such as agricultural practices, soil, climatic factors, fruit growth, harvest date, and degree of ripeness [57]; moreover, according to Tzortzakis et al. [58], the lycopene content can also be influenced by the composition of the growing substrates. In this study, only the 2BCWD treatment determined a decrease in the lycopene content in fruits tested. These results are in contrast with the literature; in fact, even if Arias et al. [59], established a direct correlation between skin redness and lycopene content, in this study, the fruits characterized by a higher redness, such as those from the 5BC treatment, did not present an equally higher content of lycopene.

Microorganisms provide an integrated measure of soil quality, driving many key processes of nutrient cycling, soil structural dynamics, and pollutant degradation and respond rapidly to natural disturbances and environmental stresses. This allows microbial analyses to discriminate soil quality status, and changes in microbial population and activity could be used as indicators of changes in soil quality [60].

In environmental microbiology, the BIOLOG system is frequently used to evaluate the effects of different agricultural management techniques on the functional diversity of soil microbial communities [61]. Results from the metabolic profile of bacterial communities indicated differences in the functional diversity of soil microorganisms among treatments. According to AWCD definition, the degree of carbon substrate utilization is directly correlated with the metabolic capacity of the individual microbial communities [62]. The increase in AWCD values for 5BCWD could indicate an enhancement in the general metabolism of soil microorganisms exposed to this type of treatment.

The application of BC and WD to the soil has contributed to changes in the degree of utilization of carbon sources. The use of some C substrates increased at the same time as the use of others decreased, the low levels of utilization suggesting that the bacteria may have adapted to new environments as reported from Macik et al. [61].

The increased utilization of particular polymers, amino acids, carbohydrates, carboxylic acids, and amines for treated samples suggests that the introduction of biostimulants to the soil may enhance the selection of microorganisms capable of metabolizing certain compounds. Having a higher metabolic function leads to a wider resource of essential nutrients that can be utilized by microorganisms but also by plants. This activity was recorded in the samples treated with 5BCWD. This phenomenon could explain the significant increase in antioxidant activity in fruits from the 5BCWD treatment mentioned above. For example, conversion of organic substrates (polymer) to small molecular weight molecules that can be further degraded to carbon dioxide and water.

Some amino acids and amines were metabolized more intensively in treatments with wood distillate. Amino acids constitute an important source of organic nitrogen in the soil. The higher utilization rates of D-glucose-1-phosphate, which can be a source of phosphorus for soil microbial communities, have been reported for 5BC samples. The increased utilization rate of the above compounds may be associated with the increased activity of microorganisms involved in the biochemical conversion of nitrogen and phosphorus [61]. The carbohydrates class, consisting mainly of sugars and their derivatives, had the highest utilization rate in the sample 2BCWD, and in the two treated with biochar. β-Methyl-D-glucoside and D-mannitol were significant carbon sources among carbohydrates. It is reported that the use of these more easily decomposable carbohydrates and carboxylic acids, favored C mineralization [37].

5. Conclusions

In this study, the potential of biochar and wood distillate as effective fertilizers in soilless tomato cultivation is highlighted. While no significant effects on vegetative growth, yield, or fruit morphology were observed, the integration of biochar and wood distillate notably influenced several quality attributes of the tomato fruits. The combination of 5% biochar and wood distillate (5BCWD) improved the fruit antioxidant activity and phenolic content, contributing to higher nutritional value, especially in terms of antioxidant capacity.

Additionally, the 5BC treatment enhanced the intensity of red color in the fruit skin, a desirable trait for both producers and consumers. These results suggest that biochar, when combined with wood distillate, can be a valuable tool for improving the functional quality of tomatoes without compromising productivity. The findings also support the sustainability of using biochar as a partial replacement for cocopeat in soilless systems, offering an eco-friendly alternative with potential benefits for both crop quality and substrate health. Encouraging results were obtained regarding the substrate microbial community, which undergoes changes depending on the substrate treatment: these changes warrant further investigation through. Further research is needed to explore the effects of different types of biochar and wood distillate to fully understand their potential in optimizing tomato production and quality as well as to investigate more thorough, through metataxonomic analyses, substrate microbial community changes.

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