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Biomass Source of Biochar and Genetic Background of Tomato Influence Plant Growth and Development and Fruit Quality

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Abstract: Maintaining healthy soils and restoring marginal lands are necessary to ensure efficient food production and food security. Biochar, a porous carbon-rich material generated from the pyrolysis of organic feedstock, is receiving attention as a soil amendment that can potentially restore soil health and enhance crop yields. However, the physical and chemical properties of biochar are influenced by pyrolysis parameters and organic feedstock sources. These determine its interaction with the soil, influencing its impact on soil health and plant productivity. While most studies report the evaluation of one biochar and a single plant cultivar, the role of genetic background in responding to biochar as a soil amendment remains unexplored. The impact of six biochars on agronomic performance and fruit quality of three tomato (*Solanum lycopersicum*) cultivars was evaluated to test the hypotheses that (1) biochars derived from different feedstock sources would produce unique phenotypes in a single cultivar of tomato, and (2) single feedstock-derived biochar would produce different phenotypes in each of the three tomato cultivars. The data supported both hypotheses. This study demonstrated that plant genetic background and biomass source are important variables that must be considered for using biochar as a soil amendment.

Keywords: biomass source; biochar; tomato; fruit quality; pyrolysis; soil amendment

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1. Introduction

Intensified crop production has resulted in a loss of organic matter and sodification in many soils, leading to the deterioration of soil health [1]. To combat depleting tilth, new soil management practices are being employed in attempts to increase soil organic matter (SOM), foster a diverse soil microbiome, improve crop productivity, and promote additional ecosystem services [2–6]. However, due to changing climatic conditions, soil organic carbon (SOC) levels are projected to decrease in the future [7]. Therefore, it is critical to pursue interventions that encourage beneficial soil practices such as implementing cover crops and reduced tillage [8–10]. Such measures will aid in the development of carbon-negative ecosystems, which focus on returning carbon assimilated by plants into the soil in a stable form. These challenges need to be addressed to ensure global food security for current and future generations.

One potential solution to address these challenges is biochar (BC), a carbon-rich, porous product generated by a thermochemical process known as pyrolysis or gasification. The production process involves the controlled thermal decomposition of feedstock under low oxygen levels at temperatures ranging from 300 °C to 800 °C [11,12]. The production of BC can be achieved using various feedstocks, the most common of which include agricultural crop residue, organic manure, and wood [13]. The feedstock source determines the final nutrient profile of the biochar. Organic waste feedstocks generate BC rich in potassium and phosphorus, low in C levels, and low in surface area. BC derived from wood feedstocks is enriched in organic matter and surface area; however, it has low N, P, and K levels and a reduced capacity for cation exchange. Generally, crop residue-derived BCs are rich in N [14–16]. The variation in nutrient profiles along with other physical properties determines how the BC interacts with the soil and collectively influences plant performance. The specific impacts of BC amendment to soil include alterations in bulk density, porosity, and water retention; these properties make the exchange of water, nutrients, and gases more efficient, resulting in enhanced crop productivity [17,18]. Productivity in a diverse range of crops, including tomatoes, lettuce and other leafy vegetables, beans, potatoes, wheat, maize, and rice, among others, has been evaluated in soils amended with BC derived from various feedstocks [19–25]. Additionally, since BC is a stable source of carbon and nutrients, it enables the proliferation of beneficial microbial communities, which in turn enhance soil tilth and health [2,26]. With improvements in automation, it is now feasible to produce consistent-quality BC; together with a growing knowledge of the utility of BC as a soil amendment for enhancing nutrient availability and facilitating long-term carbon sequestration, utilization in both research and farming is expected to increase [27–31].

The biological, chemical, and physical influence of BC and its role in enhancing soil health is well documented; however, its utilization in soils produces a spectrum of outcomes in terms of crop productivity [25,32–37]. Several recent meta-analyses investigating the role of BC on crop productivity conclude that, overall, there is a positive impact on crop yield [16,29,38,39]. However, there are studies where the BC amendment impacts one aspect of plant development but has no impact on yield or produces a detrimental outcome [21,40,41]. It is well known that the genetic background of a plant influences how it responds to a given stimulus [42–44]. Interestingly, most previous reports evaluating the impact of BC have studied one cultivar's response to biochar derived from a single feedstock. The interaction between BC type and genotype remains largely unexplored.

In this study, the impact of BC derived from six different feedstocks on the growth and development of three genotypically distinct cultivars of tomato (*Solanum lycopersicum* L.) was evaluated. Experiments were conducted to test the following hypotheses: (1) BC derived from different feedstock sources will produce unique phenotypes in a single cultivar of tomato, and (2) a single feedstock-derived BC will produce different phenotypes in each of the three tomato cultivars.

2. Materials and Methods

2.1. BC Source

Five types of BC generated from their respective feedstocks were provided by Ag Energy Systems (Spokane, WA, USA). The feedstocks used were as follows: ryegrass straw (RGS), ryegrass tailings (RGT), Russian thistle (RT), thermomechanical pulp waste (TMP), and walnut shell (W). A commercially available BC product, CoolTerra® (CT), manufactured by Cool Planet (Greenwood Village, CO, USA), was also used in the study. All experiments were conducted with 0.5% and 1% *w*/*w* rates of BC amendment.

2.2. SEM and EDX Analysis

Scanning electron microscopy (SEM) was performed on each BC at the Franceschi Microscopy and Imaging Center at Washington State University. A sample of each BC was fixed to a pin stub and sputter coated in gold. SEM samples were imaged on a Tescan Vega SEM equipped with an energy-dispersive X-ray spectroscopy (EDX) detector to make a qualitative visual assessment of the biochar samples. Images were recorded at a 1 mm and a 100 μm resolution (Figure 1A,B). Qualitative elemental composition data for each BC were collected with the EDX detector.

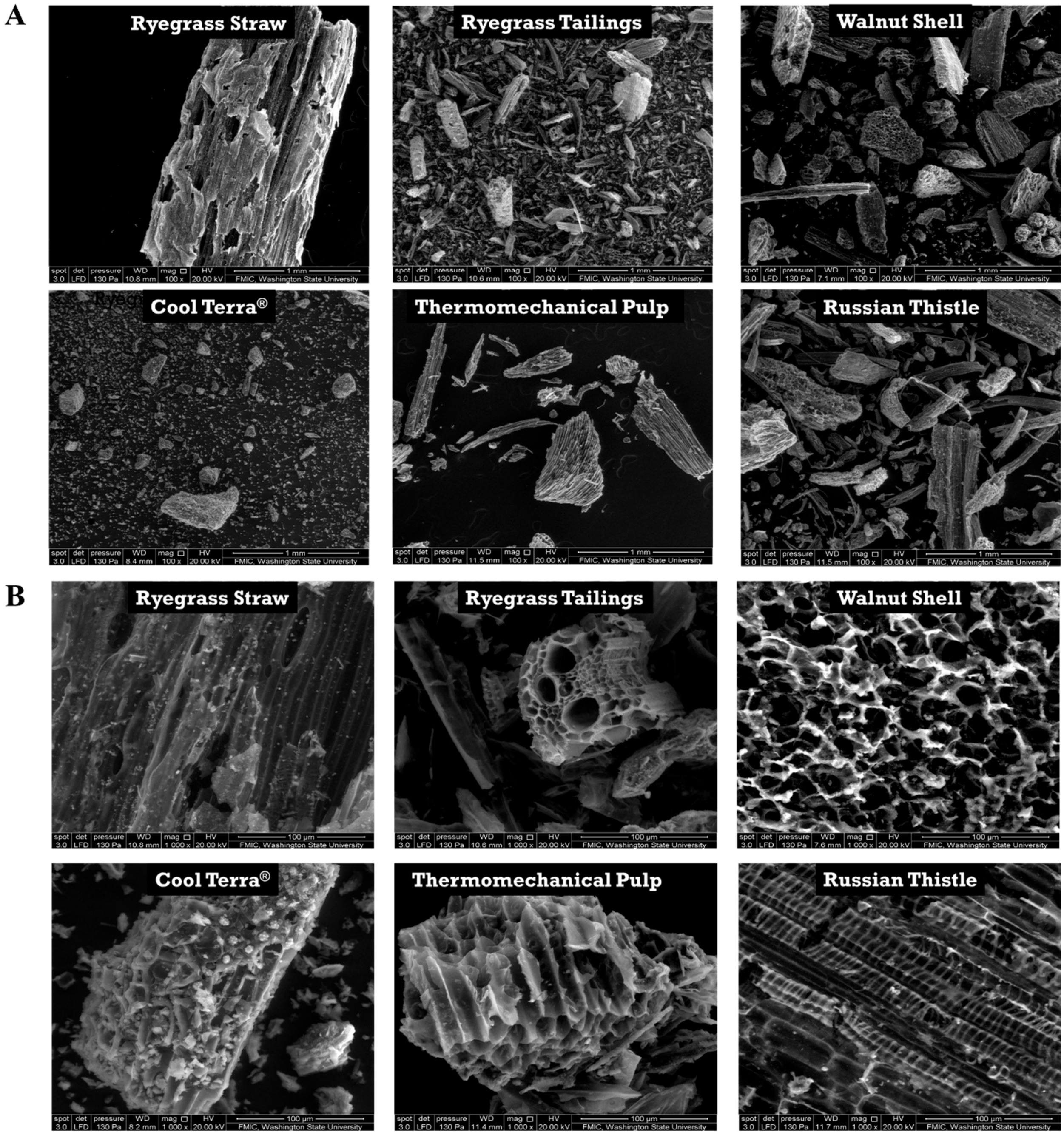

Figure 1. Ultrastructural characterization of six different types of biochars derived from different biomass using scanning electron microscopy. (**A**) Micrographs obtained at 100× resolution demonstrate the variability in the ultrastructure of the biochars. Note the variability in particle size as well as the size of the macro- and micropores. (**B**) Micrographs obtained at 1000× resolution further accentuate the variability in particle size as well as the size of the macro and micropores.

2.3. Plant Growth Conditions

Three cultivars of tomato (*Solanum lycopersicum* L.) representing unique market applications and diverse genetic backgrounds were selected for this experiment. 'Oregon Spring', an heirloom determinate variety, was selected due to its popularity in home gardening. 'Heinz 2653', also a determinate variety, is commonly used as a commercial processing tomato. 'Cobra F1', an indeterminate variety, was selected due to its commercial use as a greenhouse variety.

Seeds for the three cultivars were obtained from the Territorial Seed Company (Cottage Grove, OR, USA). Seeds were germinated in 4-inch rockwool squares and grown to 4–5 nodes (15–20 cm) in height. Afterward, plantlets were transplanted into 2.8 L pots with either organic Sunshine Mix#1/LC1 (Sun Gro Horticulture, Agawam, MA, USA) as a control or Sunshine Mix containing BC at 0.5% and 1% (*w*/*w*) rates. One week after transplant, each pot was fertilized twice a week with 450 mL of dilute (20 mL/L water) organic Alaska 5-1-1 Fish Fertilizer (Lilly Miller Brands, Atlanta, CA, USA). Plants were maintained in a glasshouse at the Washington State University Plant Growth Facilities with temperatures held at 24 °C/18 °C (day/night); relative humidity was maintained at 40–60%. High-Pressure Sodium (HPS) lights provided supplemental lighting, extending the day length to 16 h as needed. Young plants were watered every other day, while the larger, mature plants were watered daily. Plants growing on the glasshouse benches were initially arranged in a randomized design and after two weeks of growth underwent regular plant rotations and random sampling to reduce spatial variation in the glasshouse.

2.4. Experimental Design

Six independent experiments were conducted, with two experiments each for 'Oregon Spring', 'Heinz 2653' ('Heinz 2653'), and 'Cobra' F1 (Table 1). Experiments with 'Heinz 2653' and 'Oregon Spring' were conducted over 102 days while with 'Cobra' F1 for 182 days (Table 1). Each experiment consisted of 56 plants of the same genotype: eight plants were grown in soil media containing 0% BC and served as controls, while four plants were randomly assigned to each of the 12 treatment groups (Table 2).

Table 1. Planting and harvest dates for each experiment performed with three tomato cultivars.

Experiment	Cultivar	Date Planted	Date Harvested
1	'Oregon Spring'	17 February 2017	3 June 2017
2	'Oregon Spring'	20 January 2018	7 May 2018
1	'Heinz'	17 February 2017	3 June 2017
2	'Heinz'	15 May 2017	30 August 2017
1	'Cobra'	16 May 2017	10 November 2017
2	'Cobra'	8 November 2017	9 May 2018

Table 2. Experimental layout detailing the biochar treatments and number of plants used for each treatment. CT—CoolTerra, RGS—Ryegrass straw, RGT—Ryegrass tailings, TMP—Thermomechanical pulp, RT—Russian thistle, and W—Walnut. BC—Biochar.

	BC	Control	CT		RGS		RGT		TMP		RT		W	
Treatments	%	0	0.5	1.0	0.5	1.0	0.5	1.0	0.5	1.0	0.5	1.0	0.5	1.0
	n	8	4	4	4	4	4	4	4	4	4	4	4	4

2.5. Plant Growth Parameters and Assessment of Fruit Quality

Dry weight: The shoot biomass of each plant was collected at the conclusion of each experiment. Fruits were removed, plants were cut at soil level, and the shoots were completely dried in large paper bags at 60 °C for 48 h. The resulting dry tissue was weighed in grams of aboveground dry mass per plant.

Yield: To measure yield, four random fruits per plant were selected for sampling at the 'Breaker' stage [45,46]. Following the achievement of the 'Red' stage, the point in development where greater than 90% of a fruit's surface area displays color change, fruit were collected at regular intervals throughout the remainder of the experiment [46]. The yield for each plant was quantified based on the total number of fruits and cumulative fruit weight in grams.

Quality: Fruit quality parameters were assessed by quantifying total soluble solids (TSS), sugars, and organic acid content. A handheld rotary Bio-Homogenizer (model M133/1281-0 from Biospec Products Inc., Bartlesville, OK, USA) was used to extract juice from five grams of fruit pericarp tissue from each of the four sampled fruits. Juice extracted from 'Red' stage fruit was filtered through cheesecloth and used for the refractometer-based quantification of TSS. An aliquot of the juice sample was centrifuged and the supernatant was filtered using 0.45 µm pore size filters. The filtered supernatant was stored at −80 °C for later use in the quantification of sugar and organic acid profiles. Fructose, glucose, citric acid, malic acid, and fumaric acid were quantified using a Varian Prostar 230 HPLC equipped with an Aminex HPX 87H column coupled to a refractive index (RI) and UV (210 nm) detector. The column was eluted with 0.005 M of H_2SO_4 at a flow rate of 0.6 mL/min at 65 °C [47]. The identification and quantification of sugars and organic acids were performed using a previously published method [47].

2.6. Statistical Analysis

2.6.1. Three-Way and Two-Way ANOVA

For each greenhouse trial, plant dry weight, fruit yield per plant, fruit organic acid (citrate and malate), and fruit carbohydrate (glucose and fructose) data were subjected to 3-way analysis of variance (ANOVA) with variation partitioned into main effects (3 cultivars, 6 biochars, 3 rates) and interactions. The data were also analyzed using 2-way ANOVA to assess the effects of biochar, biochar rate, and their interaction separately for each cultivar. The *p*-values for main effects and interactions were calculated for the 3-way and 2-way factorial analyses. Data are plotted separately (±SE) by cultivar with means separated by LSD ($p < 0.1$) to show the effects of biochar and biochar rate (linear, deviations) on growth, yield, and fruit chemistry.

2.6.2. Correlation Plot

The correlation plot was generated by running correlation tests between fruit citric acid, malic acid, glucose, and fructose measurements using R version 4.2.2 with the R libraries stats, dplyr, and corrplot.

3. Results and Discussion

3.1. Qualitative Characterization of BC Using Scanning Electron Microscopy and EDX

BC derived from ryegrass straw and tailings (RGS and RGT) represents crop-residue biomass, while walnut shell BC (W) is derived from highly lignified biomass waste. Russian thistle (RT) represents biomass where lignification is intermediate between hardwoods and crop residue. Thermomechanical pulp (TMP) is a derivative of a process that involves heat and mechanical pressure to soften the lignin and fiberize hardwood material for the production of paper [48].

Micrographs were recorded for each BC at 100× and 1000× magnifications. A qualitative visual analysis revealed that the plant residue BC, RGT, and RT exhibited a more heterogeneous composition, exemplified by a broader range of particle sizes, in comparison with the walnut and thermomechanical pulp BC (Figure 1A,B). The CoolTerra BC featured the smallest particle size of the examined BCs, and the pores were occluded with small particles. Each feedstock in this study generated BC with distinct microscopic structures. These unique physical properties likely provide unique capabilities in regard to altering soil physical characteristics such as moisture content, bulk density, and pH [49,50] as well as microbial and nutrient uptake interactions in the rhizosphere [2] (Figure 1A,B).

Characterization with EDX spectra facilitated the qualitative estimation of the specific elements present in each BC. The EDX method is an analytical technique that relies on X-ray excitation and its interaction with a given sample. The unique atomic structure of each element in a sample corresponds to distinctive peaks on the electromagnetic emission spectrum, allowing for chemical and elemental characterization [51]. Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and silicon (Si) were the most abundant elements in all BC varieties (Table 3). Sulfur (S) and aluminum (Al) were detected in three BCs (RT, Russian thistle; W, walnut; and CT, CoolTerra®), while chlorine (Cl), molybdenum (Mo), magnesium (Mg), and sodium (Na) were only scarcely distributed among the BCs. Ryegrass tailings (RGT)-derived BC contained all analyzed elements except Cl and Na, while the only elements identified in walnut BC were N, P, Ca, and Al (Table 3). Walnut BC also demonstrated highly lignified cell walls. While this study used EDX to qualitatively assess BC elemental composition, it is feasible to use this methodology for quantitative elemental analysis [52]. The elemental composition observed is consistent with the results of other studies that examined the chemical properties of BCs; woody tissue-derived BC consistently demonstrates a less diverse and beneficial nutrient profile relative to BC derived from other sources [53] despite their generally higher surface area. These results indicate that feedstocks influence the chemical composition of their BC derivatives, which vary further based on pyrolysis temperature and retention time [54].

Table 3. Qualitative elemental composition of different biochars using EDX spectral analysis. Boxes with Y indicate the presence of elements while boxes with a dash denote that the element was either not detected or below the detection threshold.

Biochar Feedstock	N	P	K	Ca	S	Mg	Mo	Si	Cl	Na	Al
CoolTerra® (CT)	Y	–	Y	–	–	–	–	Y	–	Y	Y
Ryegrass straw (RGS)	Y	Y	Y	Y	Y	–	–	Y	Y	–	–
Ryegrass tailings (RGT)	Y	Y	Y	Y	Y	Y	Y	Y	–	–	Y
Thermomechanical pulp waste (TMP)	Y	Y	Y	Y	–	–	–	Y	–	–	–
Russian thistle (RT)	Y	Y	Y	Y	Y	–	Y	Y	Y	–	–
Walnut (W)	Y	Y	–	Y	–	–	–	–	–	–	Y

3.2. Agronomic Traits: Plant Dry Weight and Yield Per Plant

BC had a generally positive or non-significant impact on the agronomic traits explored in this study, the yield per plant (YPP) and the plant dry weight (PDW). Of the 108 groups, PDW had significantly increased over the control in 4, and remained unchanged in 104, while YPP was elevated in 14, unchanged in 88, and decreased in 2. Effect sizes varied widely, especially in terms of YPP, where decreases of as large as 151 g/plant were seen in 'Heinz' 0.5% W Trial 2 and increases of as large as 326 g/plant in 'Cobra' 0.5% RGS Trial 2 (Figures 2 and 3). RGS and RGT produced significant increases in the greatest number of trials for these traits; while very different in average particle size and pore size (Figure 1), both are crop residue BCs with diverse elemental compositions relative to the woody tissue-derived BCs (Table 3).

Three–way ANOVA indicated that PDW has a significant response according to cultivar alone in Trial 2 and both cultivar and the rate of BC application in Trial 1, but not the BC type or interactions between any of these variables. YPP saw a similar significant response not only to the cultivar and BC rate but also to the interaction between these two variables in both trials (Table 4). Broken down by cultivar, two–way ANOVA indicated that the only significant PDW response was 'Heinz' to BC type in Trial 1 and to BC rate in Trial 2; while the response of YPP to BC rate varied depending on cultivar, the BC type did not appear to be consequential (Table 5).

Previous reports on BC's impact on agronomic traits have also demonstrated mixed results, dependent upon both the biochar type and the studied plant. It was noted that there was an increase in tomato fruit diameter and yield in grapes in BC and compost-amended soils [55]. However, a field trial with tomato cultivar 'Trust' with 10 or 20% (v/v) hardwood BC generated from balsam fir and spruce showed no difference in crop yield [56]. Augmenting fertigated soilless media with citrus wood BC resulted in an increased yield in pepper, but only improved plant height and leaf size without yield gain in tomato [57]. An enhanced abundance of rhizosphere microbes in addition to a hormesis effect that stimulated plant growth was also reported [57]. Pine needle BC and Lantana BC both improved yield in wheat, but this effect was not observed in rice [58]. Negative agronomic impacts in a variety of cereal, vegetable, and fruit crops grown in soils amended with both wood and crop residue biochar have been previously reviewed [59]. The widely divergent properties of BC depending on feedstock and pyrolysis conditions combined with the rich genetic diversity of crop plants ensures that the effects of BC application on agronomic performance remain difficult to predict.

Table 4. Sources of variation and levels of significance (*p*–values) for the 3–way ANOVA of the effects of three cultivars and six biochar soil amendments (3 rates) on plant dry weight, fruit yield per plant (YPP), fruit organic acid, and fruit sugar concentrations of tomato cultivars grown in the greenhouse from February 17 2017 to 10 November 2017 (Trial 1, Figures 1 and 2) and 15 May 2017 to 9 May 2018 (Trial 2, Figures 3 and 4).

Trial Dates	Sources of Variation	Plant Wt	YPP [1]	Citrate	Malate	Glc	Fru
17 February 2017 to 10 November 2017	Cultivar (C)	0.001	0.001	0.001	0.001	0.001	0.001
	Biochar (B)	ns [2]	ns	0.001	0.001	0.001	0.001
	Rate (R)	0.05	0.001	0.002	ns	0.04	0.003
	C × B	ns	ns	0.001	0.001	0.001	0.002
	C × R	ns	0.005	0.001	0.001	0.001	0.001
	B × R	ns	ns	0.02	0.001	ns	ns
	C × B × R	ns	ns	0.002	0.001	0.04	0.04
15 May 2017 to 9 May 2018	Cultivar (C)	0.001	0.001	0.001	0.001	0.001	0.001
	Biochar (B)	ns	ns	0.02	0.001	0.001	0.001
	Rate (R)	ns	0.03	0.03	0.002	ns	ns
	C × B	ns	ns	ns	0.001	0.03	0.001
	C × R	ns	0.008	0.001	0.005	0.003	ns
	B × R	ns	ns	0.001	0.001	0.001	0.001
	C × B × R	ns	ns	ns	0.001	0.001	0.001

[1] Fruit yield (fresh wt) per plant. [2] ns, not significant.

Table 5. Sources of variation and levels of significance (*p*–values) for the 2–way ANOVA of the effects of three rates of six biochar soil amendments on plant dry weight, fruit yield per plant (YPP), fruit organic acid, and fruit sugar concentrations of tomato cultivars grown in the greenhouse in Trial 1 (Figures 1 and 2) and Trial 2 (Trial 2, Figures 3 and 4).

Trial Dates	Cultivar	Sources of Variation	Plant Wt	YPP [1]	Citrate	Malate	Glc	Fru
17 February 2017 to 3 June 2017	OS	Biochar (B)	ns [2]	ns	0.06	0.001	0.002	0.008
		Rate	ns	0.001	0.001	0.001	ns	ns
		B × Rate	ns	ns	0.03	0.002	0.09	ns
17 February 2017 to 3 June 2017	Heinz	Biochar (B)	0.03	ns	0.001	0.001	0.001	0.001
		Rate	ns	0.02	0.001	ns	ns	0.08
		B × Rate	ns	0.09	0.007	0.004	0.03	0.03
16 May 2017 to 10 November 2017	Cobra	Biochar (B)	ns	ns	0.02	0.001	0.009	0.001
		Rate	ns	0.001	0.001	0.001	0.001	0.001
		B × Rate	ns	ns	ns	0.07	ns	ns
20 January 2018 to 7 May 2018	OS	Biochar (B)	ns	ns	0.04	0.001	0.005	0.001
		Rate	ns	ns	0.009	0.001	0.007	ns
		B × Rate	ns	ns	0.05	0.001	0.07	0.03
15 May 2017 to 30 August 2017	Heinz	Biochar (B)	ns	ns	ns	0.001	0.07	0.07
		Rate	0.02	ns	0.003	ns	ns	ns
		B × Rate	ns	ns	0.002	0.001	0.001	0.001
8 November 2017 to 9 May 2018	Cobra	Biochar (B)	ns	ns	ns	0.05	0.02	0.001
		Rate	ns	0.006	0.007	ns	0.02	0.03
		B × Rate	ns	0.08	ns	ns	0.10	0.06

[1] Fruit yield (fresh wt) per plant. [2] ns, not significant.

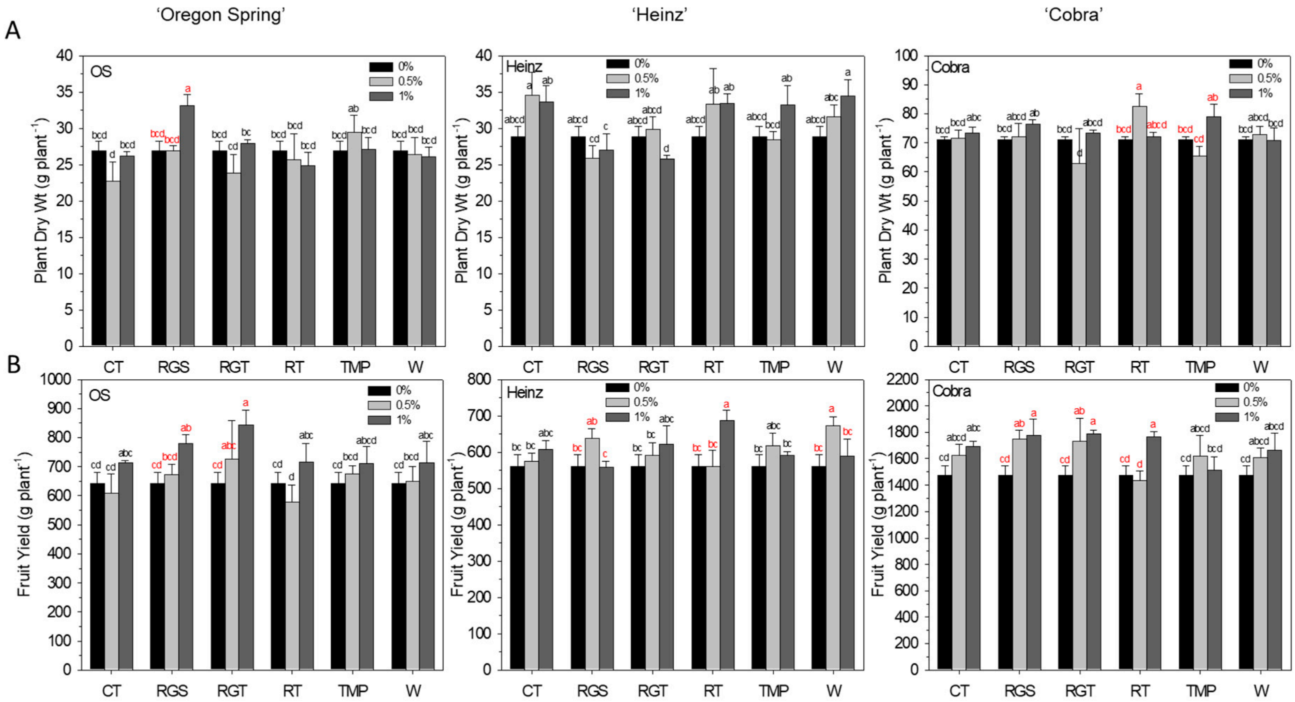

Figure 2. Trial 1—Effects of six biochar soil amendments on above-ground foliar growth (**A**) and fruit yield per plant (**B**) of greenhouse-grown tomato cultivars, Oregon Spring (OS, left), Heinz (middle), and Cobra (right). Data are means of four replicates (±SE). Letters indicate LSD $p < 0.1$ within a cultivar. Letters in red indicate significant trends (linear, deviations) with the rate of biochar. Note the differences in *Y*–axis scales. See Tables 4 and 5 for a summary of the 3–way and 2–way factorial ANOVA, respectively.

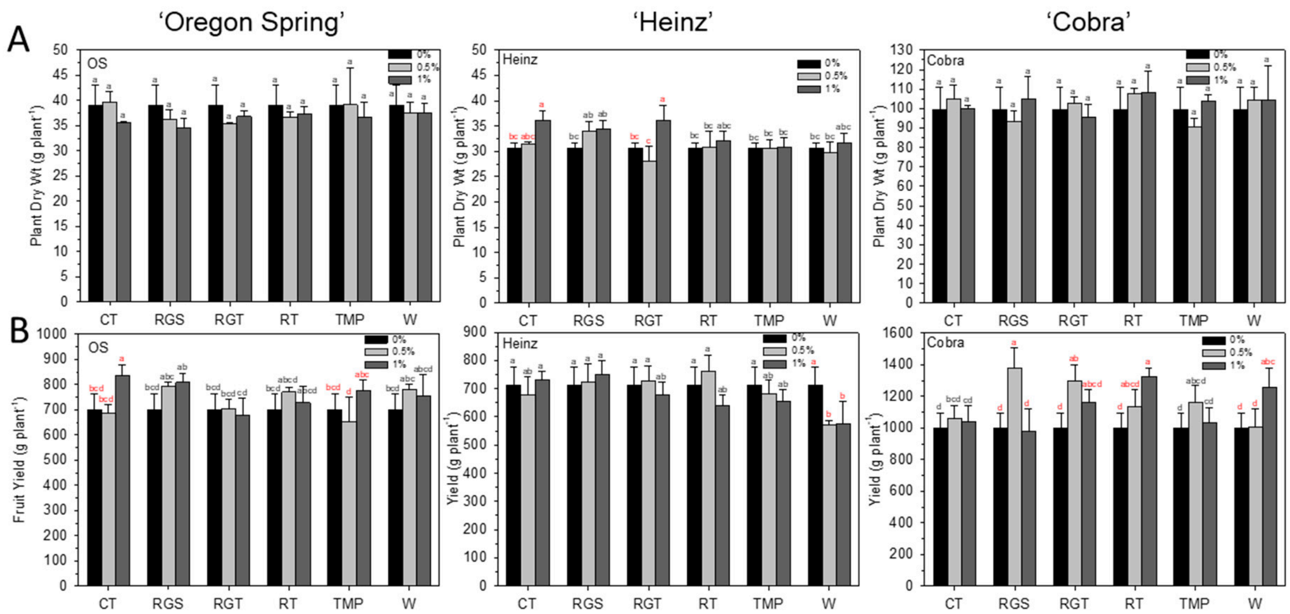

Figure 3. Trial 2—Effects of six biochar soil amendments on above-ground foliar growth (**A**) and fruit yield per plant (**B**) of greenhouse-grown tomato cultivars, Oregon Spring (OS, left), Heinz (middle), and Cobra (right). Data are means of four replicates (±SE). Letters indicate LSD $p < 0.1$ within a cultivar. Letters in red indicate significant trends (linear, deviations) with the rate of biochar. Note the differences in *Y*–axis scales. See Tables 4 and 5 for a summary of the 3–way and 2–way factorial ANOVA, respectively.

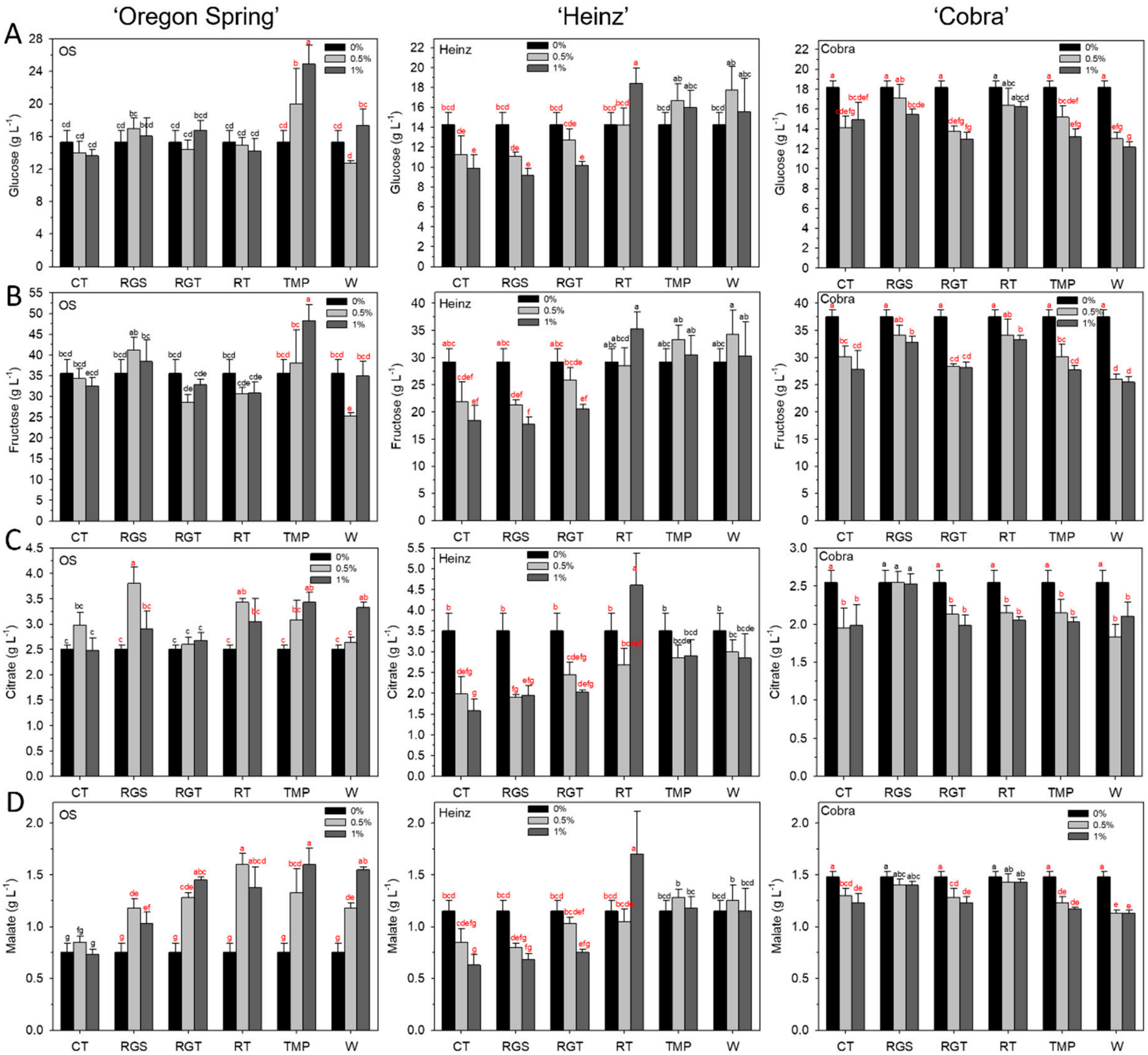

Figure 4. Trial 1—Effects of six biochar soil amendments on the concentrations of glucose (**A**), fructose (**B**), citrate (**C**), and malate (**D**) of the fruit of greenhouse-grown tomato cultivars, Oregon Spring (OS, left), Heinz (middle), and Cobra (right). Data are means of four replicates (±SE). Letters indicate LSD $p < 0.1$ within a cultivar. Letters in red indicate significant trends (linear, deviations) with the rate of biochar. Note the differences in *Y*–axis scales. See Tables 4 and 5 for a summary of the 3–way and 2–way factorial ANOVA, respectively.

3.3. Fruit Quality: Glucose, Fructose, Citrate, and Malate

In contrast with agronomic traits, fruit quality traits, as judged by representative carbohydrates and organic acids, appeared to have a greater response to BC application. A significant response to cultivar, BC type, and BC rate, and the interactions between these variables were generally observed, even when broken down by cultivar (Tables 4 and 5). Both highly positive and highly negative outcomes were observed in the trials. While the upside in some trials such as 'Oregon Spring' 1% TMP Trial 1 could be a near doubling of fruit carbohydrate and organic acid content, the downside could be as bad as a near halving of fruit carbohydrate and organic acid content as in 'Heinz' 1% CT Trial 1 (Figures 4 and 5). Additionally, the response of these traits was highly correlated; an increase or decrease in any of these compounds was generally shared with the other three (Figure 6).

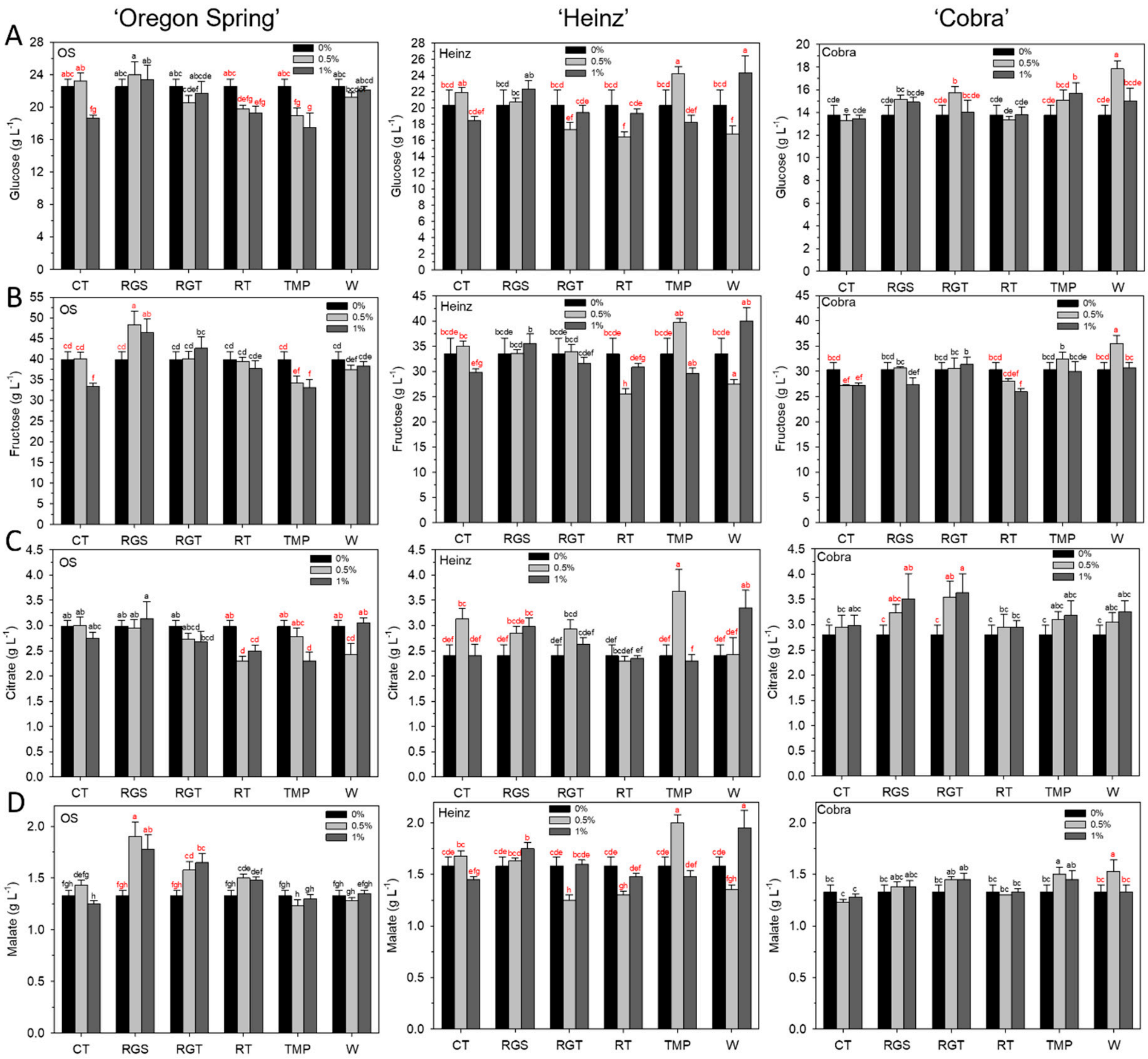

Figure 5. Trial 2—Effects of six biochar soil amendments on the concentrations of glucose (**A**), fructose (**B**), citrate (**C**), and malate (**D**) of the fruit of greenhouse-grown tomato cultivars, Oregon Spring (OS, left), Heinz (middle), and Cobra (right). Data are means of four replicates (±SE). Letters indicate LSD $p < 0.1$ within a cultivar. Letters in red indicate significant trends (linear, deviations) with the rate of biochar. Note the differences in *Y*–axis scales. See Tables 4 and 5 for a summary of the 3–way and 2–way factorial ANOVA, respectively.

While responses were varied, some general trends could be observed. Malate was generally the most responsive to BC application, while citrate was the least responsive. CT produced far more negative fruit quality outcomes than the other BC types, performing positively in only 1 trial and negatively in 16 trials, while producing some of the most precipitous drops in fruit quality. BC applications with positive outcomes were more varied between trial, BC type, rate, and cultivar. In Trial 1, the best results (or least bad, in the case of the 'Cobra' trial) were achieved with RT, along with TMP in the case of 'Oregon Spring'. In Trial 2, 'Oregon Spring' achieved the best results with RGS and RGT, 'Heinz' with TMP and W, and 'Cobra' with RGT and W. No single BC type emerged as a superior option. Despite the physical and chemical traits that make crop-residue-derived BCs appear superior to woody tissue-derived ones, this did not translate into a significant improvement in quality using crop residue-based BCs. Overall, 'Oregon Spring' experienced some of the greatest gains in fruit quality from BC application, while 'Heinz' suffered more negative effects than the other two cultivars. While there are various variables at play, under these controlled conditions the genetic variability between the cultivars is the most likely underlying reason for these observations.

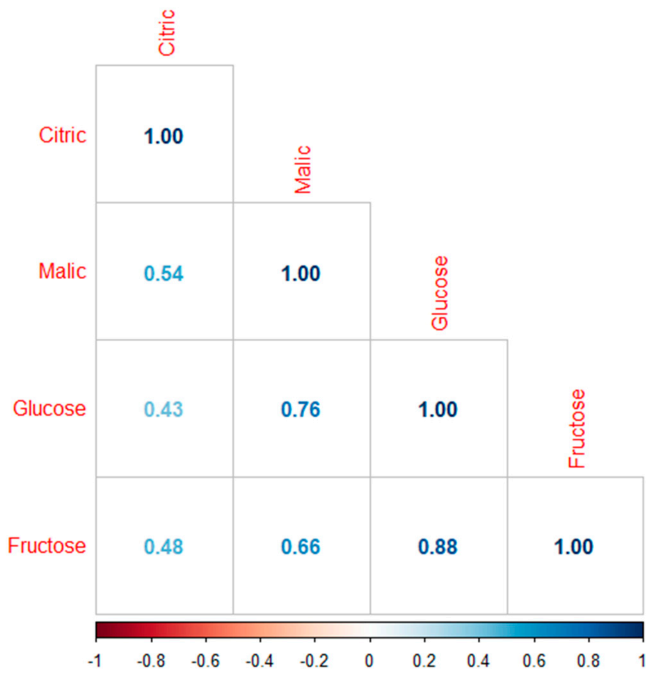

Figure 6. Correlations between tested fruit quality parameters. All significant at least $p < 0.001$.

Varied impacts on fruit quality depending on BC source and crop genetic background have been previously reported, including both increases and decreases in TSS, organic acid, and protein content, utilizing BCs as diverse as olive, bamboo, and banana in a wide array of fruit crops [60]. These previous results, further supported by this study, indicate a BC-specific effect on fruit quality that is also dependent on the genetic background of the cultivar. For example, the generally positive response to BC amendment in the 'Oregon Spring' cultivar in terms of organic acids compared to the other cultivars most likely indicates a more favorable plant–soil–genetic background interaction. These data support both hypotheses as each biochar affected fruit quality differently, and each cultivar had a unique response to each BC.

4. Conclusions

Overall, the impacts on plant dry weight and yield per plant were generally neutral or positive, while the impact on fruit carbohydrate and organic acid content was far more varied and responsive to BC application. The data presented in this study support both hypotheses: (1) BC derived from different feedstock sources will produce unique phenotypes in a single cultivar of tomato, and (2) a single feedstock-derived BC will produce different phenotypes in each of the three tomato cultivars. The use of potting soil and regular fertilization, however, limits the applicability of this study to field production; BC's positive effects on soil health and agronomic performance may be greater in poorer soils, and the vast majority of crops are not grown in rich potting soils.. Despite this limitation, the results indicate that future studies into biochar application must consider genotype, down to the cultivar level, as well as biochar source and the rate of application to make meaningful recommendations for best agricultural practices. While some cultivars grown on some BC types produced beneficial increases in yield and fruit quality, other combinations produced decidedly negative results. This further substantiates an already understood need to adopt a customized approach for BC application to enhance the yield and quality of the crop [31,39,61,62].

Future BC studies should evaluate multiple crop cultivars in conjunction with different classes of BC (e.g., manure, hardwood, or crop residue), and rates of application, to dissect the nature of the complex interactions. Additionally, the effects of BC must be examined under a wide variety of environmental conditions; for example, 'Oregon Spring' with 1% TMP application experienced highly elevated organic acids and carbohydrates in Trial 1, yet these traits were decreased in the same cultivar in Trial 2. One factor that may have contributed to the variance in the results of experiments within the same genotype is seasonality. It has been shown that in a greenhouse with supplemental lighting, seasonal variation in natural light quality, quantity, and photoperiod in northern latitudes impacts the photosynthetic performance of tomatoes [63].

While additional experimentation is required to understand the wide-ranging variability in responses, several possible variables can influence the outcomes, including feedstock, potting mix, BC characteristics, microbiome, environmental factors, and the genetic background of the plant. While responses to BC were incredibly varied between trials, and in some cases harmful, the benefit of BC was enormously positive in some trials, including YPP increases of over 30% ('Oregon Spring' Trial 1 RGS 1% and 'Cobra' Trial 2 RGS 0.5%) and a near doubling in malate, fructose, and glucose content ('Oregon Spring' Trial 1 TMP 1%). BC application can be unpredictable due to gaps in knowledge, but the potential gains are apparent. The BC types studied were produced through the pyrolysis of agricultural waste products (CoolTerra®, ryegrass, and walnut shell), industrial waste products (thermomechanical pulp), and invasive species (Russian thistle); beyond the potential to improve yield and soil health, adding economic incentive to the reuse of waste products and the control of invasive species has the potential to increase sustainability.

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Conflicts of Interest: The biochar used in this study was provided as a gift. The CoolTerra biochar was gifted to D.I. by Cool Planet Inc. while he was an undergraduate student at Heritage University, WA. All other biochars were gifted by Ag Energy Solutions, now a part of Qualterra Inc. At the time that the results of the study were being analyzed, A.D. was employed by Qualterra, Inc., which has licensed technologies from the Dhingra Research Program at Washington State University. A.D. serves as their chief scientific officer. The funders had no involvement in the collection, analysis, interpretation of data, writing, or decision to publish the results. 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.

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