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Optimizing Biochar Particle Size for Plant Growth and Mitigation of Soil Salinization

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Abstract: Pyrolyzed waste biomass, or biochar, has been suggested as a means to increase plant growth and mitigate soil salinization, which is a widespread agricultural issue and can reach extreme levels in urban soils impacted by de-icing salts. Soil mixing is enhanced by reduced biochar particle size; however, biochar properties vary with particle size, and recent studies have suggested that plant growth responses may be maximized at intermediate particle sizes. We examined the responses of two plant species (cowpea (*Vigna unguiculata*) and velvetleaf (*Abutilon theophrasti*)) to biochar amendments that spanned a wide range of particle sizes obtained by sieving, with and without de-icing salt additions. The smallest size fractions of biochar reduced plant growth relative to unamended controls. Plant biomass production was generally maximized at intermediate biochar particle size treatments, with particle sizes of 0.5–2.0 mm showing the best response. Mitigation of salt effects was also improved at intermediate biochar particle sizes in this particle size range. Our results emphasize the importance of optimizing biochar particle size to best enhance plant responses to biochar, with particular reference to saline soils.

Keywords: charcoal; plant stress; pyrogenic carbon; road salt; salinity stress; urban soils

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Citation: Tang, E.; Liao, W.; Thomas, S.C. Optimizing Biochar Particle Size for Plant Growth and Mitigation of Soil Salinization. *Agronomy* **2023**, *13*, 1394. https://doi.org/10.3390/agronomy13051394

Academic Editor: Qingling Fu

Received: 13 April 2023
Revised: 2 May 2023
Accepted: 12 May 2023
Published: 18 May 2023

1. Introduction

Soil salinization is a global economic and environmental issue with large impacts on agricultural productivity and high costs of remediation [1,2]. An estimated ~1.26 billion hectares are impacted by salinization globally [3]. High soil salinity can be induced by human activities, often through poor fertilization and water management practices [4]. However, saline soils also occur naturally, especially in arid and semi-arid regions where precipitation is low [5]. In an urban context, the use of de-icing salts, mainly sodium chloride (NaCl), in the winter months is a main cause of soil salinization [6,7]. The runoff and infiltration of de-icing salts increase soil electrical conductivity and chloride concentration in the surrounding environment [8,9], often resulting in contamination of surface and groundwater [10,11]. Additional adverse environmental impacts of de-icing salts include inhibition of soil nutrient cycling [12], alteration of aquatic and plant communities [13–15], and toxicological effects on birds [16].

Both Na^+ and Cl^- are toxic at high concentrations and stunt plant growth by displacing nutrients in the soil and reducing plant water availability through osmotic effects [5]. Urban street trees are often susceptible to road salt pollution due to their proximity to roads and high traffic areas, though some species show relatively high salt tolerance [17–19]. Plants affected by soil salinization tend to have lower leaf chlorophyll concentrations because excessive chloride is associated with chlorophyll degradation [20]. The negative impacts of de-icing salts are clear; however, their use remains widespread due to their effectiveness and low cost. Alternative de-icing agents are often costly and may themselves have adverse environmental impacts [21]. Research on practices to reduce road salt impacts on urban ecosystems remains scarce.

Biochar is an organic soil amendment product made from carbon-rich organic waste materials by pyrolysis [22]. Although much biochar research has focused on agricultural applications [23,24] and carbon sequestration [25,26], biochar has also been applied to forest restoration [27], wastewater treatment [28], and pollutant removal [29,30]. Biochar can potentially remediate salt-affected soils through salt sorption [31,32]. Specifically, biochar sorption of Na^+ in the soil solution can both reduce plant Na uptake and increase the relative uptake of Ca^{2+} and Mg^{2+} [33,34]. More broadly, biochar can generally enhance plant growth by improving soil properties, such as cation-exchange capacity (CEC), water retention capacity, and bulk density [23]. By increasing the soil CEC and water-holding capacity (WHC), biochar can reduce fertilizer and water use [35,36], which is particularly beneficial in the context of urban ecosystems.

The quality and performance of biochars depend on their chemical and physical properties, which are affected by the feedstock materials, pyrolysis method and conditions, and post-processing treatments [37,38]. Physical manipulation of biochar by post-processing treatments, such as sieving and grinding to alter the particle size and shape, can substantially change biochar porosity characteristics, WHC, bulk density, and pH [39]. For example, sieved biochar has a higher aspect ratio than ground biochar, which can increase WHC by generating increased inter-pore space [40]. Heat treatment and aeration increase surface area and reduce non-water-soluble volatile organic compounds and toxins in biochar [38,41]. In general, the properties of biochar can be manipulated to target specific applications.

Previous studies by Thomas et al. [31] and Akhtar et al. [33,34] have highlighted the importance of the ion sorption capacity of biochar in enhancing plant growth under saline soil conditions. Smaller biochar particles might be expected to better enhance biochar sorption capacity—and thus plant growth under salt stress—because smaller particles have a higher WHC [40] and improved soil-biochar contact [42]. However, recent studies suggest that there may generally be an optimum biochar particle size for enhancing plant growth responses. Large particles clearly reduce particle mixing and accessible surface area for sorption; however, very small particles may reduce soil WHC and hydraulic conductivity by filling soil inter-pores [43,44]. Small biochar particles can also show increased ash content and pH [39].

Although very few studies have examined plant growth responses across a wide range of biochar particle sizes, a recent meta-analysis presents evidence for an optimal biochar particle size of 0.5–1.0 mm [38]. Prior studies on biochar particle size effects have focused on agricultural soils; in urban ecosystems, compost-amended soils are common, and are likely to show distinct responses to both salt exposure and biochar amendments.

The present study examines soil and plant responses to a wide range of biochar particle sizes with and without additions of road salt. A greenhouse experiment was conducted over a 10-week growth period using a representative compost-amended topsoil substrate. We tested the following hypotheses: (1) biochar amendments will, in general, enhance plant growth and mitigate the negative effects of salt additions; (2) an optimum biochar particle size will exist, with biochar particle size fractions of 0.5–1.0 mm best enhancing plant growth and plant tolerance of saline soil.

2. Materials and Methods

2.1. Experimental Design and Growth Conditions

A greenhouse experiment was conducted at the University of Toronto for 67 days between 19 March and 25 May 2021. The average daily temperature was 20.1 °C with the highest at 25.0 °C and the lowest at 13.4 °C. The experiment included two treatment factors: biochar particle size and salt addition. The growth container for each plant had a volume of 0.5 L, a 10 cm depth, and a surface area of ~78 cm^2. Biochar and salt treatment dosages were calculated based on the container's surface area. A fiberglass mesh liner was added to each container to prevent soil and biochar loss. A total of 196 containers were used: 2 plant species × 6 biochar particle sizes; and a control group × 2 salinity levels × 7 replicates per treatment. Replicates were grouped using a randomized complete block designed to minimize spatial effects.

Two fast-growing plant species, *Vigna unguiculata* (L.) Walp. (cowpea), and *Abutilon theophrasti* Medik. (velvetleaf) were used in the experiment; seeds were sourced from Sprout Master and V&S Seed Supply, respectively. Cowpea is a nitrogen-fixing and salt-tolerant species suitable for hot and dry environments [45]. Velvetleaf is considered a weed in North America but is also grown as a crop plant for its edible leaves, seeds, and bast fibers [46].

Seeds were germinated in vermiculite for 17 days before being transplanted into individual containers. The growing period for cowpea was 50 days (from 19 March to 8 May 2021) and 67 days (from 19 March to 25 May 2021) for velvetleaf. Plants were watered every 3–4 days to field capacity. Both species were supplemented with ~1.5385 kg/ha (0.12 g per container) of 16-10-10 NPK slow-release fertilizer (Nutricote 16-10-10 NPK, from JCAM AGRI, Tokyo, Japan). Rhizocell C (LalRise Vita, from Lallemand Inc., Blagnac, France), a mixture of live *Bacillus velezensis* and inert *Saccharomyces cerevisiae*, was also added as a biofertilizer to velvetleaf using an application rate of 50 mL per container. Five arthropod species, *Aphidoletes aphidimyza*, *Rhopalosiphum padi*, *Neoseiulus fallacis*, *Encarsia Formosa*, and *Stratiolaelaps scimitus*, were released in the greenhouse as biocontrols to prevent pests and fungal disease.

A de-icing road salt (97% NaCl, from Sifto Safe Step, Overland Park, KS, USA) was added to the soil surface of half the pots after the seedlings were established on day 18 after the transplant on 6 April 2021. The salt dosage was 3 kg/ha (0.234 g per container) to mimic common roadside conditions [31].

2.2. Soil and Biochar Characterization

The soil used was premium topsoil from LessMess Soil (Concord, ON, Canada), a typical topsoil used in an urban setting, with mineral soil components derived from calcareous subsoil material. Soil analysis was completed by the Agriculture and Food Laboratory at the University of Guelph. Basic soil properties were as follows: total C: 26.4%; total N: 1.06% (C and N by Dumas combustion); extractable P: 130 mg/L (sodium-bicarbonate extraction); extractable Mg: 480 mg/L; extractable K: 2500 mg/L (Mg and K extractions by ammonium acetate); pH: 7.5 (saturated paste in deionized water).

The biochar used was produced by Burt's Greenhouses (Odessa, ON, Canada) from waste mixed-wood shipping pallets in a BlueFlame boiler using pyrolysis mode at 700 °C for 30 min [47,48]. The total carbon of the material was 64.5% (by Dumas combustion analysis, Activation Laboratories Ltd., Ancaster, ON, Canada). Detailed analyses of the properties of the bulk biochar have been published elsewhere [47,48]. Biochar was sieved in a mechanical sieve shaker into six particle fractions (<0.063 mm, 0.063–0.50 mm, 0.50–1.00 mm, 1.00–2.00 mm, 2.00–2.80 mm, and ≥2.8 mm) using U.S. Standard sieves. Representative biochar samples from each size fraction were mounted and sputter coated with gold-palladium. Images were taken using a scanning electron microscope (SU3500, Hitachi, Tokyo, Japan) operated at an accelerating voltage of 5.0 kV. The biochar application dosage used was 10 t/ha (7.8 g per container), comparable to dosages used in the context of restoration [49,50].

The moisture content of each size fraction was calculated using the change in dried and pre-dried masses; these values were used to adjust biochar dosages on a dry mass basis. Biochar was dried in the convection oven at 105 °C for 24 h before measurement according to the protocol in ASTM D1762-84 [51].

After harvest, pH and electrical conductivity (EC) of biochar and soil mixture from each container were measured, and the upper 2 cm of soil were collected. A pH/mV/Temp system from IQ Scientific Instruments and a conductivity meter from Hanna Instruments Inc. were used. A 1:5 (*v*/*v*) mixture of soil and deionized water was shaken on an oscillating table at 60 rpm for 24 h before measurement [40]. Similar methods were used to determine the pH and EC of biochar size fractions, but using a 1:20 (*v*/*v*) mixture of biochar and deionized water. Bulk density of biochar size fractions was determined using a graduated cylinder and analytical balance, with tap density and compression (Hausner) ratio based on manual tapping of the cylinder to achieve an equilibrium volume.

2.3. Plant Performance Measurements

Plant mortality was recorded bi-weekly, and plants dying immediately after transplant were replaced (43% of the cowpea and 37% of the velvetleaf were dead and replanted within the first 10 days). Plant height and leaf length were measured to the nearest cm on day 14 (before the fertilizer and salt treatment) and before the final harvest. Leaf area (A) in cm^2 was estimated using the leaf length (L) in cm. The allometric equation used for velvetleaf was based on a previous study: $A = 0.613 \times L^{2.204}$ [40]. An allometric equation for cowpea was developed based on the scanned leaf area and leaf length from 71 harvested leaves: $A = 0.539 \times L^{1.8729}$ (Adjusted $r^2 = 0.838$). The final total leaf area of each plant was measured using a leaf area meter (Li-3100C from Li-Cor Biosciences, Lincoln, NE, USA).

Chlorophyll fluorescence and chlorophyll content of cowpea were measured before the final harvest using a chlorophyll fluorometer (MINI-PAM, Walz GmbH) and a chlorophyll meter (CCM-200 plus, Opti-Sciences Inc., Hudson, NH, USA), respectively. Light-saturated photosynthetic rate (A_{max}), stomatal conductance (g_s) and instantaneous leaf water use efficiency (WUE_i) were measured prior to harvest on the most recently developed fully expanded leaf of each surviving cowpea using a portable photosynthesis system (LI-6400xt, Li-Cor Biosciences, Lincoln, NE, USA). Measurements were made between 7:00–14:00 local time at a light level of 1500 $\mu mol\ m^{-2}\ s^{-1}$ PPFD, a leaf temperature of 20–22 °C, and a humidity of 50–60%. Leaf physiology measurements were made on cowpea only due to leaf area constraints. At harvest, the above- and below-ground biomass of plants were separated at soil level. Stems and leaves were separated. Roots were removed from the soil and washed manually. All collected biomass was weighed after being dried in a forced-air oven at 60 °C for 48 h.

2.4. Statistical Analysis

Statistical analyses were conducted using the R programming environment (R version 4.0.2, R Core Team 2021). Analysis of variance (ANOVA) and correlation tests were used in analyses of the physical properties of biochar size fractions. For the greenhouse experiment, initial analyses including block as a random factor in a linear mixed model were run, but the block effect was not significant; therefore, a simple two-way ANOVA was used to examine the effects of biochar particle size and salt on soil parameters and plant performance. Assumptions of normality of variances and homoscedasticity of residuals were confirmed graphically.

As a supplementary test for biochar particle size effects per se, we conducted separate analyses excluding the control (no biochar) treatment. We used the Scott–Knott post hoc test clustering algorithm [52] to group means by biochar particle size, making use of the SK() function in the ScottKnott R package [53]. Pairwise tests for salt effects within a biochar particle used t-tests with *p*-values adjusted for multiple comparisons using a false discovery rate correction. Potential relationships between plant biomass and soil pH and EC were examined using linear regression and linear models that included treatments plus soil pH and EC as covariates.

In addition to species-specific analyses, we utilized meta-regression techniques to quantify general, species-pooled patterns of response. The response ratio statistic ($R = \ln(X_t/Xc)$ was used to quantify the effect size, where R is the response ratio statistic, X_t is the treatment mean, and X_c is the control mean. Pooled R values were inversely weighted by sampling variance. Response ratios were quantified for both biomass responses to biochar additions (relative to the unamended controls) and biomass responses to salt additions (relative to the biochar-amended controls without salt additions). In both cases, we quantified response patterns using a 2nd-order polynomial meta-regression function and considered a negative 2nd-order term and a positive 1st-order term consistent with an optimum curve response within the range of biochar particle sizes tested. Meta-regression analyses treated particle size classes as a ranked ordinal variable, and these were conducted using the escalc() and rma() functions in the metafor R package [54].

3. Results

3.1. Biochar Properties

The physiochemical properties of biochar fractions varied with particle size (Table 1). Biochar pH ranged from 9.0 to 9.5 and did not vary significantly with particle size (ANOVA $p > 0.05$). EC values did vary significantly (ANOVA $p < 0.001$): the smaller size fractions had a higher EC than the soil, while the larger fractions had a lower EC than the soil (>1 mS/cm). Both bulk density and tap density varied among particle size classes (ANOVA $p < 0.001$) and were negatively correlated with particle size (r = −0.881 and r = −0.860; $p < 0.001$: analysis based on mid-points of size ranges). The compression (Hausner) ratio also varied with particle size (ANOVA $p = 0.018$), being largest for the 0.063–0.50 mm size category and smallest for the 1–2 mm size category (Table 1). SEM images indicated a collapse of wood cell structure in biochar particles at the smallest size fractions (Figure 1), suggesting reduced macroporosity.

Table 1. Selected physicochemical properties of biochar by particle size fraction and of the soil used. Standard errors are given in brackets (for triplicate measurements where available).

	Biochar Size (mm)						
Size Category:	**0**	**1**	**2**	**3**	**4**	**5**	
Attribute	**<0.063**	**0.063–0.50**	**0.50–1.00**	**1.00–2.00**	**2.00–2.80**	**>2.8**	**Soil**
pH	9.1 (0.04)	9.0 (0.03)	9.0 (0.01)	9.0 (0.02)	9.2 (0.02)	9.5 (0.05)	7.5
EC (mS/cm)	1.70 (0.01)	1.47 (0.03)	1.21 (0.02)	0.83 (0.00)	0.96 (0.06)	0.89 (0.04)	1.00 (0.04)
Bulk density (g/cm^3)	0.31 (0.01)	0.25 (0.01)	0.17 (0.01)	0.14 (0.00)	0.11 (0.00)	0.12 (0.00)	0.44 (0.01)
Tap density (g/cm^3)	0.38 (0.01)	0.33 (0.01)	0.20 (0.00)	0.15 (0.01)	0.13 (0.00)	0.14 (0.01)	0.49 (0.03)
Compression ratio	1.23 (0.08)	1.33 (0.04)	1.17 (0.03)	1.07 (0.01)	1.23 (0.04)	1.23 (0.01)	1.13 (0.04)

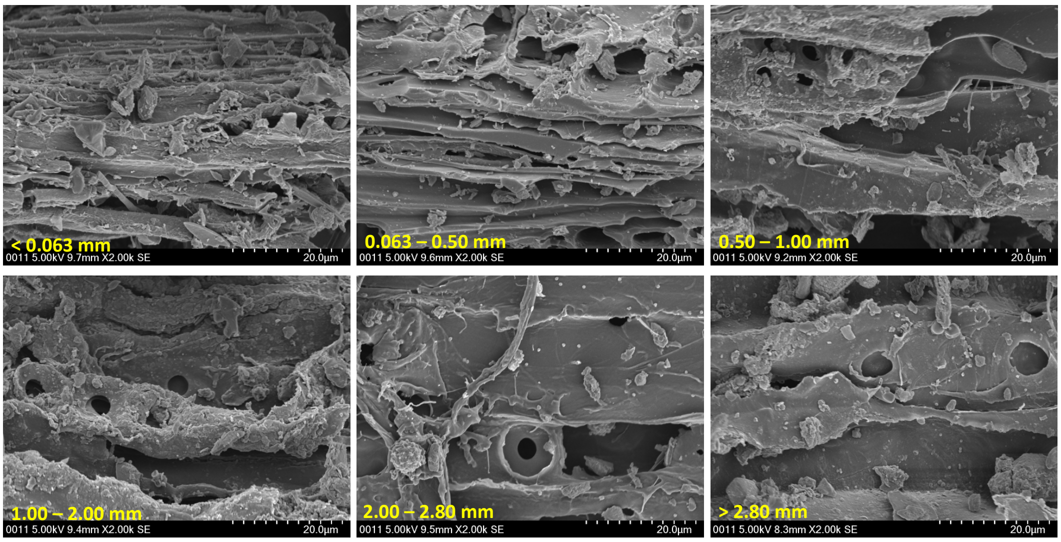

Figure 1. Representative SEM images of biochar by particle size fraction.

3.2. Soil Properties

Biochar particle size effects on soil pH were statistically significant ($p < 0.001$ for cowpea, $p = 0.043$ for velvetleaf; Table 2); however, pH values remained in a narrow range near optimum pH levels (7.2–7.4). Salt additions did not significantly affect pH (Table 2). Both salt and biochar particle size effects significantly affected soil EC in cowpea (Table 2); the smallest particle size (<0.063 mm) showed the most pronounced increase in EC relative to the control (Figure 2A). Although there were no biochar particle size effects for velvetleaf, salt additions significantly increased EC (Figure 2B; Table 2).

Table 2. ANOVA results for a greenhouse experiment examining the effects of biochar particle size and salt additions on soil properties and plant performance. The numerator degrees of freedom are 6 for biochar effects, 1 for salt effects, and 6 for the biochar × salt interaction, with 83 degrees of freedom for the denominator. Values for $p < 0.05$ are given in bold. The significance of the biochar size effect in an ANOVA omitting the control (no biochar) treatment is also indicated: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Attribute	Biochar Size F	Biochar Size *p*	Salt F	Salt *p*	Size × Salt F	Size × Salt *p*	Scott–Knott Clusters † (for Biochar Size)
Cowpea							
Soil pH	5.63	**<0.001** ***	0.09	0.760	1.09	0.370	(c,1,3) (0,2,4,5)
Soil EC (µS/cm)	2.84	**0.015**	7.06	**0.009**	1.69	0.134	(c,1,2) (0,3,4,5)
Early leaf area (cm^2)	3.11	**0.004** **	0.22	0.612	2.83	**0.015**	(c,0,1,2) (3,4,5)
Total biomass (g)	3.03	**0.010** **	0.02	0.871	1.84	0.101	(0,1) (c,2–5)
Aboveground biomass (g)	3.07	**0.009** **	0.01	0.918	1.49	0.191	(0,1) (c,2–5)
Belowground biomass (g)	2.62	**0.022** *	0.52	0.471	2.56	**0.025**	(0,1,5) (c,2–4)
Root fraction	2.47	**0.030** *	0.93	0.337	1.22	0.306	(5) (c,0–4)
Final leaf area (cm^2)	1.46	0.204	0.00	0.978	0.87	0.522	-
LMA (g/cm^2)	1.32	0.255	1.03	0.313	1.14	0.348	-
CCI	1.67	0.140	0.46	0.499	1.46	0.201	-
Fv/Fm	2.52	**0.027**	7.49	**0.008**	1.25	0.289	(c,1) (0,2–5)
A_{max} ($\mu mol\ m^{-2}\ s^{-1}$)	0.29	0.939	0.59	0.445	0.235	0.964	-
g_s ($mmol\ m^{-2}\ s^{-1}$)	0.55	0.772	0.19	0.667	0.56	0.758	-
WUE_i	2.87	**0.015**	1.72	0.194	0.47	0.827	(c,0,2,3) (1,4,5)
Velvetleaf							
Soil pH	2.61	**0.027** *	1.64	0.203	1.11	0.364	(c,1,4,5) (0,2,3)
Soil EC (µS/cm)	1.28	0.275	14.95	**<0.001**	0.59	0.740	-
Early leaf area (cm^2)	1.04	0.408	0.29	0.593	0.67	0.672	-
Total biomass (g)	3.89	**0.002** **	0.29	0.591	0.60	0.727	(c,0,1,4,5) (2,3)
Aboveground biomass (g)	3.97	**0.002** **	0.26	0.610	0.60	0.730	(c,0,1,4,5) (2,3)
Belowground biomass (g)	3.44	**0.004** **	0.36	0.548	0.62	0.714	(c,0,1,4,5) (2,3)
Root fraction	3.81	**0.002** **	0.745	0.391	0.67	0.676	(1–5) (c,0)
Final leaf area (cm^2)	3.15	**0.008** **	2.19	0.143	0.73	0.627	(c,0,1,4,5) (2,3)
LMA (g/cm^2)	1.83	0.103	1.57	0.214	1.59	0.161	-

† Clusters among biochar particle size treatments as determined by the Scott–Knott algorithm for post hoc tests at $p < 0.05$, listed in ascending order (c: control; 0: <0.063 mm; 1: 0.063–0.5 mm; 2: 0.5–1 mm; 3: 1–2 mm; 4: 2–2.8 mm; 5: >2.8 mm).

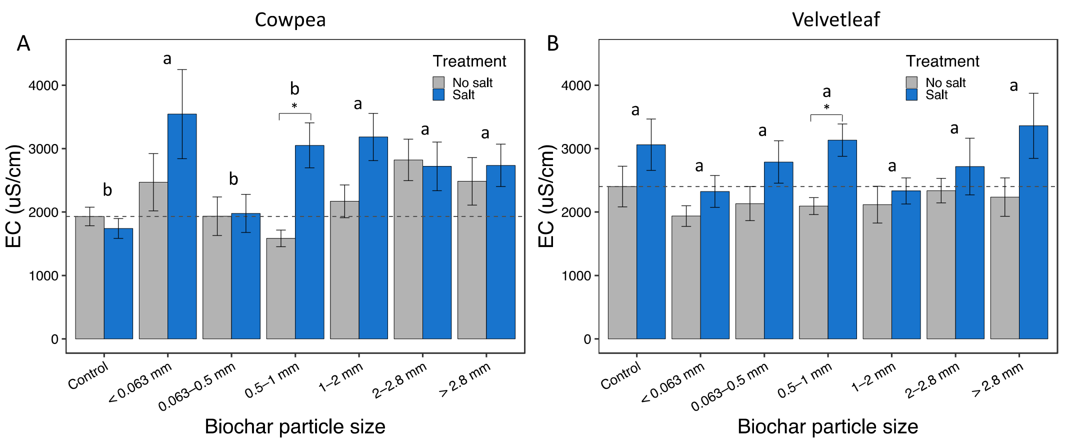

Figure 2. Biochar particle size and salt addition effects on soil EC in cowpea (**A**) and velvetleaf (**B**). Means are plotted ±1 SE. The dashed line in each panel indicates the control with no salt. ANOVA results indicate significant salt effects in both cases and a significant biochar particle size effect in the case of cowpea (Table 2). Letters indicate differences among biochar particle size treatments ($p < 0.05$) according to the Scott–Knott post hoc test clustering algorithm. Asterisks indicate the significance of salt treatments within a biochar particle size class: *, $p < 0.05$.

3.3. Plant Growth Responses

Mortality was low in the experiment, with 99% of the cowpea and 96% of the velvetleaf surviving to harvest. In early growth responses (based on non-destructive estimates of leaf area), cowpea showed a positive growth response to medium to large particle size (>1.0 mm) biochar, but no response to smaller particle size (Table 2). Biomass responses showed significant responses to biochar particle sizes for both species. Cowpea growth was highest for particle sizes > 0.5 mm (Figure 3A; Table 2), while velvetleaf showed a clearer peak in response at intermediate particle sizes (0.5–2.0 mm) (Figure 3B; Table 2). Similar trends were found for various measures of plant size, including leaf area, and aboveground, belowground, and total biomass (Table 2).

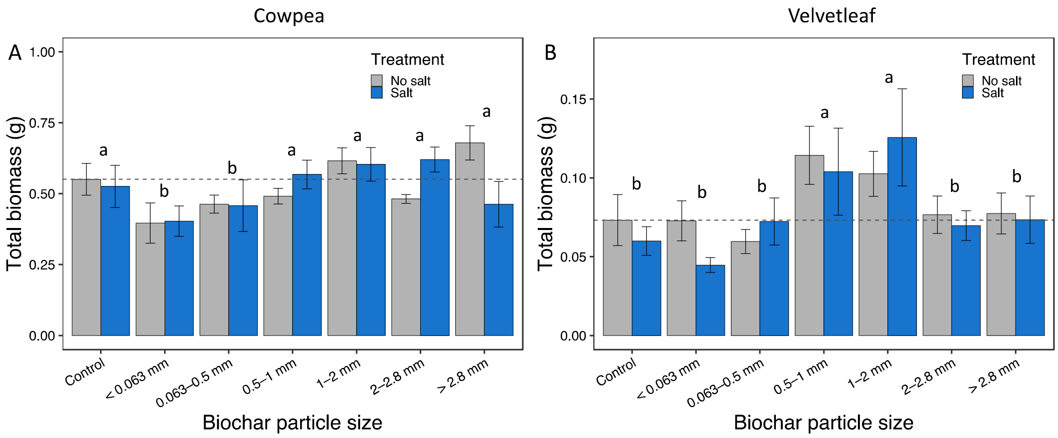

Figure 3. Biochar particle size and salt addition effects on biomass production at harvest (at 67 days) in cowpea (**A**) and velvetleaf (**B**). Means are plotted ±1 SE. ANOVA results indicate significant biochar particle size effects in both cases (Table 2). The dashed line in each panel indicates the control with no salt. Letters indicate differences among biochar particle size treatments ($p < 0.05$) according to the Scott–Knott post hoc test clustering algorithm.

For velvetleaf, biomass was enhanced relative to controls for intermediate biochar particle sizes (0.5–1 and 1–2 mm) even for the salt addition treatments (Figure 3B: post hoc comparisons signify ant at $p < 0.5$ in both cases). Leaf area at harvest showed a similar response to biomass for velvetleaf, but no significant response to treatments in cowpea (Table 2). Root fraction showed a significant response to biochar particle size in both species (Table 2), with root fraction increasing for larger biochar particle sizes. Leaf mass per area did not respond to treatments (Table 2). No visible root nodules were present on cowpea. Regressions between plant biomass and soil pH and EC were not significant for either species, and in neither case were these properties significant when included as covariates in linear models.

Meta-analysis was used to conduct species-pooled analyses for the experiment, with results supporting peak performance at intermediate biochar particle sizes (Figure 4). The pooled response ratio for the biomass response to biochar addition was negative for the smallest biochar size category (<0.063 mm), and positive for the 1–2 mm size category (Figure 4A). The overall test for the effects of moderators was significant ($p = 0.008$). The first-order term for the polynomial meta-regression was significant ($p = 0.014$), and the second-order term was marginally significant ($p = 0.067$), with the peak falling between the 0.5–1.0 mm and 1–2 mm size categories (Figure 4A).

The pooled response ratio for the biomass response to salt addition did not deviate from zero for any biochar size category (Figure 4B), and the test for effects of moderators was not significant ($p = 0.135$). However, both the first- and second-order meta-regression terms were significant in this case ($p = 0.038$ and $p = 0.041$, respectively), with the peak falling between the 0.063–0.5 mm and 0.5–1.0 mm size categories (Figure 4B).

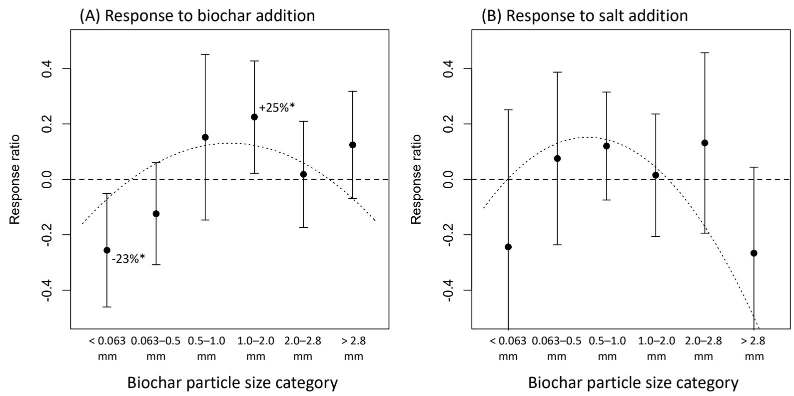

Figure 4. Meta-analyses (combining results for both species) of total plant biomass responses to biochar (**A**) and plant biomass responses to salt addition (**B**) in relation to biochar particle size. Response ratio metrics are plotted ±95% confidence limits; polynomial response curves are shown fitted using meta-regression analysis with biochar particle size categories treated as nominally ranked categories. The percent changes indicated are back-transformed from the log response ratio statistic. * Indicates significant differences.

3.4. Physiological Responses

Treatment effects on chlorophyll content index (CCI) values were not detected (Figure 5A; Table 2). Both biochar particle size and salt treatments significantly affect chlorophyll fluorescence (Fv/Fm) (p = 0.027 and p = 0.008, respectively), with positive effects of most biochar treatments and negative effects of salt addition (Figure 5B; Table 2). Increases in Fv/Fm relative to controls were particularly pronounced for biochar particles > 0.5 mm (Figure 5B).

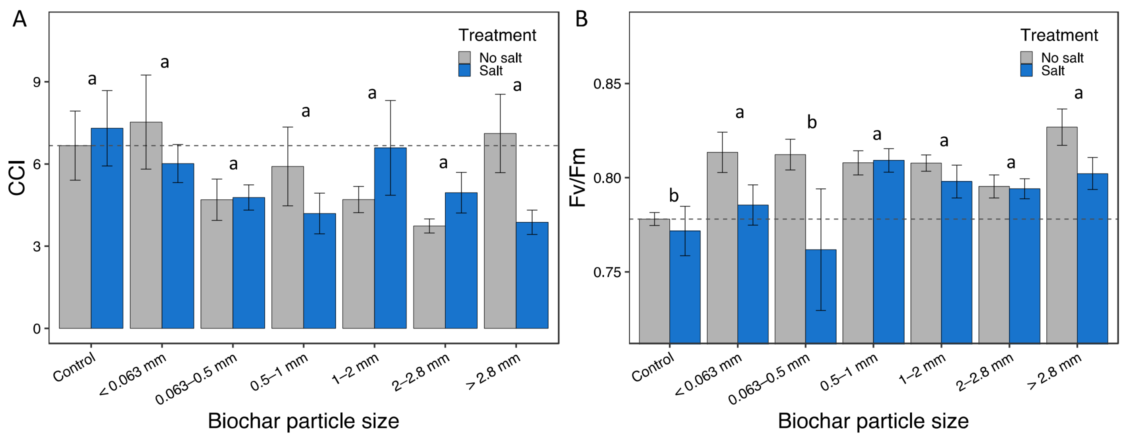

Figure 5. Biochar particle size and salt addition effects on leaf chlorophyll concentration index (CCI) (**A**), and chlorophyll fluorescence (Fv/Fm) (**B**) in cowpea evaluated prior to harvest. Means are plotted ±1 SE. ANOVA results indicate significant biochar particle size and salt effects for Fv/Fm but not CCI (Table 2). The dashed line in each panel indicates the control with no salt. Letters indicate differences among biochar particle size treatments (p < 0.05) according to the Scott–Knott post hoc test clustering algorithm.

No significant biochar particle size or salt effects were observed on light-saturated photosynthesis (A_{max}) or stomatal conductance ($g_{s;}$) (Table 2); however, leaf-level instantaneous water-use efficiency (WUE_i) was significantly reduced relative to controls at intermediate biochar particle size (0.5–2 mm) (Table 2; Figure 6).

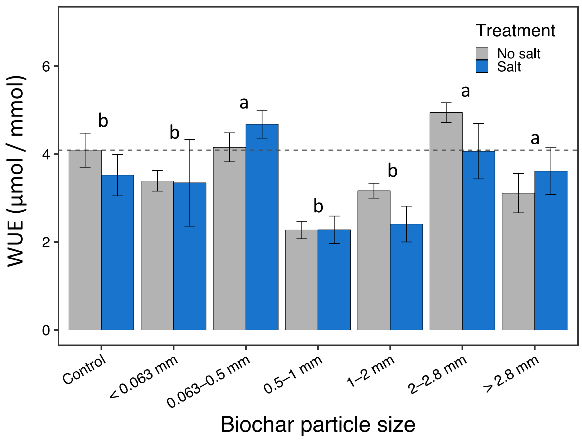

Figure 6. Biochar particle size and salt addition effects on instantaneous water-use efficiency (WUE_i) of cowpea measured using photosynthetic gas exchange. The dashed line in each panel indicates the control with no salt. Letters indicate differences among biochar particle size treatments ($p < 0.05$) according to the Scott–Knott post hoc test clustering algorithm.

4. Discussion

Our results support the hypothesis that an optimal biochar particle size for enhancing plant growth and stress tolerance exists. Intermediate biochar particle sizes (0.5–2.0 mm) enhanced plant growth and mitigated salt effects better than either smaller or larger biochar particles. At the same application dosage (10 t/ha), the smallest biochar size (<0.063 mm) generally suppressed plant growth, while the intermediate particle size categories (0.5–2.0 mm) generally enhanced growth relative to controls, even in the case of saline soil conditions.

Biochar particle size "benefits" have been quantified in a variety of ways, including soil biota responses [55,56], metal toxicity mitigation [57,58], and in terms of soil hydraulic properties, such as water retention capacity [40,59] and permanent wilting point [60]. Prior results on particle size effects on plant growth have been mixed in individual studies. Stem growth of *Salix viminalis* on a contaminated technosol was greatest at a biochar size of 0.2–0.4 mm [61], while lentil (*Lens culinaris*) had a reduced biomass improvement or even a decrease with particle sizes of <2 mm compared to 5–10 mm in a silt loam agricultural soil [62]. *Brassica chinensis* showed no biochar particle size effects among three size categories (<0.5 mm, 0.5–2 mm and 2–5 mm) on a contaminated yellow ferralsol [57], and *Hordeum vulgare* responded well to both sizes tested (<0.15 mm or >0.15 mm) in a commercial garden soil [42]. There is also evidence that plant growth responses to biochar particle size can vary among plant species [40]. However, a key limitation of these prior studies is that not more than three biochar particle size categories have been included, making detection of any optimum point unlikely.

Our results suggesting a clear optimum biochar size are consistent with recent meta-analyses. Thomas [38] pooled data from 23 studies (involving 112 comparisons) and concluded that a biochar particle size of 0.5–1.0 mm generally resulted in an optimal plant growth response regardless of soil type or texture. Edeh et al. [63] suggest a size < 2 mm is best for sandy soil because this size category best improves soil hydrological properties. Albert et al. [58] found that a biochar size of 0.9–2 mm better reduced Pb and Cd concentrations in plants compared to a size of 2–5 mm. In all cases, results are consistent in supporting an optimum biochar particle size in the range of 0.5–2 mm.

Prior work also indicates that different biochar particle sizes have distinctive physiochemical properties [39,64]. Soil bulk density increases as biochar particle size decreases in sandy, silt loam, and clay soils; small particle sizes (<0.5 mm) also generally show higher volumetric plant-available water content [65]. However, very fine biochar (<0.063 mm) is commonly hydrophobic and alters soil structure by increasing micropore and reducing macropore volumes [66]. Biochar also interacts with soil and modifies the soil pore structure, with both intrapores and interpores influencing soil hydraulic properties [66]. In the present study, scanning electron microscopy images show that the smallest size category (<0.063 mm) had a visually disrupted macropore structure (Figure 1). Only this size fraction showed visual pooling of surface water during the experiment (personal observations), consistent with low hydraulic conductivity.

NaCl is highly soluble in water, such that leaching can reduce soil salt concentrations [5]. Biochar can thus alter Na^+ and Cl^- concentrations by soil hydraulic conductivity effects as well as sorption, and drainage can be critical in mitigating salt effects. The soil EC for cowpea with biochar size < 0.063 mm was substantially (~200%) higher than the control (Figure 2A). A likely mechanism for this difference is that fine biochar physically fills the pore space between soil particles and decreases porosity [44], acting together with the water-repelling properties to hinder water movement and reduce ion leaching. However, elevated EC at small biochar particle sizes was not observed in the case of velvetleaf (Figure 2B). Prior studies have observed that biochar can increase EC at high application rates (e.g., [31,67–69]).

The inconsistent effects on EC observed here may thus be due to a balance of ion sorption and ion leaching by biochars. In spite of this variability, there is a clear pattern of intermediate biochar particle sizes (in the 0.5–2.0 mm range) acting to better mitigate salt effects on plant growth (Figure 4B).

Many urban soils are alkaline, and further increases in pH induced by biochar could be problematic. Here, we found only slight but detectable effects of biochar addition on soil pH in the context of a typical urban topsoil mix, with pH remaining within an optimal range (7.2–7.4). This result is consistent with the few prior studies examining biochar effects on the pH of neutral to alkaline urban soils, which also report only slight liming effects [70–72] or no detectable effect [73], at least for low to moderate dosages.

Neither soil EC nor pH were significant predictors of plant biomass in the experiment, and thus other factors, such as soil hydrology or nutrient availability, are more likely to explain biochar particle size effects on plant growth. Further research is needed to better understand the mechanisms for biochar particle size effects on soil properties and agronomic performance. In particular, the negative effects of small particle size and the interactive effects of biochar particle size and soil texture and physical structure deserve attention.

This short-duration greenhouse experiment is unlikely to reflect long-term field conditions. The aging of biochar will likely change its hydraulic properties [74] and impact its ability to facilitate leaching in the root zone. Biochar weathers and fragments into smaller particles naturally, and these small biochar fragments may be transported into deeper soil layers over time [75]. This suggests a strategy of adding biochar with particle sizes somewhat larger than optimal, which has additional advantages in terms of minimizing worker and public exposure to suspended biochar dust and minimizing wind erosion losses at the time of application. Our results suggest that very fine biochar is unsuitable for direct application, consistent with goals of avoiding potential human health [76] and environmental risks [77]. Along these lines, the use of granulated or pelletized biochar products may be particularly advantageous in urban environments and similar settings [40,72]. Additional research, particularly in the form of field trials, is essential to developing workable models for optimized applications that fully realize the potential benefits of biochar use in saline soils and in an urban context.

Author Contributions: Conceptualization and methodology, E.T., W.L. and S.C.T.; formal analysis, E.T. and S.C.T.; investigation, E.T.; resources, S.C.T.; data curation, E.T.; writing—original draft preparation, E.T.; writing—review and editing, W.L. and S.C.T.; visualization, E.T. and S.C.T.; supervision, project administration, and funding acquisition, S.C.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by grants from the Canadian Natural Sciences and Engineering Research Council.

Acknowledgments: We thank Melanie Sifton for input on biocontrol and greenhouse management. We also thank Audrey Chong for her advice and support with SEM imaging, and Burt's Greenhouse and LessMess Soil for donating the biochar and soil used, respectively.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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