Biochar Media Addition Impacts Apple Rootstock Growth and Nutrition

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Abstract. The effects of biochar soil amendment on perennial plant nutrition and growth are poorly understood. We investigated the effects of a green waste biochar on apple rootstock (*Malus domestica* var. M26) nutrition and growth in a series of pot trials. Apple rootstocks were grown for 5 months in both soil and sand media, with and without biochar, under a range of fertilization, pH, and soil biological regimes. Plant biomass and leaf nutrient concentration of apple rootstocks and substrate-induced respiration (SIR) of the soil were measured. Addition of biochar was associated with significant increases in leaf concentrations of calcium (Ca), boron (B), and sulfur (S) for apple rootstocks grown in sand. Increased uptake of S has not previously been reported and may be the result of the relatively high S content of the biochar used in this trial that resulted from the low temperature used to pyrolyze the biomass. Plant dry mass significantly increased by between 75% and 220% where sand was amended with biochar for every fertilization treatment. Small, but statistically significant, increases in soil pH, from pH_C (CaCl₂ extraction) 4.7 to 4.9, were found after the addition of biochar but no significant effect on soil SIR was detected. Our results indicate that biochar has positive effects on apple rootstock nutrition and growth in a sand but not in a sandy loam soil.

Biochar may be an effective management tool for increasing carbon (C) in orchard soils. Biochar is a charcoal-type material manufactured specifically for use as a soil amendment in agriculture. Biochar is created by charring organic matter by heating it under reduced oxygen conditions (pyrolysis) (Sohi et al., 2010). Interest in biochar has recently increased as a result of a number of factors: the cogeneration of C-neutral

electricity and biofuels from its manufacture, its C sequestration value, and possible soil fertility benefits (for a review, see Sohi et al., 2010). Charcoal is similar to humic substances and has rarely been differentiated from uncharred organic matter in past studies as a result of the difficulty of separating the two fractions (Chatterjee et al., 2009; Wagai et al., 2009). Hence, charcoal may contribute to benefits attributed to uncharred organic matter.

Uncharred organic matter is broken down by microbes in soil, requiring continual input to maintain soil levels. The replacement of C is rarely achieved in soils of large-scale conventionally managed orchards because the management costs of maintaining understory vegetation or applying mulches are generally considered to be uneconomically high. In contrast, biochar is highly resistant to microbial breakdown and has a residence time in soil of the order of 1000 years (Kuznyakov et al., 2009). Therefore, an orchardist may only need to invest in applying biochar once because the majority of biochar incorporated into the soil remains for many years. This long residence time in the soil potentially makes it an economically viable alternative to understory vegetation and mulching for increasing orchard soil C levels. Also, in the short term, plant-available nutrients contained within the biochar can provide fertilization benefits (Chan and Xu, 2009), particularly low-temperature-derived biochars (less than 550 °C) that favor the

retention of C, nitrogen (N), potassium (K), and S and tend to retain the structure of the feedstock (Keiluweit and Kleber, 2009; see Joseph et al., 2010, for a review of the reactions of biochar in soil). However, published research on the effects of biochar on trees is so far limited to a single-pot trial on the effect of activated C on growth, nutrition, and mycorrhizal infection rates of peach trees (Rutto and Mizutani, 2006).

Addition of biochar to the soil in some circumstances improved plant nutrition and growth of model crops such as radish and beans (Chan et al., 2007, 2008; Rondon et al., 2007). Negative effects have been reported but appear to be minor. For example, Chan et al. (2007) found a small growth decrease resulting from biochar application in unfertilized soil but found substantial growth responses to biochar with fertilizer application. The greatest benefits of biochar soil applications have been on nutrient-poor, acidic soils with low C and cation exchange capacity levels (CEC) (Chan et al., 2007; Kimetu et al., 2008; Rondon et al., 2007).

A wide range of mechanisms for the beneficial effects of biochar on plant growth has been suggested including: nutrient supply, increasing the pH of acid soils, improved soil physical attributes, reduced leaching of plant nutrients, promotion of beneficial microbes, and sorption of organic chelates (Chan et al., 2007; Deluca et al., 2009; Lehmann et al., 2003; Rondon et al., 2007). All these mechanisms may play an important role in determining the effects of biochar on plant growth and nutrition. However, these mechanisms are little understood. As a consequence, prediction of how biochar type, biochar level of application, soil, crop, and climate will interrelate to enhance crop growth is not possible at this stage. This study used apple rootstocks as a model perennial plant to assess the growth response to various root medium types and soil moisture and nutrient conditions at different biochar application rates. It was hypothesized that: 1) in acidic soil, biochar can increase apple rootstock growth through increasing soil pH; 2) biochar can improve apple rootstock growth through increasing soil microbial biomass and hence increase nutrient mineralization and availability; and 3) biochar can increase apple rootstock growth in combination with fertilization compared with fertilization alone.

Materials and Methods

Glasshouse pot trials were conducted using M26 apple rootstocks at the University of Tasmania, Hobart, Tasmania, Australia (lat. 42°52' S; long. 147°19' E). Rootstocks were grown for 5 months (15 Oct. 2009 to 8 Mar. 2010) in soil and sand media, with and without biochar, under a range of irrigation, fertilization, and biological treatments. Glass house temperature ranged between 18 and 26 °C.

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Soil and biochar treatments.

1. Impacts of biochar on soil pH—soil with 0 or 50 g·m⁻² biochar was used, with or without lime incorporated. N fertilizer was applied to these treatments.
2. Impacts of autoclaving on biological activity—soil with 0 or 50 g·m⁻² biochar was used, with soil unamended, or autoclaved. No fertilizer was applied to these treatments.
3. Fertilization effects of biochar—sand with 0 or 50 g·m⁻² biochar was used, with three fertilization treatments: 1) all essential plant nutrients except phosphorus (P), K, and Ca (-PKCa Fert); 2) N, P, K, and Ca (+NPKCa Fert); and 3) all essential plant nutrients (Total Fert); and
4. Effects of biochar application rate—soil with 0, 10, 20 and 50 g·m⁻² biochar was used. No fertilizer (No Fert), N fertilizer (N Fert), or a total fertilizer (Total Fert) was applied.

Soil and sand media. A washed river sand with particle size analysis of greater than 2 mm—29.4%, 1 to 2 mm—29.7%, 355 μ m to 1 mm—29.0%, 250 to 355 μ m—4.5%, 125 to 250 μ m—4.9%, 90 to 125 μ m—0.9%, 63 to 90 μ m—0.6%, and less than 63 μ m—1.0% was used. Particle size was determined by dry sieving (McIntyre and Loveday, 1974).

The soil used was a sandy loam, 90% sand (greater than 20 μ m), 2.5% silt (2 to 20 μ m), and 7.5% clay (less than 2 μ m). Analysis of sand, silt, and clay content was determined on dispersed samples using the hydrometer method (McIntyre and Loveday, 1974). Soil was taken from 0- to 30-cm depth from a recently ploughed field at Tahune Fields Nursery. The field had been used the previous season for raising apricot trees; however, tree growth had been very poor. The field had been previously treated with N, P, and K fertilizer and lime.

Introduction of apple replant disease with the soil could be excluded because apples had never been grown on the studied soil.

Rootstocks. A total of 400 M26 apple rootstocks of $\approx$ 8 mm collar diameter were sourced from Tahune Fields Nursery, Lucaston, Tasmania. From these, the 200 most homogeneous in size and healthy appearance were selected. The roots on the

stocks were pruned to roughly 1 cm in length and stems were cut to 35 cm total length.

Biochar. Biochar was produced from green waste feedstock using continuous slow pyrolysis at 350 °C with a PyroChar 4000 by Pacific Pyrolysis Pty, Somersby, New South Wales (<<http://www.pacificpyrolysis.com>>). The biochar had been produced within the previous year and had been sealed in a plastic container since production. The biochar was weighed $\approx$ 12 months after being produced. The particle size analysis of the biochar was: greater than 2 mm = 23.0%, 1 to 2 mm = 22.3%, 355 μ m to 1 mm = 22.7%, 250 to 355 μ m = 9.7%, 125 to 250 μ m = 8.8%, 90 to 125 μ m = 8.0%, 63 to 90 μ m = 4.6%, and less than 63 μ m = 0.9%.

Preparation of root medium. Treatment media were mixed for 2 min using a cement mixer to incorporate additives. Perlite [from Exfoliators Australia, Dandenong Victoria, Australia; pH 7.5 in a 10% (by volume) slurry] was added to all treatments at 20% by volume to improve drainage. Perlite was incorporated into all treatments for only the final 15 s of mixing to avoid compaction of the material. Perlite introduces significantly less plant nutrients than vermiculite [particularly ammonium, nitrate, phosphate, aluminum (Al), Ca, K, magnesium (Mg), manganese, and sodium (Na)] with effective CEC of only 0.40 compared with 46.3 cmol_c·kg⁻¹ in vermiculite (Dalling et al., 2013). In relevant treatments, biochar, micronutrients, and lime were also added. The application rate of lime (from Mitchell Building Supplies, Richmond, Victoria, Australia; calcium carbonate 80%, magnesium carbonate 9%, pH of 13) needed to achieve pH 6.5 was estimated through a pilot trial. Lime was incorporated into a test sample of soil at three application rates. The soil was left for 48 h and pH remeasured. From this, the rate expected to raise the pH of soil from the original pH_c (CaCl₂ extracted) of 4.9 to 6.5

was calculated. However, the estimated rate resulted in soil pH of 7.0, overshooting the target of 6.5. Soil was autoclaved before mixing at 121 °C and 220 kPa for 30 min. Each 20-cm diameter, 19-cm high pot received 3.6 L of mixed potting media.

Fertilization. Micronutrients incorporated into the root media at the beginning of the trial for relevant treatments were supplied as Osmocote Micromax fertilizer (Everris Australia Pty Ltd, Bella Vista, Australia) applied at 0.4 kg·m⁻³ and FeSO₄ applied at 1.0 kg·m⁻³. Micromax composition was: Ca 6%, Mg 3.0%, B 0.10%, copper (Cu) 1.0%, iron (Fe) 17.0%, Mn 2.5%, molybdenum 0.05%, and zinc (Zn) 1.0%. All nutrients in Micromax are in water-soluble form.

After 53 d (and 88% of rootstocks had broken dormancy), weekly macronutrient fertilization began. The fertilizer was based on modified Hoagland's solution (Hoagland and Arnon, 1950). Amounts of weekly nutrient application for each treatment are in Table 1. Nutrients were diluted in 130 mL of water.

Plant, soil, and biochar measurements. At the end of the trial (144 d after planting), a range of data and images was collected. Plants were photographed individually. Plant leaves, branches, and roots were dried at 65 °C until a constant weight was reached and then weighed individually. The original rootstock stem was excluded from analysis because stems grew little during the trial and final weight would have been highly confounded by initial weight, hence giving little information about treatment effects. For the “No Fert” soil treatments, effect of biochar on soil microbial biomass was estimated using SIR as an indicator of microbial biomass, according to the methodology of Anderson and Domsch (1978). Soil and biochar were analyzed for available nutrient concentrations, pH, and total C levels (Table 2). Soil pH (pH_c) was measured with a 1:5 soil to 0.01 M CaCl₂

Table 1. Weekly macronutrient application per plant (mg/element).

	N	P	K	Ca	S	Mg
Total -PKCa (Mg SO ₄ ·7 H ₂ O and NaNO ₃)	28.4				8.8	6.6
NPKCa Fert (CaNO ₃ ·4 H ₂ O, KNO ₃ , and KH ₂ PO ₄)	28.8	4.2	31.9	27.0		
Total Fert (CaNO ₃ ·4 H ₂ O, KNO ₃ , MgSO ₄ ·7 H ₂ O, and KH ₂ PO ₄)	28.8	4.2	31.9	27.0	8.7	6.6

N = nitrogen; P = phosphorus; K = potassium; Ca = calcium; S = sulfur; Mg = magnesium.

Table 2. Nutrient content, total carbon content, and pH of soil and biochar used in trial.

	CaCl ₂ pH	Total C	Total NH ₄ ⁺ NO ₃ ⁻		Colwell P K		DTPA Cu Fe Mn Zn			
		(%)	(mg·kg ⁻¹)		(mg·kg ⁻¹)		(mg·kg ⁻¹)			
Soil	4.90	2.7	10	4	67	166	0.99	243	20.1	2.5
Biochar	6.40	41.0	3	1	234	112	1.35	82	2.7	4.8
	Exchangeable							Hot CaCl ₂		
	Al	Ca	Mg	K	Na	S	B			
	(meq/100 g)							(mg·kg ⁻¹)		
Soil	0.15	3.0	0.3	0.37	0.05	5.3	0.3			
Biochar	<0.001	3.5	2.4	1.31	0.43	115	2.4			

DTPA = diethylenetriaminepentaacetic acid; C = carbon; P = phosphorus; K = potassium; Cu = copper; Fe = iron; Mn = manganese; Zn = zinc; Al = aluminum; Ca = calcium; Mg = magnesium; Na = sodium; S = sulfur; B = boron.

solution ratio after 1 h mixing using a combination pH electrode (Rayment and Higginson, 1992). Soil total C was measured using the Walkley and Black (1932) method. Total C of biochar was measured using a CHN/O analyzer (PerkinElmer, Waltham, MA). Total NH_4^+ and NO_3^- were measured using a Lachat Flow Injection Analyser (Searle et al., 1984). Extractable S was measured using KCl extraction (Blair et al., 1991). Available P and K were measured using the Colwell (1995) method. Available Cu, Fe, Mn, and Zn were measured using diethylenetriaminepentaacetic acid extraction (Rayment and Higginson, 1992). Exchangeable cations (Al, Ca, Mg, K, and Na) were measured using 0.1 M $\text{BaCl}_2/0.1\text{M}$ NH_4Cl extraction according to the Gillman and Sumpter (1986) method. Available B was measured by extraction with boiling CaCl_2 (Rayment and Higginson, 1992).

Plant nutrient concentration was analyzed for selected treatments with three analytical replicates used for each treatment. Leaves from the entire plant were used for nutrient analysis to obtain the minimum amount required for analysis. Dried plant leaves were finely ground. N analysis of leaf material was according to the procedure of Sweeny and Rexroad (1987). Leaf material was combusted at 950 °C in oxygen using a Leco FP-428 N Analyser (LECO Australia Pty Ltd, Castle Hill, Australia). The released N from the sample was measured as it passed through a thermal conductivity cell. For analysis of P, K, Ca, Mg, S, Fe, Cu, Mn, Zn, B, and Na, the procedure of McQuaker et al. (1979) was used. Leaf material was digested in nitric acid using a Milestone microwave oven and nutrients were measured by inductively coupled plasma atomic emission spectroscopy. For the analysis of Cl^- and NO_3^- in leaves, the procedure of Zall et al. (1956) was used. Leaf material was extracted in deionized water and the Cl^- and NO_3^- were measured simultaneously using a Lachat Flow Injection Analyser (Lachat Instruments, Loveland, CO). The nitrate was reduced to nitrite through a copperized cadmium column and the nitrite measured colorimetrically at 520 nm. The concentration of chloride was measured colorimetrically at 480 nm.

Experimental design and data analysis. A randomized block design of seven blocks was used for the pot trials. The autoclaved soil treatment was contained in a separate block to reduce microbial contamination from neighboring treatments. Within the autoclaved treatments, individual rootstock position was randomized.

All data were analyzed using SPSS software (SPSS Inc., Chicago, IL). Univariate analysis was performed using least significant difference to compare the main effects of biochar, the root medium treatments, and their interaction. All significant differences were assessed at a significance level of ($P < 0.05$) with least significant differences calculated using Fisher's protected method.

High variability was found in rootstock growth with rootstocks breaking dormancy

over a period of 70 d from first to last. Plant growth, leaf symptoms, and nutrient concentrations were also highly variable. Potential confounding sources of rootstock variability were investigated. It was found that biochar had no significant effect on root medium temperature. Root medium temperature, rootstock weight, and spatial variability (block effect) had no significant effect on rootstock growth. Hence, no confounding sources of variability were identified. To reduce the effects of rootstock variability, those that had not achieved greater than 5 cm shoot growth by the time of first measurement (14%) and those that died during the course of the trial (3%) were excluded from all analyses.

Results

Impacts of biochar on soil pH. No significant growth response was found for either lime or biochar alone (results not shown). Biochar raised pHc from 4.68 ± 0.09 to 4.86 ± 0.08 ($P = 0.017$), whereas lime raised pHc levels from 4.68 ± 0.09 to 7.03 ± 0.04 ($P < 0.0005$).

Impacts of addition of autoclaved soil on biological activity. No significant difference

in SIR was found between soil with and without biochar addition.

A significant interaction between biochar addition and autoclaved soil occurred with autoclave treatments significantly reducing rootstock total dry mass for the soil and sand without biochar by 34% and increasing it 34% with biochar compared with unaltered media (Fig. 1). For the autoclave-treated soil, addition of biochar was associated with an increase in rootstock total dry mass of 102%.

Fertilization effects of biochar. Biochar addition to sand was associated with a significant increase in rootstock total dry mass for every fertilization treatment: an increase that was not detected for sand alone. The Total -PKCa treatment increased total dry mass by 75%, the NPKCa Fert treatment increased it by 141%, whereas the Total Fert treatment increased growth by 220% (Fig. 2).

The Total -PKCa treatments resulted in leaf scorch, which was most severe for the non-biochar-treated pots (Figs. 3A and 4A). Both NPKCa Fert treatments developed misshapen and small young leaves (Figs. 3C–D and 4B). In no biochar NPKCa Fert treatments, misshapen leaves tended to be more severe and were associated with reduced

Fig. 1. Mean total dry weight of apple rootstocks. Least significant difference = 5.3. Individual SEM used for error bars. Rootstocks were grown in a soil plus 50 t·ha⁻¹ (equivalent) biochar substrate ("Biochar") or a control soil only substrate ("No Biochar") each subjected to two pre-treatments: 1) unaltered soil; and 2) autoclaved soil.

Fig. 2. Mean total dry weight of apple rootstock growth. Rootstocks were grown in a sand and 50 t·ha⁻¹ (equivalent) biochar substrate (Biochar) or a control sand-only substrate (No Biochar) each receiving three different fertilization regimes: 1) all essential plant nutrients except nitrogen (N), phosphorus (P), potassium (K) (Total-NPK); 2) N, P, K only (NPK); or 3) a total nutrient fertilization regime (Total). Least significant difference = 2.1. Individual SEM used for error bars. Biochar × fertilizer interaction ($F_{2,36}:6.1$; $P = 0.005$).

Fig. 3. Examples of rootstocks from each treatment. Rootstocks were grown in a sand and 50 t-ha⁻¹ (equivalent) biochar substrate or a control sand only substrate each receiving three different fertilization regimes: 1) all essential plant nutrients except phosphorus (P), potassium (K), and calcium (Ca) (Total-PKCa); 2) N, P, K, and Ca only (NPKCa); or 3) a total nutrient fertilization regime (Total).

stem elongation, purple veins, and severe interveinal chlorosis of young leaves (Figs. 3C and 4C). The visual symptoms and growth differences were largest for the Total Fert treatments: Total Fert non-biochar-treated plants were severely stunted and leaves developed interveinal chlorosis with much smaller leaves and narrower stems (Fig. 3E), whereas for Total Fert biochar treatments leaves were dark green and healthy (Figs. 3F and 4D).

The addition of biochar was associated with significant increases in leaf concentrations of Ca, B, and S for all treatments (Table 3). A significant biochar $\times$ fertilizer interaction was found for leaf concentration of K. Biochar increased concentrations of K in leaves for NPKCa Fert treatment, had no significant effect on K concentrations for the Total-PKCa treatment, and K concentrations were reduced in the Total-Fertilizer treatment. There was a significant interaction effect for biochar $\times$ fertilizer for leaf Fe concentration. Fe concentrations significantly decreased for Total-PKCa and Total Fert treatments but significantly increased for NPKCa Fert treatment. For all other plant nutrients, no significant effect of biochar was found.

Effects of biochar application rate. Biochar had no significant effect on rootstock growth at any application level in any fertilization treatment. However, addition of biochar was associated with a significant increase in leaf concentration of B from 24 to 28 mg·kg⁻¹ ($P = 0.036$) and a significant decrease in Fe from 122 to 95 mg·kg⁻¹ ($P = 0.048$). A biochar $\times$ fertilizer interaction was found for Mn concentrations with biochar associated with a significant decrease in Mn for both N fertilizer treatments (from 111 to 76 mg·kg⁻¹) and the Total Fert treatments (from 162 to 107 mg·kg⁻¹), whereas no significant difference was found for the No Fert treatment ($P = 0.041$).

Discussion

Impacts of biochar on soil pH. Optimal soil pH for apples is between 6.5 and 7 but in this trial, biochar only increased soil pH from 4.7 to 4.9. This contrasts with an increase of

pH from 6.3 to 7.1 as a result of the equivalent of 6 t/ha biochar to soils cropped for 5 years reported by Kimetu et al. (2008). The discrepancy may be explained by a combination of differences between the makeup of the green waste-based biochar we used and its low pH. Inherent differences occur as a result of materials used and pyrolysis processes and the biochar used in this study had pH of only 6.4 relative to pH 9.4 used by Kimetu et al. (2008). Yuan and Xu (2011) report that the soil liming effect of biochar addition was strongly correlated ($R^2 = 0.95$) with pH of the biochar used for amendment. Our findings are consistent with this. In contradiction to Hypothesis 1, biochar did not significantly increase growth of apple rootstocks through improved soil pH. However, nor did the addition of lime, which increased soil pH from 4.7 to 7.0.

Impacts of autoclaving on biological activity. In contradiction to Hypothesis 2, SIR measurements indicate that biochar did not increase plant growth through increased microbial activity and associated nutrient mineralization. This conflicts with findings of Steiner et al. (2008) in which a linear relationship between microbial population growth and biochar addition was found. However, biochar significantly interacted with autoclaved soil to increase plant growth. This may be the result of the increased availability of N and P mineralized as a result of autoclaving (Chambers and Attiwill, 1994). However, the observation of reduced growth in autoclaved treatment without biochar is not consistent with this interpretation.

Fertilization effects of biochar. Biochar addition to sand was associated with increased B, Ca, and S availability. This is consistent with Hypothesis 3, that biochar can increase plant growth through fertilization with non-volatile nutrients. According to Peryea et al. (2003), the concentration of B was deficient in the leaves of all the non-biochar treatments and they are deficient or bordering on deficient in Ca and S. We found significant increases, relative to controls, in nutrient uptake for all essential plant nutrients in the biochar treatments. The biochar contained significant levels of extractable Ca, S, B, P, K, Fe, Zn, and Cu,

suggesting that plant uptake of these nutrients was increased by biochar addition and contributed to the observed plant growth response.

Consistent with the leaf nutrient analyses, the plants of the NPKCa Fert treatments without biochar addition displayed purpling of veins, stunted shoot growth, and stunting and interveinal chlorosis of the youngest leaves, which are symptoms of B and Zn deficiency in apples. In Total Fert treatments, without biochar, chlorosis of young leaves progressed into older leaves over time, which is consistent with S deficiency. Leaf scorch seen in the N-Mg-S treatments is a classic symptom of K deficiency. These symptoms disappeared or were substantially reduced with biochar additions indicating that biochar is increasing K, S, B, and Zn availability in the root medium.

The increased uptake of S found in this trial has not been reported in previous biochar studies. This is likely to be the result of the high S in the biochar given that the pyrolysis temperature (350 °C) used to produce the biochar was low in comparison with that used in most other studies (Joseph et al., 2010). Above 375 °C, volatilization of S is known to occur (Neary et al., 1999). Also leaf biochars are higher in S than bark or wood biochars (Abdullah et al., 2010). Hence, the green waste biochar used could be expected to have higher S than wood-based biochars on which the majority of research has been done (Sohi et al., 2010).

The fertilizer $\times$ biochar interaction may give some indication of the relative importance of fertilization resulting from biochar on plant growth. The largest growth response to biochar was in the Total Fert treatment, despite being provided with all essential plant nutrients. For the Total Fert no biochar treatment, the leaves developed severe nutrient deficiency symptoms and grew very poorly. The poor growth for the no biochar treatment could be explained by nutrient leaching, because sand has a very low CEC and anion exchange capacity. Biochar could have reduced nutrient deficiency and increased plant growth through acting as a slow-release fertilizer and/or by reducing nutrient leaching. Reduced leaching resulting from biochar application could be expected to have a greater effect where all nutrients are provided in fertilizer, e.g., Total Fert treatment, and this is what was found. However, we emphasize that these are tacit results because the impact of biochar on leaching was not directly investigated in this study.

Effects of biochar application rate. Despite large growth responses found where biochar was incorporated into the sand medium, no significant growth response to biochar amendment was found in soil for any combination of biochar application level or fertilization treatment in this trial. This is likely to be partly the result of the much higher nutrient content of the soil as well as greater nutrient retention; hence, potential improvements in these properties from biochar would have less effect. For instance,

Fig. 4. Example pictures of visual deficiency/toxicity symptoms: (A) severe leaf scorch (Total-PKCa Fert.-biochar), (B) shoot stunting and undersized young leaves (NPKCa-biochar), (C) purple veins, interveinal chlorosis and leaf curling (NPKCa Fert.-biochar), and (D) healthy leaf (Total Fert. + biochar).

Table 3. Leaf nutrient concentrations for sand treatments.^z

		Total PKCa		NPKCa		Total		Effect, F and P values
		No Biochar	Biochar	No Biochar	Biochar	No Biochar	Biochar	
N	(%)	1.52	1.71	1.68	1.84	1.61	1.64	
P	(%)	0.093	0.083	0.120	0.153	0.137	0.123	
K	(%)	0.28 (a)	0.31 (a)	1.36 (c)	1.58 (d)	1.34 (c)	1.06 (b)	Biochar × Fert ($F_{2,12}=4.8$; $P = 0.03$)
Ca	(%)	0.70 (bc)	0.78 (c)	0.59 (a)	0.88 (d)	0.69 (ab)	0.78 (c)	Biochar ($F_{1,12}=20.3$; $P = 0.001$)
Mg	(%)	0.603	0.653	0.187	0.177	0.263	0.283	
S	(%)	0.107 (a)	0.123 (b)	0.103 (a)	0.127 (b)	0.12 (a)	0.133 (b)	Biochar ($F_{1,12}=5.4$; $P = 0.038$)
Cl	(%)	0.73	0.80	0.70	0.77	0.73	0.73	
B	(mg·kg ⁻¹)	16.0 (c)	22.4 (e)	8.9 (a)	16.0 (d)	10.0 (a)	12.9 (b)	Biochar ($F_{1,12}=36.8$; $P < 0.0005$)
Cu	(mg·kg ⁻¹)	6.48	6.05	3.96	4.95	4.53	4.04	
Fe	(mg·kg ⁻¹)	110 (d)	94 (c)	57 (a)	76 (b)	75 (b)	67 (ab)	Biochar × Fert ($F_{2,12}=4.1$; $P = 0.045$)
Mn	(mg·kg ⁻¹)	86.2	96.7	58.8	58.1	55.4	59.8	
Zn	(mg·kg ⁻¹)	20.7	19.4	16.3	20.5	17.5	15.1	

^zDifferent letters denote significant differences between treatment means.

N = nitrogen; P = phosphorus; K = potassium; Ca = calcium; Mg = Magnesium; S = sulfur; Cl = Chlorine; B = Boron; Cu = Copper; Fe = Iron; Mn = Manganese; Zn = Zinc.

biochar addition increased foliar concentrations of B in leaves for rootstocks in soil as it did in sand. However, although for sand many treatments produced concentrations in leaves below deficiency levels, for soil, all treatments supported plant B concentrations in the normal range and hence no growth response was observed. The fact that a significant growth response was not found in soil is at odds with other research that has found positive growth responses to biochar in similar C and nutrient-poor, acidic soils (e.g., Chan et al., 2007; Kimetu et al., 2008; Rondon et al., 2007). This may be the result of the high sand content of the soil in this trial as well as the use of perlite added to reduce hard setting and increase drainage. However, it should be noted that Chan et al. (2007) found decreases in biomass production at moderate, in contrast to high, rates in a hard setting soil that benefited from the structural advantages of incorporated biochar. Additionally, the lack of a liming effect in the current study may be the result of the inherently low pH of biochar relative to that used by Chan et al. (2007; pH CaCl₂ 9.4), Kimetu et al. (2008; pH CaCl₂ 9.4), or Rondon et al. (2007; pH water 7.00).

This study has shown that application of biochar to the growth media of apple rootstocks can significantly affect plant growth and nutrition. It appears likely that fertilization with nutrients contained within biochar was the major source of Ca, S, and B and possibly K and Zn. In interpreting these results, it needs to be noted that the fertilizer

properties of biochar will inevitably decline, whereas CEC of biochar increases over time (Cheng et al., 2006, 2008; Liang et al., 2006) and longer-term studies are warranted for investigation of effects on perennial crops. Although the effect of biochar in the soil may change over time, this research demonstrates biochar can have positive effects on plant nutrition and growth in the short term.

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