Amending Sandy Soil with Biochar Promotes Plant Growth and Root Colonization by Mycorrhizal Fungi in Highbush Blueberry

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Abstract. Biochar, a carbon-rich, fine-grained residue obtained from pyrolysis of biomass, is known to improve soil conditions and to suppress infection by soilborne pathogens. However, its use as a soil amendment has received relatively little attention by the horticulture industry. Two 12-week experiments were conducted in a greenhouse to determine the potential of using biochar, produced from mixed conifers during conversion of wood to energy, as a soil amendment for highbush blueberry (*Vaccinium* hybrid ‘Legacy’). Plants in the first experiment were fertilized once a week with a complete fertilizer solution, whereas those in the second experiment were fertilized once a month with a solution of ammonium sulfate. In both cases, the plants received the same amount of N in total and were grown in pots filled with unamended soil (sandy loam) or soil amended at rates of 10% or 20%, by volume, with biochar or a 4:1 mix of biochar and bokashi (biochar-bokashi). The bokashi was produced from fermented rice (*Oryza sativa* L.) bran and was added to increase nutrients in the amendment. Half of the plants in each soil treatment were inoculated with *Phytophthora cinnamomi* Rands, which causes root rot in blueberry. Although pH of the raw biochar was high (8.5), soil pH averaged 4.5 to 5.5 in each treatment. In the absence of *P. cinnamomi*, plants grown with 20% biochar or 10% or 20% biochar-bokashi had greater leaf area and 30% to 70% more total dry weight than those grown with 10% biochar or in unamended soil. Biochar also improved soil aggregation and increased root colonization by ericoid mycorrhizal fungi. The percentage of roots colonized by mycorrhizal fungi was 54% to 94% in plants grown with the amendments, but was $\leq 10\%$ in those grown in unamended soil. Plants inoculated with *P. cinnamomi* were stunted and showed typical symptoms of root rot. Root infection by the pathogen was unaffected by biochar or biochar-bokashi and negated any growth benefits of the amendments. Overall, amending soil with biochar appears to be a promising means of promoting plant growth and mycorrhizal colonization in blueberry, but it may not suppress phytophthora root rot.

Blueberry (*Vaccinium* sp.), a member of the Ericaceae family, is adapted to well-drained acidic soils with a high amount of organic matter (Retamales and Hancock,

2018). When blueberry is grown in mineral soils, organic materials such as bark or sawdust are often incorporated into the soil before planting to increase organic matter and

improve soil structure (Bollen and Glennie, 1961; Haynes and Swift 1986); however, these materials are expensive (Julian et al., 2011). As a result, growers are interested in alternative strategies to improve soil conditions for better growth and production in blueberry.

Recently, there has been renewed interest in using biochar as a soil amendment for production in numerous crops (Suthar et al., 2018). Biochar is a highly stable, carbon-rich residue produced by pyrolysis, a process by which biomass is thermally decomposed under low oxygen conditions and typically at temperatures $<700^\circ\text{C}$ (Lehmann and Joseph, 2009). Essentially, any form of biomass can be converted to biochar, but the most preferable forms in terms of logistics and costs include forest thinnings, crop residues (e.g., corn stover, straw, grain husks), yard waste, clean urban wood waste (e.g., roadside clearing, pallets, sorted construction debris), and manures (Wang et al., 2015). Once applied, a large fraction of the biochar is recalcitrant and can persist in soil for decades to millennia (Lehmann and Joseph, 2009). Most biochars have high ion-exchange capacities (cation and anion) and, when added to soil, improve porosity and aeration and increase retention of water and nutrients (Bruun et al., 2014; Knowles et al., 2011; Nemati et al., 2015; Sohi et al., 2010).

Plant growth and productivity have been shown to respond positively to biochar addition, especially in acidic and coarse-textured soils (Jeffery et al., 2011). However, most biochars are high in pH (>7), which could be detrimental to acidophilic plants, such as blueberry. Biochar has also been reported to increase populations of beneficial soil microorganisms, such as mycorrhizal fungi (Amendola et al., 2017; Bird et al., 2008; LeCroy et al., 2013; Lehmann et al., 2011; Solaiman et al., 2010), and suppress development of soilborne pathogens, including *P. cinnamomi* (Zwart and Kim, 2012), which is commonly associated with root rot in highbush blueberry (Bryla et al., 2008; Yeo et al., 2016).

Recent studies suggest that biochar should be combined with other sources of organic matter to increase soil fertility even when biochar is applied at a lower rate. For example, mixing biochar with compost has been shown to increase plant productivity more than the combination of biochar and mineral fertilizers (Kamman et al., 2015; Sánchez-García et al., 2016; Schulz and Glaser, 2012). Hagemann et al. (2017) determined that mixing the two results in a complex, nutrient-rich organic coating on the outer and inner surfaces of the biochar particles. This coating adds hydrophilicity, redox-active moieties, and additional mesoporosity, which strengthens biochar-water interactions and enhances nutrient retention. However, compost is often high in pH and electrical conductivity and, therefore, can be unsuitable for blueberry (Costello et al., 2019; Strik et al., 2017; Sullivan et al., 2014).

Much like composting, bokashi is a process of converting food waste and other types

of organic matter into a soil amendment. However, unlike compost, bokashi is produced by fermentation and is usually very low in pH (Christel, 2017). The process of making bokashi uses an inoculum of microorganisms called Effective Microorganisms or EM (Boechat et al., 2013). Typically, the inoculum contains a mix of microbes, such as *Lactobacillus* sp. (anaerobic bacteria), *Rhodopseudomonas* sp. (phototropic bacteria), *Saccharomycetes* sp. (yeast), actinomycetes, and various forms of filamentous fungi (Xu, 2000). Some of these microorganisms are considered antagonistic and capable of inducing systemic resistance in plants to various pathogens, including 'Candidatus phytoplasma solani' on periwinkle [*Catharanthus roseus* (L.) G. Don], bacterial blight (*Erwinia psidii* R.) on guava (*Psidium guajava* L.) fruits, *Pythium ultimum* Trow in cucumber (*Cucumis sativus* L.), and *Rhizoctonia solani* Kühn in carrot (*Daucus carota* L.) (Pierce et al., 2016; Rezende et al., 2008; Shin et al., 2017). The pores in biochar provide habitat for these microorganisms, protecting them from predation and drying (Lehmann et al., 2011). The high ion-exchange capacities of biochar can also help retain nutrients released from bokashi and other sources of organic matter (Dias et al., 2010; Prost et al., 2013).

The objective of this study was to evaluate the use of biochar alone or in combination with bokashi as a soil amendment for highbush blueberry. Two experiments were conducted, including one in which the plants in each treatment were fertigated once a week with a complete nutrient solution and another where the plants were fertigated once a month with a solution of ammonium sulfate only. Based on work in other crops, we expected that adding biochar to the soil would improve plant growth and mineral nutrition in blueberry and would be most effective under nutrient-limited conditions, such as those in the second study,

when it was mixed with bokashi. We also inoculated half of the plants in each treatment with *P. cinnamomi* to determine whether biochar or bokashi and bokashi enhanced disease suppression of the pathogen.

Materials and Methods

Soil amendments. The soil amendments used in the study included biochar and a 4:1 mix of biochar and bokashi (v/v). The biochar was produced by gasification at $\approx 750^\circ\text{C}$ (Oregon Biochar Solutions, Central Point, OR). This is a commercially available biochar manufactured from mixed conifers during conversion of wood to energy at Biomass One, a 30-MW wood-debris-fired power plant (White City, OR). The bokashi was produced from rice bran fermented with EM-1 microbial inoculant (TeraGanix, Inc., Alto, TX). Physicochemical properties of the biochar were obtained from laboratory tests provided by the manufacturer or conducted in the laboratory of KMT (Table 1).

Phytophthora inoculum. Inoculum of *P. cinnamomi* was produced in fungal spawn bags with filter patches (Fungi Perfecti, Sheldon, WA). Twenty-seven bags were filled with 3 L of medium-grade vermiculite and 1.5 L of a broth with 7.5% vegetable juice (V-8 juice; Campbell Soup Co., Camden, NJ), by volume, and $1\text{ g}\cdot\text{L}^{-1}\text{ CaCO}_3$. The bags were autoclaved three times for 55 min every 24 h. After cooling, a 100-mm-diameter petri plate of potato dextrose agar (Difco Laboratories Inc., Detroit, MI), fully colonized with a 10-d-old isolate of *P. cinnamomi*, was sliced into 100 pieces and added to each bag (i.e., one plate/bag). The isolate was obtained in 2010 from an infected, field-grown plant of 'Draper' blueberry (Vargas et al., 2015) and was used previously by Yeo et al. (2016). Bags of vermiculite were also mixed with agar plugs without the pathogen and used for the non-inoculated controls. Each bag was incubated in the dark at 20°C and shaken at 1-week intervals. After 4 weeks, the contents of each

bag were consolidated and homogenized in a cement mixer and weighed. The inoculated vermiculite was separated into five different bags, each containing 416 g fresh weight of inoculated vermiculite. The density of propagules in the vermiculite was enumerated by plating a suspension of 10 mL of inoculum in 90 mL of 0.2% water agar onto 10 plates of potato dextrose agar, incubating for 48 h in the dark, and then counting the number of *P. cinnamomi* colonies.

Soil treatments. The soil used in the experiments was a Lynden sandy loam (sandy, mixed, mesic Typic Haplorthods) collected from the top 30 cm of an area adjacent to a commercial blueberry field in Whatcom County, WA. The soil was air-dried, sieved through a 2-mm-mesh screen, and combined in a cement mixer with 10% or 20% biochar or biochar-bokashi, by volume. During mixing, a bag of inoculated (*P. cinnamomi*) or noninoculated (control) vermiculite was also added with each amendment, as well as to treatments with soil only. In total, there were 10 different soil treatments, including a combination of five levels of soil amendment (none, 10% biochar, 20% biochar, 10% biochar-bokashi, and 20% biochar-bokashi) and two levels of inoculation with *P. cinnamomi* (noninoculated and inoculated). The noninoculated treatments were mixed first, and the mixer was sanitized with 0.5% NaOCl solution between each treatment. Final density of the pathogen in the inoculated treatments was 2.7 colony-forming units (cfu) per cm^3 of soil or soil mix.

Experimental design. Experiments were initiated on 4 Apr. 2016 in a heated glasshouse located at the U.S. Department of Agriculture–Agricultural Research Service Horticultural Crops Research Unit in Corvallis, OR (lat. $44^\circ34'3''\text{N}$, long. $123^\circ17'9''\text{W}$). One hundred 'Legacy' blueberry plants were obtained from a commercial nursery (Fall Creek Farm & Nursery, Lowell, OR) as 1-year-old liners propagated from tissue culture. The plants were transplanted into 4-L plastic pots (one plant per container) filled

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Table 1. Physicochemical properties of the biochar used in the study.

Property	Value	Test method ^a
Surface area ($\text{m}^2\cdot\text{g}^{-1}$)	554	ASTM D6556-10
Bulk density ($\text{kg}\cdot\text{m}^{-3}$)	78.5	ASTM D6556-10
Total ash (%)	2.7	ASTM D1762-84
Volatile matter (%)	9.3	ASTM D1762-84
Fixed C (%)	88	Dry combustion
H:C ratio	0.25	Dry combustion
N (%)	0.94	Dry combustion
Electrical conductivity ($\text{dS}\cdot\text{m}^{-1}$)	1.21	4.10USCC:dil. Rajkovich
Liming equivalent ($\%\text{CaCO}_3$)	11.1	AOAC 955.01
Carbonates ($\%\text{CaCO}_3$)	5.1	ASTM D 4373
Tetra dioxins ($\text{ng}\cdot\text{kg}^{-1}$)	4.09	US EPA 8290
Tetra furans ($\text{ng}\cdot\text{kg}^{-1}$)	31.4	US EPA 8290
Penta furans ($\text{ng}\cdot\text{kg}^{-1}$)	4.27	US EPA 8290
Particle size class (%)		ASTM D 2862 granular
<0.5 mm	1.4	
0.5–1 mm	3.2	
1–2 mm	49.8	
2–4 mm	43.9	
>4 mm	1.8	

^aSee International Biochar Initiative (2015) for a full description of each method.

with each soil treatment (10 plants/treatment) and divided into two experiments, referred to as Expt. 1 and Expt. 2. In Expt. 1, six plants per treatment were fertigated weekly with 100 mL of a complete nutrient solution (Miracle-Gro Water Soluble Azalea, Camellia, Rhododendron Plant Food; The Scotts Company, Marysville, OH). The solution had 150 mg·L⁻¹ of N and 50 mg·L⁻¹ each of P and K. In Expt. 2, four plants per treatment were fertigated once every 4 weeks with 100 mL of ammonium sulfate solution containing 600 mg·L⁻¹ of N. The plants were also irrigated two or three times per week, as needed, in both of the experiment, with sufficient water to produce ≈20% drainage from the pots during each irrigation. Overall, the plants received the same total amount of N in both experiments, but in Expt. 2, N was applied less frequently, and no additional nutrients other than S were added.

The experiments were arranged in a completely randomized design and conducted side-by-side on the same greenhouse bench. Each pot was mulched with a 2.5-cm-layer of douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] sawdust to reduce soil evaporation and to prevent the growth of moss and algae on the soil surface. Temperature inside the glasshouse was maintained at 28 ± 2 °C during the day and 20 ± 2 °C at night. Photoperiod was extended to 14 h·d⁻¹ using two 1000-W high-pressure sodium lamps suspended ≈1.5 m above the pots.

To promote infection by *P. cinnamomi*, plants from both experiments were flooded for 48 h at 6 and 8 weeks after transplanting (Weiland et al., 2010). During flooding, each pot was placed inside a 3.8-L plastic bucket filled with 3.2 L of tap water to ≈1.5 cm below the surface of the soil. Care was taken to avoid splashing during the procedure, and all buckets were sanitized with 0.5% NaOCl solution before each flooding event (Weiland et al., 2018).

Measurements. A 4-L sample of the soil and the amendments (biochar and biochar-bokashi) was sent to a commercial laboratory (Brookside Laboratories, New Bremen, OH) for initial (preexperiment) analysis of pH, organic matter, nutrients, and texture (soil only). The pH of each was determined using a 1:1 ratio with water (McLean, 1982), and organic matter content was determined by loss-on-ignition at 360 °C (Shulte and Hopkins, 1996). Available N (NH₄-N and NO₃-N) was extracted with 1M KCl and determined using automated colorimetric methods (Dahnke and Johnson, 1990). Other nutrients were extracted using the Mehlich 3 (Mehlich, 1984) method and analyzed by inductively coupled plasma (ICP) spectrometry. Soil texture was determined using the hydrometer method (ASTM D422-63, 2007).

Leaf area was estimated weekly during the experiments by measuring the length and width of every leaf on the plants using a standard 30-cm-length ruler. Each measurement was converted to leaf area using linear model developed for highbush blueberry (Follovo et al., 2008).

Plants from both experiments were harvested destructively at 12 weeks after transplanting. At harvest, a 100-g sample of soil was collected first from each pot (non-inoculated treatments only) and sent to the commercial laboratory mentioned previously for analysis of pH, organic matter, and nutrients. Next, the leaves were removed from the plants and measured using a portable leaf area meter (LI-3000C; LICOR Biosciences, Logan, UT). Then, the stems were cut off at the soil surface, and soil was washed from the roots by rinsing them under running water. Two random samples of roots (1–2 g fresh weight each) were taken from each plant and placed in 50-mL tubes for analysis of colonization by ericoid mycorrhizal fungi and infection by *P. cinnamomi*. The leaves, stems, and remaining roots were oven-dried for 2 d at 60 °C and weighed.

Once weighed, the leaves were ground to pass through a 20-mesh screen and analyzed for N using a combustion analyzer (TruSpec CN; Leco Corp., St. Joseph, MI) and for other nutrients (P, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn) using an ICP optical emission spectrophotometer (Optima 3000DV; Perkin Elmer, Wellesley, MA). Samples were digested in a microwave with 70% (v/v) nitric acid before running them on the ICP (Gavlak et al., 2005). Reference standard apple [*Malus ×sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf.] leaves (no. 151, National Institute of Standards and Technology) were included in each run to ensure the accuracy of the instruments and digestion procedures.

Roots collected for mycorrhizal analysis were cleared with 10% KOH and stained in a solution of lactoglyceride and 0.05% trypan blue (Giovannetti and Mosse, 1980). Roots were also collected from 10 plants before planting to determine the level of initial colonization. Each root sample was examined under a microscope (×115) and quantified for percent colonization using the gridline-intersection method (McGonigle et al., 1990). Approximately 1 g of stained roots were placed onto a petri dish and spread out evenly. The dish was marked on

the bottom with horizontal and vertical gridlines. Each time a root intersected a gridline, the presence or absence of mycorrhizal structures (hyphae and hyphal coils) was recorded. Because *Phytophthora* also produces hyphae, mycorrhizal colonization was not examined in plants inoculated with *P. cinnamomi*.

Roots collected for analysis of *Phytophthora* were rinsed in 1% bleach solution (0.05% NaOCl) for 30 s, and ten 1-cm-long pieces from each sample were placed onto petri plates filled with P₁₀ARPH agar, a semiselective medium for *Phytophthora* sp. (Tsao and Guy, 1977). The agar contained 10 ppm pimarinic (Sigma-Aldrich, St. Louis, MO), 250 ppm ampicillin (Sigma-Aldrich), 10 ppm rifampicin (Sigma-Aldrich), 100 ppm pentachloronitrobenzene (Terrachlor, 75% a.i.; Chemtura, Middlebury, CT), and 25 ppm hymexazol (Tachigaren, 70% a.i.; Sankyo Co., Tokyo, Japan). The plates were incubated at 20 °C for 10 d and monitored periodically under a microscope (×400) to determine the percentage of the root sections infected by *P. cinnamomi*.

Statistical analyses. Data were analyzed separately from the two experiments. In both cases, the data were tested for normality (Shapiro-Wilk) and homogeneity of variance (Brown-Forsythe) and analyzed by analysis of variance using a statistical software package (Systat Software Inc., San Jose, CA). Fixed effects in the model included amendment type and inoculation treatment. For analysis of repeated leaf area measurements, fixed effects included amendment, inoculation, and week. To homogenize variance, root infection and mycorrhizal root colonization were transformed using the arcsine-square root transformation. After statistical analysis, data were back transformed to represent the actual means. In some instances, variance could not be homogenized successfully by transformation, and the data were reanalyzed using the Kruskal-Wallis test by ranks (soil pH, Mg, and Mg in Expt. 1 and soil SO₄-S in Expt. 2). When effects were significant, means were separated at the 5% level using Tukey's honestly significant difference test.

Table 2. Initial chemical and physical characteristics of soil and amendments (biochar and 4 biochar:1 bokashi) used in the study.

Characteristic	Soil	Biochar	Biochar-bokashi
pH	5.7	8.5	8.8
Organic matter (%)	1.6	20.5	31.3
NH ₄ -N (mg·kg ⁻¹)	1	<0.5	24
NO ₃ -N (mg·kg ⁻¹)	3	2	19
P (mg·kg ⁻¹)	88	60	673
SO ₄ -S (mg·kg ⁻¹)	26	12	29
K (meq/100 g)	1.12	1.93	7.68
Ca (meq/100 g)	1.85	1.52	1.97
Mg (meq/100 g)	0.37	0.37	2.16
B (mg·kg ⁻¹)	0.54	0.69	0.50
Cu (mg·kg ⁻¹)	2.4	0.8	1.1
Mn (mg·kg ⁻¹)	37	49	42
Zn (mg·kg ⁻¹)	2.0	2.1	6.7
Sand (%)	75.0	—	—
Silt (%)	14.7	—	—
Clay (%)	10.3	—	—

Results

Physicochemical properties of the soil, biochar, and biochar-bokashi amendments

Initial characteristics of the soil and amendments before planting. The soil used in the study was sandy and acidic with low organic matter content (Table 2). Available soil N was also low, but other extractable nutrients, including P, K, Ca, and Mg, were sufficient for production of highbush blueberry (Hart et al., 2006; Horneck et al., 2011). Biochar and biochar-bokashi, in contrast, were high in pH and organic matter, and the latter had much higher concentrations of $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, P, K, Mg, and Zn than the soil or biochar.

Characteristics of the soil after the final destructive harvest. After 12 weeks, soil pH averaged 4.6 and 5.3 in Expts. 1 and 2, respectively, and was slightly lower in unamended soil than in soil with 20% biochar or 10% or 20% biochar-bokashi in Expt. 2 (Table 3). These three amendments also increased soil organic matter in Expt. 1 and $\text{NO}_3\text{-N}$ and K in Expt. 2. Several other nutrients increased with biochar-bokashi in Expt. 2, including soil P and Zn, which were both greater with 20% of the amendment than with any other treatment, and soil Mg, which was greater with 10% or 20% of the amendment than in unamended soil.

The average concentration of most nutrients in the soil was similar between the two experiments (Table 3). However, soil $\text{NO}_3\text{-N}$ was much higher in Expt. 1 than in Expt. 2 ($\bar{X} = 87$ and $12 \text{ mg}\cdot\text{kg}^{-1}$ $\text{NO}_3\text{-N}$, respectively). Soil K was also higher in Expt. 1 ($\bar{X} = 0.50$ and $0.33 \text{ meq K per } 100 \text{ g}$ soil, respectively), whereas soil $\text{SO}_4\text{-S}$ was higher in Expt. 2 ($\bar{X} = 27$ and $44 \text{ mg}\cdot\text{kg}^{-1}$ $\text{SO}_4\text{-S}$, respectively).

Soil aggregates formed around many of the larger fragments ($> 1 \text{ mm}$ in length) of biochar in the pots (Fig. 1). The aggregates consisted primarily of silt and clay particles and were absent in the unamended soil.

Plant growth

Leaf area development. Leaf area increased over time in both experiments and was significantly affected by an interaction between the soil amendments and inoculation with *P. cinnamomi* ($P < 0.001$). In each case, leaf area was similar among the noninoculated treatments, until 10 to 11 weeks after transplanting, at which time leaf area was greater in plants grown with 20% biochar or 10% or 20% biochar-bokashi than in those grown with 10% biochar or no amendments (Fig. 2). In contrast, leaf area was unaffected by the soil amendments when the plants were inoculated with *P. cinnamomi*.

Plant dry weight. Shoot, root, and total dry weight of the plants were significantly affected by an interaction between the soil amendment treatments and inoculation with *P. cinnamomi* in Expts. 1 and 2

Table 3. Effects of soil amendments with biochar on pH, organic matter, and concentration of nutrients in soil planted with 'Legacy' blueberry in Expts. 1 and 2.^z

Soil amendment	Soil pH ^y	Organic matter (%)	NH ₄ -N			NO ₃ -N			P			SO ₄ -S ^y			K			Ca			Mg ^z	B			Cu			Mn ^y			Zn
			(mg·kg ⁻¹)			(mg·kg ⁻¹)			(mg·kg ⁻¹)			(mg·kg ⁻¹)			(mg·kg ⁻¹)			(mg·kg ⁻¹)				(mg·kg ⁻¹)			(mg·kg ⁻¹)						
Expt. 1																															
None	4.5	2.1 c ^x	3	74	121	27		0.48 b	3.3	0.55	0.58	2.7	101	1.8 b																	
10% biochar	4.2	2.3 bc	2	92	127	27		0.48 b	3.6	0.41	0.47	2.5	89	2.0 ab																	
20% biochar	4.8	2.5 ab	3	105	124	25		0.50 ab	3.7	0.56	0.50	2.4	74	2.0 ab																	
10% biochar	4.5	2.6 ab	3	110	135	29		0.54 a	4.3	0.64	0.48	2.8	89	2.3 a																	
-bokashi																															
20% biochar	4.8	2.7 a	3	121	156	25		0.52 ab	3.2	0.63	0.44	2.3	76	2.3 a																	
-bokashi																															
Significance	NS	**	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	*																	
Expt. 2																															
None	5.1 b	2.9	2	1 c	98 c	64		0.24 c	3.4	0.55 c	0.46	2.4	72	1.6 b																	
10% biochar	5.3 ab	2.5	2	9 bc	97 c	43		0.30 bc	3.7	0.64 bc	0.48	2.3	74	1.6 b																	
20% biochar	5.4 a	2.8	2	17 ab	100 bc	37		0.35 ab	4.6	0.66 bc	0.41	2.4	75	1.7 b																	
10% biochar	5.4 a	2.6	2	13 ab	113 b	38		0.36 ab	3.7	0.76 ab	0.40	2.2	78	1.8 b																	
bokashi																															
20% biochar	5.4 a	3.0	3	19 a	129 a	37		0.42 a	4.0	0.86 a	0.54	2.2	71	2.2 a																	
-bokashi																															
Significance	*	NS	NS	**	**	NS	NS	**	NS	**	NS	NS	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**			

^zSoil was collected during destructive harvest of the plants (12 weeks after transplanting), using three replicates per treatment (noninoculated treatments only) in both experiments.

^yMeans followed by the same letter within a column in each experiment are not significantly different at $P \leq 0.05$ (Tukey's test).

NS, *, **Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

($P < 0.001$). Without inoculum, plants in both experiments produced more total dry weight with 20% biochar or 10% or 20% biochar-bokashi than with 10% biochar or no amendment and had the greatest dry weight with 20% biochar in Expt. 1 and with 20% biochar-bokashi in Expt. 2 (Fig. 3). In contrast, dry weight was similar between plants grown with amendments and those grown with no amendment when the soil was inoculated with *P. cinnamomi*. Inoculation resulted in a high

percentage of red leaves on the plants and reduced total dry weight by an average of 38% in Expt. 1 and 71% in Expt. 2. None of the plants died during the experiments.

Leaf nutrient analysis

Macronutrients. The concentration of specific macronutrients in the leaves was affected by the soil amendments, as well as by inoculation by *P. cinnamomi* or interactions between the amendments and inoculation, in one or both of the experiments (Table 4). In many cases, the concentration of specific nutrients differed between plants grown in unamended soil and those grown in soil with biochar or biochar-bokashi. For example, regardless of inoculation, leaf K, Mg, and S were greater with 10% or 20% biochar-bokashi than with no amendment in Expt. 1, whereas leaf N and S were greater with most or all amendments than with no amendment in Expt. 2. Likewise, leaf P was greater with 20% biochar-bokashi, which contained a high amount of P (Table 2), than with any other treatment in Expt. 2.

Several leaf macronutrient concentrations were also greater, on average, when plants were grown in soil inoculated with *P. cinnamomi* than when they were not, including leaf P, K, and S in Expt. 1 and leaf N and K in Expt. 2 (Table 4). However, leaf P and, in some amendment treatments, Mg were lower with inoculation than with no inoculum in Expt. 2.

In general, the average concentration of N, P, K, and Ca in the leaves was higher in Expt. 1 than in Expt. 2, whereas the concentration of Mg and S in the leaves was higher in Expt. 2 than in Expt. 1 (Table 4). As mentioned, plants in Expt. 1 were fertigated weekly with a complete nutrient solution, while those in Expt. 2 were fertigated once every 4 weeks with ammonium sulfate (i.e., N fertilizer only).

Micronutrients. The concentration of micronutrients in the leaves was also affected by the soil amendments, inoculation, or their interaction in Expts. 1 and 2 (Table 5). For specific micronutrients, concentrations differed between plants grown in unamended soil and those grown with biochar or biochar-bokashi. For example, when the plants were noninoculated in Expt. 1, leaf B, Mn, and Zn decreased with 20% biochar or, in the case of B, 10% or 20% biochar-bokashi. Leaf B and Mn likewise decreased with 20% biochar (regardless of inoculation) or 10% biochar-bokashi (noninoculated plants only), respectively, in Expt. 2.

In many cases, inoculation with *P. cinnamomi* increased the concentration of B and Mn and reduced the concentration of Cu, Fe, and Zn in the leaves compared with no inoculum (Table 5). However, results varied, depending on the amendment. For example, in Expt. 1, inoculation resulted in higher concentrations of B and Mn when the plants were grown with 20% biochar-bokashi or 10% biochar, respectively, but had no effect when they were grown with the other amendments. Likewise, inoculation had no effect on leaf Cu or Zn when plants were grown with no amendment or with biochar-bokashi (10% or 20%), respectively, in Expt. 1, and led to a lower concentration of Fe in the leaves when plants were grown with 10% biochar-bokashi, as well as no effect with several amendments on leaf Fe, Mn, or Zn, in Expt. 2.

Root colonization by mycorrhizal fungi

Root colonization by ericoid mycorrhizal fungi averaged 1.5% before planting (data not shown) and, after 12 weeks, increased to $\leq 9\%$ when noninoculated plants were grown in unamended soil and $\geq 56\%$ when they were grown in soil with biochar or biochar-bokashi (Table 6). In both experiments, colonization was greater, on average, when a higher percentage of the amendments was added to the soil ($P < 0.05$) and when plants were grown with biochar-bokashi rather than biochar ($P < 0.01$).

Root infection by *P. cinnamomi*

Roots collected from the inoculated plants were heavily infected by *P. cinnamomi* (Table 7); however, infection was unaffected by the soil amendments in either experiment.

Fig. 1. Formation of aggregates (arrow) in soil amended with a woody biochar. A close-up of a large group of aggregates in soil with biochar is shown on the right. The soil was sandy and lacked structure without the biochar.

Fig. 2. Effects of a combination of soil amendments with biochar and inoculation with *Phytophthora cinnamomi* on leaf area development of 'Legacy' blueberry in Expts. 1 and 2. Asterisks indicate weeks in which leaf area differed significantly among the treatments at $P \leq 0.05$ (*) or $P \leq 0.01$ (**). Means followed by the same letter within a given week are not significantly different at $P \leq 0.05$ (Tukey's test).

None of the noninoculated plants were infected by the pathogen.

Discussion

In two experiments, biochar (alone or mixed with bokashi) increased plant growth relative to using unamended soil in highbush

blueberry. However, increases in growth varied among treatments and were dependent on the amendment type, the level of fertilization, and the rate at which the amendment was incorporated. When plants were fertigated with a complete nutrient solution (Expt. 1), the highest biomass and leaf area were obtained in soil with 20% biochar. Previous

studies indicate that biochar has the greatest influence on plant growth when it is applied in conjunction with fertilizers (Hossain et al., 2015; Meng et al., 2018; Schulz and Glaser, 2012; Zheng et al., 2015). Biochars derived from woody materials, such as conifers, generally have a high C:N ratio and low concentrations of available nutrients; therefore, they are not expected to act directly as a fertilizer source (Singh et al., 2010). This might explain why biochar did better with bokashi when the plants were fertigated with fertilizer solution containing only a N source (i.e., Expt. 2).

Regardless of the rate in which the amendments were applied, biochar and biochar-bokashi had little to no effect on soil pH in the present study. In fact, by 12 weeks after transplanting, soil pH was lower in each treatment than it was before planting. Increases in soil pH associated with the use of biochar are well documented and often correlated to the rate at which the biochar was applied (Molnár et al., 2016; Xu et al., 2012; Zhao et al., 2015); however, decreases in soil pH following the application of biochar have also been documented (Cornelissen et al., 2018; Lehman and Joseph, 2009). The influence that biochar has on soil pH depends on the liming equivalency of the amendment and the buffering capacity of the soil (Yuan and Xu, 2011). In our experiments, the acidifying effect of the ammonium fertilizer was likely greater than the liming effect of the biochar (Havlin et al., 2005; Robertson and Groffman, 2015). Likewise, van Zwieten et al. (2010) reported similar findings while investigating the effect of increasing rates of biochar and urea on wheat (*Triticum*

Fig. 3. Effects of a combination of soil amendments with biochar and inoculation with *Phytophthora cinnamomi* on shoot and root dry weight of 'Legacy' blueberry in Expts. 1 and 2. Similar letters within each inoculation treatment indicate shoot and root dry weight (lower-case letters) and total dry weight (upper-case letters) are not significantly different at $P \leq 0.05$ (Tukey's test).

Table 4. Effects of a combination of soil amendments with biochar and inoculation with *Phytophthora cinnamomi* on the concentration of macronutrients in leaves of 'Legacy' blueberry in Expts. 1 and 2.

Soil amendment	N (mg·g ⁻¹)		P (mg·g ⁻¹)		K (mg·g ⁻¹)			Ca (mg·g ⁻¹)			Mg (mg·g ⁻¹)			S (mg·g ⁻¹)	
	NI	I	NI	I	NI	I	Difference	NI	I	Difference	NI	I	Difference	NI	I
<i>Expt. 1</i>															
None	17.1 ab ^z		0.84 ab		7.8 b			3.1 ab	3.1 ab	0.0 ^{NS}	0.75 b			1.114 b	
10% biochar	16.8 ab		0.79 ab		8.6 ab			2.7 b	3.3 ab	-0.6*	0.75 b			1.170 b	
20% biochar	15.6 b		0.66 b		8.3 b			2.8 b	3.4 a	-0.6*	0.74 b			1.174 ab	
10% biochar-bokashi	18.1 a		0.87 ab		9.0 a			3.5 a	3.3 ab	0.2 ^{NS}	0.86 a			1.251 a	
20% biochar-bokashi	18.8 a		0.93 a		9.0 a			3.6 a	2.7 b	0.9**	0.89 a			1.312 a	
Avg			0.73	0.91	8.2	8.9								1.130	1.279
Significance															
Amendment	**		*		**			NS			**			**	
Inoculum	NS		**		**			NS			NS			**	
Amendment × inoculum	NS		NS		NS			**			NS			NS	
<i>Expt. 2</i>															
None	9.1 b		0.58 b		5.8 a	7.9 bc	-2.1**	2.6			1.23 a	0.93 a	0.30**	1.165 b	
10% biochar	11.4 a		0.63 b		5.2 a	5.8 d	-0.6*	2.6			1.12 a	0.93 a	0.19*	1.364 a	
20% biochar	10.4 ab		0.53 b		4.9 a	6.5 c	-1.6**	2.4			1.17 a	0.93 a	0.25**	1.311 a	
10% biochar-bokashi	11.1 a		0.59 b		5.7 a	7.8 b	-2.1**	2.5			1.06 a	0.97 a	0.08 ^{NS}	1.339 a	
20% biochar-bokashi	11.9 a		0.76 a		5.8 a	10.0 a	-4.2**	2.6			1.07 a	1.09 a	-0.02 ^{NS}	1.307 a	
Average	10.3	11.3	0.69	0.55	5.5	7.5		2.9	2.2		1.14	0.96		1.33	1.27
Significance															
Amendment	**		**		**			NS			NS			**	
Inoculum	**		**		**			**			**			**	
Amendment × inoculum	NS		NS		**			NS			**			NS	

^zMeans followed by the same letter within a column in each experiment are not significantly different at $P \leq 0.05$ (Tukey's test).

NI = noninoculated; I = inoculated with *P. cinnamomi*.

NS, *, **Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

Table 5. Effects of a combination of soil amendments with biochar and inoculation with *Phytophthora cinnamomi* on the concentration of micronutrients in leaves of 'Legacy' blueberry in Expts. 1 and 2.

Soil amendment	B ($\mu\text{g}\cdot\text{g}^{-1}$)			Cu ($\mu\text{g}\cdot\text{g}^{-1}$)			Fe ($\mu\text{g}\cdot\text{g}^{-1}$)			Mn ($\mu\text{g}\cdot\text{g}^{-1}$)			Zn ($\mu\text{g}\cdot\text{g}^{-1}$)		
	NI	I	Difference	NI	I	Difference	NI	I	Difference	NI	I	Difference	NI	I	Difference
<i>Expt. 1</i>															
None	62 a ^z	49 a	13**	6.7 a	7.0 a	-0.3 ^{NS}	42			148 a	123 ab	25*	10.6 a	14.9 a	-4.3**
10% biochar	52 ab	54 a	-2 ^{NS}	6.5 a	10.7 a	-4.2**	44			119 ab	148 a	-29*	8.8 ab	15.6 a	-6.8**
20% biochar	48 b	55 a	-7 ^{NS}	5.1 a	10.6 a	-5.5**	49			114 b	126 ab	-12 ^{NS}	7.8 b	12.4 a	-4.6**
10% biochar-bokashi	50 b	55 a	-5 ^{NS}	6.2 a	10.5 a	-4.3**	45			128 ab	129 ab	-1 ^{NS}	11.0 a	13.1 a	-2.1 ^{NS}
20% biochar-bokashi	48 b	56 a	-8*	6.2 a	10.7 a	-4.5**	48			129 ab	122 b	7 ^{NS}	11.1 a	12.2 a	-1.1 ^{NS}
Avg				6.1	9.9		42	49					9.9	13.6	
Significance															
Amendment	NS			*			NS			*			*		
Inoculum	NS			**			**			NS			**		
Amendment	**			**			NS			**			**		
× inoculum															
<i>Expt. 2</i>															
None	63 a			10.4			37 a	39 b	-2 ^{NS}	166 a	103 a	63**	10.9 a	13.4 a	-2.5**
10% biochar	53 ab			9.6			38 a	54 a	-16**	164 a	101 a	63**	11.5 a	11.9 a	-0.4 ^{NS}
20% biochar	51 b			9.0			34 a	39 b	-5 ^{NS}	135 ab	103 a	32*	10.5 a	11.5 a	-1.0 ^{NS}
10% biochar-bokashi	59 a			10.6			44 a	32 b	12*	127 b	102 a	25 ^{NS}	11.7 a	10.4 a	1.3 ^{NS}
20% biochar-bokashi	63 a			8.8			45 a	43 b	2 ^{NS}	140 ab	129 a	11 ^{NS}	11.7 a	12.0 a	0.3 ^{NS}
Avg	61	56		8.9	10.5					147	108				
Significance															
Amendment	**			NS			**			NS			NS		
Inoculum	**			*			NS			**			NS		
Amendment	NS			NS			**			*			*		
× inoculum															

^zMeans followed by the same letter within a column are not significantly different at $P \leq 0.05$ (Tukey's test).

NI = noninoculated; I = inoculated with *P. cinnamomi*.

NS, *, **Nonsignificant or significant at $P \leq 0.05$ or 0.01 , respectively.

Table 6. Effect of soil amendments with biochar on root colonization by mycorrhizal fungi in 'Legacy' blueberry in Expts. 1 and 2.^z

Soil amendment	Mycorrhizal colonization (%) ^y	
	Expt. 1	Expt. 2
None	6 d ^x	9 d
10% biochar	56 c	83 c
20% biochar	79 b	91 ab
10% biochar-bokashi	80 ab	87 bc
20% biochar-bokashi	85 a	94 a
Significance	**	**

^zNoninoculated plants only. Mycorrhizal colonization was not examined in plants inoculated with *Phytophthora cinnamomi*.

^yPercentage of total root length with mycorrhizal structures (internal hyphae and hyphal coils).

^xMeans followed by the same letter within a column are not significantly different at $P \leq 0.05$ (Tukey's test).

**Significant at $P \leq 0.01$.

Table 7. Effect of soil amendments with biochar on root infection by *Phytophthora cinnamomi* in 'Legacy' blueberry in Expts. 1 and 2.^z

Soil amendment	Root infection by <i>P. cinnamomi</i> (%) ^y	
	Expt. 1	Expt. 2
None	33	45
10% biochar	40	70
20% biochar	47	65
10% biochar-bokashi	37	60
20% biochar-bokashi	43	85
Significance	NS	NS

^zOnly plants inoculated with *P. cinnamomi* are included in the analysis. Infection was not observed in roots of the noninoculated plants.

^yPercentage of ten 1-cm-long root pieces infected by *P. cinnamomi*.

NS = nonsignificant.

aestivum L.) and radish (*Raphanus sativus* L.) in an acidic soil. In that case, soil pH was unaffected by biochar but declined with increasing rates of N application. Thus, if needed, acidifying fertilizers such as urea or ammonium sulfate could be used to moderate the liming effect of biochar on high-pH soils.

With the exception of $\text{NO}_3\text{-N}$ and K in one of the experiments (Expt. 2), biochar had no effect on soil nutrients. This was expected given that the woody materials from which the biochar was derived had low concentrations of many nutrients, including each macronutrient (Bollen, 1969). Consequently,

biochar only altered the concentration of a few nutrients in the leaves of blueberry. As expected, adding bokashi to the biochar increased the concentration of several nutrients in the leaves, including K, Mg, and S under complete fertigation (Expt. 1) and N, P, and S under limited fertigation (Expt. 2). Compared with biochar, bokashi was relatively high in nutrients and, therefore, a good replacement for fertilizer.

With or without bokashi, biochar increased both shoot and root growth relative to blueberry plants grown in unamended soil. Increased root growth with biochar has been reported in a number of crops, including cowpea [*Vigna minima* (Roxb.) Ohwi & H. Ohashi], wheat, and soybean [*Glycine max* (L.) Merr.] (Lehmann et al., 2003; Reyes-Cabrera et al., 2017; Solaiman et al., 2010). Blueberry has an extremely fine, fibrous root system and requires porous, well-drained soils for growth (Bryla et al., 2017). The physical structure of woody biochars increases pore space in soils and thereby could facilitate root penetration (Bruun et al., 2014). Based on visual observations in the present study, biochar appeared to increase aggregation of the sandy soil and, therefore, likely improved root penetration by blueberry. Absorption of nutrients by the biochar could also create nutrient patches, leading to more root growth, particularly when biochar is added with composted or fermented organic materials, such as bokashi (Hagemann et al., 2017).

Biochar and biochar-bokashi amendments also increased root colonization by ericoid mycorrhizal fungi by an average of 10-fold in the blueberry plants. Others have

reported similar increases in root colonization by arbuscular mycorrhizal and ectomycorrhizal fungi as a result of incorporating biochar into the soil before planting wheat (Solaiman et al., 2010) and larch [*Larix gmelinii* (Rupr.) Rupr.] seedlings (Makoto et al., 2010), respectively. Likewise, Duclos and Fortin (1983) reported increased root colonization of ericoid mycorrhizal fungi in lowbush blueberry (*Vaccinium angustifolium* Aiton) seedlings following the application of activated charcoal. In that case, the active carbon adsorbed toxic phenolic compounds in the rhizosphere. Excessive accumulations of such compounds in the rhizosphere might reduce the penetration of the hyphae into roots. In many ways, biochar is analogous to activated charcoal and, in fact, is known to absorb phenolic compounds that are potentially toxic to mycorrhizal fungi (Braghiroli et al., 2018; Hameed and Rahman, 2008). The highly porous nature of biochars also may provide a physical niche for mycorrhizal hyphae and offer physical protection against fungal grazers (Jaafar et al., 2014; Pietikainen et al., 2000; Warnock et al., 2007). Enriched nutrient patches from the biochar also could increase development (Cui and Caldwell, 1996), and temporary sorption and release of signaling compounds could amplify overall signaling in the rhizosphere and promote colonization in the roots in soil with biochar (Warnock et al., 2007).

Plants inoculated with *P. cinnamomi* developed root rot, regardless of whether the soil was amended with biochar or biochar-bokashi. Other studies have reported that soil pathogens, such as *Phytophthora* sp., were suppressed by low concentrations of biochar in the soil, but higher concentrations were ineffective or, in some cases, accelerated plant disease (Copley et al., 2015; Frenkel et al., 2017; Jaiswal et al., 2015; Zwart and Kim, 2012). Perhaps the rates of biochar used in the present study were too high to suppress development of *P. cinnamomi* in highbush blueberry. Biochars contain various types of organic compounds that are phytotoxic and suppress pathogens at lower dosages but could damage roots and increase susceptibility to disease at higher dosages (Bonanomi et al., 2015; Graber et al., 2014). Alternatively, the inoculation rate was high (2.7 cfu per cm³) and may have overwhelmed the ability of biochar to provide any disease suppression.

Results of this study indicate that biochar could be a suitable soil amendment for commercial production of highbush blueberry. Benefits under controlled conditions included more plant growth in soil with biochar than in unamended soil and much greater levels of root colonization by mycorrhizal fungi. Biochar also appeared to improve soil aggregation but had relatively little effect on soil pH and plant nutrition and no effect on root infection by *P. cinnamomi*. Addition of bokashi to the biochar improved plant growth and nutrition, particularly under nutrient-limited conditions. Our next step is to test biochar in a new field planting of highbush blueberry and identify the best method and

rate to apply it. Successful practices for using biochar will depend on plant response as well as the cost.

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