

AN ABSTRACT OF THE DISSERTATION OF

Bryan K. Sales for the degree of Doctor of Philosophy in Horticulture presented on June 10, 2019.

Title: Effects of Biochar and Nitrogen-Enriched Soil Amendments on Plant Growth, Mineral Nutrition, and Early Fruit Production in Highbush Blueberry (*Vaccinium* sp.)

Abstract approved: _____

David R. Bryla

The goal of the work in this dissertation was to identify alternative soil amendments to improve plant growth and yield during establishment of highbush blueberry (*Vaccinium* hybrid). Woody materials, such as sawdust and wood chips, have a high carbon to nitrogen (C:N) ratio and low water holding capacity, which can limit N availability and reduce the growth and fruit yield of highbush blueberry during establishment. This is the case in the Pacific Northwest where douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] sawdust is a readily available, low-cost source of organic matter. Growers incorporate additional fertilizer at planting to reduce N immobilization; however, this raises production costs and does not increase nutrient or soil moisture retention. The use of alternative materials such as biochar as a soil amendment could increase nutrient retention and soil moisture. Organic materials with a high C:N ratio are often used to adsorb excess N from water bodies, which increases N concentrations of the materials, allowing them to act as slow-release N fertilizers. These enriched materials could also serve as soil amendments. However, neither biochar nor N-enriched materials have been

used to grow blueberry. To address these issues, three studies were conducted from 2016 to 2018 in both greenhouse and field conditions in western Oregon. In the first study, we investigated the potential of using biochar, alone or in combination with bokashi, as a soil amendment for 'Legacy' blueberry. Bokashi is the decomposition of waste through fermentation. We found that biochar increased plant growth when fertilized weekly with a complete fertilizer (30N–10P–10K) and 600 ppm ammonium sulfate once a month. However, bokashi was more beneficial for plant growth when nutrients were limited. Biochar did not suppress infection by *Phytophthora cinnamomi* Rands, but it increased root colonization by ericoid mycorrhizal fungi. In the second study, a 2-year field experiment was conducted to determine whether amending soil with biochar or biochar and bokashi alters growth and early fruit production during establishment of 'Duke' blueberry. Plants grown in soil amended with biochar grew more in the first season, resulting in greater yield in the second season than those grown with no amendments or in soil with sawdust only. In contrast, sawdust limited plant available N, resulting in N-deficient plants. Furthermore, adding biochar to the planting hole was considerably more economical than applying it to the row and was more economical than the industry standard of incorporating sawdust in the row. In the third study, red alder (*Alnus rubra* Bong.) sawdust, douglas fir sawdust, shavings, and wood chips, biochar, and yard-debris compost were untreated or enriched with ammonium-N and evaluated as soil amendments in 'Duke'. Plants grown with enriched amendments had greater shoot dry weight and a greater concentration of N in the leaves than those grown in unenriched amendments, regardless of whether or not they received additional N fertilizer. Overall, amending soil with biochar or N-enriched organic materials appear to be a promising

means for improving plant growth, mineral nutrition, and early fruit production in highbush blueberry.

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Effects of Biochar and Nitrogen-Enriched Soil Amendments
on Plant Growth, Mineral Nutrition, and Early Fruit Production in Highbush Blueberry
(*Vaccinium* sp.)

by
Bryan K. Sales

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Bryan K. Sales, Author

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CONTRIBUTION OF AUTHORS

Dr. David Bryla was involved in the experimental design, statistical interpretation, and writing of all the chapters of this document. Dr. Dan Sullivan was involved in the experimental design of chapters 2, 3, and 4. Carolyn Scagel was involved in the experimental design and data interpretation of chapters 2, 3, and 4. Bernadine Strik was involved in the experimental design of chapters 2, 3, and 4. Kristin Trippe was involved in the experimental design of chapters 2, 3, and 4. All of the committee members provided help in editing and writing this document.

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DEDICATION

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Chapter 1 – General Introduction

1.1. Production and Physiology of Highbush Blueberry

World production of blueberry (*Vaccinium* sp.) increased by 181,000 t between 2012 and 2016 with an estimated total production of 635,000 t (Brazelton et al., 2018). Production is set to increase even more, with an estimated total of 102,000 planted hectares, essentially doubling the area of planted blueberry between 2008 and 2016 (Brazelton et al., 2018). Increased production is driven, in part, by the health benefits associated with blueberry consumption (Forney and Kalt, 2011). North America is the world's leading producer of blueberry, with the majority of production occurring in the United States (Gallardo et al., 2018). In 2017, Washington and Oregon were the highest producers of blueberry in the United States at 530,000 and 490,000 t of fruit, respectively (U.S. Department of Agriculture National Agriculture Statistics Service, 2017).

Highbush blueberry (*V. corymbosum* L.), a long-lived perennial shrub of the Ericaceae family, is native to the eastern and northeastern United States (Retamales and Hancock, 2018). Plants are adapted to well-drained, acidic soils with high amounts of organic matter. However, blueberry can be grown in a wide variety of soil textures with the addition of organic amendments that improve nutrient retention, drainage, and soil moisture retention (Strik et al., 1993). The plants have a shallow, fibrous root system lacking root hairs and form associations with ericoid mycorrhizal fungi, which aid in nutrient uptake (Cheng et al., 2012).

Ericoid mycorrhizal fungi are symbionts that colonize roots of blueberry and other plant species from the Ericaceae family (Smith and Read, 2008). The fungi utilize photosynthates from the host plant and form extensive networks of hyphae, which allow the plants to explore larger volumes of soil and improve absorption of nutrients

(Jeliazkova and Percival, 2003). The relationship has been found to improve plant growth and survival in highbush blueberry without additional fertilizers (Scagel, 2005).

However, soil nutrient availability is known to affect mycorrhizal colonization rates (Scagel and Yang, 2005). Therefore, higher mycorrhizal colonization rates are reported in sandy soils with low nutrient availability (Sadowsky et al., 2012).

The recommended soil pH for highbush blueberry is between 4.5-5.5 (Retamales and Hancock, 2018). Plants grown in soil with a high pH suffer from iron (Fe) deficiency, which can result in the yellowing of leaves, premature leaf drop, stunted growth, and, in severe cases, plant death (Polashock et al., 2017). Soil pH is more difficult to adjust after planting, and plants stunted by high soil pH usually recover slowly, if at all. Blueberry is also sensitive to high soil salinity (Machado et al., 2014). High concentrations of soluble salts in the soil create osmotic gradients that inhibit plant uptake of water and nutrients. Excessive salts, naturally or from fertilizers, can result in slow plant growth and plant death (Bryla and Machado, 2011).

Ammonium-nitrogen ($\text{NH}_4\text{-N}$), the predominant species of N found in low pH soils, is the primary form of N acquired by blueberry (Alt et al., 2017; Darnell and Hiss, 2006). Consequently, ammonium sulfate and urea are the predominant fertilizers applied to blueberry (Bryla and Strik, 2015). Both can be applied as a granular or liquid, but generally, but generally plants fertigated with liquid sources of the fertilizers are more productive than those grown with granular sources (Vargas and Bryla, 2015).

Ammonium sulfate is twice as acidifying as urea and, therefore, is recommended when soil pH is > 5.5 ; urea, on the other hand, is recommended when pH is < 5.0 (Hart et al., 2006).

Traditionally, flower buds were removed during winter pruning to prevent fruit production during the first 2 years after transplanting (Strik and Buller, 2005). However, recent trends indicate that growers are harvesting in the second season to get an earlier return on their investments (Julian et al., 2011; Strik et al., 2017). In order to harvest in the second season, without over stressing the plants, rapid plant growth in the first season is essential. Larco et al. (2013) reported a positive correlation between aboveground plant growth during the first year after transplanting and yield in the second year. Therefore, cultural practices that increase plant growth in the first season will likely increase the fruit yield in the following year or more.

Acidic plant materials, such as sawdust and bark, are applied both as a pre-plant soil amendment and as a mulch (Strik et al., 1993). However, due to an increase in demand, these materials are becoming more expensive and limited in supply. In the Pacific Northwest, sawdust from douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] trees is the most common soil amendments used to grow blueberry (Hart et al., 2006). Douglas fir sawdust has a low pH (4.0) and is low in soluble salts. As a result, plants often grow well in soils amended with the sawdust. However, unless is it well aged and partly composted, it tends to have a high carbon to nitrogen (C:N) ratio (>400), which can immobilize N during decomposition and limit plant available N (Sarker et al., 2018). Therefore, it is recommended to apply 5 lbs. N per unit of sawdust applied, which is equivalent to \approx 95 lbs. N per acre (Hart et al., 2006). Furthermore, soils amended with douglas-fir sawdust have been found to reduce availability of soil moisture during blueberry establishment (White, 2006). Thus, while douglas fir sawdust may be beneficial to blueberry in the long-term, it can limit plant growth during establishment.

Furthermore, when the sawdust is used as mulch, it decomposes rapidly and must be replenished every 2 years or so, which significantly increases production and labor costs (Julian et al., 2011).

Alternative soil amendments, such as peat moss and some composts can be used to grow blueberry. Peat moss has chemical characteristics, such as low pH and high cation exchange capacity (CEC), that are ideal for blueberry production (Spiers, 1986). However, peat is costly, a finite resource, and not considered a sustainable production practice (Sendi et al., 2013). Many composts, on the other hand, have high pH and salinity and, therefore, are unsuitable for blueberry (Strik et al., 2017; Sullivan et al., 2014). Costello et al. (2019) recently determined that highbush blueberry responds best to composts produced from woody materials (Costello et al., 2019). Typically, composts derived from woody materials have lower pH and less salts than those derived from animal manures and green plant material; however, they also tend to contain less nutrients (Hargreaves et al., 2008).

1.2. Amending Soil with Biochar

Biochar, a highly carbonaceous by-product of bioenergy production through pyrolysis or degasification of agricultural waste under low oxygen conditions, has gained attention as both a carbon sequestration strategy and potential soil amendment for crop production (Gluszek et al., 2017; Lehmann, 2009). The material can be produced from any form of organic matter, resulting in a wide range of production regions. In general, biochar is more chemically and biologically stable than the organic matter from which it was made (Spokas, et al., 2010) and, therefore, can persist in soil for decades to millennia

(Glaser et al., 2001). It also has many of the physiochemical characteristics associated with peat moss, including high CEC and porosity and low bulk density (Vaughn et al., 2015; Kern et al., 2017). Evidence to date suggests that biochar is a suitable replacement for peat moss in container production of marigold, sunflower, and tomato (Steiner and Hartung 2014; Vaughn et al., 2015). However, unlike peat moss, biochar can potentially provide benefits throughout the life of the planting.

The feedstock and temperature used to create the biochar drives the physical and chemical characteristics of the biochar (Harvey et al., 2011; Uchimiya et al., 2012). Biochars produced from nutrient-rich feedstocks such as manures will result in a higher nutrient content than those produced from low-nutrient feedstocks, such as woody materials. Additionally, as production temperature increases, easily decomposed compounds and elements (O, H, N, S) are volatilized, and C exists in thermally fixed carbon structures (Kim et al, 2012). Therefore, C becomes less labile, and plant-available nutrients exist in the ash portion in the form of salts (e.g., KOH, NaOH, MgCO_3 , CaCO_3 , and organic metal salts) (Cao and Harris, 2010). Biochar typically has a high pH (7-10), well above the recommended range for blueberry. However, the concentration of CaCO_3 in the biochar, buffering capacity of the soil, and acidifying nature of the fertilizer determine the influence biochar has on soil pH (Kloss et al, 2012).

The application of biochar to soil has been shown to improve soil conditions such as nutrient and moisture retention, moderate soil pH, and alter microbial activity; in some cases, it has also been found to inhibit soil-borne pathogens (Chan et al., 2007; Ippolito et al., 2012; Sohi et al, 2010; Van Zwieten et al., 2010). Alleviation of soil constraints with biochar has significantly improved crop production in “problem soils” that have low

fertility (Van Zweiten et al., 2010). However, in fertile soils associated with temperate climates, where these constraints are less prevalent, the role of biochar for optimization of plant growth is less clear (Jeffrey et al., 2017).

Biochar has been described as having a synergistic effect with other forms of organic soil amendments on plant growth (Bonanomi et al., 2017). Jones et al. (2016) reported that biochar added to soil in conjunction with compost increased growth of sunflower (*Helianthus annuus* L.) more than biochar or compost alone. The mechanisms proposed to explain the synergism between biochar and compost are increased plant-available nutrients and soil moisture, improved soil structure, and greater populations of beneficial microbes (Lui et al., 2012; Sanchez-Garcia et al. 2016; Schulz and Glaser, 2012). Kammann et al., (2015) found that biochar added to active compost piles becomes highly enriched with nutrients and dissolved organic C, which are slowly released into the soil. Schimmelpfenning et al. (2014) reported a 30% growth increase of ryegrass (*Lolium perenne* L.) when biochar was applied with pig slurry. In this case, increased growth response was attributed to increased N retention as a result of reduced gaseous N losses.

Biochar, used in conjunction with lacto-fermented organic materials, such as bokashi, has been shown to increase the plant growth of corn (*Zea mays* L.) (Andreev et al., 2016). Bokashi is produced using a Japanese technique in which organic matter is fermented. Fermentation of organic matter (usually flour or bran) is initiated with the addition of effective microorganisms, predominantly, *Lactobacillus* sp. (anaerobic bacteria), *Rhodopseudomonas* sp. (phototropic bacteria), *Saccharomycetes* sp. (yeast), actinomycetes, and various forms of filamentous fungi (Xu, 2001). Bokashi can be

produced from a wide range of agricultural wastes that can be prepared *in situ* at very low cost. Biochar can be added during fermentation or combined with bokashi following fermentation.

The cost of biochar differs by region and is directly related to the cost of the feedstock, which is a function of its market value for other uses and the cost of collection and transportation. Campbell et al. (2018) reported the price of biochar produced from forest biomass to range from \$71/ton to \$2512/ton, with an average cost of \$1292/ton. Estimates assume a fixed cost of wood chips at \$40/ton. However, feedstocks procured from a waste biomass stream could be available for free, significantly reducing the cost of the biochar. In addition, the adoption of policies on a regional and federal level, such as *carbon tax credits*, has potential to reduce the production cost of biochar (Campbell et al., 2018). Furthermore, combining biochar with less expensive forms of organic matter and investigating alternative incorporation strategies could make it more cost effective. For example, applying biochar directly to the root zone at planting could reduce the amount of biochar needed in a field compared to incorporating the biochar ?.

1.3. Nitrogen-enriched Soil Amendments

The adsorption of $\text{NH}_4\text{-N}$ onto organic materials, such as sawdust, compost and biochar, have been found to be an effective and low-cost method to remove N from air and water waste streams (Harmayani and Anwar, 2016; Kizito et al., 2016; Zarabi and Jalali, 2018). The use of these materials as soil amendments for crop production is a way to recycle the N while improving soil structure. Ammonium adsorption onto organic materials occurs through electrostatic retention, ion exchange, physical entrapment, and

interparticle diffusion which can result in swelling (Kizito et al., 2016; Rafatullah et al., 2010; Yu et al., 2016). Lignocellulosic materials, such as sawdust, mainly consist of lignin and cellulose, which contain functional groups such as alcohol, ketone, and carboxylic groups. These groups are natural ion-exchangers and H-bonding materials, which adsorb $\text{NH}_4\text{-N}$ onto the surface of sawdust (Wahab et al., 2010). However, adsorption of $\text{NH}_4\text{-N}$ onto lignocellulosic materials is not pH dependent, suggesting that sawdust adsorbs $\text{NH}_4\text{-N}$ through inter-particle diffusion, which results in a swelling of the materials (Jellali et al., 2011). In contrast, compost and biochar have a higher density of negatively charged functional groups than lignocellulosic materials, which increases their potential to adsorb $\text{NH}_4\text{-N}$ (Mao et al., 2018). However, not all of the $\text{NH}_4\text{-N}$ adsorbed by biochar is displaced by KCl extraction, indicating physical entrapment of N by the micropores in the biochar (Saleh et al., 2012).

Currently, there is no research investigating the effects of how soil amended with ammonium-enriched sawdust or compost alter plant growth. However, studies have reported an increase in plant growth and N uptake of plants grown in soil amended with ammonium-enriched biochar (Kocatürk-Schumacher et al., 2018; Xu et al., 2018). Chen et al., (2018) reported that cabbage (*Brassica chinensis* L.) grown in soil amended with enriched biochar produced 88% more aboveground biomass than those grown in soil with untreated biochar. Taghizade-Toosi et al. (2012) found that $\text{NH}_4\text{-N}$ adsorbed to biochar from ruminant urine increased N concentration of the shoots and roots of ryegrass.

Nitrogen enrichment of current and novel organic materials also have potential to benefit blueberry production. For example, enrichment of douglas fir sawdust with $\text{NH}_4\text{-N}$ could reduce the C:N ratio, reducing N immobilization and resulting in decreased need

for N fertilizer applications. Biochar also has a high C:N ratio but, unlike sawdust, provides little C and, therefore, has a lower potential for microbial immobilization of N. Biochars enriched with $\text{NH}_4\text{-N}$ are well positioned to act as a slow-release N fertilizer for blueberry (Chen et al., 2018). Yard debris compost has been found to be low in soluble salts and more suitable than other composts for blueberry production (Costello et al., 2019). However, this compost tends to contain a lot of woody material and, therefore, is usually low in N and other nutrients. Enrichment with $\text{NH}_4\text{-N}$ could be useful for increasing the N concentration of yard debris compost.

1.4 Study objectives

The goal of the work in this dissertation was to identify alternative soil amendments to improve plant growth and yield during establishment of highbush blueberry. First, a set of 12-week experiments were conducted in a glasshouse to evaluate the use of biochar as a soil amendment. Plants were grown in soil amended with biochar alone or in combination with bokashi at rates of 10% and 20%, by volume, and compared to those grown in soil only. Next, biochar was tested with or without bokashi under field conditions in a new planting. In this case, the use of biochar was evaluated for 2 years and compared to the conventional practice of incorporating douglas fir sawdust in the row or using soil only. Finally, another 12-week study was conducted in a glasshouse to evaluate the potential of using ammonium-enriched organic materials as soil amendments for production of blueberry. The materials included red alder (*Alnus rubra* Bong.) sawdust, douglas fir sawdust, shavings, and wood chips, biochar, and yard-debris compost. The results of this work contribute new knowledge pertaining to the use of

untreated and ammonium-enriched soil amendments such as biochar in temperate climates for fruit production.

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**Chapter 2 – Amending a Sandy Soil with a Woody Biochar
Promotes Plant Growth and Root Colonization by Ericoid Mycorrhizal Fungi
in Highbush Blueberry**

2.1. Abstract

Biochar is known to improve soil conditions and to suppress infection by soil-borne pathogens but its use as 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 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 and irrigated twice per week, while those in the second experiment were fertilized once a month with ammonium sulfate and irrigated three times per week. In both cases, the plants were grown in 4-L pots filled with soil (sandy loam) only or with soil amended at rate of 10% or 20% by volume with biochar or a 4:1 mix of biochar and bokashi. Half of the plants in each soil treatment were then inoculated with *Phytophthora cinnamomi* Rands which causes root rot in blueberry. In the absence of *P. cinnamomi*, plants amended with 20% biochar or 10% or 20% biochar-bokashi blend had greater leaf area and 30% to 70% more total dry weight than those amended with 10% biochar or unamended soil only. The biochar amendments also increased soil aggregation and root colonization by ericoid mycorrhizal fungi. The percentage of roots colonized by mycorrhizal fungi was $\leq 10\%$ in soil only and averaged 54% to 94% with the amendments. Plants inoculated with *P. cinnamomi* were stunted and showed typical symptoms of root rot. Root infection by the pathogen was unaffected by either rate of 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 highbush blueberry, but it may not suppress phytophthora root rot.

2.2 Introduction

Blueberry (*Vaccinium* sp.) is in the family Ericaceae, which is adapted to well-drained acidic soils with a high amount of organic matter (Retamales and Hancock, 2018). Organic materials such as bark and sawdust are often incorporated into the soil prior to planting a new field of blueberry, and in many cases are used as mulch afterwards. These materials are used to increase soil organic matter without increasing soil pH. However, these materials are becoming increasingly more expensive and limited in supply (Larco et al., 2013). As a result, growers are interested in alternative strategies to improve soil conditions for 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 at temperatures typically $< 700\text{ }^{\circ}\text{C}$ (Lehmann and Joseph, 2009). Essentially, any form of biomass can be converted to biochar, but the most preferable forms 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., 2017). Once applied, a large fraction of the biochar is recalcitrant and can persist in soil for decades to millennia (Lehmann and Joseph, 2009). Carbon substrates produced by this method tend to have high ion-exchange capacities (cation and anion) and, when added to soil, improve porosity and aeration and increase retention of water and nutrients (Knowles, 2011; Nemati, 2015; Sohi et al., 2010).

Plant growth and productivity has 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 (Bird et al., 2008; LeCroy et al., 2012; Lehmann et al., 2011; Solaiman et al., 2010), and suppress development of soilborne pathogens, including *Phytophthora cinnamomi* Rands (Zwart and Kim, 2012), which is commonly associated with root rot in highbush blueberry (Yeo et al., 2016).

Benefits of biochar can be further enhanced by combining it with other forms of organic material, including bokashi (Sanchez-Garcia et al., 2016; Schulz and Glaser, 2012). Bokashi is produced by fermentation of organic matter (usually flour or bran) and utilizes an inoculum of microorganisms to improve soil health called EM or “effective microorganisms” (Boechat et al., 2013). Typically, the inoculum contains species that are naturally occurring in soils and include *Lactobacillus* sp. (anaerobic bacteria), *Rhodopseudomonas* sp. (phototropic bacteria), *Saccharomycetes* sp. (yeast), actinomycetes, and various forms of filamentous fungi (Xu, 2001). Many of these microorganisms are considered antagonistic and capable of inducing systemic resistance in plants to various pathogens, including ‘*Candidatus phytoplasma solani*’ on periwinkle and bacterial blight on guava fruits (Pierce et al., 2016; Rezende et al., 2008). The pores in biochar provide good 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. We hypothesized these products would increase crop growth and nutrition, provided there was minimal or no increase in soil pH. Experiments were conducted in a glasshouse using soil that was either infested or not with *P. cinnamomi*. The amendments were incorporated at two different rates, including 10% and 20%, by volume, and compared to soil only (unamended soil).

2.3. Materials and Methods

Two 12-week experiments were conducted side-by-side on February 15, 2016 in a heated glasshouse located at the USDA-ARS Horticultural Crops Research Unit in Corvallis, OR (lat. 44°34'3" N, long. 123°17'9" W). In both experiments, biochar or a blend of 4 biochar : 1 bokashi (by volume) were each incorporated into soil at rates of 10% or 20%, by volume, and compared to un-amended soil. Half of the plants from each of these treatments were inoculated with *Phytophthora cinnamomi* to determine if biochar or biochar-bokashi provided any disease suppression against the pathogen. 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. The lamps were suspended ≈ 1.5 m above the canopy of the plants.

Soil and amendments. The soil used in the experiments was a Lynden sandy loam (sandy, mixed, mesic Typic Haplorthods) collected from a commercial blueberry field in

Whatcom County, WA. The soil was air-dried for a week in the glasshouse and sieved through a 2-mm-mesh screen to breakup large aggregates and remove rocks and debris.

Biochar and biochar-bokashi were purchased from BioLogical Carbon, LLC (Philomath, OR). The biochar was produced from hog fuel, a mixture of coarse bark chips and wood fiber from douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] trees, using gasification at 700–800 °C. The bokashi was a bran fermented with EM-1 as recommended by the manufacturer.

A 1-kg sample of the soil and each amendment (Biochar and Biochar-bokashi blend) was screened using a 2-mm sieve and sent to a commercial laboratory (Brookside Laboratories, New Bremen, OH) for initial analysis of pH, organic matter content, cation exchange capacity (CEC), nutrients, and texture (soil only).

Inoculum production. 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 of 5% to 7.5% vegetable juice (V-8 juice; Campbell Soup Co., Camden, NJ), by volume, and 1 g·L⁻¹ of CaCO₃. 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). Vermiculite bags for controls were used and inoculated with agar plugs with no pathogen. The isolate was obtained in 2010 from a ‘Draper’ blueberry plant that was infected naturally in a field located in Corvallis, OR (Vargas et al., 2015) and was used previously by Yeo et al. (2016). Each bag was incubated in the dark at 20 °C and shaken at 2-week intervals. After 4 weeks, the contents of each bag

were consolidated and homogenized in a cement mixer, weighed, and allowed to air dry. The inoculated vermiculite was separated into five different bags, each containing 416 g of inoculated vermiculite. How was pathogen enumerated?

The soils for each treatment were mixed in volumes large enough to satisfy the requirements for both experiments. A volume of 72 L of soil was combined with 8 L of biochar or biochar-bokashi to produce a mix with 10% of either amendment, and 64 L of soil was combined with 16 L of the amendments to produce mixes with 20%. An additional 80 L of soil was unamended and served as a “soil only” treatment. Homogeneity was achieved by mixing each treatment in a cement mixer for 30 min. After mixing, half of the soil from each treatment was combined with a bag of the inoculated vermiculite to achieve 100 propagules of *P. cinnamomi* per g soil or soil mix. The other half of each soil or soil mix was combined with untreated vermiculite to serve as a control for inoculation. To avoid cross contamination, the non-inoculated treatments were mixed in the following order: soil only, 10% biochar, 20% biochar, 10% biochar-bokashi, and 20% biochar-bokashi. The inoculated treatments were mixed in the same order, and the mixer was sanitized with 10% bleach solution, by volume, after each mixing to prevent cross contamination.

Experimental design. One hundred ‘Legacy’ blueberry plants were obtained from a commercial nursery as 1-year-old liners. The plants were transplanted into 4-L plastic pots (one plant per container) filled with soil or soil amended with 10% or 20% biochar or biochar-bokashi. Each pot was mulched with a 2.5-cm-layer of douglas fir sawdust to reduce evaporation and prevent moss and algae growth on the soil surface. All plants were actively growing at the time of transplanting. Six plants per treatment were assigned

to Expt. 1 and four plants per treatment were assigned to Expt. 2. Both experiments were laid out on the same greenhouse bench in a completely randomized design at a spacing of 0.1 m between the plants and 1 m between the two experiments.

Plants in Expt. 1 were fertilized weekly with 100 mL of a complete nutrient solution (30N–10P–10K) (Miracle-Gro Water Soluble Azalea, Camellia, Rhododendron Plant Food; The Scotts Company, Marysville, OH), while those in Expt. 2 were fertilized once every 4 weeks with 100 mL of liquid ammonium sulfate solution (9N–0P–0K) mixed with water at a rate of 600 mg·L⁻¹ N. The plants in Expt. 1 were also irrigated twice a week during the first 6 weeks after transplanting and three times per week during the following 6 weeks, while those in Expt. 2 were irrigated three times per week throughout the length of the study.

To promote infection by *P. cinnamomi*, the plants in 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 between flooding events (Weiland et al., 2018).

Measurements. Leaf area was estimated weekly 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).

All plants from both experiments were harvested destructively at 12 weeks after transplanting. First, a 1-kg sample of soil without roots was collected from each pot and

later air-dried, ground to pass through a 1-mm sieve, and sent to the same commercial laboratory that was used earlier for analysis of pH, organic matter, and nutrients. Next, leaves were removed by hand and measured for leaf area using a portable leaf area meter (model LI-3000C; Li-Cor Biosciences, Logan, UT). Stems were then cutoff at the soil surface, and roots were washed from the soil and rinsed under running water. Two random sample of roots (1–2 g fresh weight each) were taken from the plants and placed in 50-mL tubes for later analysis of colonization by ericoid mycorrhizal fungi and infection by *P. cinnamomi*. Finally, the leaves, shoots, and remaining roots were dried for at least 48 h at 60 °C and weighed.

Dried leaves, shoots, and roots were ground to pass through a 1-mm sieve and analyzed for N using a combustion analyzer (model TruSpec CN; Leco Corp., St. Joseph, MI) and for P, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn using an inductively coupled plasma optimal emission spectrophotometer (model Optima 3000DV; Perkin Elmer, Wellesley, MA) after microwave digestion with 70% (v/v) nitric acid (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 of all plants 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). The roots were then 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 colonization was recorded.

Roots collected for analysis of *Phytophthora* were rinsed in 1% bleach solution (0.05% NaOCl) for 30 s, and five 1-cm-long pieces from each sample were placed onto petri plates filled with P₁₀ARPH agar, a semi-selective medium for *Phytophthora* sp. (Tsao and Guy, 1977). The agar contained 10 ppm pimarinic acid (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). The plates were incubated at 20 °C for 10 d and monitored periodically under a microscope (0) to determine the percentage of the root sections infected by *P. cinnamomi*.

Statistical analyses. All 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). To homogenize variance, percent root infection and mycorrhizal root colonization were transformed using arcsine of the square root of the proportion. All transformed data was back transformed to represent the actual means. Fixed effects in the model included amendment type and inoculation treatment. For analysis on repeated measurements (manual measurement of leaf area), the fixed effects were amendment, inoculation, and week. When effects of the 5 × 2 factorial design were significant, means were separated at the 5% level using Tukey's honestly significant difference test.

2.4. Results

Physical and chemical properties of the soil, biochar, and biochar-bokashi amendments

Initial characteristics of the soil and amendments prior to planting. The soil used in the study was sandy and acidic with low organic matter content and low cation exchange capacity (Table 2.1). Nitrogen was also low in the soil, but other 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. The high N concentration in the bokashi resulted in a low C:N ratio in the biochar-bokashi blend, unlike the biochar, which had a high C:N ratio.

Characteristics of the soil after the final destructive harvest. After 12 weeks, soil pH averaged 4.6 and 5.3 in Expt. 1 and 2, respectively, and was slightly lower in unamended soil than in soil with 20% biochar or 10% or 20% biochar-bokashi in the Expt. 2 (Table 2). These latter treatments also increased soil organic matter in Expt. 1 and soil $\text{NO}_3\text{-N}$ and K in Expt. 2 (Table 2.2). Several other nutrients were likewise affected by 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 after 12 weeks between the two experiments (Table 2.2). 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; $P < 0.001$). Soil K

was also higher in Expt. 1 ($\bar{X} = 0.50$ and 0.33 meq K per 100 g soil, respectively; $P < 0.05$), while soil $\text{SO}_4\text{-S}$ was higher in Expt. 2 ($\bar{X} = 27$ and 44 mg·kg⁻¹ $\text{SO}_4\text{-S}$, respectively; $P < 0.01$).

Soil aggregates formed around many of the larger fragments (1–2 mm in length) of biochar in the pots (Fig. 2.1). The aggregates consisted primarily of silt and clay particles and were absent in soil without biochar or biochar-bokashi.

Plant growth

Leaf area development. In both experiments, leaf area increased over time ($P < 0.001$) 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 non-inoculated treatments, until 10–11 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. 2.2). 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 Expt. 1 and 2 ($P < 0.001$). Non-inoculated plants produced more dry weight with 20% biochar or 10% or 20% biochar-bokashi than when grown in unamended soil or 10% biochar in both experiments. In addition uninoculated plants had the greatest dry weight with 20% biochar in Expt. 1 and with 20% biochar-bokashi in Expt. 2 (Fig. 2.3). In contrast, dry weight was similar among plants in all amendment treatments when the plants were inoculated with *P. cinnamomi*. Plants grown in soil

inoculated with *P. cinnamomi* in Expt. 1 and Expt. 2 had 38 and 71% lower dry weights respectively.

Leaf nutrient analysis

Macronutrients. The concentration of each macronutrient 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 2.3). For specific macronutrients, the concentrations differed between plants grown in unamended soil and those with biochar or biochar-bokashi. For example, compared to plants grown in unamended soil, leaf K, Mg, and S concentrations were greater with 10% or 20% biochar-bokashi in Expt. 1, and leaf N and S concentrations were greater with all but 20% biochar or any amendment, respectively, in Expt. 2. Leaf P was also greater with 20% biochar-bokashi than with any other treatment in Expt. 2, which was probably due to the high amount of P in the bokashi (Table 2.1).

Several leaf nutrient concentrations were also greater, on average, when plants were 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 2.3). However, leaf P and, in some amendment treatments, Mg were lower in inoculated plants compared to non-inoculated plants in Expt. 2.

On average, leaf N, P, K, and Ca concentrations were greater in Expt. 1 than in Expt. 2, while leaf Mg and S were greater in the second experiment ($P < 0.05$).

Micronutrients. As with the macronutrients, the concentration of micronutrients in the leaves was also affected by the soil amendments, inoculation, or their interaction in

Expt. 1 and 2 (Table 2.4). For specific micronutrients, concentrations differed between plants grown in unamended soil and those grown with biochar or biochar-bokashi. For example, compared to non-inoculated plants grown in unamended soil in Expt. 1, leaf B, Mn, and Zn were lower with 20% biochar or, in the case of B, 10% or 20% biochar-bokashi. Leaf B and Mn were likewise greater for plants grown in soil only than with 20% biochar or 10% biochar-bokashi, respectively, in Expt. 2.

In many amendment treatments, plants inoculated with *P. cinnamomi* had lower leaf concentrations of B and Mn and higher concentrations of Cu, Fe, and Zn (Table 2.4). However, results varied, depending on the amendment. For example, compared to non-inoculated plants in Expt. 1, inoculated plants had greater concentrations of B and Mn when the plants were grown with 20% biochar-bokashi or 10% biochar, respectively, but not with the other amendments. Likewise, inoculation had no effect on leaf Cu and Zn when plants were grown in soil only or biochar-bokashi (10% or 20%), respectively. In Expt. 1, it led to a lower concentration of Fe when plants were grown with 10% biochar-bokashi (as well as no effect in several cases on Fe, Mn, or Zn) in Expt. 2.

The average concentration of micronutrients in the leaves was similar between Expt. 1 and 2 ($P > 0.05$).

Root Colonization by Mycorrhizal Fungi

Root colonization by ericoid mycorrhizal fungi averaged 1.5% prior to planting (data not shown) and, after 12 weeks, increased to only 9% or less when the plants were grown in unamended soil and $\geq 56\%$ when plants were grown in soil with biochar or biochar-bokashi (Table 2.5). In both experiments, colonization was greater, on average,

when a higher percentage of the amendments were added to the soil (i.e., 20%; $P < 0.05$) 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 2.6). On average, percent infection was lower in Expt. 1 (40%) than in Expt. 2 (65%) ($P < 0.001$). However, in both experiments, infection was not affected by the soil amendments and *P. cinnamomi* was not isolated from the non-inoculated plants.

2.5. Discussion

In two experiments, biochar (alone or mixed with bokashi) increased plant growth relative to unamended soil in highbush blueberry. However, increases in growth varied among the amendment treatments and were dependent upon the amendment type, the level of fertilization, and the rate in which the amendment was incorporated. When plants were fertigated with a complete fertilizer solution (Expt. 1), the best growth was obtained when the plants were in soil with 20% biochar. This supports previous studies that report biochar has the greatest influence on plant growth when it was applied in conjunction with an appropriate fertilizer (Hossain et al., 2015; Meng et al., 2018; Schultz and Glaser, 2012; Zheng et al., 2015). Biochar has been described as having a synergistic effect with fertilizers, increasing plant growth more than fertilizer alone (Asai et al., 2009; Chan et al., 2007; Liu et al., 2012; Steiner and Hartung, 2014). Biochar increases soil retention of nutrients and thereby improves fertilizer use efficiency (Van Zwieten et al., 2010). In most cases, improved plant growth following biochar additions are attributed to

optimization of the availability of plant nutrients (Agegnehu et al., 2016; Lehmann et al., 2003). However, some nutrients such as $\text{NH}_4\text{-N}$ may be tightly bound by biochar and, therefore, could be less available for plant uptake when certain biochars are incorporated into the soil (Wang et al., 2017).

Biochars derived from woody materials such as hog fuel generally have high C:N ratios 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 was less effective and did better with bokashi under nutrient-limited conditions (i.e., Expt. 2). Similar results were reported when biochar was paired with other forms of organic matter such as compost (Agegnehu et al., 2017; Schulz and Glaser, 2012) or vermicompost (Doan et al., 2015). Composts, which are often characterized by a high CEC and low C:N ratio, can further improve nutrient retention in soils with biochar and act as a good source of fertilizer for the plants (Kamman et al., 2015). However, many composts are also high in pH and EC and, therefore, can be unsuitable for blueberry (Costello et al., 2019; Strik et al., 2017; Sullivan et al., 2014). Bokashi, in contrast, is usually very low in pH (Christel, 2017) and is why it was chosen over compost in the present study for the mix with biochar.

Regardless of the rate in which the amendments were applied, neither biochar nor biochar-bokashi had much effect on soil pH in present study. In fact, by 12 weeks after transplanting, soil pH was lower in each treatment than it was prior to planting. Soil pH increases associated with biochar are well documented and often correlated to the rate in which the biochar is applied (Molnar 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 biochar has on soil pH depends on the liming factor of the biochar 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; Paul and Clark, 1989). Van Zwieten et al. (2010) reported similar findings while investigating the effect of increasing rates of biochar and urea on wheat and radish in an acidic soil. In that case, soil pH was not affected by biochar but declined with increasing rates of N application. Similarly, Solaiman et al. (2010) reported that significant soil pH increases required high rates of biochar in conjunction with low rates of fertilizer. Thus, if needed, higher rates of N application with urea or ammonium-based fertilizers could be used to moderate the liming effect of biochar on high pH soils or for plants adapted to low pH conditions such as blueberry.

With the exception of K in the one experiments (Expt. 2), biochar had no effect on soil nutrients. This was no surprise given that the douglas fir bark and woodchips in which the biochar was derived usually contain very little nutrients (Bollen, 1969; but see Buamscha et al., 2007). Consequently, biochar also had very little effect on nutrients in the leaves of blueberry. However, compared to plants in unamended soil leaf N was slightly higher with the lower rate of biochar under nutrient-limited conditions (Expt. 2?). In contrast, when plants were grown in unamended soil, concentrations of several micronutrients in the leaves, including B, Mn, and Zn, were lower in some inoculation treatments with the higher rate of biochar (particularly when the plants were fertigated with a complete nutrient solution). As expected, adding bokashi to the biochar resulted in higher leaf concentrations of several nutrients, including K, Mg, and S under complete

fertigation and N, P, and S under limited fertigation. Compared to biochar, bokashi was relatively high in N, P, and K and, therefore, a good source of these nutrients.

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 (*Triticum aestivum* L.), 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 (Valenzuela-Estrada et al., 2008). The physical structure of woody biochars greatly increases pore space in soils and thereby facilitates root penetration (Bruun et al., 2014). More than likely, biochar improved root penetration by blueberry in the sandy soil used in present study.

Biochar and biochar-bokashi amendments 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 prior to planting wheat (Solaiman et al., 2010) and larch seedlings (Makoto et al., 2010), respectively. Likewise, Duclos and Fortin (1983) reported increased root colonization of ericoid mycorrhizal fungi in lowbush blueberry (*V. angustifolium* Aiton) seedlings following the application of activated charcoal. In that case, the active carbon adsorbed toxic phenolic compounds in the rhizosphere, suggesting that 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 physical structure of biochar could also play a role in increasing mycorrhizal colonization. The highly porous nature of biochars provides a physical niche for mycorrhizal hyphae and offer physical protection against fungal grazers (Jaafar et al., 2015; Pietikainen et al., 2000; Warnock et al., 2007).

Plants inoculated with *P. cinnamomi* developed root rot, regardless of whether or not 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, therefore, could suppress pathogens at lower dosages but damage roots and increase susceptibility to disease at higher dosages (Bonanomi et al., 2015; Graber et al., 2014).

2.6. Conclusions

Results of this study indicate that biochar could be a good 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 improved soil aggregation and had relatively little effect on soil pH and soil and plant nutrition. Under our experimental conditions, no amendment treatment decreased 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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2.8. Tables and Figures

Table 2.1. Initial chemical and physical characteristics of the soil, biochar, and bokashi used in Expt. 1 and 2.

Characteristic	Soil	Biochar	Biochar-bokashi
pH	5.7	8.5	8.8
Organic matter (%)	1.6	20.5	31.3
CEC (cmol c /kg)	6.7	41	92
C:N ratio	n.d.	209	57
NH ₄ -N (mg·kg ⁻¹)	1.3	< 0.5	23.8
NO ₃ -N (mg·kg ⁻¹)	2.9	1.6	19.3
Bray I P (mg·kg ⁻¹)	96	46	485
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	—	—

n.d. – not determined.

Table 2.2. Effects of biochar amendments on soil pH, organic matter, and concentration of nutrients in pots planted with 'Legacy' blueberry in Expt. 1 and 2.^z

Soil amendment	Organic												
	Soil	matter	NH ₄ -N	NO ₃ -N	Bray I	SO ₄ -S ^y	K	Ca	Mg ^y	B	Cu	Mn ^y	Zn
	pH ^y	(%)	P (mg·kg ⁻¹)				----- (meq/100 g) -----			----- (mg·kg ⁻¹) -----			
<i>Expt. 1</i>													
Soil only	4.5	2.1 c ^x	3	74	90	27	0.48 a	3.3	0.55	0.58	2.7	101	1.8 a
10% biochar	4.2	2.3 bc	2	92	92	27	0.48 a	3.6	0.41	0.47	2.5	89	2.0 a
20% biochar	4.8	2.5 ab	3	105	88	25	0.50 a	3.7	0.56	0.50	2.4	74	2.0 a
10% biochar-bokashi	4.5	2.6 ab	3	110	113	29	0.54 a	4.3	0.64	0.48	2.8	89	2.3 a
20% biochar-bokashi	4.8	2.7 a	3	121	136	25	0.52 a	3.2	0.63	0.44	2.3	76	2.3 a
Significance	NS	**	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	*
<i>Expt. 2</i>													
Soil only					87								
	5.1 b	2.9	2	1 c	bc	64	0.24 c	3.5	0.55 c	0.46	2.4	72	1.6 b
10% biochar					89		0.30		0.64				
	5.3 ab	2.5	2	9 bc	bc	43	bc	3.7	bc	0.48	2.3	74	1.6 b
20% biochar							0.35		0.66				
	5.4 a	2.8	2	17 ab	98 b	37	ab	4.6	bc	0.41	2.4	75	1.7 b
10% biochar-bokashi							0.36		0.76				
	5.4 a	2.6	2	13 ab	77 c	38	ab	3.7	ab	0.40	2.2	78	1.8 b
20% biochar-bokashi													
	5.4 a	3.0	3	19 a	125 a	37	0.42 a	4.0	0.86 a	0.54	2.2	71	2.2 a
Significance	*	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 (non-inoculated treatments only) in both experiments.

^ySoil pH, Mg, and Mn failed the equal variance test in Expt. 1, while soil SO₄-S failed the test in Expt. 2. In each case, the data were analyzed using the Kruskal-Wallis test by ranks.

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

NS,*,**Non-significant and significant at $P \leq 0.05$ and 0.01, respectively.

Table 2.3. Effects of biochar amendments and inoculation with *Phytophthora cinnamomi* on the concentration of macronutrients in leaves of 'Legacy' blueberry in Expt. 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	Diff.	NI	I	Diff.	NI	I	Diff.	NI	I
<i>Expt. 1</i>															
Soil only	17.1	ab ^z	0.84	ab	7.8	b		3.1	ab	3.1	ab	0.0	NS	0.8	b
10% biochar	16.8	ab	0.80	ab	8.6	ab		2.7	b	3.3	ab	-0.6	*	0.7	b
20% biochar	15.6	b	0.66	b	8.3	b		2.8	b	3.4	a	-0.6	*	0.7	b
10% biochar-bokashi	18.2	a	0.87	ab	8.9	a		3.5	a	3.3	ab	0.2	NS	0.9	a
20% biochar-bokashi	18.9	a	0.93	a	9.1	a		3.6	a	2.7	b	1.0	**	0.9	a
Avg			0.73	0.90	8.2	8.9								1.13	1.28
Significance															
Amendment	**		*		**			NS			**			**	
Inoculum	NS		**		**			NS			NS			**	
Amendment × inoculum	NS		NS		NS			**			NS			NS	
<i>Expt. 2</i>															
Soil only	9.1	b	0.57	b	5.8	a	7.1	bc	-1.4	**	2.6			1.2	a
10% biochar	11.4	a	0.63	b	5.1	a	5.9	d	-0.8	*	2.6			1.2	a
20% biochar	10.4	ab	0.54	b	4.9	a	6.4	c	-1.5	**	2.4			1.2	a
10% biochar-bokashi	11.1	a	0.59	b	5.7	a	7.8	b	-2.1	**	2.5			1.1	a
20% biochar-bokashi	12.0	a	0.76	a	5.8	a	10.1	a	-4.2	**	2.7			1.1	a
Average	10.3	11.4	0.68	0.55	5.5	7.5			2.9	2.3	1.1	1.0		1.32	1.27
Significance															
Amendment	**		**		**			NS			NS			**	
Inoculum	**		**		**			**			**			**	
Amendment × inoculum	NS		NS		**			NS			**			NS	

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

NI = non-inoculated; I = inoculated with *P. cinnamomi*; NS,*,** Non-significant and significant at $P \leq 0.05$ and 0.01, respectively.

Table 2.4. Effects of biochar amendments and inoculation with *Phytophthora cinnamomi* on the concentration of micronutrients in leaves of 'Legacy' blueberry in Expt. 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	Diff.	NI	I	Diff.	NI	I	Diff.	NI	I	Diff.	NI	I	Diff.
<i>Expt. 1</i>															
Soil only	62 a ^z	49 a	13**	6.7 a	7.0 a	-0.3 ^{NS}	53			148 a	118 ab	30*	11 a	16 a	-5**
10% biochar	52 ab	54 a	-1 ^{NS}	6.7 a	10.7 a	-4.1**	46			119 ab	148 a	-29*	9 ab	15 a	-5**
20% biochar	48 b	55 a	-7 ^{NS}	5.1 a	10.6 a	-5.6**	49			114 b	126 ab	-11 ^{NS}	8 b	13 a	-5**
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 a	13 a	-2 ^{NS}
20% biochar-bokashi	48 b	57 a	-9*	6.2 a	10.7 a	-4.5**	50			122 ab	100 b	23 ^{NS}	11 a	12 a	-1 ^{NS}
Avg				6.2	9.9		44	53					10	14	
Significance															
Amendment	NS			*			NS			*			*		
Inoculum	NS			**			**			NS			**		
Amendment \times inoculum	**			**			NS			**			**		
<i>Expt. 2</i>															
Soil only	63 a			10.4			36 a	39 b	-3 ^{NS}	166 a	103 a	63**	11 a	13 a	-3**
10% biochar	58 ab			9.6			38 a	58 a	-20**	164 a	105 a	60**	12 a	12 a	0 ^{NS}
20% biochar	51 b			9.0			34 a	41 b	-7 ^{NS}	135 ab	103 a	32*	10 a	12 a	-1 ^{NS}
10% biochar-bokashi	59 a			10.6			44 a	32 b	12*	127 b	102 a	25 ^{NS}	12 a	10 a	1 ^{NS}
20% biochar-bokashi	63 a			9.3			46 a	45 b	1 ^{NS}	140 ab	136 a	4 ^{NS}	12 a	12 a	-1 ^{NS}
Avg	62	56		9.0	10.6					147	110				
Significance															
Amendment	**			NS			**			NS			NS		
Inoculum	**			*			NS			**			NS		
Amendment \times inoculum	NS			NS			**			*			*		

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

NI = non-inoculated; I = inoculated with *P. cinnamomi*; ^{NS,*,**}Non-significant and significant at $P \leq 0.05$ and 0.01 , respectively.

Table 2.5. Effect of biochar amendments on root colonization by mycorrhizal fungi in 'Legacy' blueberry in Expt. 1 and 2.^z

Soil amendment	Mycorrhizal colonization (% of total root length)	
	Expt. 1	Expt. 2
Soil only	6 d ^z	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	**	**

^zMycorrhizal colonization was not examined in plants inoculated with *Phytophthora cinnamomi*.

^yMeans 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 2.6. Effect of biochar amendments on root infection by *Phytophthora cinnamomi* in 'Legacy' blueberry in Expt. 1 and 2.^z

Soil amendment	Root infection by <i>P. cinnamomi</i> (% of total root length)	
	Expt. 1	Expt. 2
Soil only	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.

^{NS}Non-significant.



Fig. 2.1. *Formation of aggregates in soil amended with a woody “hog fuel” biochar. The soil was sandy and lacked any structure without the biochar.*

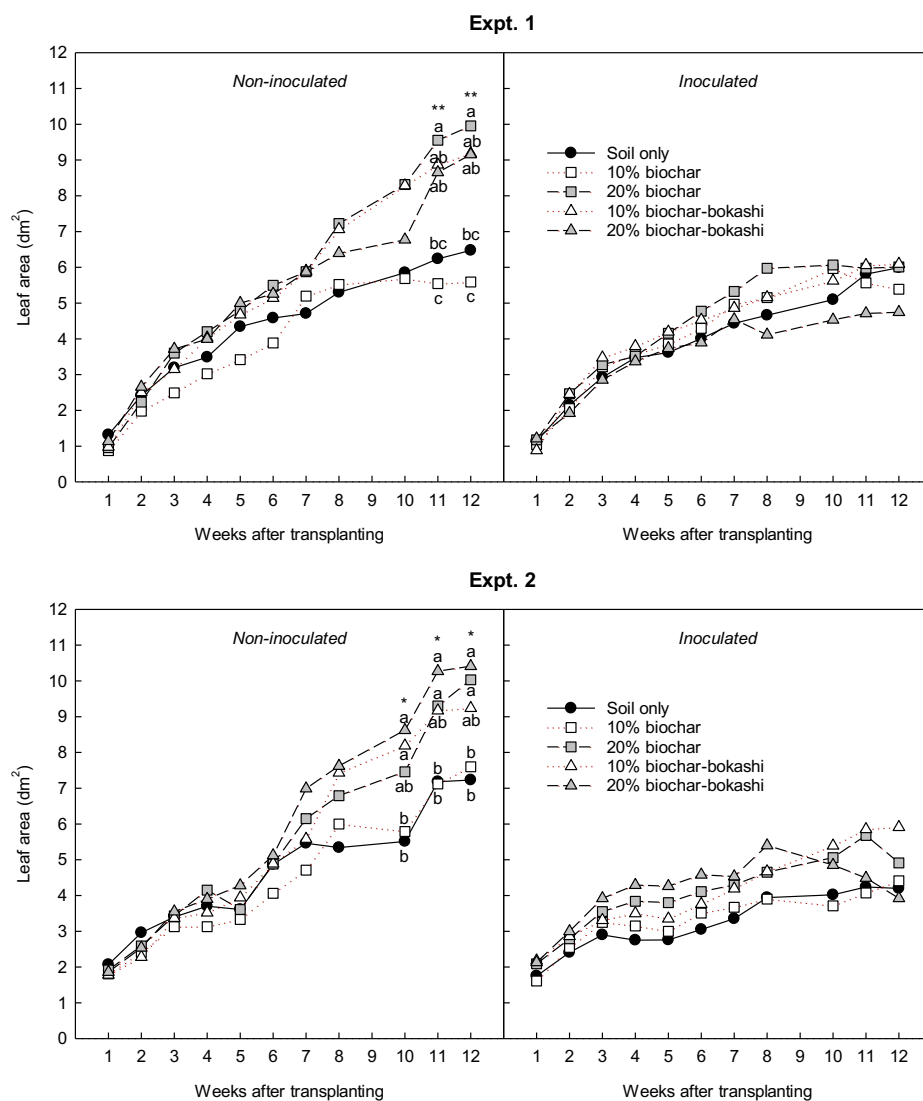


Fig. 2.2. Effects of biochar amendments and inoculation with *Phytophthora cinnamomi* on leaf area development of 'Legacy' blueberry in Expt. 1 and 2. Asterisks indicate weeks in which leaf area differed significantly among the treatments at $P \leq 0.05$ (*) or 0.01 (**). Means followed by the same letter within a given week are not significantly different at $P \leq 0.05$ (Tukey's test).

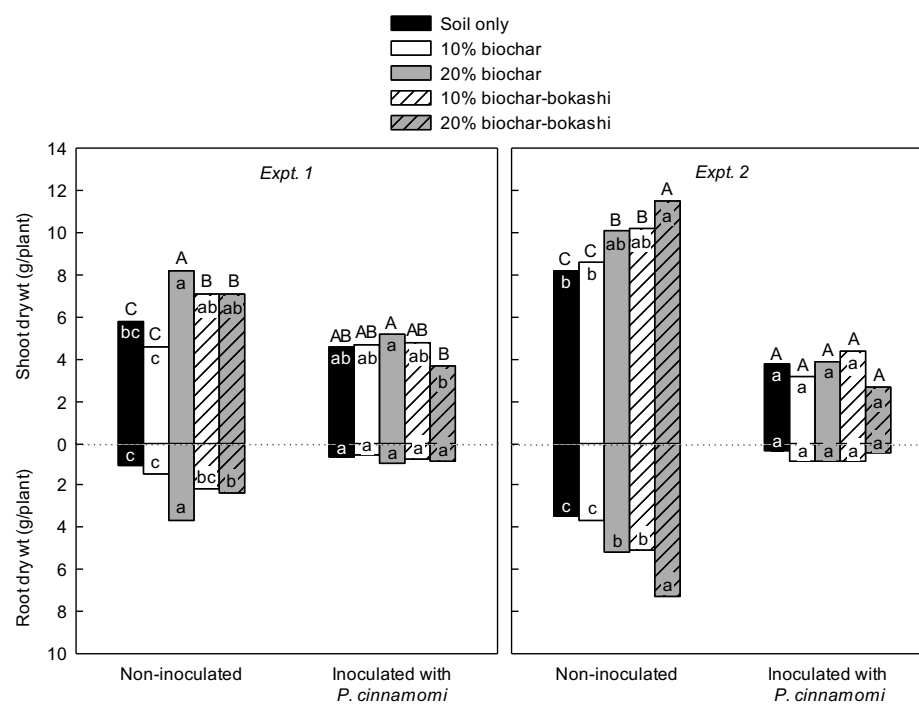


Fig. 2.3. Effects of biochar amendments and inoculation with *Phytophthora cinnamomi* on shoot and root dry weight of 'Legacy' blueberry in Expt. 1 and 2. Similar letters within each inoculation treatment indicate shoot (lower-case letters), root (lower-case letters), and total (upper-case letters) dry weight are not significantly different at $P \leq 0.05$ (Tukey's test).

**Chapter 3 – Use of Biochar as an Alternative Soil Amendment for Establishment of
Highbush Blueberry**

3.1. Abstract

Biochar, as a soil amendment, has been reported to improve plant growth by increasing soil moisture and retaining nutrients. In a previous 12-week greenhouse study, we found that amending soil with biochar alone or in combination with bokashi (fermented wheat bran) increased plant growth relative to unamended soil in highbush blueberry (*Vaccinium sp.* L.). The biochar was produced by gasification (700–800 °C) of douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] bark, wood chips, and wood fiber (referred to as “hog fuel”). In the current study, we aimed to confirm those findings under field conditions in western Oregon. The specific objectives of this 2-year study were to determine the effect of amending soil with biochar or a combination of biochar and bokashi on growth and early fruit production during establishment of highbush blueberry. To achieve these objectives, we transplanted ‘Duke’ blueberry into soil that was either left unamended or amended with biochar, 4 biochar : 1 bokashi (by volume), or 4 biochar : 1 douglas fir sawdust (by volume). Each amendment was either applied in the planting hole or incorporated into the row. A treatment with douglas fir sawdust incorporated into the row was also included and represented the industry standard for the region. Plants grown in soil amended with biochar (in the planting hole or row) had 40% to 74% greater total dry weight at the end of the first growing season and 70% to 82% greater fruit yield in the second season than those grown with no amendments or in soil with sawdust. However, leaf Mg concentrations were lower with biochar, suggesting it could limit Mg uptake in blueberry. Soil amended with sawdust, on the other hand, was higher in organic matter, microbial activity, and wet stable aggregates than the other soil treatments but plant amended with sawdust had lower leaf N concentrations during the second year after

planting. Unlike in the greenhouse, there was no benefit to using biochar with bokashi. Adding 4 L of biochar to the planting hole was considerably more economical than applying it to the row and cost \$1320/ha less than the industry standard of incorporating sawdust in the row. These findings indicate that biochar is a promising soil amendment for commercial production of highbush blueberry.

3.2. Introduction

The use of biochar as an organic soil amendment has received considerable attention in recent years. Biochar is a highly porous carbon-rich residue produced by thermal cracking (pyrolysis) of biomass under oxygen-controlled conditions (Lehmann and Joseph 2009). Like many other sources of organic matter, biochars are known to increase retention of water and nutrients in the soil and improve porosity and permeability of oxygen and other soil gases (Sohi et al., 2010; Amoakwah et al., 2017; Major et al., 2010). However, a large fraction of the C in biochar is biologically and chemically stable and, therefore, can persist in soil for many years (Glaser et al., 2001; Spokas et al., 2010). Biochars also have extremely high surface area and provide excellent sites for hosting beneficial soil microorganisms, such as mycorrhizal fungi (Herath et al., 2013; Lehmann et al., 2011; Luo et al., 2017; Molnar et al., 2016; Nemati et al., 2014). Often, plant growth and production increases in soils amended with biochar, although responses can vary depending on soil fertility and composition and quality of the biochar (Crane-Droesch et al., 2013; Hussain et al., 2017).

Composition and quality of biochars are strongly influenced by the type, nature, and origin of the feedstock. Agricultural residues, logging and wood processing residues, municipal solids, livestock/poultry waste, wastewater/sewage sludge, and biosolids are among the most common feedstocks used for biochar (Cao and Harris, 2010; Gonzaga et al., 2017; Lehman and Joseph, 2009). Major techniques for producing biochar include fast pyrolysis for a few seconds in the absence of oxygen at 425–550 °C, slow pyrolysis for minutes to hours in the absence of oxygen at 350–800 °C, and gasification in the presence of oxygen at > 800 °C (Inyang and Dickenson, 2015). Biochar can be produced

at both small and large scales, including with mobile units that can be easily moved from site to site (El Hanandeh, 2013). In general, biochars derived from woody materials are coarse and highly resistant in nature with up to 80% C (Duku et al., 2011; Zhang et al., 2008). They are also usually low in ash content and, therefore, tend to have little to no effect on soil pH (Mukome et al., 2013; Singh et al., 2010). In contrast, those produced from products with high amounts of K and other nutrients, such as food waste and animal manures, are high in ash and often increase soil pH. Ash content also generally increases with temperature and duration of pyrolysis (Cao and Harris, 2010).

Blueberry (*Vaccinium* sp.) is an acidophilic plant and adapted to well-drained soils with low pH and high organic matter (Retamales and Hancock, 2018). To increase organic matter, growers often incorporate bark or sawdust into the soil prior to planting blueberry and use them as mulch afterwards; however, the cost of these materials is increasing and availability is limited in many regions (Larco et al., 2013a). Recently, we conducted a set of preliminary trials in a greenhouse to determine whether biochar could be used as an alternative amendment for highbush blueberry (*V. corymbosum* sp. L.) (Chapter 2). The biochar in this case was produced from hog fuel, a mixture of coarse bark chips and wood fiber leftover from milling of douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] trees. Within 12 weeks, the plants grown in soil with the biochar were larger and ericoid mycorrhizal fungi more heavily colonized their roots than those grown in unamended soil. Additional growth and colonization was achieved under N-limited conditions by adding bokashi (a mix of fermented wheat bran, molasses, and naturally occurring microbes) to the biochar (Chapter 2). With or without bokashi, the biochar

increased soil pH by 0.3 units or less, even when it was incorporated at a rate as high as 20% by volume.

The objective of the present study was to determine whether biochar could improve growth and early fruit production of highbush blueberry under field conditions. Biochar, alone or in combination with bokashi or sawdust, was incorporated in the planting hole or row and compared to the conventional practice of incorporating sawdust in the row and unamended soil. Plant response as well as amendment costs were considered to identify the best practices for using biochar in blueberry.

3.3. Materials and Methods

Study site. The study was conducted in a new planting of ‘Duke’ highbush blueberry at the Oregon State University North Willamette Research and Extension Center (lat. 45°17′ N, long. 122°45′ W) in Aurora, OR. Soil [Willamette silt loam (fine-silty, mixed, mesic pachic ultic argixerolls)] at the site had an initial pH and total exchange capacity of 6.2 and 11.9, respectively, and contained 3.14% organic matter, 248 mg·kg⁻¹ P (Bray-I), and 243 mg·kg⁻¹ K. The soil was ripped (0.5-m deep), rototilled, and acidified with 110 kg·ha⁻¹ of 99.9% elemental sulfur (Harmon Systems International, Bakersfield, CA) at 10 weeks prior to planting (Hart et al., 2006).

The plants were obtained from a commercial nursery (Fall Creek Farm & Nursery, Lowell, OR) as 2-year-old container stock and transplanted on 15 Sept. 2016. Each plant was actively growing at the time of transplanting and spaced 0.76-m apart in rows of raised planting beds. The beds were 0.4-m high × 0.9-m wide and created using a

single-row bed shaper (Kennco Manufacturing, Inc., Ruskin, FL). The rows were centered 3.05-m apart and oriented in a north–south direction.

Soil amendments. Three organic amendments were used in the study, including biochar, bokashi, and douglas fir sawdust. The biochar was produced from hog fuel (a mixture of coarse bark, woodchips, and wood fiber from douglas fir trees) using gasification at 700–800 °C (BioLogical Carbon, LLC, Philomath, OR). Biochar was either applied to the soil by itself or was mixed 4:1, by volume, with bokashi (a bran fermented with EM-1 as recommended by the manufacturer); BioLogical Carbon, LLC) or douglas fir sawdust (Decorative Bark, Lyons, OR). A 1-kg sample (air-dry) of each amendment was sent to a commercial laboratory for analysis (Brookside Laboratories, New Bremen, OH).

Experimental design. Treatments were arranged in a randomized complete block design and included 1) biochar in the planting hole, 2) biochar + bokashi in the planting hole, 3) biochar + sawdust in the planting hole, 4) biochar + bokashi in the row, 5) biochar + sawdust in the row, 6) sawdust in the row, and 7) unamended soil.

Amendments containing biochar were mixed with soil in the area of the planting hole at a rate of 20%, by volume, and incorporated in the row at a rate of 10%, by volume. A lower rate was used in the latter case to reduce the cost of these treatments. However, sawdust was incorporated in the row at a rate of 20%, by volume, which is the industry standard (Hart et al., 2006). Amendments in the row were incorporated a week before planting and applied by spreading a 0.05- or 0.1-m deep by 0.9-m wide layer of biochar mix (treatments 4 and 5) or sawdust (treatment 6) on the row, respectively, prior to shaping the beds. Amendments in the planting hole were incorporated immediately prior to planting and applied by mixing 3.8 L⁻¹ of biochar (treatment 1), biochar + bokashi

(treatment 2), or biochar + sawdust (treatment 3) with 0.04 m³ of soil (an 0.30 × 0.30 m area to a depth of 0.45 m) at the location in which each plant was transplanted.

The planting including a total of seven rows of treatment plots, plus a border row on each side. The rows were divided into five blocks (based on slope), with treatments randomly assigned to a row in each block. Each treatment plot was 6.4-m long and consisted of eight plants. The middle six plants in each plot were used for measurements. Adjacent plots within a row were separated by a distance of 1.2 m in order to avoid cross-contamination between the treatments.

Management of the planting. The plants were irrigated using drip tubing (UniRam; Netafim, Fresno, CA) on each side of the row, near the base of the plants. The tubing had integrated 3.8 L·h⁻¹ pressure-compensating emitters every 0.30 m. Irrigation was scheduled based on precipitation and daily estimates of crop evapotranspiration (usbr.gov/pn/agrimet/agrimetmap/araoda.htm.) and controlled independently in each treatment using electric solenoid valves and an automatic timer (Bryla, 2011). To ensure irrigation was adequate, soil water content was measured weekly in the top 15 cm of soil profile using a Trase I time domain reflectometry (TDR) system (SoilMoisture Equip. Corp., Santa Barbara CA); values were similar among the treatments and, from May through September, ranged from 28.9% to 30.4% in 2017 (year 1) and 23.5% to 28.6% in 2018 (year 2).

The plants were fertilized with a liquid source of ammonium sulfate (8N–0P–0K–9S) at a total rate of 54 kg·ha⁻¹ N per year. The fertilizer was applied each year (2017 and 2018) from mid-April to end of July in 13 equal applications of 4.2 kg·ha⁻¹ N each using

water-powered proportional chemical injectors (Model D25F1; Dosatron, Clearwater, FL) (one unit per treatment).

Weeds were controlled using a 1-m-wide sheet of black geotextile landscape fabric (a water flow rate of $6.8 \text{ L} \cdot \text{h}^{-1}$ per m^2 and a density of $0.11 \text{ kg} \cdot \text{m}^{-2}$ as measured by the manufacturer; TenCate Protective Fabrics, OBC Northwest, Inc, Candby, OR) on each side of beds (“zippered” weed mat per Strik et al., 2017). The sheets over-lapped on top of the beds (over the drip tubing) and were tacked in place with 15-cm-long steel nails. The fabric was cut and folded back to create a $10 \times 10 \text{ cm}$ opening for each plant. Any weeds that grew through the openings were removed by hand. Grass alleyways (1.1-m wide) were planted and maintained by mowing between the beds. No chemicals were needed for pest control.

Plants were pruned in Feb. 2017 and Jan. 2018. All of the flower buds were removed in 2017 in order to encourage more vegetative growth during the first year after planting (Strik and Buller, 2005). The following year, the plants were pruned to leave between 5 to 30 floral buds per plant, depending on vigor (Strik et al., 2017).

Measurements. Five recently expanded leaves were collected from each plant on 20 July 2017 and 17 July 2018. The leaves were oven-dried for at least 48 h, ground to pass through a 1-mm sieve, and analyzed for N using a combustion analyzer (model TruSpec CN; Leco Corp., St. Joseph, MI) and for P, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn using an inductively coupled plasma optimal emission spectrophotometer (model Optima 3000DV; Perkin Elmer, Wellesley MA) after microwave digestion with 70% (v/v) nitric acid (Gavlak et al, 2005).

Ripe fruit were hand-harvested each week from 21 June to 12 July 2018. The berries were counted and weighed to determine yield and mean (weighted) berry weight in each treatment.

One randomly selected plant was harvested destructively from each plot on 7 Oct. 2017 (year 1) and 15 Sept. 2018 (year 2). First, soil samples were collected near the plants at depths of 0–15 and 15–30 cm (directly between two drip emitters) using a 2-cm-diameter soil probe (Clements Associates Inc., Newton IA). Each sample was ground to pass through a 1-mm sieve and sent to a commercial laboratory for analysis (Brookside Laboratories New Bremen, OH). Shoots were then cut at the soil surface and divided into stems and leaves. The root system was carefully removed using a shovel and rinsed under running tap water. A small sample of fresh roots (2 g) was collected from each plant and examined under a stereomicroscope (115×) for colonization by mycorrhizal fungi. Each sample was cleared in 10% KOH and stained with 0.05% Trypan blue in lactoglycerine (Giovannetti and Mosse, 1980); the percentage of roots colonized by mycorrhizal fungi were quantified using a gridline intersection technique (McGonigle et al, 1990). Leaves, stems, crown (year 2 only), and the remaining roots were oven-dried for at least 48 h at 60 °C and weighed.

Once the plants were removed, ≈ 1 kg of soil was collected immediately and the samples were laid out on greenhouse benches and air-dried. Extra care was taken to not destroy the aggregates during the process. Active C, microbial respiration, and soil aggregation. Tests of samples were determined by the Oregon State University Crop and Soil Science Central Analytical Laboratory in Corvallis, OR.

To determine active C, 2.5 g of air-dried soil was ground to pass through a 1-mm sieve and combined with 20 mL of 0.02M KMnO_4 in a 50 mL tube and shaken for 2 min. The soil was then allowed to settle for 8 min, and 0.5 mL of the supernatant was added to 49.5 mL of water and hand-shaken for 10 s. A 2 mL sample of the final solution was measured on a spectrophotometer at 550 nm (Weil et al., 2003).

To measure microbial respiration, 40 g of air-dried soil was ground to pass through a 2-mm sieve and rewet in a glass beaker to field capacity. The beaker was then placed in a glass canning jar, and a baseline reading was taken to determine the concentration of CO_2 in the headspace of the jar using an isotopic CO_2 analyzer (model G2131; Picarro, Santa Clara, CA). Readings were taken at 0, 24, and 96 h and respiration rate ($\mu\text{g CO}_2\text{-C g}^{-1}\text{ soil d}^{-1}$) was calculated by subtracting readings at 24 and 96 h from the baseline (0 h).

Wet aggregate stability was determined using a rain simulator. Approximately 20 g of air-dried soil was sieved to pass through a 2-mm sieve and collected on a 0.25-mm sieve and weighed. A rain simulator was placed 50 cm above the sieve, and 1.25 cm of rain was dropped over a 5-min period (Gugino et al., 2009). The unstable aggregates passed through the sieve and were collected on filter paper. The filter paper containing the unstable aggregates were dried at 105 °C for 24 h, weighed, and the weight of the filter paper was subtracted from the total weight to obtain the weight of unstable aggregates. The remaining particles on the sieve were washed to break up the remaining aggregates, and the sieve containing sand-sized fragments was dried at 105 °C for 24 h to determine the weight of the sand. The wet-stable aggregates are represented as the difference in weight between the sand-sized fractions and the weight of the unstable

aggregates on the filter paper. The percentage of wet-stable aggregates was calculated by dividing the weight of the stable aggregates by the weight of the total aggregates and multiplying by 100.

Once the fruit was harvested (21 June to 12 July 2018), irrigation was withheld for 10 d (4–15 Aug. 2018) to induce water stress in the plants. There was no precipitation during the period and daily high air temperature averaged 32.2 °C. Soil water content was measured at the beginning and end of the drought period using the TDR system; readings were taken using a pair of 15- and 30-m probes inserted vertically near the center of each plot. Stem water potential was also measured at midday (12:00–2:00 HR) on one representative plant in each plot using a pressure chamber (model 600; PMS Instrument Co., Albany, OR), following procedures outlined in Bryla and Strik (2007).

Statistical analysis. The data were analyzed by analysis of variance using the PROC MIXED procedure in SAS software package ver. 9.3 (SAS Institute, Cary, NC). The independent variables were amendment and block. Data were tested for normality or homogeneity of variance and log-transformed as needed. All data was back transformed to represent the actual means. Means were separated at the 0.05 level using Tukey's honestly significant difference test.

3.4. Results

Plant growth and yield. By the end of the first growing season, plants with biochar in the planting hole produced more total dry weight (sum of roots, stems, and leaves) than those in any other treatment except the one with biochar + sawdust in the row (Fig. 3.1). Plants with biochar (in the planting hole or row) also produced more fruit

than most treatments the following year and, therefore, had a higher yield than several treatments, including those with sawdust and soil only (Fig. 3.2). Yield increased as a function of the total dry weight of the plants from the previous season (Fig. 3.2, inset), but was unrelated to berry weight, which was similar among the treatments ($\bar{X} = 2.1$ g/berry).

By the end of second season, plants with biochar in the planting hole or row had a greater dry weight than those grown with biochar-bokashi in the planting hole or sawdust in the row, but they were no longer different than plants in the other treatments, including those in unamended soil (Fig. 3.1).

Leaf nutrients. The concentration of several nutrients in the leaves were affected by the soil amendments, including K, Mg, S, and B in year 1 and N and Mg in year 2 (Table. 3.2). In most cases, these nutrients were within or above the recommended range for highbush blueberry in Oregon (Hart et al., 2006). However, Mg was below normal when plants were grown in soil only in year 1 or with any of the biochar treatments in year 2. Likewise, N was below normal when plants were grown with sawdust in the row in year 2, while B was deficient (<20 ppm; Hart et al., 2006) in all but one treatment (i.e., biochar + sawdust in the planting hole in year 1) during both years of the study. Calcium, Cu, and Fe were also below normal in one or both years [the normal range is 0.41% to 0.80% Ca, 5 to 15 ppm Cu, and 61 to 200 ppm Fe; Hart et al. (2006)], but in no case were these nutrients affected by the soil amendments (see footnote “y” in Table 3.2).

Mycorrhizal colonization. The percentage of roots colonized by mycorrhizal fungi averaged 10% and was similar among the treatments (data not shown).

Soil fertility, microbial activity, and aggregation. Soil pH was similar among the treatments and, on average, was 5.5 and 5.6 at a depth of 0–15 and 15–30 cm, respectively, following the first year after planting, and 5.3 and 5.7, respectively, following the second year. Relative to sawdust in the row and unamended soil, biochar, regardless of how it was applied, had no effect on soil nutrients other than $\text{SO}_4\text{-S}$ in year 2, which was higher at 0–15 cm with biochar + sawdust in the planting hole than with biochar-bokashi in the row, sawdust in the row, or unamended soil (Table 3.3). Sawdust in the row, on the other hand, increased soil Mn in year 1 and soil organic matter and microbial activity (based on measures of active soil C and respiration) in both years relative to the other treatments (Table 3.3 and 3.4). In one or both years, sawdust in the row also increased soil aggregation relative to treatments with biochar in the row or in unamended soil (Fig. 3.3).

There were also a few minor differences in soil nutrients between several of the biochar treatments, including soil B (greater with biochar than with biochar + sawdust in the planting hole at 0–15 cm) in year 1 and soil Ca (greater with biochar + sawdust in the planting hole than with biochar-bokashi in the row at 0–15 cm) and Zn (greater with biochar-bokashi than with biochar in the planting hole at 0–15 cm) in year 2 (Table 3.3). In no case were differences in soil nutrients reflected in the concentrations measured in the leaves (Table 3.2) and fruit (data not shown).

Plant and soil water relations. Soil water content and stem water potential were similar among the treatments throughout the study and remained $> 23\%$ and -0.85 MPa, respectively (data not shown). However, when irrigation was withheld (day zero was Aug. 5, 2018) from the plants for 10 d after harvest, soil water content from 0–0.15 m was

lower in plots with sawdust in the row than those with most other treatments, including biochar, biochar-bokashi, or biochar + sawdust in the planting hole, biochar-bokashi in the row, and soil only (Table 3.5). Stem water potential was also lower at this point with sawdust in the row than with many of the other treatments (Table 3.5).

3.5. Discussion

Amending the soil with biochar, either in the planting hole or in the row prior to planting, resulted in more vegetative growth in year 1 and fruit production in year 2 of establishment than the standard practices of incorporating sawdust in the row or using soil only. Growth and yield in the present study were normal for the region and similar to a previous study in an organic planting of ‘Duke’ blueberry (Larco et al., 2013b). Increased plant growth is often reported in soils amended with biochar (De Tender et al. 2016; Headlee et al. 2014; Méndez et al. 2013). However, more growth with biochar does not always result in higher yields (Eleys et al., 2015). For example, Vacarri et al. (2015) found that adding biochar to the soil increased growth in tomato relative to soil only but had no effect on yield. Likewise, Safaei et al. (2019) recently reported that biochar increased trunk diameter and the number of shoots in a new planting of apple trees but, again, it did not increase yield. In our case, fruit production, which first occurred during the second year after planting (industry standard), was highly correlated to total plant biomass at the end of the previous year. This was expected since we removed floral buds and determined yield based on the vigor of each plant.

We should point out that adding sawdust to soil resulted in several potential benefits in the planting that were absent in other treatments relative to soil alone,

including increased organic matter content, better soil health in terms of active C and microbial activity, and a higher percentage of water-stable aggregates in the soil. However, sawdust also led to low N in the plants, which likely reduced growth and yield relative to biochar. Nitrogen is commonly immobilized by soil microbes during decomposition of woody materials such as bark or sawdust (Bünemann et al., 2006; Cesarano et al., 2017, White, 2006). Biochar, in contrast, is a recalcitrant C source and, therefore, has a minimal effect on soil N immobilization (Nelissen et al., 2015). Although this was not the case in the present study, biochar has also been shown to increase soil water holding capacity and improve crop production under adverse soil conditions such as high salinity and drought (Thomas et al. 2013; Haider et al. 2015). Bark and sawdust, on the other hand, increase soil drainage, which, depending on the frequency of rain or irrigation, could reduce water uptake and lead to water stress in blueberry (White, 2006).

The most prohibitive factor to wide-scale use of biochar in agriculture is the cost (Campbell et al., 2018). The biochar used in the present study was \$76/m³. Douglas fir sawdust, in contrast, was \$9.20/m³. In Oregon, the total estimated cost of incorporating douglas fir sawdust into the row prior to planting was \$4350/ha in 2011 (Julian et al., 2011). In comparison, the cost of amending the planting hole with 4 L of biochar was only \$1980/ha. Therefore, even after increased labor costs are considered (\$1050/ha), growers could reduce costs by replacing sawdust with biochar and, at the same time, increase returns by improving early fruit production. It is unclear whether biochar would be beneficial beyond the second season, but if it was, the common practice of replenishing the soil with sawdust mulch every few years could be eliminated potentially, reducing the production costs of blueberry even further.

Another major concern with using biochar, particularly in blueberry, is high pH. Biochar is known to act as a liming agent (Trippe et al., 2015). However, we did not find this to be the case in this or our previous study with biochar on blueberry (Chapter 2). In both cases, the biochar was produced from hog fuel. Increases in soil pH are dependent on the chemical composition of the biochar and buffering capacity of the soil (Uchimiya et al., 2013). Biochar produced from woody materials such as hog fuel are usually low in ash content and, therefore, tend to have little to no effect on soil pH (Singh et al., 2010). In contrast, those produced from products with high amounts of K and other nutrients, such as food waste and animal manures, are high in ash and often increase soil pH (Singh et al., 2010); these biochars may be less suitable for blueberry. Ash content is also affected by the temperature and duration of pyrolysis (Cao and Harris, 2010). Growers interested in using biochar in blueberry should test it on a batch-by-batch basis and ensure that it is high in C and low in ash.

The concentration of Mg in the leaves was low in each of the treatments with biochar during the second year after planting, suggesting that biochar could lead eventually to Mg deficiency in blueberry. Biochar also resulted in lower concentrations of Mg in the leaves of corn and sesame (Syuhada et al., 2016; Wacal et al., 2019). In both studies, reduced Mg concentrations were associated with increased concentrations of K in the leaves. However, in the present study, the concentration of K in the leaves was similar between treatments with or without biochar and within a normal range for blueberry. Magnesium deficiency causes the outer portion of blueberry leaves to turn yellow or red, while the middle of the leaves remains green (Polashock et al., 2017). Symptoms usually develop later in the season on leaves located at the base of the shoots. Such symptoms did

not occur in the present study. If needed, Mg deficiency could be easily corrected in plants with biochar by applying magnesium sulfate (Hart et al., 2006).

Regardless of biochar, plants from each treatment had very low concentrations of B in the leaves. Boron deficiency causes dieback of the shoot tips in blueberry and is a fairly common problem in northwestern United States and British Columbia, Canada (Hart et al., 2006). Typically, when plants are deficient, leaves close to aborted shoot tips will cup and develop a mottled chlorosis pattern; leaf and fruit buds may fail to develop on severely affected plants (Polashock et al., 2017). Again, we saw no evidence of B deficiency in the present study; however, foliar or soil applications of B fertilizer such as boric acid or sodium borate would be highly recommended when the concentration of B in the leaves is < 20 ppm (Hart et al., 2006).

As mentioned earlier, we found previously that adding bokashi to the biochar improved growth in blueberry relative to using biochar alone (Chapter 2). This was not the case in the present study. This time, whether biochar was mixed with bokashi or sawdust and incorporated into the planting hole or row, it resulted in more-or-less the same growth and yield in the plants as sawdust in the row or soil only. It is possible that the formulation of the bokashi used in the field study was different than the bokashi used in the field study. In addition, in the greenhouse experiment, we found the bokashi was a benefit under low nitrogen inputs in a sandy loam soil. However, biochar alone was more beneficial for plant growth under a complete fertigation. Therefore, in the more fertile silty loam soil under adequate nitrogen applications, the effect was not significant. Furthermore, the addition of sawdust to the biochar in the planting hole likely reduced N availability due to N immobilization associated with sawdust.

We also discovered previously that biochar or biochar-bokashi resulted in a considerable increase in root colonization by ericoid mycorrhizal fungi (Chapter 2). In the previous study, the percentage of roots colonized by mycorrhizal fungi was 56% to 91% in plants grown in soil with biochar or biochar-bokashi but $\leq 10\%$ in those grown in unamended soil. In contrast, colonization was $< 10\%$ in each treatment in the present study and was unaffected by either biochar or biochar-bokashi. The soil used in the greenhouse was sandy and low in organic matter and nutrients. Mycorrhizal colonization is often lower when blueberry plants are grown in fertile soils (Yang et al., 2002), which may explain why mycorrhizal response to biochar was so different between the present and previous study.

3.6. Conclusions

Based on the results of this study, biochar appears to have considerable potential for improving growth and fruit production in highbush blueberry. The most cost-effective method to apply biochar in the present study was adding it to the planting hole. However, this method was somewhat laborious and difficult to apply consistently. Perhaps, alternatively, biochar could be applied in a narrow band on the row (e.g., 10–20 cm wide) and incorporated into the soil prior to shaping the beds. Doing so would require slightly more biochar but reduce labor costs considerably. More research is needed to identify the best method to apply the biochar. Studies are also needed to determine whether biochar has any long-lasting effects on fruit production and mineral nutrition in blueberry.

3.7. Literature Cited

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3.8. Tables and Figures

Table 3.1. Chemical characteristics of three organic materials used as soil amendments in new planting of 'Duke' northern highbush blueberry in western Oregon.

Characteristic	Biochar	Biochar-bokashi	Sawdust
pH	10.0	8.7	4.5
C:N ratio	209	56	196
Extractable nutrients			
N (%)	0.41	1.01	0.14
P (%)	0.07	0.35	0.01
K (%)	0.81	0.85	0.04
S (%)	0.023	0.127	0.007
Ca (%)	0.74	1.49	0.06
Mg (%)	0.14	0.47	0.01
B (ppm)	18.9	47.5	2.5
Cu (ppm)	12.2	28.5	1.3
Mn (ppm)	372	642	18
Zn (ppm)	7.4	84.9	5.8

Table 3.2. Effects of soil amendments (biochar, a biochar-bokashi mix, and douglas fir sawdust) on the concentration of nutrients in the most recent fully expanded leaves of 'Duke' northern highbush blueberry during the first 2 years after planting in western Oregon.

Amendment ^x	Concn of nutrients in the most recent fully expanded leaves ^{z,y}					
	Year 1				Year 2	
	K (%)	Mg (%)	S (%)	B (ppm)	N (%)	Mg (%)
Biochar (planting hole)	0.52 b ^u	0.15 ab	0.19 b	15 bc	1.78 ab	0.11 b
Biochar-bokashi (planting hole)	0.57 ab	0.15 ab	0.19 b	23 a	1.86 ab	0.11 b
Biochar + sawdust (planting hole)	0.60 a	0.16 a	0.23 a	19 ab	1.89 ab	0.11 ab
Biochar-bokashi (row)	0.55 ab	0.13 bc	0.19 b	13 c	1.90 a	0.11 b
Biochar + sawdust (row)	0.53 ab	0.14 ab	0.19 b	12 c	1.90 a	0.12 ab
Sawdust (row) ^w	0.57 ab	0.16 a	0.19 b	15 bc	1.69 b	0.14 a
Unamended soil	0.54 ab	0.12 c	0.18 b	12 c	1.90 a	0.13 ab
Significance	0.027	0.003	0.001	<0.001	0.003	0.015
Normal range ^v	0.41–0.70	0.13–0.25	0.11–0.16	31–80	1.76–2.00	0.13–0.25

^zLeaves were sampled during the last week of July each year.

^yThe amendments had no effect on the concentration of other nutrients in the leaves, including: N (1.94%), P (0.14%), Ca (0.50%), Cu (3.4 ppm), Fe (57 ppm), Mn (62 ppm), or Zn (15.9 ppm) in year 1; and P (0.16%), K (0.62%), Ca (0.40%), S (0.15%), B (6.8 ppm), Cu (2.9 ppm), Fe (88 ppm), Mn (56 ppm), or Zn (16.5 ppm) in year 2.

^xThe amendments were incorporated in the planting hole (20% by volume) or in the entire row (10% by volume for the biochar mixes and 20% by volume for sawdust) of the planting bed (see Material and Methods for details).

^wIndustry standard.

^vHart et al. (2006).

^uMeans followed by the same letter within a column were not significantly different, according to Tukey's test ($P \leq 0.05$).

Table 3.3. Effects of soil amendments (biochar, a biochar-bokashi mix, and douglas fir sawdust) on soil organic matter content and available soil nutrients following the first 2 years after planting a new field of 'Duke' northern highbush blueberry in western Oregon.^{z,y}

Amendment ^x	Year 1			Year 2			
	Organic matter (%)	B (mg·kg ⁻¹)	Mn (mg·kg ⁻¹)	Organic matter (%)	Ca (g·kg ⁻¹)	SO ₄ -S (mg·kg ⁻¹)	Zn (mg·kg ⁻¹)
	----- 0–15 cm -----			----- 15–30 cm -----			
Biochar (planting hole)	2.4 b ^y	0.52 a	47 c	2.8	1.5 ab	102 ab	1.5 b
Biochar-bokashi (planting hole)	2.5 b	0.42 ab	50 bc	2.9	1.3 ab	107 ab	2.7 a
Biochar + sawdust (planting hole)	2.5 b	0.32 b	53 bc	3.0	1.7 a	188 a	2.0 ab
Biochar-bokashi (in row)	2.5 b	0.40 ab	50 bc	2.8	1.0 b	60 b	2.3 ab
Biochar + sawdust (in row)	2.5 b	0.40 ab	51 bc	2.8	1.1 ab	69 ab	1.8 ab
Sawdust (in row) ^w	3.1 a	0.41 ab	87 a	3.0	1.1 ab	51 b	1.8 ab
Soil only	2.4 b	0.43 ab	59 b	2.6	1.1 ab	47 b	1.9 ab
Significance	<0.001	<0.001	<0.001	NS	0.018	0.014	0.031
	----- 15–30 cm -----			----- 15–30 cm -----			
Biochar (planting hole)	2.4 b	0.38	53 b	2.6 b	1.5	47	1.6
Biochar-bokashi (planting hole)	2.5 b	0.44	52 b	2.6 b	1.3	48	1.8
Biochar + sawdust (planting hole)	2.5 b	0.45	52 b	2.6 b	1.4	55	1.8
Biochar-bokashi (row)	2.6 b	0.48	57 ab	2.8 b	1.5	22	1.7
Biochar + sawdust (row)	2.4 b	0.43	53 b	2.7 b	1.4	25	1.7
Sawdust (row) ^w	3.4 a	0.44	79 a	3.5 a	1.4	34	1.7
Soil only	2.4 b	0.35	56 ab	2.7 b	1.4	42	1.6
Significance	<0.001	NS	0.017	<0.001	NS	NS	NS

^zSoil was sampled in October each year at depths of 0–15 and 15–30 cm.

^yThe amendments had no effect on other nutrients in the soil, including NH₄-N (0–15 cm: 14.7 mg·kg⁻¹; 15–30 cm: 13.6 mg·kg⁻¹), NO₃-N (0–15 cm: 7.5 mg·kg⁻¹; 15–30 cm: 6.7 mg·kg⁻¹), P (0–15 cm: 0.17 g·kg⁻¹; 15–30 cm: 0.18 g·kg⁻¹), K (0–15 cm: 0.18 g·kg⁻¹; 15–30 cm: 0.18 g·kg⁻¹), Ca (0–15 cm: 1.6 g·kg⁻¹; 15–30 cm: 1.6 g·kg⁻¹), Mg (0–15 cm: 0.08 g·kg⁻¹; 15–30 cm: 0.07 g·kg⁻¹), SO₄-S (0–15 cm: 0.05 g·kg⁻¹; 15–30 cm: 0.05 g·kg⁻¹), Cu (0–15 cm: 1.12 mg·kg⁻¹; 15–30 cm: 1.11 mg·kg⁻¹), Fe (0–15 cm: 349 mg·kg⁻¹; 15–30 cm: 353 mg·kg⁻¹), or Zn (0–15 cm: 1.85 mg·kg⁻¹; 15–30 cm: 1.78 mg·kg⁻¹) in year 1; and NH₄-N (0–15 cm: 7.0 mg·kg⁻¹; 15–30 cm: 3.9 mg·kg⁻¹), NO₃-N (0–15 cm: 16.2 mg·kg⁻¹; 15–30 cm: 2.2 mg·kg⁻¹), P (0–15 cm: 0.15 g·kg⁻¹; 15–30 cm: 0.16 g·kg⁻¹), K (0–15 cm: 0.15 g·kg⁻¹; 15–30 cm: 0.17 g·kg⁻¹), Mg (0–15 cm: 0.12 g·kg⁻¹; 15–30 cm: 0.09 g·kg⁻¹), B (0–15 cm: 0.51 mg·kg⁻¹; 15–30 cm: 0.55 mg·kg⁻¹), Cu (0–15 cm: 1.38 mg·kg⁻¹; 15–30 cm: 1.35 mg·kg⁻¹), Fe (0–15 cm: 282 mg·kg⁻¹; 15–30 cm: 268 mg·kg⁻¹), or Mn (0–15 cm: 36 mg·kg⁻¹; 15–30 cm: 33 mg·kg⁻¹) in year 2.

^xThe amendments were incorporated in the planting hole (20% by volume) or in the entire row (10% by volume for the biochar mixes and 20% by volume for sawdust) of the planting bed (see Material and Methods for details).

^wIndustry standard.

^yMeans followed by the same letter within a column were not significantly different at $P \leq 0.05$, according to Tukey's test.

Table 3.4. Effects of soil amendments (biochar, a biochar-bokashi mix, and douglas fir sawdust) on microbial soil activity following the first 2 years after planting a new field of 'Duke' northern highbush blueberry in western Oregon.

Amendment ^z	Year 1			Year 2		
	Active soil C (mg·kg ⁻¹)	Soil respiration (mmol·m ⁻² ·s ⁻¹)		Active soil C (mg·kg ⁻¹)	Soil respiration (mmol·m ⁻² ·s ⁻¹)	
		24 h	72 h		24 h	72 h
Biochar (planting hole)	8.0 b ^x	30 b	19 b	8.1 b	18 b	12 b
Biochar-bokashi (planting hole)	11.8 b	44 b	28 b	10.2 b	30 b	19 b
Biochar + sawdust (planting hole)	11.2 b	41 b	28 b	10.4 b	22 b	14 b
Biochar-bokashi (row)	8.7 b	32 b	21 b	8.1 b	20 b	13 b
Biochar + sawdust (row)	9.2 b	34 b	21 b	7.2 b	21 b	13 b
Sawdust (row) ^y	21.4 a	79 a	47 a	19.2 a	48 a	31 a
Soil only	10.2 b	38 b	23 b	9.3 b	22 b	14 b
Significance	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

^z The amendments were incorporated in the planting hole (20% by volume) or in the entire row (10% by volume for the biochar mixes and 20% by volume for sawdust) of the planting bed (see Material and Methods for details).

^yIndustry standard.

^xMeans followed by the same letter within a column were not significantly different at $P \leq 0.05$, according to Tukey's test.

Table 3.5. Effects of soil amendments (biochar, a biochar-bokashi mix, and douglas fir sawdust) on plant and soil water relations before and after irrigation was withheld for 10 d in a new planting of ‘Duke’ northern highbush blueberry in western Oregon.^z

Amendment ^x	Well-watered (day 0) ^y			After 10 d without irrigation ^y		
	Soil water content (%)		Stem water potential (MPa)	Soil water content (%)		Stem water potential (MPa)
	0–15 cm	0–30 cm		0–15 cm	0–30 cm	
Biochar (planting hole)	28.3	27.2	-0.83	21.4 ab ^v	24.8	-0.88 a
Biochar-bokashi (planting hole)	27.0	27.7	-0.80	23.9 ab	25.9	-0.91 a
Biochar + sawdust (planting hole)	25.8	27.0	-0.77	21.5 ab	24.7	-0.94 ab
Biochar-bokashi (row)	25.6	28.3	-0.80	21.4 ab	26.7	-0.95 ab
Biochar + sawdust (row)	27.4	26.0	-0.79	19.6 bc	21.2	-0.91 a
Sawdust (row) ^w	23.5	26.9	-0.80	16.4 c	21.4	-1.03 b
Soil only	28.6	27.2	-0.76	24.7 a	22.4	-0.89 a
Significance	NS	NS	NS	0.039	NS	0.005

^zIrrigation was withheld after fruit harvest during the second season. There was no rain during this period.

^yMeasurements were made at midday.

^x The amendments were incorporated in the planting hole (20% by volume) or in the entire row (10% by volume for the biochar mixes and 20% by volume for sawdust) of the planting bed (see Material and Methods for details).

^wIndustry standard.

^vMeans followed by the same letter within a column were not significantly different at $P \leq 0.05$, according to Tukey’s test.

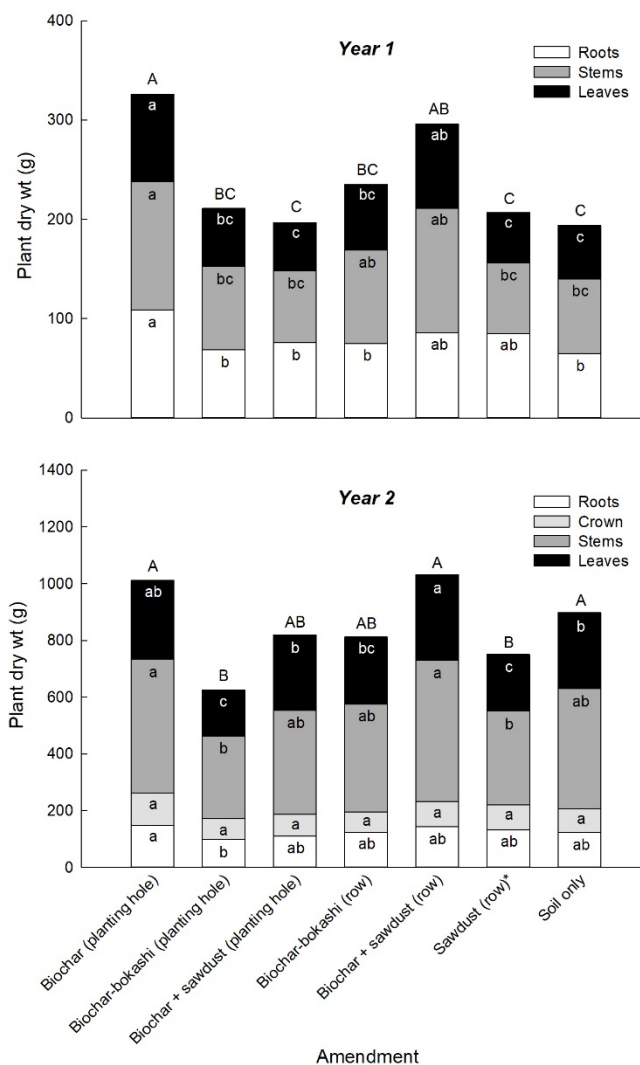


Fig. 3.1. *Effects of soil amendments (biochar, biochar mixed with bokashi or douglas fir sawdust, and sawdust only) on plant dry weight of ‘Duke’ northern highbush blueberry following the first 2 years after planting in western Oregon. The amendments were incorporated in the planting hole (20% by volume) or in the entire row (10% by volume for the biochar mixes and 20% by volume for sawdust) of the planting bed (see Material and Methods for details). Incorporation of sawdust in the row is the industry standard. The plants were excavated at the end of the growing season each year and divided into roots, crown (year 2 only), stems (new and old whips and lateral branches), and leaves. Means are separated using uppercase letters for total dry weight and lowercase letters for each plant part; those with the same letter are not significantly different, according to Tukey’s test ($P \leq 0.05$).*

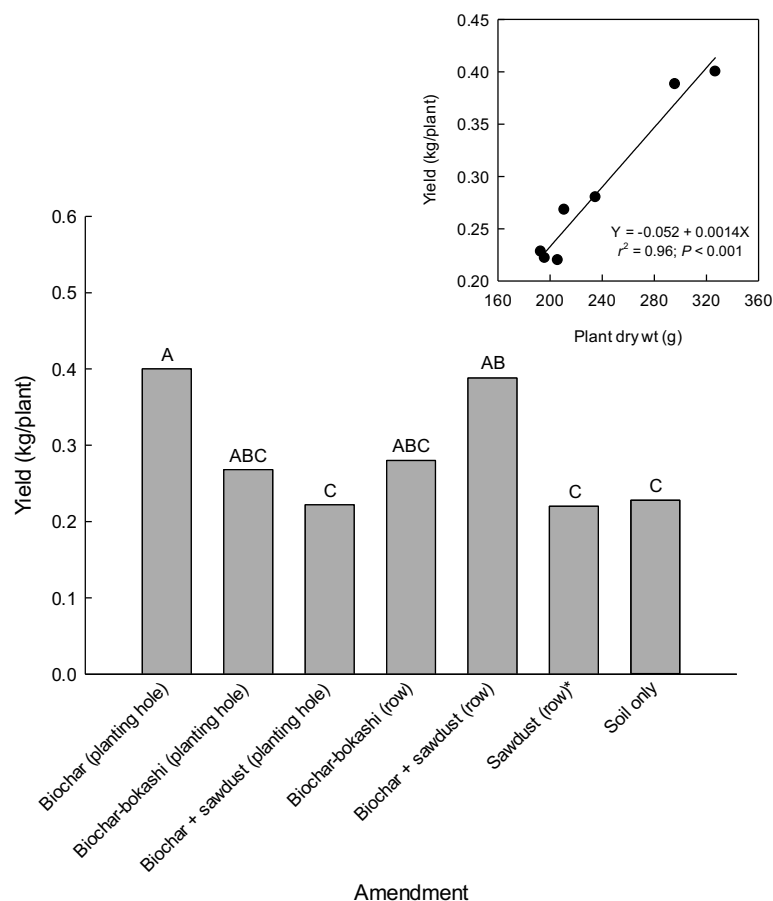


Fig. 3.2. Effects of soil amendments (biochar, biochar mixed with bokashi or douglas fir sawdust, and sawdust only) on yield of ‘Duke’ northern highbush blueberry during the second year after planting in western Oregon. The amendments were incorporated in the planting hole (20% by volume) or in the entire row (10% by volume for the biochar mixes and 20% by volume for sawdust) of the planting bed (see Material and Methods for details). Incorporation of sawdust in the row is the industry standard. Means with the same letter above the bars are not significantly different, according to Tukey’s test ($P \leq 0.05$). **Inset:** Relationship between yield (year 2) and total plant dry weight from the previous season (year 1).

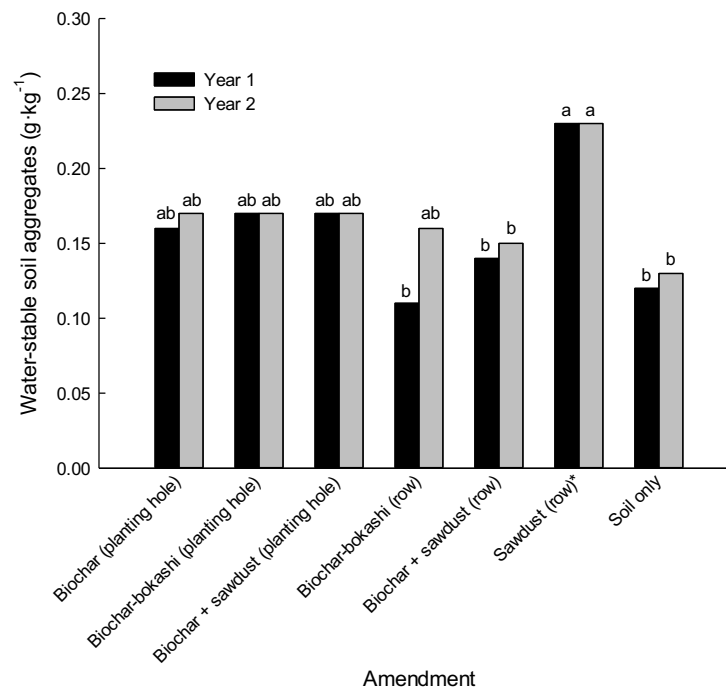


Fig. 3.3. Effects of soil amendments (biochar, biochar mixed with bokashi or douglas fir sawdust, and sawdust only) on soil aggregation following the first 2 years after planting a new field of ‘Duke’ northern highbush blueberry in western Oregon. The amendments were incorporated in the planting hole (20% by volume) or in the entire row (10% by volume for the biochar mixes and 20% by volume for sawdust) of the planting bed (see Material and Methods for details). Incorporation of sawdust in the row is the industry standard. Means with the same letter above the bars of a given year are not significantly different, according to Tukey’s test ($P \leq 0.05$).

**Chapter 4 – Growth and Nitrogen Nutrition of Northern Highbush Blueberry in
Soil Amended with Untreated and Ammonium-Enriched Organic Substrates**

4.1. Abstract

Organic substrates such as bark, sawdust, and compost are used in biofilters to remove ammonia and other odorous compounds from contaminated airstreams before release into the atmosphere. A byproduct of the process is an ammonium-rich material that must be replaced periodically. A 12-week study was conducted in a glasshouse to evaluate the potential of using such a material as a soil amendment for production of northern highbush blueberry (*Vaccinium corymbosum* L. 'Duke'). One-year-old plants were transplanted from 72-cell trays into 4-L pots filled with silty loam soil that was amended 20%, by volume, with one of six different substrates, including red alder (*Alnus rubra* Bong.) sawdust; douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] sawdust, shavings, or wood chips; biochar (gasified mix of douglas fir bark, wood chips, and wood fiber); and compost (produced from municipal yard debris). The amendments were either unenriched or enriched with ammonium-N prior to incorporating them into the soil. Plants grown with the enriched and unenriched amendments were fertigated weekly with ammonium sulfate solution at rates of 0, 25, and 50 ppm N. Overall, plants grown with the enriched amendments showed little to no response to N fertigation and had greater leaf area, shoot length, total dry weight, leaf N concentrations, and SPAD meter readings than those grown with the unenriched amendments and fertigated with 0–50 ppm N. These findings indicate that ammonium-enriched amendments can be used in blueberry to increase growth and N fertilizer use efficiency.

4.2. Introduction

Blueberry (*Vaccinium* sp.) is adapted to well-drained acidic soils with a high amount of organic matter (Retamales and Hancock, 2018). Acidic woody materials such as bark and sawdust are often incorporated into the soil prior to planting a new field of blueberry and used as mulch afterwards. However, these materials usually have high C:N ratios (> 400), which results in N immobilization during decomposition by soil microbes and a need for additional N fertilizer (Hart et al., 2006). Organic amendments high in N, such as composts derived from herbaceous materials and manures, are not suited to the edaphic requirements of blueberry due to their high pH and EC. (Sullivan et al., 2014). Increasing the N concentration of organic materials suitable for blueberry could optimize their efficacy as soil amendments in blueberry production.

Atmospheric and ground water pollution by N is a growing environmental concern and represents a significant economic loss of mineral N (Taghizadeh-Toosi et al., 2012). As a result, there is increasing research focused on reducing atmospheric N emissions and removing excess N from polluted waters. However, removal methods such as biological nitrification-denitrification, breakpoint chlorination, and chemical precipitation require high initial investment and operating costs (Huang et al., 2015; Song et al., 2011; Zhu et al., 2012). Adsorption is a simple and effective N removal process that utilizes mineral materials such as fly ash, zeolite, and sepiolite, as adsorbents (Abdulrazzaq et al., 2014; Liang et al., 2016; Wang and Wu, 2006). However, all of these materials require a secondary treatment, increasing costs and decreasing their popular use (Liang et al., 2016). Organic materials such as sawdust, wood chips, compost, and biochar are promising alternatives due to their high absorbance capacities, high

availability, and relatively low cost (Harmayani and Anwar, 2016; Kizito et al., 2016; Zarabi and Jalali, 2018). For example, Zarabi and Jalali (2018) found that the ammonium-N ($\text{NH}_4\text{-N}$) adsorption capacity of canola residue, mushroom compost, and municipal waste compost was comparable to that of zeolite and bentonite clays. Likewise, Wahab et al. (2010) found that eucalyptus (*Eucalyptus* sp.) sawdust was more efficient at $\text{NH}_4\text{-N}$ adsorption than sepioloite and some zeolites. Adsorbed N on these organic materials is plant available and, therefore, could be utilized potentially as agricultural fertilizers (Kitzo et al., 2016; Wahab et al., 2010).

Pyrogenic materials, such as biochar, have been gaining interest for potential use in agriculture and in environmental applications (Gai et al., 2014). Biochar is a highly stable, carbon-rich residue produced by the thermal decomposition of organic materials under low oxygen environment at $< 700\text{ }^\circ\text{C}$ (Lehman and Joseph, 2009). The result is a highly porous material with a high specific surface area that is dense in negative surface charge (Liang et al., 2006). Wood-derived biochars have been proven effective at removing excess $\text{NH}_4\text{-N}$ from anaerobically digested swine slurry (Kizito et al., 2016). Wang et al. (2015) reported that a woody biochar released 27% of adsorbed $\text{NH}_4\text{-N}$ in water and up to 99% with KCl extract, which suggests that $\text{NH}_4\text{-N}$ adsorbed to biochar is plant available and has potential for use as a slow-release fertilizer.

Ammonium-N is the primary form taken up by blueberry roots (Darnell and Hiss, 2006), and thus, organic materials enriched with $\text{NH}_4\text{-N}$ are potentially well suited for use in blueberry. Additionally, increasing the N concentration in woody materials such as sawdust could lower their C:N ratio, reducing additional N applications. However, the majority of research investigating the use of organic materials to adsorb $\text{NH}_4\text{-N}$ is

focused on the efficiency of N removal, with less attention given to desorption, and only a few investigating growth effects of plants grown in N-enriched amendments (Kocatürk-Schumacher et al., 2018; Taghizade-Toosi et al., 2012). The objective of this study was to investigate the response of northern highbush blueberry to ammonium-enriched amendments. We hypothesized that N adsorbed by the enriched amendments would be plant available and increase plant growth and N nutrition relative to using untreated amendments, particularly under low rates of N fertilizer application.

4.3. Materials and Methods

Plant material and treatments. One-year-old liners of ‘Duke’ northern highbush blueberry were obtained from a commercial nursery (Fall Creek Farm & Nursery, Lowell, OR) and transplanted individually into 4-L black polyethylene pots filled with Willamette silt loam (fine-silty, mixed, mesic pachic ultic argixerolls) soil that was amended 20%, by volume, with six different untreated or N-enriched substrates, including red alder sawdust (Rexius, Eugene, OR), douglas fir sawdust, shavings, or wood chips (Lane Forest Products, Eugene, OR), biochar (BioLogical Carbon, LLC), or compost (Rexius, Eugene, OR). The biochar was produced using gasification at 700–800 °C from hog fuel (coarse chips of bark and wood fiber leftover from lumbered douglas fir trees). The compost was produced from local yard waste (grass clippings, leaves, tree trimmings, and shrub prunings) and cured in aerated static air piles.

To enrich the amendments, 25 L of each was soaked for 24 h in 1% ammonium sulfate $[(\text{NH}_4)_2\text{SO}_4]$ solution. To reduce pH to < 7 , 99.7% acetic acid was also added to the solutions with biochar and compost (10 and 20 mL, respectively). After soaking, the

amendments were poured over a 0.25-mm screen and rinsed three times with tap water. The same procedure was followed for the untreated amendments, but in this case, tap water was substituted for 1% $(\text{NH}_4)_2\text{SO}_4$ solution. After rinsing, the amendments were laid out on a greenhouse bench and air-dried for 7 d prior to transplanting.

After transplanting, the plants were placed on three benches in a glasshouse (USDA-ARS Horticultural Crops Research Unit, Corvallis, OR; lat. $44^\circ 34' 3''$ N, long. $123^\circ 17' 9''$ W). Temperature inside the glasshouse was maintained at $28 \pm 2^\circ\text{C}$ during the day and $20 \pm 2^\circ\text{C}$ at night. Photoperiod was extended to $14\text{ h}\cdot\text{d}^{-1}$ using two 1000-W high-pressure sodium lamps suspended $\approx 1.5\text{ m}$ above the canopy of the plants. Plants grown in each amendment were irrigated by hand three times per week ($\approx 20\%$ drainage) and fertilized once a week with 100 mL of water (0 ppm N) or $(\text{NH}_4)_2\text{SO}_4$ solution containing 25 or 50 ppm N.

Data collection. One kg of soil was sent to a commercial laboratory (Brookside Laboratories, New Bremen, OH) prior to transplanting and analyzed for pH, organic matter content, cation exchange capacity (CEC), total C and N, NH_4^- and NO_3^- -N, and other extractable nutrients (Gavlak et al., 2005). Each sample was mixed to a 1:10 (soil:water) to determine pH. Organic matter content of the soil was determined using loss-on-ignition at 360°C . Ammonium- and nitrate-N (NO_3^- -N) were extracted from the soil with 2 M KCl and determined colorimetrically using a rapid-flow analyzer. Soil nutrients were extracted for P (Bray I) and other nutrients (Mehlich III), including K, Ca, Mg, SO_4 -S, B, Cu, Fe, Mn, and Zn, and analyzed using an inductively-coupled plasma (ICP) spectrometer.

One kg of each of the soil amendments was sent to a commercial laboratory

(Brookside Laboratories, New Bremen, OH) prior to transplanting and analyzed for pH, organic matter content, cation exchange capacity (CEC), total C and N, and total nutrients from methods described in “Test Methods for the Examination of Composting and Compost: (TMECC). The CEC of each untreated amendment was determined using the ammonium acetate displacement method at pH 7 (Sparks, 1996). Total C and N of each amendment were determined by combustion analysis (TMECC 4.01). Total N was determined by combustion analysis (TMECC method 4.02). Soil nutrients, including K, Ca, Mg, SO₄-S, B, Cu, Fe, Mn, and Zn, was analyzed using an inductively-coupled plasma (ICP) spectrometer (TMECC 4.15). Amendments were dry-ashed at 500 °C and then determined for nutrients by ICP.

Leaf greenness (reflectance) was estimated nondestructively at 12 weeks after transplanting using a SPAD-502 chlorophyll meter (Konica Minolta, Osaka, Japan). Measurements were taken at mid-canopy on single, healthy, fully expanded leaves in each pot. Triplicate readings were recorded and averaged for each plant. At harvest, the plants were then cutoff at the soil surface and separated into stems and leaves. Roots were washed to remove soil under running water. Each plant part was oven-dried for at least 48 h at 60 °C, weighed, ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass through a 20-mesh screen. Plant tissue was analyzed for total N using a combustion analyzer (TruSpec CN; Leco Corp., St. Joseph, MI). Reference standard apple [*Malus × sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf.] leaves (no. 151, National Institute of Standards and Technology) were included with each run to ensure accuracy of the N analysis.

Experimental design and statistical analysis. Treatments (6 soil amendments × 2

N enrichment levels \times 3 N rates) were arranged in a completely randomized design with five replicates per treatment (180 total plants). Data were checked for normality (Shapiro-Wilk test) and homogeneity of variance (Brown-Forsythe test) and analyzed by three-way analysis of variance (Systat Software Inc., San Jose, CA). Means were separated within N treatments (enrichment and rate) using Tukey's honestly significant difference test ($P \leq 0.05$). Effects of N enrichment on a given substrate were evaluated using Student's t test.

4.4. Results

Chemical characterization of the soil amendments. Enrichment increased N in the amendments (Table 4.1). Biochar absorbed the most N among the amendments and, along with compost, had the highest concentration following N enrichment. Alder sawdust, on the other hand, absorbed the least amount of N and had the lowest concentration of N among the enriched amendments. Douglas fir wood fractions absorbed different amounts of N but, once enriched, had a similar concentration of N in each.

In addition to N, enrichment also had a considerable effect on pH, C:N ratio, and several nutrients in the amendments, including each of the major cations (K, Ca, and Mg) and the micronutrients (Table 4.2). In each amendment, pH and C:N ratio declined as a result of enrichment, including in biochar and compost. Concentration of available K, Ca, and Mg also declined in every amendment. Total micronutrients also increased in most of the amendments, including B and Zn in biochar and Cu in all amendments, while Mn decreased in all amendments.

Plant growth. Shoot and root dry weight were significantly affected by an interaction between amendment and N enrichment, but neither were affected by N rate or any interactions with N rate (Table 4.3).

In most cases, plants grown in soils with N-enriched amendments had greater shoot dry weight than those grown with untreated amendments; however, shoot dry weight was similar between plants grown in soil with untreated and N-enriched alder sawdust (Fig. 4.1). Without enrichment, shoot dry weight was lowest with alder and douglas fir sawdust and was greatest with compost. Compost had the highest concentration of N among the untreated amendments (Table 4.2), but with enrichment, it produced a similar amount of shoot dry weight as douglas fir sawdust. Overall, N enrichment increased shoot dry by 33% to 56% when plants were amended with douglas fir shavings, douglas fir wood chips, biochar, or compost, and by 220% when they were amended with douglas fir sawdust.

Unlike the shoots, root dry weight was lower with N enrichment in 3 of the 6 amendments, including with enriched douglas fir wood chips, biochar, and compost (Fig. 4.1). Within the enrichment treatments, root dry weight was similar when the plants were grown in soil with untreated amendments but was greater with douglas fir sawdust than with biochar when the amendments were enriched.

Plant N nutrition. The concentration of N in the shoots (leaves and stems) was affected by an interaction between amendment and N enrichment but was unaffected by N rate or any interactions with N rate (Table 4.3). The concentration of N in roots, on the other hand, was affected by a three-way interaction among the treatments (Table 4.3).

Nitrogen enrichment resulted in greater concentrations of N in the leaves and stems when plants grown in soils amended with douglas fir sawdust and woodchips, biochar, and compost but had no effect when they were grown in soils with alder sawdust or douglas fir shavings (Fig. 4.2). Likewise, N enrichment resulted greater concentrations of N in the roots but only when plants were grown with douglas fir sawdust and no additional N fertilizer (i.e., 0 ppm N), with douglas fir wood chips or biochar at lower N rates (0 or 25 ppm N), or with compost at any N rate (Fig. 4.3).

Relative to the untreated amendments, total N uptake by plants more than doubled when they were grown with enriched wood chips, biochar, or compost and increased by nearly five-fold when they were grown with enriched sawdust (Fig. 4.4). Total N also increased by more than 50% when they were grown with enriched douglas fir shavings but, in this case, the value was lower than those grown with any other enriched amendment with the exception of alder sawdust. Whether the plants were grown with untreated amendments only or with enriched amendments, total N uptake was greatest when plants were grown in soil with compost.

SPAD meter readings. The SPAD meter readings were significantly affected by each two-way interaction among the treatments, including between amendment and N enrichment, amendment and N rate, and N enrichment and N rate (Table 4.3).

While the response varied among the amendments, plants grown in soils with any of the enriched amendments were greener and had higher SPAD meter readings than those grown in soils with their untreated counterparts (Fig. 4.5A). However, the readings also varied depending on the rate in which the plants were fertilized with N during the study. Overall, the readings responded positively to N fertilizer in several amendments,

including alder sawdust, douglas fir wood chips, and biochar (Fig. 4.5B), but, on average, the response to N enrichment was lower when the plants were fertilized with N (Fig. 4.5C).

The SPAD meter readings were highly correlated with leaf N concentration ($r^2 = 0.81$; $P < 0.001$).

4.5. Discussion

With exception of alder sawdust, northern highbush blueberry plants grew better in soil with N-enriched amendments than with untreated amendments. However, the best amendment tested in terms of improving plant growth response and N nutrition with enrichment was douglas fir sawdust. Relative to using untreated douglas fir sawdust, N-enriched douglas fir sawdust increased total dry weight of the plants (shoot and roots) by 177% and increased total N uptake by 460%. Furthermore, it resulted in a similar amount of growth as enriched biochar or compost. Nitrogen enrichment of douglas fir wood chips increased total N uptake and dry weight by 290% and 61%, respectively, but in this case, plants grown with this substrate had less dry weight than those grown with enriched compost.

Nitrogen enrichment increased total N uptake by plants in the amendments by an average of $4.9 \text{ mg} \cdot \text{g}^{-1}$ in the woody materials and 13 and $24 \text{ mg} \cdot \text{g}^{-1}$, respectively, in compost and biochar. The ability of biochar to adsorb N is well-documented but varies depending on its physiochemical properties (Kyoung et al., 2015; Wichuk and McCartney, 2010; Yao et al., 2012; Zheng et al., 2018). For example, Kizito et al (2015) examined adsorption of $\text{NH}_4\text{-N}$ from anaerobically digested swine slurry using three

types of biochar and found that a biochar produced from hardwood performed better than those derived from corncobs or mixed sawdust pellets due to its superior surface area and larger pore volume. However, desorption experiments using water and KCl extractions indicate that biochar releases far less $\text{NH}_4\text{-N}$ than it adsorbs (Saleh et al., 2012).

Therefore, the N concentration of enriched biochar is not necessarily representative of plant available N. Available N in enriched douglas fir sawdust was apparently highly available as it resulted in more growth than expected based on its total N.

Investigations of NH_4 adsorption by wood fractions is limited. Wahabi et al. (2010) reported that eucalyptus sawdust adsorbed $1.26 \text{ mg} \cdot \text{g}^{-1} \text{ NH}_4\text{-N}$ when it was enriched using an aqueous solution containing $50 \text{ mg} \cdot \text{L}^{-1} \text{ N}$. The lower adsorption rate reported in this case was likely due to lower concentration of $\text{NH}_4\text{-N}$ in the solution. In contrast, canola residues, municipal waste compost, mushroom compost, and bran adsorbed $7\text{--}10 \text{ mg} \cdot \text{g}^{-1} \text{ NH}_4\text{-N}$ when they were enriched with a solution containing $1000 \text{ mg} \cdot \text{L}^{-1} \text{ N}$ (Zarab and Jalali 2018). This latter result is more representative of N adsorption observed in the present study, where the amendments were enriched in a solution containing $10,000 \text{ mg} \cdot \text{L}^{-1} \text{ N}$.

The use N enriched amendments increased the concentration of N in blueberry plants, particularly in the shoots. Higher N concentrations are often reported when plants are grown in soil with N-enriched amendments (Chen et al., 2018; Mehrab et al., 2016; Xu et al., 2018). Taghizade-Toosi et al. (2012) found that $\text{NH}_4\text{-N}$ adsorbed to biochar from ruminant urine increased N concentration in shoots and roots of ryegrass (*Lolium perenne* L.). In a similar study, Kocatürk-Schumacher et al. (2018) reported that potted ryegrass grown in N-enriched biochar increased N uptake by $\approx 11 \text{ mg}$ for each g of N

applied to the pots. Much like in the present study, the largest increase in shoot N concentration in ryegrass occurred when the plants were grown in soil with N-enriched compost or biochar, and those plants had higher N concentrations than ones grown in soil with enriched woody amendments.

Despite large increases in N uptake and shoot growth, plants grown in soil with several of the N enriched amendments, including douglas fir wood chips, biochar, and compost, had less root growth than those grown in soil with corresponding untreated amendments. A similar result was reported by Chen et al., (2018), who found that cabbage (*Brassica chinensis* L.) grown in soil amended with urea-enriched biochar produced 88% more aboveground dry weight but 160% less root dry weight than those grown in untreated biochar. Root growth is often reduced with increasing N availability (Wilson, 1998; Poorter and Nagel, 2000), which was particular high in enriched biochar and compost. However, root growth was not suppressed in the present study, when the plants were grown in soil amended with N-enriched douglas fir sawdust.

4.6 Conclusions

In summary, N-enriched douglas fir sawdust and wood chips were excellent amendments for improving growth and N nutrition in northern highbush blueberry. Both substrates are highly porous in nature and work well as biofilters. Woody biofilters are highly efficient for treating odors associated with animal farms, biogas plants, and composting facilities, including ammonia gas and volatile organic S compounds (e.g., Luo and van Oostrom, 1997). Once enriched with adsorbed N, these biofilters could be used potentially as excellent sources of nutrients and organic matter for blueberry.

Biochar and compost biofilters could also work well for this purpose, but, generally, these substrates are much more expensive than sawdust and wood chips (Hort et al., 2009). Many compost are also high pH and salts, which could be detrimental to blueberry (Costello et al., 2019).

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4.8. Tables and Figures

Table 4.1. Total N in six untreated and N-enriched soil amendments.

Amendment	Total N (g·kg ⁻¹)		
	Untreated	N enriched	Increase (%)
Alder sawdust	2.2	5.4	245
Douglas fir sawdust	2.6	8.8	338
Douglas fir shavings	3.5	7.5	214
Douglas fir wood chips	1.4	7.7	550
Biochar	4.0	30.0	750
Compost	16.3	29.6	182
Average	5.0	14.8	

Table. 4.2. Chemical analysis of six untreated and N-enriched soil amendments (dry weight basis).^z

Soil amendment	pH	CEC (cmol c /kg) ^y	C:N	Total P (g·kg ⁻¹)	Total S (g·kg ⁻¹)	Cation (g·kg ⁻¹)			Micronutrients (mg·kg ⁻¹)			
						K	Ca	Mg	B	Cu	Mn	Zn
Untreated												
Alder sawdust	6.5	n.i.	229	0.1	0.1	1.3	1.0	0.3	3	2	30	8
Douglas fir sawdust	4.5	23	196	0.1	0.1	0.4	0.6	0.1	3	1	18	6
Douglas fir shavings	4.5	32	143	0.1	0.1	0.4	1.0	0.1	2	1	16	6
Douglas fir wood chips	5.0	13	350	0.1	0.1	0.6	0.6	0.1	3	1	22	6
Biochar	10.0	32	209	0.7	0.3	8.1	7.4	1.4	19	12	372	7
Compost	7.5	49	23	2.7	1.6	10.2	18.2	4.9	22	58	592	208
N enriched												
Alder sawdust	3.3	—	90	0.1	0.1	0.1	0.6	0.1	4	11	6	10
Douglas fir sawdust	3.3	—	59	0.1	0.0	0.1	0.2	0.03	2	5	3	6
Douglas fir shavings	4.2	—	60	0.2	0.2	0.6	0.7	0.2	3	17	3	11
Douglas fir wood chips	3.6	—	68	0.1	0.0	0.1	0.1	0.1	2	10	5	5
Biochar	4.0	—	28	0.6	0.9	0.8	7.2	0.9	78	31	204	30
Compost	4.7	—	9	2.4	2.2	1.2	8.8	2.2	21	56	535	200

^zEach amendment was mixed with four parts sandy loam soil and planted with northern highbush blueberry. The soil had an initial pH of 5.0 (1 soil : 1 water) and contained 3.34% organic matter, 61 mg·kg⁻¹ NH₄-N and 2 mg·kg⁻¹ NO₃-N, 120 mg·kg⁻¹ P (Bray I), 21 mg·kg⁻¹ SO₄-S, 344 mg·kg⁻¹ K, 1360 mg·kg⁻¹ Ca, 178 mg·kg⁻¹ Mg, 0.22 mg·kg⁻¹ B, 1.08 mg·kg⁻¹ Cu, 67 mg·kg⁻¹ Mn, and 1.42 mg·kg⁻¹ Zn.

^yMeasurements were made using buffered ammonium acetate (pH 7), which can underestimate the CEC of alkaline materials such as biochar and compost and overestimate the CEC of low pH materials such as douglas fir sawdust, shavings, and wood chips. The measurement on alder sawdust had a CV > 50 and, therefore, was not included (n.i.) in the table.

Table 4.3. Results from analysis of variance from a $6 \times 2 \times 3$ factorial experiment involving six organic soil amendments (alder sawdust, douglas fir sawdust, wood chips, or wood shavings, biochar, and yard debris compost), two levels of ammonium-N enrichment (untreated and N enriched), and three N rates (0, 25, and 50 ppm N) arranged in a completely randomized design with five replications.

Source	Dry wt (g/plant)			N concn (%)			Total N ^y (g/plant)	SPAD meter reading
	Shoot ^z	Roots	Total	Leaves	Stems	Roots		
Amendment (A)	<0.001	0.251	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
N enrichment (E)	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
N rate (R)	0.427	0.647	0.446	0.179	0.640	<0.001	0.151	<0.001
A × E	<0.001	0.048	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
A × R	0.098	0.994	0.212	0.098	0.301	<0.001	0.423	0.009
E × R	0.133	0.530	0.210	0.894	0.532	<0.001	0.192	0.005
A × E × R	0.577	0.925	0.680	0.927	0.223	<0.001	0.168	0.921

^zShoots includes leaves and stems.

^yTotal N included N in the leaves, stems, and roots.

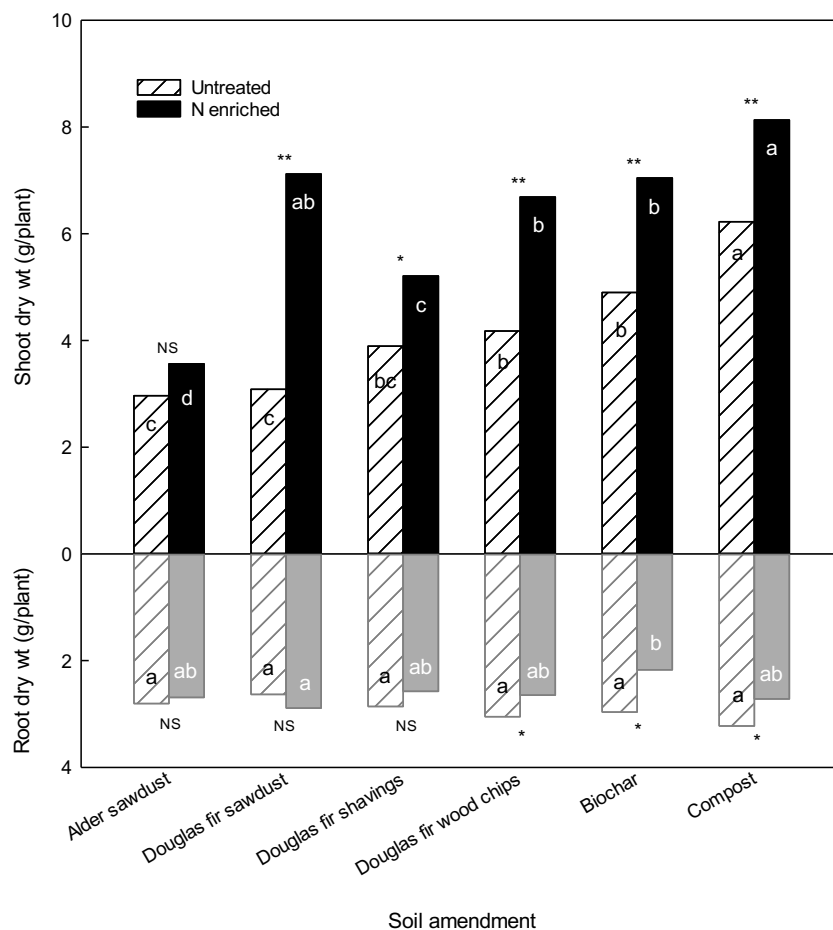


Fig. 4.1. Interactive effects of soil amendment \times N enrichment on shoot (leaves and stems) and root dry weight of 'Duke' blueberry. Data were pooled across three rates of N fertigation (0, 25, and 50 ppm N). Each bar represents the mean of five replicates. Means with the same letter within a given enrichment treatment are not significantly different, according to Tukey's test ($P \leq 0.05$). Asterisks above or below the bars indicate dry weight within a given amendment was affected by N enrichment at $P \leq 0.05$ (*) or 0.01 (**), according to Student's t test. NS – non-significant.

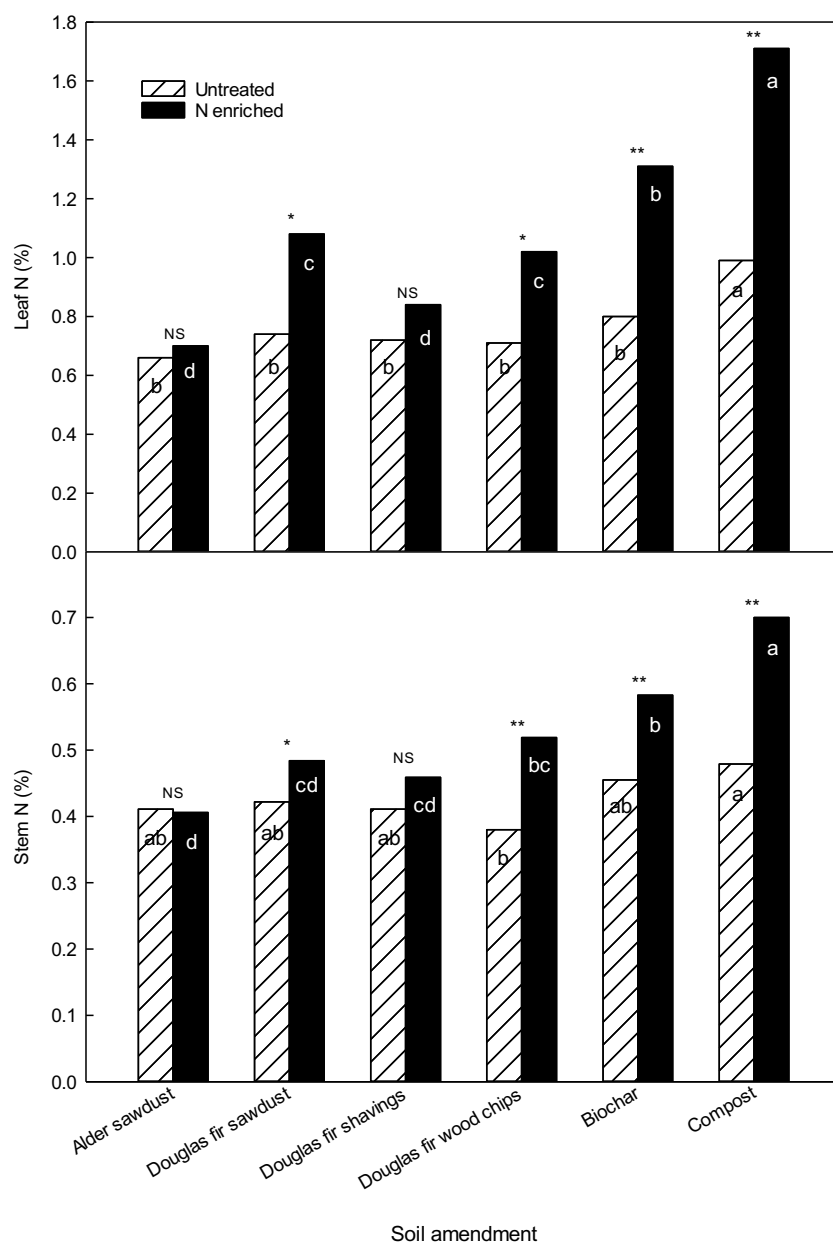


Fig. 4.2. Interactive effects of soil amendment \times N enrichment on concentration of N in the leaves and stems of 'Duke' blueberry. Data were pooled across three rates of N fertigation (0, 25, and 50 ppm N). Each bar represents the mean of five replicates. Means with the same letter within a given enrichment treatment are not significantly different, according to Tukey's test ($P \leq 0.05$). Asterisks above the bars indicate N concentration within a given amendment was affected by N enrichment at $P \leq 0.05$ (*) or 0.01 (**), according to Student's *t* test. NS – non-significant.

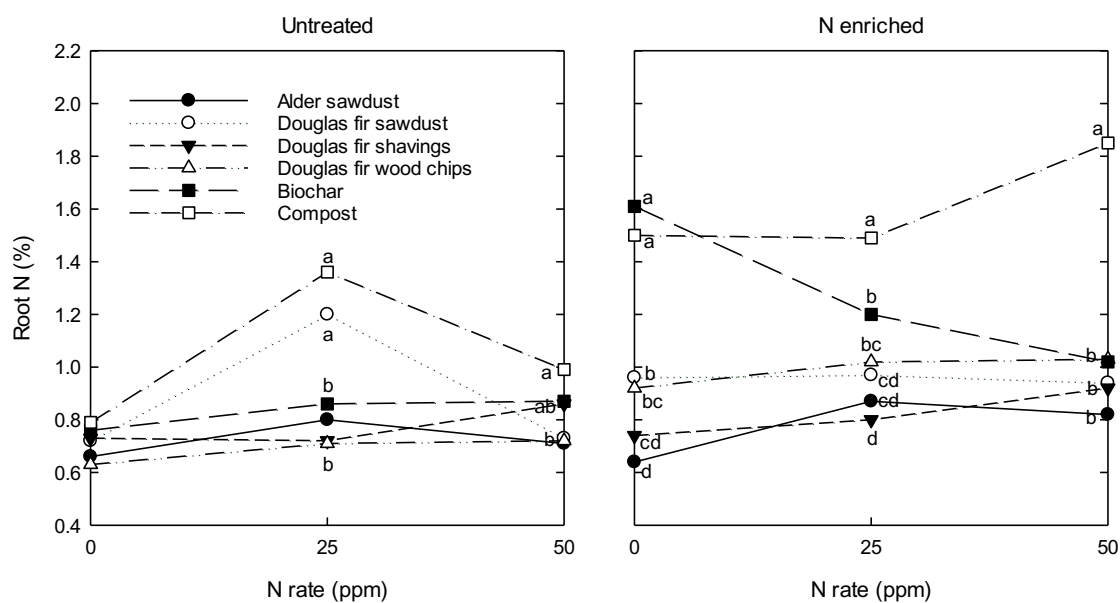


Fig. 4.3. Interactive effects of soil amendment \times N enrichment \times N rate on concentration of N in the roots of 'Duke' blueberry. Each symbol represents the mean of five replicates. Means with the same letter within a given N rate are not significantly different, according to Tukey's test ($P \leq 0.05$).

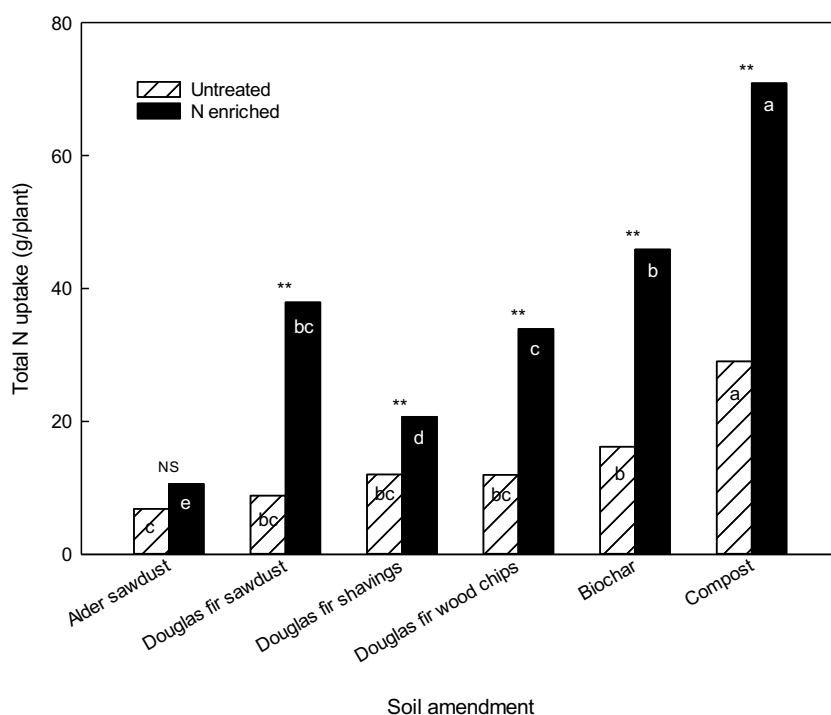


Fig. 4.4. Interactive effects of soil amendment \times N enrichment on total N content in ‘Duke’ blueberry. Data were pooled across three rates of N fertigation (0, 25, and 50 ppm N). Each bar represents the mean of five replicates. Means with the same letter within a given enrichment treatment are not significantly different, according to Tukey’s test ($P \leq 0.05$). Asterisks above the bars indicate N content within a given amendment was affected by N enrichment at $P \leq 0.01$, according to Student’s t test. NS – non-significant.

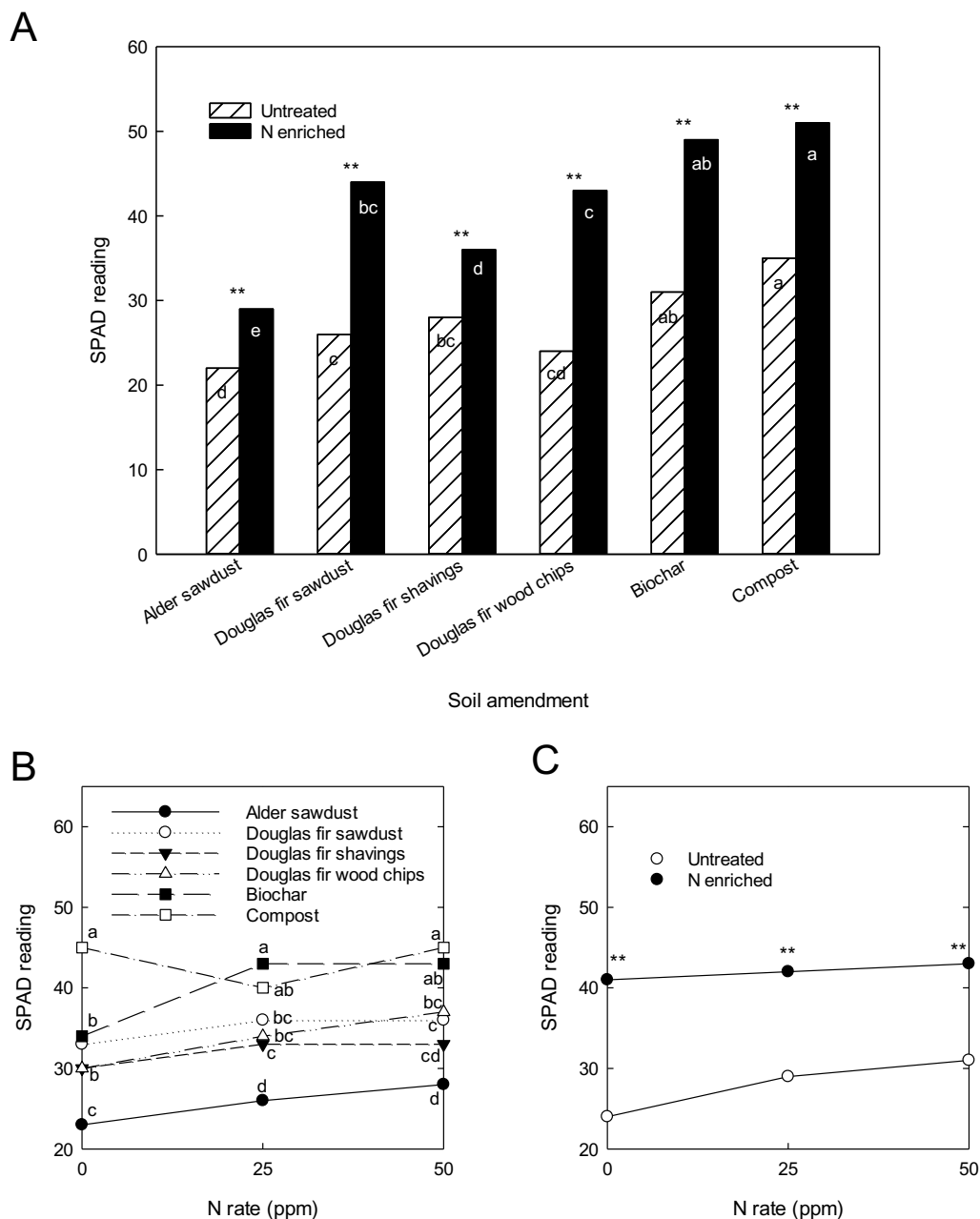


Fig. 4.5. Interactive effects of soil amendment \times N enrichment (A), soil amendment \times N rate (B), and N enrichment \times N rate (C) on SPAD meter readings measured on new, fully expanded leaves of 'Duke' blueberry. Each bar or symbol represents the mean of five replicates. Means with the same letter within a given enrichment treatment (A) or N rate (B) are not significantly different, according to Tukey's test ($P \leq 0.05$). Asterisks above the bars or symbols indicate readings within a given amendment (A) or N rate (C) were affected by N enrichment at $P \leq 0.01$, according to Student's *t* test.

Chapter 5 – General Conclusions

The work revealed that biochar produced via gasification of douglas fir wood materials between 700–800 °C could be used as a soil amendment for production of highbush blueberry. Under controlled greenhouse conditions, biochar not only improved plant growth but also greatly increased root colonization by ericoid mycorrhizal fungi. Growth was further enhanced under nutrient-limited conditions when bokashi (fermented wheat bran) was added to the biochar. However, with or without bokashi, biochar did not suppress root infection by *P. cinnamomi*, and in fact, increased soil moisture availability, which could contribute to increased occurrence of root rot in the presence of the pathogen. While biochars are often considered liming agents, the one used in the current project had little effect on soil pH, particularly when it was used in conjunction with NH₄-N fertilizer. Lack of a large increase in soil pH was likely due to low ash content of the woody biochar and the acidifying effect of the fertilizer. Therefore, biochar and the source of fertilizer should be considered when selecting biochar as a soil amendment for blueberry.

Biochar also improved plant growth as well as early fruit production under field conditions in a new planting of highbush blueberry. In fact, by the second season, yield was more than 70% higher in plants grown in soil with biochar than in those grown with douglas fir sawdust in the row or in soil without any amendments. Unlike douglas fir sawdust, which is commonly used as a soil amendment for blueberry in the Pacific Northwest, biochar did not result in any problems with N immobilization or water limitations in the new planting. The most cost-effective method to apply biochar was

adding it to the planting hole, which was approximately \$1300/ha less than using sawdust in the row. However, this method was somewhat laborious and difficult to apply consistently. Perhaps, biochar could be applied in a narrow band on the row (e.g., 10–20 cm wide) and incorporated into the soil prior to shaping the beds. Doing so would require slightly more biochar but reduce labor costs considerably. More research is needed to identify the best method to apply the biochar. Studies are also needed to determine whether biochar has any long-lasting effects on fruit production and mineral nutrition in blueberry.

Enrichment of woody materials with $\text{NH}_4\text{-N}$ lowered the C:N ratio by 270%, on average, and produced materials that acted as a slow-release fertilizer or compost. In general, ‘Duke’ blueberry grew better in soil with N-enriched amendments than with untreated amendments. Six different potential amendments were tested in the greenhouse, and the best in terms of improving plant growth and N nutrition was douglas fir sawdust. Relative to using untreated douglas fir sawdust, N-enriched douglas fir sawdust increased total dry weight of the plants by 177% and increased total N uptake by 460%. It also resulted in more-or-less the same amount of growth as enriched biochar or compost. Enriched douglas fir wood chips were also effective for improving growth but less so than enriched sawdust or compost. Both sawdust and wood chips work well as biofilters and are highly efficient for treating odors associated with animal farms, biogas plants, and composting facilities, including ammonia gas and volatile organic S compounds. Once fully loaded, these biofilters could be used potentially as excellent sources of nutrients and organic matter for blueberry. Biochar and compost biofilters could also

work well for this purpose, but, generally, these substrates are much more expensive than sawdust or wood chips.

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LIST OF APPENDICES

5.2 Appendix

- A. Fig. 1. Effects of biochar amendments and inoculation with *Phytophthora cinnamomi* on pH and electrical conductivity (EC) of the soil solution in pots grown with 'Legacy' blueberry in Expt. 2 (Chapter 2).
- B. Fig 2. Effects of biochar amendments on the relative uptake of macro- (N, P, K, Ca, Mg, and S) and micronutrients (B, Cu, Mn, Zn) by non-inoculated 'Legacy' blueberry plants in Expt. 1 and 2 (Chapter 2). An asterisk indicates the amendment resulted in an increase or decrease in uptake of a given nutrient relative to soil only ($P < 0.05$).

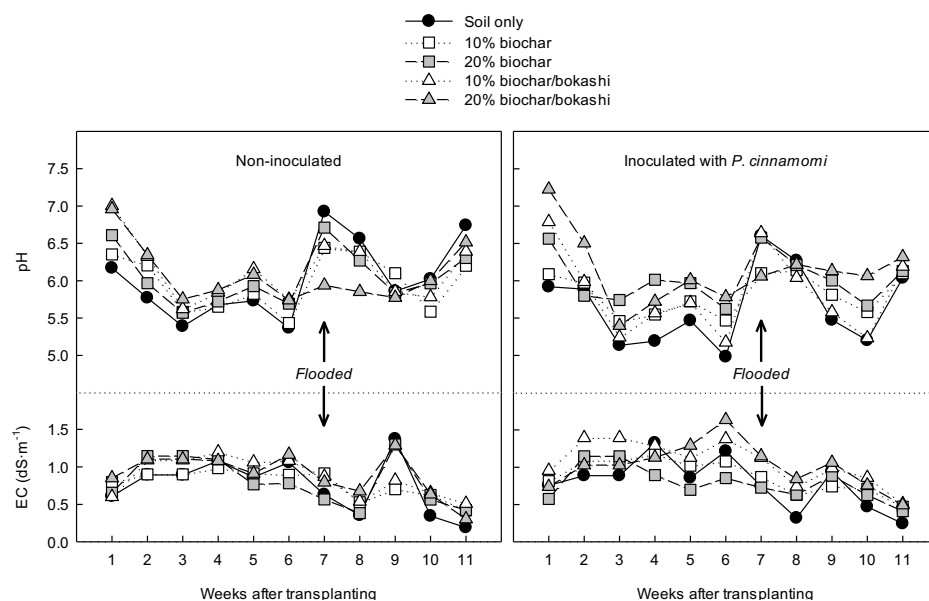


Fig. 1. Effects of biochar amendments and inoculation with *Phytophthora cinnamomi* on pH and electrical conductivity (EC) of the soil solution in pots grown with 'Legacy' blueberry in Expt. 2 (Chapter 2).

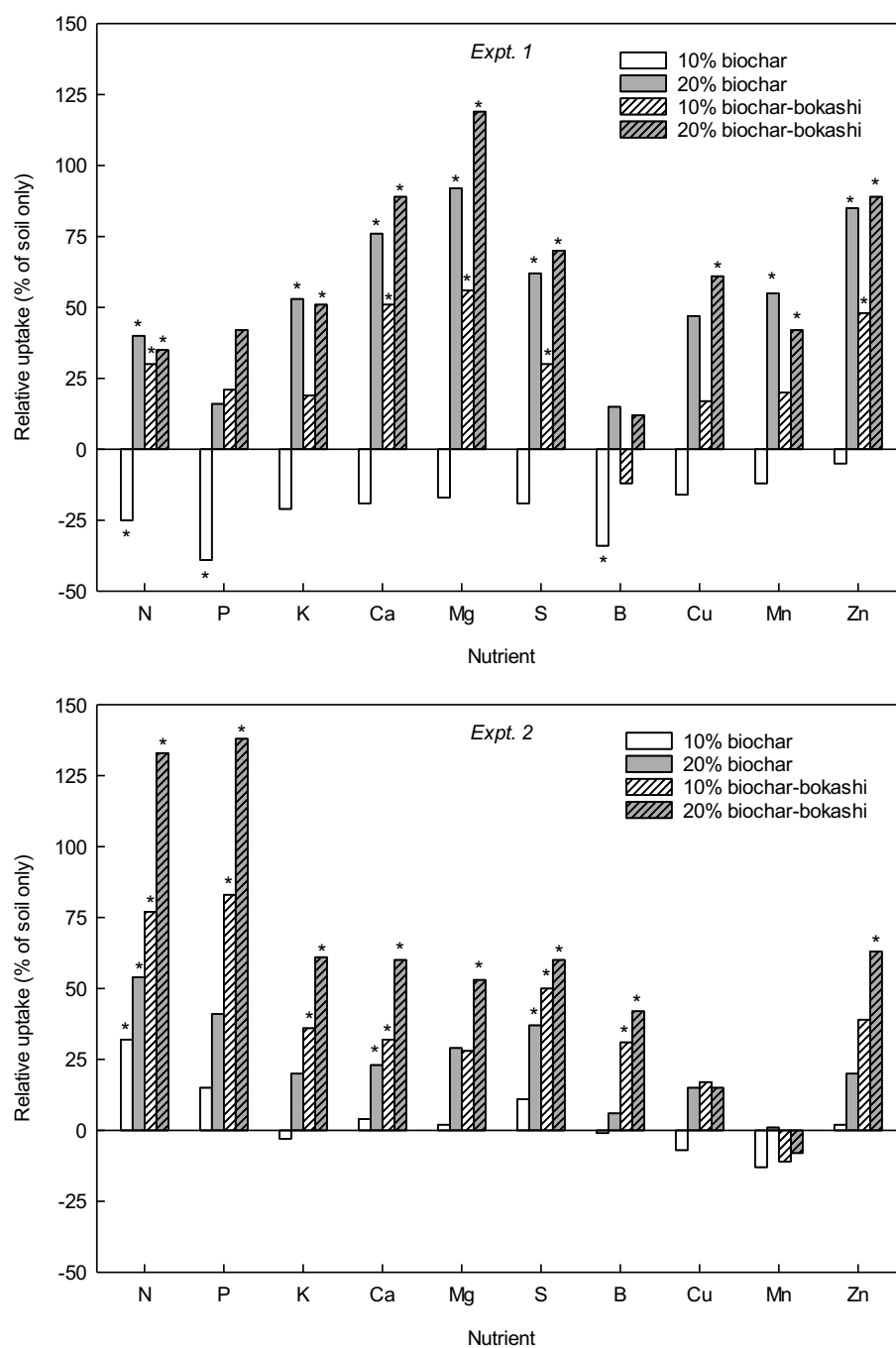


Fig 2. Effects of biochar amendments on the relative uptake of macro- (N, P, K, Ca, Mg, and S) and micronutrients (B, Cu, Mn, Zn) by non-inoculated 'Legacy' blueberry plants in Expt. 1 and 2 (Chapter 2). An asterisk indicates the amendment resulted in an increase or decrease in uptake of a given nutrient relative to soil only ($P < 0.05$).