Douglas-fir seedling quality in biochar-amended peat substrates

Jessica L Sarauer[1], Mark D Coleman[2]

[1]State University of New York at Morrisville, Morrisville, USA
[2]Department of Forest, Rangeland and Fire Science, University of Idaho, Moscow, USA

✉ mcoleman@uidaho.edu

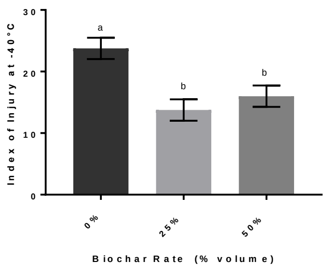
Abstract

Artificial forest regeneration using nursery produced growing stock is commonplace in the Pacific Northwest. High quality seedlings are needed for outplanting success, which depends on a seedling's ability to establish new roots and overcome stress. Containerized seedling stock is typically grown in artificial growing media. Peat, a popular component of growing media, is a non-renewable resource. Biochar has similar physical attributes to peat, which makes it a potential alternative. In our study, we grew Douglas-fir seedlings in containers with biochar-amended peat-based growing media to determine if biochar could improve seedling quality. Douglas-fir seeds were sown in March 2016 and seedlings were grown under standard light and temperature conditions at an operational forest nursery for nine months. After nine months, seedling quality was assessed for height, diameter, cold hardiness, and root growth potential. Using biochar did not improve Douglas-fir seedling quality, except for slightly increasing cold hardiness and root growth potential for equivalently sized seedlings. Seedlings grown without biochar had increased height and diameter compared to seedlings with biochar and they had higher root growth potential (all dependent on fertilizer rates). Douglas-fir seedling quality might be improved with biochar amendment if negative growth impacts of soil reaction can be overcome.

Keywords

Conifer; Electrolyte leakage; Fertilizer; Root growth potential index; Forest regeneration

Contents



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1 Introduction

Following harvest, Idaho forests are typically planted with seedlings produced in containerized seedling nurseries. In 2015, over 154 million nursery-grown seedlings were produced in Idaho, Oregon, and Washington (Hernandez et al. 2016). Nursery-grown seedlings receive adequate water and nutrients but after outplanting, seedlings are often exposed to many environmental stressors. Therefore, production of high-quality seedlings that can tolerate transplant stress and rapidly establish new roots is essential for successful reforestation (Grossnickle 2005; Haase et al. 2006).

Seedling quality can be assessed using morphological or physiological metrics (Mattsson 1997). Seedling height and stem diameter are evaluated most often because they are easy to measure and have been shown to be good estimates of overall outplanting success (Mexal and Landis 1990; Long and Carrier 1993; Rose and Ketchum 2003). There are additional morphological seedling quality measures that can be used to assess plant vigor or field performance, but none are as common as seedling height and diameter measurements (Haase 2008). Physiological measurements of seedling quality provide information about the seedling's response to stress (Haase 2008). One common physiological measure is cold hardiness (Haase 2008), which measures the seedling's ability to survive sub-freezing temperatures and is an indicator of overall stress resistance (Burr 1990). Cold hardiness fluctuates with temperature, photoperiod, and precipitation but can be manipulated in the nursery via irrigation, fertilization, and other culturing practices (Burr 1990). Root growth potential (RGP) is another physiological assessment of seedling quality. RGP is a measure of a seedling's ability to grow roots when put in an ideal environment for a set period of time (Simpson and Ritchie 1997). A seedling's capacity to grow new roots can aid in overcoming root confinement, poor root-soil contact, and low root permeability, all of which can cause water stress (Burdett 1990). Root growth potential has been shown to be a good predictor of field performance for Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings, even though RGP only reflects root growth under ideal conditions (McCreary and Duryea 1987; Simpson and Ritchie 1997).

Container seedlings are typically grown in peat-based growing medium due to the favorable physical and chemical attributes of peat that allow for gas exchange, plant support, and water provision (Michel 2010). Recently, there has been a call for a substitute for peat (Abad et al. 2001) because of the environmental and economic concerns associated with harvesting and utilizing peat.

A possible alternative or amendment to peat, is biochar, yet its effects on seedling quality are unknown. Biochar is charcoal produced during pyrolysis (Bridgewater 2004) with potential for soil application (Lehmann and Joseph 2009). Biochar could be a suitable replacement or amendment to peat because it has some of the same physical attributes as peat including low bulk density (Blok et al. 2017), high total air space (Blok et al. 2017), and high water retention (Laird et al. 2010). When added to peat, biochar increases air space, water-holding capacity, and total porosity (Mendez et al. 2015). Biochar amendment to peat also results in higher growing medium nutrient concentrations both with (Nemati et al. 2015) and without fertilizer (Locke et al. 2013), increased pH (Nair and Carpenter 2016), and increased cation exchange capacity (CEC) (Headlee et al. 2014). Although biochar alone can supply some cations, it does not always provide adequate P or K for container crops (Locke et al. 2013) and can actually decrease P availaiblty for acid-loving conifers (Sarauer and Coleman 2018). Pre-treating biochar with fertilizer can promote plant growth and increase functional chemical groups that create electrostatic charge for cation binding (Joseph et al. 2013). Nonetheless, biochar attributes vary depending on feedstock and pyrolysis conditions (Masek et al. 2013) and these differences must be considered when assessing plant response (Chan et al. 2008). Other research has found plant growth responses to biochar to vary by finding biochar to decrease (e.g. Bi et al. 2009; Gravel et al. 2013; Matt et al. 2018), cause no change (e.g. Locke et al. 2013; Matt et al. 2018), or increase plant growth (e.g. De Tender et al. 2016; Headlee et al. 2014). When specifically looking at conifer species, Dumroese et al. (2018) found biochar can replace up to 25% peat in container grown ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson) and Matt et al. (2018) found ponderosa pine to have similar growth in both biochar-amended and peat control containers.

In this nursery study, biochar from a mixed conifer feedstock was used as an amendment to a peat-based media to grow interior Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco *var. glauca* (Beissn.) Franco) seedlings for forest regeneration. Douglas-fir is a valuable commercial species that is regularly grown in forest nurseries. We examined the effects of biochar and fertilizer treatments on morphological and physiological seedling quality during a nine-month growing period. An accompanying paper (Sarauer and Coleman 2018) reports effects on Douglas-fir seedling biomass and photosynthetic capacity throughout the growth period.

2 Materials and methods

2.1 Growing media

The media consisted of varying proportions of biochar (Evergreen Forests Products biochar New Meadows, ID), peat-based medium (Metro Mix, SunGro, Agawam, MA), and controlled-release fertilizer (Osmocote Plus 15-9-12 NPK, 6-month release formula, Scotts Company, Marysville, OH). The peat had a much lower surface area than the biochar used in this experiment (Sarauer and Coleman 2018). Half of the biochar received a pretreatment of 100 mg N L^{-1} of Peters Professional Soluble Plant Food 20-20-20 (The Scotts Company LLC, Marysville, OH) (pre-treated) while the other half did not (untreated). All biochar was rinsed, agitated with equal volumes of water, and gravity drained through paper filters. All biochar went through the rinsing, agitating, and draining cycle three times. Pretreatment of biochar resulted in the pre-treated biochar having greater N content than the untreated biochar (Sarauer and Coleman, 2018). Biochar rates were 0, 25, or 50% by volume. The controlled-release fertilizer was applied at three rates: full rate (0.790 g N L^{-1}), half rate (0.395 g N L^{-1}), and quarter rate (0.198 g N L^{-1}), based on product recommendations and no other fertilizer was applied. Growing medium components were mixed for 10 minutes in a 170 L cement mixed and then manually added to 45-cell Styroblock containers

(Stuewe and Sons, Tangent, OR). Each treatment filled two, 45-cell Styroblock containers, resulting in 90 seedlings per treatment. Treatments were applied in a full factorial design resulting in a total of 18 treatments (2 pre-treatments x 3 biochar rates x 3 fertilizer rates). Sarauer and Coleman (2018) report media analyses for pH and nutrient concentrations with pH and extractable P being the most notable. Media pH increased with biochar rate but depended on fertilizer treatment. No fertilizer effect on media pH was observed at 50% biochar while the fertilizer effect was most apparent without biochar addition. Extractable P concentration was negatively correlated with pH so less extractable P was available in biochar treatments compared with no biochar additions. Greater extractable P was available at the highest fertilizer rate without biochar, but the opposite response to fertilizer occurred at 50% biochar.

2.2 Seedlings and nursery conditions

In March 2016, interior Douglas-fir seedlings (Coeur d'Alene Indian Reservation, 800 m above sea level) were sown in 340 ml cells in 45-cell Styroblock containers, thinned to the single, most-vigorous germinant per cell, and grown under standard light and temperature conditions at the University of Idaho Pitkin Forest Nursery (Moscow, ID, 46.7254° N, 116.9560° W). Containers were placed on one bench in the greenhouse and were rearranged monthly to minimize any potentially confounding effects. Irrigation frequency was based on container weights (Dumroese et al. 2015). Designated weight containers were randomly chosen after each rearrangement from the 25% biochar rate Styroblocks. Once the designated Styroblocks contained 85% of their initial weights, all Styroblock containers were irrigated via overhead injection. In September 2016, once budset occurred, watering was reduced to 70-75% of initial weight.

2.3 Cold hardiness

Cold hardiness was measured in early December 2016 on Douglas-fir needles using the freeze-induced electrolyte leakage (FIEL) method (Colombo et al. 1984) on four seedlings per treatment. Ten needles were cut into 1 cm segments and put into 20 ml vials filled with 1 ml deionized (DI) water. Vials were put into a programmable freezer (Lo-Cold, Scientemp Corp., Adran, MI) and the temperature was lowered and held at each of six set points (2°C (control), -7°C, -14°C, -21°C, -28°C, -35°C, -40°C). After two hours at each set point, subsamples were removed and stored at 2°C. After freezing, the volume in each vial was brought to 10 ml and vials were put on an orbital shaker (Model 361, Fisher Scientific, Waltham, MA) for one hour. After shaking, electrical conductivity (EC) of the needle solution was measured using a conductivity meter (Seven compact S230, Mettler Toledo, Switzerland). To determine maximum electrolyte leakage, vials were then autoclaved for 20 minutes, cooled to room temperature, placed on the orbital shaker for one hour, and then measured again for EC. The proportion of electrolytes released due to freezing compared with total post-autoclave electrolytes released was calculated (Colombo et al. 1984). The index of Injury (I_T), the measurement of cold hardiness was calculated on a percentage basis according to (Flint et al. 1967). The I_T values were compared across treatments. A lower I_T indicates lower electrolyte leakage or greater cold hardiness of the needles.

2.4 Measurements

Seedlings were removed from the styroblocks in late December 2016 and stored at -2°C for three months. Seedlings were then thawed for one week and the growing medium was removed from the roots by washing. Seedlings were measured for height to the nearest 0.1 cm and root collar diameter to the nearest 0.1 mm. After morphological measurements, seedlings were put into an aeroponic chamber where they were misted with water every five minutes for 16 days. Temperature inside and outside the aeroponic chamber was 20°C. Artificial lighting created a 14-hour photoperiod. After 16 days, newly elongated white roots greater than 1 cm were counted. Seedlings were classified based on root growth potential index classes: 0 = no new root growth, 1 = some new roots, but none > 2 cm long, 2 = 1 to 3 new roots > 1 cm long, 3 = 4 to 10 new roots > 1 cm long, 4 = 11 to 30 new roots > 1 cm long, 5 = more than 30 roots > 1 cm long (Burdett 1978).

2.5 Statistical analysis

The effect of biochar pre-treatment, biochar rate, fertilizer rate, and their interactions on seedling quality were analyzed using the generalized linear mixed model (GLIMMIX) with SAS Software version 9.4 (SAS Institute Inc, Cary, NC). Type III tests of fixed effects were used to examine main effects and their interactions for each model. Differences were considered significant at $p \leq 0.05$. If a significant effect was found, Tukey-Kramer tests were performed for multiple comparisons. To meet normality and homoscedasticity assumptions for analysis of variance, cold hardiness data were log transformed for statistical analyses. Correlations of log transformed root count with diameter and with height were analyzed using PROC CORR with SAS Software version 9.4 (SAS Institute Inc, Cary, NC).

3 Results

3.1 Cold hardiness

The I_T values at -40°C (I_T40) of seedlings grown with pre-treated biochar did not differ among fertilizer rates, while the I_T40 of those grown with untreated biochar was 35% higher among seedlings given the lowest fertilizer rate compared to those given the highest fertilizer rate (Table 1, Figure 1). The I_T40 was also influenced by biochar rate and averaged 37% higher for seedlings grown with 0% biochar compared with those grown with 25% or 50% biochar (Table 1, Figure 2). Similar trends among treatment were observed at other test temperatures (Table S1). The temperature of 50% index of injury, a common measure of plant cold hardiness known as LT_{50}, was not reached at our lowest test temperature and, therefore, could not be used to compare treatments.

Table 1. P-values of the tested effects of biochar pre-treatment (T), biochar rate (B), and fertilizer rate (F) for seedling quality. Boldface indicates significance at *p* ≤ 0.05.

Effect	I_T (-40°C)	RGP Index	Height (cm)	Diameter (mm)
T	0.36	**0.01**	0.37	0.51
B	**<0.01**	**<0.01**	**<0.01**	**<0.01**
F	0.09	**<0.01**	**<0.01**	**<0.01**
TxB	0.13	0.62	0.94	0.40
TxF	**0.01**	0.19	0.19	**0.04**
BxF	0.10	**<0.01**	**<0.01**	**<0.01**
TxBxF	0.87	0.38	0.07	0.67

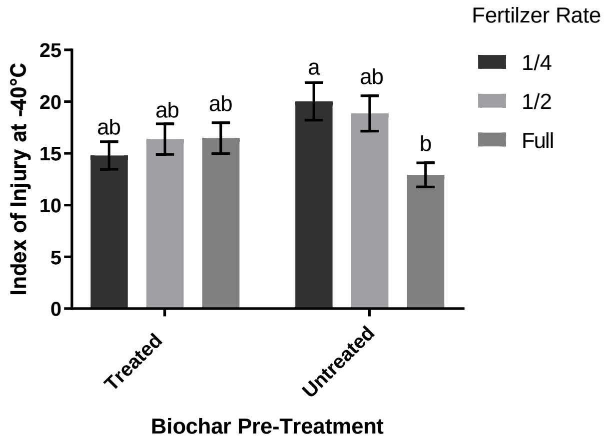

Figure 1. Index of Injury (I_T) at -40°C (I_T40) of Douglas-fir seedling needles in response to biochar treatment and fertilizer rate. Bars represent standard error, n=30. Bars having the same letter above are not significantly different (α = 0.05).

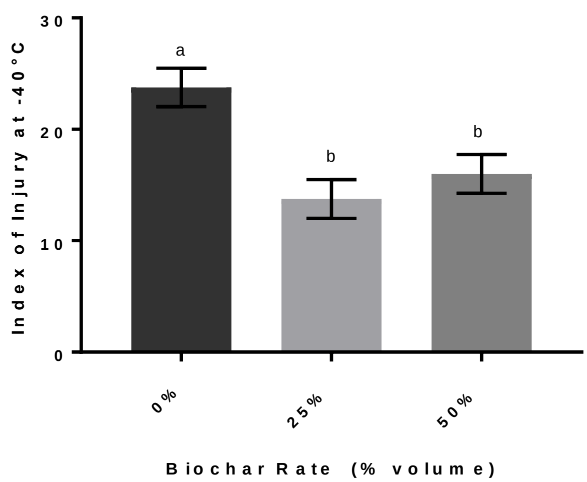

Figure 2. Index of Injury (I_T) at -40°C (I_T40) of Douglas-fir seedling needles in response to biochar rate. Bars represent standard error, n=60. Bars having the same letter above are not significantly different (α = 0.05).

3.2 RGP index

RGP averaged highest with the highest fertilizer rate in both the 0% and 25% biochar rates (Table 1, Figure 3). Additionally, seedlings grown in pre-treated biochar had 6% higher RGP Index than seedlings grown in untreated biochar (Table 1).

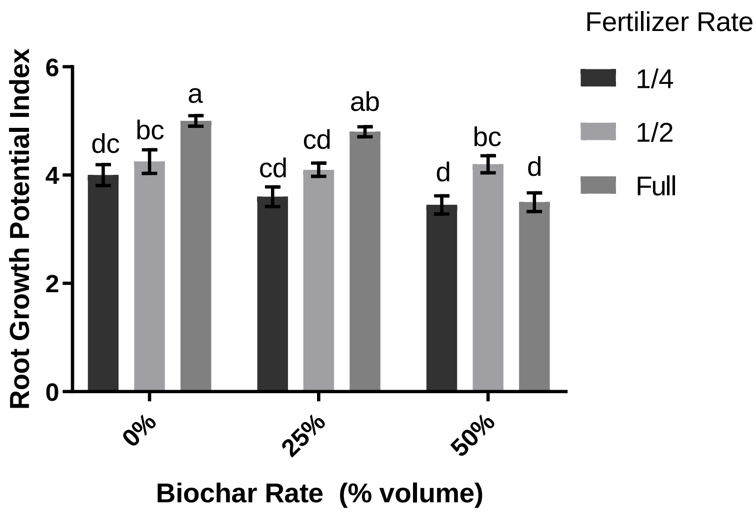

Figure 3. Root Growth Potential Index of Douglas-fir seedlings in response to biochar rate and fertilizer rate. Bars represent standard error, n=20. Bars having the same letter above are not significantly different (α = 0.05).

3.3 Root count

The number of new roots longer than 1 cm was positively correlated with stem diameter (r^2=0.53, Figure 4) and the slopes of the lines were significantly different for each biochar rate (p=0.034, Figure 4a), for biochar pre-treatment type (p=0.04, Figure 4b), but not for fertilizer rate (p=0.07, Figure 4c). There was also a significant correlation between number of new roots and height (r^2=0.45, data not shown). The slopes of the lines for height did not differ by biochar rate (p=0.087) or biochar pre-treatment type (p=0.08), but did differ for fertilizer rate (p=0.0008).

3.4 Seedling morphology

Height tended to increase with increasing fertilizer rates, and within each fertilizer rate, height decreased with increasing biochar rate (Table 1, Figure 5). For seedlings grown with 0% biochar, seedlings in the highest fertilizer rate compared with the quarter rate. The fertilizer effect lessened at the 25% and 50% biochar rates.

Seedlings grown with treated biochar had equivalent stem diameters at the full and half fertilizer rates, yet those were significantly greater than the quarter rate. However, among seedlings grown with untreated biochar, stem diameter decreased significantly between each fertilizer rate (Table 1, Figure 6a). Similar to height, stem diameter tended to increase with increasing fertilizer rates (Table 1, Figure 6). Additionally, stem diameter tended to decrease with increasing biochar rates (Table 1, Figure 6b).

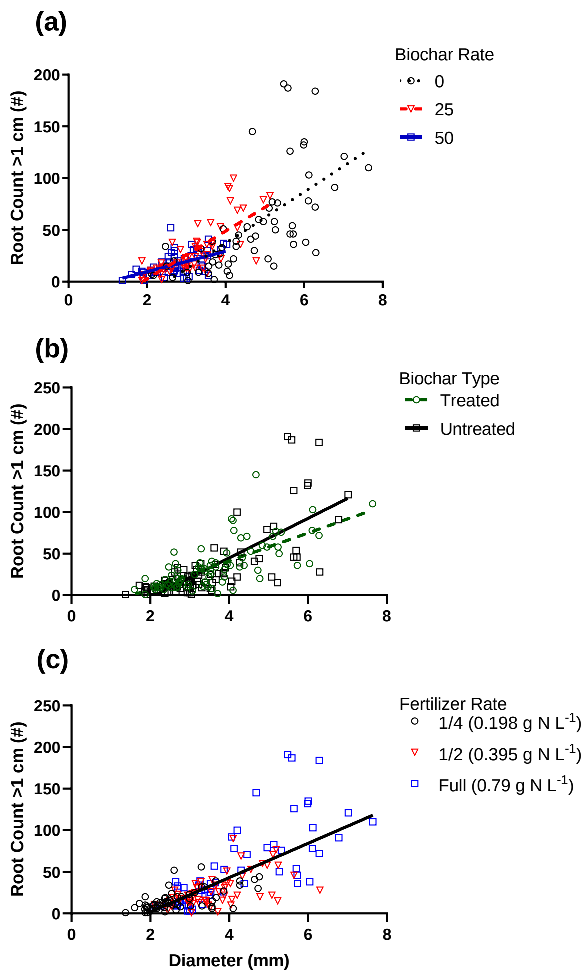

Figure 4. Root count as a function of diameter determined by biochar rate (a), biochar type (b), and fertilizer rate (c). The slopes of the lines significantly differ among biochar rates and biochar type, but not for fertilizer rate.

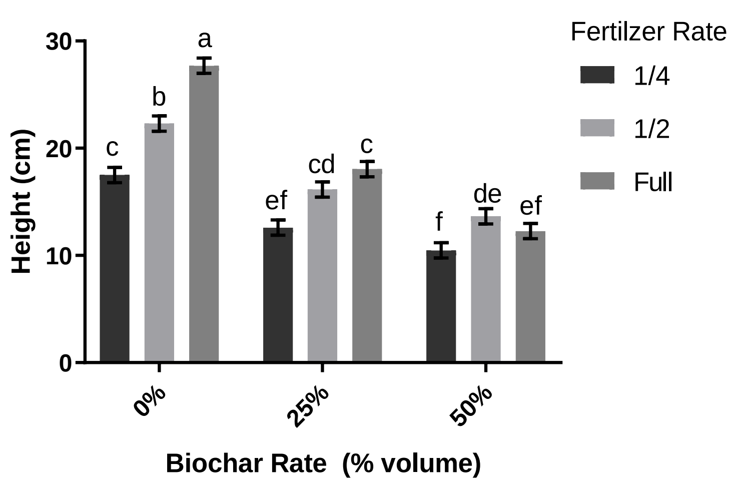

Figure 5. Height (cm) of Douglas-fir seedlings in response to biochar rate and fertilizer rate. Bars represent standard error, n=20. Bars having the same letter above are not significantly different (α = 0.05).

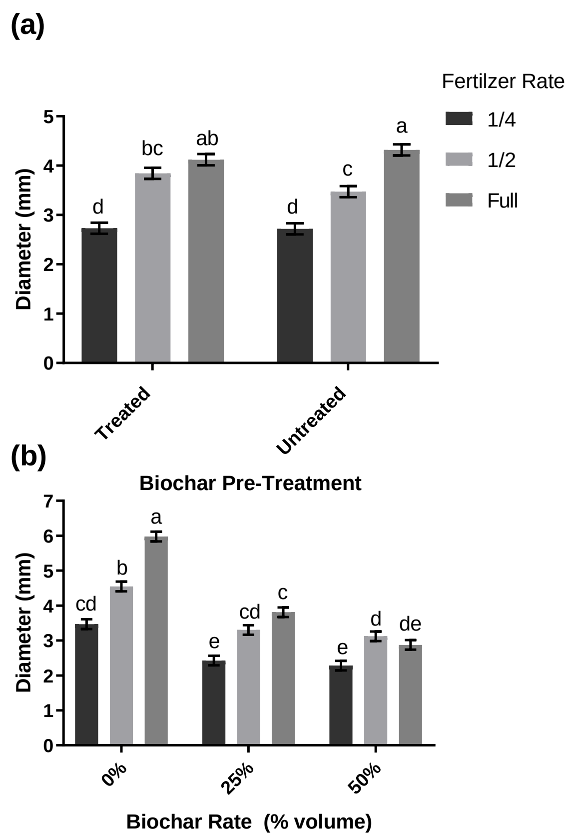

Figure 6. Diameter (mm) of Douglas-fir seedlings in response to (a) biochar treatment and fertilizer rate and (b) biochar rate and fertilizer rate. Bars represent standard error, n=30 for (a) and n=20 for (b). Bars having the same letter above are not significantly different (α = 0.05).

4 Discussion

Despite similarities between biochar and peat in nutrient and water holding capacity, amending growing media with biochar did not produce high-quality Douglas-fir seedlings in this study. Morphologically, seedlings grown in biochar were smaller than seedlings grown without biochar. Although cold hardiness slightly improved with biochar addition (Figure 1), biochar did not improve RGP.

4.1 Seedling morphology as influenced by biochar

Biochar decreased Douglas-fir height and stem diameter in our study. Sarauer and Coleman (2018) concluded that the most likely cause of reduced growth of Douglas-fir seedlings grown in the Evergreen Forest Products biochar was unfavorable conditions in the biochar-amended media. The pH of the amended media was 7.5 for pre-treated biochar and 7.7 for untreated biochar, which is higher than the range of 5.2-6.2 where conifers grow best (Binkley and Fisher 2013). The increased pH was correlated with decreased P availability attributable to calcium fixation reactions (Lucas and Davis 1961). Phosphorus deficiency can limit photosynthesis in conifers (Ben Brahim et al. 1996) and therefore growth. This illustrates the challenges of comparing different growing media because each may require specific culturing regimes to optimize their efficacy for growing seedlings.

Stem diameter increased with fertilizer rates, particularly for seedlings grown in the absence of biochar or with biochar that had not been pre-treated with fertilizer. The lack of stem diameter response to full versus half fertilizer rates within treated biochar could be due to the pre-treatment providing extra nutrients. Pre-treating biochar increases nutrient availability due to more functional chemical groups increasing electrostatic charge for cation binding (Joseph et al. 2013). Treated biochar had 2.9 mg N g^{-1} while untreated biochar had 1.8 mg N g^{-1} after biochar pretreatment (Sarauer and Coleman, 2018). Untreated biochar tends to adsorb NH_4^+ and NO_3^-, potentially decreasing N availability (Clough et al. 2013).

4.2 Cold hardiness and biochar

Cold hardiness was measured in late fall when seedlings had hardened-off following the growing season. Cold hardiness is lowest during the growing season (Burr 1990) and a hardiness increase is triggered by shorter photoperiod and cooler temperatures (Beck et al. 2004). Sakai and Weiser (1973) found interior Douglas-fir from Idaho (seeds native to this experiment's location) and Colorado to withstand temperatures of -30 to -50°C, and leaf tissues were not injured at -40°C. It has been shown that conifers in the boreal region and in the Rocky Mountains can withstand temperatures as low as -80°C (Sakai and Weiser 1973).

In this experiment, biochar may have influenced cold hardiness in Douglas-fir seedlings. Cold hardiness of rice seedlings also increases when seedlings are treated with high concentrations of biochar leachate containing the organic molecule 6-(Methylthio) hexa-1, 5-dien-3-ol, which functions as an activator protein ligand to encourage cold resistance functions in the rice seedlings (Yuan et al. 2017). Even though the organic molecules are found on biochar's surface and can be washed off when rinsed, rinsing may not have removed all organic molecules. This type of residual molecule could have resulted in increased cold hardiness in Douglas-fir seedlings in our study.

Increased cold hardiness in response to fertilizer in seedlings grown with untreated biochar could be due to increased N availability, which has been shown to increase cold hardiness in some studies (Taulavuori et al. 2014). Similar increases in cold hardiness with fertilization have been observed in container-grown *Pinus palustris* Mill. seedlings (Davis et al. 2011), containerized *Picea mariana* (Mill.) Britton, Sterns & Poggenb. seedlings (Bigras et al. 1996), and containerized *Eucalyptus globulus* Labill cuttings (Fernandez et al. 2007). Pre-treated biochar may have added enough N to negate fertilizer rate effects on cold hardiness.

4.3 Root growth potential and biochar

For the 0% and 25% biochar treatments, root growth potential declined as fertilizer rate decreased, which could be due to an associated reduction in photosynthate and stored carbohydrates. Thompson and Puttonen (1992) suggest that a reduction in current photosynthate could result in a lower RGP as they found a correlation between carbon allocation to roots and an increase in the number of new roots in Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) seedlings. In our study, a trend of declining photosynthesis rates with decreasing rates of biochar and fertilizer was evident, but only during the active growth phase (Sarauer and Coleman 2018). The photosynthate produced during active growth could have been stored for future use.

Seedlings grown in pre-treated biochar had greater RGP, which may be due to the nutrient status of the treated biochar. Pre-treating biochar improves nutrient availability (Joseph et al. 2013) since biochar tends to adsorb NH_4^+, which can decrease available N by saturating exchange sites (Clough et al. 2013). Without pre-treatment saturation, nutrients added with fertilizer may be less available.

Seedlings that were taller and that had larger stem diameters also produced more new roots (> 1 cm in length) in this study, which corresponds to the documented correlation between seedling size and RGP (Ritchie 1984). Thus, it is possible that seedlings grown with 0% biochar and given the highest fertilizer rate had higher RGP simply because they were larger with improved physiological condition. It is interesting that seedlings grown in 25% biochar with the highest fertilizer rate had the same RGP Index value (5) as those grown with 0% biochar, even though seedlings treated with 25% biochar were significantly smaller in both height and diameter. For a given seedling diameter, RGP was marginally improved in moderate biochar amendment. Even though the 25% biochar seedlings were smaller, biochar could have increased the rhizosphere microbial diversity and stimulated plant systemic defense (Kolton et al. 2017), which could have resulted in more root growth. Or, root growth could have been stimulated in the biochar amended seedlings due to recalcitrant organic compounds from the biochar interacting with the roots to stimulate plant growth (Kolton et al. 2017). Alternatively, removing biochar from the roots (roots were washed before seedlings were put into the aeroponic chamber) could have resulted in increased RGP of biochar-amended seedlings. Being in a biochar-free environment, without high, growth inhibiting pH conditions, could have stimulated root growth, even though height and diameter were smaller. These possible explanations for increased RGP at a given diameter suggest future investigations that were beyond the scope of the current study.

The RGP Index values indicate that seedlings in all treatment combinations were able to grow new roots in RGP testing. Most treatments in this study produced seedlings with high RGP Indexes, ranging from 3 to 5. An RGP Index value of 3 indicates that the seedling has 4-10 new roots greater than 1 cm in length, suggesting the seedlings will have high field survival rates because they had more than five new roots (Simpson et al. 1994). Even though biochar seedlings were smaller with relatively low RGP, it is likely they would produce new roots in the field.

5 Conclusions

For biochar to improve Douglas-fir seedling establishment using containerized stock in the Pacific Northwest, negative growth impacts from the biochar must be overcome. Root growth potential was higher in biochar amended seedlings for a given seedling size and if we are able to grow equivalently sized trees in biochar then we should expect to see improved RGP and increased outplanting success. We attribute positive impacts of biochar on cold hardness to favorable nutrition in pre-treated biochar. If biochar can help maintain or improve nutrition, then we would expect greater cold hardiness to result. It will be necessary to produce biochar with favorable pH or treat alkaline biochar to create favorable pH to result in equivalently sized Douglas-fir seedlings to those grown with only peat-based growing media. We expect improved soil reaction to improve seedling nutrition and therefore greater cold hardiness and RGP. For biochar to benefit conifer reforestation it will be necessary to understand the effect that different types of biochar have on seedling growth, physiology, and the measures of seedling quality. Types of biochar considered should include those prepared from different biomass feedstocks, pyrolysis conditions, or pre-treatment approaches.

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